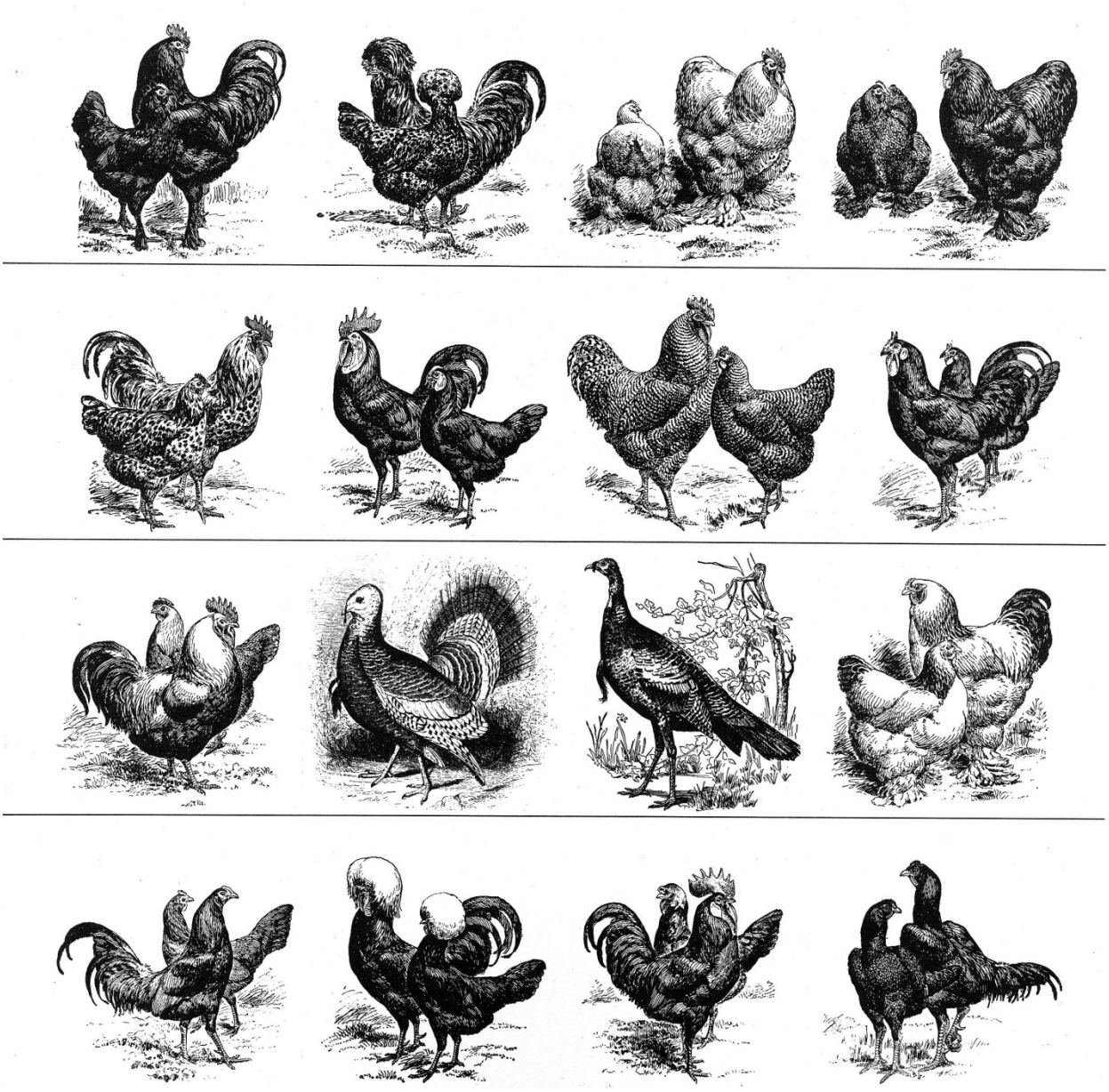
PROCEEDINGS OF THE SIXTY-EIGHTH WESTERN POULTRY DISEASE CONFERENCE
April 3 to 6, 2019
Puerto Vallarta, Jalisco, Mexico

MEMORIAS DE LA XLIV CONVENCIÓN ANUAL ASOCIACIÓN NATIONAL DE ESPECIALISTAS EN CIENCIAS AVÍCOLAS
3 al 6 de abril de 2019
Puerto Vallarta, Jalisco, México



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EL GUAJOLOTE DORADO (ANECA)

HÉCTOR MANUEL CERVANTES CAMBEROS

Nacido en la Ciudad de México, cuenta con el título de médico veterinario zootecnista de la UNAM. Hizo su maestría en la Universidad de Georgia, además de haber revalidado su título en EUA y de haber cursado otros estudios en Medicina Avícola en la Universidad de Estatal Carolina del Norte. Es diplomado, Ex-Presidente y miembro reconocido del Colegio Estadounidense de Veterinarios Avícolas y también Ex-Presidente de la Asociación Americana de Patólogos Aviares (AAAP) y el primer Presidente de un país de habla española.

En la actualidad es Profesor Adjunto de Medicina Avícola en las Escuelas de Medicina Veterinaria de las Universidades de Georgia y Estatal de Carolina del Norte, además de gerente senior de servicios veterinarios avícolas de Phibro Animal Health. Ha trabajado en Pfizer, Peterson Farms, Champion Agri-International, Cargill y Ralston Purina en EUA y México.

Además de recibir numerosos premios en EUA y Latinoamérica, ha presentado unos 135 trabajos como conferencista invitado en EUA, Latinoamérica y otras partes del mundo, además de más de 80 resúmenes y trabajos en línea. Su mayor contribución ha sido la invención del método de Cervantes, para evaluar de manera más objetiva y uniforme la calidad del pollito recién nacido, que incluye los parámetros físicos y microbiológicos más importantes, adoptado en la mayor parte de las empresas latinoamericanas.

Hector was born in Mexico City and received his veterinary medicine degree from UNAM. He received his master's degree at the University of Georgia besides reactivating his US accredited veterinary licensure. He also took additional courses in avian medicine at North Carolina State University. Dr. Cervantes is a member and Diplomate of the renowned American College of Poultry Veterinarians, and served as its president. Hector also served as president of the American Association of Avian Pathologists (AAAP), being the first AAAP president from a Spanish-speaking country.

Besides being Senior Manager of Poultry Veterinary Services at Phibro Animal Health, Dr. Cervantes is also an adjunct professor of avian medicine in both the School of Veterinary Medicine at the University of Georgia and School of Veterinary Medicine at North Carolina State University. Hector has worked for Pfizer, Peterson Farms, Champion Agri-International, Carghill, and Ralston Purina both in the US and Mexico.

Dr. Cervantes has received numerous awards in the US and Latin America; has given some 135 presentations as an invited speaker in the US, Latin America, and other parts of the world; and has given more than 80 online presentations. His greatest contribution has been the development of the "Cervantes Method" of objectively and uniformly evaluating the quality of newly hatched chicks, integrating the most important physical and microbiological parameters. This method has been adopted by the majority of Latin American poultry enterprises.

68th WPDC SPECIAL RECOGNITION AWARD

GREGG J. CUTLER

Dr. Gregg Cutler was born in Los Angeles, CA. His grandparents settled in LA after emigrating from Eastern Europe in about 1911. His paternal grandfather established an egg distribution business called Covina Egg Farms. He traveled to small producers then sold the eggs to restaurants and small grocery stores.

As a small child, Gregg remembers going with his grandfather or father or uncle to pick up eggs. Gregg also remembers being fascinated by the chickens - especially the dead ones. As the San Fernando Valley had become too developed for chicken farms, the operation was moved to Moorpark and the brothers entered into an agreement with S&K in Moorpark. After a few years, Julius Milling started to expand into what became Julius Goldman's Egg City. Gregg's father, Bernard, worked at Egg City, where he handled all sales and marketing until his retirement.

As he studied a UC Santa Barbara and Ventura College, Dr. Cutler changed his mind about a major. Originally, he was going to study marine biology. While working at a summer camp, he met a girl named Jody who wanted to become a veterinarian. She was a zoology major at UC Davis. That occupation sounded like a good idea so he tried it out by working in a mixed practice after school and weekends. After Ventura College, Gregg transferred to UC Davis as a zoology major. The girl never made it to veterinary school but became his wife of nearly 50 years.

After receiving his AB in zoology, Gregg went to graduate school in avian nutrition as a graduate student of Dr. Fred Hill. During that time he also worked in the avian physiology lab of Dr. Ray Berger. At the end of that first year in graduate school, he was admitted to veterinary school at UC Davis.

After Dr. Cutler graduated from veterinary school, he practiced for over a year in the mixed practice he had worked for as an undergraduate. They treated anything non-human that walked, crawled, swam, slithered, or flew. After a little more than a year, Dr. Cutler received a call from Dr. Henry Adler. There was going to be a residency in poultry at Davis - was he interested? He couldn't say yes fast enough. Because Jody had a great job and they had just bought a house, Dr. Cutler commuted every week to Davis for almost two years. Dr. Dick McCapes supervised his residency. He worked closely with Drs. Art Bickford and Yan Ghazikhanian, and shared an office with Dr. Ray Bankowski. The field time introduced him to the Northern California Poultry Industry. By 1977, Dr. Cutler had completed a residency and a MPVM.

After the completion of his time in Davis, Dr. Cutler went to work at Egg City as a staff veterinarian. Working with Dr. John Allen, he was responsible for bird health and care in addition to food safety of processed egg product. The lab made some of their own vaccines and did most of their own diagnostic testing.

Dr. Cutler left Egg City in 1980 and worked as the Western Regional Veterinarian for DeKalb Ag Research. He provided technical service, customer training and supervised the vaccine manufacturing plant. During that time he met most of the Southern and Central California producers. In 1983-84, Dr. Cutler was president of WPDC.

By 1985, Egg City had undergone several ownership changes. Dr Allen had died and the ranch needed a veterinarian and an experienced manager. Dr. Cutler became Executive Vice President/ COO. He was responsible for

all operations of the 3.5 million bird integrated egg producer, two million per year hatchery and rearing, five hundred ton-per-day feed mill and egg processing and breaking plants.

In 1988, Dr. Cutler founded his consulting practice for poultry medicine, Cutler Associates International. The four veterinarians are involved with poultry health, management, food safety, and animal welfare for their many clients. During this time, Dr. Cutler became a Diplomate of the American College of Poultry Veterinarians and in 1996-97 served as president of AAAP.

In addition to his practice, Dr. Cutler has served on the American Veterinary Medical Animal Welfare and Legislative Committees and currently serves in the House of Delegates. He also continues to serve on the California Animal Health and Food Safety Board, which he has since its inception in 1986.

In his spare time, Gregg and his wife restore and drive antique carriages. They also officiate in carriage driving shows all over the United States. Not long ago, Dr. Cutler fulfilled a lifelong dream to become a private pilot. He has earned his license and is working on an instrument rating. The UC Davis School of Veterinary Medicine recently awarded Dr. Cutler the Alumni Achievement Award for his recognized leadership and dedicated service to protecting the health and safety of poultry in California, the United States, and internationally.

We are extremely proud to present the 68th Western Poultry Disease Conference Special Recognition Award to Dr. Gregg J. Cutler. We wish him well in his continued dedicated involvement in the poultry industry, community, and family.

IN MEMORIUM

CRAIGMYLE RIDDELL

October 19, 1933 – November 18, 2018

Born and raised in Scotland, Craig first attended St. Andrew's University graduating with a BSc in General Science in 1954. He and his wife, Pat, then immigrated to Canada where he later completed his DVM (honors) at the Ontario Veterinary College in Guelph, Ontario. After graduation, he worked as a large animal practitioner in Alberta, then relocated to Ontario where he worked as a diagnostic pathologist and a veterinarian at a poultry breeding company. He continued his education completing a MSc in Veterinary Pathology at the University of Connecticut in 1967 and PhD at the Western College of Veterinary Medicine (WCVM), University of Saskatchewan in 1975.

During his PhD studies at the WCVM, Craig was recruited as an assistant professor in the Department of Veterinary Pathology in the same institution, and received tenure in 1977. Along with teaching both agriculture and veterinary medicine undergraduate and graduate students, he was actively involved in the WCVM, volunteering his time and expertise to various committees including strategic planning, college review, audio visual, research task force, animal resources, admissions, awards, library, tenure, student-faculty, and electron microscopy. He also served on the University Committee on Animal Care and Supply. Even after his retirement, Dr. Riddell regularly visited the department to converse with old colleagues and former students and attended noon hour seminars presented by current research and diagnostic pathology students.

Not only dedicating his time to the WCVM, Dr. Riddell was also a founding member of the Saskatchewan Poultry Extension Program in 1978 – a program first of its kind and to this day the only of its kind in Canada. This program, initially funded by the Saskatchewan government, was a two-year trial period monitoring flock performance, identifying problem areas, extension and field work to alleviate problems. The trial period was a success, and after 41 years the now-industry funded program is still in operation.

In addition to his teaching, college and extension services, research was an important aspect of his career, mainly poultry diseases with special reference to leg weakness and nutritional pathology. Publishing over 96 peer-reviewed papers, he received the Research Paper Award for best research paper in *Avian Diseases* (1976) and the PP Levine Award for Best Paper Published in *Avian Disease*s (1984). He authored a handbook on Avian Histopathology while on sabbatical at the University of Georgia, conducted research on poultry vaccination, bone and liver diseases at the Houghton Poultry Research Station, England and the Victorian Institute of Animal Science, Victoria, Australia, and worked at the diagnostic laboratory in San Bernardino, California during the course of his other sabbatical leaves.

Sharing his research and knowledge was important to Dr. Riddell. He was on the American Association of Avian Pathologists editorial boards, as well as other associations such as the Saskatchewan Poultry Industry Council, Western Meeting of Poultry Clinicians and Pathologists, Western Conference of Veterinary Diagnostic Pathologists, and Non-Ruminant Committee of Farming for the Future. He was a large contributor to the Western Poultry Disease Conference, serving as proceedings editor, program chair and president, as well as contributing oral presentations.

Craig and Pat kept busy with their two daughters, Morag and Cara, coaching their soccer teams, teaching them to ski, Pony Club, or recitals. Later, he welcomed a grandson, Keiran. He loved the outdoors, skiing, camping and hiking, so much that he hiked the Continental Divide in Alberta and the British Isles. Outdoor trips were enjoyed by all who joined him, being an avid geologist, he was always keen to point out interesting facts about the landscape. After retirement, he served as councillor for the Rural Municipality of Corman Park and trained border collies to work with sheep. Being a true Scotsman, he loved loud bagpipes. Craig left an enormous legacy and will be missed by all who knew him.

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68th WPDC CONTRIBUTORS LIST

(As of March 21, 2019)

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Poultry Health Services, Ltd.
Airdrie, AB, Canada

SUSTAINING MEMBERS

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Masakazu Matsumoto, DVM, PhD
Corvallis, OR

Richard Yamamoto, PhD
Davis, CA

SPECIAL ACKNOWLEDGMENTS

The 68th Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions and support to the Conference. The financial contributions provide support for outstanding presentations and to help pay for some of the costs of the Conference, thus helping us to maintain a relatively low registration fee for an international conference. Many companies and organizations, including some that also contribute financially, send speakers at no expense to the Conference. We thank all these people, and acknowledge their support and contribution.

Once again, the WPDC is forever grateful to our distinguished contributors and supporters of the conference who are vital in making the conference a success. All our contributors and supporters are listed on the following pages. We greatly appreciate their generosity and sincerely thank them and their representatives for supporting this year's joint meeting of WPDC and ANECA.

We thank Sarah Mize for her enthusiastic willingness to accept the position of Program Chair for this 68th WPDC meeting, which is being jointly held with the XLIV Annual ANECA Convention. Dr. Mize would like to thank the executive committee and Felicia Pohl for their extraordinary support.

The Executive Committee of the Western Poultry Disease Conference extends a heart-felt thanks to the ANECA group-in-charge of organizing this meeting and serving as local arrangement coordinators. Deserving special recognition include Maritza Tamayo and Julio Arellano Rodriguez. And a special thanks to all those working for ANECA of whom we may not even be aware. Thank you so very much!

Many have provided special services that contribute to the continued success of this conference. For this year's joint meeting, the WPDC and ANECA have contracted CIC mundiales for providing accommodation and registration support as well as the web site for the conference. We thank Dr. David Frame for editing and producing another outstanding Proceedings of this meeting. Dr. Frame is indebted to Mr. Dana Frame for his meticulous proofreading and formatting of the Proceedings for publication. We acknowledge Bruce Patrick (Graphic Communications, Brigham Young University) for providing the illustrations appearing on the opening page. We express our gratitude to all authors who submitted manuscripts, and are especially appreciative of those who submitted their manuscripts on time.

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68th WPDC PROCEEDINGS

Please note that the proceedings of the 68th Western Poultry Disease Conference are not refereed, but are presented as a service and a source of information to those attending the conference and to others who wish to gain some insight as to the information presented.

The proceedings of the 68th WPDC/XLIV ANECA are available in electronic format only. They can be downloaded from the American College of Poultry Veterinarians website (www.acpv.info).

WESTERN POULTRY DISEASE CONFERENCE (WPDC) HISTORY

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
1st WPDC – 1952		A. S. Rosenwald		
2nd WPDC – 1953	P. D. DeLay	A. S. Rosenwald		
3rd WPDC – 1954	C. M. Hamilton	Kermit Schaaf		
4th WPDC – 1955	E. M. Dickinson	W. H. Armstrong		
5th WPDC – 1956	D. E. Stover	E. E. Jones		
6th WPDC – 1957	D. V. Zander	H. E. Adler		
7th WPDC – 1958	H. E. Adler	E. E. Jones		
8th WPDC – 1959	R. D. Conrad	L. G. Raggi		
9th WPDC – 1960	L. G. Raggi	A. S. Rosenwald		
10th WPDC – 1961	A. S. Rosenwald	D. V. Zander		
11th WPDC – 1962	D. V. Zander	R. V. Lewis		
12th WPDC – 1963	R. V. Lewis	Walter H. Hughes		
13th WPDC – 1964	W. H. Hughes	Bryan Mayeda		
14th WPDC – 1965	B. Mayeda	R. Yamamoto		
15th WPDC – 1966	R. Yamamoto	David S. Clark (1st sign of Contributors)		
16th WPDC – 1967	D. S. Clark	Roscoe Balch		
17th WPDC – 1968	R. Balch	Richard McCapes		
18th WPDC – 1969	R. McCapes	Dean C. Young		
19th WPDC – 1970 4th Poultry Health Sym. (PHS)	D. C. Young	W. J. Mathey	1st combined WPDC & PHS	1st listing of distinguished members
20th WPDC – 1971 5th PHS	W. J. Mathey	Ramsay Burdett		
21st WPDC – 1972 6th PHS	R. Burdett	Marion Hammarlund		
22nd WPDC – 1973 7th PHS	M. Hammarlund	G. W. Peterson		
23rd WPDC – 1974 8th PHS	G. W. Peterson	Craig Riddell		
24th WPDC – 1975 9th PHS	C. Riddell	Ralph Cooper		
25th WPDC – 1976 10th PHS	R. Cooper	Gabriel Galvan		
26th WPDC – 1977 11th PHS	G. Galvan	Don H. Helfer	Hector Bravo	
27th WPDC – 1978 12 PHS	D. H. Helfer	Art Bickford		
28th WPDC – 1979 13th PHS	A. Bickford	J. W. Dunsing		
29th WPDC – 1980 14th PHS 5th ANECA	J. W. Dunsing Angel Mosqueda T.	G. Yan Ghazikhanian	P. P. Levine	
30th WPDC – 1981 15th PHS	G. Y. Ghazikhanian	Mahesh Kumar		
31st WPDC – 1982 16th PHS	M. Kumar	Robert Schock		
32nd WPDC – 1983	R. Schock	George B. E. West		
33rd WPDC – 1984	G. B. E. West	Gregg J. Cutler		
34th WPDC – 1985	G. J. Cutler	Don W. Waldrip		Bryan Mayeda

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
35th WPDC – 1986	D. W. Waldrip	Duncan A. McMartin	J. A. Allen	
11th ANECA	Jorge Basurto	Mario Padron	A. Tellez-G. Rode	
36th WPDC – 1987	D. A. McMartin	Marcus M. Jensen		
37th WPDC – 1988	M. M. Jensen	Barry Kelly	A. S. Rosenwald	
38th WPDC – 1989	B. Kelly	Masakazu Matsumoto		Louise Williams
39th WPDC – 1990	M. Matsumoto	Jeanne M. Smith		Dean Young
40th WPDC – 1991	J. M. Smith	Richard P. Chin	A. S. Rosenwald	
16th ANECA	Martha Silva M.	David Sarfati M.	A. S. Rosenwald	
41st WPDC – 1992	R. P. Chin	Rocky J. Terry	Marcus Jensen	Henry E. Adler * *(posthumous) R. A. Bankowski C. E. Whiteman
42nd WPDC – 1993	R. J. Terry	A. S. Dhillon	W. W. Sadler	Royal A. Bagley
43rd WPDC – 1994	A. S. Dhillon	Hugo A. Medina		G. B. E. West
44th WPDC – 1995	H. A. Medina	David D. Frame	W. M. Dungan* *(posthumous)	A. J. DaMassa Gabriel Galvan Walter F. Hughes W. D. Woodward R. Yamamoto
45th WPDC – 1996	D. D. Frame	Mark Bland	Don Zander	Pedro Villegas
21st ANECA	R. Salado C.	G. Tellez I.	M. A. Marquez	Ben Lucio M. Mariano Salem Victor Mireles Craig Riddell
46th WPDC – 1997	Mark Bland	James Andreasen, Jr.	Bryan Mayeda	Roscoe Balch Paul DeLay J. W. Dunsing Don Helfer D. E. Stover
47th WPDC – 1998	J. Andreasen, Jr.	H. L. Shivaprasad	W. J. Mathey	Marcus Jensen Duncan Martin
48th WPDC – 1999	H. L. Shivaprasad	R. Keith McMillan		
49th WPDC – 2000	R. K. McMillan	Patricia Wakenell	R. P. Chin	Ralph Cooper Robert Tarbell
50th WPDC – 2001	P. Wakenell	Ken Takeshita		Don Bell Art Bickford
51st WPDC – 2002	K. Takeshita	Barbara Daft	Hiram Lasher	Bachoco S.A. de C.V.
27 ANECA	J. Carillo V.	Ernesto P. Soto		Productos Toledano S.A.
52nd WPDC – 2003	B. Daft	David H. Willoughby		Roland C. Hartman
53rd WPDC – 2004	D. H. Willoughby	Joan Schrader		G. Yan Ghazikhanian
54th WPDC – 2005	J. Schrader	Stewart J. Ritchie	W.D. Woodward	R. Keith McMillan
55th WPDC – 2006	S. J. Ritchie	Peter R. Woolcock		M. Hammarlund
56th WPDC – 2007	P.R. Woolcock	Bruce Charlton	R. Keith McMillan	M. Matsumoto
57th WPDC – 2008	B. Charlton	Rocio Crespo	A. S. Rosenwald* *(posthumous)	B. Daft
33rd ANECA	M. A. Rebollo F.	Maritza Tamayo S.	A. S. Rosenwald*	Ernesto Ávila G.
58th WPDC – 2009	R. Crespo	Victoria Bowes		G.L. Cooper
59th WPDC - 2010	V. Bowes	Nancy Reimers		
60th WPDC - 2011	N. Reimers	Larry Allen		John Robinson
61st WPDC - 2012	L. Allen	Vern Christensen		
62nd WPDC - 2013	V. Christensen	Portia Cortes	Víctor Manuel Mireles M.	A. Singh Dhillon

YEAR	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
63rd WPDC – 2014 39th ANECA	P. Cortez Néstor Ledezma M.	Ernesto Soto Ernesto Soto	Hugo Medina Benjamin Lucio Martínez	
64th WPDC – 2015	Ernesto Soto	Shahbaz Haq	Bruce R. Charlton	David Willoughby
65th WPDC – 2016	S. Haq	Susantha Gomis		
66th WPDC – 2017	S. Gomis	C. Gabriel Sentíes-Cué	Richard McCapes	Peter Woolcock Richard Chin
67th WPDC – 2018	C.G. Sentíes-Cué	Rodrigo A. Gallardo		David D. Frame
68th WPDC – 2019 44th ANECA	R. Gallardo Ricardo Cuetos Collado	Sarah Mize Maritza Tamayo		Gregg Cutler

MINUTES OF THE 67TH WPDC ANNUAL BUSINESS MEETING

Western Poultry Disease Conference President, Dr. Gabriel Senties-Cué, called the meeting to order on Monday, April 16, 2018, at 5:15 PM, at the Marriott City Center, Salt Lake City, hotel. There were 25 people in attendance.

APPROVAL OF THE 66th WPDC BUSINESS MEETING MINUTES

The minutes of the 66th WPDC Business Meeting were reviewed by members of the Executive Committee during the Executive Committee meeting and recommended approval of the minutes as written. The minutes were approved.

ANNOUNCEMENTS

President Senties-Cué acknowledged all the contributors, in particular, Ceva Animal Health, which contributed at the Super Sponsor level, and the American Association of Avian Pathologists and Zoetis, which contributed at the Benefactor level. All the contributors were acknowledged and thanked for their generous support.

The efforts of the current WPDC officers were acknowledged for their work and participation in the organization of this year's meeting.

REPORT OF THE SECRETARY-TREASURER

Dr. Richard Chin presented the Secretary-Treasurer report. For the 2017 meeting, we had an income of approximately $69,117 and expenses of approximately $54,797, which resulted in a net gain of approximately $14,320. Consequently, this resulted in WPDC's balance with Conference & Event Services to be $66,818. Additionally, Rosy's old agency account within the UCD Vet School has $46,127. Therefore, WPDC has a total of $127,264 in reserves. For the 2018 meeting, Dr. Chin estimates that we should come out even. Finally, Dr. Chin noted that this was his last report as WPDC's secretary-treasurer and thanked the group for their support for the past 20 years.

REPORT OF THE PROCEEDINGS EDITOR

Dr. David Frame presented the Proceedings Editor report. There were 80 papers submitted for publication in the proceedings, and the process went smoothly. He thanked the authors for their timely submissions, noting that 50% of the papers were received prior to the due date.

WPDC continues to be grateful to the American College of Poultry Veterinarians for providing space on their website to host the WPDC proceedings. They are still working to upload all proceedings. As approved last time, all WPDC proceedings on the ACPV website are not password-protected, and open to the public.

WPDC EXECUTIVE COMMITTEE

Dr. Simone Stout was nominated for Program Chair for the meeting in 2020. There were no other nominations and Dr. Stout was elected unanimously as Program Chair-elect. The following officers were nominated for 2018-2019:

Program Chair: Dr. Sarah Mize
President: Dr. Rodrigo Gallardo
Past-President: Dr. Gabriel Senties-Cué
Contributions Chair: Dr. Richard Chin
Proceedings Editor: Dr. David Frame
Secretary-Treasurer: Dr. Rodrigo Gallardo
Program Chair-elect: Dr. Simon Stout

Nominations for all officers were closed and all nominees were approved unanimously.

FUTURE MEETINGS

2019: 68th WPDC (joint meeting with ANECA), Puerto Vallarta, Mexico, April 2-6, 2019 (Note that the dates were changed to earlier in the month.) ACPV will sponsor their workshop on April 2, 2019.

2020: 69th WPDC and ACPV-sponsored Workshop, Sacramento, CA, March 29 – April 1, 2020.

2021: 70th WPDC and ACPV-sponsored Workshop, Sacramento, CA (dates to be determined)

NEW BUSINESS

Dr. Chin stated that CE credits will be sent to every registrant from ACPV (Bob Bevans-Kerr). There were no additional items for discussion.

ADJOURNMENT

Dr. Gabriel Senties-Cué turned the presidency over to Dr. Rodrigo Gallardo who acknowledged and thanked all those involved in organizing this year's meeting. Dr. Gallardo adjourned the 67th WPDC annual business meeting at 5:33 PM.

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USO DEL INHIBIDOR CABERGOLINA DE LA PROLACTINA PARA DISMINUIR EL COMPORTAMIENTO DE ANIDACION EN PAVAS CRIOLLAS

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RESUMEN

Con el objetivo de evaluar el efecto de la cabergolina como alternativa contra la cloquez en las guajolotas criollas, se utilizaron 45 guajolotas criollas en cloquez: 34 (experimentales) que se les administró cabergolina y 11 (testigos) sin cabergolina; Se utilizaron 8 tratamientos en los cuales se administró vía oral .125 mg y .250 mg de cabergolina por aplicación por medio de 1 a 3 aplicaciones. Se revisó diariamente a las guajolotas si estaban de pie o echadas y la presencia de huevos. Los resultados indican que a las 4 semanas del experimento el 8.82% de las guajolotas tratadas retornaron al siguiente ciclo de postura, en comparación con 0% de las guajolotas sin tratar. Como conclusión, la administración de cabergolina representa una alternativa al problema de la cloquez en las guajolotas criollas pero son necesarios estudios posteriores en los cuales se utilice una mayor dosis o durante más días para obtener mejores resultados.

SUMMARY

With the objective of evaluating the effect of cabergoline as an alternative against nesting behavior in creole turkey hens, were used 45 creole turkey hens with incubation behavior: 34 (experimental) that were administered cabergoline and 11 (controls) without cabergoline. Eight treatments were used in which .125 mg and .250 mg of cabergoline were orally administered by application though 1 to 3 applications. The turkey hens were checked daily if they were standing or lying down and the presence of eggs. The results indicate that at the 4 weeks of the experiment, 8.82% of the treated turkey hens returned to the next layings cycle, compared to 0% of the untreated turkey hens. In conclusion the administration of cabergoline represents an alternative to the problem of behavior nesting in creole turkey hens, but further studies are needed in which a higher dose is used or for more days to obtain better results.

INTRODUCTION

After the laying of the egg, mediated by its genetics, environment and hormonal production, birds can manifest the nesting behavior, which is a natural instinct that makes them stay on the egg to incubate it, until the chick is born. This behavior is not desirable in the breeders in the intensive productions, since there is a decrease in the consumption of food and water, they resist to move from the nests, the ovulation stops, so the production of eggs is paralyzed (1). Consequently, it is tried to avoid, either by genetic selection that in recent years has managed to reduce considerably the nesting behavior in hens, or by the forced abrupt interruption, intervening at the beginning of the behavior, placing these birds in an inadequate environment for the incubation of eggs as gridded floors, individual cages, intense light, noise and frequent changes of site every 7 or 10 days while the use of hormones to avoid the nesting behavior has achieved discreet results (5).

The presence of nests and eggs inside are very important components in the induction and continuity of the incubation behavior. The turkey hens are optimal mothers, so much so those in rural productions are used to incubate not only the eggs of their species, but also others of different birds (5).

The predisposition and ease of certain breeds and strains to manifest the nesting behavior, is determined by the greater or lesser production of prolactin, which inhibits the production of follicle stimulating hormone, luteinizing hormone and turn there is a decrease in the levels of estrogen and progesterone stopping the production of eggs (1).

It has been observed that turkey hens identified as broody had higher plasma prolactin concentrations than females than if they were laying (2).

Evidence indicates that dopamine is the most important inhibitory factor of prolactin secretion in hens that are incubating (9). Bromocriptine is a dopamine agonist that stimulates receptors (D2) and has been used in hens resulting in a decrease in prolactin levels, decreasing the days of pause between laying sequences by improving egg production (7).

Cabergoline is also a dopamine agonist that is used to decrease prolactin in mammals (4). The cabergoline used in birds is only limited to reports in zebra finches, which were administered orally but without having an effect on prolactin plasma concentrations and without causing adverse effects (10).

We hypothesized that cabergoline would have the potential to inhibit the concentration of prolactin in creole turkey hens with nesting behavior.

MATERIALS AND METHODS

The test was developed from September to October 2018 with 45 creole turkey hens with nesting behavior, from a 52-week old flock maintained by the small species center of Ixtacuixtla Tlaxcala. It began with the identification and separation of the turkey hens with nesting behavior; for this, all the females were separated that in the mornings before opening the nests were lying under the nests or in the corners of the huts and those that at the beginning of the evening, were inside the nests without laying eggs. The day after we separated them, we checked that there were no eggs and that they were still lying in the corners of the house. Then, two groups were randomly formed: the experimental group that was integrated with 34 turkey hens and the control group composed of 11 turkey hens to which cabergoline was not administered. The females of the experimental group were numbered metal ring in the left tarsus as a means of identification. The treatments of the experimental group were formed in the following way:

Treatment 1: 3 females to which they were given 1 single application of 0.125 mg orally cabergoline (at day 1).

Treatment 2: 4 females to which they were given 2 applications with a one-day interval of 0.125 mg orally cabergoline (at day 1 and 3).

Treatment 3: 4 females to which were given 3 applications with a 1-day interval of 0.125 mg orally cabergoline (at day 1, 3 and 5).

Treatment 4: 3 females to which were given 3 applications (1 a day for days in a row) of 0.125 mg orally cabergoline (at day 1, 2 and 3).

Treatment 5: 5 females to which were given 1 single application of 0.250 mg orally cabergoline (at day 1).

Treatment 6: 5 females to which they were given 2 applications with a one-day interval of 0.250 mg orally cabergoline (at day 1 and 3).

Treatment 7: 5 females to which they were given 3 applications with a 1-day interval of 0.250 mg orally cabergoline (at day 1, 3 and 5).

Treatment 8: 5 females to which they were given 3 applications (1 a day for days in a row) of 0.250 mg orally cabergoline (at day 1, 2, 3).

The identification of the turkey hens that were raised was recorded daily and the presence of eggs was checked.

RESULTS

It was observed that the administration of cabergoline reduces the interval of return to the next cycle of posture. It can be seen that 8.82% of turkey hens treated with cabergoline returned to posture at 4 weeks, while those that were not administered all continued unproductive.

DISCUSSION

Studies have been conducted in hens using bromocriptine as an inhibitor of prolactin improving egg production and decreasing the days of pause between laying sequences (7). Our results suggest that the administration of cabergoline orally represents an alternative solution to the problem of nesting behavior in creole turkey hens, since 8.82% of treated turkey hens returned to posture.

The precocity with which the nesting behavior is identified and treated directly conditions the effectiveness of the operation and, consequently, the restarting of the laying (8); son that in the results obtained in the present study what could influence, is that some females probably had been in the nesting behavior for several days and by not identifying them early prolactin was at its maximum peak and therefore the result was that only 8.82% of treated turkey hens returned to the place instead of a higher percentage.

In order to combat the nesting behavior, it is advisable to move the animal out of its usual facilities, since apparently the stress caused by change of facilities decreases the concentration of prolactin with a return to the laying more quickly (6). It has also been shown that in cage turkey hens in nesting behavior 100% of these turkey hens returned posture at 5 weeks after the start of the test (3). What did not happen with the totality of females in the present study due to the fact that the turkey hens were kept in a usual facility for them.

Our results with cabergoline suggest that the doses used were low compared with studies with hens

with the difference that they used bromocriptine there (7).

In our study, no adverse effects were observed in turkey hens after consuming cabergoline, as in a previous study in which cabergoline was administered orally to zebra finches without presenting adverse effects (10).

CONCLUSIONS

The administration of cabergoline orally is an alternative for control of nesting behavior and when observing that there are no adverse effects, later studies could be done using a higher dose or a higher frequency of application in which better results could be observed than in the present study.

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FIELD EXPERIENCE WITH A NEW LIVE COCCIDIOSIS VACCINE USED IN A BIO-SHUTTLE WITH A CHEMICAL ANTICOCCIDIAL IN COMMERCIAL NAE (NO ANTIBIOTICS EVER) US BROILERS

EXPERIENCIA DE CAMPO CON UNA NUEVA VACUNA DE COCCIDIOSIS EN UN PROGRAMA BIO-DUAL CON UN ANTICOCCIDIANO QUIMICO EN POLLOS DE ENGORDA COMERCIALES NAE (NUNCA ANTIBIOTICOS) EN E.U.A.

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RESUMEN

Una nueva vacuna viva de coccidiosis aplicada a través de gotitas de gel al día de edad en la incubadora (Immucox® 3) fue utilizada para vacunar a más de 600,000 pollos de engorda comerciales NAE (nunca antibióticos). Las aves también recibieron un anticoccidiano químico (Deccox®) en el alimento de los 14 a los 28 días de edad. Se determinó la ingesta de la vacuna por la presencia de la tinción azul en las

lenguas de los pollitos. En 6 casetas se determinó el ciclado de la vacuna por medio de la calificación de lesiones macroscópicas y microscópicas en 5 aves/caseta por semana, y por medio de la excreción de ooquistes en una muestra fecal compuesta/caseta por semana. El desempeño de los pollos de engorda y la viabilidad se comparó con pollos de engorda criados en semanas previas y posteriores. La ingesta de la vacuna excedió a un 95% con una excreción uniforme de ooquistes a los 7 días, aumentando a los 14 días con lesiones leves de *E. acervulina*, disminuyendo de 21 a 28 días debido al uso de Deccox ®. Las lesiones y la excreción de la coccidiosis fueron inconsistentes a partir de los 28 días en adelante. No se notó coccidiosis, enteritis necrótica, o cambios en el desempeño.

SUMMARY

A new live coccidiosis vaccine applied trough gel droplets at day of age in the hatchery (Immucox® 3) was used to vaccinate more than 600,000 commercial NAE broilers. Birds also received a chemical anticoccidial (Deccox®) in the feed from 14 to 28 days of age. Vaccine intake was determined by the presence of blue dye in chicks' tongues. Vaccine cycling was determined in 6 houses by gross and microscopic lesions scoring in 5 birds/house per week, and by oocyst shedding in a pooled fecal sample/house per week. Broiler performance and livability were compared to broilers raised on previous and following weeks. Vaccine intake exceeded 95% with uniform oocyst shedding at 7 days, increasing at 14 days with mild *E. acervulina* lesions, decreasing from 21 to 28 days due to Deccox®. Coccidiosis lesions and shedding were inconsistent from 28 days onward. No coccidiosis, necrotic enteritis, or performance changes were noticed.

INTRODUCTION

Coccidiosis is a disease of great economic significance in commercial chickens around the world. It is caused by protozoan parasites of the genus *Eimeria*. Current coccidiosis control strategies are based on the use of anticoccidial drugs and/or live coccidiosis vaccines (7). The prevention of coccidiosis based on vaccination of chicks at day of age in the hatchery has become more popular in the recent years. There are several coccidiosis vaccines commercially available in the USA, most of them containing different numbers of live sporulated oocysts of three *Eimeria* species: *E. acervulina*, *E. maxima*, and *E. tenella*. These vaccines are usually diluted in water and mass-applied in the hatchery using a spray cabinet. Recent studies have shown that gel-based diluents are effective delivery methods for coccidiosis vaccines for day-old chicks (1, 3, 4, 6, 8). This study documents the use of a new coccidiosis vaccine applied through gel droplets in commercial broilers raised in the USA.

MATERIALS AND METHODS

Vaccine mixing and application. A new live coccidiosis vaccine available in the USA, Immucox 3 from Ceva Animal Health, was used for this study. Immucox 3 contains live sporulated oocysts of *E. acervulina*, *E. maxima*, and *E. tenella* (225 oocysts/dose). A gel powder, CevaGel® from Ceva Animal Health, was reconstituted following manufacturer's recommendations. Batches of containing five gallons of distilled water and 700 grams of CevaGel were mixed using an Electromaster 6-gallon tilting blender for two minutes. A total of 160 ml of sterile blue dye and 80,000 doses of Immucox 3 were added to the gel mix and blended for another 30 seconds. Vaccine mix was applied using a gel droplet applicator (DesVac Duo from Ceva Animal Health) delivering 25 mL per chick box containing 100 chicks. Vaccine mix intake was determined by observing blue dye in chicks' tongues three to five minutes after vaccine application.

A total of total of 666,700 chicks were vaccinated following this protocol on Monday, Tuesday, Thursday, and Friday of the same week at the integrator's hatchery. Chicks were placed on built up litter in commercial broiler farms and raised following integrator's guidelines. Birds received a chemical anticoccidial, Decoquinate – Deccox 0.003%, in the feed from 14 to 28 days of age as part of a bio-shuttle coccidiosis control program.

Vaccine cycling. Immucox 3 cycling was monitored in six houses representing 25% of the birds placed that week. Monitoring consisted on gross and microscopic coccidiosis lesions scoring in five birds/house per week, and on measuring oocyst shedding in a pooled fecal sample/house per week. Gross lesions were scored using the methodology described by Johnson and Reid (5). Microscopic lesion scoring was performed by scrapping the intestinal mucosa in the duodenum, mid jejunum, and ceca separately. Intestinal scrapings were examined using a microscope (x10) and based on the number of oocysts observed per filed, a score was assigned (Score 1: 1-10, Score 2: 11-20, Score 3: 21-40, and Score 4: >40 oocysts per field). The number of oocysts per gram of feces (OPG) was determined following the McMaster chamber method (2).

Broiler performance. Performance indicators normally used by broiler integrators in the USA: Livability, Age, Body weight, Weight gain, Feed Conversion, Adjusted Feed Conversion, Calorie

Conversion, Adjusted Calorie Conversion, and Condemnations were recorder for Immucox 3-vaccinated birds. These indicators were compared to birds placed two weeks prior and two weeks after the Immucox 3 trial. All those birds had also received a live coccidiosis vaccine including precocious strains of the same three *Eimeria* species (HatchPak Cocci III®) mixed with water and applied through a spray cabinet delivering 21 mL/chick box. These birds received similar feed, including Decoquinate-Deccox® from 14 to 28 days of age.

RESULTS AND DISCUSSION

Vaccine intake and cycling. A total of 5,600 chicks were examined and the overall vaccine intake was 95.8% (95.8% on Monday, 95.4% on Tuesday, 95.5% on Thursday, and 96.9% on Friday). No gross lesions due to *E. maxima* or *E. tenella* were observed. Mild gross *E. acervulina* lesions were observed in 13% of the birds at seven days, increasing to 67% of the birds at 14 days. No gross *E. acervulina* lesions were noticed while birds received Deccox in the feed. Some mild lingering gross *E. acervulina* lesions were observed from 35 days to 49 days. Microscopic scoring of *E. acervulina* followed a similar pattern. The percentage of birds with *E. maxima* oocysts detected microscopically was 7, 17, 0, 0, 3, 10, and 10% at 7, 14, 21, 28, 35, 42, 49 days of age respectively. Oocyst shedding curves by house are shown in Figure 1.

These results indicate uniform vaccine intake and oocyst shedding at seven days of age, effective re-infection resulting in increased oocyst shedding at 14 days, followed by strong anticoccidial effect of Deccox. From 28 days onward, there is an inconsistent lesion and oocyst shedding pattern. This is most likely due to the interruption of the immunity development when using an effective chemical anticoccidial between 14 and 28 days.

Broiler performance. No coccidiosis or necrotic enteritis outbreaks were reported during the trial. Performance of broilers receiving Immucox 3-Deccox bio-shuttle was comparable to broilers receiving HatchPak Cocci III-Deccox bio-shuttle (Table 1). Advantages in average daily gain, adjusted feed conversion, and adjusted calorie conversion occurred consistently along all four weeks.

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Figure 1. Weekly fecal oocyst shedding in broilers vaccinated with Immucox 3® receiving Deccox® from 14 to 28 days and placed in 6 different houses.

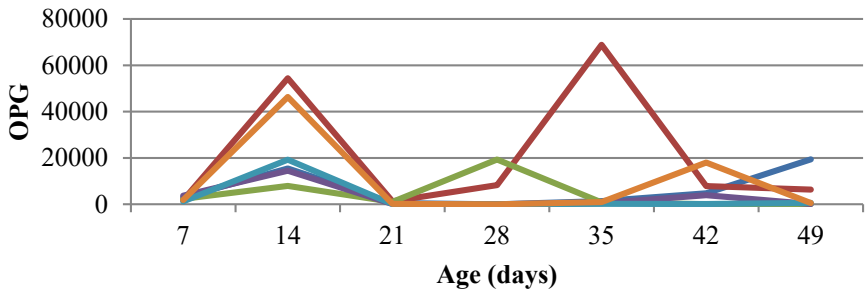

Table 1. Performance differences between broilers vaccinated with Immucox 3® via gel droplets and broilers vaccinated with HatchPak Cocci III via water spray in the previous (Weeks -1 and -2) and following weeks (Week 1 and 2).

	Week 2	Week 1	Week -1	Week -2
Livability %	0.34*	-0.17	-0.36	0.52
Age (days)	0.26	0.64	-1.01	-3.55
Average weight (lb)	0.32	0.16	0.16	-0.16
Average daily gain (lbs/day)	0.0052	0.0014	0.0056	0.0061
Feed Conversion	-0.02	0.00	-0.02	-0.09
Feed Conversion Adjusted	-0.05	-0.01	-0.04	-0.08
Calorie Conversion	-35.00	-2.00	-31.00	-127.00
Calorie Conversion Adjusted	-71.00	-20.00	-50.00	-109.00
Total Condemnations %	-0.03	0.00	0.05	-0.06

*Performance differences calculation example: Weekly livability of Immucox 3®-vaccinated broilers minus Livability in Week 2 of HatchPak Cocci III®-vaccinated broilers.

EVALUACIÓN DE LOS RESULTADOS DE *SALMONELLA* EN DIFERENTES PARVADAS

RESULTS ASSESMENT OF *SALMONELLA* IN DIFFERENT FLOCKS

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SUMMARY

Some types of *Salmonella* can cause severe disease in birds, such as fowl typhoid and pullorum disease, with a severe clinical picture. These entities are caused by specific serotypes, highly adapted to birds. Or in other situations the presence of this bacteria in birds could passed unperceived, without causing symptoms; such as the case of paratyphoid salmonella infections. However, depending on the management conditions these salmonellas can altered the delicate balance of the intestinal microflora, affecting the productive parameters. In the public health context, *Salmonella,* is very related to poultry products (mainly chicken meat and eggs), being the paratyphoid salmonellas a serious public health issue because many of them are zoonotic (1). Due to the latter is key to implement integral control programs to allow keeping the prevalence in minimum values in the primary production (farms, hatcheries, slaughter plants, etc.) (2). But to be able to implement the control measures, it is necessary to have a constant monitoring of the situation in the different links. The objective of the following work was to evaluate the environmental prevalence of the paratyphoid Salmonellas in commercial farms.

INTRODUCCIÓN

Algunas bacterias del genero *Salmonella* pueden causar enfermedades graves en las aves como tifoidea aviar y pulorosis, con manifestación clínica severa. Estas entidades son causadas por serotipos específicos, altamente adaptados a las aves. O en otras ocasiones la presencia de esta bacteria en las aves puede pasar desapercibida, sin causar sintomatología; como en el caso de las infecciones con salmonellas paratíficas. Sin embargo dependiendo de las condiciones de manejo estas salmonellas pueden alterar el delicado balance del micro flora intestinal, afectando los parámetros productivos. En el contexto de salud pública, *Salmonella* está muy ligada a los productos avícolas (principalmente la carne de pollo y los huevos), siendo las salmonellas paratíficas un serio problema para la salud pública ya que varias de ellas son zoonoticas (1).

Debido a esto resulta clave implementar programas de control integrales que permitan mantener la prevalencia, en la producción primaria, (granjas, *plantas* de incubación, planta de faena, etc.) en valores mínimos (2). Pero para poder implementar medidas de control, es necesario un monitoreo constante de las situación en los distintos eslabones. El objetivo del siguiente trabajo fue evaluar la prevalencia ambiental de Salmonellas paratíficas en granjas de producción comercial.

MATERIALES Y METODOS

Para la evaluación de salmonellas paratíficas es importante comenzar evaluando el ambiente. En caso de ambientes positivos, evaluaremos la excreción y colonización de salmonella a través del muestreo de las aves. Para evaluar ambientes en los que los animales que se encuentran a piso, el método de elección será el de cubre calzados o hisopos de arrastre. Si los animales se encuentran en jaulas debemos utilizar hisopados de tráquea y polvo del galpón. Si los muestreos nos dan negativos, deberemos establecer un programa de control periódico. Si los controles de ambiente nos dan positivos a *Salmonella* spp. debemos evaluar la excreción por parte de las aves, para ello utilizaremos hisopados cloacales y materia fecal fresca. Así mismo también evaluaremos la colonización bacteriana para lo cual utilizaremos el contenido cecal de las aves.

Para ambos tipos de muestreo, piso y jaula, utilizaremos la metodología propuesta por el Servicio Nacional de *Sanidad* Agroalimentaria Argentina (SENASA) dentro el marco del Plan Nacional de Sanidad Avícola, Res. SENASA N° 86/2016, en el Manual de Procedimientos Operativos del Programa de Vigilancia y Control de Salmonella (3).

Aves a piso. El monitoreo se realizará en una integración de Argentina, en la provincia de Entre Rios, de pollos de engorde. Se seleccionaron cinco granjas de producción, cuya capacidad total de alojamiento alcanza las 270.000 aves, con historial positivo a *Salmonella* Typhimurium. Se las evaluó a lo largo de tres ciclos productivos consecutivos. Además, en dichas granjas se utilizó una vacuna a subunidad contra *Salmonella* por lo que también se evaluó la prevalencia para monitorear la eficacia de la vacuna.

Aves a jaula. Se evaluó una granja de postura comercial ubicada en la provincia de Buenos Aires, Argentina. En la misma se evaluaron seis lotes de producción de 120.000 aves cada uno durante 89 semanas.

RESULTADOS

Aves a piso. A continuación se muestran los resultados obtenidos (Tabla 1). **Granja 1**, 6 de los 7 galpones *continuaron* siendo positivos (**85%**), pero solo 1 galpón de los 6 a *Salmonella Typhimurium*, en el resto solo se halló *Salmonella spp.* Para el segundo ciclo solo 3 de los 7 galpones resultaron positivos a *Salmonella* (42%) pero en este caso solo *Salmonella* spp. Por último, en el tercer ciclo, solo 1 de los 7 galpones mantuvo una positividad a *Salmonella* Typhimurium (14%). **Granjas 2 y 4**: relativizaron en todos sus galpones al primer ciclo de vacunación. **Granja 3** no se aisló *Salmonella* hasta el tercer ciclo, en donde se encontró *Salmonella* Typhimurium en 1 galpón de los 3 (33%). **Granja 5** mantuvo un 50% de positividad a *Salmonella* spp. Durante el primer y segundo ciclo. Al tercer ciclo logro negativizar la totalidad de sus galpones. Evaluando las granjas como parte de la integración podemos observar que el porcentaje de positividad en el primer ciclo fue del 41%, en el segundo del 23% y en el tercero tan solo del 11%.

Aves a jaula. En el caso de animales que estén a jaula los muestreos fueron negativos hasta la semana 89 en donde uno de los lotes dio positivo a *Salmonella* Enteritidis en la muestra de polvo del galpón e hisopados de tráquea, pero los muestreos de hisopado de cloaca y guano arrojaron resultados negativos.

CONCLUSIONES

El sistema de monitoreo permitió no solo evaluar la situación epidemiológica de las granjas sino también la eficacia del programa de control establecido con la vacuna. *En* el caso de las aves a piso se pudo observar como con el pasar de los ciclos productivos bajo la utilización de la vacuna se pudo disminuir la prevalencia. Mientras que en el caso de las aves a jaula se pudo observar que si bien había un desafío ambiental, debido a la positividad de los hisopados de tráquea, no había replicación de Salmonella en el ave ya que los hisopados de cloaca dieron negativos.

Es importante establecer un buen programa de monitoreo de *Salmonella*, en todos los eslabones de la cadena avícola, los que deben incluir no solo presencia o ausencia, sino también cuantificación e identificación de las salmonellas presentes. Esto nos permitirá saber la prevalencia de salmonella en cada sector, como también establecer programas de bioseguridad y vacúnales adecuados para controlarla. También un buen programa de monitoreo, nos servirá para evaluar objetivamente esos programas de control implementados. Con el objetivo de bajar la

prevalencia de salmonellas móviles no solo en las granjas, sino en el producto final.

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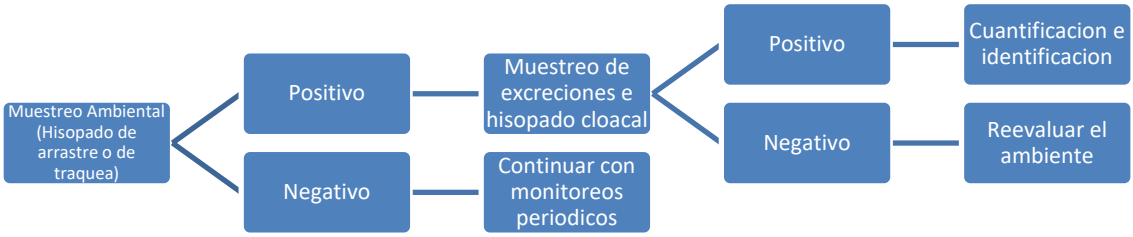

Tabla 1. Distribución de los resultados de los muestreos de *Salmonella* en las distintas granjas.

Distribución de los resultados de los muestreos de Salmonella en las distintas granjas

Granja	Galpón	Ciclo 1	Ciclo 2	Ciclo 3
1 (130000)	**1**	Positivo (spp)	Negativo	Negativo
	2	Positivo (spp)	Positivo (spp)	Negativo
	3	Positivo (Typhimurium)	Negativo	Negativo
	4	Positivo (spp)	Negativo	Negativo
	5	Negativo	Positivo (spp)	Positivo (Typhimurium)
	6	Positivo (spp)	Positivo (spp)	Negativo
	7	Positivo (spp)	Negativo	Negativo
2 (50000)	**1**	Negativo	Negativo	Negativo
	2	Negativo	Negativo	Negativo
3 (26000)	**1**	Negativo	Negativo	Negativo
	2	Negativo	Negativo	Positivo (Typhimurium)
	3	Negativo	Negativo	Negativo
4 (40000)	**1**	Negativo	Negativo	Negativo
	2	Negativo	Negativo	Negativo
	3	Negativo	Negativo	Negativo
5 (26000)	**1**	Negativo	Negativo	Negativo
	2	Positivo (spp)	Positivo (spp)	Negativo

THE RESPIRATORY SYNDROME ETIOLOGY OF LAYING HENS BY PCR

LA ETIOLOGIA DEL SINDROME RESPIRATORIO EN GALLINAS DE POSTURA POR PCR

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RESUMEN

El desarrollo técnico de la producción de huevo contribuyó a la productividad y a la eficiencia del sector, pero favorece a la aparición de enfermedades respiratorias en gallinas de postura. Este trabajo busca el objetivo del diagnóstico etiológico por PCR y RT-PCR de los principales agentes etiológicos involucrados en el Síndrome Respiratorio en gallinas de postura con signos clínicos respiratorios. Los agentes de las enfermedades respiratorias causan signos clínicos similares y pueden ocurrir simultáneamente, principalmente en granjas de postura debido a las altas densidades de alojamiento, fallas en la bioseguridad y el manejo. Se ha demostrado que el diagnóstico molecular es efectivo y rápido para detectar uno o más agentes respiratorios en las gallinas de postura comerciales. El síndrome respiratorio en gallina de postura se confirma como una etiología mixta y el agente más frecuentemente encontrado era el MS, seguido del MG, IBV y ORT con asociaciones entre ellos.

SUMMARY

The technical development of egg production contributed to the productivity and efficiency of the sector, but it favors the appearance of respiratory diseases in laying hens. This work aimed at the etiological diagnosis by PCR and RT-PCR of the main etiological agents involved in the Respiratory Syndrome in laying hens with respiratory clinical signs. The agents of respiratory diseases cause similar clinical signs and can occur simultaneously, mainly in farms of posture, due to the high densities in the lodging, biosecurity and handling failures. Molecular diagnosis has been shown to be effective and rapid in detecting one or more respiratory agents in commercial laying hens. The respiratory syndrome in laying hens was confirmed as being of mixed etiology and had as its most frequent agent MS, followed by MG, IBV and ORT, with associations among them.

INTRODUCTION

Egg production has evolved greatly in Brazilian agribusiness (1) and around the entire world. According to the Brazilian Association of Animal Protein, the production of eggs in Brazil in 2018 approached 40 billion units and the country remains among the ten largest egg producers in the world. The intensification of production contributed to an improvement in the efficiency of the sector, however it favored an increase in the spread of infectious diseases. Among these diseases, those that affect the respiratory system stand out due to the high impact in the sector (5). In addition to this, the synergism among different infectious agents can cause respiratory syndrome, reducing the zootechnical potential of the laying hens and increasing costs of treatment. The current situation of the etiology of respiratory syndrome in laying birds is unknown, so studies to determine their true frequency are important for the adoption of control measures (1). Respiratory pathogens such as *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS), *Ornithobacterium rhinotracheale* (ORT) and avian infectious bronchitis virus (IBV) may interact synergistically increasing the severity and duration of respiratory disease (8). Among the different diagnostic methods, polymerase chain reaction (PCR), associated with the analysis of zootechnical data and clinical signs, have been of great value in the epidemiological diagnosis of these diseases. In addition to your economic viability, greater specificity among serological and isolation techniques has contributed in the implantation in diagnostic laboratories (10). The aim of this study was the etiological diagnosis by PCR and RT-PCR of the main etiological agents involved in the Respiratory

Syndrome in laying hens with respiratory clinical signs.

MATERIALS AND METHODS

Trachea swabs were collected from 140 layer hens of six different farms (A, B, C, D, E and F) in the Brazilian southeast region. In farm A, 10 tracheal swabs were collected from four flocks, while in the others five farms (B, C, D, E and F) these swabs were collected from two flocks (Table 1). All samples were put in 1 ml of Frey medium, incubated at 37 ° C for 12 h and then routed for DNA or RNA extraction (11). After extraction, the material was quantified in Biodrop Touch® (Biochrom, UK) and amplified for the agents MG (9), MS (7), ORT (4), and IBV (3). Each sample was homogenized with loading buffer and GelRed®, applied in 1.5% agarose gel layered in Tris- Borate- EDTA (TBE) 0.5X, and finally submitted to the electrophoresis conditions (14). After the electrophoresis, the amplicons were visualized under ultraviolet light transilluminator.

RESULTS AND DISCUSSION

Among the investigated agents, MS was observed in 68.57% (96/140) of hens evaluated, being the agent of higher frequency in farms evaluated and reinforcing their high prevalence in laying hens (12); (2). Then, it was possible to observe the presence of MG in 16.42% (23/140), IBV in 10.71% (15/140) and ORT in 5% (7/140) of the chickens studied. Both ORT and IBV were diagnosed on farm A with frequencies of 10% (4/40) and 35% (14/40) and on farm B with frequencies of 15% (3/20) and 5% (1/20) respectively (Table 1). The frequency of positive samples for IBV on farm A may be the result of vaccination of these birds. In the MG infection it was detected on farms A (0/40) and C (0/20). Farms C and D had the highest rates of MS infection in 95% (19/20) and 100% (20/20) of the hens, but in farm C there was no case of mixed infection between the agents analyzed (Table 1)

In this study, it was still possible to observe mixed infection among the analyzed microorganisms, with the highest frequency between MG and MS being 10.71% (15/140) of the chickens studied (Table 1). Among farms evaluated, farms A and B were the ones that showed more cases of association between the microorganisms. Farm A presented 5% (2/40) mixed infection frequencies for MS + IBV and IBV + ORT and 2.5% (1/40) MS + ORT, while in farm B there was a frequency of synergism in 5% for MG + IBV, MG + ORT, MG + MS and 10% (2/20) for MS + ORT in the analyzed birds (Table 1). The synergistic action of IBV with MS and with ORT in 5% (2/40) of birds in farm A leads us to believe that this virus, even being the vaccine strain, may contribute to the exacerbation of respiratory signals (10). Farms D, E and F presented only a combination of GM and MS with frequencies of 5% (1/20), 30% (6/20) and 35% (7/20) respectively (Table 1). Associations between mycoplasmas and other pathogens can therefore both aggravate and prolong respiratory disease (6, 10). Researches focusing on the etiology, prevalence and degree of involvement and association of microorganisms in cases of respiratory diseases are fundamental for the development of preventive measures and adequacy of the legislation in poultry health.

CONCLUSION

Molecular diagnosis was effective in detecting one or more respiratory disease agents in commercial laying hens. Rapid and sensitive methods to detect and differentiate pathogens from respiratory diseases in hens are critical for the adoption of preventive measures in the control of respiratory syndrome. MG, MS, ORT, and IBV agents have demonstrated that they can occur alone or in association with respiratory syndrome in laying hens. The respiratory syndrome in laying hens, as a result of the mixed infection in this study, had MS as the most frequent agent, followed by MG, IBV, and OR. The associations between these agents in the six farms were the most found. These results suggest the need for a more detailed study on the etiology of respiratory diseases.

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Table1. Frequency of infection by *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), *Ornithobacterium rhinotracheale* (ORT), and avian infectious bronchitis virus (IBV) in commercial laying hens.

Agents / **Simple Infection**	**FARM A**	**FARM B**	**FARM C**	**FARM D**	**FARM E**	**FARM F**	**TOTAL**
MG	0/40 (0%)	2/20 (10%)	0/20 (0%)	1/20 (5%)	7/20 (35%)	13/20 (65%)	23/140 (16,42%)
MS	15/40 (37,5%)	15/20 (75%)	19/20 (95%)	20/20 (100%)	16/20 (80%)	11/20 (55%)	96/140 (68,57%)
ORT	4/40 (10%)	3/20 (15%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	7/140 (5%)
IBV	14/40 (35%)	1/20 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	15/140 (10,71%)
Mixed Infection	**FARM A**	**FARM B**	**FARM C**	**FARM D**	**FARM E**	**FARM F**	**TOTAL**
MG +IBV	0/40 (0%)	1/20 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/140 (0,71%)
MG + ORT	0/40 (0%)	1/20 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	1/140 (0,71%)
MG + MS	0/40 (0%)	1/20 (5%)	0/20 (0%)	1/20 (5%)	6/20 (30%)	7/20 (35%)	15/140 (10,71%)
MS + ORT	1/40 (2,5%)	2/20 (10%)	0/20 (0%)	0/20 (0)	0/20 (0%)	0/20 (0%)	3/140 (2,14%)
MS + IBV	2/40 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/140 (1,42%)
IBV + ORT	2/40 (5%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	2/140 (1,42%)

EFFECT OF *IN OVO* IBDV VACCINES ON THE SIZE AND INTEGRITY OF THE BURSA IN COMMERCIAL BROILERS

EFECTO DE LAS VACUNA CONTRA EL IBDV *IN OVO* SOBRE EL TAMAÑO E INTEGRIDAD DE LA BOLSA EN POLLOS DE ENGORDA COMERCIALES

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RESUMEN

Se evaluó el efecto de una vacuna nueva dual recombinante de HVT(HVT-IBD-ND) y dos vacunas vivas atenuadas (cepas 89/03 y ST-12, respectivamente) *in ovo* sobre la integridad de la bolsa de Fabricio en pollos de engorda comerciales. Se obtuvieron dos cientos setenta huevos fértiles de una planta incubadora de pollo de engorda comercial. A los 18 días del desarrollo del embrión, los huevos fértiles fueron divididos en forma equitativa y se formaron grupos de 45 huevos/ grupo y se asignaron a seis tratamientos diferentes; a la vacuna dual recombinante HVT-ND-IBD (Innovax ND-IBD), a la cepa 89/ 03, a la ST-12, a la HVT-ND-IBD y 89/ 03, a la HVT-ND-IBD y ST-12 y a un grupo control no vacunado. Después de incubar, los pollos de engorda fueron alojados en aislamientos tipo Horsfall Bauer bajo presión negativa. A los 7, 14 y 21 días de edad, se aplicó eutanasia a doce aves de cada grupo por medio de inhalación de CO_2 y se les practicó la necropsia. Se registraron los pesos corporal y de la bolsa, y se estimó la relación de peso corporal/ bolsa. Se colectaron tejidos de la bolsa y se evaluaron por histopatología, se realizó en RT-PCR en tiempo real y re-aislamiento del virus. La bolsa de la aves vacunadas con la vacuna recombinante HVT-ND-IBD no mostró lesiones macroscópicas (inflamación, hemorragias o atrofia) o histopatológicas (degradación de linfocitos o cambios císticos) durante el periodo de evaluación. Las lesiones macroscópicas y los cambios histopatológicos en la bolsa de los pollos de engorda vacunados con la 89/03 y la ST-12 mostraron las características típicas asociadas con las vacunas suaves atenuadas vivas

SUMMARY

The effect of a novel dual recombinant HVT (HVT-IBD-ND) and two live attenuated (89/03 and ST-12 strains, respectively) *in ovo* vaccines on the integrity of the bursa of Fabricius in commercial broilers was evaluated. Two hundred and seventy fertile eggs were obtained from a commercial broiler hatchery plant. At 18 days of embryonic development fertile eggs were equally divided in seven groups with 45 eggs/group and assigned to six different treatments; dual recombinant HVT-ND-IBD (Innovax ND-IBD), 89/03 strain, ST-12, HVT-ND-IBD and 89/03, HVT-ND-IBD & ST-12 and non-vaccinated control group. After hatching, the broiler chickens were housed in Horsfall Bauer type isolators under negative pressure. At 7, 14, and 21 days of age, twelve birds from each group were euthanized by CO_2 inhalation and necropsied. Body and bursal weights were recorded and bursal/body weight ratios were estimated. Bursa tissues were collected and evaluated by histopathology, real time RT-PCR and virus re-isolation. Bursas from birds vaccinated with the recombinant HVT-ND-IBD didn't show gross (inflammation, hemorrhages or atrophy) or histopathological (lymphocytic depletion or cystic changes) lesions during the evaluation period. Gross lesions and histophatological changes in bursas from 89/03 and ST-12 vaccinated broilers showed typical characteristics associated with mild live attenuated vaccines.

INTRODUCTION

Infectious bursal disease (IBD) is a viral disease widely disseminated in poultry that induces dysfunction of the immune system, especial the immune mechanisms involved in antibody production. Birds suffering from this disease are immunosuppressed and therefore they are more susceptible to opportunistic infections or they will develop a poor response to vaccinations. Because of the negative effects of this disease in poultry health and performance, control measures are widely carried out in the poultry industry.

The most effective way to control this disease is by vaccination, and there are different types of vaccines, that have different characteristics. In the

present study, the effect of different IBDV vaccination treatments on the size and integrity of the bursa were evaluated.

EXPERIMENTAL DESIGN

Two hundred and seventy fertile eggs will be obtained from a commercial broiler hatchery plant. At 18 days of embryonic development fertile eggs will be equally divided in seven groups with 45 eggs/group, and they will receive six different treatments as follows:

a) One group will be in *ovo* vaccinated with the recombinant vaccine HVT-ND-IBD

b) A second group will be vaccinated with the live attenuated vaccine 89/03,

c) A third group will receive ST-12

d) A fourth group will receive both HVT-ND-IBD & 89/03

e) A fifth group will receive both HVT-ND-IBD & ST-12

f) Groups six will remain unvaccinated

Vaccines will be diluted and applied, following the recommendations of the manufacturer.

After hatching, the birds will be housed in Horsfall Bauer type isolators under negative pressure.

At 7, 14 and 21 days of age, twelve birds from each group be euthanized by CO2 inhalation and necropsy procedures will be carried out.

Body and bursal weights will be registered to calculate bursal/body weight ratios to determine bursal atrophy. Bursal tissues will be collected for histopathological evaluation and virological procedures.

EFFECT OF THE ADDITION OF A YEAST FERMENTED PRODUCT AND COCCIDIA CHALLENGE IN BROILER CHICKENS

EFECTO DE LA ADICIÓN DE PRODUCTOS DE FERMENTACIÓN DE LEVADURAS Y DESAFÍO CON COCCIDIAS EN POLLOS DE ENGORDA

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RESUMEN

El objetivo del estudio fue evaluar el desempeño productivo, la medición de la tibia, histopatología del intestino delgado y respuesta inmune de pollos de engorda alimentados con dietas adicionadas con producto de fermentación de levaduras (PFL) y desafiados con una vacuna de coccidia (COC). Se alojaron ciento ochenta pollos de engorda en baterías de jaulas. De los días 1 a 20 de edad (fase 1), los pollos fueron asignados a dos tratamientos: 1) Control sin la adición de PFL, y 2) Grupo adicionado con PFL en la dieta. La dieta fue adicionada con nicarbazina. En el día 20, 30 aves de cada tratamiento se sacrificaron, y el resto de los pollos de engorda fueron divididos en dos subgrupos; un subgrupo fue desafiado con una vacuna de coccidia, mientras que el otro no fue desafiado. La vacuna fue mezclada en el alimento. Del día 21 al 28 de edad (fase 2) se retiró el fármaco anticoccidiano del alimento. Los resultados fueron sometidos a ANOVA. En la fase 1, los pollos de engorda adicionados con PFL tuvieron un diámetros mayor en la epífisis inferiores de las tibias ($P< 0.10$), y grupos de linfocitos mayores ($P < 0.10$), hiperplasia de las placas de Peyer ($P< 0.05$), y mayor concentración de IgA ($P< 0.10$) pero menor hiperemia/ hemorragias ($P< 0.10$) en el duodeno. En la fase 2, fueron menores la CA ($P< 0.01$), la longitud de la tibia ($P< 0.01$) y el peso de cenizas ($P< 0.05$) en los pollos desafiados con coccidias. El FI, el diámetro de la epífisis inferior y la diáfisis mejoraron mientras que el conteo de ooquistes se redujo en los pollos desafiados

adicionados co PFL (interacción COC y PFL, P < 0.05). En resumen, la adición con PFL mejoró el FI y la mineralización de la tibia y redujo los conteos de ooquistes de los pollos de engorda adicionado con la vacuna de coccidia, pero no mostró ningún efecto sobre la IgA duodenal y las concentraciones de MBL en suero

SUMMARY

The objective of the study was to evaluate the productive performance, tibia measurements, histopathology of the small intestine, and immune response in broiler chickens fed diets added with a yeast fermented product (YFP) and challenged with a coccidia vaccine (COC). One hundred and eighty chicks were allocated in battery cages. From 1 to 20 d of age (phase 1), chicks were assigned to two treatments: 1) Control with not addition of YFP, and 2) Group with addition of 800 ppm of YFP in the feed. The diet was added with nicarbazine. On day 20, 30 birds from each treatment were killed, and the rest of the broilers were divided in two subgroups: one subgroup was challenged with a coccidia vaccine, while the other one was not challenged. The vaccine was mixed in the feed. From 21 to 28 d of age (phase 2) the anticoccidial drug was withdrawn from the feed. Results were subjected to ANOVA. In phase 1, YFP-fed broilers had higher diameter of the lower epiphysis of the tibia ($P < 0.10$), and higher lymphoid clusters ($P < 0.10$), hyperplasia of the Peyer patches ($P < 0.05$) and IgA concentration ($P < 0.10$) but had lower hyperemia/hemorrhage ($P < 0.10$) in the duodenum. In phase 2, the FCR ($P < 0.01$), length of the tibia ($P < 0.01$) and weight of ashes ($P < 0.05$) were lower in coccidia challenged broilers. The FI, the diameter of the inferior epiphysis and diaphysis were improved while the oocyst counts were reduced in coccidia challenged YFP-fed broilers (COC and YFP interaction, $P < 0.05$). In summary, the addition of YFP improved the FI and tibia mineralization and reduced the oocyst counts in broilers challenged with a coccidia vaccine, but it did not show any effect on the duodenal IgA and serum MBL concentrations.

INTRODUCTION

Whole yeast cell products and several derivatives, such as yeast culture, yeast extracts, yeast fermentation products and other products that retain the cell wall components, known as mannan-oligosaccharides (MOS) exert beneficial effects on the health and growth of poultry (4, 6, 7). It has been suggested that several yeast compounds have immunomodulatory effects such as increased mucosal IgA secretions and humoral and cell-mediated immune responses by binding pathogenic bacteria and blocking their adhesion to the intestinal cells, and by interacting with mucosal dendritic cells and macrophages, shaping innate and adaptive immune responses (4, 7, 12). For these reasons, yeast products are promoted as alternatives to substitute the antibiotics growth promoters. Furthermore, in recent years, several studies have been carried out to test the effectiveness of yeast products when fed to naturally or planned coccidia challenged broilers with encouraging results (4, 7, 12). Different mucosal secretions and humoral and cell-mediated immune responses elicited by yeast products in coccidia infected broilers have been associated to reduction of the oocysts excretion and to diminishment of the coccidia infection.

The mannan-binding lectin (MBL), plays a crucial role in innate immunity against different pathogenic microorganisms. In chickens suffering from viral diseases or inoculated with *E. coli*, a two- to three-fold increments in serum levels of MBL have been reported. It appears that MBL plays a major role in the first-line innate immune defense against bacteria, viruses, and parasites, acting directly as an opsonin, or by initiating a pathway of complement activation (11). It has been hypothesized that dietary yeast may initiate enhanced production of MBL, which may directly neutralize or enhance the opsonization of pathogenic bacteria (9). Whether this mechanism is involved in the reduction of coccidia infections in broilers fed yeast products is unknown. Therefore, the objective of this study was to evaluate the productive performance, tibia measurements, oocyst excretion, histopathology of the small intestine and immune response in broiler chickens fed diets added with a yeast fermented product and challenged with a coccidia vaccine.

MATERIALS AND METHODS

One hundred and eighty male Ross B308 1-d old chicks were housed in groups of two in battery cages. From 1 to 20 d of age (phase 1), chicks were randomly assigned to two treatments: 1) Control with not addition of a yeast fermented product (YFP) and, 2) Group with addition of 800 ppm of YFP in the feed. The diet was added with nicarbazine. On day 20, 30 birds from each treatment were killed, and the rest of broilers were divided in two subgroups: one subgroup received a challenge with a coccidia vaccine, and the other one was not challenged. The vaccine was mixed in the feed, with sterile distilled water, using a 16x dosage/bird containing 9,600, 3,200, 6,400 and 3,200 sporulated oocysts of *E. acervulina, E. maxima, E. mivati*, and *E.* tenella, respectively, to cause a mild coccidia challenge. The feed of unchallenged broilers

was mixed with the same volume of sterile distilled water. From 21 to 28 d of age (phase 2) the anticoccidial drug was removed from the feed.

At 20 and 28 d the weight gain (WG, g), feed intake (FI, g) and feed conversion ratio (FCR) were calculated. On days 20 and 28, six pooled droppings samples per treatment were taken for evaluation of oocysts using the McMaster counting chamber technique. At 20 and 28 days of age, blood samples were taken for analysis of MBL by ELISA technique. On day 28, all broilers were killed by cervical dislocation. Broilers killed at 20 and 28 days were subjected to the same sampling procedures. The breast, legs, thighs and frame were weighed to get the carcass components and whole carcass weight. The length, weight and the diameter of the upper and lower epiphysis and diaphysis of the left tibia were recorded. Then, the tibias were dried, defatted and incinerated to determine the ash content. One-cm samples from the duodenum and jejunum were taken from six broilers per treatment for histopathologic evaluations; the tissues were stained and the histopathological changes were observed under light microscope. A 10-cm section of the duodenum was excised and rinsed with phosphate buffered water for IgA determination using an ELISA kit. The results were subjected to ANOVA using the procedures of the General Linear Models of SAS.

RESULTS AND DISCUSSION

Period from 1 to 20 days of age. At the beginning of the experiment (day 1) broilers were randomly assigned to the treatments, but chicks fed diets without YFP had higher initial body weight ($P< 0.01$) compared to that of the YFP-fed group, being the weight difference of approximately 5%. Because of this, the initial weight was used as a co-variable in subsequent analysis of the growth performance responses. At 20 days of age, the body weight was similar between treatments; the initial weight difference was reduced to 1%. The FI, WG and FCR were also similar between treatments. The carcass weight and its components, as well as the tibia measurements, with the exception of the diameter of the lower diaphysis did not differ between treatments. The diameter of the lower diaphysis of the tibia was higher ($P < 0.10$) in YFP-fed broilers. In two previous trials carried out in our laboratory, higher ashes retention (5) and higher ashes apparent ileal digestibility (6) were observed in broilers consuming diets added with a hydrolyzed yeast. Gao *et al*. (3), found that broilers fed increasing levels of a yeast culture showed lineal increments in calcium and phosphorus digestibility. The reported increases in the retention and digestibility of ashes, calcium and phosphorus of broilers fed diets added with different yeast products may explain the larger diameter of the lower diaphysis found in the present work.

No oocysts in excreta were detected in 20-d old broilers. In the histopathologic evaluation of the intestine, it was found that the lymphoid clusters ($P < 0.10$) and the hyperplasia of the Peyer patches ($P < 0.05$) were higher but the hyperemia/hemorrhage ($P < 0.10$) was lower in the duodenum of YFP-fed broilers. We may hypothesize that these histopathological responses may be related to immune stimulation of the gut lymphoid tissue by an infectious microorganism, other than coccidia, that lead to higher cell infiltration or hyperplasia of lymphoid tissues. The higher lymphoid clusters and hyperplasia of the Peyer patches may indicate a higher immune response in YFP-fed broilers. These suggestions are back up by higher ($P < 0.10$) IgA concentration in the liquid washed from the duodenum of YFP-fed broilers, which showed an increment of 38% compared to the group without YFP, and also agrees with previous reports (3, 7). However, the serum concentrations of MBL did not show differences between treatments. This result agrees with a previous experiment where broilers fed increasing levels of YFP did not show any change in the MBL concentrations from 17 to 35 d of age (2).

Period from 21 to 28 days of age. The body weight of 28-d broilers and the WG from 21-28 d did not show statistical differences among treatments. In the FI, a YFP and COC interaction ($P < 0.05$) was observed since the FI of coccidia challenged broilers without added YFP was the lowest, whilst the FI of the rest of the treatments was similar. The FCR was lower in coccidia challenged broilers ($P < 0.01$) compared to non-challenged broilers. Opposite to our results, Nollet *et al*. (10) and Gao *et al*. (4) found depressed WG and increased FCR in coccidia challenged broilers. In agreement to our findings, in the studies of Gao *et al*. (4) and Nollet *et al.* (10) the FI of coccidia infected broilers was depressed. The addition of YFP in the diet of coccidia challenged broilers improved the FI, which was closets to that of non-challenged broilers, but did not have any effect on the WG and FCR. Opposite to this, Shanmugasundaram *et al*. (12) and Gao *et al*. (4) observed lower WG, FI and FCR in yeast-fed broilers challenged with coccidia; whereas, Nollet *et al*. (10) found that the WG, FI and FCR were not affected in MOS-fed broilers challenged with coccidia. In a meta-analysis of data to study the relationship of the variation in FI and WG in coccidia infected broilers, it was concluded that the magnitude of the effects on WG and FI varied with the type of *Eimeria*, animal age, sex, and genetic line (8).

The length of the tibia ($P < 0.01$) and weight ($P < 0.05$) of ashes were lower in coccidia challenged broilers. In a recent study, *Eimeria* challenged broilers

had decreased tibia relative weight of ash content and ash concentration at 14 d of age (1). The COC and YFP interaction was significant ($P < 0.05$) for the diameter of the inferior epiphysis and the diameter of the diaphysis of the tibia. The diameter of the inferior epiphysis in coccidia challenged non-YFP added broilers was the lowest, which is in agreement to the lower length and weight of ashes of the tibia seen in coccidia challenged broilers; and the diameter of the inferior epiphysis of the non-challenged YFP-fed broilers was the highest, which also agrees with the higher value of this response seen in YFP-fed broilers in the previous period, and other reports (5, 6). Furthermore, the coccidia challenged YFP-fed broilers also showed greater diameter of the inferior epiphysis compared to the coccidia challenged non-YFP added broilers. This result is also supported by other studies (1). In line to the previous result, the diameter of the diaphysis in coccidia challenged non-YFP added broilers was the lowest and that of the coccidia challenged YFP-fed broilers was the highest, whereas the other treatments had intermediate values.

No oocysts were found in non-challenged broilers at 28-d of age; while in coccidia challenged birds a positive excretion of oocysts was observed, being higher for the group without YFP compared to the YFP-fed broilers (COC and YFP interaction, $P < 0.05$). This agrees with previous studies (4, 7). The COC and YFP interaction was significant ($P < 0.05$) for the lymphoid clusters in the duodenum since in non-challenge YFP-fed broilers were the lowest and for the non-challenge non-YFP added broilers were the highest, while in the other treatments intermediate values were observed. This finding is opposite to the response seen in the previous period. It is surprising that the highest lymphoid response was observed in broilers in which no oocysts in excreta were detected. In the duodenum, the hyperemia/hemorrhage was lowest in non-challenged non-YFP added broilers and was highest in challenged YFP added broilers. It is also contradictory that the hyperemia/hemorrhage was higher in coccidia challenged broilers which had the lower oocyst excretion. These results need further clarification. The similar IgA concentration among treatments is opposite to previous finding (3, 7). The MBL concentrations in non-challenge YFP-fed broilers was the lowest and for the challenge YFP-fed broilers was the highest; the other treatments had intermediate values. In coccidia challenged YFP-fed broilers the MBL concentration was greater, which could be related to the lower excretion of oocysts in these birds compared to those not added with YFP; however, the MBL concentration of the challenged YFP-fed broilers was similar compared to that of chickens not challenged and not added with YFP. This suggests that the differences in MBL concentrations were independent of the coccidia challenge and the addition of YFP. In summary, the addition of YFP improved the FI and tibia mineralization and reduced the oocyst counts in broilers challenged with a coccidia vaccine, but it did not show any effect on the duodenal IgA and serum MBL concentrations.

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EVALUATION OF THE EFFICIENCY OF PREPARATIONS OF QUINFAMIDE FOR THE COCCIDIOSIS TREATMENT IN BROILER CHICKENS

EVALUACION DE LA EFICIENCIA DE LAS PREPARACIONES DE QUINFAMIDE PARA EL TRATAMIENTO DE LA COCCIDIOSIS EN POLLOS DE ENGORDA

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RESUMEN

Existe una necesidad creciente para los fármacos nuevos para el control de la coccidiosis en la avicultura. Por lo tanto, es justificable la investigación acerca del desarrollo de las nuevas opciones farmacológicas. Hay evidencia de que quinfamida tiene una eficacia anticoccidiana baja en ovinos, lo cuál puede incrementarse cuando se combinan con un compuesto gastro-retentivo. Por esta razón, la investigación de quinfamida se propuso como una nueva opción para el tratamiento y el control de la coccidiosis aviar. El objetivo de esta prueba fue evaluar la eficacia anticoccidiana de quinfamida en pollos de engorda solos y la combinación correcta con quitosan o carbopol como vehículos gastro-retentivos en pollos de engorda para un ciclo de producción, administrándolos en combinación con el alimento por una nueva forma de dosificación. Los resultados fueron favorables, con la primera dosis de quinfamida sola se obtuvo la mejor eficacia anticoccidiana y subsecuentemente se determinó que esta eficacia no se aumentó cuando se combinó con quitosan, pero la combinación con carbopol fue favorable, así que se escogió para manejarse en combinación con el alimento en un ciclo de producción. Al final del estudio se determinó que la quinfamida tiene efectividad anticoccidiana en pollos y esta eficiencia puede aumentarse cuando se administra en combinación con carbopol y muestra un residuo bajo.

SUMMARY

There is a growing need for novel drugs to the control coccidiosis in poultry. It is therefore justifiable research on the development of new pharmacological options. There is evidence that quinfamide has low anticoccidial efficacy in sheep, which can be increased when combined with a gastro-retentive compound. For this reason, quinfamide research was proposed as a new option for the treatment and control of avian coccidiosis. The aim of this trial was to evaluate the anticoccidial efficacy of quinfamide in broilers alone and the right combination with chitosan or carbopol as gastro-retentive vehicles in broilers for a production cycle, administering them in combination with food by a new dosage form. The results were favorable, first dose quinfamida alone with the best anticoccidial efficacy was obtained and subsequently determined that this efficacy was not increased when combined with chitosan, but the combination with carbopol was favorable, so it was chosen to manage in combination with the food over a production cycle. At the end of the study it was determined that the quinfamida has limited effectiveness anticoccidial in chickens and this efficiency can be increased when administered in combination with carbopol and shows a low residual.

INTRODUCTION

Coccidiosis is one of the most common and economically important diseases of chicken and has a major economic impact on the global poultry industry (3,20). All *Eimeria* species tend to develop drug resistance to most anticoccidial drugs (2,15,19,21). Consequently, the search for new drugs is always welcomed (5,6). Quinfamide, is widely used to treat intestinal *Entamoeba* infections in humans (13,14,17).

Has low bioavailability and acts at the luminal level, immobilizing trophozoites of *Entamoeba histolytica* causing somehow their destruction and their elimination with faeces (11,17). Amoebas and coccidia do not belong to the same taxonomic phylum. The anticoccidial effect of quinfamide on *Eimeria*-infected sheep was reported as moderate and enhanced by chitosan (1). This effect due to the vehicle anticipated that efficacy of quinfamide could be affected by formulation. For this reason, the aim of this trial was to evaluate the effectiveness of quinfamide compared with that of quinfamide formulated with a polymer matrix-vehicle (carbopol) and utilizing decoquinate as a standard in the treatment of avian coccidiosis caused by *Eimeria maxima*, *E. tenella* and *E. acervulina*.

MATERIALS AND METHODS

Quinfamide was pelleted with and without carbopol. This latter carrier was added because it has mucoadhesive properties and slows down the transit time of quinfamide in the gut (16). Feed was first prepared without the experimental compounds. The necessary amount of pellets was added to the daily required quantity of food and then homogenized. Three-hundred one-day-old female Cobb healthy broiler chickens were raised in a test house with clean wood shavings in the Department of Aviculture, at the *Faculty of Veterinary Medicine* of the *Universidad Nacional Autónoma de México* (UNAM). The floor-pen trial was performed according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (7). Two-week old, ten coccidian-free Cobb-500 seeder chickens were inoculated with a mixture containing sporulated oocysts of *Eimeria acervulina*, *E. maxima* and *E. tenella* (4). They were placed into each pen, except in the uninfected-group pens, on days 4, 5 and 6 after their inoculation. One-day old broiler chickens were weighed and randomly allotted to one of three experimental treatments and two control groups in separated pens (20 chickens/replicate and 3 replicates per group), with seven weekly measurements. In order to determine quinfamide anticoccidial efficacy in broilers, three different treatments for experimentally infected broilers were assayed throughout the duration of this trial, *i.e.,* from day one to 49. Two control groups were also included in this study: an uninfected and untreated control group (UU) and an infected and untreated group (IU). The UU group was set aside in a clean separated space. Treatments were provided as an in-feed mix, using the following inclusion rates: 30 ppm of quinfamide at 0.1% (group Q); 30 ppm of quinfamide at 0.1% mixed with carbopol at 0.1% (group QC). The third experimental group received 30 ppm of decoquinate prepared at 0.1% (group D) and was considered the golden standard. Body weight gain was measured individually on days 1, 7, 14, 21, 28, 35, 42 and 49. On days 1, 7, 14, 21, 28, 35, 42 and 49, litter samples were collected from each pen in order to quantify the number of oocysts per gram of litter (7). Oocysts counts were expressed as oocyst per gram of litter and were determined for each pen obtaining three repetitions per group. The McMaster technique was carried out (10,12). On days 21, 35 and 49, four broiler chickens were randomly selected from each pen and were humanely euthanized, based on Mexican regulations (18). Numerical ranking of gross lesions was developed, setting a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) and 4 (extremely severe lesions) (8). Coccidial intestinal lesions were scored in the upper, middle, and caecal regions. Statistical analysis was carried out based on a Generalized Linear Model (GLM). All significant differences were based on $P < 0.05$. The analyses were performed with package software IBM SPSS® Statistics 20.

RESULTS

At the end of this trial, body weight gain from the quinfamide (**Q**) and quinfamide + carbopol (**QC**) groups were statistically higher than the corresponding values for decoquinate (**D**) and the infected-untreated (**IU**) groups (Figure 1). Starting from day 21 until the end of the study, the group that received quinfamide + carbopol (**QC**) shed significantly ($P < 0.05$) less oocysts than infected groups treated with quinfamide and infected birds that remained untreated. Oocyst excretion was similar in broilers that consumed decoquinate (**D**) and the combination of quinfamide + carbopol (**QC**). As far as lesions scores in the D group is concerned, the coccidial challenge induced first-degree lesion scores. The QC group showed most of the first-degree lesions confined to the cecal region, where walls were thickened, and lumen size was reduced as compared to UU animals. Statistically significant differences in lesion scores frequencies were detected among all groups ($P = 0.001$).

DISCUSSION

This study shows that quinfamide alone has limited anticoccidial activity, while the combination of quinfamide plus carbopol reduces, in a statistically significant manner, the oocyst per gram of litter shedding rate in broiler chickens. It is interesting to notice that carbopol increases activity of quinfamide against *Eimeria.* In the current study, unaltered body weight gain, low mortality rate and low degree of lesions of the GI tract observed in treated birds,

suggest that intestinal integrity was preserved. One interesting and unexpected result obtained, was the growth enhancement effects observed for Q and QC groups. Their body weight gain was like that of the UU group, in which chickens were likely to possess unaltered intestinal integrity. Results demonstrated that the quinfamide-treated groups gained significantly more body weight than the D and the UU groups. This preliminary report describes that, without a clear-cut effect of quinfamide increasing feed intake, groups treated quinfamide and quinfamide-carbopol increased body weight gain and reduced both, oocyst output and intestinal lesion scores. Hence, utilization of quinfamide, quinfamide-carbopol and/or chemical analogs as alternatives to control coccidiosis in broiler chickens, merit further research.

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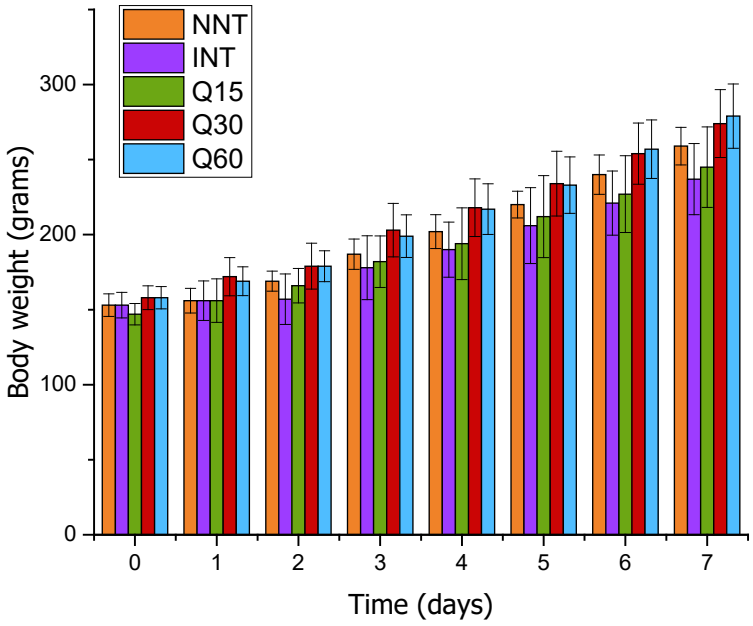
Figure 1. Anticoccidial efficacy of three treatments based on broiler body weight (g) (mean ± SE, n = 60 per group). UU = uninfected-untreated control group; IU = infected-untreated control group; Q = quinfamide 30 ppm; QC = quinfamide 30 ppm, mixed with carbopol at 0.1% and D = decoquinate 30 ppm.



HIPÓTESIS SOBRE LA ESPONDILOLISTESIS COMO UNA DE VARIAS CONSECUENCIAS DEL MANEJO ALTO DEL BEBEDERO DE NIPPLE

HYPOTHESES ABOUT THE SPONDYLOLISTHESIS AS ONE OF SEVERAL CONSEQUENCES OF THE HIGH MANAGEMENT OF THE NIPPLE DRINKER

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SUMMARY

Nipple drinkers have come to revolutionize the way drinking water is presented to broilers, providing the main benefits of water hygiene and less transmission of diseases compared to channel-shaped waterers that are contaminated with dust and feces. If the producer raises them very high, the chickens must stretch to reach them, attaining an almost erect or upright posture. The anatomy of the chicken from their ancestors up to modern strains intensively used today is not designed for this uncomfortable position, sometimes resulting in deviation, slippage, or misalignment of the only moveable portion of the vertebral column, which located between the notarium and the synsacrum.

RESUMEN

El bebedero de niple, ha venido a revolucionar la forma de proveerles agua a los pollos de engorda, con grandes beneficios principalmente de higiene del agua y menos transmisión de enfermedades por este conducto. Comparado con los bebederos en forma de

canal que se contaminan más con el polvo y partículas de excremento que caen en su interior. Si el personal los eleva mucho, el pollo se tiene que estirar para alcanzarlos, llegando a adoptar una postura casi erecta y/o vertical. La anatomía del pollo desde sus ancestros hasta las estirpes explotadas intensivamente en la actualidad, no está diseñada para esta incómoda postura. Llegando como consecuencia en algunos casos a desviar, resbalar o desalinear la única vertebra móvil que se encuentra entre el notarium y el sinsacro.

INTRODUCCIÓN

En condiciones normales, la anatomía de la región torácica de la columna vertebral de las aves domésticas y concretamente la del pollo de engorda, ha estado diseñada por la naturaleza para mantenerse en forma casi horizontal con una muy ligera inclinación de adelante hacia atrás cuando el pollo se desplaza o se mantiene de pie. Y no vertical, como sería el caso del ser humano. Y solo las vértebras cervicales están diseñadas para trabajar en diferentes posiciones, incluida la vertical, solo que su trabajo es diferente y la carga que soportan, es mucho menor comparado con las vértebras torácicas. Y cuando está comiendo, las vértebras torácicas se mantienen en forma horizontal. O con una ligera inclinación hacia adelante.

Espondilolistesis.-(del griego *spóndylos*, vértebra y *olisthesis*, resbalón). El significado de este término es resbalón o deslizamiento de una vértebra con relación a la adyacente.

Esta afección puede aparecer en cualquiera de las regiones vertebrales de las aves domésticas; pero en el pollo de engorda, marcadamente se ha venido observando en las vértebras de la región del tórax. Concretamente algunos autores refieren dicho resbalón o deslizamiento a la 6ª vertebra torácica que es la vértebra móvil y además queda entre el notarium y el sinsacro.

El padecimiento va desde un esguince muy leve, pasando por una desalineación mínima, en la mayoría de las veces imperceptible a simple vista, hasta la total desalineación de una vértebra con otra. Todas las presentaciones anteriores, invariablemente cursan con dolor, desde leve hasta incapacitante. Llegando incluso a la postración. Este padecimiento ha sido poco o muy poco estudiado en aves, pero puede llegar a ser de importancia productiva y, por lo tanto, económica, porque implica no solo la postración de los pollos con un daño muy evidente en la columna vertebral; sino también el hecho de solo sentir dolor por la fatiga de las estructuras propias de las vértebras y estructuras complementarias de las articulaciones vertebrales como son: cartílago, ligamentos, músculos, membrana sinovial por la posición anormal constante por querer alcanzar el bebedero de niple colocado alto donde el pollo se tiene que estirar y casi ponerse en forma casi erecta y/o vertical.

Esta hipótesis, deriva de la observación de varias parvadas en los cuales a partir de la cuarta semana comenzaban a aparecer pollos con una incoordinación desde muy leve, hasta postración sin ninguna causa aparente. Acentuándose más durante la sexta semana. Derivando lo anterior en un porcentaje de hasta un 2% de animales condenados al sacrificio prematuro. Con sus respectivas consecuencias económicas para las empresas.

Participación del bebedero alto en el daño. El bebedero de niple ha venido a revolucionar la manera de proporcionar Agua de bebida a las aves explotadas intensivamente. Y en este caso que nos ocupa, al pollo de engorda. Siendo muchos los beneficios que podemos encontrar y entre ellos son: suministro de agua casi en cualquier lugar de la caseta; Ahorro de horas hombre de trabajo; por lo tanto, en mano de obra, debido a que de hecho solo se lavan al término de la parvada; por su estructura, posición y funcionamiento, mayor higiene del agua de bebida, debido a que se contaminan menos y a su vez, contaminan menos el agua que beben los pollos. Sin embargo, cuando el personal que labora en granjas por ignorancia de la anatomía y fisiología de los pollos, lo maneja alto o muy alto, el pollo se tiene que estirar mucho para alcanzarlo. Y si agregamos a lo anterior el peso que le hemos venido exigiendo al pollo, la desalineación o resbalón de la vértebra puede suceder con mayor facilidad en algunos individuos en particular. Y es en esta circunstancia, que sospechamos se genera el daño mecánico a la 6ª vertebra torácica que es la única vertebra móvil que existe entre el notaruim y en sinsacro.

Comentario: En nuestro quehacer cotidiano, nos enfrascamos tanto en el manejo de todo lo que interactúa para lograr nuestros objetivos de consumos, pesos, conversión, coeficiente de variación o uniformidad, índice de productividad, etc. Que nos olvidamos de la gran importancia que tiene la interacción en armonía entre la mecánica y la biología.

PROPUESTA

Una vez formulada esta hipótesis, se propone comenzar a realizar estudios en parvadas de pollos de engorda para confirmar o descartar la participación del manejo alto del bebedero de niple en esta afección; realizando:

Monitoreos semanales del peso corporal, del coeficiente de variación o uniformidad.

Mortalidad, conversión, ganancia de peso diaria e Índice de productividad.

De 1 a 6 semana, probar con diferentes alturas del bebedero de niple, en pollos de engorda

A las 6 semanas se sacrificará una muestra estadística de aves:

Sanas, sospechosas y afectadas

Toma de radiografías de las vertebras involucradas.

Buscar lesiones histopatológicas de cartílago y de hueso de las vertebra involucradas.

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CORRELATION OF *SALMONELLA* ENUMERATION OF BOOTSOCKS, CECA, AND CARCASS RINSES FROM BROILER CHICKENS

CORRELACIÓN DE LA ENUMERACIÓN DE *SALMONELLA* EN LAVADOS DE TORULAS DE ARRASTRE, CIEGO, Y CANALES DE POLLOS DE ENGORDA

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RESUMEN

En la industria avícola de engorda el abordaje regulatorio del control de patógenos de origen alimenticio en los EE. UU. se enfoca principalmente en las plantas de procesamiento. Las pruebas regulatorias identificaron un decremento en la prevalencia positiva de *Salmonella* en un 20% en 1996 a un 6.5% en el 2011, sin embargo, la presentación de infecciones humanas de *Salmonella* permaneció primariamente sin cambios durante el mismo periodo de tiempo (1). Las intervenciones en las plantas de procesamiento son efectivas en la reducción de la prevalencia de patógenos de origen alimenticio sobre las canales de pollo de engorda, aunque las reducciones proporcionadas por estas intervenciones son limitadas. El control pre-sacrificio de los patógenos en los pollos de engorda pueden ser clave en la reducción de la cantidad de contaminación microbiana de las canales. Esto incluye: medidas de bioseguridad y medidas de sanidad, controlar la humedad de la cama, usar retiro de alimento bien calendarizado antes del sacrificio, usar ácido en el agua de beber durante el periodo de retiro de alimento, usar programas de vacunación, y monitorear las parvadas por patógenos antes del procesamiento. Comprender que la carga de *Salmonella* que entra a la planta de procesamiento ayudará a saber que tan bien están trabajando las intervenciones, y ayudar a las compañías avícolas a cumplir los requerimientos de prueba regulatorios. En el 2013, Berghaus *et al*., encontraron en un estudio en granjas que la prevalencia de *Salmonella* y la carga en torulas de arrastre para correlacionar con la carga de *Salmonella* en los lavados de las aves completas con plumas (1). En el actual estudio controlado de 32 corrales, las aves fueron muestreadas para evaluar la correlación de la prevalencia y la carga de *Salmonella* en los lavados de torulas de arrastre, el ciego y la canal. Se les aplicó *Salmonella* Heidelberg al 50% de las aves en el corral a los cuatro días de edad. Diez aves del desafío horizontal por corral fueron procesadas en una planta piloto de procesamiento a pequeña escala a los 42 días de edad. Después del procesamiento, se lavaron las canales y los ciegos de las diez aves de desafío horizontal. Se tomaron cinco ciegos adicionales de aves de desafío directo de cada corral, y se colectaron torulas de arrastre de cada corral.

SUMMARY

The regulatory approach to foodborne pathogen control in the U.S. broiler chicken industry is focused largely on processing plants. Regulatory testing identified the prevalence of *Salmonella*-positive broiler carcasses decreased from 20% in 1996 to 6.5% in 2011, however, the occurrence of human *Salmonella* infections remained primarily unchanged during the same period of time (1). Processing plant interventions are effective at reducing prevalence of foodborne pathogens on broiler carcasses, although the reductions provided by these interventions are limited. Pre-harvest pathogen control in broiler chickens can be key in reducing the amount of microbial contamination of carcasses. These include: biosecurity measures and sanitation practices, controlling litter moisture, using well-timed feed withdrawal prior to slaughter, using acid in drinking water during feed withdrawal, using vaccination programs, and screening flocks for pathogens prior to processing. Understanding the load of *Salmonella* coming to the processing plant will aid in determining how well pre-harvest interventions are working, and help poultry companies meet the regulatory testing requirements. In 2013, Berghaus *et al*. found in an on farm study that *Salmonella* prevalence and load of bootsocks to correlate to the *Salmonella* load in whole bird rinses with feathers on (1). In this current controlled 32 pen study, birds were sampled to evaluate the correlation of prevalence and load of *Salmonella* in bootsocks, ceca, and carcass rinse samples. *Salmonella* Heidelberg was given to 50% of the birds in each pen at four days of age. Ten horizontally challenged birds per pen were processed at a small-scale pilot processing plant at 42 days of age. After processing, carcass rinses and ceca (post-rinse) were collected from the ten horizontally challenged birds. Additional ceca were taken from five directly challenged birds per pen, and bootsocks were collected from each pen.

INTRODUCTION

Salmonellosis remains a major cause of human foodborne illness, and continues to be a significant health concern. The Centers for Disease Control and Prevention has estimated that nontyphoidal *Salmonella* species are second only to norovirus as a leading cause of foodborne illness in the United States, causing approximately 11% of all domestically-acquired foodborne illnesses, and that Salmonella species are the leading cause of hospitalizations (35%) and deaths (28%) from foodborne illnesses (4). The main obstacle to *Salmonella* control in the poultry industry is the abundance of the bacteria. Once *Salmonella* gets onto a farm, they spread rapidly due to infected chickens and rodents serving as carriers. *Salmonella* carriers constantly shed the bacteria and contaminate the environment (3). The continuing problem of contamination of retail poultry products with *Salmonella* produces important public health concerns, especially considering the global increase in chicken consumption. In response, the U.S. Department of Agriculture (USDA) implemented the Hazard Analysis of Critical Control Points (HACCP) program in meat- processing plants to provide quality control and surveillance in attempt to reduce the amount of *Salmonella* contamination associated with poultry (2).

MATERIALS AND METHODS

Experimental design. In this thirty-two-pen study eight hundred Ross x Ross male broilers were assigned to two treatment groups, with twelve replicate blocks per treatment. Birds received routine vaccinations (HVTSB1) at hatchery, and were vaccinated with an approved broiler coccidiosis vaccine. Twenty-five broiler chicks were allocated into each floor pen. Water and feed were provided *ad libitum*.

***Salmonella* challenge.** At four days of age *Salmonella* Heidelberg was given to 50% of the birds (13 birds per pen) in each pen by oral gavage with a $2.2x10^7$ CFU/chick nalidixic acid resistant *Salmonella* Heidelberg. Horizontally exposed chicks were tagged and color-coded (for identification).

Sample collection. On day 42, ten horizontally challenged birds per pen were processed at a small-scale pilot processing plant. After processing, carcass rinses and ceca (post-rinse) were collected from the ten horizontally challenged birds. Additional ceca were taken from five directly challenged birds per pen, and bootsocks were collected from each pen. Carcass rinses were collected from defeathered birds by placing the birds in individual plastic bags with 100 mL of buffered peptone water. Birds were shaken for one minute in a mechanical carcass shaker. After removal from the carcass shaker, the buffered peptone water was poured into prelabeled sterile sample cups. Cecal samples were collected aseptically using disinfectant between each bird, and placed into sterile whirlpak bags. Bootsocks were taken from each pen by placing bootsocks over bootcovers and walking the entire surface of the pen.

Laboratory/Sample processing. Upon arrival at the lab 10 mL of a 10X tetrathionate brilliant green broth was added. Bootsocks were stomached in 100 mL of 1X tetrathionate brilliant green broth. Cecal samples were weighed, stomached and 50 mL of 1X tetrathionate brilliant green broth was added. Three

1ml aliquots were removed from cecal, carcass rinse, and bootsock samples for MPN analysis. All samples (carcass rinses, bootsocks and ceca) were incubated overnight at 42ºC. A 10 uL loopful was struck from the incubated samples to XLT-4 + nalidixic acid agar and incubated overnight at 37ºC. Up to 3 black colonies were selected and confirmed as *Salmonella* heidleberg using poly-O *Salmonella* specific antiserum. For all samples, the three 1 mL sample of stomachered tetrathionate brilliant green broth were transferred to three adjacent wells in the first row of a 96 well 2-mL deep block. A 0.1 mL aliquot of sample was transferred to 0.9 mL of tetrathionate broth in the second row, and the process was repeated for the remaining rows (to produce 5 ten-fold dilutions), and the blocks incubated (24 h at 42º C). A 1 uL sample from each well of the blocks was transferred onto XLT-4 agar containing nalidixic acid with a multi-channel pipettor, incubated plates (37º C for 24 h), recorded final dilution of each sample, and entered in MPN calculator (1). Suspect *Salmonella* isolates were confirmed by poly-O *Salmonella* specific antiserum.

Statistical analysis. Spearman's nonparametric correlation coefficient was used to quantify the association between MPN values obtained for boot sock samples with the pen-level mean MPN values for ceca and carcass rinse samples, and to quantify the association between boot sock MPNs and pen-level *Salmonella* prevalences for ceca and carcass rinse samples. Pens with no *Salmonella* identified in carcass rinse samples were arbitrarily assigned a mean of 1.0 log10 MPN/carcass, and pens with no *Salmonella* identified in ceca samples were assigned a mean of -0.5 log10 MPN/g, to facilitate graphical presentation of the data. Analyses were performed using commercially available statistical software (Stata version 15.1, StataCorp LLC, College Station, TX).

RESULTS AND DISCUSSION

The data from this study was used to evaluate if the bootsock most probable number (MPN) could be used as a predictor of ceca MPN and carcass rinse MPN. The relationship between bootsock MPN and carcass rinse MPN was not statistically significant, however there was a statistically significant positive association between bootsock MPNs and pen-level mean ceca MPNs and prevalences.

In summary, the goal of *Salmonella* control for broiler companies is to lower the level of *Salmonella* entering the processing plant. HACCP was developed in an attempt to improve food safety and reduce human illnesses caused by poultry through mandating in-plant changes that would reduce contamination product with food-borne pathogens. While chicken carcass contamination with *Salmonella* has declined since the implementation of HACCP, the incidence of human illnesses associated with *Salmonella* has remained relatively unchanged (2). Therefore, enumeration of *Salmonella* level in ceca usually provides an indication of an intervention's effectiveness to lower the load of *Salmonella* entering the processing plant. The data from this study show that bootsocks could be used to predict the incoming load of *Salmonella.*

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Figure 1. Scatter plot of boot sock *Salmonella* MPNs versus pen-level mean ceca MPNs for 32 pens. The line in the plot represents the best fit for a simple linear regression with boot sock MPN as a predictor of mean ceca MPN.

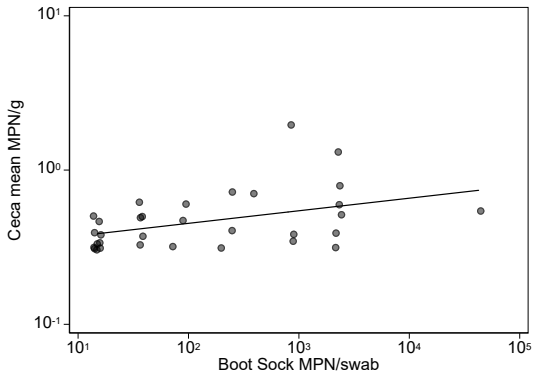
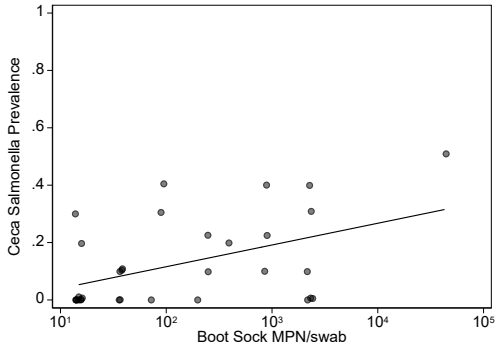


Figure 2. Scatter plot of boot sock *Salmonella* MPNs versus pen-level *Salmonella* prevalence in ceca samples for 32 pens. The line in the plot represents the best fit for a simple linear regression with boot sock MPN as a predictor of *Salmonella* prevalence in ceca samples.



A CASE REPORT OF HIGH MORTALITY IN PIGEONS DUE TO VIRAL HEPATITIS

UN REPORTE DE CASO DE ALTA MORTALIDAD EN PALOMAS DEBIDO A HEPATITIS VIRAL

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RESUMEN

De abril a noviembre de 2018, el Laboratorio de Salud Animal e Inocuidad Alimentaria de California – Turlock branch, recibió seis envíos de palomas de competencia y pichones debido a un aumento en la mortalidad. Los envíos contenían palomas adultas con una historia de depresión, diarrea y debilidad. La morbilidad de la parvada tuvo un pico de 80%, y la mortalidad a 25%. Las palomas enviadas demostraron tener una debilidad severa y dificultad respiratoria ante mortem. A la necropsia, se notó que los hígados estaban moderadamente congestionados y aumentados de tamaño, con cambios esplénicos y pancréaticos leves. Microscópicamente, se observó una necrosis de leve a severa y una degeneración de hepatocitos con hiperplasia de leve a severa del conducto biliar, inflamación mononuclear, congestión sinosoide y hemosiderosis. Se notó que había infiltración leve de células mononucleares inflamatorias en el pancreas y riñones. Con microscopía electrónica directa se identificaron partículas tipo reovirus en el hígado. Los hígados eran positivos a rotavirus por RT-rtPCR. La hepatitis en palomas debida a una infección por rotavirus ha sido poco reportada y representa un patógeno significativo para la industria de las palomas debido al potencial de alta mortalidad de la parvada y pérdida de la producción.

SUMMARY

From April to November 2018, the California Animal Health and Food Safety Laboratory-Turlock branch received six submissions of racing pigeons and squab due to increased mortality. Submissions contained adult pigeons with a history of depression, diarrhea, and weakness. Flock morbidity peaked at 80%, and mortality at 25%. Submitted pigeons demonstrated severe weakness and labored breathing ante mortem. At necropsy, moderately congested and enlarged livers were noted, with mild splenic and pancreatic changes. Microscopically, mild to severe necrosis and degeneration of hepatocytes was observed with mild to severe bile duct hyperplasia, mononuclear inflammation, sinusoidal congestion, and hemosiderosis. Mild mononuclear inflammatory cell infiltration of the pancreas and kidney was noted. Direct electron microscopy identified reovirus-like particles from liver. Livers were positive for rotavirus by RT-rtPCR. Hepatitis in pigeons due to rotavirus infection has been infrequently reported and represents a significant pathogen for the pigeon industry due to the potential for high flock mortality and lost production.

INTRODUCTION

Rotaviruses are known to cause viral enteritis in mammals and avian, and are sometimes associated with economically significant nonspecific enteric disease in the commercial poultry sector (2). Avian rotaviruses A, D, F, and G have been isolated worldwide and associated with disease in turkeys, chickens, pheasants, partridges, pigeons, ducks, parrots, and parakeets (3,5,8,9,12). Avian rotaviruses are composed of 11 double-stranded RNA segments and are members of the *Reoviridae* family (2). While rotavirus infection is typically associated with diarrhea, rotavirus viremia has been reported sporadically (1,7,11). Rotavirus A was isolated from racing pigeons suffering from diarrhea in 1992, and recent reports of rotavirus A causing vomiting, diarrhea, hepatitis, and increased mortality in domestic pigeon flocks in Australia and Europe have been published (4,7,11).

CASE REPORT

From April to December 2018, six submissions of domestic pigeons (*Columba livia domestica*) were presented to the CAHFS Laboratory, Turlock branch, for diagnostic evaluation. Both males and females were represented in submissions, with ages ranging from eight weeks to four years of age. Flock morbidity was reported as high as 80%, and mortality varied from 7% to 28%. Mortality occurred over a seven to nine-day period, affecting both young breeder candidates and adult breeder pigeons. Owners reported severe depression and weakness, extreme thirst, vomiting water and whole corn kernels, and diarrhea.

Gross lesions observed at necropsy were minor and inconsistent, however most pigeons had crops distended with water. Some livers appeared normal in coloration and consistency while others appeared enlarged, darkly mottled, congested, and friable. Spleens were inconsistently enlarged and mottled, and kidneys variably pale and enlarged. Microscopically, mild to severe interconnecting hepatocellular necrosis with vesicular degeneration was observed in liver sections, with amorphous eosinophilic intracytoplasmic inclusion bodies (presumed councilman bodies) present in areas of severe necrosis. Minimal lymphoplasmacytic infiltration was observed periportally, with mild to severe biliary duct hyperplasia present. Variable sinusoidal congestion and erythrophagocytosis was present in most liver sections. Rotavirus A immunohistochemistry revealed positive staining in liver sections from all cases. Rotavirus RT-rtPCR targeting the *NSP4* gene was positive from liver and spleen in all submissions. Reovirus, influenza A virus, and avian paramyxovirus-1 RT-rtPCRs were negative from all submissions. Viral particles resembling those of the family *Reoviridae* were observed in liver preparations by direct electron microscopy.

The electronic database of CAHFS was searched for necropsy cases in which reovirus-like viral hepatitis was diagnosed in pigeons system wide between January 1, 2000 to December 31, 2018. Eight cases were identified, with four originating from racing flocks, one from squab producing flocks, and three with no specified use. Case history, gross lesions, and microscopic lesions were similar to those reported for the current outbreak. Reovirus-like viral hepatitis was diagnosed by transmission electron microscopy or direct electron microscopy of liver.

DISCUSSION

We have identified rotavirus A from the livers of pigeons suffering from severe hepatic disease. Reovirus-like particles were visualized in all cases by direct electron microscopy from liver samples, and are compatible with rotavirus A as it is in the family *Reoviridae* and shares similar morphologic features with reovirus (2). Rotavirus RT-rtPCR was positive from liver in each submitted case, and rotavirus A immunohistochemistry was positive on liver sections from each case. The six submissions were comprised mainly of squab breeder candidates and one racing flock. The squab breeder owners belonged to a co-operative where squab were delivered on a weekly basis. The role of horizontal transmission is suspected in this case. Wild birds may have also played a role in the transmission of rotavirus A, as rotavirus A seroprevalence in feral and domestic pigeons is reported to range from 10-68% (4,6,10,13). Reports of increased wild bird mortality were not noted during these outbreaks by owners, however the first case in squab breeders did report wild birds living in the pigeon lofts.

Previous to this outbreak in 2018, reovirus-like viral hepatitis was rarely diagnosed in CAHFS pigeon submissions. One case every few years was diagnosed until 2018, where nine cases were diagnosed at CAHFS system wide (including the discussed Turlock cases). These retrospective cases were termed “reovirus-like viral hepatitis” due to the fact that diagnosis was made based off of direct and transmission electron microscopy from liver, where “reovirus-like” viral particles were observed. The authors hypothesize that these cases were actually due to rotavirus A infection based off of similar histories, gross and microscopic lesions, and electron transmission results, however definitive diagnostics for rotavirus A were not performed in these retrospective cases.

In summary, we conclude that the histologic, molecular, and electron microscopy results are supportive of rotavirus A hepatitis in the submitted pigeons. Rotavirus A hepatitis in pigeons is a relatively new disease, with outbreaks reported in Australia and Europe in 2017 (7,11), and the currently described outbreak in California pigeons. Further research is required to elucidate the possible transmission routes, pathogenicity, and reservoirs of rotavirus A causing hepatitis in pigeons.

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MONTANIDE™ ISA 71 R VG FOR LONG TERM PROTECTION VACCINE AGAINST INFECTIOUS CORYZA

LA MONTANIDE™ ISA 71 R VG COMO VACUNA PARA PROTECCIÓN A LARGO PLAZO CONTRA LA CORIZA INFECCIOSA

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RESUMEN

La coriza infecciosa (CI) es una enfermedad aguda causada por el *Haemophilus paragallinarum* el cual afecta el sistema respiratorio aviar. Para desarrollar una vacuna trivalente de CI para combatir los tres serovares, con adyuvante que mejora la eficacia de la vacuna, se requiere que resista el medio desestabilizante bacterial, y permite una tasa flexible de adyuvante en vacunas multivalentes.

En una pequeña prueba con 30 pollos y en una prueba en gran escala con 100,000 pollos, fueron evaluadas vacunas trivalentes de CI basada en Montanide™ ISA 71 R VG o un adyuvante estándar. Las vacunas basadas en el ISA 71R fueron estables; no se observaron efectos adversos en el sitio de inyección después de la aplicación. La producción de huevo y la tasa de mortalidad diaria de las parvadas vacunadas con el ISA 71R también fue similar a lo que fue observado en una parvada sana de cada producción.

Los resultados muestran que Montanide ™ ISA 71 R VG es un adyuvante seguro y eficaz para la protección de pollos contra enfermedades bacteriales en el campo.

SUMMARY

Infectious coryza (IC) is an acute disease caused by *Haemophilus paragallinarum* which affects poultry respiratory system. To develop inactivated trivalent IC vaccine to combat all three serovars, an adjuvant that improves vaccine efficacy, resists to destabilizing bacterial media, and allows flexible adjuvant ratio in the multivalent vaccines is required.

In a small trial with 30 chickens and a large scale trial with 100000 chickens, IC trivalent vaccines either based on Montanide™ ISA 71 R VG or standard adjuvant were evaluated. ISA 71R based vaccines were stable; no untoward effects at the injection site were observed after injection. The egg production and the daily mortality rate of vaccinated flocks with ISA 71 R was also similar to what is observed in a healthy flock for each farm.

Result shows that Montanide ISA 71 R VG is a safe and efficacious adjuvant to protects chickens against bacterial disease in the field.

MONTANIDE™ ADJUVANTS FOR MUCOSAL DELIVERY OF LIVE AVIAN VACCINES

EL ADYUVANTE MONTANIDE™ PARA APLICACIÓN EN MUCOSAS DE VACUNAS VIVAS AVIARES

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RESUMEN

Se han utilizado adyuvantes específicos para la vacunación en mucosas. Aquí demostraremos la importancia de la aplicación de vacunas vivas en la mucosa aviar por medio de un adyuvante de polímeros y micro-emulsión en pollos.

Se utilizaron el Montanide™ IMS 1313 NVG (adyuvante en micro-emulsión) y el Montanide™ Gel 01 (adyuvante basado en polímeros) como diluyentes para una vacuna de Bronquitis Infecciosa atenuada viva liofilizada (cepa H-120) aplicada en pollos de un día de edad.

Ambas vacunas intranasales con adyuvante contra la Bronquitis Infecciosa mostraron una mejora significativa en títulos de anticuerpos comparadas con vacunas comerciales sin referencia de adyuvantes. Después del desafío, la aplicación intranasal de las vacunas con adyuvante redujo bastante la calificación de los signos clínicos. La administración por aspersión de la vacuna viva con adyuvante de polímero indujo una más alta protección comparada con las vacunas sin referencia de adyuvante.

Nuestros datos demuestran que los adyuvantes de polímero y micro-emulsión de Montanide™ pueden mejorar la eficacia de las vacunas, y que la aplicación de la vacuna con adyuvante confiere a los animales vacunados una protección significativamente mejorada contra los patógenos.

SUMMARY

Specific adjuvants must be used for mucosal vaccination. Here we demonstrate the improvement of mucosal live avian vaccines by polymer and micro-emulsion adjuvants in chicken.

Montanide™ IMS 1313 NVG (micro-emulsion adjuvant) and Montanide Gel 01 (polymer based adjuvant) were used as diluents for a lyophilized attenuated live infectious bronchitis vaccine (H-120 strain) in day old chickens.

Both adjuvanted live intranasal vaccines against infectious bronchitis showed significantly improved antibody titres compared to a commercial non-adjuvanted reference. After challenge, intranasal delivery of adjuvanted vaccine strongly reduced the clinical signs scoring. Spray administration of the polymer adjuvanted live vaccine induced a significantly higher protection compared to the non-adjuvanted reference vaccine.

Our data demonstrates that Montanide polymeric and micro-emulsion adjuvants can improve mucosal live vaccines efficacy, and that delivery of adjuvanted vaccine confers to vaccinated animals a significantly improved protection against pathogens.

USING SOCIAL NETWORK ANALYSIS FOR NETWORK-SMART OUTREACH AND TO BETTER UNDERSTAND LIVE BIRD MOVEMENT IN ENGLISH AND SPANISH SPEAKING COMMUNITIES IN CALIFORNIA

USANDO EL ANÁLISIS DE REDES SOCIALES PARA UN ALCANCE CON REDES INTELIGENTES PARA UN MEJOR ENTENDIMIENTO DEL MOVIMIENTO DE AVES VIVAS EN LAS COMUNIDADES DE HABLA INGLESA Y ESPAÑOLA EN CALIFORNIA

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RESUMEN

El análisis de redes sociales (SNA, por sus siglas en inglés) es una forma científica y cuantitativa de estudiar y visualizar las relaciones. Desde la perspectiva del control de enfermedades, el SNA puede ser usado en combinación con los Sistemas de Información Geográfica (GIS, por sus siglas en inglés) para: 1) crear y analizar una red del movimiento de la avicultura de traspatio (BYP, por sus siglas en inglés) en California y 2) crear y analizar una red de intercambio de conocimientos entre los propietarios de BYP y contactar a los profesionales para mejorar la diseminación de la información acerca de la bioseguridad y acerca de brotes de enfermedades.

SUMMARY

Social network analysis (SNA) is a scientific and quantitative way of studying and visualizing relationships. From a disease control perspective, SNA can be used in combination with Geographic Information Systems (GIS) to 1) build and analyze the network of live backyard poultry (BYP) movement in California and 2) to build and analyze the network of knowledge sharing between BYP owners and outreach professionals to improve the dissemination of biosecurity and disease outbreak information.

INTRODUCTION

Based on a 2010 USDA survey, backyard poultry (BYP) ownership is increasing in California and nationally (7). However, the location and number of backyard poultry flocks remains unclear and it is difficult to communicate with BYP owners in a coordinated fashion in part due to the lack of regulation. In addition, survey and anecdotal observations indicate that BYP owners have poor biosecurity practices (10). Furthermore, California's resources to educate BYP owners are limited. As an example, there are only two poultry extension specialists and no poultry farm advisors in California. This combination of poor biosecurity practices (e.g. exposure to wild birds) and unregulated bird movement can facilitate the spread of disease among BYP flocks as evidenced by the 2003 and 2018 virulent Newcastle Disease (vND) outbreak in Southern California (9). The ongoing 2018 vND outbreak in Southern California shows us that a better understanding of live bird movement from Mexico is important for the security of BYP and commercial poultry.

In this study, GIS-based surveys and social network analysis (SNA)— a quantitative way of studying and visualizing networks (3)— were used to map out and quantify live BYP movement and to design networks-smart outreach plans. The study aimed to address two critical issues in California: 1) limited BYP poultry education and outreach resources and 2) decrease in infectious disease monitoring (e.g. avian influenza monitoring discontinued by USDA).

MATERIALS AND METHODS

Survey design and data collection. A survey was created in English and Spanish using the survey instrument Qualtrics. The survey consisted of four sections:

1. Information about the BYP owner.
2. Biosecurity practices.
3. Bird health.

4. Movement network: BYP owners were asked to identify who and where they buy, sell and trade BYP with.

5. Advice network: BYP owners were asked to identify who they reached out to for poultry advice.

Recruitment of BYP owners. Surveys were sent out to BYP owners via email and social media. Specifically, participants of the California Backyard Poultry Census (online survey in English and Spanish hosted on UC Cooperative Extension Poultry website) were emailed and invited to participate in the survey. The survey was also shared with farm advisors, UC Davis media coordinators, UC Agriculture and Natural Resources media coordinators and California Department of Food and Agriculture (CDFA) to recruit BYP owners via email and social media.

Social network analysis. Using the participants' information and the contacts and affiliations the indicated they bought, sold or traded poultry with, a relational, binary matrix and attribute file were built. The matrix was analyzed and visualized using the statistical program R and SNA software ORA. For the movement network, nodes represent individuals or places (e.g. feed stores hatcheries) and ties represent live bird movement. For the advice network, nodes represent individuals or organizations (e.g. University of California Agriculture and Natural Resources) with links representing knowledge sharing. Network size, measures of centrality and measures of cohesion parameters were calculated for both networks (movement and advice) using ORA and R such as node count, edge count, centrality measures (e.g. total, in and out-degree) and density.

RESULTS AND DISCUSSION

Social networks play an important role in accelerating the adoption of innovations and cooperation in agriculture (2, 4). Yet, social networks have been under-researched as a tool toward optimizing agricultural outreach efforts (5). In states as large and diverse as California, well-connected social networks are essential for the deployment and adoption of innovations. In this study, SNA was used to understand the network between BYP owners and outreach professionals (e.g. poultry extension specialists). By understanding the structure of the advice network of BYP owners, outreach professionals can more effectively design network interventions to make the network more connected and conducive for knowledge sharing (1, 11). The ability to communicate disease outbreak and biosecurity information in a coordinated fashion can help mitigate disease outbreaks.

While SNA is not commonly used to improve outreach efforts to poultry owners (5), SNA has been used to understand disease transmission among poultry flocks (6, 8). In this study, SNA was also used to map live BYP movement in order to better understand the risk of disease transmission among BYP and in turn commercial flocks. Moreover, results from the English and Spanish survey were compared in order to gain insights on differences in movement patterns and the spread of disease among these flocks.

CONCLUSION

SNA results in combination with geographical data can help optimize outreach and disease mitigation efforts in California.

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BACTERIOLOGICAL STUDY OF THE EGG SHELL OF LAYING HENS HOUSED IN CAGE AND FLOOR

ESTUDIO BACTERIOLOGICO DEL CASCARON EN GALLINAS DE POSTURA ALOJADAS EN JAULA Y PISO

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RESUMEN

Se seleccionaron huevos de 10 marcas comerciales en diferentes tipos de alojamiento: jaula y en piso (libre de jaula) obtenidos de 4 cadenas de supermercados para compararlos en el laboratorio de bacteriología del Departamento de Medicina y Zootecnia de Aves de la FMVZ de la UNAM, para poder determinar la presentica de Enterobacterias en el cascarón. Se llevaron a cabo lavados del cascarón con PBS en un ambiente estéril, para luego colocar 1 ml del lavado en un caldo nutritivo. Luego fueron sembrados en Agar Bilis Rojo y Violeta. Las muestras fueron teñidas con Gram, 3% KOH y pruebas bioquímicas (TSI, Urea, Citrato, LIA, SIM y Ornitina) para su identificación. Los géneros que estuvieron más frecuentemente presentes en los huevos de gallinas en piso fueron: *Escherichia coli*, *Citrobacter freundi*, y *Enterobacter aerogenes*.

SUMMARY

Eggs from 10 commercial brands were selected in different types of housing: cage and on the floor (free of cage) obtained from 4 supermarket chains to compare them in the bacteriology laboratory of the Department of Medicine and Bird Husbandry of the FMVZ of the UNAM, in order to determine the presence of Enterobacteria in shell. Shell washes were performed with PBS in a sterile environment, to then place 1 ml of the wash in nutritious broth. They were sown in Agar Bilis Red and Violet. The samples were stained with Gram, 3% KOH and biochemical tests (TSI, urea, citrate, LIA, SIM and ornithine) for identification. The genera most frequently present in eggs of hens on the floor were: *Escherichia coli*, *Citrobacter freundi*, and *Enterobacter aerogenes*.

INTRODUCTION

Poultry farming in Mexico is constantly growing due to an increase in the human population, which in turn is reflected in a growth in the consumption of chicken and egg. The preference of poultry products for the population is due to the fact that they are low-cost, healthy and high-quality food (Ruiz, 2018).
Data from the National Union of Poultry Farmers indicate that poultry farming represents 63.8% of livestock production where 6 out of 10 people include poultry such as chicken, eggs and turkeys in their diet. It is estimated that there is a per capita consumption of 23.2 kg. of egg.

The egg is one of the foods of high quality animal origin for its excellent source of proteins, amino acids, minerals and vitamins. It is formed by three main structures: yolk (33%), albumin (58%) and shell (9%). It has chemical and physical barriers that protect it naturally against contamination by microorganisms (Gutiérrez, 2015).

Contamination of the shell has different origins, but the main one is the lodging system of the hens, due to the type of egg collection. The conventional cage

system allows to obtain a clean egg since at the time of oviposition it slides thanks to the slope (10%) that the cages have, in exchange for the cage-free system a dirty egg is obtained because the egg remains in the nests, leaving it exposed to contamination by feces (Calvo, 2013). This type of cage-free housing system can favor shell contamination and cause foodborne diseases.

The World Health Organization (WHO) mentions that unhealthy foods are the most common cause of diarrheal diseases and that each year 550 million people are ill, of which 220 million are children under 5 years of age.

At present, there is a tendency for consumers to acquire free-range chicken eggs, which is why a greater vigilance of this type of production is necessary in order to avoid foodborne diseases.

MATERIALS AND METHODS

For the development of this study, lots of eggs of hens were analyzed on the floor (free of cage) and in a cage of ten different egg brands acquired from different self-service stores.

The present study was carried out in the Laboratory of Bacteriology, Department of Medicine and Animal Husbandry of the Faculty of Veterinary Medicine and Zootechnics, UNAM. The technique described by Dr. Gentry was used to identify the presence of Enterobacteria in the egg shell.

Self-sealing (sterile) bags were used and nine mL of phosphate buffered saline (PBS) was added to each bag. The egg was rubbed (through the bag) for two minutes to suspend surface materials. The egg was then expelled from the top of the bag.

One mL of the solution was taken, placed in nutritious broth and subsequently incubated for 24 h. With a bacteriological loop, a sample of the nutrient broth tube was obtained to be sown on MacConkey agar and incubated for 24 h. This process of seeding was carried out repeatedly on MacConkey agar until the colonies were purified.

To determine the morphology of the colonies, smears and 3% KOH test were performed. Once the results were obtained, it was determined at which samples the biochemical tests were performed (TSI, Urea, Citrate, LIA, SIM and Ornithine), and the isolated microorganisms were identified.

RESULTS

The presence of *E. coli* was identified in two chicken egg brands in a cage. In the hen egg brands on floor (free of cage), *E. coli*, *Citrobacter freundi*, *Enterobacter aerogenes* were found.

DISCUSSION

The results obtained coincide with that reported by Loaiza *et al*. 2011 that mention that it is necessary to incorporate to the food industry different tests for the identification of pathogenic microorganisms that can be a source of foodborne diseases.

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EFFICACY AND ECONOMICS OF ORAL FLURALANER FOR THE TREATMENT OF NORTHERN FOWL MITES IN COMMERCIAL LAYERS

EFICACIA Y ECONOMIA DEL USO DE FLURALANER ORAL PARA EL TRATAMIENTO DE ACAROS AVIARES DEL NORTE EN GALLINA DE POSTURA COMERCIAL

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RESUMEN

Hay poco acuerdo acerca del tratamiento más efectivo y seguro para los ácaros aviares del norte en las gallinas de postura comerciales. Los protocolos pueden consistir en azufre en el alimento y aplicaciones repetidas de un plaguicida tópico en intervalos semanales. Estas aplicaciones implican mano de obra itnensa, y el costo de la mano de obra no siempre se considera en el costo del tratamiento. Los tratamientos repetidos con frecuencia estresan a las aves, y la eficacia puede variar. El desecho del huevo durante el tratamiento tópico es también una preocupación. Este reporte de caso describe el uso exitoso de fluralaner en el tratamiento de tres granjas de postura comerciales infestadas con el ácaro aviar del norte (Ornithonyssus sylviarum) en Canadá en el 2018. Se administró Fluralaner en solución acuosa al 1% (Exzolt®) dos veces con siete días de diferencia a una dosis de 0.5 mg/kg de peso corporal por tratamiento. El producto se aplicó en el agua de bebida, reduciendo tanto la mano de obra como la molestia a las aves requeridas por las aplicaciones tópicas. Los parámetros de desempeño permanecioern dentro del objetivo de la parvada y no se observaron efectos secundarios.

SUMMARY

There is little agreement on the most effective and safest treatment for northern fowl mites in commercial layers. Protocols may consist of sulfur in the feed and repeated topical pesticide applications at weekly intervals. These applications are labor intensive, and the cost of the labor is not always considered in the treatment cost. Repeated treatments often stress the birds, and the efficacy may vary. Egg disposal during topical treatment is also a concern. This case report describes the successful use of fluralaner in treating three commercial layer farms infested with northern fowl mite (*Ornithonyssus sylviarum*) in Canada in 2018. Fluralaner 1% aqueous solution (Exzolt®) was given twice 7 days apart at a dose of 0.5 mg/kg of body weight per treatment. The product was applied by drinking water, reducing both labor and bird disruption required for topical applications. Performance parameters remained within flock target and no side effects were observed.

INTRODUCTION

Northern fowl mites (*Ornithonyssus sylviarum*) are occasionally observed in replacement pullets, breeders, and commercial layer operations. Once mites are observed in one area of the barn they tend to progressively spread to the whole barn and subsequently to the next barns of the site in a few months. Infestations affect bird's comfort and work environment before causing skin lesions and reducing egg quality and production. Once mites are observed producers increase biosecurity to keep the mites on the site and may at some point add sulfur to the diet as it anecdotally reduces mite activity and/or reproduction rate. Once the farm personnel have complained enough, topical pesticides are typically applied on and under the birds, although with reluctance. The topical application is very labor intensive, the work is very unpleasant, the birds are soaked and stressed, and multiple weekly applications are often required. Disposition of the eggs that are on the belt during the application is also a topic for debate. However, the most concerning fact is that multiples of the label dose are occasionally used to achieve control and this practice is not allowed by the Health Canada Pest Management Regulatory Agency.

Fluralaner (Exzolt® - MSD Animal Health) is a systemic poultry acaricide indicated for the treatment of poultry red mite (Dermanyssus gallinae) and northern fowl mite infestation in pullets, breeders and layer hens with a zero egg and 14 days meat and offal withdrawal in several countries (1.) It is not registered currently in Canada. The dose is 0.5 mg fluralaner per kg body weight (equivalent to 0.05 mL of product) administered twice, seven days apart. The complete course of treatment must be administered to maintain a continued therapeutic concentration for 14 days and obtain the therapeutic effect for approximately two mite life cycles. Fluralaner is applied via drinking water, is absorbed rapidly and the mites die shortly after feeding.

MATERIALS AND METHODS

Two independent practicing veterinarians following three commercial layer farms with northern fowl mites infestations elected to ask for Exzolt under Health Canada Veterinary Drug Directorate Emergency Drug Release program. Farms ranged from three to five barns joined by a front hallway and housing a total of 38000 to 200000 hens in either conventional or enriched housing. Birds were 38 to 60 weeks old at treatment. Farms had been mite-free for one, 18, and 20+ years prior to the current infestation. Lines represented include Lohmann white, Dekalb white, ISA brown, Hy-Line brown.

During the week before the treatment water lines were flushed, leaking drinker and water filters replaced if deemed necessary. Birds that were not deemed healthy or fit to drink normally by the farm personnel were culled prior to treatment. Fluralaner 1% aqueous solution (Exzolt) was given twice 7 days apart at a dose of 0.5 mg/kg of body weight per treatment. First treatments were given June 13, October 25, and November 23, 2018. The product was applied by drinking water using dosing pumps set at either 1:100 or 1:128. Sulfur was inadvertently left in the feed for ~11 days post-treatment in Farm 2.

Diagnosis was confirmed by a combination of visual macroscopic and microscopic observations of the mites from the environment and from the birds' vent area (3/3 farms) and mite identification by a third-party laboratory (1/3 farm). Treatment efficacy was assessed by sporadic visual macroscopic and microscopic observations of the mites from the environment and/or from the birds' vent area. Post-treatment safety and production impact was determined from the review of the regular farm records.

RESULTS

Every farm was mite free 22 days following the first treatment and remains mite-free as at January 2019. Fluralaner was consumed within 3-10 hours. Farm 1 reported seeing no live mites on birds 14 days following the first treatment. Farm 2 reported checking 50 birds 2 days post treatment and saw no live mites. Farm 3 reported seeing no live mites either on the birds or on the eggs 5 days post-treatment start; however they were still seeing live mites in the floor dust in the front of one barn and they sprayed this area with a pesticide. Farm 3 took 30 dust samples on day 17 and observed one mite from the dust falling from the feed saver at the back of one barn; that dust came from the manure rollers. A second round of samples was done that afternoon and two mites were found in the dust that was collected after blowing the manure rollers. It was then decided to spray the front and the back end of that barn (the floor, the walls as well as the material, the hallway, the stairs, the washroom and the electrical room). No mites were found at the next check done on day 22. Economic analysis and scenarios are presented in the Tables 1 - 4. Production remained within flock target and no side effects were observed.

DISCUSSION

Farm 1 had their last infestation in the mid-90s, which they managed to eradicate over a two-year period by spraying all barns twice and pullets once before they came. They reported a production cost to the chronic infestation they endured. They qualified the 2018 infestation as the worst they ever had, and they sprayed the worst barn three times at seven days interval with permethrins starting mid-May. Following each application, they observed ~2% production drop for five days. This intervention did not eliminate mites on bird and they decided to use Exzolt about a month later.

Farm 2 had a mite infestation in 2017 and 4 consecutive weekly pesticide treatments at four times the concentration was required to eliminate the infestation. The workers reported suffering from shoulder and back issues following this chore and none of them was willing to repeat the task.

Farm 3 did a particularly thorough post treatment environmental sampling that lead to the discovery of three live mites from the dust of the manure roller and elected to add a premise treatment as a precaution. *Ornithonyssus* completes its full life cycle on the bird in less than seven days although it is reported that they may survive without a host for three to four weeks. It is our understanding that mites will seek a host when available, but we suppose that it is unfortunately possible that a few mites did not make it to the hen

during the14-day period when a meal would have been lethal.

The practicing veterinarians involved and their clients found that the benefits of a systemic treatment applied via the water include a reduced food safety concern with egg exposure to aerosolized insecticide particles despite best efforts to avoid egg exposure; reduced risk of workers injury associated with application, topical exposure, and respiratory exposure to the insecticide despite providing adequate personal protective equipment; reduced biosecurity risk associated with bringing in the additional help to spray birds; reduced stress on birds from being sprayed, soaked, and exposed insecticide vapors; maintaining compliance with Canadian regulatory agencies.

Treatment cost was ~17 cents ($C) per kilogram of hens. When considering the ease of administration, the labor cost, the predictable efficacy, and the intangible yet sizeable benefits cited above farmers considered the fluralaner regimen a good value and would choose it again over traditional topical pesticide spray.

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Table 1. Farm 1 – Economic analysis, effective vs ineffective treatment.

Farm 1	Exzolt	Pesticide (1X dose)
Treatment ($/1000 birds)	265	10
Labor (h/1000 birds)	0	1
Wage $/h	0	15
Labor cost	0	15
Cost/treatment	265	25
# treatment	1	3
Cost	265	75

Table 2. Farm 1 –Potential egg production opportunity cost associated with pesticide spray treatment.

Egg production loss assumptions per 1000 birds				
base egg production	95%	95%	95%	95%
day of treatment egg diversion*	100%[a]	50%[b]	10%[c]	0%[d]
treatment stress production drop	2%	2%	2%	2%
# days before return to base production	5	5	5	5
# of spray treatment required	3	3	3	3
# eggs lost	3135	1710	570	285
producer table egg price ($/dz)	1.96	1.96	1.96	1.96
potential loss ($)	512	279	93	47

Table 3. Farm 2 – Economic analysis, effective elimination.

Farm 2	Exzolt	Pesticide (4X dose)
Treatment ($/1000 birds)	350	40
Labor (h/1000 birds)	0	1
Wage $/h	0	20
Labor cost	0	20
Cost/treatment	350	60
# treatment	1	4
elimination cost	350	240

Table 4. Farm 2 - Potential egg production opportunity cost associated with pesticide spray treatment.

Egg production loss assumptions per 1000 birds				
base egg production	95%	95%	95%	95%
day of treatment egg diversion*	100%[a]	50%[b]	20%[c]	0%[d]
treatment stress production drop	2%	2%	1%	1%
# days before return to base production	5	5	2	2
# of spray treatment required	4	4	4	4
# eggs lost	4180	2280	836	76
producer table egg price ($/dz)	1.96	1.96	1.96	1.96
potential loss ($)	683	372	137	12

* scenario a=discard 100% of the eggs on day of pesticide spray; scenario b=sell the eggs at 50% of the full value if sell for break out for example; scenario c=start spraying when belt cleared and pick up eggs manually after finished spraying and assume discard 10%-20% of the day's production; scenario d=say nothing and sell all eggs at full value despite direct pesticide exposure to some eggs.

COMPATIBILITY EVALUATION OF TWO FOWL POX VECTORIZED VACCINES AGAINST THE AVIAN INFLUENZA VIRUSES H7N3 AND H5N2

EVALUACIÓN DE LA COMPATIBILIDAD DE DOS VACUNAS VECTORIZADAS DE VIRUELA AVIAR CONTRA LOS VIRUS DE INFLUENZA AVIAR H7N3 Y H5N2

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RESUMEN

En México, desde la década de los 90 del siglo pasado, se detectaron brotes del virus de influenza aviar (VIA) H5N2. En junio del 2012 se identificó el virus de IA H7N3 altamente patogénico como el responsable del brote en gallinas ponedoras comerciales que causó una severa enfermedad y alta mortalidad. Incluso las vacunas muertas inactivadas han sido cruciales para el control de la enfermedad producida por estos virus, la vacuna recombinante de viruela aviar (VA) contra el virus de la influenza aviar H5N2 ha demostrado ser una herramienta clave en el control de esta enfermedad al estimular la respuesta celular. La administración de las vacunas recombinantes de viruela aviar contra los virus de IA H5 y H7 están recomendadas para pollos al día de edad. En este estudio se utilizó un modelo de desafío de eficacia diseñado para evaluar la protección conferida de las vacunas contra la mortalidad y signos clínicos y así poder demostrar la compatibilidad de estas dos vacunas cuando son administradas simultáneamente.

SUMMARY

In Mexico, since the 90s decade of the last century, outbreaks of avian influenza virus (AIV) H5N2 were detected, in June of 2012 highly pathogenic AIV H7N3 was identified as the responsible of the outbreak in commercial laying hens causing a severe disease with high mortality. Even the inactivated killed vaccines have been crucial for the control of the diseases produced by those viruses, the recombinant fowl pox (FP) vaccine against the avian influenza virus H5N2 has demonstrated to be a key tool in the control of this disease by stimulating the cellular response. The administration of the fowl pox recombinant vaccines against the AIV H5 and H7 is recommended at one day of age in order to demonstrate the compatibility of this two vaccines when they are administered simultaneously, in this study a challenge model efficacy study was designed evaluating the protection conferred by the vaccines against mortality and clinical signs.

INTRODUCTION

Evidence of AIVs infection was found in most Latin America countries, with Mexico as the country with the largest number of conducted studies and reported cases (3).

In Mexico in 1993, the first cases of avian influenza virus (AIV) H5N2 were detected (5, 10). From these events, the Animal Health Department (SENASICA) strengthened the biosafety measures and authorized the development, production and use in risk areas of an emulsified vaccine based on inactivated virus. To complement the vaccination schedules and ensure the control of this disease, a recombinant vaccine that express the H5 hemagglutinin protein came up to the market. This vaccine has proven to be a valuable tool in stimulating cellular immunity in commercial laying hens and broilers (1, 2, 11, 12).

The outbreaks of highly pathogenic avian influenza (HPAI) of the H7N3 subtype began in 2012 and this virus has circulated in farms within Mexican territory causing a several damage in the poultry and affecting poultry works (7, 8). For this reason, the authorities have implemented vaccination as a control measure. Now days in Mexico, the recombinant vaccine of the avian fowl pox virus that expresses the H7 protein with the genomic construction of the Mexican H7 sequence, is the option to stimulate the cellular response and offer protection to the birds in the face of an avian influenza virus challenge H7.

The use of two recombinants fowl pox vaccines, to express H5 and H7 can provide early cellular immunity. The feasibility of the use of both vaccines administered simultaneously has to be evaluated by efficacy challenge, but the main purpose of this study is demonstrate the compatibility of the simultaneous administration in Mexican commercial broilers

considering that in Mexico the breeders receive at least two vaccinations against fowl pox virus.

MATERIALS AND METHODS

Viruses. The HPAI challenge viruses used to evaluate the efficacy were replicated and standardized by the Mexican government authorities at CENASA according to Mexican Normativity NOM-055-ZOO-1995 the HPAI H7N3 virus was identified as: IA SUBTIPO H7N3 CPA 743-15. The HPAI H5N2 virus used for the challenge was identified as: IA SUBTIPO H5N2 QUERETARO 020-94. The titer used for the challenge in both cases is 10^6 DIE/50% / 0.2 mL

Vaccines. A FP recombinant H5 vaccine TROVAC VIA H5, (Boehringer Ingelheim Animal Health MEXICO) was constructed using as a vector the fowl pox (FP) derived from the vaccine strain contained in the DIFTOSEC fowl pox vaccine and by inserting the HA gene of the A/turkey/Ireland/1378/83 H5N8 isolate. The FP recombinant H7 vaccine TROVAC Pr1me 7, (Boehringer Ingelheim Animal Health Mexico) was constructed using as a vector the fowl pox (FP) derived from the vaccine strain contained in the DIFTOSEC fowl pox vaccine and by inserting the Mexican sequence A/chicken/Guanajuato/074375/2015(H7N3).

Vaccination and challenge experiment. To evaluate the efficacy of the vaccines 70 SPF chickens (Specific Pathogen Free) and 30 commercial birds (broiler) were included in this study. All vaccinated birds received experimental treatment at day-of-age subcutaneously with a volume of 0.2 mL in the middle third back of the neck according their group:

Group 1, 10 SPF chickens treated with FP recombinant H5 vaccine. At 21 d post-vaccination, this group was challenged with the HPAI H5N2 avian influenza strain. Group 2, 10 SPF chickens treated with FP recombinant H7 vaccine. At 21 d post-vaccination this group was challenged with HPAI H7N3 strain. Group 3, 20 SPF chickens treated with FP recombinant H5 and FP recombinant H7 administered simultaneously in the same diluent. At 21 d post vaccination 10 chickens of the group were challenged with HPAI H5N2 and the remaining 10 chickens challenged with HPAI H7N3. Group 4, 20 SPF chickens were vaccinated with diluent without vaccine. At 21 d post vaccination 10 birds of the group were challenged with the HPAI H5N2 and the remaining 10 birds were challenged with the HPAI H7N3. Group 5, 20 commercial broilers vaccinated with FP recombinant H5 and FP recombinant H7 vaccine administered simultaneously in the same diluent. At 21 d post vaccination 10 chickens of the group were challenged with the HPAI H5N2 and the remaining 10 chickens were challenged with HPAI H7N3. Group 6, 20 commercial broilers were vaccinated with diluent without vaccine. At 21 d post vaccination, 10 chickens of the group were challenged with the HPAI H5N2 avian influenza virus and the remaining 10 birds were challenged with the HPAI H7N3.

The challenge procedure for all the groups was by intranasal route with a volume of 0.2 mL. After the challenge according to the Mexican Normativity, the vaccine has demonstrated 80-100% protection of the birds during 14 d post-challenge (end of the study), and for the test to be valid, at least 80% of the unvaccinated control animals must die. During the study the chickens were maintained at Mexican Government facilities CENASA (National Center of Diagnostic Service in Animal Health).

The chickens in the clinical study were placed at one-day-of-age in breeding pens in the isolation room. At 21 days of age, the chickens were moved to the isolation units; the isolation units operate independently, under a negative pressure and with individual air intake and exhaust filters.

RESULTS

The results showed 100% of protection in the commercial broiler chickens vaccinated simultaneously with FP recombinant H5 and FP recombinant H7 at one day old when challenged with HPAI H5N2 and HPAI H7N3 (Group 5).

The SPF chickens vaccinated simultaneously with FP recombinant H5 and FP recombinant H77 at day old (Group 3) showed 100% of protection when challenged with HPAI H5N2 and 80% of protection when challenged with HPAI H7N3. In this group, two chickens died, at five and six days post challenge.

The SPF chickens vaccinated with FP recombinant H7 challenged with HPAI H5N2 and the SPF chickens vaccinated with FP recombinant H7 challenged with HPAI H7N3 showed 100% of protection.

All the SPF chickens and commercial broilers not vaccinated and challenged (Controls) with HPAI H5N2 and HPAI H7N3 died between two and five days post-challenge.

DISCUSSION

Different studies reported that, FP recombinant H5 vaccine can provide protection from lethal Mexican H5N2, and prevent shedding in the feces and transmission to contact birds at one day old SPF chickens (1, 2, 10, 12), but knowledge of the efficacy of the vaccine in commercial chickens from breeders vaccinated against FP is limited. The results obtained in this study confirm the excellent level of protection

induced by FP recombinant H5 vaccine in commercial broilers and in SPF chickens against the Mexican challenge strain identified as IA SUBTIPO H5N2 QUERETARO 020-94.

Although the results obtained in this study showed 100% of protection against the HPAI H5 challenge virus used, it is necessary to obtain more information on protection against challenges of emerging strains of influenza virus challenge in Mexico, since there is evidence of heterogeneity in the hemagglutinin gene (4, 5, 7).

The protection of the oil emulsion vaccines produced with the Mexican H7 LPAI was demonstrated in SPF chickens (6); but due the genetic and antigenic distance the protection is limited, and better options has been studied (9).

In the field the perception of the protection is low in production commercial broilers and layers, for this reason vaccines with recombinant technologies with the insert of the sequence provided by the Mexican government has been authorized.

The FP recombinant H7 vaccine demonstrated in this study to be an excellent option for the control of the HPAI H7 virus.

No information is available related to the simultaneous vaccination with two FP recombinant vaccines expressing different hemagglutinin, in this efficacy study the protection of the FP recombinant H7 vaccine and the FP recombinant H5 administered simultaneously by subcutaneous route in commercial broilers at one day old demonstrated to be 100% of protection.

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Table 1. Percentage of protection after 14 days post challenge of the commercial broilers vaccinated and non-vaccinated.

Treatment 0.2 mL subcutaneously at 1 day old	% of protection after challenge with HPAI H5N2	% of protection after challenge with HPAI H7N3
FP recombinant H5 + FP recombinant H7	100	100
Placebo	0	0

CARACTERIZACIÓN DE LA PRODUCCIÓN DE EXCRETAS DE CODORNIZ JAPONESA REPRODUCTORA

CHARACTERIZATION OF BREEDER JAPANESE QUAIL FECES

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SUMMARY

In order to characterize the feces produced by breeder Japanese quail, feed consumption, egg weight, and fresh as well as sun-dried feces weight were recorded during a 6-month laying cycle. Feed consumption per quail was 26.87 ± 3.64 g, average egg weight was 11.90 ± 0.65 g, water content of feces was 58.4 ± 8.55%, and on average each quail produced 20.76 ± 4.37 g of fresh and 8.64 ± 1.66 g of dry feces per day. Of the total feed consumed per group per day, feces represented 32.83 ± 5.77%. Based on average fecal production per day, it was estimated that 100 quails produced 2.076 kg of fresh excreta and 0.864 kg of dry matter.

RESUMEN

Para caracterizar la producción de excretas de codorniz japonesa en producción de huevo se registró durante un ciclo de postura de 6 meses el consumo de alimento, peso del huevo y peso de las heces en fresco así como deshidratadas al sol durante 2 días. El consumo de alimento por codorniz fue de 26.87±3.64 g, peso promedio del huevo 11.90±0.65g, el contenido de agua de las heces fue de 58.4±8.55%, en promedio por día cada codorniz produjo 20.76±4.37 g de heces en fresco y 8.64±1.66 g de heces en seco. Del consumo total de alimento por el lote por día las heces representaron el 32.83±5.77%. Con base en la producción de heces promedio por día se estimó que cada 100 codornices produjeron 2.076 kg de excretas en fresco y 0.864 kg en seco.

La codorniz japonesa (*Cotournix cotournix japonica*) se caracteriza por un ciclo de rápido crecimiento, buen índice de conversión alimenticia, precocidad, elevada productividad y resistencia a enfermedades (6). La producción de carne de codorniz se concentra en España y Francia; Estados Unidos y de huevo en China, Japón y Brasil (7). Entre los estados de inicio y de madurez, 1000 codornices producen 704 kg de excretas en 30 días (11). El manejo inadecuado del excremento puede contaminar el medio ambiente principalmente las aguas superficiales y subterráneas (10). Sin embargo, las excretas contienen nutrientes que se utilizan como fertilizante orgánico. Por lo tanto, los nutrientes, como el nitrógeno, el fósforo y el potasio pueden reciclarse. El objetivo del estudio fue caracterizar la producción de excretas de codorniz japonesa en producción de huevo.

MATERIAL Y MÉTODOS

El estudio se realizó en la Unidad Avícola de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Sinaloa, en Culiacán Sinaloa, México (24 46’ 13’’ LN y 107 21’ 14’’ LO). El clima de la zona es BS (h’) w(w)(e), semiseco muy

cálido, con lluvias en verano, con 25.9 ºC de temperatura promedio anual; humedad relativa promedio de 68%, máxima de 81% y mínima 51%; precipitación anual promedio de 688.5 mm. En el periodo de febrero a julio de 2018, se utilizaron 120 codornices durante un ciclo de postura de 6 meses, alojadas en jaula en batería. El alimento en forma de harina (Tabla 1) se ofreció *ad libitum*. El programa de iluminación fue de 16 h. Diariamente se midió el consumo de alimento, la producción de huevo y se colectaron las heces, se pesaron y se colocaron al sol durante dos días, y se pesaron de nuevo. Los datos se analizaron mediante estadística descriptiva. Los resultados de las características de excretas de codorniz japonesa se muestran en la tabla 2.

DISCUSIÓN

El consumo de alimento promedio fue de 26.87. Este valor es inferior al calculado por Al-Daraji *et al*. (1) (34.44 a 37.8 g) y Danuta *et al.* (3) quienes reportan 40.29 g. Sin embargo, el peso del huevo fue de11.9 g, similar al reportado por Al-Daraji *et al.* (1) y Danuta *et al.* (3) con valores de 11.0 a 11.4 y 12.0 g respectivamente. Las excretas tuvieron 58.2% de humedad. Las excretas de gallinas de postura contienen 40.4% (Brown, 2013), mientras que García *et al*., (5) reportan 79.24%. Edwards y Daniel, (4) mencionan que un factor limitante en el manejo de excretas de aves es la humedad, que puede variar de 36.9 a 77.0%. En el presente estudio se encontró que en promedio por día cada codorniz produjo 20.76 g de heces en fresco, Suppadit, (11) (12) reporto que 1000 codornices producen 704 kg de excremento es igual a 704 g por codorniz al ciclo de 30 días dando como resultado 23.46g al día de excremento. Las excretas secas producidas por codorniz, fueron 8.64 g, y respecto al total de alimento consumido por el lote de codornices por día, las heces representaron el 32.83 ± 5.77%, similar a lo reportado en gallinas ponedoras en base seca de un 31.8 ± 1.1% del alimento consumido (13). Se concluye que las excretas de codorniz japonesa contiene 52.2% de agua, producen por ave por día 20.8 g de excretas frescas y 8.7g al secarlas al sol.

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Tabla 1. Composición y aporte nutricional calculado de la dieta, g por 1000 g.

Ingrediente:		Ingrediente:	
Pasta de soya	361.6	DL-Metionina	2.8
Maíz	542.0	Pigmento (Forafil™)	2
Aceite de soya	443.0	Vitaminas y minerales	2.0
Piedra caliza	58.8	Adsorbente de micotoxinas	1.0
Monofosfato dicálcico	9.6	Prebiótico	2.0
Sal de mar	2.9	Fitasa	2.0
Composición nutricional calculada			
Proteína cruda, %	20.06	P, disponible, %	0.35
EM Mcal/kg	2935	Metionina, %	0.50
Ca, %	2.58		

Tabla 2. Consumo de alimento, peso del huevo y características de la producción de excretas de codorniz japonesa reproductora [A].

Días en producción	Consumo alimento, g día	Peso del huevo, g	Agua en heces, %	Heces por codorniz, g	Heces secas por codorniz, g	Heces secas con respecto a consumo, %
1-28	28.0±3.6	11.9±0.7	60.7±5.8	24.3±3.9	9.5±1.8	34.3±4.2
29-56	27.7±3.8	12.0±0.7	58.1±10.1	21.5±3.3	8.8±2.0	31.0±9.2
57-84	27.3±3.5	12.3±0.6	56.4±9.8	20.3±3.6	8.7±1.8	31.9±4.8
85-112	26.7±3.1	12.0±0.6	54.5±6.2	19.0±3.9	8.6±0.9	32.6±4.3
113-140	26.3±4.7	12.1±0.3	56.5±8.3	22.4±5.1	9.1±1.3	32.1±2.6
141-168	23.4±2.3	11.6±0.6	59.8±5.6	18.9±4.5	7.7±1.3	34.7±4.8
169-188	-	11.3±0.3	63.1±5.8	18.3±2.5	7.2±0.9	-
General	26.9±3.6	11.9±0.6	58.2±7.9	20.8±4.4	8.7±1.7	32.8±5.8

[A] Los valores representan la media ± desviación estándar.

GEORGIA 08 VACCINATION STUDIES ASSESSING PROTECTION AGAINST IB VARIANTS GA-13 AND DMV/1639 IN SPF LEGHORNS

ESTUDIOS DE VACUNACIÓN CON GEORGIA 08 PARA EVALUAR LA PROTECCIÓN CONTRA LAS VARIANTES DE BI GA-13 Y DMV/1639 EN LEGHORNS SPF

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RESUMEN

En esta década, las tres variantes más prevalentes del virus de la bronquitis infecciosa (BI) que emergieron en los Estados Unidos son el GA08, Ga13 y DMV/1639. El serotipo DMV/1639 se ha diseminado geográficamente en Pennsylvania, en el Valle Shenandoah, Arkansas e incluso en Ontario. Los reportes de "ponedoras silenciosas" han coincidido con el aumento en los aislamientos de DMV/1639 en ponedoras comerciales de Ontario. De estos tres serotipos, las vacunas comerciales solo se derivan de la variante del VBI GA08. Estos dos estudios fueron conducidos el Leghorns SPF albergadas en aislamientos horsfall para evaluar la protección cruzada de la vacuna GA08 contra las variantes de BI GA13 y DMV/1639.

INTRODUCTION

In the last decade, the three most prevalent variant infectious bronchitis (IB) viruses to emerge in the United States are GA08, GA13 and DMV/1639. The DMV/1639 serotype has spread in geography into Pennsylvania, the Shenandoah Valley, Arkansas and even Ontario. Reports of "silent layers" have coincided with the rise in DMV/1639 isolations in Ontario commercial layers. Of these three serotypes, commercial vaccines are currently only derived from the GA08 variant IBV. These two studies were conducted in SPF leghorns housed in horsfal isolators to evaluate the cross-protection of GA08 vaccine against IB variants GA13 and DMV/1639.

MATERIALS AND METHODS

Study 1. One hundred thirty-five SPF leghorns were placed into nine isolators after being divided at hatch into three treatment groups: No Vaccine, GA08 Vaccine A by eye drop or by coarse spray (14mL/100 chicks). At weekly intervals starting at day 7, birds in the non-challenged isolator for each treatment had choanal clefts swabbed for IBV using quantitative PCR to check for vaccine "takes". At 21 days of age, the other two isolators per treatment were challenged with 3.5 EID50/dose of GA13 IBV. The study was terminated five days later as all birds were evaluated for clinical signs and scored for internal lesions; tracheas were swabbed for IBV PCR and preserved in formalin for histopathology (3 and 4 scores = moderate to severe lesions).

Study 2. Two hundred twenty-five SPF leghorns were placed into 15 isolators after being divided into three treatment groups: No vaccine and GA08 Vaccines A and B applied by coarse spray (14mL/100 chicks). Choanal clefts were swabbed to monitor for vaccine takes (real time PCR) at weekly intervals from the two isolators per treatment designated for no challenge. At 25 days of age, the other three isolators per treatment were challenged with 3.5 EID50/dose of DMV/1639 IBV. The study was terminated five days later as all birds were evaluated as in Study 1 but with the additional metric of body weights.

RESULTS

GA08 vaccine takes were strongly positive for all treatments at seven days of age. By the three-week sampling, the mean CT value had risen by 10 cycles in each of the Vaccine A treatments—about a 3-log reduction (base-10) in IBV RNA. Clinical signs were very mild and not significantly different between treatments after challenge with either variant virus. However, Grade 3 and 4 tracheal lesions (moderate to severe) were consistently higher in non-vaccinated controls than in the vaccine treatments. The 60% reduction in Grade 3-4 lesions of eye drop or spray application of GA08 Vaccine A was not significant in the GA13 study but the 88-95% reduction in Grade 3-4 lesions of Vaccine A and B, respectfully, was significant in the DMV/1639 study. Body weights were significantly depressed in DMV/1639 controls compared to both vaccine treatments in Study 2. Finally, the percentage of tracheas containing little to

no live IBV post challenge (CT>35) was significantly different between vaccinates and controls. In the GA13 challenge study, all controls fell below this line while 73-80% of eye drop and spray vaccinated birds were above this standard. In the DMV/1639 study, 9% of controls were above this line compared to 95% of Vaccine A and 91% of Vaccine B (**see** Table 1).

DISCUSSION

These two SPF leghorn studies have demonstrated that GA08 vaccine given by coarse spray at hatch can achieve significant cross protection against variant IBVs GA13 and DMV/1639. In Study 1, eye drop and coarse spray applications of GA08 Vaccine A were comparable in both vaccine "take" and GA13 protection levels. In Study 2, both commercially available GA08 vaccines gave solid "takes" and high levels of protection against tracheal colonization, lesions and body weight suppression caused by DMV/1639 challenge. Because IBV in broilers is a "different animal" altogether, similar studies should be conducted in commercial broilers before drawing any conclusions about the cross protection potential of GA08 vaccines in this market segment.

Table 1. Summary of GA13 and DMV/1639 challenge study results in GA08 vaccinated SPF leghorns.

	Vaccine Treatments		Pre-challenge Results			Post challenge Results		
	IBV Vaccine	Route	Mean CT values and % ≤ 35			Histopath scores >2	Birds CT>35	Body Wt. (g)
			7 days	14 days	21 days			
Study 1	Control	--	--	--	--	54%	0%a	--
GA13 challenge	GA08 Vaccine A	Eye Drop	23.9 (100%)	30.4 (100%)	33.9 (93%)	22%	73%b	--
	GA08 Vaccine A	Coarse Spray	24.5 (100%)	32.4 (100%)	34.8 (53%)	20%	80%b	--
Study 2	Control	--	--	--	--	74%a	9%a	313a
DMV/1639 challenge	GA08 Vaccine A	Coarse Spray	27.1 (100%)	35.9 (48%)	37.9 (17%)	9%b	95%b	350b
	GA08 Vaccine B	Coarse Spray	27.5 (100%)	35.3 (52%)	35.8 (43%)	4%b	91%b	356b

BENEFITS OF THE LONG TERM USE OF A SINGLE DOSE VECTOR HVT-IBD VACCINATION IN BROILERS

BENEFICIOS DEL USO A LARGO PLAZO DE UNA SOLA DOSIS DE LA VACUNA VECTORIZADA HVT-IBD EN POLLOS DE ENGORDA

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RESUMEN

La vacunación temprana contra la enfermedad infecciosa de la bolsa (por sus siglas en Ingles IBD) es una práctica común; sin embargo, el uso de vacunas de pobremente atenuadas puede comprometer la integridad bursal de las aves jóvenes. Vectores virales utilizados para la expresión transgénica de proteínas inmunogénicas proporcionan protección sin el daño inicial a la bolsa de Fabricio. El objetivo de este trabajo fue demostrar la idoneidad de la vacunación con un vector HVT+IBD vaccination (Boehringer Ingelheim) para pollos de engorde en Ecuador se han vacunado un total de 65 millones de pollos durante los últimos 10 años demostrando una continua mejora en el estatus sanitario y productivo en comparación el promedio nacional del país. Se han observado mejores resultados en los indicadores productivos, sugiriendo una adecuada protección contra los desafíos de campo de IBD. Un total de 5.16 gramos adicionales en ganancia diaria de peso, menor conversión alimenticia (0,19 puntos), mejor índice de eficiencia Europeo (+64.42 puntos) y un mejor índice de productividad (+14.59 puntos). Estos resultados demuestran la idoniedad de una dosis única de esta vacuna vectorizada HVT+IBD como estrategia de control para IBD en broilers.

ABSTRACT

Early vaccination against infectious bursal disease (IBD) is a common practice; however, the use of poorly attenuated vaccines can compromise the bursal integrity in young birds. Viral vectors for transgenic expression of immunogenic proteins can provide adequate protection without the initial bursal damage. The objective of this work was to demonstrate the suitability vector HVT+IBD vaccination (Boehringer Ingelheim) for broilers in Ecuador. A total of 65 million the broilers have been vaccinated over the last ten years showing a continuous improvement in health status and productive parameters when compared with the Ecuador national average. Better results for all the productive indexes have been observed, suggesting adequate protection against the IBD field challenges. A total of 5.16 grams more in daily weight gain; lower feed conversion rate (-0,19 points); higher European efficacy index (+64.42 points) and higher productivity index (+14.59 points). These results indicate the suitability of single dose of this vector HVT+IBD vaccine control strategy for IBD in broilers.

INTRODUCTION

Poultry vaccines are widely applied to prevent and control contagious diseases aiming to avoid or minimize the emergence of clinical outbreaks at the farm level, therefore maintaining or increasing production. Vaccines and vaccination programs vary broadly in regard to several local factors (e.g. costs, type of production, local pattern of disease, facilities and access to technology), nevertheless they follow general trends on strains use, timing and inoculation routes that are established by an active research in the area. Currently, infectious bursal disease (IBD) control is mostly attempted using live and/or killed vaccines for the dams and progeny (1). A different approach to improve IBD vaccination is the immunization of chickens with viral vectors expressing the viral protein 2 (VP2) of the IBDV, this can be achieved using avian Herpesvirus (2,3). The vector HVT-infectious bursal disease vaccine (vHVT-IBD) assessed in this trial, expresses the VP2 from a IBDV classical strain, Faragher 52/70 and has been proven to protect against MD and classical and variant IBD strains (2). The objective of this work was to demonstrate the suitability of long-term use (ten years) of a vector HVT+IBD vaccine (Boehringer Ingelheim) for IBD control for broilers in Ecuador.

EXPERIMENTAL DESIGN

The vHVT-IBD vaccine virus used at 0.2 ml per dose following the manufactures instructions, is a genetically modified HVT (strain FC126), the genome of which contains the VP2 gene of the 52/70 Faragher strain of IBDV(2). The resulting strain vHVT013-69 is commercially known as VAXXITEK® (Boehringer Ingelheim). The replication-competent herpesvirus vectors can express multiple antigens and both humoral and cellular immune response can be induced in the birds with the potential for long term induction of protective immunity thanks to the persistent infection of the viral vector (3). The criteria to evaluate performance included the assessment of the consolidated results for the productive parameters in the flocks and their comparison with the Ecuador Poultry industry national average, based on the premise that healthier flocks (Gumboro and Marek disease under control) will provide better results obtaining their full potential.

RESULTS

A total of 65 million the broilers have been vaccinated over the last ten years showing a continuous improvement in health status and productive parameters when compared with the Ecuador national average. Better results for all the productive indexes were observed, suggesting adequate protection against the IBD field challenges: a total of 5.16 grams more in daily weight gain; lower feed conversion rate (-0,19 points); higher European efficacy index (+64.42 points) and higher productivity index (+14.59 points). The evolution of the improvements on the time line for daily weight gain and feed conversion rate after the implementation of the Vector HVT+IBD program during the last 10 years can be observed in Figure 1, while progressive improvement of the European efficiency index and productive index are shown in Figure 2.

CONCLUSIONS

Current poultry industry management includes the use of built up litter, leading to early, high and persistent viral exposure of the chickens to both classical and variant strains. The long-term use of a unique dose of a vector HVT IBD vaccine for Marek disease and IBD has proven to be an alternative to avoid immunosuppression and the productive cost associated with it. These results indicate the suitability of single dose of this vector HVT+IBD vaccine control strategy for IBD in broilers.

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Figure 1. Progressive improvement of daily weight gain and feed conversion rate after the implementation of the Vector HVT+IBD program during the last 10 years.

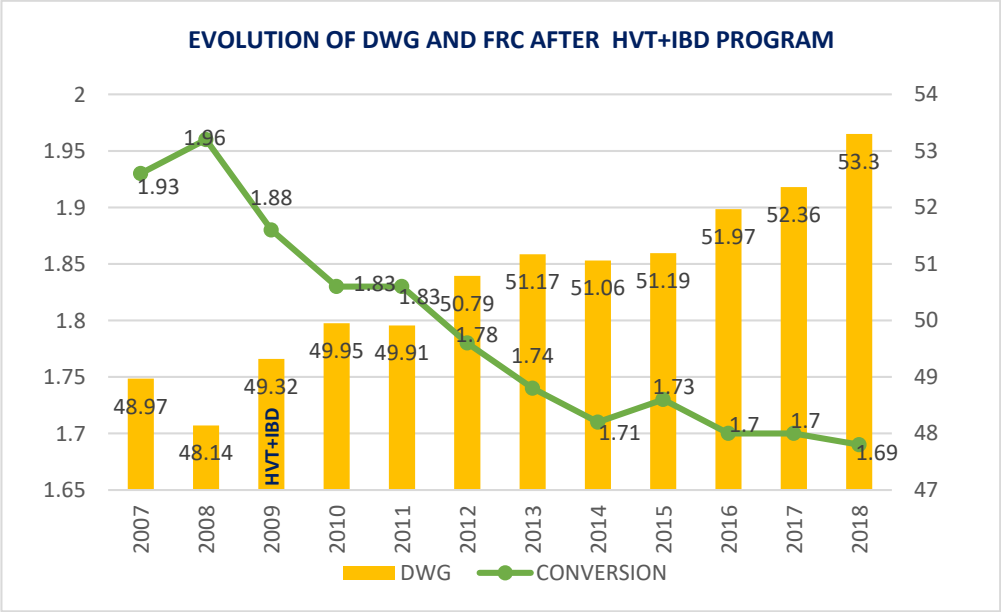
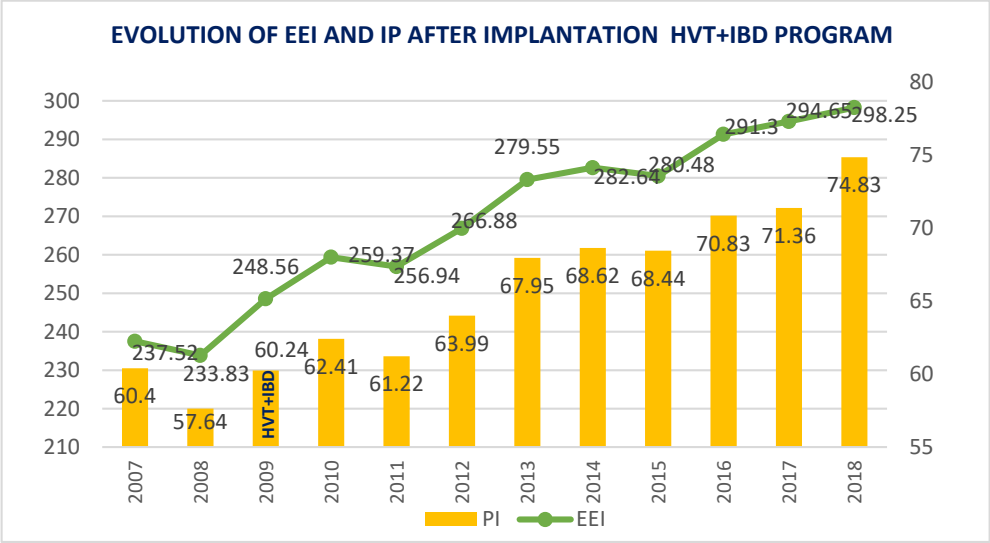


Figure 2. Progressive improvement of the European efficiency index and productive index after the implementation of the Vector HVT+IBD program during the last 10 years.



CARACTERIZACIÓN CLÍNICA Y MOLECULAR DE UN ADENOVIRUS AVIAR GRUPO D FADV-11 EN COLOMBIA

CLINICAL AND MOLECULAR CHARACTERIZATION OF AN AVIAN ADENOVIRUS GROUP D FADV-11 IN COLOMBIA

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SUMMARY

The avian adenoviruses represent one of the most important emerging pathogens in poultry, being the causal agent of the inclusion bodies hepatitis (IBH). Previous reports of serotype 4 and 8 show the sporadic presentation of the disease in Colombia. However, this is the first clinical and molecular description of a virus belonging to group D (FADV-11) in broiler breeders for the country. Together with soft, enlarged and hemorrhagic livers there was an unusual increase in mortality between days 2 and 17 (3.27%) with a peak on day 7. Tissues for histopathology and molecular analysis were submitted to the laboratory. The results showed inclusion bodies consistent with IBH in liver cells and the molecular amplification and sequencing showed the presence of a FADV-11. The presence of new serotypes of the disease in Colombia indicate an increase in the genetic pool and the challenge level, suggesting the need of increasing the control measures.

RESUMEN

Los adenovirus aviares representan uno de los más importantes patógenos emergentes en la avicultura, siendo el agente causal de la hepatitis por cuerpos de inclusión (por sus siglas en ingles IBH). Reportes previos de serotipo 4 y 8 demuestran la aparición esporádica de la enfermedad en Colombia. Sin embargo, esta es la primera descripción clínica y molecular de un virus perteneciente al grupo D (FADV-11) en reproductoras de engorde para el país. Conjuntamente con hígados suaves aumentados de tamaño y hemorrágicos se observó un incremento inusual de la mortalidad entre los días 2 y 17 (3.27%) con un pico al día 7. Se enviaron tejidos al laboratorio para histopatología y análisis molecular: Los resultados mostraron cuerpos de inclusión consistentes con IBH en las células hepáticas y la amplificación molecular y secuenciación demostraron la presencia de un FADV-11. La presencia de nuevos serotipos de la enfermedad en Colombia indica un incremento en el pool genético y el nivel de desafío, sugiriendo la necesidad de incrementar las medidas de control.

INTRODUCCIÓN

Los adenovirus aviares (FAdVs), producen enfermedades severas en las aves comerciales, siendo las más comunes la hepatitis por cuerpos de inclusión (IBH) y el síndrome de hidropericardio (HPS). Los adenovirus aviares se clasifican en cinco especies diferentes (FAdV-A a FAdV-E) debido a su estructura molecular y también en 12 serotipos (6). Los brotes de hepatitis por corpúsculos de inclusión (IBH) se asocian principalmente con FAdV-2, FAdV-7, FAdV-8a, FAdV-8b y FAdV-11, mientras que el síndrome de hidropericardio (HPS) es causado por FAdV-4 (2). Se ha observado IBH en pollos infectados verticalmente tan jóvenes como de 5 días de edad con mortalidad de hasta 5% y se reprodujo experimentalmente la infección en pollos libres de patógenos específicos de 1 día de edad que desarrollaron signos de la enfermedad 3 días después (2, 7). En general el diagnóstico de rutina de la infección por FAdV generalmente se lleva a cabo mediante una combinación de aislamiento viral e histopatología, a veces apoyado con microscopía electrónica o reacción en cadena de la polimerasa -PCR- (5). El presente reporte se realiza por la presentación de Hepatitis por Cuerpos de Inclusión en reproductoras pesadas. el diagnóstico de la enfermedad se realizó por histopatología, apoyado por PCR y secuenciación del genoma del virus en un equipo de nueva generación (Next Generation Sequencing [NGS] (5). El resultado obtenido de la secuencia indica que el virus responsable del proceso infeccioso fue un Adenovirus aviar perteneciente al serotipo 11 grupo D, este es el primer reporte de este serotipo en Colombia.

HISTORIA DEL CASO

El 13 de agosto de 2017 fueron alojadas en una granja de 18.363 hembras reproductoras y 2.756 machos reproductores, el peso promedio a la recepción fue de 39,5gr y 38,8gr con 3 y 5 aves de mortalidad durante el transporte, para hembras y machos respectivamente. Las aves fueron alojadas en piso usando cascarilla de arroz como cama y en criaderos cubiertos en papel a una densidad de 60 aves por m^2. La evaluación microbiológica de la muestra de aves enviadas al laboratorio fue negativa para hongos y salmonella en ciego e hígado, se aisló *E. coli* en el saco vitelino del 40% de las aves muestreadas considerándose un valor aceptable dentro de los rangos de tolerancia establecidos. Durante los primeros dos días el comportamiento de las aves fue normal, sin embargo, después del tercer día se presentó un incremento atípico en la mortalidad de la hembra que hizo pico en el día 7 con un posterior descenso estabilizándose después del día 18. Las aves afectadas presentaron signos de depresión, plumas erizadas, palidez, renuencia al desplazamiento e inanición. Se tomaron 20 aves del lote con mortalidad y se realizó la necropsia, las aves fueron sacrificadas por electrocución, después de una inspección externa fueron pesadas y sangradas por corte de la vena yugular y colocadas en una solución desinfectante, se realizó la inspección de todos los tejidos y órganos internos, se tomaron muestras de hígado y bazo para análisis histopatológico, prueba de PCR y posterior secuenciación

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DETECCIÓN MOLECULAR

A partir de improntas de hígado en tarjetas FTA, la reacción de PCR se llevó a cabo tomando como referencia lo descrito por De La Torre, et al (1). Para el alineamiento múltiple de las secuencias consenso se utilizó el software Bioedit para Windows, versión 7 (IBIS BIOSCIENCES, USA) (3). El árbol filogenético para el gen hexón de FADV se construyó utilizando el programa MEGA versión 7 (4), con 18 secuencias utilizando el algoritmo de Neighbor-joining, con 1000 réplicas y modelo de máxima similitud.

RESULTADOS

Los hallazgos a la necropsia mostraron una evidente hepatomegalia, con bordes redondeados, friables al tacto, con áreas pálidas junto con hemorragias petequiales y equimoticas. Se descartó la presencia de un agente bacteriano por el resultado negativo al cultivo bacteriológico, razón por la que se consideró como principal diagnóstico diferencial la hepatitis viral que fue confirmada por los hallazgos de los corpúsculos de inclusión en las muestras enviadas a histopatología de los hígados con lesiones. La mortalidad acumulada durante la evolución del caso sumo 3.27%, en condiciones normales a esta edad la mortalidad no supera el 1% (Fig. 1).

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Figura 1. Mortalidad diaria lote problema.

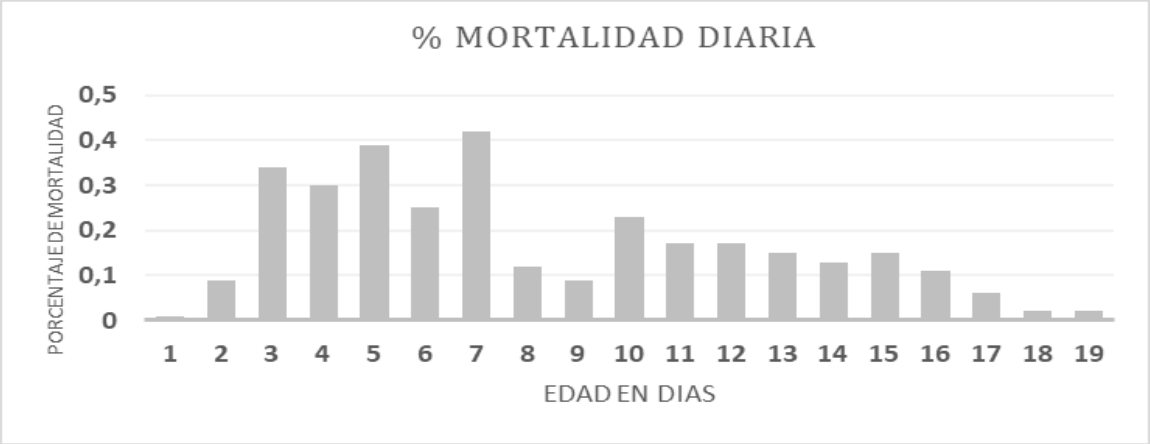
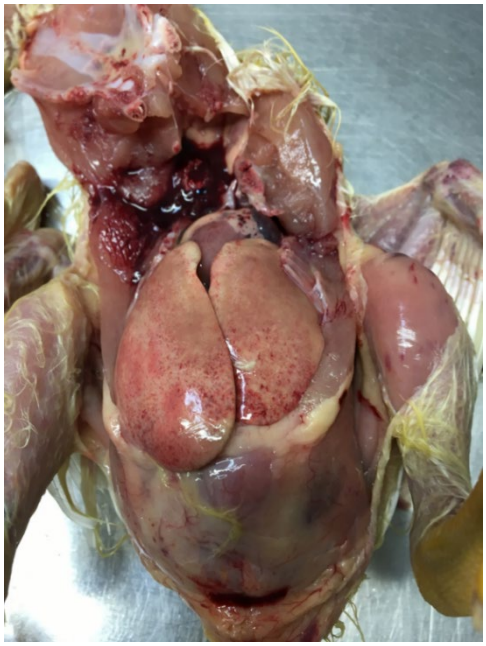


Figura 2. Lesiones hepáticas en aves enfermas.



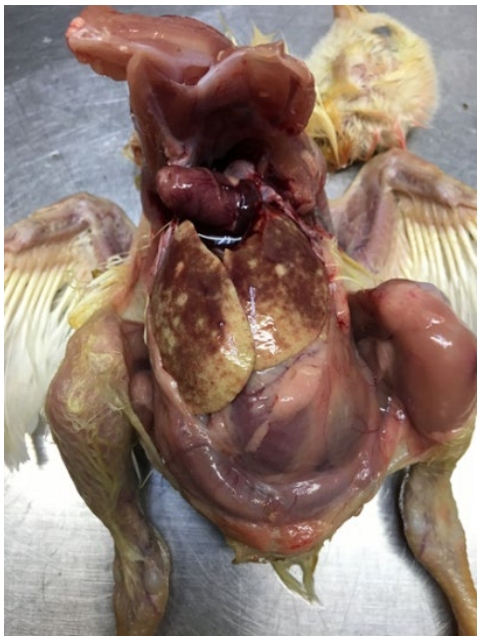
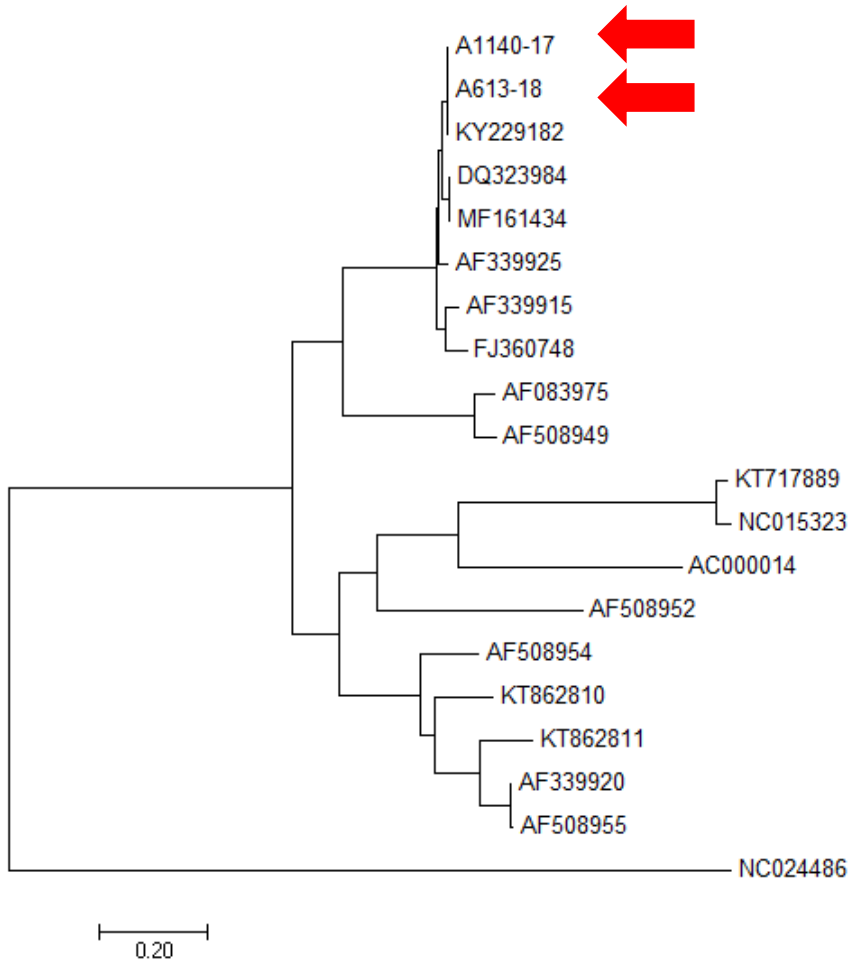


Figura 3. Árbol filogenético para el gen hexón de FADV. Numero de acceso y nombre de los virus usados en el grafico 2.
1 NC024486 Adenovirus de pato Cepa GR. AC000014 Adenovirus A. KT717889, Adenovirus 10 Cepa C-2B. NC015323 Adenovirus C. AF508952 Adenovirus 5 Cepa 340. AF339920 Adenovirus 11 Cepa XII. AF508955 Adenovirus 7 Cepa YR36. KT862811 Adenovirus 8b Cepa 764. KT862810 Adenovirus 8a Cepa TR59. AF508954 Adenovirus 6 Cepa CR 119. AF508949 Adenovirus 3 cepa 75. AF083975 Adenovirus 9. AF339915 Adenovirus 2 Cepa VR-82. FJ360748 Adenovirus 11 Brazil. DQ323984 Adenovirus 11 aislado 1047. MF161434 Adenovirus 11 USP-EC-02. AF339925 Adenovirus 12 Cepa 380 KY229182.Aviadenovirus Cepa GR. A1140-17 Santander – Piedecuesta. A613-18 Santander – Lebrija



DEVELOPMENT OF A VACCINE AGAINST APEC TO PREVENT EGG LAYER PERITONITIS IN CHICKENS

DESARROLLO DE UNA VACUNA CONTRA APEC PARA PREVENIR LA PERITONITIS EN LA GALLINA DE POSTURA

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RESUMEN

La *Escherichia coli* aviar patogénica (APEC) es un patógeno primario de las gallinas de postura que causa mortalidad aguda y peritonitis resultando en pérdidas significativas de producción en la industria avícola. Mientras que las vacunas de *E. coli* actualmente disponibles están basadas en célula-completa un método alternativo es vacunar contra las proteínas bacterianas de adquisición de hierro. Estas proteínas son esenciales para el crecimiento bacteriano y se sabe que contribuyen a la virulencia de APEC. Por lo tanto, enfocando la inmunidad sobre estas proteínas esenciales deberá resultar en una respuesta inmune más efectiva y relevante. Las vacunas de receptor sideróforo y de proteína porina (SRP®) enfocan al sistema immune en las proteínas de adquisición de hierro y se ha demostrado la efectividad de la tecnología para varios patógenos.

Para demostrar la eficacia contra APEC, se condujo un estudio placebo-controlado, doble ciego vacunación-desafío en gallinas de postura de 22 semanas de edad (WOA, por sus siglas en inglés) para evaluar los datos de mortalidad y de colonización en un modelo de peritonitis. La mortalidad resultó ser del 76% en las gallinas tratadas con el placebo y de 0% en las vacunados-SRP. La colonización en los tejidos evaluados tuvo un rango del 76 al 88% en las gallinas tratadas con el placebo y de 0 al 12% en las vacunadas-SRP. En conclusión, la tecnología de la vacuna-SRP propició una protección substancial de las gallinas ponedoras contra la mortalidad y la colonización bacteriana asociadas con la peritonitis.

SUMMARY

Avian pathogenic *Escherichia coli* (APEC) is a primary pathogen of laying chickens causing acute mortality and peritonitis resulting in significant losses of production in the poultry industry. While currently available poultry *E. coli* vaccines are whole-cell based, an alternative approach is to vaccinate against bacterial iron-acquisition proteins. These proteins are essential for bacterial growth and are known to contribute to APEC virulence. Therefore, focusing immunity on these essential proteins should result in a more effective and relevant immune response. Siderophore receptor and porin protein (SRP®) vaccines focus the immune system on iron-acquisition proteins and effective demonstration of the technology has been shown for several pathogens.

To demonstrate efficacy against APEC, a placebo-controlled, double-blind vaccination-challenge study was conducted in 22-week-of-age (WOA) laying hens to assess mortality and colonization data in a peritonitis model. Resulting mortality was 76% in placebo-treated hens and 0% in SRP-vaccinates. Colonization in assessed tissues ranged from 76% to 88% in placebo-treated hens and 0% to 12% in SRP-vaccinates. Conclusively, SRP vaccine technology elicited substantial protection of laying hens against mortality and bacterial colonization associated with peritonitis.

BACKGROUND

APEC is associated with peritonitis in commercial breeder and layer chickens and is a frequent contributor to morbidity, mortality, and loss of production (10, 12). Peritonitis is characterized by infection of the oviduct and ovary where atrophy and yolk fusion are observed and often accompanied by yellow fibrin-like clots on the air sacs, liver, and throughout the abdomen (9, 12, 15). The route of infection appears to be largely by the ascending movement of APEC via the cloaca to the oviduct and into the peritoneal cavity but intratracheal introduction and infection may also occur (9, 10, 12). It is thought that the former route may be due to the relaxation of the inner circular vaginal musculature as a consequence of intensive egg production (10, 12, 15). This is consistent with the general industry observation that large surges in the frequency of mortality and peritonitis are observed shortly after

peak egg production in broiler breeder and egg-producing stock.

Vaccination continues to be a preferred approach in disease control and available *E. coli* vaccines are whole-cell derived and have varying degrees of efficacy (8). An alternate approach for developing a more effective *E. coli* vaccine may be to specifically target the ability of bacteria to acquire iron that is essential for growth - one of the main pathogenic mechanisms of bacteria (1, 7, 11, 14). In the environment of the host, where iron is largely sequestered, bacteria produce siderophores for iron acquisition and siderophore receptor proteins (SRP) on their cell surface that are coordinately responsible for the active transport and internalization of iron into the cell (1, 7, 11). Vaccines directed at SRP have been shown to be effective in cattle against *E. coli* O157:H7 (13), *Salmonella enterica* Newport (4, 6), and most recently against mastitis caused by *Klebsiella pneumoniae* (5). In the latter three reports, it is noteworthy that vaccinated cows had significantly increased milk production and a decrease in somatic cell counts within produced milk. These additional benefits are thought to be related to the reduction of bacterial burden and stress in the vaccinated animal. SRP vaccine technology has also been successfully applied in chickens for protection against *Pasteurella multocida* (2) and *Salmonella* Enteritidis (3). Here, we report the successful development of an SRP vaccine for *E. coli* peritonitis in layer chickens.

MATERIALS AND METHODS

Chickens. Fifty Specific Pathogen Free leghorn hens were obtained from Valo BioMedia, (Adel, IA) and grown to 10 WOA prior to study initiation. Hens were housed in a single room within a floor pen containing wood shavings and sunflower seed hulls and were fed and watered *ad libitum*. Roosts and environmental enrichments were provided to the hens and housing density was lower than that recommended in the USA National Chicken Council Animal Care Guidelines. Lighting was provided as 12L:12D and intensity set at 15 LUX until the hens were 18 WOA when lighting was increased to 16L:8D and 30 LUX to support egg laying. The hens were tagged with numbered wing bands, randomly allocated into two groups and commingled for the duration of the study.

Vaccine. We determined the SRP protein profile of 107 isolates of APEC and other *E. coli* sourced from across the USA from dead chickens, diagnostic labs and other locations. From these isolates, two were chosen on the basis of their coverage of most of the SRPs known to exist in *E. coli* to provide the broadest possible protection in the field. SRP antigen was prepared from each isolate and formulated in a water-in-oil emulsion.

General protocol. This study was a placebo-controlled, randomized, and double-blind study to demonstrate the effectiveness of an *E. coli* SRP vaccine to protect chickens from *E. coli* mortality and peritonitis. Hens were vaccinated subcutaneously with 0.25 mL of vaccine in the back of the neck at 10 and 18 WOA. Control hens received a placebo vaccine containing all components except antigen. Hens were challenged at 21 WOA and necropsied one week later. All necropsy samples were tested for the presence of the challenge organism by plating on eosin-methylene blue nalidixic acid (NAL) agar. Data were analyzed by Preventive Fraction and Fishers exact test for statistical significance (alpha threshold set at < 0.05).

Challenge. The *E. coli* challenge organism was sourced from Dr. T. Johnson at the University of Minnesota and was selected for NAL resistance to facilitate isolation from chickens. Overnight cultures were propagated from a frozen stock and grown to log phase on the day of challenge. Hens were challenged by the intraperitoneal route.

RESULTS AND DISCUSSION

After challenge, 19 of the 25 hens from the placebo-treated group died prior to scheduled necropsy seven days later. During this time, no hens from the SRP-vaccinated group died or were moribund. All hens were necropsied and tested for the presence of the challenge *E. coli* when they died or at the end of the seven-day challenge period. Four hens from the vaccinated group were challenge-*E. coli* positive in one or more tissues. In contrast, 19 of the 25 placebo-treated hens were positive in each tissue tested and three other hens were positive in one or more tissues. Prevented fractions range from 84-100% protection (Fishers Exact test; all $p< 0.00001$).

E. coli infection continues to be a problem in the poultry industry, and its incidence is expected to increase as the use of antibiotics declines and the movement to cage-free aviaries increases. Therefore, as part of effective control programs, the use of vaccines will intensify, and it is important to provide the industry with current technologies that will be effective in this changing environment. An SRP vaccine is such a technology, eliciting immunity directly focused on iron acquisition - a main pathogenic mechanism of *E. coli*, and eliminating much of the irrelevant immunity and reactivity elicited by bacterins and live vaccines.

In this study, the *E. coli* SRP vaccine was demonstrated to prevent mortality and significantly reduce colonization after severe *E. coli* challenge and

confirms the utility of this vaccine in the control of peritonitis.

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Table 1. Summary of *E. coli* mortality and colonization of vaccinates vs. placebo-treated hens.

Mortality or Tissue	Vaccinates (% positive)	Placebo (% positive)	Prevented Fraction	P value
Mortality	0	76	100	.0000000116*
		Tissue Colonization		
Liver	0	76	100	.0000000116*
Heart	0	76	100	.0000000116*
Spleen	8	76	89.6	.0000016181*
Air Sac	12	76	84.4	.0000095457*
Oviduct	8	88	91	.0000000115*
Ovary	8	84	90.5	.0000000713*

***** indicates vaccinates are significantly different from placebo-treated hens.

HVT CONSTRUCT VACCINE VERIFICATION & MONITORING OF FIELD VIRUS CHALLENGE USING VIRAL FLEX-SEQ®

VERIFICACIÓN Y MONITOREO DE UNA CONSTRUCCION DE VACUNA HVP ANTE UN DESAFÍO DE VIRUS DE CAMPO USANDO EL VIRAL FLEX-SEQ®

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RESUMEN

Un mayor desafío práctico con la aplicación de vacunas de HVP vectorizadas (rHVT) ha sido la incapacidad de las compañías avícolas para verificar y monitorear definitivamente el éxito de la vacunación usando estos productos. El Viral Flex-Seq® usa la siguiente generación para la detección de la vacuna rHVP y para la caracterización de patógenos asociados en el caso de un desafío de campo. Además, el Viral Flex-Seq® permite la detección de la Innovax® contra los HVP no Innovax®, un mejoramiento en el control de calidad clave para las incubadoras que desean optimizar la vacunación. Un ejemplo de real práctico de campo de una vacunación deficiente o fallida, detección de errores en productos vacunales y el origen y evolución del virus de desafío en un sitio avícola serán presentados. Será discutida la aplicación de esta prueba en programas de aseguramiento de la calidad, así como la comparación del desempeño de los programas de incubadoras y de vacunación entre y dentro de las compañías.

SUMMARY

A major practical challenge with the application of HVT vector vaccines (rHVT) has been the inability of poultry companies to definitively verify and monitor the success of vaccination using these products. Viral Flex-Seq® uses next-generation sequencing for rHVT vaccine detection and for the characterization of associated pathogens in the case of field challenge. In addition, Viral Flex-Seq allows for the detection of Innovax® vs. non-Innovax origin HVT, a key quality control improvement for hatcheries wishing to optimize vaccination. A practical real-life field example of poor or missed vaccination, detection of vaccine product errors and the origin and evolution of challenge virus on a poultry site will be presented. Application of this testing in quality assurance programs as well as for the comparison of hatchery and vaccine program performance across and within companies will be discussed.

INTRODUCTION

Following the introduction of RFLP based methods to characterize molecularly ILT field viruses (1) sequencing-based approaches to more accurately describe alterations in ILT field strains have been developed (2). Until now, molecular testing solutions that may be conducted on a mass scale with rapid turnaround times have not been available.

Viral Flex-Seq (RAPiD Genomics) uses next generation sequencing with triplex genetic marker testing to demonstrate the presence of rHVT-ILT vaccine, and 8-plex multiplex genetic marker testing to identify ILT field virus. The assay is conducted using samples deposited on Flinders Technology Associates (FTA) cards. For detection of rHVT-ILT vaccine, the recommended samples are spleen at eight days, or feather pulp at 15 days, post-vaccination (based upon peak vaccine sequence recoveries in studies conducted at the MSD Animal Health Center for Diagnostic Solutions). Field virus is detected from FTA card tissue impressions collected during an outbreak from an appropriate site determined by field virus tissue tropism (tracheal samples for infectious laryngotracheitis or bursa for infectious bursal disease). The assay includes a housekeeping gene (DAGLA) that ensures sufficient sample (enough "chicken") has been applied to the FTA card to eliminate the possibility of a false negative result. This new assay was used to manage ILT vaccination programs on a multi-age commercial layer pullet site.

MATERIALS AND METHODS

The first study was conducted on a commercial layer farm with five multi-age pullet barns housing 431,000 pullets. Chicken embryo origin (CEO) vaccine had long been used by eyedrop at six weeks of age to control infectious laryngotracheitis (ILT), but the farm continued to experience sporadic clinical signs and mortality in one or more of the pullet houses. During a period of six months of 2017, this farm suffered repeated mortality and clinical signs of ILT that resulted in mortality totaling at least 12,500 pullets despite increasing vaccination dose up to 4X, in an attempt to achieve better coverage and protection. Mortality occurred as young as five weeks of age, or as late as 15 weeks of age.

Twenty baseline tracheal swab samples were taken to characterize and quantify the field ILT challenge on the farm using Viral Flex-Seq.

The vaccination program was changed to an rHVT-ILT vaccine (Innovax-ILT, Merck Animal Health) given by subcutaneous inoculation at the hatchery followed by tissue culture origin ILT vaccine (LT-Ivax®, Merck Animal Health) by eye-drop at six weeks of age. The subsequent pullet placements were monitored with 20 samples of feather pulp and 20 tracheal swab samples per sampled barn every 3-4 months from late 2017 through 2018. At least two barns were sampled at each time point.

In a second case, to demonstrate the specificity of the Viral Flex-Seq assay for the particular rHVT-ILT vaccine under investigation, 40 birds vaccinated with rHVT-IBD and CVI 988 were sampled via the feather pulp between 17 and 21 days of age.

A preliminary study to compare quantities of ILT field virus circulating in a population of birds of 53 weeks of age vaccinated with different ILT vector vaccines and secondary live ILT vaccination programs was also conducted. The flocks were vaccinated with rFP-ILT or rHVT-ILT (Innovax-ILT, Merck Animal Health) plus CVI 988 at day one, followed by secondary vaccinations between weeks 36-39 with ILT CEO vaccines via the drinking water.

RESULTS

The initial sampling of axillary feather pulp (to evaluate HVT-construct vaccine take) was taken during the transition to the new program. Two pullet houses were vaccinated with HVT-construct-ILT vaccine, while another, pullet flock was on the older program and had been vaccinated with an HVT-construct-IBD vaccine. Viral Flex-Seq is highly sensitive, with the negative cut-off for this evaluation at only 29 gene copies. The initial samples indicated that there was some mixing of HVT-construct-ILT and HVT-construct-IBD birds in the same cage. This resulted in a hatchery audit that revealed both vaccines in the same liquid nitrogen tank, and the same needle being used for all products in the same equipment (Figure 1). Hatchery operating procedures were improved for subsequent vaccinates.

As new pullets were vaccinated and added to the farm, subsequent sampling demonstrated improved sampling technique (more “chicken” on the FTA card) and improved vaccination take with the HVT-construct-ILT vaccine, indicating improved hatchery vaccination performance (Figure 2).

In February 2018, once the entire site had transitioned to HVT-construct-ILT vaccine (five months post-CEO vaccine use), field virus sampling still demonstrated the persistence of CEO-vaccine-type ILT virus on the farm. In June 2018, field virus could not be detected in tracheal samples submitted. The sample quality was considered poor (not enough “chicken” on the FTA card), so the results were labeled potential false negatives. In August 2018, the field virus samples were identified as extremely good quality with clear, true negative results for field strain ILT virus.

Analysis of samples specifically vaccinated with rHVT-IBD and CVI988 vaccines confirmed the complete absence of the rHVT-ILT from the samples. Sample quality was good with only three out of 40 samples that did not contain enough material. The quantity of the non-ILT-construct origin HVT was low and variable with 10 out of 37 birds testing true negative.

Comparison of 53 weeks old layers vaccine with rFP-ILT (RGCAN_19) and rHVT-ILT (RGCAN_20) vaccine indicated differences in field virus quantity between individual birds with a non-significant trend towards lower quantities of field virus in the rHVT-ILT samples (Figure 3). Other rHVT-ILT vaccinated flocks demonstrated negligible quantities of ILT field virus (RGCAN_8 and RGCAN_9).

DISCUSSION

Viral Flex-Seq provides a tool that can be used to monitor hatchery vaccination technique and to detect errors in hatchery vaccination with HVT-construct vaccine, detecting both missed birds and birds that have been vaccinated with a different HVT or HVT-construct vaccine.

One of the most difficult questions when dealing with a multi-age facility is “when has field virus challenge declined to a point where it’s safe to rely only upon an HVT-construct vaccine?”. This question requires two answers: “Is the hatchery administration effective?” and “Has the field virus challenge become negative?”. Viral Flex-Seq can be used to answer both

questions when high quality samples yielding sufficient DNA are taken. From this field experience, there is a learning curve involved in developing the technique to take good field samples…both from feather pulp for vaccine take analysis and from tissues appropriate for field virus sampling.

Sequential sampling on this multi-age commercial pullet facility demonstrates that it is possible to transition from CEO ILT vaccination accompanied by clinical signs and ILT mortality due to CEO-vaccine-type ILT virus, to rHVT-ILT vaccination, ultimately resulting in true negative results for ILT virus on the farm.

The absolute quantitative nature of the Viral Flex-Seq assay facilitates on-going monitoring programs to ensure target quantities of circulating vaccine are recovered, the vaccines prescribed are actually administered and monovalent HVT is not administered in error. Quantities of field virus may be monitored to assess the ability of rHVT-ILT vaccines to reduce or eliminate shedding of field virus in the field situation.

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Figure 1.

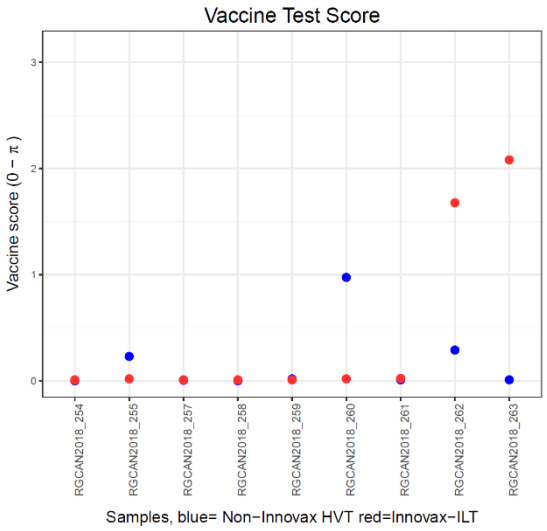


Figure 2.

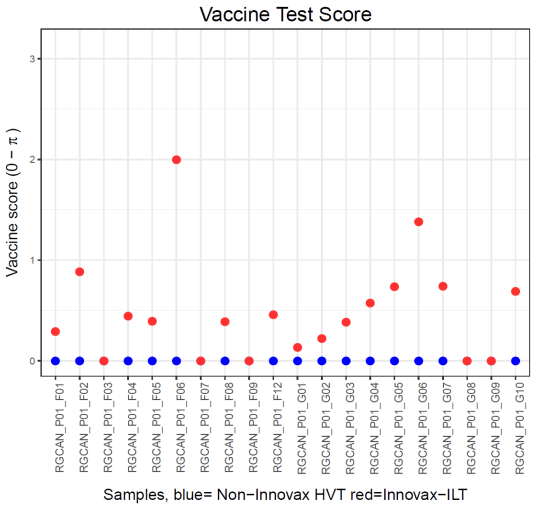
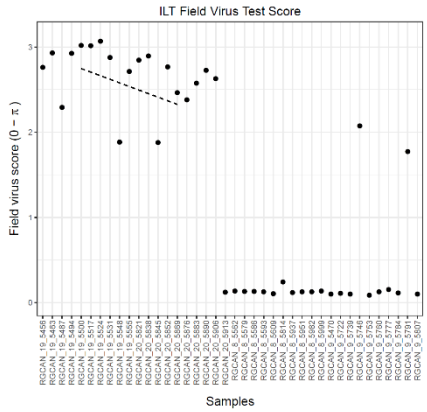


Figure 3.



LIVE *SALMONELLA* TYPHIMURIUM VACCINATION OF BROILERS RESULTS IN CROSS-PROTECTION AGAINST OTHER *SALMONELLA* SEROTYPES AT DIFFERENT AGES OF EXPOSURE

VACUNACIÓN CON *SALMONELLA TYPHIMURIUM* VIVA EN POLLOS DE ENGORDA Y LOS RESULTADOS EN PROTECCIÓN CRUZADA CONTRA OTROS SEROTIPOS DE *SALMONELLA* EN DIFERENTES EDADES DE EXPOSICIÓN

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Zoetis, U.S. Poultry

RESUMEN

El Servicio de Inocuidad Alimentaria e Inspección (FSIS, por sus siglas en inglés) anunció la implementación de nuevos estándares de desempeño de *Salmonella spp.* para las plantas de procesamiento avícola el 14 de mayo de 2010. Desde entonces, la industria avícola ha estado adoptando y probando varias estrategias que apuntan a reducir los niveles de *Salmonella spp.* Las intervenciones del lado vivo ayudan a reducir tanto la prevalencia como la carga de *Salmonella spp.* son el componente clave de un programa de control de *Salmonella* exitoso. La vacunación de pollos de engorda con vacunas vivas de *Salmonella typhimurium,* es una estrategia que ha ganado una amplia atracción y éxito en años anteriores. El objetivo de seguir cuatro estudios fue el evaluar el efecto de la vacuna viva de *Salmonella typhimurium* [Poulvac® ST(PVST)] sobre la protección cruzada contra desafíos de tres serotipos de *Salmonella* dados en edades tempranas y tardías de pollos de engorda.

INTRODUCTION

The Food Safety and Inspection Service (FSIS) announced the implementation of new *Salmonella spp.* performance standards for poultry processing plants in May 14th, 2010. Since then, the poultry industry has been adapting and testing several strategies that aim to reduce the levels of *Salmonella* spp. Live-side interventions that help reduce both the prevalence and the load of *Salmonella* spp. are a key component of successful salmonella control programs. Vaccination of broilers with live *Salmonella* Typhimurium vaccines is a strategy that has gained ample traction and success in the past few years. The objective of the following four studies was to evaluate the effect of a live *Salmonella* Typhimurium vaccine [Poulvac® ST (PVST)] on cross-protection against three *Salmonella* serotype challenges given at early and late ages to broiler chickens.

MATERIALS AND METHODS

Four broiler studies were conducted to evaluate the cross-protective effect of PVST against four salmonella serotypes when exposed to either early or late challenges. For the early challenge studies, *Salmonella* spp. load (enumeration) was determined by most probable number (MPN) methodology in liver/spleen and cecae samples. For the late challenge studies, *Salmonella* spp. prevalence was determined in liver/spleen and load in cecae samples. The four studies had the following designs:

Early challenge studies

Study 1 (*Salmonella* Enteritidis). One hundred fifty broiler chicks were placed into six different isolators (25 per) at day of age. Three isolators received birds vaccinated with PVST by spray at hatch and boosted by oral gavage at 14 days. Birds from the three remaining isolators were not vaccinated (control group). At four days of age, all birds were challenged by oral gavage with *Salmonella* Enteritidis at a target dose of 10^4 CFU per bird. At 21 days (17 d post-challenge), all birds were terminated and sampled.

Study 2 (*Salmonella* Kentucky). One hundred ten broiler chicks were placed in an isolation room into two different pens (55 birds per pen) at day of age. One of the pens received birds vaccinated with PVST by spray at hatch whereas the other received birds not vaccinated (control group). At 5 days of age, all birds were commingled into one big pen and challenged by oral gavage with *Salmonella* Kentucky at a target dose of 10^6 CFU per bird. At 14 days (9 days post-challenge), all birds were terminated and sampled.

Late challenge studies

Study 3 (*Salmonella* Heidelberg). Two hundred seventy broiler chicks were divided into three isolation rooms with two different pens each (45 birds per pen)

at day of age. In each room, one of the pens received birds vaccinated with PVST by spray at hatch and boosted by gavage at 14 days whereas the other pen received birds not vaccinated (control group). At 35 days of age, all birds were challenged by oral gavage with *Salmonella* Heidelberg (SH) at a target dose of 10^9 CFU per bird. At 49 days (14 d post-challenge), all birds were terminated and sampled.

Study 4 (*Salmonella* Kentucky and *S.* Infantis). One hundred twenty-eight broiler chicks were placed in an isolation room into two separate pens (64 birds per pen) at day of age. One of the pens received birds vaccinated with PVST by spray at hatch and boosted by gavage at 14 days whereas the other pen received birds not vaccinated (control group). At day 34, half of the birds from each pen were transferred into another isolation room and comingled in one big pen (64 birds total with 32 birds per treatment) and were challenged with *Salmonella* Kentucky (SK) at a target dose of 10^6 CFU per bird. The remainder birds (64 birds total with 32 birds per treatment) that stayed in the room were comingled and challenged with *Salmonella* Infantis at a target dose of 10^6 CFU per bird. At 46 days (12 d post-challenge), all birds were terminated and sampled.

All statistical analyses were conducted at a 0.05 level of significance ($P < 0.10$ considered as a trend) using two-sided tests. Studies 1 and 3 were conducted with progeny from breeders vaccinated with a killed *Salmonella* Enteritidis vaccine, whereas study 2 and 4 had progeny from breeders not vaccinated for *Salmonella*.

RESULTS

The early and late challenge results are presented in Tables 1 and 2 respectively.

Study 1. Upon early *Salmonella* Enteritidis challenge, the load of *Salmonella* spp. in liver/spleen samples tended ($P = 0.077$) to be lowered (-0.57 log) by PVST vaccination. In addition, cecal load was 0.92 log numerically (P = 0.234) lower in PVST vaccinated birds.

Study 2. On the early *Salmonella* Kentucky challenge, the cecal load of *Salmonella* spp. tended ($P = 0.072$) to be lower in PVST vaccinated birds. There was no statistical difference (P=0.489) in the liver/spleen enumeration results.

Study 3. Upon late *Salmonella* Heidelberg challenge, both liver/spleen prevalence ($P = 0.043$) and cecal load ($P = 0.003$) were significantly lower in samples obtained from vaccinated birds. When compared with the controls, the vaccinated birds had Salmonella prevalence and load reduced by 75.00% and 1.54 log, respectively.

Study 4. Upon late *Salmonella* Kentucky challenge, both the liver/spleen prevalence ($P = 0.001$) and cecae load (P=0.027) were reduced by PVST. Vaccination was associated with a 75.78% reduction of liver/spleen prevalence and 0.49 log lower cecal load. For the late *Salmonella* Infantis, cecal *Salmonella* spp. enumeration ($P = 0.064$) and prevalence (P=0.196) by MPN (limit of detection ≥ 4 colony forming units) tended to be lowered by PVST vaccination. The PVST vaccinated birds had 75.73% less positive cecae than birds not vaccinated. There were no statistically significant differences ($P = 1.000$) in liver/spleen prevalence.

DISCUSSION

After spray vaccination of day old birds with live *Salmonella* Typhimurium vaccines, the bacteria are ingested and rapidly colonize the gut epithelial cells. At a first stage, populating the gut mucosa with the vaccine strain prevents the attachment and colonization of wild salmonella types that might be present at the hatchery, transport trucks, or brooding areas. Simultaneously, innate and humoral (especially IgA – mucosal immunity) responses are triggered in order to protect and mount an immune response to the vaccine strain. Knowing that broiler chicks are more susceptible to *Salmonella* colonization at day of age than at later stages of life (2), it is crucial that the *Salmonella* vaccination takes place right after hatch allowing not only a better take and more efficient vaccination, but also a better competitive exclusion.

One of the goals of the studies presented herein was to evaluate the capability of a live *Salmonella* Typhimurium vaccine to respond to early *Salmonella* challenges where little time was allowed to construct a full immune response. Study 1 showed that PVST was able to reduce the number of *Salmonella* Enteritidis in the liver/spleen when birds were challenged at four d of age and sampled at day 21. This finding indicates that even though there was a high load of *Salmonella* present in the cecae, the birds that were vaccinated had less *Salmonella* colonizing these organs. In study 2, PVST reduced the load of *Salmonella* Kentucky in the cecae nine d post-challenge. The liver/spleen *Salmonella* enumeration results were low overall suggesting that there was a poor organ colonization. The results of these two studies show the value of early vaccination with PVST on cross-protecting against *Salmonella* from groups D and C.

Studies 3 and 4 with the associated late *Salmonella* challenges, were intended to evaluate the cross-protective effects of live *Salmonella* vaccination close to processing age in broilers. Overall, PVST effectively reduced the colonization of the three

serotypes tested. Both *Salmonella* Heidelberg and *Salmonella* Kentucky had significant reductions in prevalence and load in the liver/spleen and cecae, respectively. The *Salmonella* Infantis challenge had a lower take compared with the other two serotypes which could have limited the ability to detect treatment effects. Nevertheless, PVST vaccination tended to reduce the *Salmonella* Infantis load and prevalence in the cecae.

In a comprehensive review on host immunity to *Salmonella*, Lillehoj *et al*. (1) emphasize that each serotype triggers different cellular and humoral immune responses and that, despite these differences, *Salmonella* vaccines have shown encouraging results in controlling *Salmonella* infection. The data presented herein reiterate the observations by Lillehoj *et al*. (1) since the live *Salmonella* Typhimurium vaccine was shown to cross-protect against three other serotypes. In conclusion, the administration of live *Salmonella* vaccination in broilers was shown to be an effective live-side intervention against early and late challenges of salmonellas belonging to the three most common *Salmonella* O-groups (B, C, and D).

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Table 1. Effects of PVST vaccination against *Salmonella* Enteritidis and *Salmonella* Kentucky challenges on liver/spleen and cecae *Salmonella* spp. load.

	Early challenge			
	Salmonella Enteritidis		*Salmonella* Kentucky	
	Liver/spleen enumeration (Log CFU/ml)	Cecae enumeration (Log CFU/ml)	Liver/spleen enumeration (Log CFU/ml)	Cecae enumeration (Log CFU/ml)
Control	1.18	5.47	0.31	5.61
PVST	0.61	4.55	0.37	5.23
P-value	0.077	0.234	0.489	0.072

Table 2. Effects of PVST vaccination against *Salmonella* Heidelberg, *Salmonella* Kentucky and *Salmonella* Infantis challenges on liver/spleen prevalence and cecae load of *Salmonella* spp.

	Late challenge					
	Salmonella Heidelberg		*Salmonella* Kentucky		*Salmonella* Infantis	
	Liver/spleen prevalence (%)	Cecae enumeration (Log CFU/ml)	Liver/spleen prevalence (%)	Cecae enumeration (Log CFU/ml)	Liver/spleen prevalence (%)	Cecae enumeration (Log CFU/ml)
Control	50.00	1.87	51.61	1.21	0.00	0.39
PVST	12.50	0.33	12.50	0.72	3.13	0.31
P-value	0.043	0.003	0.001	0.027	1.000	0.064

THE EFFECTS OF DRINKING WATER ADDITIVES ON PERFORMANCE AND INTESTINAL MICROBIOTA IN BROILERS

LOS EFECTOS DE LOS ADITIVOS EN EL AGUA DE BEBIDA SOBRE EL DESEMPEÑO Y LA MICROBIOTA INTESTINAL EN POLLOS DE ENGORDA

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[B] Trouw Nutrition Mexico

RESUMEN

Los aditivos en el agua de bebida (DWA, por sus siglas en inglés) basados en ácidos grasos de cadena corta (SCFA, por sus siglas en inglés) reducen de manera efectiva el pH en el agua y en el estómago, apoyando la digestión (1). Los ácidos orgánicos pueden ser parcialmente amortiguados, esto apoya a los ácidos para trabajar de manera efectiva, no sólo en el agua de bebida, sino también en todo el tracto gastrointestinal, puesto que parte de los ácidos se vuelven disponibles en la última parte del intestino delgado. Pueden esperarse modificaciones de la microbiota intestinal debidas al uso de aditivos en el agua de bebida (2). El objetivo de este estudio fue determinar la eficacia de los aditivos en el agua de bebida sobre el desempeño en el crecimiento y en la microbiota intestinal de pollos de engorda criados bajo condiciones de investigación y de baja higiene.

INTRODUCTION

Drinking water additives (DWA) based on short chain fatty acids (SCFA) effectively reduce the pH in the water and stomach, supporting digestion (1). Organic acids may be partially buffered, this supports acids in working effectively, not only in the drinking water, but also throughout the gastro-intestinal tract since part of the acids become available in the latter part of the small intestine. Modifications of the intestinal microbiota due to the use of drinking water additives can be expected (2). The objective of this study was to determine the efficacy of drinking water additives on growth performance and intestinal microbiota of broilers reared under research and low hygiene conditions.

MATERIALS AND METHODS

A total of 2120 Ross 308 cockerels were divided over 4 dietary treatments (10 replicates/treatment; 53 birds/pen, final stock density 37 kg/m^2) in a 2x2 full factorial design: Control vs. Drinking Water Additive (Selko-pH, Trouw Nutrition, The Netherlands) and research vs. low hygiene conditions (LHC). Low hygiene conditions were created by litter removal from the previous study without cleaning the barn. DWA was provided at 1.0 l/m3 (day 1-3), 1.3 l/m3 (day 4-6) and 1.5 l/m3 (day 7-35). Titrations were performed prior to the start of the study to determine the dose for application during the whole trial. The dose used was the maximum dose that can be used by keeping the pH of the water at 3.8; the control water had a pH of 9.1. A standard three phase feeding program meeting all nutritional requirements was fed to all treatment groups, and water was provided *ab libitum*. At the hatchery, the birds were vaccinated against coccidiosis, infectious bronchitis, Marek, and infectious bursal disease (Gumboro). Body weight and feed intake were determined at day 10, 25 and 35. At day 19, 10 birds per treatment of the LHC group (1 per pen) were necropsied and the intestinal content of the duodenum, ileum, and cecum was collected. The microbial composition of these samples was determined by analyzing 16S rRNA gene amplicons via Illumina sequencing. Data were analyzed using a combination of mothur and USEARCH. Growth performance data were analysed using PROC GLM procedure of SAS.

RESULTS

Performance. No differences were observed in water intake during the starter period. However, during the grower and finisher period, control birds showed a reduction in water intake. The results show that hygiene conditions affected performances during the starter period, birds performed better (around 1.0%) under more clean conditions. Hygiene conditions also influenced performance during the grower period. Body weight, daily weight gain and intake were higher (around 4%) in birds reared under better cleaning conditions. For the overall study period, the results of Low Hygiene Conditions

negatively affected (P<0.001) the growth of the birds (62.0 g/day vs. 63.3g/day). Regarding the water treatments, control birds tended to have a reduced daily weight gain and performed worse than those receiving DWA. Over the complete study period (0-35 days), DWA showed a significantly higher (P<0.001) daily weight gain than the control group (63.9 g/day vs. 60.3 g/day, respectively). Additionally, daily feed intake increased by 5.5% (P<0.001) (Table 1). Mortality was not affected by DWA treatments or hygienic conditions.

Microbiota. The results from the analyses of microbial composition in the duodenum, ileum, and cecum showed a higher diversity in cecum samples compared to small intestinal samples. DWA administration significantly lowered the abundance of *Streptococcus* compared to the control group at duodenum, ileum, and cecum (Figure 2). Microbial community structures of the duodenum and ileum showed strong correlation with the body weight of the individual animals, mainly due to specific members of the *Lactobacillus* population (P=0.0002). *Lactobacillus aviarius* has been reported to be potential performance related (2).

DISCUSSION AND CONCLUSION

The results show that the water additive reduced water pH, but did not negatively affected water intake. Control birds showed a lower water intake than those receiving the water additive. Birds reared under better hygiene conditions (complete cleaning) showed a higher body weight, daily weight gain, feed intake but also feed conversion ratio than those reared under less hygiene conditions (simple cleaning). This was more pronounced during the starter period, when no effect of water treatments was observed. During the grower and finisher period, a positive effect on body weight, daily weight gain and feed intake was observed in birds receiving DWA. In conclusion, DWA based on short chain fatty acids can effectively improve growth performance in both hygiene conditions. These improvements may partially be attributed to DWA induced changes of the intestinal microbiota.

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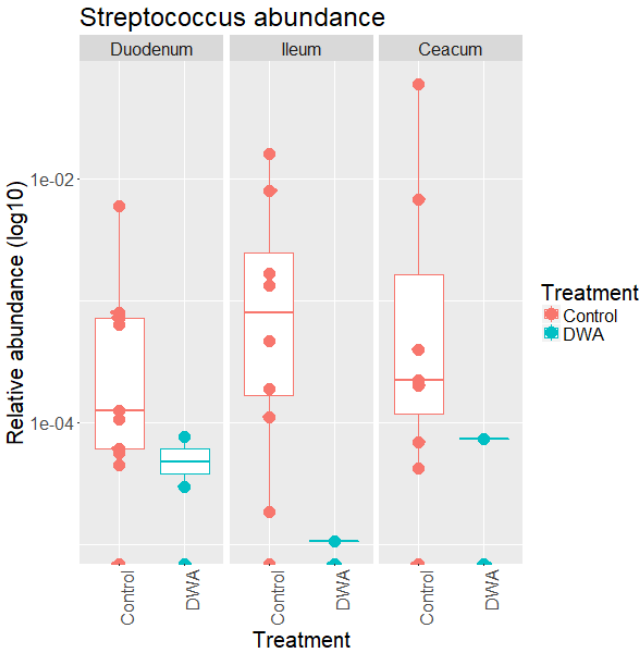
Table 1. Performance results for the control vs drinking water additive in the complete study period.

Item	*DWA*	*Control*	*SEM*	*P-value*
BW day 35, g	2274[a]	2147[b]	17.89	<0.0001
ADG day 0-35, g	63,9[a]	60,3[b]	0.51	<0.0001
ADFI day 035, g	98,7[a]	93,5[b]	0.91	<0.0001
FCR	1.55	1.55	0.01	0.41

Note: SEM means standard error of the mean. BW: body weight; ADG: average daily gain; ADFI: average daily feed intake; FE: feed efficiency. [a-b] Different superscripts within a row indicate a significant difference (P< 0.05).

Figure 2. Relative abundance of *Streptococcus* in both treatments at day 19.



EFFECTS OF RESIQUIMOD ON INNATE IMMUNE RESPONSE IN EARLY POST-HATCH CHICKENS CHALLENGED WITH INFECTIOUS BRONCHITIS VIRUS INFECTION

EFECTOS DEL RESIQUIMOD SOBRE LA RESPUESTA INMUNE INNATA EN POLLOS RECIEN ECLOSIONADOS DESAFIADOS CON UNA INFECCION CON EL VIRUS DE BRONQUITIS INFECCIOSA

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RESUMEN

Está en aumento el uso de los ligandos tipo receptores toll (TLR) que estimulan la inmunidad innata del hospedador vía la activación de las células inmunes ante el involucramiento con sus receptores como agentes inmunomodulares para combatir enfermedades. De estos TLRs, el Imiquimod y el resiquimod (R-848), que pertenecen al grupo de los compuestos de imidazoquinolina han sido estudiados por su potencial inmuno-estimulante contra varios patógenos. Sin embargo, su papel en la activación immune contra un desafío con el virus de bronquitis infecciosa (IBV, por sus siglas en inglés) en pollos, especialmente el R-848, no ha sido bien documentado. En este estudio, nos dirigimos a identificar varios mecanismos de R-848 induciendo la activación immune contra el IBV en pollos cuando se les dio R-848 pre-eclosión, para ver si proporcionaría protección contra la infección post-eclosión que indujera morbilidad y mortalidad.

Encontramos una sobre-regulación significativa de IL-1β y del IFN-γ mRNA y reclutamiento de distintas células inmunes que son vitales para el inicio

y mantenimiento de una respuesta inmune fuerte tipo Th1.

En conclusión, R-848, cuando se administró pre-eclosión, es capaz de iniciar respuestas inmunes innatas en el pulmón tan temprano como el día de edad. Con experimentos posteriores para determinar el efecto protector del resiquimod en una dosis óptima puede ser benéfico en el desarrollo de mejores estrategias de control para IBV.

SUMMARY

Toll like receptor (TLR) ligands that enhances host innate immunity via immune cell activation upon engagement with its receptors are being increasingly used as immunomodulatory agents to combat disease. Of these TLRs, Imiquimod and resiquimod (R-848), belonging to a group of imidazoquinoline compounds have been studied for their immune stimulatory potential against several pathogens. However, their role in immune activation against an infectious bronchitis virus (IBV) challenge in chicken, specially R-848, has not been well documented.

In this study, we aimed at identifying several mechanisms of R-848 induced immune activation against IBV in chicken and to ascertain when R-848, given pre-hatch, would provide protection against a post-hatch infection induced morbidity and mortality.

We found a significant upregulation of IL-1β and IFN-γ mRNA and recruitment of distinct immune cells that are vital for initiation and maintenance of a strong Th1 type immune response.

In conclusion, R-848, when delivered pre-hatch, is able to initiate innate immune responses as early as day one in the lungs of chicken. With further experiments into determining the protective effect of resiquimod in an optimal dosage may be beneficial in development of better IBV control strategies.

BACKGROUND

The immune system of a host is armed with a range of pattern recognition receptors (PRRs) including TLRs, to mount host responses against invading pathogens (6). These receptors recognize and bind with pathogen associated molecular patterns (PAMPs), certain molecules present on the surface and within microbes. The TLR receptor-ligand interactions then lead to the activation of signaling pathways that ultimately result in upregulation of several genes responsible for immune responses (6).

Understanding of these interactions and its pathways had let to the application of natural or synthetic TLR ligands in hosts to induce activation and release of immune cells, eliciting antiviral responses (1, 3, 8). Among other TLR ligands, naturally occurring TLR 7 ligand, ssRNA and its synthetic analogs such as imiquimod and resiquimod have been used in this regard against avian pathogens. Imiquimod and resiquimod are two of the members belonging to the group of imidazoquinolinamines shown to poses abilities as immune response modifiers (4). As a vaccine adjuvant, resiquimod has been used along with inactivated Newcastle disease virus and infectious bronchitis virus in chickens (7, 10) and its mechanisms of action in chicken peripheral blood mononuclear cells have been evaluated (2, 9).

Nonetheless, its prophylactic potential as a stand-alone agent in a chicken model against a post hatch IBV infection has not been studied so far. Thus, we directed our studies at identifying resiquimods protective effect against a post hatch IBV infection and to analyse its mechanisms of action in the respiratory system when delivered in ovo.

MATERIALS AND METHODS

To determine mechanisms of *in ovo* delivered resiquimod in young chicken, ED 18 SPF eggs were inoculated with either (400 µg in 200 µL PBS) resiquimod or PBS (200 µL PBS alone) and incubated until hatch. On the day of hatch, chickens from both groups (resiquimod pre-treated = 6, PBS pre-treated = 6) were euthanized and lung tissue preserved in Optimum Cutting Medium (OCT, Leica Biosystems, Wetzlar, Germany) for immunofluorescent assay, in 10% neutral buffered formalin for histopathological examination, and in RNASave® (Biological Industries, Beit Haemek, Israel) for cytokine mRNA analysis.

To evaluate resiquimod induced protection assessed by morbidity and mortality, 13 eggs were injected *in ovo* with resiquimod while 15 eggs were injected with PBS alone. On day 1 post-hatch, 10 birds from the resiquimod pre-treated group and 12 birds from PBS pre-treated group were infected with IBV M41 strain intra-trachealy at a dose rate of 2.75 X 10^4 EID_{50} per bird and remaining birds were maintained as uninfected controls (Resiquimod control = 3, PBS control = 3). Birds were monitored for 14 days post-infection (dpi) to detect clinical signs and associated mortality. Body weight measurments were obtained daily until end of experiment. At 3 and 7 dpi, oropharyngeal and cloacal swabs were collected from infected birds using Puritan® Unitranz-RT transport system (Puritan Medical Products LLC, Maine, USA) and stored in -80^0 C until analyzed. The clinical score of each bird was evaluated twice daily and the humane end point of the birds determined. A clinical score of 5 was considered the humane endpoint.

Viral RNA from lung tissue was extracted using Trizol reagent (Ambion, Invitrogen Canada Inc., Burlington, ON, Canada) and two step RT-PCR was

occupied for the purpose of IBV-N gene detection and IL-1β, iNOS and IFN-γ mRNA gene analysis. Extraction of viral RNA from swabs were performed by the use of E.Z.N.A.® viral RNA kit (Omega Bio-tek Inc., Norcross, GA, USA).

Immunofluorescence detection of CD4+, CD8α+ T cells and KUL01+ macrophages were performed with the use of CD4 (Southern Biotech, Birmingham, Alabama, USA), CD8α (Southern Biotech, Birmingham, Alabama, USA) and unlabeled monoclonal antibody specific for chicken macrophages/monocytes, KUL01 (Southern Biotech, Birmingham, Alabama, USA) primary antibodies. For CD8 cells and macrophages, Dylight® 550 conjugated goat anti-mouse IgG (H+L) (Bethyl Laboratories Inc., Montgomery, TX, USA) was used as secondary antibody and for CD4 cells, biotinylated goat anti-mouse IgG (H+L) (Southern Biotech, Birmingham, Alabama, USA) followed by Dylight® 488 was used.

Formalin fixed, lung tissues were processed and stained with hematoxylin and eosin (H&E) at the Diagnostic Services Unit of the University of Calgary's Faculty of Veterinary Medicine. Histological changes observed were scored as follows. A score of 0 was given to lung tissue exhibiting an inner ring of interrupted epithelia in para-bronchioles, indicating presence of many air spaces close to the lumen with the presence of multiple air exchange areas in the surrounding outermost tissue and mononuclear cell infiltration only at 25%. A score of 1 recognizes loss of air exchange areas closest to the lumen of the para-bronchioles and mononuclear cell infiltration is at 50%. A score of 2 is given when the mononuclear cell infiltration reaches 75% resulting in a loss of most air exchange areas and a highest lesion score of 3 is given when a massive mononuclear infiltration above 75% is seen and little to no air exchange areas visible (5).

RESULTS AND DISCUSSION

An upregulation of IL-1β and IFN-γ genes was seen in the day old lungs of in ovo resiquimod delivered birds compared with controls. Coinciding with this upregulation a significant recruitment of two immune cell types were also evident in the resiquimod pre-treated lungs compared to control lungs. Post infection, the mortality rates or the morbidity did not differ among resiquimod pre-treated and PBS treated groups. Moreover, lung lesion score of resiquimod pre-treated birds was significantly higher than that of the control group in day old lungs suggesting a detrimental effect of resiquimod pre-treatment. Collectively, we can conclude that although capable of inducing upregulation of several cytokine genes and the recruitment of key immune cells, high dose resiquimod delivery in ovo may be deleterious to the host in the face of an IBV infection.

(This article will be submitted as a full length manuscript to a peer reviewed journal.)

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MEDIATORS OF CPG ODNS INDUCED EARLY PROTECTION OF POST-HATCH CHICKENS INFECTED WITH INFECTIOUS BRONCHITIS VIRUS

MEDIADORES DE CPG ODNS INDUJERON PROTECCION TEMPRANA EN POLLOS POST-ECLOSION INFECTADOS CON EL VIRUS DE BRONQUITIS INFECCIOSA

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RESUMEN

La bronquitis infecciosa (IB, por sus siglas en inglés) es una de las enfermedades más importantes en pollos (junto con la Influenza Aviar (AI, por sus siglas en inglés) y la enfermedad de New Castle (ND, por sus siglas en inglés)) por el fuerte impacto económico en la industria avícola. CpG oligodeoxinucleótidos (ODNs) han sido implicados por inducir una resistencia natural contra muchos patógenosen varios modelos animales incluyendo especies aviares. En el estudio actual, administramos in ovo CpG ODNs en embriones de 18 días (ED), y fueron desafiados con el virus de bronquitis infecciosa cepa Massachusetts (M)41 vía oro-faríngea una vez que las aves eclosionaron. Se colectaron muestras de pulmón, traquea y sangre a los 3 y 7 días post-infección (dpi) para evaluar varias células inmunes y varias expresiones de genes de citocina, la carga del genoma viral del IBV y la producción de anticuerpos. Encontramos una reducción significativa en las cargas del genoma viral, expresión estimulada de IL-1β y de IFN-γ mRNA y un aumento en el reclutamiento de macrófagos, células T CD4+ y células T CD8α + en pulmones a los 3 dpi de las aves tratadas con CpG ODNs cuando se compararon con los controles. Más aún, fue evidente que hubo una reducción significativa en la morbilidad, mortalidad y excreción viral inducidas por el IBV a través de la vía oral tanto a 3, como a 7 dpi en las aves tratadas con CpG ODNs, implicando que es una aplicación que vale la pena considerar en el desarrollo de nuevas estrategias de control como alternativas o junto con los métodos de control de IBV actuales.

SUMMARY

Infectious bronchitis (IB) is one of the leading diseases in chickens (alongside avian influenza (AI) and new castle disease (ND)) that imparts major economic importance to the poultry industry. CpG oligodeoxynucleotides (ODNs) have been implicated in inducing natural resistance against many pathogens in various animal models including avian species. In the present study, we delivered CpG ODNs in ovo to embryo day (ED) 18 eggs and, challenged with infectious bronchitis virus Massachusetts (M)41 via oro-pharyngeal route once the birds hatched. Lung, tracheal and blood samples were collected at 3 and 7 days post-infection (dpi) to evaluate several key immune cells and several cytokine gene expression, IBV viral genome load and antibody production. We found a significant reduction in viral genome loads, enhanced expression of IL-1β and IFN-γ mRNA and increased recruitment of macrophage, CD4+ T cells and CD8α + T cells in 3 dpi lungs of CpG ODNs treated birds when compared with controls. Moreover, a significant reduction in IBV induced morbidity, mortality and viral shedding through oral route at both 3 and 7 dpi, was evident in CpG ODNs treated birds implying CpG ODNs, an applicant worthy considering in developing novel control strategies as alternate or adjunct to current IBV control methods.

INTRODUCTION

Infectious bronchitis, as the name implies is mainly an acute and severe disease of the upper respiratory system (8). The causative agent infectious bronchitis virus (IBV) infects primarily the epithelial surfaces of the respiratory tract and also the female reproductive tract and kidneys with varying severity depending upon the type of strain that infects and replicates in the aforementioned tissues (11). The adverse effects of the infection are reflected upon the poultry industry as major production losses in broilers due to carcass condemnation, poor weight gain and mortality and in breeder and commercial layers due to down grading of eggs and sub-optimal levels of egg production during and after infection with IBV (4, 9).

Although the mortality associated with primary IB disease is low as 10 - 25%, highly infectious nature of the agent has led to high morbidity in flocks reaching near 100% (1, 9). Owing to frequent mutations and recombinations, new strains and serotypes have emerged in vaccinated flocks. For the control of IBV, live attenuated and killed vaccines are currently being used and their reliability has become a concern against different mutant strains as the current vaccines only provide protection against standard field strains (2, 3). Thus, the emergence of these variant IBV strains/serotypes has led to IB outbreaks. Considering above factors, the development of novel approaches as an alternative or adjunct to current control measures against IBV has become essential.

One such approach is to exploit the innate arm of the immune system to enhance the host defences directed against inciting pathogens. A crucial component in this regard that had been the subject of research in the last few decades is the use of synthetic compounds that mimic pathogen components such as Cytosine-guanosine deoxynucleotides (CpG) oligodeoxynucleotides (ODNs). In chickens, the use of CpG ODNs as a prophylactic or a therapeutic agent has been studied extensively against many bacterial and viral agents (7, 10). Moreover, the immunostimmulatory effects of CpG ODNs against a pre-hatch IBV infection in chicken embryos have been studied recently (5, 6). However, these effects and its protective ability of CpG ODNs against a post-hatch IBV challenge in chickens has not been evaluated. Thus, we aimed at identifying the association of immune cells (CD4, CD8+ T cells and macrophages) and pro inflammatory cytokines (iNOS, IFN-γ, and IL-1β) in CpG ODNs induced innate and adaptive immunity. Also we aimed at determining whether *in ovo* delivery of CpG ODNs will limit IBV infection encountered post hatch in chickens *in vivo*.

METHODS AND MATERIALS

CpG ODNs protective effect was evaluated by assessing the morbidity and mortality subsequent to an IBV infection, quantifying several immune cells stimulated through CpG ODNs delivery in ovo, assessing upregulation of few mRNA cytokines, detecting and quantifying IBV-N gene in lung tissue, oropharyngeal and cloacal swabs and lastly by demonstrating reduced IBV-N antigen levels in tracheal mucosal epithelium.

First, we delivered CpG ODN 2007 to embryo day (ED) 18 SPF eggs *in ovo* via chorioallantoic route, while another subset of eggs was delivered with PBS. On the day of hatch, intra-tracheal inoculation of IBV M41 strain at a dose rate of 2.75 X 10^4 EID_{50} was performed on a subset of birds while remaining were kept as controls. Birds were monitored daily for clinical signs and survival. On 3 and 7 days post infection (dpi), subsets of the birds were euthanized to collect tracheal and lung tissue for the purpose of viral RNA quantification, detection of immune cell recruitment, to observe tracheal histopathology and to quantify several cytokine gene expression. Oropharyngeal and cloacao swabs were also obtained on 3 and 7 dpi.

Viral RNA was extracted from lung tissues using trizol reagent (Ambion, Invitrogen Canada Inc., Burlington, ON, Canada) and two step RT-PCR was occupied for the purpose of IBV-N gene detection. Pfaffle method was used to quantify changes in cytokine genes in 3 dpi lung.

Immunofluorescence detection of CD4+, CD8α+ T cells , KUL01+ macrophages and B cells were performed with the use of CD4 (Southern Biotech, Birmingham, Alabama, USA), CD8α (Southern Biotech, Birmingham, Alabama, USA), unlabeled monoclonal antibody specific for chicken macrophages/monocytes, KUL01 (Southern Biotech, Birmingham, Alabama, USA), and IgM (Southern Biotech, Birmingham, Alabama, USA) primary antibodies. For CD8 cells, macrophages and IgM, Dylight® 550 conjugated goat anti-mouse IgG (H+L) (Bethyl Laboratories Inc., Montgomery, TX, USA) was used as secondary antibody and for CD4 cells biotinylated goat anti-mouse IgG (H+L) (Southern Biotech, Birmingham, Alabama, USA) followed by Dylight® 488 was used.

Histology sections of trachea was prepared and stained with hematoxylin and eosin (H&E) by Histopathology Diagnostic Services Unit at the University of Calgary Faculty of Veterinary Medicine to identify IBV induced pathology.

RESULTS

We found that, when delivered to ED 18 embryos *in ovo*, TLR-21 ligand CpG ODNs significantly improve IBV induced mortality, morbidity and histopathology with associated increased recruitment of immune cells such as macrophages, and T cells. Further we noticed a reduction in viral genome loads in oropharyngeal swabs at 3 and 7 dpi and in lungs at 3 dpi.

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AVIAN REOVIRUSES, MOLECULAR CHARACTERIZATION LOOKING FOR BETTER CLASSIFICATION METHODS

REOVIRUS AVIARES, BUSCANDO MEJORES METODOS DE CLASIFICACION POR CARACTERIZACION MOLECULAR

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RESUMEN

El reovirus aviar (ARV, por sus siglas en inglés) es la causa principal de la artritis viral y la tenosinovitis en pollos y pavos (1, 2), disparando pérdidas económicas en la industria avícola debido a tasas de conversión alimenticia afectadas, falta de uniformidad y un aumento en decomisos en las

parvadas afectadas a nivel mundial. Los ARVs son virus no envueltos. Su material genético está formado por diez segmentos de doble cadena de ARN. Estas características les permiten tener una tasa elevada de mutación y recombinación, escapando de la inmunidad producida por las vacunas. Las cepas vacunales clásicas tales como la S1133, 1733 y 2408, han sido usadas desde los años 70 y las homologías en las variantes moleculares a estas vacunas están frecuentemente por abajo del 80%.

Por años las variantes moleculares han sido detectadas y clasificadas en cinco (3, 4) y seis (5, 6, 7) grupos genotípicos basados en el gen S1, que codifica para la proteína Sigma C. Sin embargo, sabemos que las caracterizaciones basadas en el S1 no proveen el cuadro completo y no se correlacionan con la antigenicidad y patogenicidad de estas cepas, pero es un método rápido y fácil para agrupar estos aislamientos. Puesto que los virus incorporados en las vacunas autógenas se seleccionan, en parte, basados en la caracterización del S1, es necesario reconsiderar el método. En estudios previos en nuestro laboratorio (8, 9) se ha sugerido que otros genes i.e. M2 y L3, codificando para las proteínas estructurales externas, tienen una gran variabilidad y serían buenas candidatas para la clasificación de las variantes moleculares del ARV y pueden estar asociadas con la patogenicidad y antigenicidad. Desde el 2015 comenzamos el esfuerzo de la caracterización molecular basado en la caracterización S1 y el agrupamiento de los aislamientos obtenidos por el Laboratorio de Salud Animal e Inocuidad Alimentaria de California. Además, hemos seleccionado los diferentes aislamientos para llevar a cabo la secuenciación completa del genoma buscando diferencias y variabilidad de los diferentes genes del reovirus.

INTRODUCTION

Avian reovirus (ARV) is the main cause of viral arthritis and tenosynovitis in chickens and turkeys (1, 2), triggering economic losses in the poultry industry due to impaired feed conversion rates, lack of uniformity and increased condemnations in the affected flocks worldwide. ARV's are non-enveloped viruses. Its genetic material is formed by ten segments of double-stranded RNA. These features allow a high mutation and recombination rates, escaping the immunity elicited by vaccines. Classical vaccine strains such as S1133, 1733 and 2408, have been used since the 1970's and molecular variants homologies to these vaccines are frequently under 80%.

For years, molecular variants have been detected and classified into five (3, 4) and six (5, 6, 7) genotypic clusters based on the S1 gene, that encodes for the Sigma C protein. Nevertheless, we know that characterizations based on S1 don't provide the full picture and do not correlate with antigenicity and pathogenicity of these strains is a quick and easy method to group these isolates. Since the viruses incorporated in autogenous vaccines are selected, in part, based on S1 characterization, it is necessary to reconsider the approach. Previous studies in our laboratory [8,9] have suggested that other genes i.e. M2 and L3, coding for outer structural proteins, have a high variability and would be good candidates for classification of molecular variants of ARV and might be associated with pathogenicity and antigenicity. Since 2015 we have started a molecular characterization effort based on a S1 characterization and grouping of the isolates obtained by the California Animal Health and Food Safety Laboratory. In addition, we have selected distinct isolates to perform full genome sequencing looking for differences and variability of the different reovirus genes.

MATERIALS AND METHODS

One hundred and fifty reovirus isolates were selected based on clinical importance, year, tissue of isolation and cytopathic effect in cell culture (CPE). Confirmed isolates were submitted to an S1 gene RT-PCR and sequencing using the forward and reverse primers (3). The resulting sequences were aligned with the commercial vaccine S1133 in order to compare homologies. Phylogenetic trees were crafted in order to visualize the classification of the ARV molecular variants. Seventeen representative isolates were selected and whole genome sequenced and classified in order to check gene associations. Statistical data analysis and graphs were made using PRISM Graph Pad.

RESULTS

Eighty-five out of 150 (56,6%) ARV S1 segment (1,088 bp) were effectively RT-PCR amplified, sequenced and classified into six genotypic clusters (GC). In 2016 the GC1 strains were predominant with approximately 80% of the variants for that year. In 2017, CG6 increased it representation with 31% of the sequences while GC1 was still the most predominant with 40% (Figure 1). The average homology of each GC to S1133 was calculated and shown in table 1. The percent homologies to S1133 of the S1 sequences by year showed consistency. GC1 was the group with the highest homology to S1133 while GC4, GC5 and GC6 the groups with the lowest homologies. In order to assess the similarity of the S1 sequences within each cluster we calculated the homology within GC. From high to low, these homologies were GC1 (96.4%),

GC2 (77.5%), GC4 (77.0%), GC5 (97.7%) and GC6 (94.8%) (Table 2). A summary of the sequence distribution on the different genetic clusters and their homologies by year are shown in Figure 2. A considerable reduction of sequences clustered in GC1 was followed by an increase in GC2, GC4 and GC6. GC3 was first identified in 2016, GC5 was first identified in 2017. Seventeen ARV isolates associated with clinical signs in the field, were selected for full genome and serotype studies. These results will be presented at the meeting.

DISCUSSION

Molecular surveillance is crucial to control and prevent pathogenic ARV outbreaks. Using the primers described by Kant *et al*. (3), only 85 out of 150 (56.6%) partial S1 genes were detected, amplified and sequenced. We attribute the lack of amplification of more than 30% partially to the molecular divergence on ARV variant strains. Kant *et al*., using the same primers between 1980 to 2000, had a slightly higher amplification success (70%) (3). Partial S1 gene characterization methods have classified ARV strains into five (3, 4) and six (5, 6, 7) genotypic clusters. Our research showed that strains isolated in California belong to all six distinct genotypic clusters. The most predominant clusters were GC1 (51.8%), GC6 (24.7%) and GC2 (12.9%). Similar results were described in Europe (3). While Lu *et al*. (5) stated that most of their sequences clustered in GC2, followed by GC4 and GC1, Palomino *et al*. (7) affirmed that their most predominant sequences were from GC5, followed by GC4 and GC1. The "cluster" nomenclature is used to compare the viruses detected in different parts of the country and the world even though, we need to take into consideration the fragment size, number of sequences in the analysis, sequences selected as backbone and the subjectivity of the analysis. These factors play a role in the conformation of the clusters. Between 2015 and 2018, the ARV isolates genotypic cluster representation in the State of California has changed. A decrease on the representation of GC1 and an increase of GC6 classified strains have occurred (Table 2). Multiple factors might be influencing this shift, including the use of autogenous vaccines. The use of certain GC's as a predominant antigen in autogenous vaccines might be important in driving the change in the representation of that genetic cluster. Our hypothesis relies in the fact that inactivated non-homologous vaccines provide partial protection to the field challenge not eliminating viral shedding from infected birds. This allows selection of strains different than the vaccine altering the representation of ARV's in the environment. Homologies to a reference commercial vaccine strain showed that GC1 had the highest homology. Even though, GC1 is the group that encompasses the vaccine strains, the average homology of this group was under 78%. The rest of the GCs had average homologies to S1133 between 58.6 and 53.2%, very distant from the viruses that are used in commercial live and inactivated vaccines explaining the lack of effectiveness of these vaccines in the generation of protection. Based on the homologies over time, we see that each of the clusters have maintained their homologies to S1133 since 2016 (Figure 2). The results on the whole genome sequencing of seventeen selected isolates will be discussed during the meeting.

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Figure 1. Phylogenetic tree depicting 85 ARV S1 sequences (883 bp). Sequences were obtained from reoviruses isolated from tenosynovitis cases in CA between 2015 and 2018. The backbone sequences (black) were obtained from other authors. Sequences were grouped into six genotypic clusters (GC). Commercial vaccine strains are labeled by asterisks.

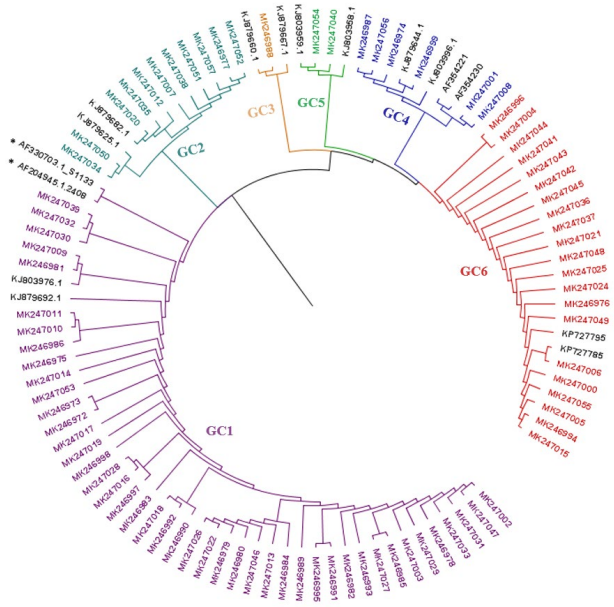


Table 1. Sequence frequencies by genotypic cluster (GC) and year from 2015 to 2018, arithmetic sum and percentage of the total sequences by genotypic cluster (ND= non-detected).

Genotypic cluster	Total sequences by year				Sum	Total (%)
	2015	2016	2017	2018		
GC1	2	23	18	1	44	51.8
GC2	ND	2	5	4	11	12.9
GC3	ND	1	ND	ND	1	1.2
GC4	ND	2	2	2	6	7.1
GC5	ND	ND	2	ND	2	2.4
GC6	ND	2	15	4	21	24.7

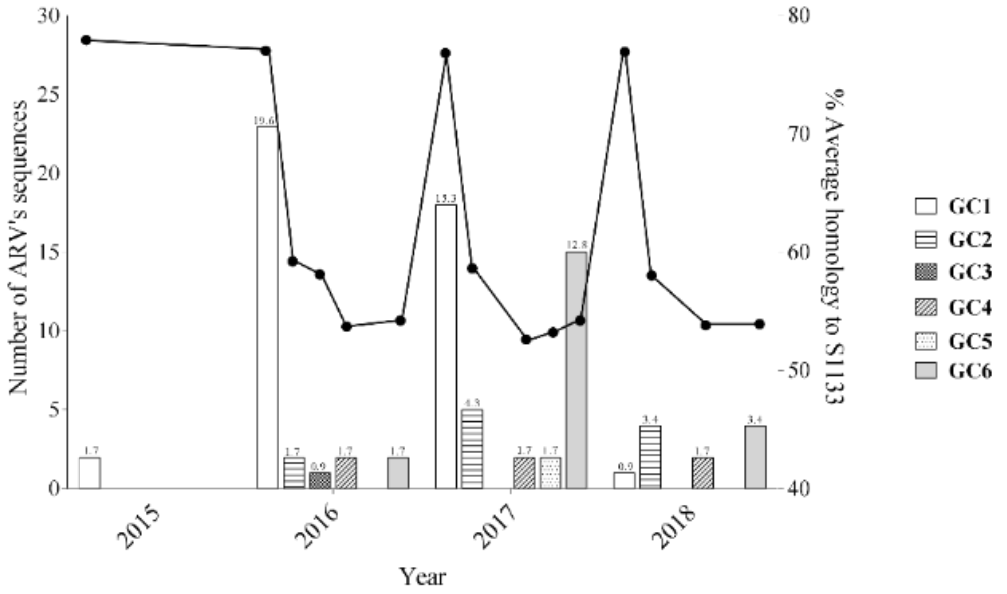
ND = not detected

Table 2. Average homologies to S1133 of each of the genotypic clusters by year and homologies within GC (ND= non-detected).

Genotypic cluster	Homology to S1133 (%)				Total Average (%)	Homology within GC (%)
	2015	2016	2017	2018		
GC1	77.9	77.0	76.8	76.9	77.2	96.4
GC2	ND	59.2	58.6	58.0	58.6	77.5
GC3	ND	58.1	ND	ND	58.1	ND
GC4	ND	53.7	52.6	53.8	53.4	77.0
GC5	ND	ND	53.2	ND	53.2	97.7
GC6	ND	54.2	54.2	53.9	54.1	94.8

ND = not detected

Figure 2. Frequencies and average homologies based on 85 ARV S1 sequences (883 bp) obtained from reovirus (ARV) isolates from tenosynovitis clinical cases. Bars are showing the isolate frequencies in each Genotypic cluster (GC) per year. Numbers above bars represent the percentage (%) from the total samples (85). The bold line represents the average homology to S1133 based on the S1 sequences. GC (genotypic clusters).



UNIQUE *EIMERIA* FROM NON-COMMERCIAL CHICKENS

EIMERIA DISTINTIVAS DE POLLOS NO-COMERCIALES

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RESUMEN

Recientemente, la coccidia que parecía morfológicamente como una Eimeria conocida de pollos comerciales se ha observado en poblaciones no-comerciales. La Eimeria de pollos no comerciales mostró patrones similares a la coccidia de los pollos comerciales en lo que se refiere a la patogenicidad y

susceptibilidad a los fármacos anticoccidianos. El aislamiento de *E. tenella* pareció ser totalmente controlado usando una vacuna viva de coccidia comercial.

SUMMARY

Recently, coccidia that appeared morphologically like known Eimeria from commercial chickens have been observed in non-commercial populations. The Eimeria from non-commercial chickens showed similar patterns to the coccidia from commercial chickens concerning pathogenicity and susceptibility to anticoccidial drugs. The *E. tenella* isolate appeared to be fully controlled using a commercial live coccidia vaccine.

INTRODUCTION

The commercialization of poultry has made meat protein more affordable for many. At the same time, raising birds was a time honored-activity that provided food and other forms of entertainment. Today, noncommercial or backyard poultry production is growing in popularity, with backyard owners keeping birds for pleasure, meat and eggs. As this trend and the number of animals increase, there is a need for better knowledge of the animal care, welfare and diseases. Enteric diseases such as coccidiosis is not only associated with chickens in commercial environments. The eradication or elimination of coccidia is challenging, primarily due to the hard oocyst protective wall and relatively high fecundity of the organism. These parasites invade the intestines and ceca of chickens of all ages unless the animals are protected via medication or immunity.

MATERIALS AND METHODS

Small scale non-commercial chicken operators noticing unthrifty birds or dead birds sought professional help to obtain answers or explanation for the state of their animals. Day of age commercial broiler chickens were placed in wire-floor cages and provided water and feed *ad libitum* and provided supplemental heat.

Coccidia isolates were expanded via oral inoculation of 0.5 mL per bird. Fecal droppings for each isolate was checked for oocysts started at 85 h post inoculated (pi) and checked regularly to determine first presence of oocysts.

In another test, the coccidia isolated and prepared for sporulation were inoculated *per os* into eight day of age coccidia naïve chickens. These birds were medicated with one of eight anticoccidials; medicated feed was provided 36 h prior to inoculation and each bird was given 5,000 sporulated oocysts. Birds that were hyper-immunized with a commercial live coccidia vaccine were each challenged with 5,000 sporulated oocysts to determine the level of protection against that isolate.

RESULTS

The *Eimeria* noticed in the samples were *E. necatrix, mivati, maxima, tenella*, and *mitis*. Several isolates were evaluated to determine the pathogenicity. These isolates from non-commercial sources showed relative pathogenicity of which was comparable to organisms from commercial sources. One of the *E. tenella* from the non-commercial group caused 20% mortality in an inoculated, non-medicated chickens. Mortality occurred at approximately 123 h post inoculation. Severe parasitemia was noticed in the ceca and rectum. Of the drugs tested, only one provided good efficacy, three were moderate and four provided marginal to poor efficacy. Birds that were hyper-immunized and challenged with the *E. tenella* isolate were 100% protected.

DISCUSSION

These results show that coccidia from non-commercial chickens may be pathogenic and did not show complete susceptibility to anticoccidials commonly used commercial poultry. Based on the findings, *E. tenella* from a commercial coccidia vaccine appeared to be fully protective against non-commercial isolate.

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Table 1. The impact of three lesser species of coccidia in young chickens.

Eimeria	Growth(g)	% Growth
acervulina/mivati	101	46
Mivati	116	53
mitis-1	139	63
mitis-2	130	59
Control	220	100

COCCIDIOSIS CONTROL: ANTICOCCIDIALS, BIOLOGICS, OR BOTH

CONTROL DE LA COCCIDIOSIS: ANTICOCCIDIANOS, BIOLOGICOS, O AMBOS

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RESUMEN

La coccidiosis es una enfermedad importante en la producción avícola comercial, millones de doláres se gastan anualmente para el control y la prevención. El control de la coccidiosis es esencial vía inmunidad protectora, anticoccidianos (AC) o más recientemente usando una vacuna más un AC (bio-dual). Los datos de las pruebas de sensibilidad anticoccidiana (AST, por sus siglas en inglés) apoyan la disminución en la eficacia de varios medicamentos. Usando las vacunas vivas de coccidiosis en rotación con los anticoccidianos parece que ha ayudado a proteger la eficacia de los fármacos, pero con el uso del programa bio-dual podría fracasar este propósito.

SUMMARY

Coccidiosis is an important disease in commercial poultry production, millions of dollars are spent annually for the control and prevention. Controlling coccidiosis is essential via protective immunity, Anticoccidials (AC) or more recently using a vaccine plus an AC (bio-shuttle). The anticoccidial sensitivity testing (AST) data supported the decline in the efficacy of several drugs. Using live coccidiosis vaccines in rotation with anticoccidials appeared to have spared the efficacy of the drugs, but with the use of the bio-shuttle might be defeating that purpose.

INTRODUCTION

Coccidia are generally present in almost all poultry-producing facilities and could cause significant threat to the health and or productivity of the animals. Dependent on the species of coccidia, a single oocyst ingested can result in approximately 900,000 new oocysts. Coccidiosis control is paramount in maintaining bird health and productivity. This control is achieved via the use of in-feed anticoccidial drugs (AC) or biologics. In recent times, a third option has been practiced, use of an AC in conjunction with the live coccidia vaccine. This practice is known as a “Bio-shuttle”

MATERIALS AND METHODS

Broiler chickens 10-12 days of age were used in these experiments and grown in metal cages. Prior to the testing period, all birds were placed on un-medicated broiler ration consisting of approximately 20-22% protein. Birds were randomized and placed into experimental pens 36-48 h prior into inoculation, then assigned their respective treatments. All studies were terminated on days six or seven post infection (PI), dependent on the predominant *Eimeria* species.

Eimeria species obtained from commercial poultry farms; litter or fecal samples or intestinal tracts were submitted. Each coccidia isolate was expanded in to coccidia naïve week of age chickens and fed un-medicated feed. The levels for each isolate in the test phases varied based on the types of organism present. The treatment groups were un-medicated feed and inoculated with coccidia or fed medicated feed and given coccidia. Each medication was provided at the recommended levels. Index score (anticoccidial index – ACI) was determined using growth, gross lesions, microscopic parasitism and livability. Each anticoccidial was ranked based on the ACI score for each isolate. (Scale: ≤ 30 = fail; 31-40 = poor, 41-50 = fair, 51-70 = marginal, 71-85 = good and ≥ 86 = very good).

RESULTS

The current data has shown that the decline in anticoccidial is still occurring. The group that has the lowest ACI was the group in which vaccines were not used. The group that had the highest ACI was the group in which only LCV was used. The group with the intermediate ACI was the group that had used a vaccine in conjunction with an AC, Table 1. The current data agrees with the previous findings of Mathis and Broussard; the use of a vaccine allows a

replacement of drug resistant coccidia with sensitive organisms.

DISCUSSION

The data showed erosion in the effectiveness of the commonly used drugs. However, in using vaccines the trend is moving in the direction of improved efficacy. The pattern noticed with the use of the vaccine along with the drug appeared to be encouraging a reversal to being less effective.

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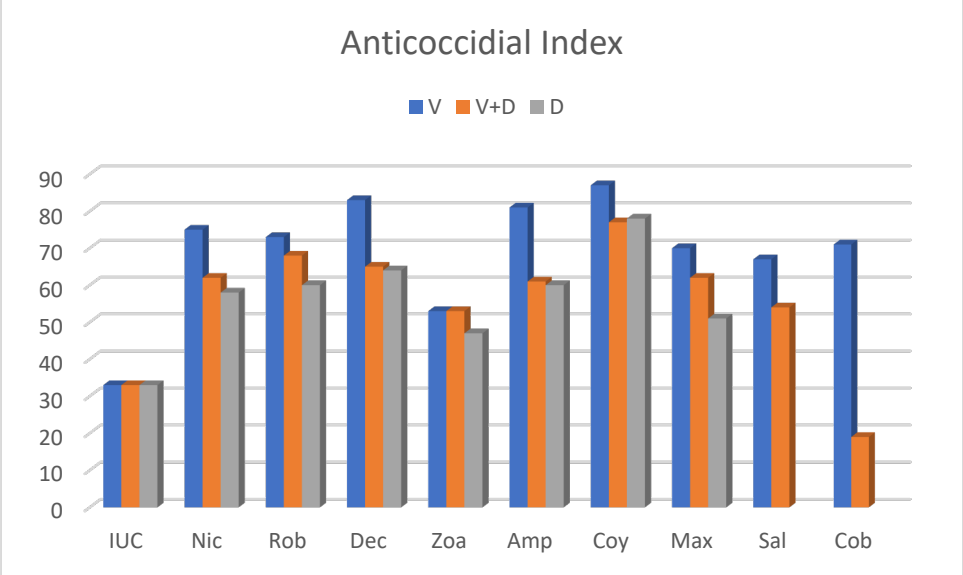
Figure 1. Summary of several trial to assess anticoccidials effectiveness.



PRODUCTIVE RESPONSE OF LAYING HEN TO THE APPLICATION OF TILVALOSINA 3AT

RESPUESTA PRODUCTIVA DE LA GALLINA DE POSTURA PARA LA APLICACIÓN DE TILVALOSINA 3AT

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RESUMEN

Para poder evaluar la tilvalosina 3AT en gallinas de primer ciclo, se llevó a cabo un experimento. Se usaron 1056 gallinas de postura Bovans con 19 semanas de edad, que se alojaron en una caseta con ambiente natural en aulas de 3 niveles. Las aves se dividieron en 2 tratamientos: tratamiento 1.- Pulsos mensuales de la medicación por una semana a una dosis de 75 ppm de tilvalosina 3AT + 300 ppm de

clortetraciclina. Tratamiento 2.- El programa usual de la granja. Cada tratamiento tenía 11 repeticiones de 48 aves cada uno. Las variables que se evaluaron durante la fase de experimentación fueron la producción de huevo, el promedio del peso del huevo, el consumo de alimento por día, la conversión alimenticia, la masa de huevo por día, el porcentaje del huevo roto y sucio, y la mortalidad semanal. Al final del período experimental, el número de huevos acumulados por gallina alojada, los kilogramos de huevo por ave alojada, la mortalidad semanal. Al final del periodo experimental, se midieron el número de huevos acumulados por gallina alojada, los kilogramos de huevos por gallina alojada, la mortalidad acumulada y la conversión acumulada. Los resultados delas variables fueron sometidos a un análisis de regresión tomando como indicador las variables del tratamiento. Los resultados obtenidos en 51 semanas de experimentación demostraron que el uso de la tilvalosina 3AT + clortetraciclina mejoró la producción de huevo en un 2.8%, así que el número de piezas por ave en un 14.8 %. La conversión alimenticia acumulada disminuyó en 170 g por kilogramo producido. Así como 9.5 piezas de huevo por ave alojada se requirió para el pago de tratamiento para un ciclo de producción de 51 semanas (P <0.01).

SUMMARY

In order to evaluate tilavalosin 3AT in first cycle hens, an experiment was carried out. We used 1056 laying hen of Bovans stock with 19 weeks of age, which were housed in a natural environment house in cages of 3 levels. The birds were divided into two treatments: treatment 1.- Monthly pulses of medication for one week at a dose of 75 ppm of tylvalosin 3AT + 300 ppm of chlortetracycline. Treatment 2.- The usual program of the farm. Each treatment had 11 repetitions of 48 birds each. The variables that were evaluated during the experimentation phase were egg production, average weight of egg, consumption of bird feed per day, feed conversion, egg mass per day, percentage of broken and dirty egg, weekly mortality. At the end of the experimental period, the number of eggs accumulated per packaged hen, kilograms of eggs per bird encased, cumulative mortality and cumulative conversion was measured. The results of the variables were subjected to a regression analysis taking as indicator variables the treatments. The results obtained in 51 weeks of experimentation showed that the use of tylvalosin 3AT + chlortetracycline improved egg production by 2.8%, as well as the number of pieces per bird in 14.8. The accumulated feed conversion decreased by 170g per kilogram produced. As well as 9.5 pieces of egg per perched bird are required to pay the treatment for a production cycle of 51 weeks (P <0.01).

INTRODUCTION

Tilvalosin 3AT is a second-generation macrolide antibiotic, based on a very active lactone ring. It has both bacteriostatic and bactericidal activity as well as having the additional benefit of the antimicrobial activity of the main metabolite 3-AT against Gram-positive and some Gram-negative organisms and against *Mycoplasma* acts to inhibit protein synthesis in the bacterial cell reaching relatively high intracellular concentrations in phagocytic cells (macrophages and neutrophils), likewise it can specifically increase the activity of macrophages, helping the innate (non-specific) immune system to eliminate foreign particles, such as pathogens. It is used in the treatment of respiratory diseases associated with mycoplasmosis (*Mycoplasma gallisepticum*, *M. sinoviae*, Mg and Ms respectively), ORT and enteritis caused by *Clostridium perfringens*. With this background, the objective of this work was to evaluate the productive response of the hen laying in the first cycle to the addition of pulse of Tilvalosin 3AT in high challenge conditions.

MATERIALS AND METHODS

The test was carried out in a commercial farm in Tepatitlán de Morelos Jalisco, which has raised three-level booths. The vaccination schedule was a high challenge of pathogens that include: Newcastle, avian influenza serotype H5 and H7, infectious coryza, laryngotracheitis, infectious bronchitis, EDS, Gumboro, smallpox, reovirus, and hepatitis C7.

We used 1056 birds of the Bovans White line, of 19 weeks of age which were divided into two treatments, each treatment had 11 replications and each replica was 48 birds each. All the birds had the same management and feeding that was carried out on the farm.

The treatments were:

Treatment 1 was using the pulses program of tylvalosin medication, which consisted of monthly pulses throughout the production phase for a week at a dose of 75 ppm + 300 ppm chlorhetracycline.

Treatment 2 was the usual antimycoplasmic program of the farm which is directed when respiratory signs are present.

During the 51 weeks of experimentation, the percentage of production, the weight of the egg (g) was calculated, the egg mass was calculated day (g), in addition the percentage of dirty, broken egg was calculated, the food consumption was measured bird / day (g) to calculate the weekly and cumulative feed

conversion. Mortality was also recorded weekly. At the end of the test, the cumulative conversion, the number of eggs per chicken housed, the kg of egg accumulated per bird, the cumulative conversion and the cumulative mortality were calculated. To the variables, a multiple linear regression analysis was carried out, having as independent variable the weeks of age of the hen and as an indicator variable to the treatments.

RESULTS

The number of eggs during the period evaluated (Table 1) was observed, 14.8 pieces per hen lodged in the group treated with the monthly pulses (247.9pza) which was reflected in the egg kg per bird encasetada in 830g more (14,513 kg) than the control birds (233.1 pza and 13,683 kg) ($P < 0.05$), in addition to decreasing the feed conversion in the whole period studied (2,471 vs 2,641 kg: kg) ($P < 0.05$). The variable egg mass per bird / day, bird / day feed consumption, cumulative feed consumption, weekly feed conversion, percentage of dirty egg, broken, weekly and cumulative mortality percentage, no statistically significant difference was found between any of the treatments employees ($P > 0.05$). For the resistance of the shell and the internal quality of the egg evaluated at 56 weeks of age (Table 1), no significant difference was found in favor with the use of the monthly pulses of Tilvalosina 3AT + Chlortetracycline in the laying hen. ($P > 0.05$). There was a tendency to have greater egg production in the birds that were treated with the monthly pulses of Tilvalosina 3AT and Chlortetracycline (70.3%) and a lower average egg weight (56.4g) with respect to the control diet (67.5% and 56.9g respectively) ($P < 0.09$).

During the 51 weeks of experimentation to treatment 1, 10 pulses were applied with a total cost of $ 7140.0, and to treatment 2, 3 treatments against mycoplasmosis were applied with a total cost of $ 1,110.0.

Finally, using the data in Table 1, the cost per treatment per hen was calculated, resulting in a cost of $ 11.01, considering the market price of the egg kg at $ 19.0 and that one kg of egg contains 16.4 pieces that required 9.5 eggs per egg. bird to pay for all the treatment with the monthly pulses of Tilvalosina 3AT + Chlortetracycline resulting in a net gain of 5.3 eggs per hen enrolled From the results obtained in 51 weeks of experimentation it can be concluded that the use of monthly pulses of Tilvalosina 3AT + Chlortetracycline, increased the percentage of production in 2.8%, the kilograms of egg in 0.83 kg and the number of pieces in 14.8 eggs. The average egg weight decreased by 0.5g and the cumulative feed conversion by 170g per kilogram of egg produced.

The use of the monthly pulses of Tilvalosina 3AT + Chlortetracycline did not show any benefit over the egg mass per day, the consumption of food, the percentage of broken egg, dirty, or weekly mortality. 9.5 pieces of egg per bird are required to pay for all the treatment for a production cycle of 51 weeks.

Table 1. Results of the productive variables obtained in 51 weeks of production of hens treated with monthly pulses of Tilvalosin + chlortetracycline.

	Tilvalosin + chlortetracycline	Control	SEM
Egg production, %*	70.3[a]	67.5[b]	3.6
Egg weight, g *	56.4[b]	56.9[a]	0.61
Egg mass bird / day, g	41.1	39.7	2.44
Consumption of bird feed / day, g	98.9	98.7	0.9
Weekly feed conversion per bird, kg: kg	4.368	4.690	1.365
Accumulated feed / bird consumption, kg	35.927	35.869	0.357
Conversion accumulated per bird, kg: kg	2.471[b]	2.641[a]	0.152
Eggs per bird, kg	14.513[a]	13.683[b]	0.93
Eggs per bird, pieces	247.9[a]	233.1[b]	14.9
Dirty egg,%	6.25	6.23	0.86
Cracked egg, %	1.84	2.34	0.75
Weekly mortality,%	0.14	0.21	0.10
Cumulative mortality,%	7.51	11.0	5.55
Shell resistance, g / cm^2 (56 weeks)	3989.8	3863.4	383.3
Yolk color with DSM fan (56 weeks)	10	9	0.5
Haugh Units (56 weeks)	87.3	87.3	3.48
Price / kg feed, $	5.581	5.275**	NA
Production cost / kg feed, $	13.79	13.93	NA

SEM = standard error of the mean.
* p <0.09
** $ 60.0 / ton of the 3 treatments with tylosin + chlortetracycline is contemplated
a, b Show statistically significant difference in row (P <0.05)

WOODEN BREAST IN COMMERCIAL BROILERS IS ASSOCIATED WITH MORTALITY, "TURTLE BIRDS," AND PULMONARY DISEASE

LA PECHUGA DE MADERA EN POLLOS DE ENGORADA COMERCIALES ESTÁ ASOCIADA CON MORTALIDAD, "AVES TORTUGA", Y ENFERMEDADES PULMONARES

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RESUMEN

Las miopatías, la cuales afectan negativamente la calidad de la carne, se han incrementado (12). Entre estas, la "Pechuga de Madera" (PM) ha aparecido a nivel mundial como una enfermedad muscular económicamente significativa (13). El musculo pectoral afectado se endurece, se ve gomoso, y más pálido de lo normal (12). Histológicamente, se encuentra degeneración muscular asociada con

regeneración polifásica, flebitis linfocítica, y expansión y fibrosis de los espacios intersticiales (11). El musculo dañado en los pollos de engorda modernos está relacionado con al rendimiento muscular y el rápido crecimiento (4). Ya que ambos factores están genéticamente programados (5) estos también son influenciados por el manejo y el ambiente (3).

Este estudio, se les hizo la necropsia a los pollos de engorda comercial, criadas para la enseñanza, desechadas o muertas durante los 16 días del periodo de crecimiento. En el mismo periodo se presentaron "aves tortuga" sin signos clínicos o con problemas respiratorios leves y las aves que murieron de espaldas. Los hallazgos presentados en el reporte de este estudio mostraron que la mortalidad tardía en la parvada es más evidente en machos pesados con pechuga de madera, esto está asociado con las aves tortuga y enfermedades pulmonares, y la pechuga de madera tiene un impacto mucho mayor sobre la parvada que solo la calidad de la carne.

SUMMARY

Myopathies, which adversely affect meat quality, have increased (12). Among these, "Wooden Breast" (WB) has emerged worldwide as an economically significant muscle disease (13). Affected breast muscle is hardened, rubbery, and paler than normal (12). Histologically, degeneration of muscle cells associated with polyphasic regeneration, lymphocytic phlebitis, and expansion and fibrosis of interstitial spaces are seen (11). Muscle damage in modern broiler chickens is related to muscle yield and rapid growth (4). While both factors are genetically programmed (5) they can also be influenced by management and environment (3).

To our knowledge, the negative concerns surrounding WB have been those of meat quality (8) and economic losses due to rejection of affected breast meat by the consumer (7). Lack of an efficient method to detect WB in live birds (6), may explain why no studies have been conducted on the association of WB with clinical disease or mortality.

No recent study on the causes of mortality in broiler chickens in commercial flocks is available.

A recognized cause of mortality in fast growing broilers found dead on their backs is sudden death syndrome (SDS) or "flip-over". Heavy, healthy-looking males are most frequently affected. At necropsy, they show full gastrointestinal tracts, slightly enlarged livers and kidneys, and occasional subperitoneal renal hemorrhages. Other lesions include enlargement of the left ventricle and pulmonary congestion and edema (10). The physiological mechanism causing SDS is not fully understood even though the syndrome has been well described. Affected birds suddenly squeak, extend their neck, flap their wings, lose their balance, and flip over onto their backs (1). Death occurs within a few minutes. Prior to death, no clinical signs are seen (9).

In this study, cull and dead birds during the last 16 days of the growing period of a commercial broiler chicken flock raised for teaching were necropsied. Increased mortality in heavy males that had wooden breast and concurrent pulmonary disease was found. Turtle birds without clinical signs or with mild to moderate respiratory distress and birds that died on their backs occurred during the same time period. Findings presented in this case report show that late flock mortality is mostly heavy males with wooden breast, wooden breast is associated with turtle birds and pulmonary disease, and wooden breast has a much greater impact on flocks than just meat quality.

CASE REPORT

Case history. Mortality between days 40 to 56 (last 16 days of the growing period) in a broiler flock raised in the Teaching Animal Unit (TAU) at North Carolina State University College of Veterinary Medicine for teaching purposes is reported. The flock consisted of 4000 as-hatched Ross 708 broilers grown on contract for a local integrator. Birds were placed on the day of hatch and processed at 56 days. They were housed on pine shavings in a 5472 square feet curtain-sided barn equipped with radiant brooders, forced air furnaces, and paired feed and water lines.

The flock was checked at least twice daily for mortality (cull and dead birds) and birds that were on their backs and unable to right themselves ("turtle birds") were turned over. Finding dead birds on their backs was recorded for 22 birds. Position of other dead birds was either not recorded or they were not on their backs. Mortality was necropsied to determine the most probable cause of death or reason for culling. Muscle samples were taken from the left *pectoralis major* muscle of each bird. Lungs were fixed *in situ* by removing the heart and separating the thoracic cavity from the rest of the carcass without disturbing the lungs and fixing the entire sample in NBF. Following fixation, lungs were carefully removed, trimmed, and processed for histopathology as described for muscle samples.

Clinical Findings. Total flock mortality was 174 birds (61 culls, 113 dead; 4.35%). Mortality during the last 16 days was 63 (14 culls, 49 dead; 1.6% of flock; 36% of total mortality).

Beginning in week 2, dead birds that lacked gross lesions were found on their backs. These were diagnosed as "flip overs". In contrast, most birds found on their backs during the last 16 days were alive. A few of these showed mild to moderate dyspnea, but

most did not show any signs of distress. They tended to be bright, alert, and responsive, but were unable to right themselves. When righted, they were able to walk, but some continued to show variable degrees of respiratory distress, which gradually returned to normal. These birds were called "turtle birds", as they were unable to right themselves after accidentally being turned onto their backs (Fig. 1).

Gross pathology. The majority (73%) of birds that died during the last 16 days were large males. Fifty-six percent of the birds that died had severe pulmonary disease (congestion and edema). Sixteen percent of the birds had cardiac lesions including excess pericardial fluid or an enlarged right heart. Seventy percent of the birds that died had marked hardness of the *pectoralis major* muscles typical of WB (Fig. 2). Twenty-one of 22 birds recorded as being found dead on their backs had concurrent WB and pulmonary disease.

Histopathology. Muscles were scored as normal (score 0) or having (score 1) lesions typical of WB (myofiber degeneration and regeneration, expansion of interstitial spaces, fibrosis, necrosis) (Fig. 3). Muscles with lesser degrees of broiler breast myopathy were also scored 0, as these would not be identified as WB at processing. Lungs were scored based on airway patency as normal (score 0) or having pulmonary disease (congestion, edema, pneumonia, score 1) (Fig. 4).

Forty-three of the 63 broilers (68.3%) that died or were culled (38 of 49 dead broilers, [77.5%] and 5 of 14 culled broilers [35.7%]) had concurrent WB and pulmonary disease. Fifteen broilers did not have either WB or pulmonary disease. The remaining 5 broilers had either WB or pulmonary disease.

Statistical findings. Data were evaluated using a nominal logistic regression to express the relationship between WB and pulmonary disease, groups, and sex in the birds necropsied during the last 16 days of the growing period. Pulmonary disease was highly correlated with WB ($p<0.0001$), WB increased significantly over time ($p = 0.0018$), and sex correlated with WB (p value=0.0456). A Student's t-test was used to compare the weight of turtle birds to the targeted weight given by the 2014 Aviagen Performance Manual (http://en.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-708-Broiler-PO-2014-EN.pdf) according to the age and sex of the bird. Turtle birds were significantly heavier than the target values ($p =0.002592$).

DISCUSSION

The incidence and association of WB and pulmonary disease in broiler chickens during the late growing period were investigated in this study. Thirty-eight of 49 dead broilers (77.5%) and five of 14 culled broilers (35.7%) had concurrent WB and pulmonary disease. Except for a single bird, dead birds found on their backs were males that had both WB and pulmonary disease and were significantly heavier than target weights. These findings indicate that heavy birds with WB may accidentally fall over onto their backs and be unable to right themselves. The number of birds with both WB and pulmonary disease increased with the age of the flock as the birds gained weight.

A new syndrome, "turtle birds", was identified in which commercial broilers that were close to processing age and had WB accidentally fell onto their backs and were unable to right themselves. Some showed mild to moderate dyspnea but were otherwise bright, alert, and responsive. When turtle birds were found alive and righted, they stood up and walked away normally. If they had dyspnea, it gradually disappeared with time after the birds were upright. However, if not discovered in time, they probably died. Severe pulmonary congestion and edema and excess pericardial fluid at necropsy indicated death resulted from cardiopulmonary failure most likely from pressure on the heart and lungs by the weight of the breast.

SDS matches our necropsy findings as birds were on their backs and had pulmonary edema without other significant lesions (10). However, turtle birds found alive on their backs did not have SDS, as they were clinically normal. Birds that die from SDS have interstitial edema and degeneration of cardiac muscle (10).

Why birds with WB cannot right themselves when they are on their backs is unclear, but this is likely related to damage of the *pectoralis major* muscle. When placed on their backs, normal birds rapidly right themselves by elevating their wings and flipping over. Wing movement requires coordinated actions of both the *pectoralis major* muscles, which lower the wings, and *supracoracoideus* muscles, which elevate the wing. Both muscles act in concert with each other as agonist and antagonist – when one contracts, the other relaxes. As the *pectoralis major* is in a state of permanent contracture due to WB and cannot relax, contraction of the *supracoracoideus* to elevate the wings so the bird can right itself is ineffective and prevented from occurring. Inability of chickens with WB to raise their wings above horizontal has been seen in broilers during clinical evaluation of another flock. WB was confirmed histologically in the birds that could not raise their wings, but was not present in birds that could raise their wings normally (unpublished results). Ability to raise the wings was not determined for the birds in this

study, but should be evaluated in future clinical studies on WB.

Inability of a chicken to right itself also is a characteristic clinical finding in inherited muscular dystrophy in New Hampshire chickens (2). Affected birds cannot completely raise their wings and are also unable to right themselves. The possible relationship between WB and inherited muscular dystrophy also needs to be investigated.

To confirm WB is a predisposing factor for mortality in this flock, the prevalence of WB in the overall flock would be required. Currently, concerns about WB are limited to protein quality (8) and rejection by the consumer (7); however, the association between late mortality, turtle bird syndrome, WB, and pulmonary disease needs to be investigated further.

In conclusion, the prevalence of WB in late mortality in a conventional broiler chicken flock was assessed at necropsy. WB was significantly correlated with male birds and pulmonary disease. Almost all dead birds found on their backs were heavy males with concurrent WB and pulmonary disease. During the same period, turtle birds that were on their backs and unable to right themselves were seen in the flock, They tended to be clinically normal except for mild to moderate respiratory distress in some of them. These findings indicate that turtle birds are a consequence of WB, particularly in heavy males. Pulmonary congestion and edema characteristic of cardiopulmonary failure likely resulted from compression of the thorax by the heavy breast. Based on the findings in this report, the importance of WB is likely greater than just a problem with meat quality. Future studies are needed to confirm and extend these findings.

(The full length article will be published in *Avian Diseases*.)

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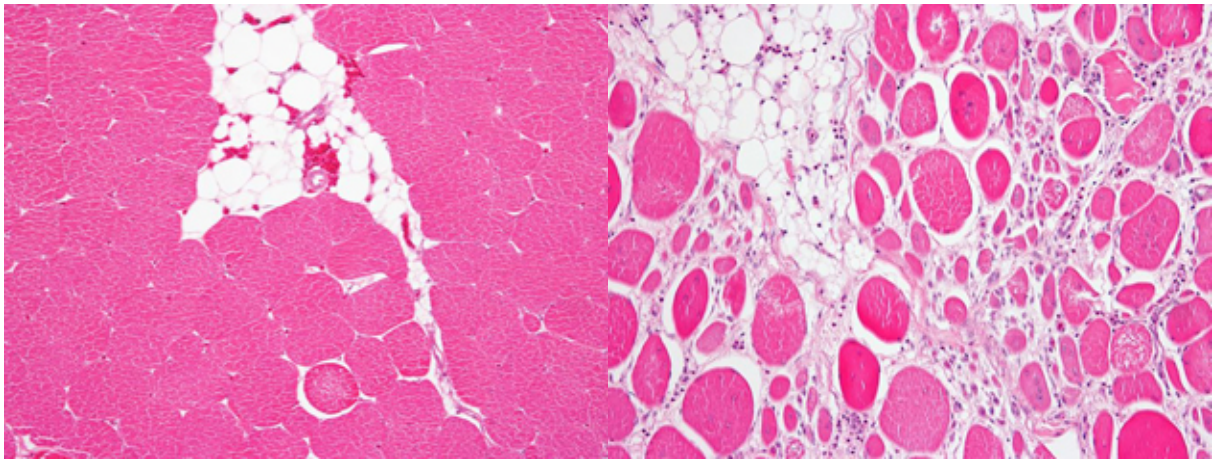
Figure 1: Turtle Bird. Broiler chicken is on its back and unable to right itself, but otherwise is bright, alert, and responsive. This bird is not showing dyspnea.

Figure 2. Wooden breast myopathy scores. A. Score 0. Normal muscle. Score 0 also included chickens with mild to moderate myopathy, as these would not be identified as wooden breast when processed. The interstitial spaces are almost invisible; muscle fibers have a polygonal shape and uniform eosinophilic color. B. Score 1. Wooden Breast. Severe muscle disease that would be rejected as wooden breast when processed. There is degeneration, regeneration, muscle cell atrophy and fiber splitting, and expansion and fibrosis of interstitial spaces.



INFECTIOUS CORYZA IN VACCINATED LAYERS: ARE VACCINES FAILING TO PROTECT?

CORIZA INFECCIOSA EN GALLINAS DE POSTURA VACUNADAS: ¿ESTAN FALLANDO LAS VACUNAS EN LA PROTECCION?

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RESUMEN

Durante el 2017 y 2018, en California se detectó coriza infeccioso en varias parvadas de gallina de postura comerciales vacunadas. Las cepas de *Avibacterium paragallinarum* aisladas de las gallinas infectadas fueron caracterizadas subsecuentemente como genotipo C y serotipo C-2. Este serovar y genotipo se incluyó en las vacunas que están disponibles comercialmente y que se usaron en las ponedoras comerciales en California. El objetivo de esta investigación fue determinar la eficacia de las vacunas comerciales y autógenas utilizadas actualmente para el control de *A. paragallinarum*, el agente causal de la Coriza Infecciosa. Las gallinas comerciales vacunadas con una vacuna comercial inactivada y una vacuna autógena, se desafiaron con *A. paragallinarum* utilizando diferentes protocolos de vacunación. El aislamiento de *A. paragallinarum* utilizado para el desafío fue seleccionado de una cepa de campo del 2017 recuperada de ponedoras infectadas durante el brote. Se colectó información sobre la enfermedad clínica, los signos respiratorios, la producción de huevo y la excreción bacteriana post infección.

INTRODUCTION

During 2017 and 2018 infectious coryza was detected in several commercial vaccinated egg layer flocks in California. The *Avibacterium paragallinarum* strains isolated from infected layers were subsequently characterized as genotype C and serotype C-2. This serovar and genotype is included in the vaccines that are commercially available and used in commercial layers in California. The objective of this investigation was to determine the efficacy of currently used commercial and autogenous vaccines for the control of *A. paragallinarum*, the causative agent of infectious coryza. Commercial hens vaccinated with an inactivated commercial and an autogenous vaccine, were challenged with *A. paragallinarum* using different vaccination protocols. The *A. paragallinarum* isolate utilized for challenge was selected from a 2017 field strain recovered from layers infected during the outbreak. Information was collected on clinical disease, respiratory signs, egg production and bacterial shedding post infection.

MATERIALS AND METHODS

Birds. One hundred and twenty, 21-week old leghorn HyLine W-36 hens were received at UC Davis poultry medicine research facility. Birds were divided in 12 groups of 10 hens each.

Pathogen and antibody screening. Blood samples were collected, and sera screened for the presence of antibodies against AI, IBD, IBV, MS, MG and NDV. Swabs were collected for the detection of AMPV, ILT, MG, MS, IBV and *A. paragallinarum (AP).*

Bacterial culture. A bacterial culture of *AP* was obtained from clinical cases isolated on blood agar streaked with *S. aureus.* Titration was performed using a qPCR.

Experimental design. 21-week-old hens were divided in 12 groups of 10 hens each. AP vaccines were applied at 6 and 10 weeks of age. Groups were administered either: one dose of autogenous bacterin, two doses of autogenous bacterin, one dose of the commercial bacterin, two doses of the commercial bacterin or one dose of commercial bacterin followed by a dose of autogenous bacterin and were subdivided into challenged and non-challenged groups. In addition, we evaluated non-vaccinated control groups, that were either challenged or non-challenged with *AP*. Challenge was performed at 22 weeks of age using *AP* with a titer of 5 x 10^3 cfu in 200uL. Fifty microliters of the inocula were applied on each eye and nostril. Choanal swabs were collected at 3- and 8-days

post challenge (dpc) to measure *AP* load. We evaluated daily egg production, clinical and respiratory signs. Birds were necropsied at 8 dpc. Gross pathology information was collected. Clinical signs were scored from 0 to 3: 0= no signs; 1= watery foamy eyes and or nasal exudate; 2= nasal discharge and swelling of the infraorbital sinus and 3= severe swelling with or without conjunctivitis and apathy (1). Respiratory signs were scored from 0 to 3: 0= no signs, 1= mild nasal sounds, 2= tracheal rales and 3= tracheal rales that can be heard from distance (3).

RESULTS

Before challenge, commercial hens were screened serologically for the presence of antibodies against pathogenic agents that could potentially influence the clinical outcome of the experimental challenge. Positive titers against IBD, IBV and NDV reflected the vaccination protocol administered to the hens. MS was positive to antibodies in 95% of the tested samples while MG was positive in 5%. There was molecular detection of ILT in 10% of the samples, MG in 3%, MS in 96%, IBV in 2.5%. All birds were *AP* negative before challenge.

While no statistical differences in egg production were detected, differences in clinical sign indexes were detected. The groups vaccinated with one dose of commercial bacterin followed by the autogenous vaccine recorded significantly reduced clinical signs ($P<0.05$) (Figure 1).

While bacterial load 3dpc did not show a reduction in bacterial shedding, at 8 dpc we detected a significant reduction in shedding in the group vaccinated with two doses of the commercial strain at 6 and 10 weeks of age ($P<0.05$), demonstrating the effectiveness of the commercial vaccine. A shedding reduction suggestive of significance was detected in the group of hens vaccinated with one dose commercial and two doses of the autogenous vaccine ($0.05<p< 0.01$) (Figure 2). Respiratory signs started two days post challenge and persisted for the whole experimental period. No differences were detected between the groups (vaccinated vs non-vaccinated challenged). Gross pathology was characterized by peritonitis (between 20-40% of birds) and perihepatitis (between 10 and 50%) in all vaccinated and non-vaccinated *AP* challenged groups.

DISCUSSION

Complex upper respiratory diseases are common in production settings. Prior to challenge, birds were antigenically positive for ILT, 19 weeks after the last ILT vaccination, and IBV, 12 weeks after the last vaccine. This illustrates a complex scenario in which vaccine viruses might be manifesting as a rolling reaction on affected premises. In our experiment, clinical signs support the complex scenario of the synergistic effect of co-challenges additional to the controlled coryza exposure. Bacterial load at 8 dpc demonstrated a neutralizing effect of the two-dose commercial vaccine application. This result correlates with the genotyping and serotyping of the bacteria that was characterized as a C genotype, serovar C-2 strain, the same serovar of *AP* included in the vaccine. Clinical signs described in the field were synonymous with complicated coryza cases that are manifested pathologically with septicemia lesions. Those cases are usually related to co-infection with a combination of pathogens and usually not only by a coryza challenge. Special attention needs to be taken to alternative systems where higher particulate matter and ammonia (2, 4) and higher gram-negative bacterial concentrations (5) have been detected. These changes in the environment might induce irritation and inflammation of the upper respiratory tract, changing the respiratory microbiota and ultimately facilitating co-infection with agents such as IBV, *AP*, and ILT thus generating a complex upper respiratory tract infection. The gross pathology seen 8 dpc is characteristic of a complex infectious coryza case.

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Figure 1. Clinical sign indices by group post infection with *Avibacterium paragallinarum.* *statistical significance.

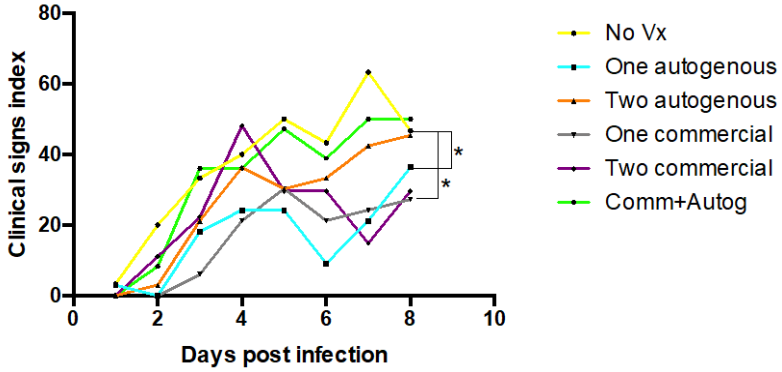
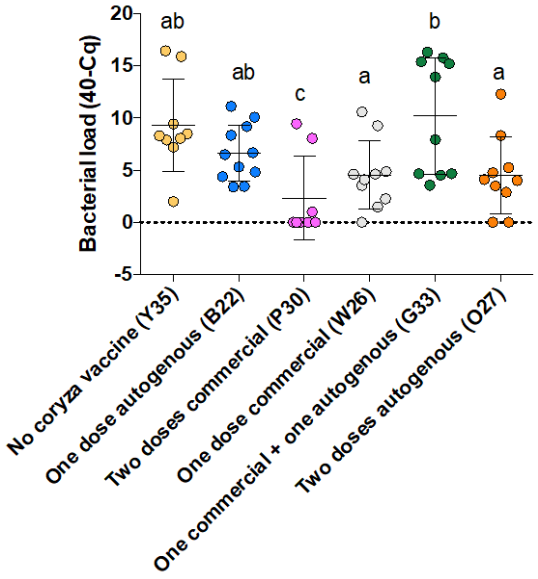


Figure 2. *Avibacterium paragallinarum* bacterial load (40-Cq) 8 dpc measured from infraorbital swabs. Superscript letters represent statistical significance.



APPLICATION OF A FOURTH-GENERATION AMMONIUM QUATERNARY THROUGH THERMO FOGGING AS A DISINFECTANT IN EMPTY FACILITIES AND IN PRESENCE OF LIVE ANIMALS

APLICACION DE UN CUATERNARIO DE AMONIO DE CUARTA GENERACION A TRAVES DE TERMONEBULIZACION COMO UN DESINFECTANTE EN INSTALACIONES VACIAS Y EN PRESENCIA DE ANIMALES VIVOS

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RESUMEN

Es esencial desinfectar las instalaciones avícolas entre cada ciclo de producción, y la posibilidad de aplicar productos directamente sobre los animales abre la posibilidad de incrementar las herramientas disponibles para mejorar el desempeño productivo de las parvadas. Los cuaternarios de amonio son moléculas altamente versátiles que permiten diversificar sus usos y son de gran ayuda en la industria avícola.

SUMMARY

Disinfecting poultry facilities between each production cycle is essential, and the possibility of applying products directly on animals opens up the possibility for increase the tools available to improve the productive performance of the flocks. The ammonium quaternaries are highly versatile molecules that allow to diversify their uses and are of great help in the poultry industry.

INTRODUCTION

The disinfection of facilities between production cycles is fundamental for the good start of the commercial flocks nowadays (2), considering the high presence of challenges to the health of the birds and the enormous variety of pathogens there is a great risk when this process is not carried out correctly.

In addition to the above, there is a high presence of challenges once the birds are installed in the houses, so that the decrease of the environmental pressure derived from pathogenic microorganisms is an important factor to consider in the current intensive productions.

There is a wide variety of disinfectants that can be used on inert surfaces, however, there are few molecules that can be applied in the presence of live animals without generating negative effects on the health and productivity of the birds; this is why the application of an ammonium quaternary (AQ) that does not generate these adverse effects is a reliable option (according to the provisions of Directive 98/8 / EC of the European Parliament and the Council of February 16, 1998 relative to the marketing of biocides.).

MATERIALS AND METHODS

A AQ of fourth generation to 48% concentration ready to thermo fogging will be used (no dilution required).
In empty facilities it will be applied 1 mL for each $3m^3$ of total volume.

With live animals it will be applied 1mL for each $5m^3$ of total volume of the chicken house.

For empty facilities, the disinfectant will be used after the removal of the poultry litter and the removal of organic matter (OM) residues by means of pressurized water, since the presence of OM affects the effectiveness of the disinfectants (3). Once dry, it was applied to the aforementioned dosage. The effectiveness evaluation of the disinfectant was done by means of sampling swabs; 3 (floor, curtain and feeder) were taken prior to washing and 3 after disinfection at the same spots.

With live animals will be applied five times, first immediately after the reception of the birds in the

chicken house and then at days 8, 15, 22, and 29 of age. The product will be used in a natural environment house with 15000 birds inside.

RESULTS

Disinfection of empty facilities. The results in the disinfection of the facilities are Table 1.

In the three sampled areas we can observe a practically total decrease of the CFU compared to the initial sampling, this shows us that the AQ is an effective molecule for the decrease of the bacterial loads of the houses, which helps us have a better start of the flocks that will be placed in these facilities.

It is worth mentioning that the TF quaternary cloud can reach places that are not normally disinfected, such as the spaces between the roof isolation of the chicken house, for example, and can also be applied to motors, sensors and parts of the equipment that cannot get wet.

Application with live animals. After performing five applications of the product by TF, a 2% reduction in the accumulated mortality rates at 35 days of life of the birds is reported in comparison with the untreated huts.

We need to mention that the initial TF was the one that caused the most disturbance in the behavior of the birds, but subsequent applications showed that they quickly adapted to the sound of the machine and the product cloud.

DISCUSSION

AQ have various uses, from the disinfection of fertile eggs (Brake and Sheldon, 1990) to the disinfection of large-scale installations of poultry facilities, and they are one of the most widely used molecules due to their high effectiveness, even in low concentrations (3).

With the application of the AQ without causing adverse effects on the animals and improving the productive parameters, the range of possibilities of the use of this molecule is extended even more, for example Ramesh et al (2002) evaluated various disinfectants in the transports for chicks of the incubator to the farm, with the quaternary efficiencies up to 92% in the elimination of *Salmonella* biofilms, without leaving negative residual effects in the birds, which has been confirmed with this evaluation.

Finally, the effectiveness of disinfectants is linked to several factors, as established by Stringfellow *et al*. (5) in a study that simulated various field conditions, in the case of this test was conducted under the best possible conditions, thus obtaining results favorable in the application on empty facilities, however at the time of applying it on live animals during the productive cycle we face a volatile panorama since variables cannot be controlled as in the previous situation, however the results were favorable and can be attributed to the reduction of environmental pressure that microorganisms normally exert on birds, with evaluations on a larger scale, we could more accurately find the benefits of this use of the quaternary, for example, its effect on daily weight gain since, in theory, by decreasing the presence of pathogens, the expenditure of resources that animals usually use to defend themselves can be used into an improvement of this and other parameters.

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Table 1. Results of disinfection of facilities.

Área	Initial CFU	Final CFU	% of reduction
Floor	2 400 000	16 700	99.3
Curtain	850 000	30	99.99
Feeder	190 000	1400	99.26

DRIED EGG ALBUMEN ADDITION IN MALE BROILER CHICKEN DIETS AND ITS IMPACT ON PRODUCTIVE PERFORMANCE

ADICION DE ALBUMINA DE HUEVO DESHIDRATADA EN DIETAS PARA POLLOS DE ENGORDA MACHOS Y SU IMPACTO SOBRE EL DESEMPEÑO PRODUCTIVO

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RESUMEN

El siguiente experimento se realizó con el fin de obtener información sobre el comportamiento productivo de pollos de engorda alimentados con dietas adicionadas con 5% de albúmina de huevo deshidratada durante la fase de pre-inicio (1-7 días). Se utilizaron 100 pollitos machos Ross 308 de un día de edad, los cuales fueron asignados al azar en dos tratamientos con 5 repeticiones (10 pollitos cada una), las aves se colocaron en jaulas de batería Petersime. Las dietas de pre-inicio fueron las siguientes: (T1) Dieta basal con base en sorgo y pasta de soya; (T2) dieta basal adicionada con 5% de albúmina de huevo deshidratada. Se evaluaron los parámetros productivos, el rendimiento de la canal y la alometría del tracto gastrointestinal. Los resultados no mostraron diferencias significativas (P>0.05) en los parámetros productivos ni en el rendimiento de la canal. Sin embargo, las aves alimentadas con albúmina mostraron una reducción en el tamaño proporcional del hígado (P<0.05) en comparación con la dieta basal (T1: 2.36 vs T2: 2.26%), esto sugiere que la albúmina de huevo deshidratada se podría absorber intacta, promoviendo la movilización de ácidos grasos y disminuyendo su almacenamiento en el hígado.

SUMMARY

The aim of this study was to obtain information about the productive performance of broilers fed with diets added with 5% of dried egg albumen during Pre-Starter phase (1-7 days). One hundred Ross 308 1-day-old male broilers were randomly assigned into two treatments with 5 repetitions (10 chicks each), birds were allocated in Petersime cages. Pre-Starter diets were as follows: (T1) sorghum-soybean meal basal diet; (T2) basal diet added with 5% dried egg albumen. Broiler performance, carcass yield, and gastro-intestinal tract allometry were measured. Results showed no significant differences (P>0.05) in productive performance nor carcass yield. However, birds fed with albumen showed a reduction in their proportional liver size (P<0.05) compared with basal diet (T1: 2.36 vs T2: 2.26%), this suggest that the dried egg albumen may be absorbed intact, promoting fatty acids mobilization and diminishing its storage in the liver.

INTRODUCTION

Albumen is considered an excellent source of natural protein that contains essential amino acids of high bioavailability. This bioavailability increases up to 90% during the thermal process and pasteurization used in the production of dehydrated albumen (7).

Egg albumen contains 88% water, 11.5% protein and 0.5% carbohydrates (free or bound with proteins). The proteic fraction includes ovalbumin (54%), ovotransferrin (12%), ovomucoid (11%), lysozyme and ovomucin (3.5%); avidin, cystatin, ovomacroglobulin and ovoinhibitor are also found but in lower proportions (1). Glucose represents 98% of total free carbohydrates; the remaining percentages consist of ashes, traces of lipids and inorganic ions (potassium, sodium, calcium, magnesium and iron) that are used during the embryo development. Albumen also contains ovoflavin, considered a water soluble pigment and vitamins (C, A and choline) (6).

Albumen proteins have been used in the food industry and in the manufacturing of pharmaceutical products because of its specific properties (e.g. anti-inflammatory, anti-viral). Lysozyme, for instance, is able to cause lysis of the cell wall of gram-positive bacteria such as *Listeria monocytogenes* and

Clostridium botulinum; and ovotransferrin have a chelating effect (5). Some peptides derived from egg albumen protein hydrolysis possess features like cytotoxic and anti-cancer effects, angiotensin-converting-enzyme (ACE) inhibitors, antimicrobials and antioxidants.

The use of dried egg albumen may improve broilers intestinal health associated with the antimicrobial properties of its proteins and peptides, reaching a better growth and productive performance.

MATERIALS AND METHODS

This study was performed at poultry experimental facilities of Centro de Enseñanza, Investigación y Extensión en Producción Avícola (CEIEPAv-FMVZ-UNAM, México). One hundred Ross 308 1-day-old male broilers were randomly assigned into two treatments with five repetitions (ten chicks each), birds were allocated in Petersime battery cages. The experiment was divided in two phases: a Pre-Starter (1 to 7 d) and a Grower (8 to 21 d). Pre-Starter diets were as follows: (T1) sorghum-soybean meal basal diet; (T2) basal diet added with 5% dried albumen. Birds were fed with the same commercial basal diet in Grower phase, no dried albumen was supplemented.

Broiler performance was measured from 1 to 21 d (weight gain, feed consumption, and feed conversion ratio). On the twenty-one day, chicks were slaughtered according to the Official Norm (NOM-033-SAG/ZOO-2014), carcass yield and allometry of the gastro-intestinal tract was evaluated.

Data were analyzed to verify the fulfillment of normality and homogeneity of variances assumptions. Experimental data were submitted to t and Z test, setting a significant level of 0.05 for estimating differences between treatments. JMP® Design of Experiments Software (JMP. 2013. Version 11.0.0. Cary, NC: SAS Institute) was used for statistical analysis.

RESULTS

No significant differences ($P>0.05$) in productive performance (weight gain, feed consumption, and feed conversion ratio) were found between treatments throughout the experiment. There was no significant effect ($P>0.05$) of dried albumen over carcass yield (T1: 66.48 vs T2: 66.97%). However, birds fed with dried albumen showed a reduction in the proportional liver size ($P<0.05$) compared with basal diet (T1: 2.36 vs T2: 2.26%). No effect on other allometry variables was observed ($P>0.05$).

DISCUSSION AND CONCLUSIONS

Currently, there is little research regarding to the use of dried egg albumen as source of protein in comparison of those that use plasma powder to replace soybean meal in poultry diets. Martinez (3) reports the use of egg albumen in weaned pigs, mentioning an increase in body weight during the first week of experimentation compared with the use of whey. At the end of the test, the treatment added with egg albumen showed an inferior performance compared to the treatment enriched with whey, this behavior was similar to the findings obtained in the present study.

Noy *et al*. (4) suggest that the re-esterification of fatty acids in the intestinal mucosa is not complete in birds and a high proportion (above 50%) is released in the portal vein as non-esterified fatty acids, these are transported in the blood-stream bound to albumin. It is proposed that the albumin added in the Pre-Starter phase in broilers can be absorbed intact, promoting the fatty acids transport, and increasing their concentration in target organs (e.g. muscles) for their storage and oxidation (2). The above, could explain the reduction of the liver size in the treatment added with dried egg albumen, reducing the storage of lipids in this organ.

This experiment was a first approach to the use of dried egg albumen in poultry diets and may be used as a model in other species.

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USE OF RHVT-ILT AND CEO VACCINE TO REDUCE VIRAL SHEDDING AND IMPROVE PROTECTION UNDER STRONG CHALLENGE CONDITIONS

USO DE UNA VACUNA RHVT-ILT Y CEO PARA REDUCIR LA EXCRECION VIRAL Y MEJORAR LA PROTECCION BAJO CONDICIONES FUERTES DE DESAFIO

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RESUMEN

La laringotraquitis infecciosa (ILT, por sus siglas en inglés) es una enfermedad respiratoria aguda de las aves que es controlada principalmente a través de la vacunación con las vacunas vivas atenuadas y las vacunas recombinantes. Las vacunas vivas atenuadas de ILT son capaces de proteger a los pollos contra los signos clínicos, la mortalidad y detener la replicación del virus de desafío. Sin embargo, las vacunas vivas atenuadas, en particular las de origen de embrión de pollo (CEO, por sus siglas en inglés), retienen la virulencia inherente e inducen las reacciones vacunales asociadas con pérdidas en la producción. Las vacunas recombinantes con Herpesvirus de Pavo (rHVT) -LT no recuperan la virulencia, pero son menos efectivas para detener la replicación viral después del desafío. Desde la introducción de las vacunas recombinantes de ILT, los complejos de postura multi-edad han adoptado el uso de las vacunas de ILT recombinantes y CEO en un programa combinado para mejorar la seguridad y expander la protección en las gallinas de postura. Aunque esta estrategia ha demostrado ser exitosa bajo condiciones de campo, el fundamento de estos beneficios no ha sido estudiado. El objetivo de este estudio fue evaluar el efecto de la vacunación con la vacuna HVT Innovax®-ILT (rHVT-LT) sobre la replicación de la CEO y la eficacia en la protección de la vacuna rHVT-LT cuando se administró sola o en combinación con la vacuna CEO vía gota ocular y en el agua de bebida para pollos libres de patógenos específicos (SPF).

SUMMARY

Infectious laryngotracheitis (ILT) is an acute respiratory disease of poultry that is mainly controlled through vaccination with live-attenuated and recombinant vaccines. ILT live attenuated vaccines are capable to protect chickens against clinical signs, mortality, and halt replication of the challenge virus. However, the live attenuated vaccines, in particular the chicken embryo origin (CEO), retain inherent virulence and induce vaccination reactions associated with production penalties. Recombinant Herpesvirus of Turkeys (rHVT) -LT vaccines do not regain virulence but are less effective to halt virus replication after challenge. Since the introduction of ILT recombinant vaccines, multi age layer complexes have adopted the use of recombinant and CEO ILT vaccines in a combined program in order to improve safety and expand protection in laying hens. Although this strategy has demonstrated to be successful under field conditions, the foundation of its benefits has not been studied.

The objective of this study was to evaluate the effect of HVT Innovax-ILT vaccine (rHVT-LT) vaccination on CEO replication and the protection efficacy of a rHVT-LT vaccine when administered alone or in combination with a CEO vaccine via

eyedrop and drinking water to specific pathogen free (SPF) chickens.

MATERIALS AND METHODS

Vaccination. The HVT Innovax-ILT vaccine was administered at 1 day of age subcutaneously followed by CEO vaccination administered at 38 days of age via eye-drop or drinking water. Three groups of 30 chickens were manually vaccinated subcutaneously (SC) with a standardized dose of rHVT-LT (3000 PFU/dose) vaccine, two groups were mock inoculated with a commercial vaccine diluent subcutaneously and two groups were not inoculated at all. At 38 days of age, four groups of 24 chickens were vaccinated with the CEO vaccine via drinking water ($10^{4.6}$ $TCID_{50}$) or via eye drop ($10^{4.4}$ $TCID_{50}$).

CEO vaccination monitoring. Body weight gain was measured over a seven-day period, before and after CEO vaccination (38 – 45 days). At five days post CEO vaccination clinical signs were recorded to evaluate vaccine reactions. Clinical signs were scored at as previously described (1.) Briefly, signs of dyspnea, conjunctivitis and lethargy were scored on a scale of 0 to 3, indicating normal (0), mild (1), moderate (2), and severe (3). CEO vaccine viral genome load in trachea and conjunctiva were quantified at four and seven days post CEO vaccination.

Challenge. At 55 days of age, vaccinated groups (rHVT-LT, CEOdw, CEOed, rHVT-LT + CEOdw, rHVT-LT + CEOed) and one non-vaccinated (NVx/Ch) group of chickens were challenge with virulent strain 1874C5 at a dose of $10^{3.8}$ $TCID_{50}$. The challenge virus was administered in a total volume of 200 µL; 50 µL was delivered in each eye and 100 µL was delivered intra-tracheally. The non-vaccinated non-challenged (NVx/NCh) group of chickens was mock-inoculated with tissue culture media in a similar fashion. At three to seven days post-challenge (dpch) clinical signs were scored, and tracheal swabs were collected at three and five dpch to quantify challenge virus genome load by real-time PCR

RESULTS

Monitoring of CEO. Chickens vaccinated solely with CEO via eye drop (CEOed) developed transient conjunctivitis which was not observed in any of the other CEO vaccinated groups (CEOdw, rHVT-LT + CEOdw, rHVT-LT + CEOed) including the group of chickens that was vaccinated with CEO via the drinking water. Groups vaccinated with CEO alone (CEOdw, CEOed) or the combined vaccination program rHVT-LT + CEO (rHVT-LT + CEOdw, rHVT-LT + CEOed) showed no significant differences ($P > 0.05$) in body weight gain after 7 days post CEO vaccination. ILTV viral genome load post CEO vaccination were quantified at 4 and 7 days post CEO vaccination in trachea (Figure 1a) and conjunctiva (Figure 1b). At four days post CEO vaccination, viral genome load in trachea was significantly higher ($P < 0.05$) in groups vaccinated with CEO alone (CEOed, CEOdw) than in rHVT-LT + CEOdw and rHVT-LT + CEOed) vaccinated groups. While at seven days, the CEOed group showed higher ($P < 0.05$) genome load than the rHVT-LT + CEOed group (Figure 1a). In the conjunctiva viral genome load at four and seven days post CEO vaccination was significantly higher ($P < 0.05$) in the CEOed group than in the CEOdw, rHVT-LT + CEOdw, and rHVT-LT + CEOed groups (Figure 1b).

Challenge. All the vaccinated challenged groups (rHVT-LT/Ch, CEOdw/Ch, CEOed/Ch, rHVT-LT + CEOdw/Ch, rHVT-LT + CEOed/Ch) showed significant reduction ($P < 0.05$) in clinical signs compared to the NVx/Ch group from three to seven days post challenge (Figure 2a). Body weight gain by seven days post challenge (62 days of age) was maintained ($P > 0.05$) in all vaccinated challenged groups as compared to the NVx/NCh group (Figure 2b). Challenge virus genome load in trachea at three and five days post challenge for all vaccinated challenged groups showed a significant ($P < 0.05$) reduction as compared to the NVx/Ch group of chickens (Figures 2c & 2d). However, the rHVT-LT/Ch group exhibited higher viral genome load ($P < 0.05$) than those detected for the CEO and rHVT-LT + CEO challenged groups. During the peak of virus replication, at day three post challenge, viral genome load percent reduction in relation to the NVx/Ch group was 63.5% (rHVT-LT), 93.0% (CEOdw), 89.0% (rHVT-LT + CEOdw), 94.4% (CEOed) and 94.2% (rHVT-LT + CEOed).

DISCUSSION AND CONCLUSIONS

Multi-age layer facilities that use CEO-type infectious laryngotracheitis (ILT) vaccines can develop very significant ILT challenge virus populations after recycling the vaccine type virus for many years. These complexes present a unique challenge when transitioning to recombinant type vaccination programs. This study explored the use of rHVT-ILT and CEO ILT vaccines in a combined program to determine whether a combination could demonstrate improved control of ILT. After CEO vaccination, greater clinical signs scores were observed in groups of chickens that received CEO vaccine by itself than in groups of chickens that received rHVT-LT + CEO. In particular, the group of chickens that received the CEO vaccine via eye drop

by itself developed a transient conjunctivitis which appeared by day four and disappeared around seven days post vaccination. Compared to the conjunctivitis reaction observed for the CEOed group, the CEOdw vaccinated group showed a minimal vaccination reaction. It is important to emphasize that, unlike in the field where incomplete vaccination coverage by drinking water administration occurs frequently, in this experiment, vaccination coverage reached at least 98% of the birds achieving a satisfactory uniformity. Therefore, lesser opportunities for vaccine back passages in poorly vaccinated chickens was allowed. With regards to CEO vaccine replication, both rHVT-LT + CEOdw and rHVT-LT + CEOed vaccinated groups of chickens showed lower CEO viral genome load in trachea than chickens vaccinated only with the CEO vaccine. This result confirmed that prior vaccination with rHVT-LT reduced the CEO vaccine virus replication in trachea and conjunctiva which indicates that the rHVT-LT + CEO vaccination strategy is a safer alternative than the use of CEO vaccine by itself.

Vaccine protection after challenge was assessed by the ability of vaccinated chickens to prevent clinical signs of the disease, to lessen challenge virus replication in the trachea, and to avoid body weight loss after challenge. Several studies have demonstrated that both CEO and rHVT-LT vaccines significantly decrease clinical signs of the disease after challenge (1,2,3). Similarly, in this study, a significant reduction in clinical signs was observed for rHVT-LT, CEO, and rHVT-LT + CEO vaccinated groups of chickens. Reduction of challenge virus in trachea after challenge in CEO-vaccinated chickens has been estimated to range from 87 to 100% (1,4,5). While for rHVT-LT, vaccinated chickens the challenge virus reduction rate ranges from 25 to 65% (1,6.) In this study rHVT-LT-vaccinated chickens showed a 63.5% reduction, while CEOdw, CEOed, rHVT-LT + CEOdw, rHVT-LT + CEOdw vaccinated groups of chickens achieved challenge virus reduction rates of 93.0%, 94.2%, 89.0%, and 94.2%, respectively. Therefore, the addition of CEO vaccination to previously rHVT-LT vaccinated chickens reduced challenge virus replication in the trachea as compared to vaccination with rHVT-LT alone. The route of CEO vaccine administration, either by drinking water or via eye drop did not influence the level of protection when they were applied alone or in combination with a rHVT-LT vaccine.

Overall this study found that priming with rHVT-ILT reduced reactions to the CEO ILT vaccines. Priming with rHVT-ILT also reduced CEO virus replication. The addition of CEO vaccine provided more robust protection than rHVT alone. Providing robust protection while reducing the overall CEO reaction and shedding may be a strategy to gain better control of ILT on a multi-age facility.

(The full length article will be published in *Avian Pathology*.)

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Figure 1. Trachea and conjunctiva viral load post CEO vaccination. a) Mean trachea viral load (log_{10} $^{2-\Delta\Delta Ct}$) at 4 and 7 days post CEO vaccination. b) Mean conjunctiva viral load (log_{10} $^{2-\Delta\Delta Ct}$) at 4 and 7 days post CEO vaccination. Mean viral load represented by bars with standard deviation (SD) error lines plotted from the mean and individual values represented by geometric symbols. Different letters indicate significance differences among groups ($P < 0.05$)

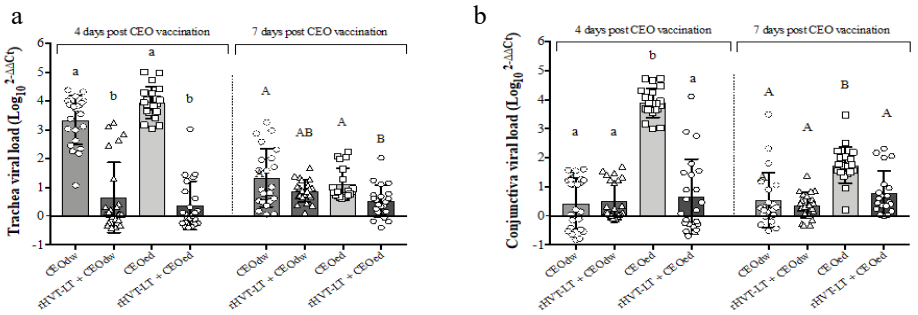
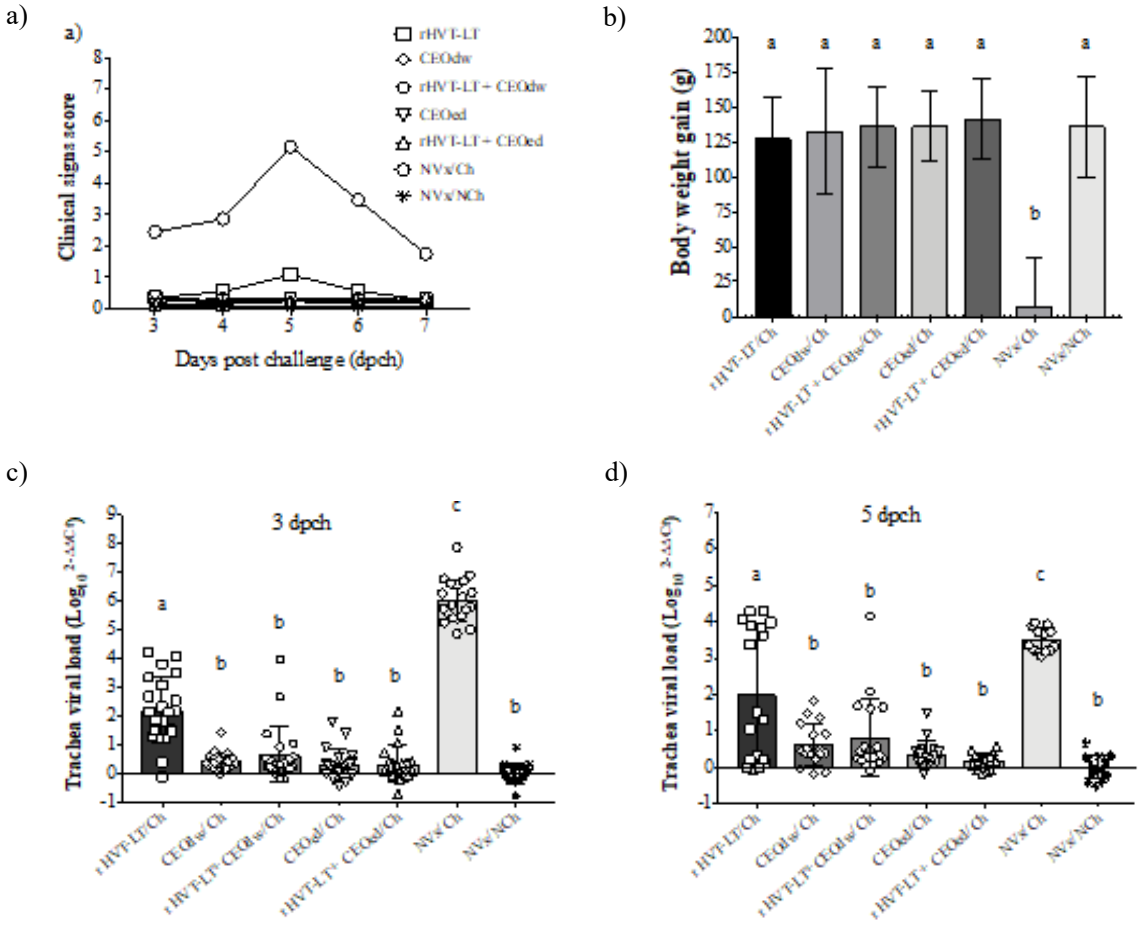


Figure 2. Clinical signs body weight gain and trachea viral load after challenge. a) Mean clinical signs scores represented by geometric symbols at 3 to 7 days post challenge. b) Mean body weight gain (g) 7 days post challenge represented by bars with standard deviation (SD) error lines plotted from the mean. c) Mean trachea viral load (log_{10} $^{2-\Delta\Delta Ct}$) at 3 days post challenge. d) Five days post challenge. The mean represented by bars with standard deviation (SD) error lines plotted from the mean and individual values represented by geometric symbols. Different letters indicate significance differences among groups ($P < 0.05$).



LO BUENO, LO MALO Y LO FEO DE LA INFLUENZA AVIAR Y TLC EN LA AVICULTURA MEXICANA

David Gastélum C.

Mexico es el tercer país con más población en America y el undécimo en el mundo, se ha crecido de 1990 con 90 millones de habitantes consumidores a 125 millones en 2018 (*estimado) con un crecimiento anual del 1.8%, Mexico a negociado un tratado que entró en vigor en 1993 (aún no les exportamos a nuestros socios Americanos y Canadienses), hoy convivimos en algunas regiones del País con I.A. donde se Vacuna, y cada año se importa más....

Lo bueno

Nuestras costumbres/el tamaño del mercado-consumo / la vacunas / la bioseguridad/ la tecnología-inversiones /....

Lo malo

Los costos/ las ineficiencias productivas/ Las importaciones en Vol y valor de pollo, huevo, reproductoras y huevo fértil / los riesgos sanitarios y dependencia / las no exportaciones / oportunidades de la cadena productiva no aprovechada/ ....

Lo feo

La I. A. y efectos en el consumo y en el consumidor / relaciones tensas con autoridades, y la no exportación / la importación creciente del pie de cría /importaciones de Pierna muslo de Usa y Pechuga de Brasil / ....

EFFECT OF THE TYPE OF DIET AND THE ADDITION OF HUMIC SUBSTANCES AS GROWTH PROMOTER IN BROILER CHICKENS

EFECTO DEL TIPO DE DIETA Y LA ADICIÓN DE SUSTANCIAS HÚMICAS COMO PROMOTOR DE CRECIMIENTO EN POLLOS DE ENGORDA

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RESUMEN

El objetivo fue evaluar los parámetros productivos, rendimiento en canal, histopatología y microbiología del intestino delgado e hígado en pollos de engorda alimentados con dietas altas y bajas en fibra (AF y BF) y la adición con extractos de sustancias húmicas (ESH). Un grupo de 240 pollos de engorda Ross 308 fueron colocados en jaulas de retención de los 21 a los 42 días de edad y asignados aleatoriamente a tres tratamientos de dietas: CP) Control Positivo adicionado con un antibiótico promotor de crecimiento y un fármaco anticoccidiano; CN) Control Negativo sin antibiótico, ni fármaco anticoccidiano, y ESH igual que el CN pero añadido con un 0.5% de ESH, en dos juegos de dietas: bajo y alto contenido de fibra (BF y AF). Al final de la prueba, la pechuga y la canal fueron pesadas y se tomaron muestras del intestino delgado e hígado para histopatología y análisis microbiano. Los resultados fueron sometidos a ANOVA. El peso corporal final ($P< 0.10$) y la ganancia de peso ($P < 0.01$) fue mayor y la conversión alimenticia ($P < 0.05$) fue menor en los pollos con ESH, sin importar el tipo de dieta. El total de las bacterias mesofílicas (TBM) y *E. coli* fueron menores ($P< 0.01$) en el contenido del yeyuno y ciego en los pollos de engorda CP, comparado con los CN y los adicionados con ESH. El conteo de TBM en el hígado fueron mayores en los pollos de engorda adicionados con ESH que recibieron la dieta AF. En general, hubo dos respuestas claras sobre el número y severidad de lesiones de tejido entre los tratamientos. En resumen, la adición de ESH en las dietas de los pollos de engorda mejorando las respuestas productivas pero no redujo los conteos de TBM y *E. coli* en el yeyuno, ciego e hígado ni disminuyeron el número o severidad de las lesiones en el duodeno, yeyuno e hígado.

SUMMARY

The objective was to evaluate the productive parameters, carcass yield, histopathology and microbiology of the small intestine and liver in broilers fed low and high fiber diets (LF and HF) and added with an extract of humic substances (EHS). A group of 240 Ross 308 male broilers were allocated in holding cages from 21 to 42 d of age and randomly assigned to three dietary treatments: PC) Positive control added with an antibiotic growth promoter and anticoccidial drug, NC) Negative control without antibiotic growth promoter and without anticoccidial drug, and EHS) Same as NC) but added with 0.5% of EHS, in two set of diets: low and high fiber content (LF and HF). At the end of the trial, the breast and carcass were weighed and samples of the intestine and liver were taken for histopathology and microbial analysis. The results were subjected to ANOVA. The final body weight ($P < 0.10$) and weight gain ($P < 0.01$) were higher and the feed conversion ($P < 0.05$) was lower in EHS-fed broilers, regardless of the type of diet. The total mesophilic bacteria (TMB) and *E. coli* were lower ($P < 0.01$) in the content of jejunum and ceca of PC broilers, compared to NC and EHS-fed broilers. The TMB counts in liver were higher in EHS-fed broilers receiving the HF diet. Overall, there were no clear responses on the number and severity of tissue lesions among treatments. In summary, the addition of the EHS in the diet of broiler chickens improved the productivity responses, but did not reduce the counts of TMB and *E. coli* in jejunum, ceca and liver nor diminished the number and severity of lesion in duodenum, jejunum and liver.

INTRODUCTION

In veterinary medicine, humic substances (HS) have been used as antidiarrheal agents, pain relievers, immunomodulators and antimicrobials, after the recommendations of the Veterinary Committee of the European Medicine Agency for the Evaluation of Medicinal Products (EMEA, 1999). In recent years, HS have been tested as growth promoters in animal production and are one of the promising options to face the globally ban of antibiotics in feeds (6, 7). Humic substances as part of humus-soil organic matter, and are compounds arising from the physical, chemical and microbiological transformation (humification) of biomolecules. Approximately 80% of the total carbon in terrestrial media and 60% of the carbon dissolved in aquatic media are made up of HS; hence they are a complex mixture of many different acids containing carboxyl and phenolate groups (6, 7).

In broiler chickens and laying hens, a growth-promoting effect of HS when supplemented in the feeds or drinking water has been documented. In broiler chickens, improvements in body weight, feed conversion, ash content of the tibia, ash retention and digestibility, as well as reduction in crypt depth and increased length of the villi of the jejunal mucosa due to the inclusion of HS have been reported (4, 8, 9). Most of the HS tested in poultry are commercially available or purified products. It is well known that worm composts originating from animal manures are also good sources of HS. In a previous study, it was found that the addition of an extract of HS (EHS) from a worm compost reduced the mucosal permeability and the bacterial translocation to the liver in feed restricted broiler chickens; the feed restriction model was used to cause intestinal inflammation (7). These responses were not observed in non-challenged broilers or under *in vitro* conditions (6). One of the proposed mechanisms of action of HS is related to the ability to create protective layers over the epithelial mucosal membrane of the digestive tract against toxic and bacterial contaminated substances (10). Whether this mechanism is involved in the enhanced growth through the reduction of the microbial load and lesion scores in broilers added with HS is unknown. The objective of this work was to evaluate the productive parameters, carcass yield, histopathology and microbiology of the small intestine and liver of broilers fed low and high fiber diets (LF and HF) and added with an extract of humic substances.

MATERIALS AND METHODS

The isolation and extraction of the EHS from worm compost was performed as described by (6, 7). A group of 240 Ross 308 male broilers were allocated in holding cages (30 cm wide x 38 cm deep x 37 cm height) providing 1140 cm^2/bird from 21 to 42 d of age. Cages were arranged in batteries and were provided with gas heaters, equipped with a plastic feeder and a cup waterer. Birds were randomly assigned to three treatments: PC) Positive control added with bacitracin methylene disalicylate as antibiotic growth promoter (AGP) and salinomycin as anticoccidial drug (AC); NC) Negative control without AGP and AC drug; and EHS) Same as NC) but added with 0.5% of EHS, and two set of diets with low and high fiber content (LF and HF). The LF diet was formulated with corn and soybean meal and the HF was formulated with corn, soybean meal, distillers dried grain with solubles and wheat bran. Feed and water were offered ad libitum throughout the experiment. Broilers were weighed at the beginning and end of the trial to calculate the daily weight gain (WG, g/d). Feed offered and refused was registered to calculate the daily feed intake (FI, g/d). The feed conversion ratio (FCR) was estimated by dividing the FI between the WG. At the end of the trial, all broilers were killed and the carcass and breast were weighed.

For measurement of microbiota, digesta from the jejunum and ceca were taken as well as liver samples from six broilers per treatment. The total mesophilic bacteria (TMB) were determined using the standard plate count method. Counts of viable *E. coli* in the samples were conducted by plating serial 10-fold dilutions onto MacConkey agar plates. Counts of viable *Lactobacillus* (LAC) in the jejunum samples were also conducted using lactobacilli medium III agar plates. The *E. coli* and LAC plates were incubated for 24 h, both under aerobic conditions. Colonies on each agar plate were expressed as colony-forming units per gram (log_{10} CFU/g). In the jejunum samples, the total fungi and yeast were also cultured on Sabouraud glucose agar with chloramphenicol and incubated at 37 C for 48 h.

One-cm samples from the duodenum, lower jejunum and liver were taken from six broilers per treatment for histopathologic evaluations; the tissues were stained and the histopathological changes were observed under light microscope by an experienced avian veterinarian who was blind to treatment allocations. Based upon severity of the lesions, a score of 0 (no lesions), 1 (mild lesions), 2 (moderate lesions), 3 (severe lesions) or 4 (extremely severe lesions) were recorded for each chicken. The total number of lesion scores were recorded per each tissue per treatment. Additionally, the severity of lesion, from 0 to 4, was calculated as the sum of lesion scores in the three tissues (duodenum, jejunum, and liver).

The results were subjected to ANOVA. The number of lesions and severity of lesion scores were transformed to log_{10} before the analysis.

RESULTS AND DISCUSSION

The final body weight ($P < 0.10$) and WG ($P < 0.01$) were lower and the FCR ($P < 0.05$) was higher in NC broilers, without APC and AC, regardless of the type of diet; these variable responses were not different between PC broilers, added with an AGP and AC, and EHS-fed broilers. The weight of the breast and carcass were lower in NC broilers compared with that of PC and EHS-fed broilers; the breast and carcass weight of PC and EHS broilers was similar. These results agree to previous reports in which improvements in body weight, FCR and carcass measurements due to the inclusion of HS have been reported in broiler chickens (4, 8, 9).

The TMB and *E. coli* counts were lower ($P < 0.01$) in the jejunum and ceca of PC broilers compared to NC and EHS-fed broilers; the TMB and *E. coli* in jejunum and ceca were similar between NC and EHS broilers. These results do not agree with previous reports based on *in* vitro and *in vivo* assays in which different sources of HS showed antimicrobial activity against many human pathogenic bacteria (2), as well as inhibition of *S. aureus*, *Candida*, *E. coli* and *S. Enteritidis* (12). It was also reported that broiler chickens fed diets with HS showed lower *E. coli* counts in the intestinal content (1). Opposite to this, the coliforms and *E. coli* counts in the caecum were not affected in broilers added with humates (5).

In the liver, the *E. coli* counts were not different among treatments; but the TMB counts were similar in broilers fed the LF diet, while in broilers fed the HF diet the TMB were higher in EHS compared to PC broilers (Interaction of treatment and diet, $P < 0.01$). This finding is also opposite to the lower bacterial translocation found in the liver of broilers fed a HS extracted from the same worm compost as the EHS used in the present study (7). The LAB counts were higher in PC and NC compared to EHS-fed broilers ($P < 0.05$). In previous studies, a lack of response (5, 7) or increased LAC counts (1) were reported in broilers supplemented with HS. The fungi were similar among treatments but the yeast were lower in EHS-fed broilers compared to NC, while yeast in PC broilers were intermediate.

The number of lesions in duodenum were highest ($P < 0.05$) in broilers receiving the EHS and were lowest in NC broilers, whereas in the jejunum the lesions were highest ($P < 0.05$) in PC broilers and were lowest in EHS-fed broilers. In liver, the number of lesions were higher ($P < 0.05$) in NC broilers compared to PC and EHS-fed broilers; no differences in the lesion number were observed between PC and EHS-fed broilers. These findings do not agree with the microbiological analysis in which the PC broilers had reduced TMB and *E. coli* counts in duodenum, jejunum and liver compared to EHS-fed broilers. The results of the severity of lesion, indicate that there were no statistical differences among treatments in the frequency of score 0, or no lesions, based in the sum of lesion in the duodenum, jejunum, and liver. The frequency of score 1 (mild lesion) was higher ($P < 0.05$) in NC broilers compared to PC and EHS-fed broilers. The frequency of score 2 (moderate lesions) was higher ($P < 0.05$) in EHS-fed broilers compared to PC and NC broilers. The frequency of score 3 (severe lesions) were higher ($P < 0.05$) in PC broilers compared to broilers of NC and EHS-fed broilers. Score 4, extremely severe lesions, were not detected in any tissue. One of the proposed mechanisms of action of HS is related to the ability to create protective layers over the epithelial mucosal membrane of the digestive tract against toxic and bacterial contaminated substances (10); however, the results of the number and severity of lesion do not support this suggestion because the supplementation of the EHS did not reduce the overall lesions compared to the PC and NC groups.

Regarding the diet effect, it was found that the final body weight, FI, WG, and the breast and carcass weight were higher ($P < 0.05$) in LF-fed broilers; however, the TMB and LAB counts, the number of lesions in liver and the frequency of score 3 lesions were higher ($P < 0.05$) in HF-fed broilers. When chickens are fed alternative ingredients such as distillers dried grain with solubles and wheat bran that are high in non-starch polysaccharides (NSPs), poor performance and unmanageable litter conditions caused by sticky droppings are observed. High dietary NSPs increases the digesta viscosity and reduces the digestibility and contributes to undesirable fermentations in the intestine and, hence to higher bacterial counts and bacterial translocation to the liver (11). Higher digesta viscosity was also reported in broilers fed a source of HS fed a LF diet (7); from this finding it was expected that broilers on the EHS treatment and fed a HF diet would show impaired growth responses, higher microbiological counts and higher lesion scores in all tissues. This assumption was only supported by the higher TMB counts in the liver of EHS-fed broilers receiving a HF diet.

In summary, the addition of the EHS in the diet of broiler chickens improved the productivity responses, but did not reduce the counts of TMB and E. coli in jejunum, ceca and liver nor diminish the number and severity of lesion in duodenum, jejunum and liver.

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INTRAPULMONARY DELIVERY OF CPG-ODN USING A LARGE SCALE PROTOTYPE NEBULIZER UNDER FIELD CONDITIONS PROTECTS NEONATAL BROILER CHICKENS AGAINST BACTERIAL SEPTICEMIA

ADMINISTRACION INTRAPULMONAR DE CPG-ODN USANDO UN NEBULIZADOR PROTOTIPO A GRAN ESCALA BAJO CONDICIONES DE CAMPO QUE PROTEGEN A LOS POLLOS DE ENGORDA NEONATOS CONTRA LA SEPTICEMIA BACTERIANA

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RESUMEN

Previamente ya se había demostrado la protección significativa de los pollos de engorda neonatos contra la septicemia bacteriana letal por medio de una administración intrapulmonar (IPL) de CpG-ODN al momento de la eclosión usando una cámara de nebulización en escala de laboratorio. El objetivo de este estudio fue probar la eficacia de la administración IPL de CpG-ODN usando un nebulizador prototipo a gran escala y explorar las condiciones ambientales óptimas requeridas para la eficacia de CpG-ODN, y mantener el bienestar de las aves en situaciones industriales. Los experimentos de campo a gran escala se condujeron en dos incubadoras de pollo de engorda comerciales del oeste de Canadá. Los pollitos neonatos (7000-8000) recibieron CpG-ODN o agua destilada (DW, por sus siglas en inglés) durante 30 minutos usando el nebulizador prototipo a gran escala. Los pollitos se muestrearon estratégicamente a partir de diferentes lugares del prototipo (abajo y a la izquierda = 40, abajo ya la derecha = 40, en medio izquierda = 40, en medio derecha = 40, arriba izquierda = 40, arriba derecha = 40) y se desafiaron con dosis letales de *Escherichia coli*. A una humedad óptima, humedad relativa (RH) y temperatura, IPL CpG-ODN administrado a las aves demostró dar, significativamente, una mejor sobrevivencia ($P<0.05$) en comparación con el grupo control DW. Identificamos que la administración IPL CpG-ODN utilizando un nebulizador prototipo a gran escala es una técnica efectiva en situaciones industriales.

ABSTRACT

We have previously demonstrated significant protection of neonatal broiler chickens against lethal bacterial septicemia by intrapulmonary (IPL) delivery of CpG-ODN at hatch using a laboratory scale nebulizing chamber. The objective of the current study was to test the efficacy of IPL CpG-ODN delivery using a large scale prototype nebulizer and explore the optimum environmental conditions required for CpG-ODN efficacy while maintaining welfare of the birds at industrial settings. Large scale field experiments were conducted at two commercial broiler hatcheries in Western Canada. Neonatal broiler chicks (7000-8000) received CpG-ODN or distilled water (DW) for 30 minutes using a large scale prototype nebulizer. Broiler chicks were sampled strategically from different locations of the prototype (bottom left = 40,

bottom right = 40, middle left = 40, middle right = 40, top left = 40, top right = 40) and challenged with lethal doses of *Escherichia coli*. At optimum humidex, relative humidity (RH) and temperature, IPL CpG-ODN administered birds showed significantly better survival ($P<0.05$) compared to the DW control group. We have identified that IPL CpG-ODN delivery using a large scale prototype nebulizer is an effective technique in the industrial settings.

INTRODUCTION

Bacterial infections in the poultry industry is a leading cause of first week mortality that results in economic losses to the producers (6). Canadian poultry producers have started to voluntarily withdraw from the use of prophylactic antibiotics due to public health concerns such as the emergence of antibiotic resistant bacteria. As a result, the need for alternative strategies has increased in order to safeguard poultry health and wellbeing. We have recently demonstrated that IPL delivery of CpG-ODN in the laboratory scale could induce protective immunity in neonatal broiler chicks and protect against lethal *E. coli* septicemia (2). Upon identification of this technique as industry feasible, we developed a large scale prototype nebulizer through engineering collaborations. The objective of this study was to investigate the efficacy of IPL CpG-ODN delivery using the large scale prototype nebulizer at industrial settings.

MATERIALS AND METHODS

IPL delivery of CpG-ODN. A large scale prototype nebulizer was constructed with an environment controlled chamber ($7.55m^3$) that can hold 8000 chicks. The nebulizer component was an ultrasonic type with the ability to deliver 0.5-5 □m particles. Field experiments were performed in a commercial broiler hatchery in Saskatchewan and a broiler farm in British Columbia. Newly hatched broiler chicks were arranged into 102 or 104 chicks/basket and stacked up to 10 baskets in height (9 containing chicks and one top empty basket). Eight of those stacks were arranged inside the chamber in 2X4 configuration. CpG-ODN^{2007} dissolved in sterile distilled water was administered to the chicks for 30 minutes. An equal volume of distilled water was aerosolized as a negative control to another group of birds. In both experiments, intra-chamber environment was maintained at optimum humidex, temperature and RH.

Animal model and *E. coli* challenge study. To test the efficacy of CpG-ODN delivery, chicks were strategically collected from different areas of the chamber; bottom-left (n=40), bottom-right (n=40), middle-left (n=40), middle-right (n=40), top-left (n=40) and top-right (n=40). Birds (n=40) were randomly collected from the DW control. Two days following treatment, birds in each group were challenged using a field isolate of *Escherichia coli* (*E. coli)* by subcutaneously injecting $1X10^5$ colony forming units (cfu)/bird (n=20) and $1X10^6$ cfu/bird (n=20). They were evaluated closely three times during the critical stage and twice thereafter up to 8-10 days. Survivability was evaluated and clinical scoring of the birds was performed as described previously (1,2).

Statistical analysis. A cumulative clinical score (CCS) for each bird was generated as previously described (1). The significance of difference of survival and CCS were analyzed with Prism (Prism 6.0, GraphPad Software Inc; San Diego, CA, USA) with a significance level of $P<0.05$. The significance of differences among groups in survival patterns and median survival times were analyzed using the log-rank test. Significance of differences of CCS among groups was tested using Kruskal Wallis nonparametric analysis of variance.

RESULTS

The groups of chicks that received CpG-ODN using the large scale prototype nebulizer had significantly better survival ($P<0.05$) in both experiments conducted in Saskatchewan and British Columbia compared to the DW control. In Saskatchewan experiment, average survival of the CpG-ODN treated birds was 68.75% whereas the control group had only 42.5% survival. CCS of the groups that received CpG-ODN was significantly better compared to the DW control group. In the experiment conducted in British Columbia, IPL CpG-ODN administered birds had an average survival of 51.7% compared to the DW control group that had 25% survival. CCS values of the CpG-ODN received groups were significantly low compared to the DW control ($P<0.05$).

DISCUSSION

Emergence of antibiotic resistant bacteria has become a global concern and antibiotic use in the poultry industry has been linked to it (5). The commercial poultry industry in Canada has initiated to voluntarily withdraw from the use of prophylactic antibiotics. This decision has challenged the capacity to ensure health and wellbeing of poultry birds particularly during the most susceptible first week of life (3). As a result, research focused on the development of alternative strategies has become vital. CpG-ODN has been identified immunostimulatory

against bacterial infections in broiler chickens when administered intramuscularly, subcutaneously and injected *in ovo* to day 18 old embryos (1,4). Recently, we reported that IPL delivery of CpG-ODN as aerosolized micro induces protective immunity against *E. coli* septicemia in neonatal broiler chicks (2). We identified this technique adaptable to the fast paced nature of the commercial poultry industry, particularly in the commercial broiler hatcheries where a large number of chicks are handled on a daily basis. As a result, we collaborated with engineers and constructed a large scale prototype nebulizer of 8000 bird capacity to deliver CpG-ODN as an aerosol in the IPL route.

The prototype consists of an ultrasonic nebulizer component that generates 0.5-5µm particles of the CpG-ODN solution. An air handling system directs the CpG-ODN aerosolized air through a duct system into a chamber (7.55mm^3) with 8000 chicks' capacity which has a control panel that regulates the intra-chamber environment. Upon conducting the experiments at two provinces with drastically different climatic conditions, we understood that maintaining an intra-chamber optimum humidex, RH and temperature were critical for successful nebulization of CpG-ODN and protection of neonatal broiler chicks against *E. coli* septicemia. Although we nebulized chicks for 30 minutes in the large scale experiments, we have learnt in our laboratory scale experiments that exposure for 15 minutes is able to induce a significant immunoprotection (2). In future we anticipate conducting more experiments to explore the potential of reducing the exposure time using the large scale prototype nebulizer to improve its efficiency. Our experience in the field experiments have proved that the IPL delivery of CpG-ODN using a large scale prototype nebulizer is an applicable technique in the commercial poultry industry as an alternative to antibiotics. It has the potential to improve poultry health and wellbeing, ensure public health and minimize the emergence of antibiotic resistant bacteria in the environment.

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TURKEY ARTHRITIS REOVIRUS – DIAGNOSTIC STRATEGIES

REOVIRUS DE LA ARTRITIS EN PAVOS- ESTRATEGIAS DE DIAGNÓSTICO

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RESUMEN

Del 2010-2011, fueron reportados varios casos severos de artritis/ tenosinovitis (cojeras y tarsos inflamados) en pavos comerciales en los estados del medio oeste. Esta condición parece ser el resurgimiento de cojeras que fueron reportadas hace 30 años (1, 3). Hemos aislado el VARP de los tendones y fluido de articulaciones de estos pavos con cojeras (2). La enfermedad causa pérdidas económicas substanciales a los productores de pavos. La administración de vacunas autógenas a las reproductoras de pavos fue capaz de desacelerar la infección, pero ahora múltiples parvadas de pavos presentan la artritis viral y la enfermedad continúa sin disminuir. También ha cambiado la imagen clínica; los signos de erosión del cartílago y la presencia de la enfermedad en pavos más jóvenes a una edad de 5-7 semanas. Los objetivos principales del estudio fueron el caracterizar algunos de estos nuevos aislamientos de VARP y desarrollar un diagnóstico molecular y pruebas serológicas para la detección del virus y sus anticuerpos, respectivamente. Basados en los datos, encontramos que los diferentes estados o diferentes presentadores/ parvadas no tenían diferentes virus; al contrario, estos compartían virus en común aunque se observó una variación basada en los datos temporales.

SUMMARY

Avian reoviruses (ARVs) belong to the genus orthoreovirus in the family *Reoviridae*. They are ubiquitous in domestic poultry and are often isolated from apparently healthy birds. However, ARVs have also been implicated in several disease conditions in both chickens and turkeys including enteritis, hepatitis, neurological disorder, myocarditis, respiratory distress and viral arthritis/tenosynovitis. Viruses isolated from chickens and turkeys are commonly known as chicken reovirus (CRV) and turkey reovirus (TRV), respectively. The CRVs were initially isolated in 1957 from naturally occurring cases of synovitis in chickens. Their role in viral arthritis of chickens is well defined and its pathogenesis has been well established.

The TRVs are arbitrarily divided into turkey enteric reovirus (TERV) and turkey arthritis reovirus (TARV) depending on the disease condition and tissues from where they are isolated. For many years, turkey enteric reoviruses (TERVs) have been isolated from apparently healthy poults as well as from turkeys with poult enteritis complex (PEC). Currently, no molecular marker exists that can differentiate TERV from TARV although efforts are under way in our laboratory to do so.

In 2010-2011, several cases of arthritis/tenosynovitis (lameness and swollen hock joints) were reported in commercial turkeys from the Midwestern states. This condition appeared to be re-emergence of lameness that was reported over 30 years ago (1, 3). We isolated TARV from the tendons and joint fluids of these lame turkeys (2). Koch's postulates were fulfilled by experimental inoculation of these newly isolated TARVs in turkey poults (4). From 2011 to 2018, we at the Minnesota Veterinary Diagnostic Laboratory (MVDL) have isolated hundreds of TARV from such cases. The disease caused substantial economic losses to turkey producers. Autogenous vaccines administered to breeder turkeys were able to slow down the infection at first but multiple turkey flocks are now presenting with viral arthritis and the disease continues unabated. The clinical picture has also changed; signs of cartilage erosion and presence of disease in younger turkeys at the age of 5-7 weeks.

The main objectives of this study were to characterize some of these newly isolated TARVs and to develop molecular diagnostic and serological tests for the detection of the virus and its antibodies, respectively. We have recently developed protocols for complete genome sequencing of avian reoviruses along with a bioinformatics pipeline for in-depth sequence analysis. This was done by characterizing, by whole genome sequencing, 87 'old' and 'new' strains of TARV isolated between 2011 and 2017 from 11 different U.S. states. These strains were selected on the basis of state/geographical area, year of isolation,

turkey age group, flocks type (breeder/ commercial) and different submitters. Six isolates of TERV were also included. The Illumina MiSeq 250 paired end cycle was used for whole genome sequencing (WGS). The obtained MiSeq files underwent quality check assembly by using a pipeline developed in our lab.

Based on the WGS data, we found that different states or different submitters/flocks do not have distinct viruses; rather they share common viruses although variation based on temporal data was observed. Based on M2 (μB) gene, three different genetic clusters of TARV were observed. *In silico* epitope mapping indicates that these three M2 based genetic clusters, fortuitously, overlap with epitope mapping. The divergence plot based on M2 also indicates the formation of three clusters. The antigenic nature of the μB (coded by M2 segment) could be attributed to its location on virus capsid. Being the major outer capsid protein, it is very likely to have the neutralization epitopes. These observations strongly support that M2 could be a major serotype determinant. This indicates that, in theory, we can select three isolates (one from each cluster) for preparing a multivalent vaccine, which should be protective against a large majority of currently circulating TARVs.

Virus isolation from gastrocnemius tendon remains the gold standard for definitive diagnosis of TARV infection, but this procedure is time consuming and hence the need for rapid molecular tests cannot be overemphasized. Thus, we have developed a real time RT-PCR (qPCR) for the detection of TARV and a universal avian reovirus qPCR for detection of all avian reoviruses. These tests are currently being used at MVDL. We have also developed a whole-virus enzyme-linked immunosorbent assay (ELISA) for the detection of TRV antibodies in infected turkeys. This ELISA is capable of detecting antibodies from different TRV strains currently circulating in the US turkey flocks. This test should be useful for monitoring antibody titers in both breeder turkeys that are vaccinated for reovirus and the offspring from those breeders. Detection of high anti-reovirus titers at a later age might allow producers to identify a field challenge, giving them time to alter the processing schedule and salvage more birds.

Additionally, we are in the process of developing cross neutralization assays to characterize newly isolated TARVs and determine if antigenic and genetic differences among viral variants can be correlated. For this purpose, we have developed hyperimmune sera against 17 different TARV isolates, which will be used in the development of cross neutralization assays.

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EVALUATION OF THE HUMORAL IMMUNE RESPONSE AND PROTECTION AT CHALLENGE, ASSOCIATED WITH THE APPLICATION OF DIFFERENT BACTERINS OF INFECTIOUS CORYZA IN SPF BIRDS

EVALUACION DE LA RESPUESTA INMUNE HUMORAL Y LA PROTECCION AL DESAFIO ASOCIADO CON LA APLICACIÓN DE DIFERENTES BACTERINAS DE LA CORIZA INFECCIOSA EN AVES SPF

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RESUMEN

Se evaluaron tres bacterinas comerciales trivalentes y una bivalente *in vitro* contra la coriza infeccioso aviar (AIC, por sus siglas en inglés). Las aves SPF se vacuanron a las 7 y 10 semanas de edad, con 0.5 mL; 15 aves con el producto A, 15 aves con el producto B, 15 con el producto C, 15 con el producto D y 15 más se usaron como controles. Se tomaron muestras de Suero de todos los grupos experimentales cuando las aves tenían 7,8,9,10,11 y 12 semanas de edad y se evaluaron por medio de la prueba HI con los serovares A, B y C de la AIC. A la semana 12, todos los grupos fueron desafiados con los serovares A, B y C de la AIC. Vimos que hubo diferencias en la respuesta inmunológica contra el serovar A de la AIC de la 10ª semana de edad en adelante entre los cuatro grupos vacunados. Sin embargo, no hubo diferencias estadísticas en el desempeño de las cuatro vacunas en la respuesta inmunológica contra el serovar B y C. Mientras que para la evaluación de la protección clínica, sólo la vacuna D mostró un menor porcentaje de protección contra el serotipo A.

Avibacterium paragallinarum es el agente causal de la Coriza Infecciosa Aviar (AIC). Se conocía anteriormente como *Haemophilus paragallinarum*. Pertenece al género *Haemophilus* que es un miembro de la familia Pasteurellaceae. La familia es conocida por los microorganismos pleomórficos, gram negativos, no móviles, bacilos y cocobacilos que son capaces de reducir a los nitratos y usar carbohidratos (1). El impacto de la infección de *A. paragallinarum* en la producción avícola es muy significativo, y las pérdidas económicas se deben a la reducción en el desempeño en el crecimiento en aves en crecimiento y una marcada reducción (10%-40%) en la producción de huevo en gallinas de postura (2).

SUMMARY

Three trivalent and one bivalent commercial bacterines were evaluated *in vitro* against avian infectious coryza (AIC). SPF Birds were vaccinated at 7 and 10 weeks of age, with 0.5 mL; 15 birds with product A, 15 birds with product B, 15 with product C, 15 with product D and 15 more birds were used as controls. Serum samples were taken from all the experimental groups when birds were 7,8,9,10,11 and 12 weeks old and were evaluated by HI test with A, B and C serovars of AIC. At week 12, all the groups were challenged with A, B and C serovars of AIC. We found that there were differences in the immunological response against serovar A of AIC from the 10th week of age and onwards among the four vaccinated groups. However, there were no statistical differences in the performance of the four vaccines in the immunological response against serovar B and C. While for the clinical protection assessment, only vaccine D showed a lower percentage of protection against serotype A.

Avibacterium paragallinarum is a causal agent of avian infectious coryza (AIC). It was previously known as *Haemophilus paragallinarum*. It belongs to the genus *Haemophilus* which is a member of the family Pasteurellaceae. The family is known for its pleomorphic, gram negative, non-motile, bacilli and coccobacilli organisms that are able to reduce nitrates and use carbohydrates (1). The impact of *Av. Paragallinarum* infection in poultry production is very significant, and economic losses are due to reduction in growth performance in growing birds and a marked reduction (10%-40%) in egg production in layers (2).

MATERIALS AND METHODS

Birds. 75 seven-week old SPF birds were used. Free access water and food were given. Birds were

kept in isolation units with filtered air during the experiment.

Vaccination. Four experimental groups were formed; A(bivalent), B(trivalent), C(trivalent) and D(trivalent) with 15 birds each; the groups were vaccinated according with the dose and administration route recommended by the producer (Table 1) all the birds were given an individual number. In addition, five controls per group (A, B and C) were used for the potency-challenge test.

Potency-challenge test. When birds reached 12 weeks old, 15 experimental groups were formed to perform the potency-challenge test. three seeds of *Avibacterium paragallinarum* were used; serotype A (W), serotype B (022) and serotype C (Modesto). These were inoculated intranasally with 0.2 mL per bird with a concentration of 10^7 ufc/mL. (Table 1)

Lesions scale. The clinical signs of AIC were registered during seven days considering the following scale; 0 no signs, 1; nasal exudate only or mild facial swelling; 2, nasal exudate and mild facial swelling; 3, exudate and moderated facial swelling (3).

Bacterial reisolation. At necropsy (seven days after challenge) sinus swabs were streaked out on blood agar and cross streaked with a *Staphilococcus* feeder strain. After incubation for 24 h at 37ºC in an atmosphere with CO2, the plates were inspected for satellite growth (4).

Hemagglutination inhibition (HI) test. All the serum samples were sent to the SANFER-IASA Biology laboratory where the HI test was performed according to the procedure described by Jacobs, 1992.

Statistics. Software "R" was used to perform all the statiscal tests and to plot the results. HI titers were converted into Log2 values and a non-parametric test (Kruskal-Wallis test with an α=0.05) was used to assses difference among vaccinated groups at each determined week. When statistical difference ($p<0.05$) was detected, post-hoc Nemenyi pairwise comparision test was performed.

RESULTS

For the serovar A screening, no specific antibodies were detected until the 9th week of the bird´s life. At 10 weeks of age, statistical difference was observed in the groups A and C compared to the groups D and B. Positive levels of antibodies were detected in birds that received vaccine A and C, obtaining a mean of HI titers (log2) of 5.5 and 3.9 respectively. At this same week, birds vaccinated with vaccines D and B remained negative (2 Log2). At the 11th week, group C HI titers (5.6 Log2) were statistical different than the HI results of the birds of the group D (2.0 Log2) and B (2.3 Log2) which resulted negative. Birds vaccinated with product A obtained more dispersed titers with a lower mean (4.8 Log2) which caused to be statistical equal to groups B, C and D. Finally, at the 12th week groups treated with vaccines A, B and C elicited the same level of specific antibodies, obtaining HI means of 8.2, 7.9, 7.7 Log2 respectively. However, vaccine D did not produce the same effect, since at this time point it also remained negative. (Figure 1.A)

Contrastingly, during all the duration of the trial it was not possible to detect specific antibodies against serovar B and C within the four different groups (Figure 1B and 1C)

Vaccine A was found to protect 100% against serovar A, B and C**. Vaccine B** protected 100% against serovar A, B, C. **Vaccine C** showed to protect 100% for A, B and C serovar. **Vaccine D** protected 80% against serotype A, 100% against serotype B and C. 100% of the control birds showed clinical signs of AIC.

DISCUSSION

In 2001 (5) Soriano found that birds vaccinated with bivalent bacterines and challenged with serovar A, had a lower percentage (40%) and media of circulating antibodies. However, birds in the same group showed high circulating titers and protection when challenged with B serovar. Jacobs *et al*. did not fount cross- protection in vaccinated birds with bivalent bacterines and challenged with B serovar. Kume *et al*. (6) found that vaccinated birds with serovars A and C were protected to challenge with strains of B serovar. In this study the bivalent vaccine was able to protect against challenge with B serovar.

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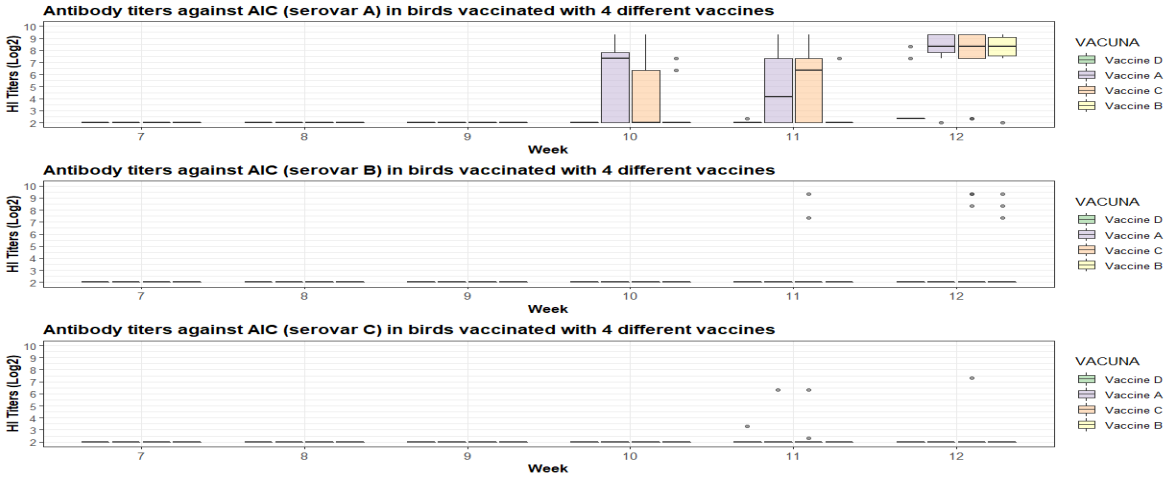
Table 1. Summary of the experimental design.

VACCINE	# OF BIRDS	VACCINATION/VIA/DOSE	SEROLOGY	CHALLENGE SEROTYPES 12TH WEEK	EXPERIMENTAL GROUPS		OBSERVATION
A	15	7 and 10 weeks /IM/0.5 mL	7,8,9,10, 11 y 12 weeks	Serotype A (W)	1	5 birds vaccine A	7 days after challenge and Bacterial reisolation
					2	5 birds vaccine B	
					3	5 birds vaccine C	
					4	5 control birds	
B	15	7 and 10 weeks/SC/0.5 mL		Serotype B (022)	5	5 birds vaccine A	
					6	5 birds vaccine B	
C	15	7 and 10 weeks/SC/0.5 mL			7	5 birds vaccine C	
					8	5 control birds	
D	15	7 and 10 weeks/IM/0.5 mL		Serotype C (Modest)	9	5 birds vaccine A	
					10	5 birds vaccine B	
					11	5 birds vaccine C	
					12	5 control birds	

IM: intramuscular
SC: subcutaneous

Figure 1. Boxplot of the serological screening from 7th to 12th weeks of age, of birds vaccinated with four different vaccines against AIC. (A)Serovar A HI results. (B) Serovar B HI results. (C) Serovar C HI results.



IN OVO DELIVERY OF SYNTHETIC CPG-ODN ENRICHES IMMUNE COMPARTMENTS TO INDUCE PROTECTIVE IMMUNITY IN NEONATAL CHICKENS

LA ADMINISTRACION *IN OVO* DE CPG-ODN SINTETICO ENRIQUECE LOS COMPARTIMENTOS INMUNES PARA INDUCIR LA INMUNIDAD PROTECTORA EN POLLOS NEONATOS

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RESUMEN

CpG-ODNs sintéticos han mostrado efectos inmunoprotectores contra las infecciones bacterianas en pollos. Sin embargo, los mecanismos inmunes de CpG-ODN involucrados en esta protección no se ha estudiado con detalle en pollos. Aquí, investigamos los cambios en el sistema immune después de la administración *in ovo* de CpG-ODN en pollos de engorda. Se inyectaron huevos embrionados de dieciocho días de edad con 50 µg de CpG-ODN o solución salina. Luego, examinamos los efectos de CpG-ODN sobre las poblaciones de las células inmunes. Se colectaron los bazos y los pulmones de embriones o pollitos y se hizo un análisis de citometría de flujo para examinar los cambios en las poblaciones de células T CD4+ y CD8+ T, las células presentadoras de antígenos (APCs-monocitos/macrófagos) y su expresión de los marcadores de maduración (CD40 y CD86). Estos datos indicaron un aumento significativo de macrófagos y los subgrupos de células T CD4+ and CD8+ T en tanto el bazo, como en los pulmones de los embriones y pollitos tratados con CpG-ODN. Los macrófagos en el bazo y los pulmones mostraron una sobrerregulación de CD40, pero no de CD86. Aquí, se demostró por primera vez que la administración *in ovo* de CpG-ODN en pollitos neonatos estimula y enriquece los nichos inmunológicos en el bazo y los pulmones.

ABSTRACT

Synthetic CpG-ODNs have shown immunoprotective effects against bacterial infections in chickens. However, the immune mechanisms of CpG-ODN involved in this protection is not studied in detail in chickens. Here, we investigated the changes of immune system following *in ovo* CpG-ODN administration in broiler chickens. Eighteen-day-old embryonated eggs were injected with either 50 µg of CpG-ODN or saline. Then, we examined the effects of CpG-ODN on immune cell populations. Spleen and lungs from embryos or chicks were collectted and flow cytometry analysis was done to examine the changes in CD4+ and CD8+ T-cell subsets, antigen presenting cell (APCs-monocyte/macrophage) populations and their expression of maturation markers (CD40 and CD86). This data indicated a significant increase of macrophages, CD4+ and CD8+ T-cell subsets in both spleen and lungs of CpG-ODN treated embryos and chicks. Macrophages in spleen and lungs showed an upregulation of CD40 but not CD86. Here, we demonstrate for the first time that *in ovo* delivery of CpG-ODN in neonatal chickens, stimulate and enrich immunological niches in spleen and lungs.

INTRODUCTION

Synthetic oligodeoxynucleotides containing CpG-motifs (CpG-ODNs) are well known as pathogen-associated molecular patterns (PAMPs) that stimulate immune cells and elicit immune responses (1, 9). The *in vivo* immunoprotective effect of CpG-

ODN in chickens against a bacterial infection was first reported from our lab (6), against an *E. coli* infection. Since this report, many groups have shown immunoprotective effects of CpG-ODN in chickens agianst bacterial (14), viral (3) and protozoal (2) diseases.

Several studies have been carried out to investigate different aspects of immune changes caused by CpG-ODN in chickens. Most are cytokine gene expression studies (4, 11, 15), while few studies also investigated changes in heterophils (7), macrophages and lymphocytes (5). However, the mechanisms by which CpG-ODN alone provides protection in chickens against bacterial infections are not completely understood, and a better understanding of CpG-ODN immune mechanisms in chickens will help in maximizing therapeutic potential of CpG-ODNs against diseases.In the present study, we investigated cellular immune changes in spleen and lungs following *in ovo* CpG-ODN administration.

MATERIALS AND METHODS

Synthetic CpG-ODN. CpG-ODN (CpG 2007) with 5'-TCGTCGTTGTCGTTTTGTCGTT-3'sequence was used in this study. These ODNs were synthesized with a phosphorothioate backbone (Operon Biotechnologies, Inc. Huntsville, AL).

Chicken embryos. Fertilized hatching eggs were obtained from a commercial broiler breeder operation in Saskatchewan, Canada. Eggs were incubated until hatch at the Animal Care Unit (ACU) at the Western College of Veterinary Medicine, University of Saskatchewan. This work was approved by the University of Saskatchewan's Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use.

Cells for flow cytometry. Cell preparation and antibody staining for flow cytometry was done as previously described with some modifications (8) Briefly, spleen and lung were collected at 24, 48 and 72 hr post *in ovo* injection from chicken embryos (n=3/group) and processed for cell isolation. Each spleen was gently pushed through a metal strainer by manual pressure to obtain a single cell suspension with ~3 mL of phosphate buffered saline (PBS) and collected in a 15 mL centrifuge tube. For lung, each tissue was manually dissected and incubated with ~1 mL of collagenase type I from Clostridium histolyticum (Sigma-Aldrich, St. Louis, Missouri, USA, C9891) dissolved in Dulbecco's Modified Eagle Medium (1 mg/mL) for 30 min in 37°C. After incubation, these tissues were pushed through a metal strainer to obtain a single cell suspension and washed twice with PBS. Then both spleen and lung cells were incubated with red blood cell lysis buffer. Following three washes with wash buffer (PBS containing 2% fetal bovine serum and 0.1% sodium azide) cells were used for the flow cytometry after staining with appropriate antibodies

Experimental design. In ovo *injections*: Either sterile saline (100 µL) or synthetic CpG-ODNs that were diluted in sterile, pyrogen-free saline (50µg/100µl) were administered in a 100µL volume per bird by the *in ovo* route into the amniotic cavity of ,18 day old embryonated eggs using 22-gauge-1-inch, hypodermic needles (n=10/group). Following injections, a drop of melted wax was placed on the pore created to seal the egg. All eggs were then transferred to the incubator until taken for tissue sample collection.

Flow cytometry. One set of spleen and lung cells (~$5x10^5$ cells) were incubated with mouse anti-chicken monocyte/macrophage phycoerythrin (KUL01-PE) antibody at 4 C for 30 min for detecting APCs. Then, cells from the previous step were washed and incubated with either mouse anti-chicken CD40 or CD86 primary antibodies separately at 4 C for 30 min. After three washings with PBS, the cells were stained with PerCP/Cy5.5 goat anti-mouse IgG secondary antibody at 4 C for 30 min. Another set of ~$5x10^5$ cells were incubated with mouse anti-chicken CD8 (FITC) and CD4 (PE) together at 4 C for 30 minutes. Lastly, the washed cells were suspended in 300 µL buffer in flow tubes and processed for flow cytometric analysis. Flow cytometry data were acquired by Epics XL (Beckman Coulter) and FACS Caliber (BD Bioscience), and data were analyzed with FlowJo software (Tree Star).

Statistical analysis. Cell populations from flow cytometry analysis were graphed and analyzed with the use of Prism (Prism 5.0, GraphPad Software Inc., San Diego, CA) with a significance level of $P<0.05$. For testing difference of APC percentages, CD4+ and CD8+ expression between groups, a two way ANOVA followed with Bonferroni post-test and Student-t test with Welch's correction for unequal variance was done, with a significant difference of $P<0.05$.

RESULTS AND DISCUSSION

CpG-ODN treated birds showed a marked increase in APCs (monocyte/macrophage), expression of CD40 costimulatory marker on APCs and T lymphocyte populations in both spleen and lung at all three time points. The percentage of APCs in CpG-ODN treated embryos in spleen at 24, 48 and 72 hours post treatment were 5.88, 12.6 and 11.2 %, respectively. Whereas, in the saline treated group they were, 2.9%, 1.1% and 1.1%. Lungs showed the same increase with CpG-ODN treatment. CD40, which is a

co stimulatory molecule on APCs, known to activate APCs and facilitate CD8+ T-cell priming (13) and generate protective CD8+ cytotoxic T cell (CTL) immunity (10) was expressed in significantly higher percentages on APCs, with CpG-ODN compared to saline in both spleen and lung. These findings suggest that *in ovo* CpG-ODN traetment result in more equipped APCs that could provide protective immunity in chickens against non specific infections. CD4+ (T helper cells) and CTLs are involved in more humoral and cell mediate specific immunity, respectively (12). At 72 hours post *in ovo* injections (day of hatch), the percentage of CD4 and CD8 T cells in spleen of CpG-ODN treated embryos were 1.89% and 1.09%, respectively compared to 0.22% CD4+ T cells and 0.23% CD8+ T cells in saline control. Similarly, the percentage of CD4+ and CD8+ T cells in lungs of CpG treated embryos were 0.67% and 6.01%, respectively compared to 0.43% CD4+ T cells and 0.79% CD8+ T cells in saline control. These data suggest that CpG-ODN promotes the enrichment of T-cell immunological niches both in lymphoid (spleen) and non-lymphoid (lungs) organs in chickens. In conclusion, *in ovo* delivery of CpG-ODN enriches immunological niches in spleen and lungs by preparing the chicken embryo to tolerate pathogenic insults more efficiently, at hatch.

(The full length article will be published in *Scientific Reports*.)

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COMPARATIVE EVALUATION OF ADJUVANT EFFECT OF EMULSIGEN-D AND CPG-ODN ON IMMUNE RESPONSE TO INACTIVATED FADV VACCINE IN BROILER BREEDERS

EVALUACION COMPARATIVA DEL EFECTO ADYUVANTE DE EMULSIGEN-D Y CPG-ODN SOBRE LA RESPUESTA INMUNE PARA LA VACUNA FADV INACTIVADA EN REPRODUCTORAS PESADAS

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RESUMEN

La hepatitis por cuerpos de inclusión (IBH, por sus siglas en inglés) es una enfermedad adenoviral aviar (FAdV, por siglas en inglés) económicamente importante de los pollos de engorda a nivel mundial. Actualmente, no existe una vacuna comercial disponible para el control de la IBH en Canadá. El objetivo de este estudio fue caracterizar el perfil inmune de las reproductoras pesadas después de la vacunación con la vacuna FAdV-8b con el adyuvante Emulsigen-D o CpG-ODN. Se vacunaron cuatro grupos (n=24/grupo) de reproductoras pesadas a las 16 semanas de edad con FAdV-8b (1x106 TCID50/ave) con el adjuvante con 20% de Emulsigen-D o 50 µg de CpG-ODN. A los grupos control se les administró solución salina o FAdV-8b sin adjuvante. Las reproductoras fueron reforzadas 3 semanas después con las vacunas respectivas. Ambos grupos con adyuvante Emulsigen-D y CpG-ODN indujeron anticuerpos a un nivel de aproximadamente 2 log10 en reproductoras pesadas. Una respuesta mixta de células T ($P<0.05$) se observe con los grupos con vacuna con adyuvante Emulsigen-D y CpG-ODN. En resumen, ambos adyuvantes mejoraron el perfil inmune de la vacuna inactivada FAdV-8b.

ABSTRACT

Inclusion body hepatitis (IBH) is an economically important fowl adenoviral (FAdV) disease of broiler chickens worldwide. Currently, there is no commercial vaccine available to control IBH in Canada. The objective of this study was to characterize the immune profile of broiler breeders following vaccination with a FAdV-8b vaccine adjuvanted with Emulsigen-D or CpG-ODN. Four groups (n=24/group) of broiler breeders were vaccinated at 16 weeks of age with FAdV-8b (1x106 TCID50/bird) adjuvanted with either 20% Emulsigen-D or 50 µg CpG-ODN. Control groups were administered either saline or FAdV-8b with no adjuvant. Broiler breeders were boosted three weeks later with respective vaccines. Both the Emulsigen-D and CpG-ODN adjuvanted groups induced antibodies level approximately 2 log10 in broiler breeders. A mixed T-cell response ($P<0.05$) was observed in the Emulsigen-D and CpG-ODN adjuvanted vaccine groups. In summary, both the adjuvants improved the immune profile of the inactivated FAdV-8b vaccine.

INTRODUCTION

FAdVs are double-stranded DNA viruses of the family Adenoviridae and genus Aviadenovirus (1). FAdVs are classified into five species (A to E) and twelve serotypes (1-7, 8a, 8b, 9-11) (1). Of these, FAdV-2, -7, -8a, -8b and -11 cause inclusion body hepatitis (IBH) (1). IBH is the most widespread FAdV disease. Its incidence is rising in several continents including North America. Unavailability of effective vaccines against IBH in North America leaves the industry to rely on autogenous vaccines in broiler breeders as a preventative measure (3). Vaccines can be made efficacious by using good quality antigens or by using improved adjuvants.

Oil-emulsion adjuvants such as Emulsigen-D are commonly used in poultry vaccines. However, their effects on immune profiles are not well characterized in chickens with FAdV vaccines. Modern adjuvants, like CpG-ODN, have also shown promise in experimental avian vaccines (2, 5). In chickens, CpG-ODN has been shown to induce specific humoral immunity (IgG and IgA) against enteric and respiratory pathogens (5). However, CpG-ODN has not been evaluated as a vaccine adjuvant with FAdV vaccines (4). Therefore, the objective of this study was to characterize humoral and cellular immune responses of a FAdV-8b vaccine adjuvanted with Emulsigen-D or CpG-ODN in broiler breeders.

MATERIALS AND METHODS

Adjuvants, virus and vaccine. CpG-ODN was commercially synthesized and reconstituted in TE buffer before use. Emulsigen-D was purchased from MVP Technologies (Omaha, NE). The FAdV-8b inactivated vaccine (1x106 TCID50/dose) and was adjuvanted with either Emulsigen-D (20%/dose) or CpG-ODN (50 µg/dose).

Animals and experimental design. Day-old broiler breeders (Ross, Aviagen Inc., Huntsville, AL) were reared and used in animal experiments as per Aviagen guidelines. All experiments were conducted according to the Canadian Council of Animal Care guidelines and were approved by the University Animal Ethics Board. FAdV seronegative broiler breeders were divided into four groups (n=24) which were designated as; Group 1 = FAdV-8b (1x106 TCID50/bird) +20% Emulsigen-D; Group 2 = FAdV-8b 1x106 TCID50/bird) +50 µg CpG-ODN; Group 3 = unadjuvanted FAdV-8b (1x106 TCID50/bird) and Group 4 = saline. Broiler breeders were vaccinated intramuscularly at 16 weeks of age and boosted at three weeks later with respective vaccines. Antibodies were (n=5) were determined weekly for six weeks post-vaccination in sera. Blood samples (n=5) were collected at four days post-vaccination (dpv) and 9 days post-booster vaccination (dpbv) to measure the cytokine expression of T-cells.

Detection of antibodies by virus neutralization assay. Antibodies against FAdV were measured by virus neutralization test in 96 well plates as described previously (6).

Cytokine expression in peripheral blood mononuclear cells. IFN-γ and IL-4 expression in T-cells in PBMCs was determined by flow cytometry. Briefly, PBMCs were stimulated *in vitro* for 8 h with Concanavalin-A (5 µg/mL). Following incubation, the cells were washed twice with 1X PBS and stained for anti-chicken T- cell marker. For intracytoplasmic staining of IL-4 and IFN-γ, cells were fixed by adding 250 µL permeabilization solution/sample (BD Biosciences, San Jose, CA) during vortexing and incubating for 20 min on ice. Following fixation, cells were incubated with biotinylated-rabbit-anti-chicken IL-4 (LS Bio, Seattle, WA) or rabbit-anti-chicken IFN-γ (Thermo Fisher Scientific, Waltham, MA) primary antibodies. The samples were washed twice with 1X BD wash-buffer and incubated with Streptavidin-PE/CY5.5 (BioLegend, San Diego, CA) and goat-anti-rabbit IgG-PE/CY5.5 (Thermo Fisher Scientific, Waltham, MA) secondary antibodies. Subsequently, the cells were washed and resuspended in 200 µL for flow cytometry. Positive, negative and isotype controls were processed simultaneously.

Statistical analysis. The experimental data was analyzed in Prism 7 (Graph Pad Inc. San Diego, CA). The results were analyzed using ANOVA test. The results were considered significant at $P<0.05$.

RESULTS

Serum antibody response in broiler breeders. Vaccination with FAdV-8b antigen alone or saline administration does not induce detectable antibodies in broiler breeders. Antibodies were detected three weeks post-vaccination in broiler breeders when Emulsigen-D or CpG-ODN were used as vaccine adjuvant with FAdV-8b vaccine. The mean antibody levels in either of the groups were greater than 2.90 log10±0.30. The antibody levels peaked six weeks later and were >3.10 log10±0.32 in both the groups. The antibody levels consistently remained at similar levels until the experiment was ended when the broiler breeders were 48 weeks of age.

Cytokine expression on peripheral blood mononuclear cells. The percentage of T-cells which expressed IL-4 and IFN-γ were increased significantly ($P<0.05$) in the FAdV-8b vaccine adjuvanted with Emulsigen-D or CpG-ODN. A mixed response was observed in both the groups, whereas IFN-γ

expressing T cells were observed in FAdV vaccine alone group compared to saline.

DISCUSSION

Adjuvants are added to vaccines to enhance the immune response against vaccine antigens. In this study, we have antibody and cellular immune responses of broiler breeders by measuring antibody levels in serum and IFN-γ and IL-4 production on T-cells following vaccination with FAdV-8b vaccine adjuvanted with Emulsigen-D or CpG-ODN.

Antibodies deactivated the FAdVs in blood and visceral organs (6). Significant antibody response was observed in broiler breeders following vaccination with Emulsigen-D or CpG-ODN adjuvanted FAdV vaccine. Irrespective of the adjuvant type, the mean antibody levels were ≥2.90 log10. The results were in agreement with the previous studies that used inactivated FAdV-8 vaccine (7). We further evaluated cytokine profile of T-cells to determine the type of cellular immunity. We determined IL-4 and IFN-γ expression of T-cells at 9 dpv. We noticed that vaccine antigen alone favors T-cells that produce IFN-γ producing, while formulating FAdV-8b with Emulsigen-D or CpG-ODN guide T-cells to secrete both significantly higher IL4 or IFN-γ. In summary, both the adjuvants improved the immune profile of the inactivated FAdV vaccine in broiler breeders.

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EIMERIA SPP. IN CHICKENS: EFFICACY OF TWO ALTERNATIVE PRODUCTS AND INFLUENCE ON THE GUT MICROBIOTA

EIMERIA SPP. EN POLLOS: EFICACIA DE DOS PRODUCTOS ALTERNATIVOS Y SU INFLUENCIA SOBRE LA MICROBIOTA DEL INTESTINO

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RESUMEN

Eimeria spp. son patógenos intestinales importantes en los pollos. Hay parásitos apicomplexan en la subclase de las coccidias – el nombre que coloquialmente se usa con frecuencia cuando se refieren a la *Eimeria* spp.. Los aditivos anticoccidianos en el alimento, incluyendo antibióticos se han utilizado tradicionalmente para el control de las infecciones de *Eimeria* en la producción de pollos de engorda. Por ende, las tendencias en la producción libre de antibiótico y orgánica requiere de nuevos enfoques en la prevención de la coccidiosis. Hay dos métodos que no son mutuamente exluyentes que son el uso de extractos de plantas con actividad antiparasitaria y la manipulación de la microbiota intestinal pro pre- y probióticos.

Se ha demostrado que algunos pre- y probióticos reducen los efectos adversos después del desafío con varias coccidias, así como la enteritis necrótica. Sin embargo, el conocimiento acerca de la interacción entre la coccidia y la microbiota intestinal es vago y prácticamente depende de los estudios en los que la microbiota se investigó por aislamiento de bacterias.

El objetivo de este experimento fue probar el uso de té verde y vinagre de sidra de manzana que son efectivos contra la infección con *Eimeria* spp.. Además, se investigó la influencia de la infección con *Eimeria* spp. sobre la microbiota del intestino.

Los resultados demostraron que el té verde y el vinagre de sidra de manzana en concentraciones seleccionadas no tuvo un efecto anticoccidiano significativo. Más aún, un cronograma de los cambios en la microbiota del yeyuno se estableció mostrando que la disimilitud de la comunidad tuvo un pico a los 10 dpi cuando la infección fue prácticamente eliminada de las aves.

SUMMARY

Eimeria spp. are important intestinal pathogens of chickens. They are apicomplexan parasites in the subclass coccidia – the name that is often colloquially used when referring to *Eimeria* spp.. Anticoccidial feed additives, including antibiotics, have traditionally been used to control *Eimeria* infections in broiler production. Thus, the trend to antibiotic-free and organic production requires new approaches to coccidiosis prevention. Two not mutually exclusive methods are the use of plant extracts with antiparasitic activity and the manipulation of the intestinal microbiota by pre- and probiotics.

Many home remedies against coccidiosis are used by owners of backyard chicken flocks. Evidence for the activity of these remedies is anecdotal at best. In this study some of these were tested using scientific approaches. The results will not only help backyard flock owners find an effective “natural” treatment and avoid the useless ones, but also direct research into identifying compounds with antiparasitic activity for commercial broiler flocks. One popular treatment is green tea, which is the aqueous extract of the leaves of the tea plant *Camellia sinensis*. It has been suggested that polyphenols, which are abundant in green tea, have antiparasitic activity. The addition of organic, unfiltered apple cider vinegar to the drinking water is another home remedy against coccidiosis. As is the treatment using green tea, the vinegar is not entirely implausible, since acetic acid has been shown to have some anticoccidial activity.

A few pre- and probiotics have been shown to reduce adverse effects after challenge with various coccidia as well as necrotic enteritis. However, knowledge about the interaction between coccidia and the intestinal microbiota is vague and mostly relies on dated studies in which the microbiota were investigated by isolation of bacteria. Very few studies

using current molecular techniques have been published on the topic. However, the information is pertinent for the targeted development and evaluation of pre- and probiotics.

The aim of this experiment was to test if the use of green tea and apple cider vinegar are effective against infection with *Eimeria* spp.. In addition, the influence of the infection with *Eimeria* spp. on the gut microbiota was investigated.

Two hundred forty one-day-old broiler chicks not vaccinated against coccidia were divided into eight groups with five replicates. Each replicate was kept in one cage. *Ad libitum* access to starter feed, not containing anticoccidials or antibiotics, and water were provided during the entire experiment. Four of the eight groups were infected when 14 days old with a five-fold dose of a fully virulent coccidia vaccine containing *E. acervulina*, *E. maxima* and *E. tenella*, while the other four groups were left uninfected. The four tested treatments were green tea, apple cider vinegar, amprolium and an untreated control.

The dosages for the apple cider vinegar and green tea were obtained by employing our backyard poultry advisory council. They reached out to hundreds of their constituents to determine the dosages they used. The dosages we indicated below were those most often used by the majority of backyard flock owners. Freshly prepared green tea prepared with two teabags per liter and diluted 1:2 was dispensed in lieu of drinking water every morning. Apple cider vinegar was added to the drinking water at a dose of 5 ml/L. Amprolium was given at a concentration of 0.012% active substance in the drinking water.

Birds were monitored daily and weighed before the start of the experiment. They were again weighed on the day of the infection as well as 7 and 14 days post infection (dpi). No bird showed clinical signs including diarrhea during the study. Differences in body weight between the groups were not significant ($P < 0.05$). When lesions in duodenum, jejunum and ceca of ten birds per group were scored at 6 dpi, only mild and unspecific lesions were observed. These results reflect the low infection dose that was selected in order to not mask any potential anticoccidial effects of the treatments.

Water consumption was determined daily to make sure that it was not depressed by the treatments. There was no significant difference in water consumption between the groups.

Oocyst counts in the feces from 5 to 9 dpi showed significant reduction by treatment with amprolium 5 dpi, but otherwise the differences in opg between groups were not found to be significant.

Jejunal contents were collected from three birds per group on the day of the infection and 1, 2, 4, 6, 10 and 14 dpi for investigation of the intestinal microbiota by 16S rDNA gene sequencing. Comparison of the untreated-uninfected and the untreated-infected groups showed the maximum community dissimilarity 10 dpi. Close examination of bacterial taxonomic composition revealed that the Clostridia class was significantly enriched in infected compared to uninfected birds.

In conclusion, the results demonstrated that green tea and apple cider vinegar at the selected concentrations did not have a significant anticoccidial effect. Furthermore, a timeline of the changes in the jejunal microbiota was established, showing that the community dissimilarity peaked 10 dpi when the infection was mostly cleared from the birds.

(The full-length manuscript will be submitted to *Avian Diseases*.)

CARACTERÍSTICAS FISICOQUÍMICAS Y MICROBIOLÓGICAS DE LAS EXCRETAS DE GALLINAS DE POSTURA EN JAULA

PHYSICO-CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF EXCRETA OF CAGED HENS

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SUMMARY

To find the physicochemical and microbiological properties of the manure produced by White Lohmann hens maintained in an automated laying house, two subsamples were collected to obtain two compound samples, which were measured in the laboratories of food and bacteriology and mycology at the FMVZ – UAS. Physicochemical properties: pH (5.99), electrical conductivity (15.34 dS / cm 3), bulk density (0.42 g / cm 3), total nitrogen (2.84%), organic matter (74.8%) C / N (19.46), phosphorus (2.1%), potassium (4.09%), calcium (8.03%), magnesium (0.79%), sulfur (0.46%), iron (0.23%), zinc (0.035%), copper (0%), manganese (0.04%), boron (0.01%). The biological properties measured were: nematodes (absent), fungi (absent), Escherichia coli (log 8.4), aerobic bacteria (log 8.3), anaerobic bacteria (log 5.71), nitrifying bacteria (log 6.18) Pseudomonas fluorescent (absent) Bacillus spp (log 7.11). Based on NPK content, it is expected that hen feces is a good residue that can be a good fertilizer prior composting.

RESUMEN

Para conocer las propiedades fisicoquímicas y microbiológicas de las excretas producida por gallinas Lohmann blancas mantenidas en una caseta automatizada, se recolectaron submuestras para obtener dos muestras compuestas, en las que se midieron en los laboratorios de análisis de alimentos y de bacteriología y micología de la FMVZ-UAS, las propiedades físico químicas: pH (5.99), conductividad eléctrica (15.34 dS/cm^3), densidad aparente (0.42 g/cm^3), nitrógeno total (2.84%), materia orgánica (74.8%), relación C/N (19.46), fósforo (2.1%), potasio (4.09%), calcio (8.03%), magnesio (0.79%), azufre (0.46%), fierro (0.23%), zinc (0.035%), cobre (0%), manganeso (0.04%), boro (0.01%). Las propiedades biológicas medidas fueron: nematodos (ausentes), hongos (ausentes), *Escherichia coli* (log 8.4), bacterias aeróbicas (log 8.3), bacterias anaeróbicas (log 5.71), bacterias nitrificantes (log 6.18), *Pseudomonas* fluorescentes (ausentes), *Bacillus* spp (log 7.11). Con base en el contenido de NPK, la gallinaza es un residuo con buena expectativa como fertilizante, previo compostaje.

Las granjas avícolas generan cantidades de estiércol sólido que contienen significativos porcentajes de proteína, materiales lignocelulósicos y resulta de la mezcla de las deyecciones, plumas, residuos de alimentos y huevos rotos, que caen al suelo; a esta acumulación de materia se le denomina gallinaza (Piad, 2001). La gallinaza está compuesta por nitrógeno 3.8%, fósforo 0.65%, potasio 3.79 %, calcio 7.37 %, magnesio 0.96%, carbono 39.9% y materia orgánica 71.9%; contiene 74.53% de humedad, pH 7.18, CE 5.69 dS/m, relación C/N 6:01, (4), además contiene xantofilas, antibióticos, antiprotozoarios, antioxidantes, inhibidores de moho, probióticos, fenoles policlorados, dioxinas, hormonas y bacterias *Actinomycetes, Clostridia/ Eubacteria, Bacilli / lactobacilli* así como bacterias patógenas *Escherichia coli, Salmonella* spp*., Campylobacter jejuni, Listeria monocytogenes y Clostridium perfringens* (3), es por ello que de acuerdo al incremento en el número de animales y la regionalización de la producción se han generado fuertes presiones sobre los productores de huevo de acuerdo a que si las operaciones de producción no son manejadas adecuadamente, la emisión de gases a través de los desechos puede causar contaminación del agua y el aire así como la emisión de gases como amoniaco, metano, sulfuro de hidrógeno, monóxido de carbono, dióxido de carbono, ácidos orgánicos volátiles y compuestos fenólicos, estos olores generados pertenecen a los más ofensivo para la salud humana. Por lo cual el objetivo de este estudio fue evaluar las características fisicoquímicas y

microbiológicas de las excretas de gallinas de postura en jaula.

MATERIALES Y MÉTODOS

Se recolectaron submuestras de excretas producida por gallinas Lohmann blancas mantenidas en una caseta automatizada, en las que se midieron características físico-químicas y microbiológicas, las determinaciones físico-químicas fueron pH (potenciómetro Hanna Instrument con la solución de CaCl 20.01 M), conductividad eléctrica (CE) a partir del extracto de saturación en una relación de 1:2 suelo y agua, se cuantificó (conductímetro modelo Soul-Bridge), materia orgánica (MO) se determinó por la técnica de Walkley y Black y la NOM-021-RECNAT-2000, 2002, posteriormente, el material fue secado a 70 °C y cribado en tamiz No.2, las ceniza se determinaron por incineración (mufla a 600 °C por 2h), macroelementos (Cationes Intercambiables: Ca+2, Mg+2, Na+ y K+) y micro elementos (Fe, Mn, Zn y Cu) mediante el método AS- 12 (NOM-021-RECNAT-2000) y AS-14 (NOM-021-RECNAT-2000) y la determinación de Nitrógeno total fue mediante la técnica Microkjeldhal. Las determinaciones microbiológicas se realizaron por el método de diluciones seriadas, caracterizando e identificando por grupo funcional, los microorganismos (bacterias aeróbicas, anaeróbicas, bacterias fijadoras de nitrógeno, hongos, *Actinomycetes, Pseudomonas* fluorescentes) y nematodos.

RESULTADOS

Los resultados obtenidos se muestra en Tabla 1.

DISCUSIÓN

El contenido de nitrógeno en las excretas (2.84%) es menor al reportado por W.Czekala *et al*., (4) (3.8%). Se considera que el nitrógeno existe en varias formas y se transforma constantemente por la actividad microbiana, cambios en temperatura, pH, humedad y concentración de oxígeno al igual que modifican la disponibilidad del fósforo y del potasio. Se estimó para la gallinaza una relación C/N 19:46 y MO de 74.80%. Estos valores son mayores que los indicados por W. Czekala *et al*., (4), de 10:5 y 71.9%. Por lo cual este material representa un valioso aporte energéticamente para la actividad microbiana Quiroa et al., (2). Respecto a los resultados microbiologicos basados en el cultivo los *Bacillus* spp., *Escherichia coli*, bacterias aeróbicas, bacterias nitrificantes predominaban en las muestras de gallinaza de igual manera que los datos reportados por Lu *et al*., (1).

Por lo cual se concluye que la gallinaza demuestra ser un material que se puede venderse comercialmente y considerarse como uno de los materiales más completos por su contenido en nutrimental y la diversidad de microorganismos, sin embargo, para su buen aprovechamiento, se debe considerar un proceso de descomposición ya que la primera desventaja que debe abordarse es la pérdida de nitrógeno, lo que resulta en un material de menor valor comercial y práctico.

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Tabla 1. Características físico-químicas y microbiológicas de la gallinaza.

Parámetros físico-químicos		Parámetros microbiológicas	UFC/g
pH	5.99	Nematodos	0
CE (dS/m)	15.34	Hongos	0
Densidad (g/cm^3)	0.42	*Escherichia coli* (Log 10)	8.35
Humedad, %	74.75	Bacterias aeróbicas (Log 10)	8.45
Cenizas, %	25.19	Bacterias anaeróbicas (Log 10)	5.88
Relación C/N	19.46	Bacterias nitrificantes (Log 10)	6.47
Materia orgánica, %	74.80	*Pseudomonas* fluorescentes	0
Nitrógeno total, %	2.84	*Bacillus sp* (Log 10)	7.37
Fósforo, %	2.1	*Trichoderma* spp.	0
Potasio, %	4.09		
Calcio, %	8.03		
Magnesio, %	0.79		
Azufre, %	0.46		
Fierro, %	0.23		
Zinc, %	0.035		
Cobre, %	0		
Manganeso, %	0.04		
Boro, %	0.01		

DIAGNOSTICO SITUACIONAL DE LA MYCOPLASMOSIS AVIAR EN MÉXICO

DIAGNOSIS OF AVIAN MYCOPLASMAS IN MEXICO

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SUMMARY

Mycoplasma gallisepticum and *Mycoplasma synoviae* are two diseases that are very important in the poultry industry around the world, the presence or absence of these illnesses means a very big difference in the poultry production results. This is especially important in Central México, where the viral challenges are frequently dangerous by avian influenza (low pathogenesis), Newcastle disease, infectious bronchitis and laryngotracheitis because the virus challenges plus mycoplasmas produce a very high mortality.

There are no official data in México enumerating how many birds or how many flocks are positive to *Mycoplasma gallisepticum* (Mg) and/or *Mycoplasma synoviae* (Ms) in the Mexican poultry industry. This paper evaluates the serologic results of many samples of broiler breeders, broilers, and layers from different states in Mexico using the following tests: plate agglutination, hemagglutination-inhibition, and ELISA. In this way we know if these samples are positive or not, and if they are in our country.

RESUMEN

Mycoplasma gallisepticum y *Mycoplasma synoviae* son dos entidades muy importantes en la Avicultura mundial, su presencia o ausencia implican una gran diferencia en los resultados productivos que consiguen las parvadas en la Industria Avícola, esto es especialmente importante en México en donde en el centro del país son muy frecuente los desafíos virales por Influenza Aviar (de baja patogenicidad), Enfermedad de Newcaste, bronquitis infecciosa y/o laringotraqueitis y la presencia de los desafíos virales y los micoplasmas complican de manera severa los resultados productivos (elevan la mortalidad y la duración del proceso infeccioso).

En México no existen datos oficiales que nos digan cuantas aves o parvadas del inventario nacional se encuentran afectadas por Mg y/o Ms por lo que en el presente trabajo se presentan resultados de evaluaciones serológicas (AP, HI Y ELISA) en diversas parvadas en diferentes regiones del país de aves pesadas y ligeras que nos permiten conocer si efectivamente se encuentran en nuestro País.

MATERIALES Y METODOS

Se utilizaron sueros de parvadas de Reproductoras pesadas, pollo de engorda y de ponedoras ligeras (15 sueros por lote de cada una de las parvadas en todas las Empresas muestreadas y de todos sus lotes) de diferentes partes de la República (San Luis Potosí, Querétaro, Morelos, Puebla, Chiapas e Hidalgo) y se enviaron a un laboratorio de referencia en la Cd. De Tehuacán Puebla, en donde les hicieron a todos los sueros las siguientes pruebas: aglutinación de placa, inhibición de la hemoaglutinación y ELISA contra Mg y Ms.

De ésta manera se puede valorar sin ninguna duda si las aves son positivas o no, es un sistema muy sencillo y practico, pero debe de mantenerse éste orden para checar los sueros: AP primero HI segundo y ELISA en tercer lugar contra cada uno de los diferentes Mycoplasmas y relacionar los resultados de cada una de las diferentes pruebas y la coincidencia entre las pruebas es casi exacta en la mayoría de los casos.

RESULTADOS

Reproductoras Pesadas

Parvada 1 (Sin vacuna)

No. Sueros evaluados 150: AP MG 1+(Dil 1/10 0+) MS 4+(Dil 1/10 0+) HI MG 0+ MS 6+(Dil 1/20 6+) ELISA MG 2 S MS 5 S

Parvada 2 (Sin vacuna)

No. Sueros evaluados 58: AP MG 4+ (Dil 1/10 0+) MS 39 + (Dil 1:10 12+) HI MG 6 1//20 MS 11 1/20 ELISA MG 0+ MS 18 S

Parvada 3 (Con vacuna)

No sueros evaluados 56: AP MG 9+(Dil 1/10 0+) MS 26+(Dil 1/10 8+) HI MG 4 1:20 5 1:40 MS 3 1:20 5 1:40 4 1:80 MS 1 1:20 4 1:40 6 1:80 5 1:160 4 1:320 ELISA MG 4 S MS 1 P

Pollo de engorda

Parvada 4: Muestreados al finalizar el ciclo

No. Sueros evaluados 150: AP MG 0+ MS 16+(Dil 1/10 0+) HI MG 7 1/20 MS 5 1/20 ELISA MG 0+ MS 0+

Parvada 5: Muestreados al finalizar el ciclo

No. Sueros evaluados 24: AP MG 24+(Dil 1/10 14+) MS 24+(Dil 1/10 6+) HI MG 1:20 8+ 1:40 12+ 1:80 7+ 1:160 1+ MS 1:20 2+ 1:40 3+ 1:80 12+ 1:160 6+ 1:320 1+ ELISA MG 17 S MS 4S 8P

Ponedoras

Parvada 6: Sin vacuna

No. sueros evaluados 60: AP MG 25+(Dil 1/10 3+) MS 31+(Dil 1/10 16+) HI MG 1:20 19+ 1:40 10+ 1:80 4+ MS 1:20 12+ 1:40 16+ 1:80 8+ 1:160 12+ 1:320 3+ ELISA MG 5S MS 10 S

CONCLUSIONES

1. En las parvadas estudiadas se encontró una positividad Serológica en todas ellas tanto a MG como a Ms, en diferente grado, pero sí con elementos para decir que ambos gérmenes se encuentran diseminados en los diferentes estados que se muestrearon de la República y en las diferentes funciones zootécnicas (Reproductoras, pollo de engorda y ponedoras).

2. En todos los casos son infecciones mixtas en ningún caso se encontró alguna Empresa que tuviera uno solo de los dos gérmenes.

3. En base a los resultados podemos decir que es mayor la presencia de Ms que de Mg en general en todos los casos.

4. En algunos casos el % de infección es muy bajo sobre todo en 1 parvada de reproductoras y en 1 de pollo de engorda.

5. Se recomienda hacer muestreos con mayor cantidad de sueros para que sea más significativo el resultado (difícil por el costo).

6. Se recomienda establecer un plan Nacional de erradicación de éstos gérmenes. Saber en caso de Reproductoras o ponedoras si son aves vacunadas.

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A CASE OF FATTY LIVER HEMORRHAGIC SYNDROME IN A BACKYARD LAYER

UN CASO DE SÍNDROME DE HÍGADO GRASO HEMORRAGICO EN PONEDORAS DE TRASPATIO

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RESUMEN

El síndrome de hígado graso hemorrágico (SHGH) afecta a las ponedoras comerciales, pollo de engorda comercial y es considerado como una causa de mortalidad no infecciosa en la avicultura de traspatio (2,3). En ponedoras comerciales, los signos clínicos pueden incluir una baja en la producción de huevo y grandes coágulos de sangre encontrados en la mortalidad. En las parvadas de traspatio, este síndrome está asociado con una muerte súbita. Las aves afectadas con frecuencia están obesas y postradas con coágulos de sangre en la cavidad celómica, con un hígado agrandado, de color amarillo pálido con evidencia histopatológica variable de degeneración vacuolar (1,2,4). La causa exacta de esta condición no está definida pero con frecuencia los casos documentados incluyen una combinación de factores de la dieta, hormonales, genéticos y ambientales (1,2). Comúnmente las patogénesis propuestas incluyen: 1) trastornos del marco reticular debido a los hepatocitos agrandados que contienen depósitos de lípidos; 2) peroxidación de lípidos por la cantidad excesiva de estos, causando daño al marco de reticulina y afectando la reparación celular (2). Este caso investiga los descubrimientos en macroscópicos e histopatológicos del SHGH, así como una visita al sitio que ofrece una visión del desarrollo del SHGH en esta gallina.

INTRODUCTION

Fatty liver hemorrhagic syndrome (FLHS) affects commercial layers, commercial broilers and is considered a common cause of non-infectious mortality in backyard poultry (2,3). In commercial layers, clinical signs can include a drop in egg production and large blood clots found in the mortality. In backyard hobby flocks, this syndrome is associated with sudden death. Affected birds are often obese and in lay with coelomic blood clots and an enlarged, pale yellow liver with variable histopathologic evidence of vacuolar degeneration (1,2,4). The exact cause for this condition is not defined but frequently documented causes include a combination of dietary, hormonal, genetic, and environmental factors (1,2). Commonly proposed pathogeneses include: 1) disruption of the reticular framework by swollen hepatocytes containing lipid deposits 2) lipid peroxidation of excessive lipid causing damage to the reticulin framework and affecting cell repair (2). This case investigates the gross and histopathologic findings of FLHS, as well as a site visit that offers insight into the development of FLHS in this hen.

CASE HISTORY

A nine-month-old buff Orpington hen was submitted for necropsy in February 2018 following a history of sudden death with no previous clinical signs per the owner. The hen was part of a small, same-age, mixed breed backyard flock purchased from a hatchery. At the time of death, the remaining flock was healthy.

Gross necropsy. On presentation, the hen was of adequate feathering and weighed 2.46 kg. The coelomic cavity fat pad measured 2-2.5 cm thick at the base. Abundant adipose surrounded coelomic viscera and significant stores were found around the heart apex, heart base and extended up into the pectoral girdles. Both liver lobes were diffusely friable and pale

yellow-tan in color with rounded edges. Surrounding the left liver lobe was a large, dark red, gelatinous collection of clotted blood. Reflection of this clot revealed a hepatic tear surrounded by subcapsular ecchymotic and petechial hemorrhages.

Histopathology. Sections of liver found a focally extensive area of hemorrhage and fibrin adhered to the liver capsule. The hepatic architecture was diffusely disrupted by swollen hepatocytes containing variable sized, discrete, clear vacuoles (lipid). Multifocally, the sinusoids were congested and infiltrated by moderate numbers of macrophages, lymphocytes and plasma cells.

Site observations. Following finalization of the report, a site visit was organized to evaluate the flock thanks to support from the Indiana State Poultry Association. The most relevant finding was the variety of feedstuff offered including meal worms, a homemade treat composed of molasses, corn and oats, and an organic, mixed layer feed containing corn, oats, wheat, and peas. The remaining flock was healthy sans a mild mite infestation. All birds were assessed to be of adequate weight or obese.

DISCUSSION

The clinical history, gross findings and histopathologic findings of this case follow literature on fatty liver hemorrhagic syndrome. A high-energy diet, observed during the site visit, is regularly cited as a contributing cause leading to the development of this syndrome. FLHS is more frequently diagnosed in hens, who are often obese and in lay at the time of death. Typical lesions were observed on necropsy and on histopathology, though it has been noted that vacuolar degeneration is a poor indicator of FLHS severity (2,4). The client was advised to switch the main diet to a pelleted layer feed stored in a leak proof container, as aflatoxins have been implicated as possible causes (2). Other recommendations included removing high-energy treats, careful handling of birds, and observance of biosecurity to prevent introduction of infectious disease as inflammation and other diseases have been suggested in the pathogenesis of FLHS (2). The dietary choices and coop environment designed by the client were determined by online research. With the growing interest and trend in owning backyard poultry, there is a need for quality information on nutrition, non-infectious diseases, and infectious diseases that can be distributed to clients to protect the flock, client and commercial producers.

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MORTALITIES IN CALIFORNIA'S BACKYARD POULTRY CAUSED BY GASTROINTESTINAL IMPACTIONS

MORTALIDADES EN LA AVICULTURA DE TRASPATIO EN CALIFORNIA DEBIDO A IMPACTACIONES GASTROINTESTINALES

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RESUMEN

En contraste a la avicultura comercial convencional, en dónde las aves son criadas principalmente en ambientes controlados en interiores, la avicultura de traspatio es típicamente criada en un entorno menos restringido, potencialmente exponiéndolas a una mayor variedad de sustancias ingeribles, incluyendo muchos tipos de forrajes. Consecuentemente, los problemas divergentes como la impactación gastrointestinal causada por la ingestión de forrajes y otros materiales se han tomado en cuenta en la avicultura de traspatio. Para poder determinar la prevalencia de estas impactaciones en la avicultura de traspatio, se realizó una investigación de las bases de datos en retrospectiva para las presentaciones de necropsias en el sistema de laboratorios de Salud Animal y Seguridad Alimentaria de California (sus siglas en ingles CAHFS) y revelando que las impactaciones gastrointestinales estaban asociadas a muertes de 41 casos de parvadas de traspatio de enero del 2013 a julio del 2018. En un 75.61% (n=31) de estos casos, la impactación fue causada por materiales fibrosos, el 17.07% (n=7) por alimento compactado, y un 7.32% (n=3) por ingestas misceláneas (pedazos de tortilla, plástico suave y viruta de madera). La porción relativamente grande de impactaciones por pasto indica que el comportamiento de forrajeo es la fuente predominante de material de impactación en la avicultura de traspatio y los pastos grandes podrán ser un peligro significativo de salud en la avicultura criada en traspatio.

ABSTRACT

In contrast to conventional commercial poultry, which are primarily raised in a controlled indoor environment, backyard poultry are typically raised in a less restricted setting, potentially exposing them to a greater variety of ingestible substances, including multiple types of forage. Consequently, divergent problems such as gastrointestinal impactions caused by the ingestion of forage and other materials has been noted in backyard poultry. In order to determine the prevalence of these impactions in backyard poultry, a retrospective database search for necropsy submissions to the California Animal Health and Food Safety (CAHFS) laboratory system was performed and revealed that gastrointestinal impaction was associated with the death of 41 backyard flock cases from January 2013 to July 2018. In 75.61% (n=31) of these cases, the impaction was caused by fibrous plant material, 17.07% (n=7) by compacted feed, and 7.32% (n=3) by miscellaneous ingesta (tortilla pieces, soft plastic, and wood shavings). The relatively large proportion of grass impactions indicate that foraging behavior is the predominant source of impaction material in backyard poultry and that long grasses may be a significant health hazard for backyard raised poultry.

INTRODUCTION

The popularity of raising backyard poultry has significantly increased together with the public's growing interest in local food production and less intensive rearing systems (1, 10). Due to differences in husbandry, these emerging backyard poultry owners face new and unique difficulties that are typically not encountered in conventional systems. Conventional poultry producers keep large flocks of birds confined within poultry houses or barns. This allows these producers to have strict control with regard to the types of material the birds may consume and regulate other important aspects of poultry husbandry such as temperature, humidity and biosecurity. In contrast,

backyard poultry owners have small flocks –often only single digits— and follow practices closer to that of free-range poultry, where birds have access to an outdoor environment and forage material during the day. While many diseases such as salpingitis and Marek's disease can be seen in all poultry rearing systems (3, 4, 6), the exposure to the outdoor environment often puts backyard poultry at greater risk for predation and transmissible diseases.

In free-range pastured poultry, gastrointestinal impactions caused specifically by ingested roughage (i.e. grass impactions) have been noted occasionally in the literature (1, 2, 5, 6, 13, 15). In conventional poultry production, grass impactions are not a large concern since flocks do not have access to forage. However, gastrointestinal impactions resulting from ingestion of other materials such as feathers by caged layers (9), bale net wrap by laying hen pullets (14), and litter material by broiler breeders (12) have been reported. This ingestion of non-feed material may be attributed to stress, overcrowding, nutritional deficiencies, and boredom and are not exclusive to commercial poultry (12). In contrast, grass impactions are unique in that they are not due to abnormal behavior, but can be attributed to an overconsumption of forage, consumption of forage that is excessive in length, sudden access to fibrous vegetation, or hydrophilic fiber sources (13). These gastrointestinal impactions are exacerbated and may be more easily elicited by an underlying gastrointestinal or neurological pathology. This report documents the prevalence of gastrointestinal impactions as a cause of mortality in backyard poultry in California, evaluates the risk factors contributing to impactions, and identifies preventative husbandry practices for owners.

MATERIALS AND METHODS

Data were compiled from backyard poultry necropsy cases seen at the California Animal Health and Food Safety (CAHFS) Laboratory System between January 1, 2013 and July 17, 2018. A retrospective accession search was performed using the laboratory database search engine (STARLIMS 10.5.111) to find any necropsy cases of backyard poultry matching the diagnosis key words of "crop impaction", "intestinal obstruction", "intestinal stasis", "crop stasis", "crop obstruction", "duodenal impaction", "proventricular impaction", "ventricular impaction", "grass impaction", and/or "intestinal impaction". Results matching these key words were then manually reviewed to remove any cases where the primary cause of death was not found to be gastrointestinal impaction caused by ingested material.

RESULTS

Case coordinators from 41 out of 6779 submissions found the primary cause of death to be gastrointestinal impaction specifically caused by ingested material. Gastrointestinal impaction cases secondary to neoplasia (Marek's disease lymphomas or carcinomatosis) or other gastrointestinal pathologies (necrotizing enteritis, ventriculitis, etc.) or secondary to lead exposure were not included in this case study (n=57). Case coordinators of the 41 cases with impactions due to ingesta did not indicate underlying or concurrent conditions causing the gastrointestinal stasis including microscopic neuropathology often associated with Marek's disease in the case reports thus these cases were evaluated as is for the purposes of this study.

The 41 gastrointestinal impaction necropsy reports were analyzed to determine the material causing the impaction, of which grass forage material determined to be the most common cause of impaction followed by compact feed (either dry, doughy, or pasty in texture), then miscellaneous food and non-food material (Table 1). Overall, grass impactions made up 0.46% (n=31/6779) of all backyard poultry necropsy cases submitted to the CAHFS laboratories during the study period. Of the 31 grass impaction cases, 16.1% (n=5) of the impactions occurred only in the crop, 3.2% (n=1) only in the gizzard, 51.6% (n=16) only in the small intestine, and 29.0% (n=9) in multiple sections of the gastrointestinal tract.

Information regarding the clinical signs, grit content, and body condition score were also extracted from the 41 ingesta-related gastrointestinal impaction cases. Clinical signs noted in the necropsy reports were anorexia (n=6), lethargy (n=10), seclusion (n=1), coming off lay (n=1), ataxia (n=1), cyanotic combs (n=1), diarrhea (n=1), constipation (n=1), and/or labored breathing (n=1). Four birds presented with no clinical signs noted before sudden death. Clinical signs leading to the spontaneous death of the birds ranged from 0 days (i.e. signs were noted the day of death) to 60 days, lasting an average of 7.57 days. Grit content was only noted in 16 case reports, delineating 5 cases without any grit content, 9 with minimal grit content, and 2 with moderate grit content. Of the reports indicating the body condition of the birds, the majority were in poor/emaciated (n=18) and the rest were in moderate (n=15) condition. Of the 5 cases where the housing of the birds was specified, all were free-ranged (n=3) or pastured (n=2). However, "free-range" is a broad term that can encompass pastured poultry as well, so it is not clear if all these free-range birds had access to forage, though it can be assumed that at least two of the free-range birds were pastured as they passed due to grass impaction. The ages of the

birds ranged from several days to 6 years in the 27 impaction cases where age was noted, with the average age of birds being 1.38 years, and the median age being 1 year.

Of the ingesta-related gastrointestinal impaction cases, 39 occurred in chickens, 1 in a goose, and 1 in a turkey. Breeds that were specified on the necropsy report included Ameraucana (n=4), Araucana (n=1), Plymouth Barred Rock (n=2), Rhode Island Red (n=2), Black Slate Turkey (n=1), Chinese Goose (n=1), Cuckoo Maran (n=1), Dominique (n=1), Leghorn (n=1), Lohmann Brown (n=1), Nankin (n=1), Swedish Flower (n=1), and Wyandotte (n=1).

DISCUSSION

The consumption of long forage material is more likely to lead to impaction in the gizzard or small intestine, whereas the over-consumption of forage is more likely to lead to impactions in the crop (2). This is due in part to the function of the crop and gizzard, which act as the food storage and grinder of the bird, respectively. In order to break down less digestible material (e.g. grass) into smaller particle sizes, the gizzard retains the material for a longer period. Because the crop stores ingesta for the gizzard to digest, the over-consumption of forage leads to an accumulation of material in the crop, which increases the risk of impaction (1). The majority of the necropsy cases seen had impactions in the small intestines as opposed to the crop or gizzard, which may suggest that the management of the available forage is a more significant problem for backyard poultry owners than the amount of forage. Therefore, maintaining a mowed forage area to minimize birds' exposure to long strands of grass or considering preventing birds from foraging altogether should be considered (13).

Grit also has the potential to help prevent impactions (7), although too few necropsy reports specified the grit content found in the birds for an association between grit content and impactions to be made in this study. If grit is not supplemented by owners, backyard poultry will consume grit naturally available in their foraging area, such as small pebbles. However, this natural grit may not be beneficial to help break down grass and may actually hinder the process, as the surface texture, size, and quantity of grit is crucial to its ability to assist with grass breakdown (7). Grit should be rough in texture and small (e.g. 1-1.6mm quartzite particles) to have the highest surface area to volume ratio, and must be supplemented in enough quantity to allow for an adequate grit to grass ratio in the gizzard (7). It must also be noted that grit has been found to aid only in the breakdown of grass that is already in small or narrow pieces (7), so the maintenance of forage length is still crucial. While impactions in the small intestine are not affected by supplementing grit (2), in the case of forage accumulation in the crop or gizzard, supplementing grit may be sufficient to break down the material and prevent impactions.

Different genetics affect the average forage intake of a bird (15), so it is possible there are certain breeds that are more likely to develop impactions, but a breed predilection could not be determined in this study due to the wide variety of breeds affected and the lack of a controlled study to assess breed differences. In addition, the cases described are limited to the population of poultry cases that were submitted to the CAHFS laboratory system, and not all necropsy reports specified the breed.

As noted in this case study, various ingesta such as compacted feedstuff, tortilla pieces, soft plastic and wood shavings can cause obstructions and impactions as well. Therefore, it is also important for owners to practice proper husbandry methods to prevent or minimize these exposures. Certain types of ingesta such as dried grains or tortilla tend to expand in the gastrointestinal tract, especially if this feed consumption is followed shortly by water consumption (9). Owners may choose to provide grit as described above to help hens process these feeds and/or consider soaking these feeds before feeding to prevent expansion in the gastrointestinal tract, or avoid them altogether. The consumption of inedible material (e.g. wood shavings) is often a result of stress, thus birds that are placed in novel environments or otherwise stressful situations are more prone to eating non-feed substances (12). For this reason, owners should do their best to mitigate stressful situations for their flock as well as ensure there are no easily consumed small objects in the poultry area to minimize the potential of ingesting materials which can lead to impactions.

In addition to management of forage and feed, backyard poultry owners are encouraged to monitor for potential impactions in birds by observing for signs of impaction such as anorexia and lethargy, as well as palpating crop contents periodically to ensure the crop is not swelling (5). If potential impactions are found, owners may opt to try and decrease the pH of the diet to help pass compacted material through the gastrointestinal tract (13) or supplement the diet with Epsom salts and molasses to create a laxative effect (2). However, it should be noted that surgical intervention may be required to remove impactions (8). To reduce the chances of gastrointestinal impactions being triggered by Marek's disease, owners should also strongly consider obtaining birds that are vaccinated against Marek's (11). As pastured and free-range poultry become more common both commercially and in backyard settings, optimizing

foraging conditions to mitigate gastrointestinal impactions is an important and unique husbandry consideration. In addition, a greater understanding of poultry genetics, forage selection, and welfare (i.e. alternative enrichments) may help mitigate gastrointestinal impactions in the future.

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Table 1. Ingested material causing impactions in backyard poultry necropsies.

Material	Percent of Cases
Grass/Forage	75.6% (31)
Feed	17.1% (7)
Tortilla	2.4% (1)
Soft Plastic	2.4% (1)
Wood Shavings	2.4% (1)
Total	100% (41)

THE INDUSTRY APPROACH FOR VELOGENIC GENOTYPE XII NEWCASTLE DISEASE OUTBREAKS CONTROL IN PERU

EL ENFOQUE DE LA INDUSTRIA PARA EL CONTROL DE LA ENFERMEDAD DE NEWCASTLE VELOGÉNICO TIPO XII EN PERÚ

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RESUMEN

La enfermedad de Newcastle (EN) es una preocupación mayor en los países endémicos y su control requiere de intervenciones de bioseguridad y múltiples vacunaciones. Un virus de EN genotipo XII actualmente circula en Perú, generando brotes continuos y pérdidas económicas. El objetivo de este trabajo fue el evaluar la protección proporcionada por los diferentes programas de vacunación usados por la industria peruana. El Grupo A= vector comercial HVT + EN, vivo (aspersión) e inactivado (SQ); Grupo B = el mismo programa usando un diferente vector HVT + EN (Boehringer Ingelheim); Grupo C = vector comercial HVT + EN, vivo (aspersión) y el Grupo D = desafiados sin vacunar. Las aves fueron desafiadas con virus de EN velogénico genotipo XII. El rango de protección va del 83 al 100% en los grupos vacunados. Las aves en el Grupo B alcanzaron los niveles de protección más altos cuando se compararon con los otros grupos y los grupos con o sin vacuna inactivada. Los resultados sugieren que la presencia del desafío del genotipo XII agresivo del vHVT-EN, las vacunas inactivadas y vivas del EN necesitan tener protección complementaria.

SUMMARY

Newcastle disease (ND) is a main concern for endemic countries and its control requires biosecurity interventions and multiple vaccination. A genotype XII ND virus currently circulates in Peru generating continuous outbreaks and economic losses. The aim of this work was to assess the protection provided by the different vaccination programs used by the Peruvian industry. Group A= commercial vector HVT+ND, live (spray) and inactivated (SQ), Group B= same program using a different vector HVT+ND (Boehringer Ingelheim); Group C= commercial vector HVT+ND, live (spray) and group D= Unvaccinated challenged. The birds were challenged with a velogenic genotype XII ND virus. The protection ranged between 83 and 100% in the vaccinated groups. The birds in group B reached the highest protection levels when compared with the other groups with or without inactivated vaccine. The results suggest that in the face of a harsh genotype XII challenge vHVT-ND, inactivated vaccines and live ND are needed to complement protection.

INTRODUCTION

Newcastle disease infects many species and different types of birds, mainly affecting fighting birds, broilers and commercial layers. The Newcastle disease virus (NDV) is a member of the genus Avulavirus of the family Paramyxoviridae. Only APMV-1, can cause Newcastle disease (ND) in domestic poultry (6). The clinical signs and lesions of the disease are not pathognomonic, vary with viral strain, host, age, level of immunological protection and other factors and can go from 100% mortality in unvaccinated birds to egg production drops in seemingly healthy layers (5).

The disease control is based on proper diagnostics, biosecurity measures and vaccination. Inactivated and live vaccines, including HVT vectorized vaccines, are widely used for vaccination. In high-risk countries, broilers are vaccinated with programs that also include traditional live and inactivated vaccines. Vaccines vectorized in HVT containing the fusion gene (F) are used with great success; this type of vaccine induces both humoral and cellular immunity and reduces viral excretion after exposure (4). The objective of this study was to compare the protection against a lethal ND challenge (Genotype XII) provided by commercial vaccine combinations used in Peru: vHVT+ND and live (with or without inactivated).

EXPERIMENTAL DESIGN

Group A= commercial vector HVT+ND (SQ), live (spray) and inactivated (SQ), Group B= same

program using a different vector HVT+ND (Boehringer Ingelheim); Group C= commercial vector HVT+ND, (SQ) live (spray) and group D= Unvaccinated challenged. All vaccinated groups were raised in the field brought to the laboratory to be challenged at 30 days of age by eye drop (50uL/bird) with a velogenic viscerotropic genotype XII ND virus (108 DIE50). The strain used Chicken/Arequipa-Perú/VFAR81/2015 (GenBank accesion no. KU594618) has a ICPI of 1.88 (3).

RESULTS AND DISCUSSION

The clinical signs in the susceptible birds included periorbital inflammation, depression, greenish diarrhea and nervous signs. No torticollis was observed on the surviving vaccinated birds. Mortality in groups A and D started as early as day 4. Group B had no mortality after a 10 days observation period, while mortality was observed in the other groups: C (5/30 birds = 17%), A (3/30 birds = 10%) and D (30/30 = 100%) as shown in graph 1.

The protection to a lethal genotype XII Peruvian ND virus provided by vector HVT+ND and live hatchery vaccination (with or without inactivated) was measured mortality as criteria. The unvaccinated challenged control showed 100% mortality while the inactivated, live and HVT+ND groups (A & B) showed a viability of 90 and 100% protection, respectively. The protection in the group without the inactivated vaccine dropped to 83%. Previous studies in our laboratory has demonstrated that in Peru are circulating highly virulent ND viruses leading to the necessity to built up on the protection using inactivated vaccines (2,3). This as shown in this study where the groups with inactivated vaccines had better performance and in concordance with our previous results for Peru (1). Until a few years ago, with strains of lower pathogenicity such as the peacock strain (ICPI: 1.82), we achieved acceptable protection without the inactivated vaccine, but currently with strains of greater virulence (1.88) we cannot, perhaps, besides the greater virulence of strains, genetics, inadequate infrastructure and inadequate vaccination procedures contribute to increased stress, susceptibility and reduced vaccine protection.

CONCLUSIONS

The results suggest that using inactivated vaccines in the hatchery can provide between 7 y 17% higher protection in a combined program including live (spray) and vector HVT+ND (SQ) vaccines and that a difference can be observed in the protection provided by different vector vaccines within the program. Under a stringent highly virulent challenge like the one present for the Peru poultry industry, it is necessary the use of inactivated vaccines to booster the protection levels provided by the broiler vaccination programs.

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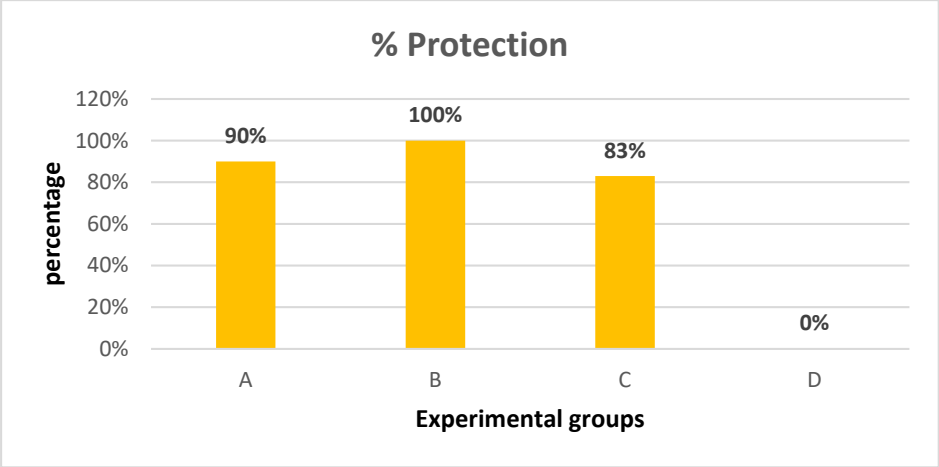
Graph 1. Cumulative viability (percentage protection) up to 10 days post challenge.



INFECTIOUS BRONCHITIS VIRUS AND ITS VARIANTS: DIFFERENT VACCINATION STRATEGIES FOR PREVENTION AND CONTROL

VIRUS DE BRONQUITIS INFECCIOSA Y SUS VARIANTES: DIFERENTES ESTRATEGIAS DE VACUNACIÓN PARA LA PREVENCIÓN Y EL CONTROL

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RESUMEN

El virus de la bronquitis infecciosa (VBI), un coronavirus, es un virus ARN envuelto que causa una enfermedad del aparato respiratorio superior altamente contagiosa en pollos. Debido a que es un virus ARN, por naturaleza puede cambiar muy rápidamente cuando se replica en el hospedador, VBI existe como múltiples tipos diferentes y variantes de dichos tipos, los que comparten muy poca o ninguna reacción cruzada. En consecuencia, las vacunas desarrolladas contra un tipo de virus es muy probable que no provean una protección adecuada contra otro tipo. El VBI fue inicialmente tipificado usando los ensayos de anticuerpos en la neutralización del virus pero ahora el tipo de VBI es identificado generalmente usando la secuencia viral del pico de glicoproteína. El tipo genético de virus circulante en el campo puede informarnos sobre la selección de una o más vacunas comerciales disponibles para la prevención y el control, reconociendo que una vacuna homóloga al virus circulante es la mejor estrategia para el éxito. Si una vacuna homóloga no esta disponible, entonces una combinación de tipos de VBI algunas veces puede presentar una protección aceptable con la meta de reducir la replicación del virus de campo así que la transmisión se puede prevenir o minimizar. Se han hecho numerosos estudios examinando diferentes combinaciones de tipos vacunales de VBI contra las variantes del virus y que información puede ser extremadamente valiosa cuando se formule la estrategia de vacunación. Sin embargo, es imposible en este momento el predecir cual tipo de vacuna de VBI o combinación de tipos podrían proveer una protección aceptable contra una nueva variante del virus que circula en el campo. La única forma segura de saber si una vacuna o combinación de estas proveerán una protección adecuada es conducir estudios de desafío de vacunas en pollos. Esta presentación revisará los tipos de VBI y sus variantes circulando en el campo y discutirá sobre varias estrategias de vacunación usadas contra estos.

ABSTRACT

Avian coronavirus infectious bronchitis virus (IBV) is an enveloped RNA virus that causes a highly contagious upper-respiratory tract disease in chickens. Because RNA viruses by nature can change rapidly when they replicate in the host, IBV exists as multiple different types and variants of those types, which share little to no cross-reactivity. Consequently, a vaccine developed against one type of the virus will likely not provide adequate protection against another type. IBV was initially typed using antibodies in virus neutralization assays but now IBV type is identified genetically using the sequence of the viral spike glycoprotein. The genetic type of the virus circulating in the field can inform the selection of one or more commercially available vaccines for prevention and control, recognizing that a homologous vaccine to the circulating virus is the best strategy for success. If a homologous vaccine is not available, then a combination of IBV vaccine types can sometimes provide acceptable protection with the goal of reducing the replication of the field virus so transmission can be prevented or minimized. There have been a number of studies examining different combinations of IBV vaccine types against variant viruses and that information can be extremely valuable when formulating a vaccine strategy. However, it is impossible at this time to predict which IBV vaccine types or combinations of types will provide acceptable protection against a new variant virus circulating in the field. The only sure way to know if a vaccine or combination of vaccines will provide adequate

protection is to conduct vaccine challenge studies in chickens. This presentation will review the IBV types and variant viruses circulating in the field and discuss the various vaccine strategies used against them.

INTRODUCTION

Avian infectious bronchitis is found worldwide and is an economically important disease, costing the poultry industry millions of dollars annually due to decreased production, condemnations at processing and mortality. The etiologic agent is avian infectious bronchitis virus (IBV), an enveloped positive-sense single stranded RNA virus that causes a highly contagious upper-respiratory tract disease in chickens. Currently, the best and only strategy to control IBV is the use of attenuated live and killed vaccines (6). In general, live vaccines are given to broilers at one-day of age in the hatchery and sometimes in the field at 14 to 18-days of age. Killed IBV vaccines, which must be injected are used after live priming vaccines in breeders and layers to provide long-lived immunity. Regardless of the kind of vaccine used, complete protection is difficult to establish because different IBV types do not cross-protect. In addition, application of live and killed vaccines is difficult to do properly, and equipment failures, mishandling of the vaccine, poor technique and cutting the dose can lead to flocks that are not adequately protected.

The purpose of vaccinating against IBV is to prevent clinical signs of the disease but it is also important to reduce pathogenic field virus replication and subsequent transmission. Like all positive sense RNA viruses, IBV has an enormous capacity for developing genetic diversity when it replicates. Coronaviruses are single stranded positive sense RNA viruses. The viral genome, which is very large (~32Kb), codes for the viral RNA dependent RNA polymerase (RdRp, genes 1a and 1ab), 4 structural proteins (Spike [S], Membrane [M], Envelope [E], and Nucleocapsid [N]) and numerous regulatory proteins.

Surveillance of IBV types circulating in the field is a critical and necessary component of a successful control strategy. Diagnosis of IBV is almost exclusively accomplished using molecular techniques. Viral RNA can be detected by reverse transcriptase-polymerase chain reaction (RT-PCR) and by real time RT-PCR. Tests are designed to detect all IBV types followed by sequencing the S1 gene to determine genetic type or by type specific real time RT-PCR. Determining the current IBV type(s) circulating in the field is necessary to select effective vaccines and design appropriate vaccine strategies to control the virus.

IBV VARIANTS

Genetic diversity among IBV types is generated through mutations, insertions, deletions and recombination events as the virus replicates. The very large viral genome has a mutation rate as high as 1.2 X 10^{-3} substitutions/site/year. This high rate of mutation contributes to the many variant types of the virus in the field. The S glycoprotein is the most studied structural protein found in coronaviruses. The S glycoprotein consists of two subunits designated S1 and S2 and forms club shaped projections on the surface of the virus particles (2). The S1 subunit mediates host cell attachment and is for the most part responsible for host cell specificity. In addition neutralizing antibodies are directed against the S1 subunit (1). The S2 subunit is anchored in the envelope of the virus and is non-covalently connected to S1. Antigenic diversity (variant viruses) are generated when mutations, insertions, deletions and recombination events occur in the spike gene. This can result in a significantly different spike protein on the surface of the virus and consequently a new IBV genetic and antigenic type. A review of the literature reveals a number of viruses with evidence of recombination, but by and large new IBV types almost always emerge through mutations in S accumulating over time, referred to as genetic drift. When the virus is free to replicate, and transmit to naïve or partially protected birds, mutations accumulate and those mutations that provide a fitness advantage will be maintained. If the mutations are in the S gene, the result will eventually be the emergence of a new IBV type.

Some of the important IBV types in the USA and Mexico are listed in Table 1, and their relationship to each other in the S1 glycoprotein can be seen in the phylogenetic tree in Figure 1. In the USA, the predominant viruses circulating in commercial poultry are GA08, GA13 and DMV/1639. Arkansas and Mass types are also isolated but they are largely vaccine related isolations. In Mexico, the most recent reports of IBV types in the literature and in GenBank (www.ncbi.nlm.nih.gov) are for IBV types isolated between 1998 and 1999, 20 years ago. Those viruses genetically aligned with Conn, Mass, Mexico/1765/99 (similar to Mexico/7277/99 in table 1) and BL56/96. This lack of information regarding current IBV types in Mexico is concerning since it makes it extremely difficult to design effective vaccination control programs.

IBV AND TRANSMISSION

To develop an effective vaccine strategy to control IBV, it is necessary to know what types of the

virus are circulating in commercial poultry. It is also important to maintain a timely surveillance program to keep track of this ever-changing virus so vaccines can be updated appropriately. Effective vaccination against IBV significantly reduces virus replication and stops virus transmission. The average number of new infections caused by one infected individual, is referred to as the R0 number and for IBV it is 19.95. This means that 1 infected chicken can spread the virus to almost 20 susceptible chickens. However, the R0 number for a fully protected chicken is 0.69 (4). When R0 is greater than 1, the outbreak will continue to spread, however; when R0 is less than 1 the outbreak will die out. Therefore, vaccination has two important outcomes. It lowers R0 to a number less than 1 so the outbreak doesn't spread, and it reduces replication of the virus thereby diminishing the possibility of new viruses emerging.

IBV CONTROL

No protection or only partial protection against field viruses is not uncommon and can be due to poor cross-protection from the vaccine(s) being utilized or improper vaccination technique. In addition, vaccines if improperly applied can persist in the flock and potentially revert to virulence. It is well established that IBVs with completely different spike proteins do not cross protect and that homologous attenuated live vaccines provide the best protection (6). However, we do not have homologous attenuated live vaccines for all the different IBV types found in chickens. An IBV type is defined by cross-reactive neutralizing antibodies (serotype), S1 protein sequence (genetic type) or cross-protection in chickens (protectotype). Most all diagnostic efforts now identify the genetic type of the virus and genetic type for the most part correlates with serotype although there are a few exceptions. Generally cross-protection decreases with declining percent similarity in the sequence of the S1 protein, however; some vaccine types have been shown to provide better cross-protection than others. Likely the extent of conserved regions in the spike protein between different IBV types contribute to the cross-protection observed. In addition, the strength of the immune response can also contribute to cross-protection, and the number of vaccinations received and the combination of different IBV vaccine types can maximize the development of cross-protective antibodies. It should be recognized that cross-protection in chickens, cannot be predicted with confidence and must be tested *in vivo* (3).

Developing a vaccination strategy with either homologous vaccine types or with a combination of heterologous vaccine types given multiple times is important for the control of IBV because it can reduce field virus replication below transmission levels, which prevents or at least slows the emergence of new antigenic variants of IBV, which may be capable of causing disease.

SUMMARY

Avian coronavirus IBV is worldwide in distribution and causes significant economic losses. Control of IBV is largely through the use of live attenuated and killed vaccines but protection is difficult to establish because different IBV types do not cross-protect. Different types of IBV are generated when the virus replicates, and although recombination can play a role, for the most part, replication leading to mutations, deletions and insertions in the spike glycoprotein gene, accumulating over time (genetic drift), most often leads to the emergence of new IBV types. Vaccination if not done correctly or if the wrong vaccine type or combination of vaccine types are used, only partial protection is achieved which allows field viruses to continue to replicate and transmit among chickens providing an opportunity for mutations to occur. If those mutations occur in the S gene, they can lead to the emergence of new variant viruses potentially capable of causing disease. Key to a control program for IBV is widespread and timely surveillance. Knowing the types of IBV currently circulating in a given area is critical and will ensure that the vaccines and the vaccine program selected is the best strategy to control this economically important disease.

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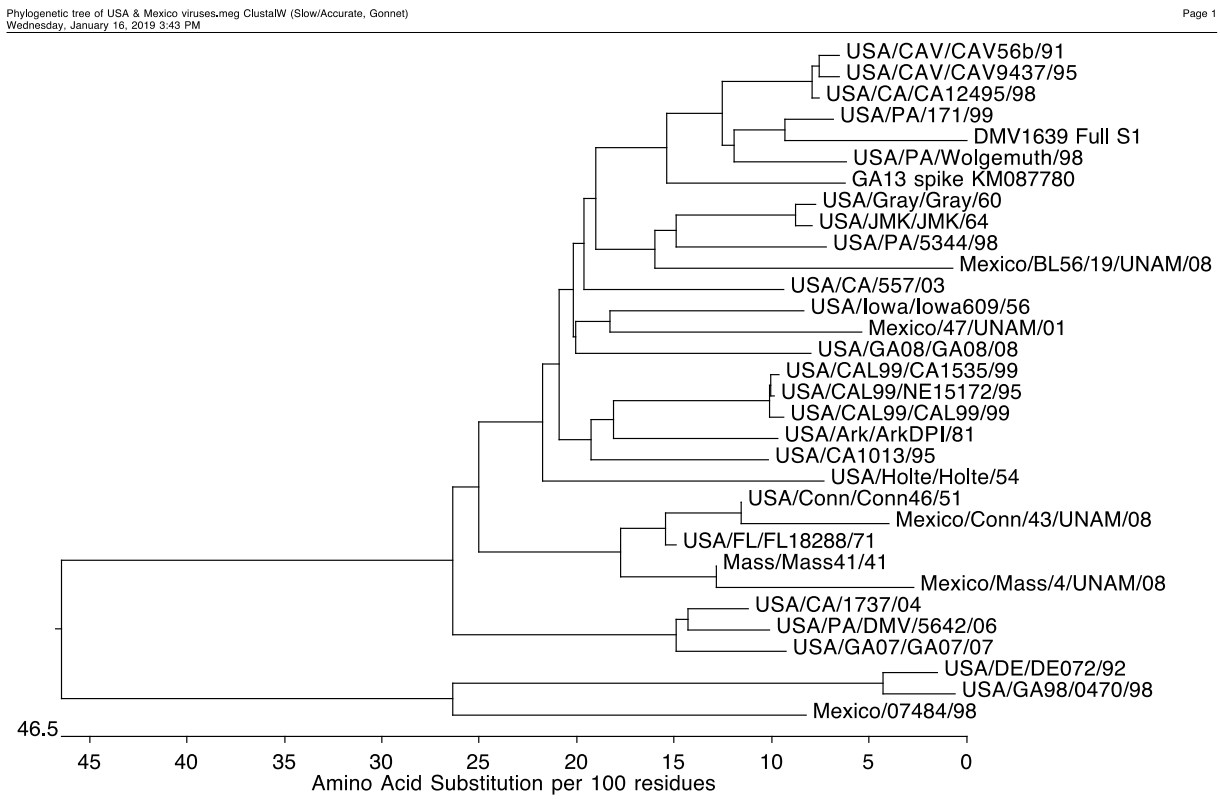
Table 1. Some important infectious bronchitis virus types in the USA and Mexico

Country	Strain	Type	GenBank #
United States of America	Ark/ArkDPI/81	Arkansas	AF006624
	CA/557/03	California	DQ912828
	CA/1737/04	California	DQ912830
	CA/CAL99/CAL99/99	Cal99	DQ912831
	CA/CAV/CV56b/91	California Variant	AF027509
	Conn/Conn46/51	Conn	L18990
	DE/DE072/92	Delaware	U77298
	DMV/1639/14	Delaware	KR232396
	GA/GA11/124/11	GA11	NA[B]
	GA/GA08/GA08/08	GA08	GU361606
	GA/GA07/GA07/07	GA07	JN160805
	GA/GA98/0470/98	GA98	AF274437
	Mass/Mass41/41	Mass	AY561711
	PA/Wolgemuth/98	Wolgemuth	AF305595
	PA/171/99	PA/171	AF419314
Mexico	Mexico/Ark type	Arkansas	NA
	Mexico/Conn/43/UNAM/08	Conn	EU526403
	Mexico/Mass/4/UNAM/08	Mass	EU526411
	Mexico/47/UNAM/01	?[B]	EU526405
	Mexico/7277/99	?	AF363596
	Mexico/BL56-19/UNAM/08	BL56	EU526407
	Mexico/07484/98	?	AF288467
	Mexico/UNAM-97/97	?	NA

Figure 1. Phylogenetic analysis showing the genetic relationships for the S1 protein among IBV types from the USA and Mexico.



MANAGEMENT OF CHICKEN WASTE

MANEJO DE LOS DESECHOS DE LOS POLLOS

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RESUMEN

El manejo correcto de los desechos de las aves y en general de varias especies, presenta por sí mismo una serie de ventajas, y al no hacerlo estamos contribuyendo a la contaminación del ambiente ya que es un desecho que contiene elementos que pueden contaminar al ambiente.

SUMMARY

The correct handling of waste of birds and in general of any species, brings in itself a series of advantages, and by not doing so we are contributing to the contamination of the environment since it is a waste that contains some elements that can contaminate to the environment.

INTRODUCTION

The laying hen produces an average of 150 to 200g of fresh feces, which is considered a source of pollution due to the high content of nitrogen and phosphorus present, as well as containing a large amount of moisture. With the proper management of chicken manure, volume can be reduced, odors eliminated and possible pathogens destroyed. The beneficial effect of using manures of different species including birds has been known for some time. Until the end of the 19th century, agriculture depended mainly on manures to obtain good harvests; however, this changed rapidly with the production of chemical fertilizers. With natural gas sources, cheap and abundant for the synthesis of ammonia, chemical fertilizers became so affordable that the manures were displaced.

But due to current organic, ecological or sustainability trends, organic fertilizers have once again become important (1).

Previously, the animals were raised dispersed over large areas of land, with an immediate and economic incorporation in situ of their manure to the land where they grazed. Currently with the structuring of production basins, livestock tends to centralize in smaller areas, this procedure, although productively is more efficient, lower cost and with a more adequate control and prevention of diseases, causes the accumulation of large quantities of manures which is far from the agricultural fields where it could be used, this results in the production of unfavorable environmental conditions by contamination of odors, nitrates, salts, biological, bacteriological and landscape among others. In Mexico, manure is obtained mainly from cattle, followed by laying hens, pigs, cunicels, goats, among others, the quality of manure varies according to its origin, laying hens dung is twice as rich in nitrogen and about five times richer in phosphorus than bovine (2). Manures can be obtained in semi-solid semi-liquid state, fresh with little or no dilution of water, in any of these forms can be applied directly to agricultural land, which improves or degrades soil fertility if not handled properly. The stacking of manure outdoors until the time of application, which in Mexico is usually once a year, causes loss of nutrients by the action of the sun, rain and wind also causing a serious problem of environmental contamination and damage to the inhabitants (3). In Mexico, it is estimated that in 1920 there was a waste of manure of 50% due to poor management and application, according to the livestock census. Currently it is feasible to obtain 49 million tons of manure per year. The degradation and stabilization of manures and any type of organic matter occurs spontaneously in nature with the participation of organisms present in the soil, which take their energy and food from it.

With this background, it was proposed to carry out an adequate management of the waste from the experimental area of egg-producing birds for the dish of the Poultry Center of the UNAM, which has a flock of 1,600 laying hens of light race, the purpose of these birds is to perform thesis of Master and Doctorate, fulfill an important function in the teaching of subjects that the curriculum of the FMVZ-UNAM contemplates on the other hand it supports the poultry industry in its investigations.

In the present work we will explain what is the management that is done to the waste generated by these birds in the area since it produces an average of 240kg-320kg of fresh feces per day, when properly handling this waste we will reduce the volume in less 50% of the original volume, reducing odors, propagation of pathogens and noxious fauna. The final destination of the laying hens manure is to fertilize the fields, if this material is handled correctly we are producing a fertilizer rich in nutrients for the soils with the benefits that this entails both for the center and the final consumer. For the handling of waste, the most important thing is to have a roofed fenced area with wide doors since, as we mentioned previously, if the process is done in an open place, we can lose nitrogen and phosphorus among others that are important for the development of what we are going to fertilize, we have 8 containers of 6m³ each, although we could do without them, the next thing is to have carbon sources or substrate can be leaf litter, wood chips, sawdust, straw among others and most importantly the excreta, starts with a bed of 20 cm of substrate and then a bed of 10 cm of chicken droppings is placed, repeating until reaching a height of less than one meter and above 80 cm (4 layers). The layers can be made (photo 1) or they can be mix the materials trying not to exceed the m³ (photo 2). Between day two and three the temperature increases until reaching 70° C (photo 3), with this temperature we eliminate pathogens and a seed that contains the substrate in a static form the biopile. If the turning process is carried out two or three times a month, this has the advantage of accelerating the microbiological process, we have done it in both ways, what we are doing is an aerobic and anaerobic composting the fermentation process lasts between 30 and 40 days at the end of which what is done is to wait for the biopile to mature which is between 3 and 4 months and can already be used as a fertilizer. We can conclude, the proper management of solid waste generated by laying hens in the research area, will allow us to continue producing food of animal origin in addition to showing future doctors the responsible use of solid waste and being more environmentally friendly ambient.

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Figure 1. Presentation of a static biopile in layers of sawdust compost.

Figure 2. Mesh of sawdust substrate, dry leaves, and laying hen feces.

Figure 3. Temperature reached during the composting process (68°C).

FIELD SAFETY OF *IN OVO* AND SUBCUTANEOUS ADMINISTRATION OF INNOVAX®-ND-IBD (HVT-NDV-IBD) AND INNOVAX®-ND-ILT (HVT-NDV-ILT) VACCINE IN COMMERCIAL BROILERS

SEGURIDAD EN CAMPO DE LA ADMINISTRACIÓN *IN OVO* Y SUBCUTÁNEA DE LAS VACUNAS INNOVAX®-ND-IBD (HVT-NDV-IBD) E INNOVAX®-ND-ILT (HVT-NDV-ILT) EN POLLO DE ENGORDA COMERCIAL

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RESUMEN

Se han conducido estudios para establecer la seguridad en campo de las vacunas Innovax-ND-IBD y la Innovax-ND-ILT, administradas por ruta *in ovo* en embriones de 18 a 19 días de edad o por ruta subcutánea a pollos de un día de edad bajo condiciones de campo. Los estudios de seguridad de campo fueron conducidos en cuatro diferentes estados de los EE. UU. en cuatros sitios de ubicación. Todos los pollos fueron observados buscando mortalidad o signos sistémicos y localizar eventos adversos hasta los 21 días de edad. La tasa de mortalidad se presentó en un rango aceptable para cada productor así como las tasas de decomiso en el rastro para todos los sitios. No se presentaron eventos sistémicos adversos relacionados a la vacunación. No se presentaron reacciones localizadas en el sitio de inyección de ninguna de la vacunas probadas en los pollos, cuando se evaluaron los sitios de inyección después de la vacunación. Estos datos proporcionaron apoyo para la seguridad de las vacunas Innovax-ND-IBD e Innovax-ND-ILT para ser utilizadas por las rutas *in ovo* y subcutánea bajo condiciones de campo.

SUMMARY

Studies were conducted to establish the field safety of Innovax-ND-IBD and Innovax-ND-ILT, administered by the *in ovo* route in 18- to 19-day-old

chicken embryos or by the subcutaneous route in day-old chickens under field conditions. The field safety studies were conducted in four different states in the US with four site locations. All chicks were observed for mortality and signs of systemic and localized adverse events until 21 days of age. Mortality rates were within the acceptable range by each producer as were condemnation rates at slaughter for all sites. There were no systemic adverse events related to vaccination. There were no localized injection site reactions in any of the test vaccinated chicks when injection sites were evaluated following vaccination. These data provide support for the safety of Innovax-ND-IBD and Innovax-ND-ILT vaccine for use by the *in ovo* and subcutaneous routes under field conditions.

INTRODUCTION

Immunization of chickens with live, inactivated or recombinant vaccines, is extensively used in the poultry industry world-wide and vaccination is considered the cornerstone of infectious disease control in all segments of poultry production (3). Several recombinant HVT-based vaccines have been developed and used successfully to protect poultry flocks against a number of important avian pathogens (1). However, to date, these vaccines offer a limited range of protection and are seldom combined and used for concurrent vaccination against multiple diseases due to the suspected interference among different HVT-based vaccines. Until now, the development of recombinant vaccines that can protect against multiple diseases from one vector with a single dose delivered by the mass application has proven difficult especially in the case of HVT (2). To overcome the shortcomings, we successfully constructed a double recombinant HVT-vectored vaccine (HVT-NDV-IBD) and (HVT-NDV-ILT) that provide excellent protection against three key poultry pathogens from a single HVT backbone with a single dose given by mass application.

The present report describes the field safety studies of Innovax-ND-IBD (HVT-NDV-IBD) and Innovax-ND-ILT (HVT-NDV-ILT) vaccine administered by the *in ovo* route in 18- to 19-day-old chicken embryos or by the subcutaneous route in day-old chickens under field conditions. The recombinant HVT vector vaccine, designated HVT-NDV-ILT, contains both the ILTV gD plus gI genes, and the NDV-F gene. The recombinant HVT vector vaccine, designated HVT-NDV-IBD, contains both the IBDV VP2 gene, and the NDV-F gene.

STUDY DESIGN

For the HVT-NDV-IBD field study, a total of 264,400 birds (167,800 *in ovo*; 96,600 subcutaneous), representing 4 distinct US geographical locations were vaccinated with the test vaccine HVT-NDV-IBD and placed at grower farms. For the HVT-NDV-ILT field study, a total of 186,800 birds (94,200 *in ovo*; 92,600 subcutaneous), representing 3 distinct US geographical locations were vaccinated with the test vaccine HVT-NDV-ILT and placed at grower farms. Control chicks were vaccinated with commercially available Marek's vaccines in the same manner and at the same age as the test groups and placed in separate houses on the same commercial broiler farm. All chicks were observed for mortality and signs of systemic and localized adverse events until 21 days of age.

RESULTS AND DISCUSSION

For the HVT-NDV-IBD field study, the daily mortality rate during the first 21 days from hatching was no more than 0.53% and total 21-day cumulative mortality was no more than 2.3% in the tested vaccine (HVT-NDV-IBD) group. The daily mortality rate during the first 21 days from hatching was no more than 0.57% and total 21-day cumulative mortality was no more than 2.5% in the control group. Condemnation rates ranged from 0.03% to 0.34% in the tested vaccine (HVT-NDV-IBD) and control groups.

For the HVT-NDV-ILT field study, the daily mortality rate during the first 21 days from hatching was no more than 0.31% and total 21-day cumulative mortality was no more than 2.0% in the tested vaccine (HVT-NDV-ILT) group. The daily mortality rate during the first 21 days from hatching was no more than 0.30% and total 21-day cumulative mortality was no more than 1.8% in the control group. Condemnation rates ranged from 0.08% to 0.15% in the tested vaccine (HVT-NDV-ILT) compared to 0.04% to 0.51% in the control group.

For both studies, there were no systemic adverse events related to vaccination. There were no localized injection site reactions in any of the test vaccinated chicks when injection sites were evaluated following vaccination. Both mortality and condemnation rates were reported to be within a range accepted as normal for the type of bird, the flock history, and the management conditions.

CONCLUSION

These data provide support for the safety of Innovax-ND-IBD and Innovax-ND-ILT vaccine for use by the *in ovo* and subcutaneous routes under field conditions.

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AN OVERVIEW AND ANALYSIS OF AVIAN INFLUENZA H9N2 OUTBREAK IN CHICKEN FARMS IN INDONESIA

VISIÓN GENERAL Y ANÁLISIS DE BROTES DE INFLUENZA AVIAR H9N2 EN GRANJAS DE POLLO EN INDONESIA

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RESUMEN

La presencia del virus de Influenza Aviar H9N2 es enzootica en aves domesticas en Asia y África (3, 5) pero no ha sido reportada en Indonesia. Este subtipo viral también es categorizado como una Influenza Aviar de baja patogenicidad (1) y es capaz de facilitar la emergencia de nuevas cepas debido a sus segmentos de genes de codificación de proteína interna (4).

SUMMARY

Presence of avian influenza virus H9N2 is enzootic in domestic poultry in Asia and Africa (3, 5) but has not been reported in Indonesia. This viral subtype although categorized as low pathogenic avian influenza (1) is able to facilitate emergence of new strains due to its internal protein-encoding gene segments (4).

OVERVIEW

At the end of 2016 and in 2017 several cases of low pathogenic AIV-H9N2 avian influenza infection have been diagnosed in several areas throughout Indonesia. This is a new finding since circulating avian influenza virus in the country has been of HPAI H5N1 subtype.

Production sectors 1, 2, and 3 (2) of broiler, layer, as well as breeder flocks reported increased mortality and decreased egg production. Out of recorded total of 99 suspect cases 49 cased were confirmed to be infected with AIV.

To determine cause of disease pharyngeal and cloacal swabs were collected. RNA isolation, reverse transcriptase PCR, and sequencing were conducted to confirm presence of Newcastle disease virus, infectious bronchitis virus, avian influenza virus H5N1 virus, and AIV-H9N2 virus. Attempts for avian influenza virus isolation and characterization were further carried out in samples tested positive for the disease.

Case data consisting of type of chicken farm, age of chicken, egg production decrease as well as combined infection with NDV, IBV, and AIV-H5N1 are also analyzed. Of the 49 cases positive for AIV-H9N2, 35 cases were diagnosed with single infection while 14 cases were mixed with one or two other viruses (AIV-H5N1, IBV, and/or NDV). Infected chicken's average age in layer and breeder farms' was 43.17 ± 16.56 weeks. Decrease in egg production

range was 35.85 ± 17.80% lower than the farm baseline.

While HA gene forms a cluster distinct from Vietnam and China lineage, NA-encoding-gene shows variations which can be grouped into three distinct clusters. This suggests possible multiple ancestries of the AIV-H9N2 in Indonesia.

Presence of AIV-H9N in the country, co-circulating with AIV-H5N1 poses a serious economical as well as human health threat. Good biosecurity as well as vaccination measures are needed for future disease prevention.

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MITIGATION OF NECROTIC ENTERITIS WITH USE OF COMBINATION OF ESSENTIAL OILS AND PROBIOTICS

MITIGACIÓN DE LA ENTERITIS NECRÓTICA CON EL USO DE UNA COMBINACIÓN DE ACEITES ESENCIALES Y PROBIÓTICOS

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RESUMEN

Como la producción avícola libre de antibióticos continua en incremento, se han introducido una multitud de alternativas en los últimos años. Mientras que muchos productores han demostrado beneficios en la mitigación de la enteritis necrótica, unos pocos han añadido un coctel para evaluar el potencial de relaciones sinérgicas. Una prueba de 42 días fue conducida con 500 pollos de engorda machos Cobb en cuatro corrales (30 aves/ corral, n = 2160 aves) con 8 réplicas incluyendo un control (sin aditivos), GDP, y siete productos probados (combinación de probióticos y aceites esenciales).

El objetivo del estudio fue el evaluar los efectos de estos aditivos sobre el desempeño y la respuesta de los pollos de engorda expuestos a una vacunación temprana contra un desafío de coccidiosis como factor predisponente para la enteritis necrótica. En el día 8 se tomaron aves de cada corral para evaluar las lesiones de enteritis necrótica en el duodeno, yeyuno e íleon. La mortalidad fue medida durante todo el estudio. El desempeño y la CA fue medida en los Días 0, 14, 28 y 42.

Basado en los hallazgos del estudio, podemos concluir que bajo un modelo de desafío de enteritis necrótica que se presenta naturalmente, la suplementación con EP en las dietas de pollos de engorda puede reducir significativamente las lesiones al Día 8 y mejorar en general la CA durante un periodo acumulativo (Día 0-42) del periodo de crecimiento. Además, las aves alimentadas con dietas que fueron suplementadas con EP tuvieron un GDP significativamente más alto durante los Días 0-42. Por lo tanto, entre los aditivos, la combinación de productos resultó en un desempeño general mejor bajo

este modelo de desafío natural de EN comparado con los productos solos.

SUMMARY

As antibiotic free poultry production continues to increase, a multitude of alternatives have been introduced in the past few years. While many of the products have been shown to demonstrate a benefit in mitigating necrotic enteritis, few have been added to a cocktail to evaluate potential synergistic relationships. A 42-day trial with Cobb 500 male broiler chicks was conducted in floor pens (30 birds/pen, n=2160 birds) with 8 replicates including a control (no additive), AGP, and seven test products (probiotic combinations and essential oils). The study aimed to assess the effects of these additives on performance and response of broilers exposed to an early coccidiosis vaccine challenge as a predisposing factor to necrotic enteritis. On Day 8 three birds from each pen were lesion scored for necrotic enteritis in the duodenum, jejunum, ileum. Mortality was measured for the entire study. Performance and FCR were measured on Day 0, 14, 28 and 42.

INTRODUCTION

Antibiotic-free poultry production is continuing to grow in market share and in order to maintain the low-cost of the world's most popular protein, viable antibtioic alternatives need to be elucidated (1, 2). Often assoicated with antibiotic free production, Necrotic Enteritis and Coccidiosis cost the poultry industry a combine US $9 Billion annually (3, 4). While extensive research has been done with individual products, there is less with combination products. Therefore, we sought to determine the potential benefit of additive cocktails compared to single additives during a natural necrotic enteritis challenge.

MATERIALS AND METHODS

Birds, diets and husbandry. A total of 2,300 day-old Cobb 500 male broiler chickens were acquired from a local hatchery to select 2,160 birds for the trial based on uniform average weight and healthy appearance. Birds were assigned to one of the nine dietary treatments (8 pens/treatment) as follows:

1) **Negative Control (NC)**: corn-soybean meal basal diet
2) **Positive control (PC)**: NC + Virginiamycin (1 lb/ton)
3) **Bacillus Licheniformis A (BLA)**: NC + (0.5 lb/ton from 0-42 days of age)
4) **Essential Premix (EP)**: NC + Essential Premix (1 lb/ton)
5) **Bacillus Licheniformis B (BLB)**: NC + (1 lb/ton from 0-42 days of age)
6) **Bacillus subtilis A (BSA)** (1 lb/ton)
7) **Saponin product (SP)** (1 lb/ton from 0-42 days of age)
8) **BLA+ SP**: NC + BLA (0.5 lb/ton from 0-42 days of age) + SP (1 lb/ton from 0-42 days of age)
9) **Probiotic mix (PM)**: NC + (1 lb/ton from 0-42 days of age)

Necrotic enteritis challenge. A concentrated commercial coccidiosis vaccine was sprayed ~24 h after bird placement, which in conjunction with the presence of *C. perfringens* spores and appropriate barn environment leads to the development of an NE outbreak one-week post vaccine application. On Day 8, three birds were selected based on average body weight of each pen, euthanized by cervical dislocation, and the small intestines examined for NE lesions and scored based on a 0-4 scale system. Each section of the small intestine, i.e. duodenum, jejunum and ileum, was scored separately by personnel blinded to the treatments.

Performance parameters. Upon arrival (Day 0), birds were weighed in groups of 30 and assigned to each pen. Subsequently, birds were weighed on Day 8 (7 days after coccidiosis challenge with peak NE mortality) and at the end of starter (Day 14), grower (Day 28) and finisher phases (Day 42). Additionally, feed consumption was also recorded on per pen basis on days 8, 14, 28 and 42. Finally, adjusted body weight gain, feed intake and feed conversion ratio were calculated for each phase (days 0-8, 9-14, 15-28 and 29-42) and also for the cumulative experimental period (Day 0-42).

Statistical analyses. Statistical analysis for all data was performed using the ANOVA procedure of JMP software (2013) and significance between treatments ($P < 0.05$) determined by the LSD test.

RESULTS AND DISCUSSION

As expected, NE lesion scores were mostly prevalent in the duodenum portion and less pronounced in the distal sections of the small intestine. Supplementation of EP, PM and SP significantly reduced lesion scores in the duodenum compared to the PC; however, only EP resulted in significantly lower scores than those of control group.

EP was the only additive that reduced mortality significantly during Day 0-14 period. None of the treatments reduced mortality significantly during Day 0-28 and Day 0-42 period. However, when compared to the control group, PC, EP and BSA reduced Day 0-

42 mortality by 38%, 33% and 21% respectively (although not statistically different due to pen variability).

The data demonstrate that PC and EP groups had the highest body weight at the end of the study. EP group had significantly higher body weight on Day 8, 14 and 28 compared to the NC. On Day 42, only the PC group had higher body weight compared to NC. Although EP birds were significantly heavier than BLB and BLA & SP groups on Day 42, and although these birds were heavier than NC, there was not any significant difference among these groups.

Average daily gain (ADG) was significantly higher for EP and BLA during the starter period compared to Control. In the grower phase, EP and PC had significantly higher ADG compared to NC and BLB. Although ADG was statistically similar in all treatments during finisher phase, cumulatively (Day 1-42) PC and EP groups had significantly higher ADG compared with NC, BLB and SP.

For Days 0-28, birds fed diets containing any of the additives had significantly lower FCR compared to Control. However, for the overall experimental period (Day 0-42) PC, BLA, EP, BSA and SP had significantly lower FCR compared with NC. Better FCR with PC and EP was in accordance with better ADG in these groups.

Based on the study findings, we can conclude that under a naturally occurring necrotic enteritis challenge model, supplementation of EP to broiler diets could significantly reduce Day 8 lesion scores and improve overall FCR during the cumulative (Day 0-42) grow-out period. Additionally, birds fed diets that were supplemented with EP had significantly higher ADG during Day 0-42. Therefore, among the additives, combined products resulted in better overall performance under this natural NE challenge model compared to single products.

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Table 1. Weight gain (g) of broiler chickens fed different dietary treatments.

Treatments	d1-8	d8-14	d1-14	d14-28	d1-28	d28-42	d1-42
NC	174.68^{bc}	248.61^{c}	422.63d	1082.9^{c}	1501.51^{c}	1613.75	3094.95^{c}
PC	175.16^{bc}	266.02^{ab}	441.18bcd	1192.54^{a}	1631.15^{a}	1634.50	3259.83^{a}
BLA	184.26^{a}	279.14^{a}	463.52a	1124.95^{abc}	1587.99^{ab}	1563.83	3150.28^{abc}
EP	182.4^{a}	279.40^{a}	462.01a	1171.55^{ab}	1634.69^{a}	1584.15	3240.30^{ab}
BLB	171.51^{c}	253.32^{bc}	424.11cd	1106.77^{bc}	1523.37^{bc}	1540.73	3051.98^{c}
BSA	172.02^{c}	270.61^{a}	442.24bc	1179.15^{a}	1609.30^{a}	1528.82	3169.04^{abc}
SP	179.06^{ab}	270.73^{a}	450.06ab	1130.19^{abc}	1581.05^{ab}	1534.62	3096.04^{bc}
BLA+SP	182.50^{a}	276.71^{a}	457.96ab	1129.77^{abc}	1582.06^{ab}	1478.80	3050.81^{c}
PM	179.14^{ab}	265.86^{ab}	445.43ab	1148.92^{abc}	1593.87^{ab}	1513.89	3139.06^{abc}
P-value	*0.0007*	*0.0009*	*<0.0001*	*0.038*	*0.013*	*0.21*	*0.045*

INTERACTION OF ENROFLOXACIN AND WATER SANITIZERS IN BROILER CHICKENS

INTERACCIÓN DE LA ENROFLOXACINA Y DESINFECTANTES DEL AGUA EN POLLOS DE ENGORDA

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RESUMEN

Este experimento fue conducido para determinar si los tres desinfectantes más comunes usados en agua (cloro, iodo y citrato) en la producción de pollos de engorda afectan la actividad antimicrobiana de la enrofloxacina (ENR). Se prepararon varias diluciones de enrofloxacina para interactuar con los desinfectantes de agua, para identificar los posibles cambios *in vivo* a través de evaluaciones relativas a la biodisponibilidad (Fr) en 500 pollos de engorda Cobb sanos.

Los resultados muestran un decremento de la actividad antimicrobiana cuando la concentración del cloro era de dos veces la dosis máxima recomendada para desinfectar el agua. Sin embargo, no se ha repetido a concentraciones recomendadas, de ahí que, se observó un incrementó en Fr. Por otra parte, a una dosis de 4 a 8 veces la dosis máxima recomendada para el iodo redujo el Fr. Finalmente, de 1 a 8 veces la dosis maxima recomendada para el citrato como desinfectante de agua incremento la actividad antimicrobiana y el Fr. Estos resultados revelan que hay muchas interacciones no probadas de los antimicrobianos con los desinfectantes de agua.

SUMMARY

This experiment was conducted to determine if the three most commonly used water sanitizers (chlorine, iodine, and citrate) in broiler chicken production affected the antibacterial activity of enrofloxacin (ENR). Serial dilutions enrofloxacin were prepared to interact with water sanitizers, to identify possible changes *in vivo* throught evaluations of relative bioavailability (Fr) in healthy Cobb 500 chickens.

Results show decrease the antimicrobial activity when chlorine concentrations are two times the maximum recommended dose of chlorine as water sanitizer. However, this is not repeated at concentrations recommended, then, an increase in Fr is observed. On the other hand, 4 to 8 times the maximum recommended dose of iodine reduced the Fr. Finally, 1 to 8 times the maximum recommended dose of citrate water-sanitizer increased the antimicrobial activity and the Fr. These results reveal that there are many untested interactions of antibacterials with water sanitizers.

INTRODUCTION

The modern poultry industry requires precise dosing protocols and adequate equipment to ensure delivery of the chosen dose of drug to the flock and eventually to each chicken. This should be carefully taken into consideration when administering antibacterial drugs. The preferred manner for administering antibacterial drugs is through their drinking water. However in practice, considerable variations in bioavailability should be expected due to diverse factors, incluuding possibly the presence of sanitizers added to reduce bacterial load in water (15). The possible interaction of a sanitizer added to drinking water with a given antibacterial drug has not been addressed in formal literature. The efficacy of most water sanitizing agents is based on their high reactivity with chemical and organic entities(8); therefore it is reasonable to think that they can modify F of antibacterial drugs (6).

MATERIALS AND METHODS

This study complied with Mexican regulations for use of experimental animals, as laid out by the

Universidad Nacional Autonoma de Mexico and Mexican government regulations in NOM-062 ZOO-1999. In all, 1,365 healthy, 6-week-old, Cobb 500 female chickens, weighing 2.5 kg ± 12 g, were included in this trial. Four F studies were carried out for each ESP with 3 replicates per group. Also, an F study with 3 replicates, administering only ENR in sterile de-ionizedwater. In all cases, the dose of ENR was 10 mg/kg alone or as ESP and the volumen adjusted to deliver 1 mL/2.5 kg of body weight, as a single oral bolus dose. After treatment, blood samples were taken from the wing or jugular vein at time 0, 0.25, 0.5,0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 h, using five chickens per sampling period and drawing 3 mL of blood per chicken. Blood samples were immediately centrifuged, and approximately 1.0mL of serum was collected, identified, and frozen until analyzed. The concentration of ENR in serum samples was measured by HPLC as proposed by Idowu and Peggins (7). Mean value of serum concentrations of ENR vs. time for all treatments was analyzed by means of Shapiro–Wilk (13) to test for normal distribution and PK parameters with normal distribution using a GLM. Bonferroni multiple comparison tests for marginal means and standard errors were adjusted for the model considered and were performed at a significance level of $P < 0.05$. This model was analyzed by means of least squares, using the SPSS package.

RESULTS

Figure 1 presents serum profiles of ENR from ESP that differ from values obtained for the reference control (E), in a statistically significant manner ($P < 0.05$) and resulted in higher Cmax and/or AUC values. Figure 2 depicts the opposite, i.e., lower Cmax and AUC values of ESP, as compared to the control.

DISCUSSION

There appears to be no clear explanation for higher Cmax of ENR obtained from the ECl+ treatment, a possible explanation of these results may be related to changes in the pH of the gastrointestinal contents caused by chlorinated water. Aside from ECl+, all other treatments of the chlorine series, showed statistically lower Cmax values than the control ($P < 0.05$). ESP from 2 of the highest concentrations of the citrate-based sanitizer produced statistically higher Cmax, AUC, and Fr values. The citrate-based sanitizer utilized is manufactured from extracts of orange, grapefruit seed and other vegetable sources (3). Grapefruit seed extracts are known to be capable of interacting with gpG in the GI epithelium, allowing better bioavailability of some drugs (1). Consequently, it is likely that the active principles of citrate-based sanitizer contributed to the increased F of ENR obtained in this experiment. It is known that iodine is less reactive tan chlorine (10), and this may explain why pharmacokinetic parameters could be altered only at high concentrations of iodine. In this work, the chlorine, iodine, and citrate-based studied sanitizers included the maximum recommended concentrations found in the literature, and even they were eight times more concentrated (14). It is not uncommon that the drinking water in poultry coops contain high concentrations of iodine, chlorine, or citrate products as a result of accidental accumulation or due to an inadequate dosing (9).

The rational use of antimicrobials has been worldwide prioritized due to the increase of microbial resistance to these agents and the lack of new families of antimicrobial agents destined to poultry medicine (4,11). One of the obvious strategies that emerge from this situation is the use of antibacterial drugs based on PK/PD ratios (5). For example, for ENR pharmacokinetics/pharmacodynamics (PK/PD) ratios establish that a maximum Cmax value is required for optimal performance of this drug (2,12). In summary, the water sanitizers (iodine, chlorine, or citrate)-ENR interaction products induce changes in the Fr of antibacterial drug. These changes are dependent on both the nature of the sanitizer and its concentration.

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Figure 1. Mean ± SD of serum concentrations of enrofloxacin after a single bolus administration of 10 mg/kg delivered directly into the proventriculus (treatment E), and serum concentrations of enrofloxacin derived from administering enrofloxacin plus different concentrations of water sanitizers treatment ECL+: enrofloxacin 10 mg/kg plus chlorine 0.002 mg/kg (from sodium hypochlorite); treatment EC+++: enrofloxacin 10 mg/kg plus citrate-based sanitizer 3.2 mg/kg; and treatment EC++++: enrofloxacin 10 mg/kg plus citrate-based sanitizer 6.4 mg/kg.

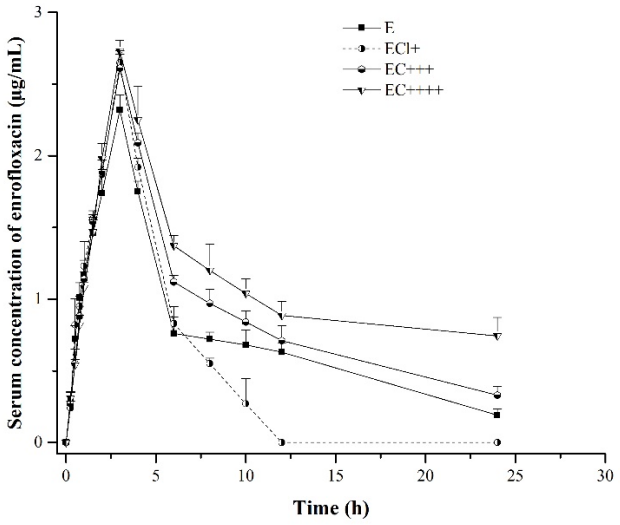
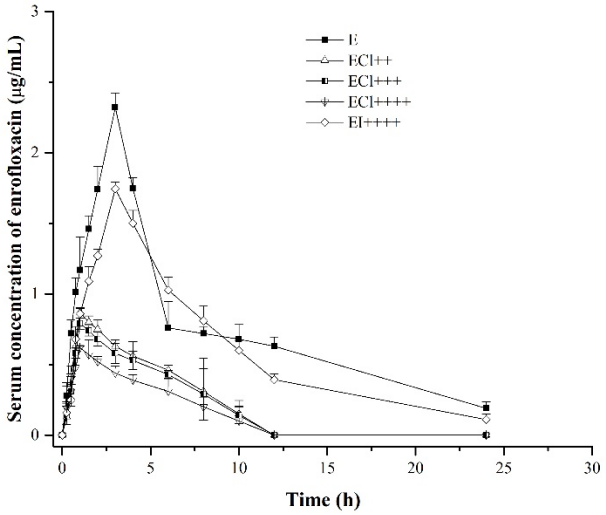


Figure 2. Mean ± SD of serum concentrations of enrofloxacin after a single bolus administration of 10 mg/kg delivered into the proventriculus (treatment E) and serum concentrations of enrofloxacin derived from administering enrofloxacin plus different concentrations of water sanitizers treatment ECL++: enrofloxacin 10 mg/kg plus chlorine 0.004 mg/kg (from sodium hypochlorite); treatment ECL+++: enrofloxacin 10 mg/kg plus chlorine 0.008 mg/kg; treatment ECL++++: enrofloxacin 10 mg/kg plus chlorine 0.016 mg/kg; and treatment EI++++: enrofloxacin 10 mg/kg plus iodine 0.0256 mg/kg.



PREVALENCE OF *SALMONELLA* SPP., *CAMPYLOBACTER* SPP., *ESCHERICHIA COLI*, AND *ENTEROCOCCUS* IN RETAIL POULTRY PURCHASED FROM GREATER LOS ANGELES, CA, USA

PREVALENCIA DE *SALMONELLA* SPP., *CAMPYLOBACTER* SPP., *ESCHERICHIA COLI,* Y *ENTEROCOCCUS* EN POLLO A LA VENTA ADQUIRIDOS EN EL ÁREA DE LOS ANGELES, CA, EE. UU.

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RESUMEN

El Sistema de Nacional de EE. UU. de Monitoreo de Resistencia Antimicrobiana (siglas en ingles NARMS) es un programa de vigilancia integrante de salud pública que monitorea y difunde información sobre la prevalencia y las tendencias de resistencia a los antimicrobianos (RAM) de los patógenos con origen en los alimentos y bacterias comensales encontradas en los alimentos para animales, carne a la venta, y en humanos. La Universidad de California, Davis se unió al programa NARMS en enero de 2018 para apoyar a la Administración de Alimentos y Fármacos con el aislamiento de los organismos objetivo de la carne en venta. De enero a diciembre de 2018, se adquirieron cuatrocientos ochenta muestras de aves (pollo y pavo), carne de res, cerdo en el área de Los Ángeles. En las muestras de aves compradas, la prevalencia de *Salmonella, Campylobacter, Escherichia coli,* y *Enterococcus* fue del 6.4% (23/ 360), 8.3% (20/ 240), 70.0% (77/ 110) y 80.9% (89/ 110), respectivamente. La colección de muestras en curso y el análisis estadístico son conducidos para

evaluar las tendencias temporales y otros predictores de la observación de la prevalencia de las bacterias.

SUMMARY

The U.S. National Antimicrobial Resistance Monitoring System (NARMS) is an integrative public health surveillance program that monitors and disseminates information on the prevalence and antimicrobial resistance (AMR) trends of foodborne pathogens and commensal bacteria found in food animals, retail meat, and humans. The University of California, Davis joined the NARMS program in January 2018 to facilitate the Food and Drug Administration with isolation of target organisms from retail meat. From January to December 2018, four hundred and eighty samples of poultry (chicken and turkey), beef, and pork were purchased from the Greater Los Angeles region. In retail poultry samples, the prevalence of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* was 6.4% (23/360), 8.3% (20/240), 70.0% (77/110), and 80.9% (89/110), respectively. Ongoing sample collection and statistical analysis are being conducted to assess temporal trends and other predictors of observed bacterial prevalence.

INTRODUCTION

Salmonella spp. and *Campylobacter* spp. are the most frequently reported organisms responsible for foodborne illnesses (6). In the United States (US), *Salmonella* and *Campylobacter* account for an estimated 1.2 and 1.3 million illnesses each year, respectively (1, 2). The higher risk of infection from consumption of poultry products relative to other meat commodities (3) is concerning, especially as demand for poultry meat in western developed countries like the US continue to increase and surpass that of other countries (8). Thus, in considering food safety, poultry meat is of particular importance.

The gastrointestinal tract of infected poultry and livestock can serve as a reservoir for *Salmonella* and *Campylobacter* (7, 12). Stringent biosecurity and disease management strategies are implemented at the farming and processing steps to minimize fecal dissemination and contamination of poultry flocks, equipment, and commercial products (12). In 2012, USDA Food Safety and Inspection Services surveyed *Salmonella* and *Campylobacter* prevalence and counts in raw chicken parts at the end of production lines. Of 2496 samples tested, 657 and 534 samples had quantifiable concentrations of *Salmonella* and *Campylobacter*, respectively, with 94.2% of *Salmonella* positive samples containing less than 3.00 MPN/mL and 83.5% of *Campylobacter* samples containing less than 10 CFU/mL (5). Due to the generally low counts of *Salmonella* and *Campylobacter* present in contaminated poultry carcasses, infectious doses responsible for human sickness are likely attained through proliferation of small quantities of pathogens between production and consumption (4). Thus, surveillance of poultry products at the retail level may provide insight on where intervention measures can be taken to reduce risk of foodborne illnesses.

Under the FDA, the NARMS program tracks prevalence and antimicrobial resistance trends of *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* from retail chicken, turkey, pork, and beef (11). The University of California, Davis, joined the NARMS program in January of 2018 to assist with isolation of target organisms from retail meat in the Greater Los Angeles region. Alongside prevalence data, information such as store location, packaging type, source of origin, claims of antibiotics use, brand, and distributor were collected. The purpose of this study was to assess temporal trends and other predictors of observed *Salmonella*, *Campylobacter*, *Escherichia coli*, and *Enterococcus* in retail poultry samples.

MATERIALS AND METHODS

Sample collection and processing. A total of 480 meat samples were collected from 64 retail stores in southern California including West Los Angeles, East Los Angeles, Irvine, and Ontario between January and December 2018. Grocery store locations were selected based on primary and secondary store lists generated by FDA-CVM (9). Forty samples were collected each month on a bimonthly basis, with 20 samples comprising of 10 skin-on/bone-in chicken, five ground turkey, two to three ground beef, and two to three pork chops being collected each trip. Samples were kept on ice during transportation and refrigerated in the laboratory until processing.

Bacterial isolation. A minimum of 25g of each sample – one piece for chicken or pork, and 25g for ground beef or ground turkey – was aseptically added to 250 mL of buffered peptone water and manually massaged for three minutes. The resulting rinsates were used for isolation of *Salmonella*, Campylobacter, *E. coli*, and *Enterococcus* using methods described in the FDA NARMS Retail Meat Surveillance Laboratory Protocol (10). Briefly, 50 mL of each rinsate was mixed with equal volumes of double strength lactose, Bolton, MacConkey, and enterococcosel broth for enrichment of *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus*, respectively. Overnight cultures were streaked onto selective agar plates to obtain pure cultures of target

organisms. Deviations from the NARMS protocol include implementation of Eosin methylene blue agar alongside MacConkey for more efficient *E. coli* isolation. PCR was also utilized in addition to biochemical tests for confirmation of *Salmonella*, *Campylobacter*, and *E. coli.* Ground beef and pork chop samples were not processed for Campylobacter.

Data analysis. Excel and Stata 15 were used to analyze data (descriptive statistics and logistic regression).

RESULTS

Overall bacterial prevalence. Prevalence of Salmonella, *E. coli*, and *Enterococcus* in ground beef and pork chop samples was 1.7% (2/120), 29.5% (33/112), and 83.9% (94/112), respectively. In poultry (chicken and ground turkey), *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcu*s prevalence was 6.4% (23/360), 8.3% (20/240), 70% (77/110), and 80.9% (89/110), respectively.

Bacterial prevalence in chicken and ground turkey. Figure 1 depicts the prevalence of the four target organisms in retail chicken samples. Highest prevalence of *Salmonella* was observed in August (20%, 4/20) and absence was observed in June and July. *Campylobacter* prevalence was highest in August (40%, 4/10) and October (40%, 4/10), and absence was observed in February, April, May, and December. *E. coli* prevalence ranged from 40% to 100%, while Enterococcus prevalence ranged from 33.3% to 100%.

Figure 2 depicts the prevalence of S*almonella*, *Campylobacter*, *E. coli*, and *Enterococcus* in retail ground turkey samples. Prevalence of *Salmonella* was only observed in March (20%, 2/10), September (20%, 2/10), and November (10%, 1/10), while prevalence of *Campylobacter* was only observed in January (10%, 1/10). *E. coli* prevalence ranged from 40% to 100%, while *Enterococcus* prevalence ranged from 40%, to 100%. Notably, for *Enterococcus*, 100% prevalence was observed in eight months (January, February, March, July, August, September, November, and December).

Prior NARMS data. NARMS reports from FDA Center for Veterinary Medicine for 2015-2017 indicate that Salmonella prevalence in retail chicken, turkey, pork, and beef collected in California was 5.2%, 4.4%, and 2.3%, for each year, respectively. In these same years, Campylobacter prevalence was 8.1%, 13.5%, and 6.5%, respectively.

DISCUSSION

Chicken, turkey, beef, and pork were selected for survey due to their ubiquity and large range of selection in retail stores. Furthermore, stores were selected in the Greater Los Angeles area due to the large and diverse residing population. *Salmonella* and *Campylobacter* are two major foodborne pathogens of concern; due to their lower isolation rates, *E. coli* and *Enterococcus* were also isolated as gram-negative and gram-positive indicator organisms for contamination, respectively.

This study showed that the four organisms of interest are prevalent in retail samples, particularly *E. coli* and *Enterococcus*. Notably, poultry samples contained much higher prevalence of *Salmonella* and *E. coli* compared to that in beef and pork. Between chicken and turkey samples, both *Salmonella* and *Campylobacter* were more frequently observed and higher in prevalence for chicken samples. This suggests that controls and interventions could be improved for broiler production and distribution to reduce contamination. However, due to the small sample size and limitation to ground meat for turkey, an increased sample size and type of turkey products surveyed is necessary to provide a more accurate representation of bacterial prevalence. Although *Salmonella* and *Campylobacter* prevalence in 2018 increased relative to that in 2015-2017, NARMS data from prior years correspond to samples purchased in the Contra Costa region. Thus, the increased prevalence may be accounted for by regional differences and generally higher prevalence of *Salmonella* and *Campylobacter* in retail meats sold in southern California.

Ongoing statistical analysis is being conducted to build logistic regression models which will provide more insight on predictors for observed bacterial prevalence.

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Figure 1. Prevalence of *Salmonella* (n=240), *Campylobacter* (n=120), *E.coli* (n=56), and *Enterococcus* (n=56) in retail chicken purchased from southern California from January to December 2018.

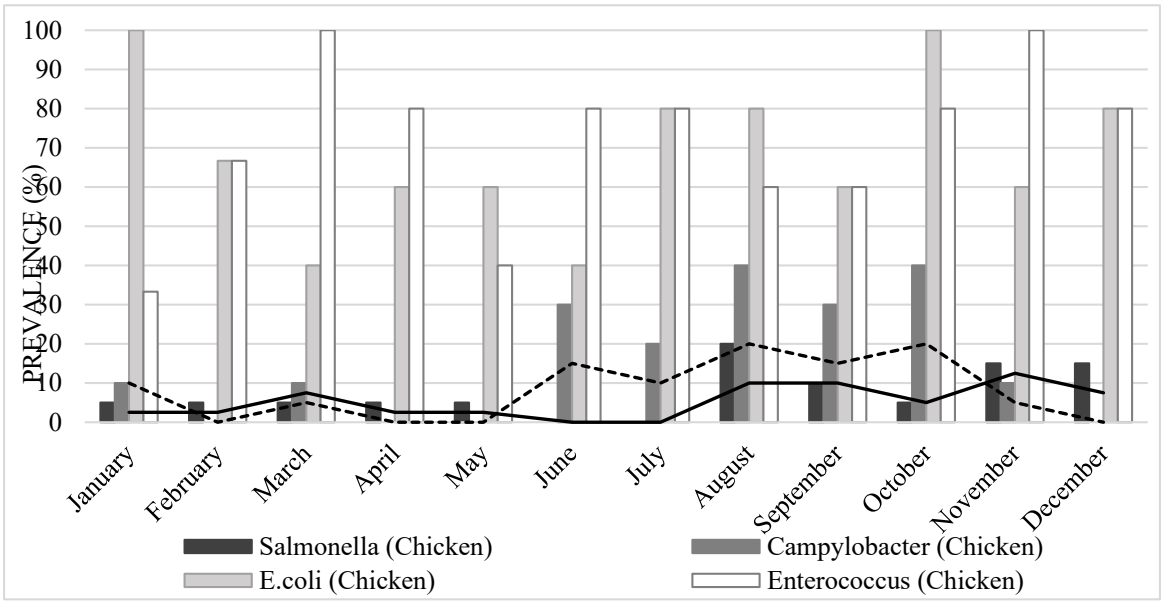
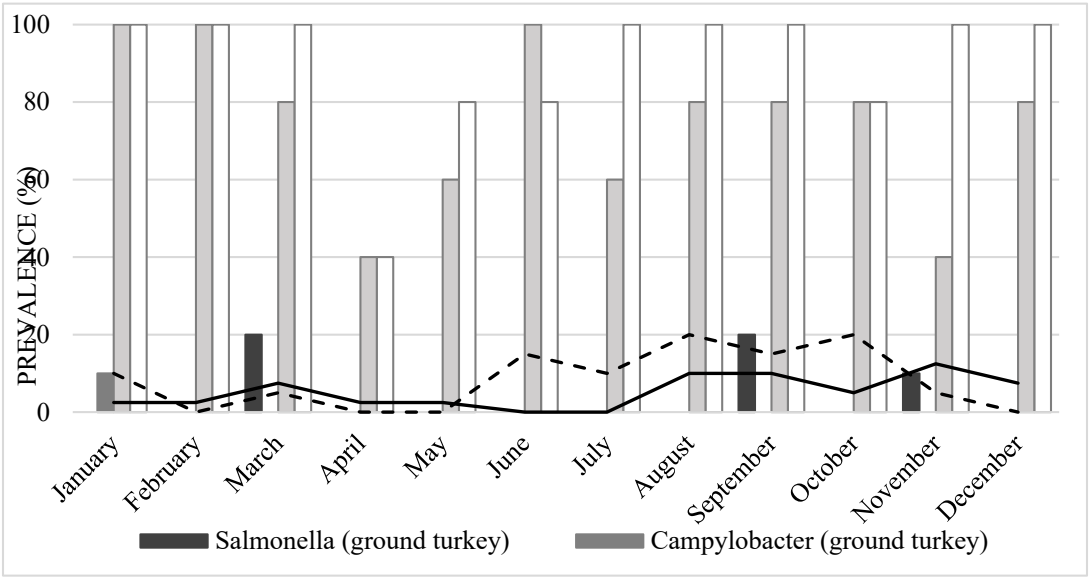


Figure 2: Prevalence of *Salmonella* (n=120), *Campylobacter* (n=120), *E. coli* (n=54), and *Enterococcus* (n=54) in retail ground turkey purchased from southern California from January to December 2018.



VARIANT INFECTIOUS BURSAL DISEASE VIRUS (VARIBDV) - SK09: A POTENTIAL VACCINE CANDIDATE TO CONTROL IBDV INFECTION IN CANADA

VARIANTE DEL VIRUS DE LA ENFERMEDAD INFECCIOSA DE LA BOLSA (VARIBDV)-SK09: UNA CANDIDATA POTENCIAL PARA VACUNA PARA EL CONTROL DE LA INFECCION DE IBDV EN CANADÁ

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RESUMEN

En Norte América, las cepas variantes de la Enfermedad Infecciosa de la Bolsa (varIBDV) son capaces de pasar a través de la inmunidad establecida por el programa actual de vacunación comercial e inducir una inmunosupresión. Anteriormente, se habían identificado cinco cepas como las más prevalentes en el Oeste de Canadá, y la cepa más predominante, la SK09 (60%), demostró un buen potencial para ser un candidato para vacuna. El objetivo de este estudio es aplicar la SK09 en diferentes formas de vacunas para reproductores de pollo de engorda para el control de las infecciones de varIBDV en Canadá, particularmente, como una vacuna de complejo inmune. Cinco grupos de reproductoras (n = 15/ grupo) fueron tratadas con una sola dosis de la vacuna de complejo inmune, dos vacunaciones con el complejo inmune, una dosis de complejo inmune más una vacuna inactivada, una vacuna viva o una vacuna viva más una vacuna inactivada basada en la SK09. El grupo control recibió una dosis de solución salina. La descendencia de pollo de engorda (n= 15/ grupo) de los seis grupos de reproductoras y otro grupo de pollo de engorda comercial (n=15) de padres que estaban bajo un programa de vacunación comercial fueron desafiados con la SK09 a los 6 días de edad. Un grupo adicional de progenie (n= 15) de reproductoras tratadas con la solución salina se mantuvieron como control, no vacunados sin desafío. La detección de la carga viral en la bolsa de Fabricio indicó que la protección fue conferida a la progenie vacunada con la SK09 contra su desafío homólogo.

SUMMARY

In North America, variant infectious bursal disease virus (varIBDV) strains are able to break through the immunity established by current commercial vaccine program and induce immunosuppresion. Previously, we identified five most prevalent strains in Western Canada, and the most predominant strain, SK09 (60%), demonstrated good potentials to be a vaccine candidate. The objective of this study is to apply SK09 in different forms of broiler breeder vaccines to control varIBDV infections in Canada, particularly as an immune complex vaccine. Five groups of breeders (n=15/group) were treated with a single dose of immune complex vaccine, immune complex vaccine twice, immune complex plus inactivated vaccine, live vaccine or live plus inactivated vaccine developed based on SK09. The control group received saline. Broiler progeny (n=15/group) from six breeder groups and another group of commercial broilers (n=15) whose parents undergone a commercial vaccine program were challenged by SK09 at day 6 of age. An additional progeny group (n=15) from breeders treated with saline was kept as no vaccine no challenge control. Viral load detection in bursa of Fabricius indicated that protection was conferred in SK09 vaccinated progenies against homologous challenge.

INTRODUCTION

As one of the most significant immunosuppressive disease in poultry industry around the world, infectious bursal disease (IBD) has caused huge economic loss since its first identification. The etiological agent, infectious bursal disease virus (IBDV), is categorized under *Birnaviridae* and classified into two serotypes, serotype 1 and serotype 2. Up to date, only strains from serotype 1 are reported to be pathogenic and are assorted to classical, variant (varIBDV) and very virulent (vvIBDV) strains based on virulence (1).

Although classical and vvIBDV infections are controlled by commercially available strains, varIBDV has emerged as prevailing strains in North America due to the antigenic changes. Compared to vvIBDV, varIBDV does not lead to huge mortality and morbidity. However, varIBDV leads to subclinical infections and severe immunosuppression. Consequently, infected birds become easily susceptible to secondary infection leading to considerable economic loss. Previously, our research revealed that 43% broiler chicken farms in Saskatchewan were tested positive for varIBDV, which also correlated with high mortality and higher condemnation and lower meat production. The data indicated an annual loss of 3.9 million kilograms of meat production as well (7). Despite a dedicated vaccination program, commercial vaccines in Canada failed to provide full protection for broiler chickens due to the antigenic difference between the vaccine and circulating varIBDV strains (4). Five prevalent varIBDV strains have been isolated from Western Canada, namely SK09, SK10, SK 11, SK12 and SK13. The most prevalent strain, SK09 (60%), has been proved to have potentials to be a vaccine candidate (3).

Different types of vaccines and different procedure has been applied in IBDV control in poultry industry. Generally, live attenuated, recombinant and immune complex vaccines are utilized for broiler vaccination. For broiler breeders, hyperimmunization by live vaccine priming and inactivated vaccine boosting is widely practiced to ensure adequate transfer of neutralizing maternal antibody to broiler progeny (8).

The application of an immune complex vaccine against IBDV was first carried out by Haddad et *al*., in 1994 (6). This immune complex vaccine was capable of providing guarded protection for broiler chicks with reduced bursal damage and working in the presence of preexisting antibody. Therefore, the objective of this study was to develop effective breeder vaccines to control varIBDV infections by applying SK09 in different forms, particularly as an immune complex vaccine.

MATERIALS AND METHODS

Vaccination preparation and experiment groups. Virus used for broiler breeder vaccination (SK09) were propagated in specific pathogen free leghorns. Bursae were pooled and homogenized to obtain a virus stock with 40% (w/v) suspension in sterile saline (4). The virus stock was titrated by embryo infective dose 50 (EID_{50}).

Broiler breeders (n=20/group) were randomly allocated into six groups [group 1 = saline; group 2 = immune complex (Icx) (1×10^3 EID_{50} live SK09 + antisera with ELISA titer of 200/bird); group 3 = immune complex twice (Icx-Icx) (1×10^3 EID_{50} live SK09 + antisera with ELISA titer of 200/bird/time); group 4 = immune complex – inactivated (Icx-K) (1×10^3 EID_{50} live SK09+ antisera with ELISA titer of 200/bird for Icx priming, 1×10^4 EID_{50} inactivated SK09/bird for boosting); group 5 = live (1×10^2 EID_{50} live SK09/bird) and group 6 = live plus inactivated (L-K) (1×10^2 EID_{50} live SK09/bird for live priming, 1×10^4 EID_{50} inactivated SK09/bird for boosting)].

For the inactivated vaccine, the virus was inactivated in 0.2% formalin solution followed by three dialysis (2). The immune complex vaccine was prepared by mixing virus and antibody, and was incubated at room temperature for 1 hour before administration. Broiler breeders were vaccinated at 13 weeks of age (live and first immune complex vaccine) and 16 weeks of age (inactivated and second immune complex vaccine).

To determine the efficacy of different types of SK09 vaccines, broiler progenies were obtained from each broiler breeder group (n=15/group). Commercial broiler chickens (n=15) were paralleled to compare current vaccination programs in the field. All broiler progenies were challenged with SK09 (1×10^2 EID50/bird) orally at 6 days of age. An additional group of broilers (n=15) hatched from saline treated breeders was kept as no vaccine no challenge control. Bursae were collected for viral load detection at 3, 6, 9 and 13 days post challenge (dpc) (n=3/group/time point). All animal experiments were approved by the University Committee on Animal Care and Supply, Animal Research Ethics Board, University of Saskatchewan.

Viral load detection. Total RNA was extracted with RNeasy Mini Kit (Qiagen) from RNAlater (Invitrogen) stabilized bursae tissue. cDNA was reverse transcribed afterwards with QuantiTect Rverse Transcription Kit (Qiagen) and viral load was detected by real-time PCR (Mx3000P qPCR system, Agilent Technologies). IBDV VP2 gene fragment was amplified using 5'-GGACACAGGGTCAGGGTCAAT-3' as forward primer and 5'-GCAGTGTGTAGTGAGCACCCA-3'

as reverse primer. The TaqMan probe for VP2 was labelled with FAM and ZEN/IBFQ at 5' and 3', respectively. Chicken 18S RNA was amplified concurrently as a reference gene with a forward primer 5'-CGGCTACCACATCCAAGGAA-3' and a reverse primer 5'-GCTGGAATTACCGCGGCT-3'. The 18S RNA TaqMan probe was labelled with HEX reporter dye at 5' and ZEN/IBFQ at 3'. The IBDV VP2 and 18S RNA amplifications were performed in the same tube (20 µL total volume) using Prime Time-Gene Expression Master Mix (IDT) and 2 µL of cDNA template. The PCR amplification cycles were initialed at 95 C for 10 min, followed by 40 cycles of denature at 95º C for 15 s and annealing at 60º C for 60 s. Serially diluted cDNA samples were used to determine PCR efficiency. The experiment was performed in triplicates with appropriate controls. IBDV viral loads in bursal tissues were determined by ΔΔ Ct method. Negative Ct values were set to be 39.

RESULTS

Based on viral load quantification by qRT-PCR, no virus was detected in bursa from the no vaccine no challenge broiler chickens, whereas virus peaked at day 3 post challenge in no vaccine challenged progeny and decreased to nearly undetectable level at day 13 post challenge. For all SK09 vaccinated progenies, no virus was detected for all sampled time points. In comparison, although no virus was detected in bursa from commercial broilers until day 6 post challenge, a higher viral load than the peak of no vaccine challenged group was detected at day 13 post challenge.

DISCUSSION

The viral load data demonstrated a sound protection in broiler chickens elicited by SK09 breeder vaccination against homologous challenge, while the commercial breeder vaccination program is only able to provide the protection in progeny until day 12 post hatch.

The circulating varIBDV are not amenable to antibodies stimulated by classical strain applied in commercial vaccines, which resulted in subclinical infection and immunosuppression. SK09 manifests to be a new vaccine candidate to for controlling the prevalence of varIBDV in Canada. Nevertheless, further experiments such as cross-protection test are to be carried out to fully examine SK09 strain as a promising vaccine candidate.

Meanwhile, though not completely understood yet, immune complex vaccine might have better antigen processing and presentation mechanism.(5) Future investigation of the working mechanisms of immune complex vaccine may be conducted.

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KINETICS OF THE IMMUNOLOGICAL RESPONSE TO IBD FIELD VACCINATION OF COMMERCIAL LAYERS IN MEXICO

CINÉTICA DE LA RESPUESTA INMUNOLÓGICA A LA VACUNACIÓN DE CAMPO CONTRA IBD EN PONEDORAS COMERCIALES EN MÉXICO

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RESUMEN

El objetivo de este estudio fue el evaluar la eficiencia de la vacunación en campo contra la Enfermedad Infecciosa de la Bolsa (IBD), también conocida como Enfermedad de Gumboro, en parvadas de gallinas ponedoras comerciales en el estado de Jalisco, México. Un total de 6 operaciones de ponedoras comerciales fueron incluidas en esta evaluación representando una población de más de 1.5 millones de aves. Se midieron los anticuerpos maternos de IBD, al día de edad. Se colectaron aleatoriamente muestras de sangra y de tejido de la bolsa de forma semanal de 10 aves de cada parvada seleccionada entre la semana 2 y 6 de edad, si importar el programa de administración de la vacuna viva de IBD, para evaluar los valores serológicos de la IBD, se hizo histopatología de las bolsas de cada toma de muestras y también RT-qPCR para la detección de IBD y secuenciación sobre tejidos individuales de la bolsa. La ruta más frecuente de administración de la vacuna fue a través del agua de bebida o en combinación de agua de bebida y gotas oculares. Todas las parvadas recibieron tres vacunaciones de IBD vivo en campo, excepto una que recibió 2 vacunaciones vía aspersión. Se realizó la serología usando un equipo convencional comercial de ELISA para IBD. Las observaciones a través de la examinación histopatológica mostraron una bursitis aguda consistente con la enfermedad aguda de la bolsa que se presenta de los 28 a 42 días de edad. El análisis RT-qPCR realizado se basó en la secuencia del nucleótido de 408 pares de bases de largo (721-1128) de la región hipervariable del gen vp2 del IBD. El análisis filogenético detectó variantes del IBDV en 50% de las parvadas de ponedoras examinadas, un 33% de cepas intermedias de IBDV (similar a Likert/ D-78) y 17% fueron negativas. Este estudio mostró una reducción significativa en la eficiencia de la vacunación contra IBD con los métodos actuales usados en las parvadas de ponedoras evaluadas.

SUMMARY

The objective of this study was to evaluate the field vaccination efficiency against infectious bursal disease (IBD), also known as Gumboro disease, in commercial layer flocks in the state of Jalisco, Mexico. A total of 6 commercial layer operations were included in this evaluation representing a layer population of over 1.5 million birds. Maternally derived antibodies to IBD were measured at day of age. Weekly blood and bursal tissue sampling were randomly collected from 10 birds from each selected flock between 2 to 6 weeks of age, regardless of their IBD live vaccination program administered, to evaluate the IBD serological values, histopathology of the bursas at each sampling time and RT-qPCR for IBDV detection and sequencing on individual bursal tissues. The most frequent route of vaccination was drinking water or a combination of drinking water and eye drop. All flocks received 3 live IBDV vaccinations in the field, except one that received 2 vaccinations via spray. Serology was performed using a conventional commercial IBD Elisa kit. Observations through histopathological examination showed acute bursitis consistent with acute bursal disease that ocurred at 28 - 42 days of age. RT-qPCR analysis performed was based on the 408 base pairs long (721-1128) nucleotide sequence of the hypervariable region of the vp2 gene of IBDV. The phylogenetic analysis detected IBDV variants in 50% of the layer flocks examined, 33% IBDV intermediate strains (Lukert/D-78-like) and 17% were negative. This study showed a significant IBD vaccination efficiency reduction with the current field methods used in the layer flocks evaluated.

INTRODUCTION

The aim of this study was to evaluate the field vaccination efficiency against IBD in commercial layer flocks in the state of Jalisco, Mexico. Monitoring field vaccination is critical to ensure the proper immunization of commercial layer flocks to prevent

and control field IBDV infections that will impair the health status and production performance of the affected flocks. Numerous variables in vaccine management (proper storage, correct preparation, water quality, etc.) and methods of vaccine administration in the field such as route of vaccination, supervision of consistent and uniform vaccine uptake, passive immunity, etc. may reduce or impair the proper immunization of the flock, leaving birds susceptible and exposed to field infections. Regular monitoring of field vaccination efficiency is a key procedure to evaluate the proper and consistent immunization of any given flock.

MATERIALS AND METHODS

Location. Six different commercial layer operations in the state of Jalisco, Mexico.

Birds. Over 1,5 million layers from different genetic lines (mainly Bovans and Hy-Line) were represented in this study. Random collection of 290 blood samples and bursas from all six layer flocks was carried out.

Vaccination program. Except one, all layer flocks in this study received three live IBDV vaccinations starting at around seven days of age and repeated twice on a weekly basis. Only one flock was vaccinated twice via spray.

Sampling schedule. Weekly blood and bursal tissue sampling were randomly collected from 10 birds from each selected flock between two to six weeks of age, regardless of their IBD live vaccination program.

Parameters measured. IBD Serology, histopathology and RT-qPCR for IBD. A commercial conventional IBD Elisa kit was used for the serological values. Histopathological examination of the individual bursas at each sampling time and RT-qPCR for IBDV detection and sequencing on individual bursal tissues was performed.

RESULTS

Serology. Serum samples collected from day old chicks from different genetic lines were tabulated (Figure 1). High variability was observed in the passive immunity transfered from different layer breeder flocks to the progeny, ranging from 1669 to 10,331. All serum samples from day old chicks were 100% IBD positive.

Blood samples were collected from pullets at two to six weeks of age from the six commercial layer companies. Serological assessment using a conventional commercial IBD Elisa kit showed the catabolic rate of the passive immunity having the lowest values at 21-28 days of age (Figure 2). Last field IBD vaccination was conducted between 19 to 26 days of age. High serological values were observed between 35 and 42 days of age.

Histopathology. Each bursa collected for this study, was divided into two halves. One half was immersed in buffered formalin at 10% for histopathological analysis and the other half was stored frozen for RT-qPCR analysis. Bursas with high histopathological lesion score results showing acute necrotizing bursitis were likely to have a high viral load and were the preferred specimens for IBDV detection by molecular methods. Acute bursitis, consistent with acute bursal disease, occurred at 28 to 42 days of age (Table 1). Bursa lesions were graded as follows: 0: Normal; 1: Minimal; 2: Mild; 3: Moderate; 4: Marked. 5: Severe. Curiously, normal bursae were still observed in some bursal tissue from birds at 35 days of age.

RT-Qpcr. RT-qPCR analysis performed was based on the 408 base pairs (721-1128 bp) nucleotide sequence of the hypervariable region of the vp2 gene of IBDV. Bursal tissues with high histopathological lesion score results were selected for RT-qPCR analysis. The phylogenetic analysis detected IBDV variants in 50% of the layer flocks examined, 33% IBDV intermediate strains (Lukert or D78-like) and 17% were negative. This study showed a significant IBD vaccination efficiency reduction with the current field methods used in the layer flocks evaluated.

CONCLUSION

Vaccination efficiency of IBD field vaccine administration in the commercial layer flocks evaluated was significantly reduced and unable to allow induction of immunity against IBDV field variants in 50% of the flocks evaluated. Effective IBD field vaccination was observed in 33% of the layers flocks evaluated. The remaining 17% of the flocks were negative by PCR. Results indicated that 67% of the layer flocks evaluated were susceptible to field IBDV infection.

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Figure 1. Passive immunity in day old chicks from different genetic lines.

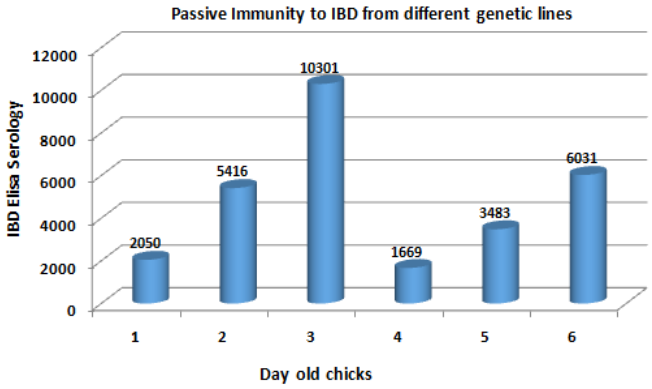
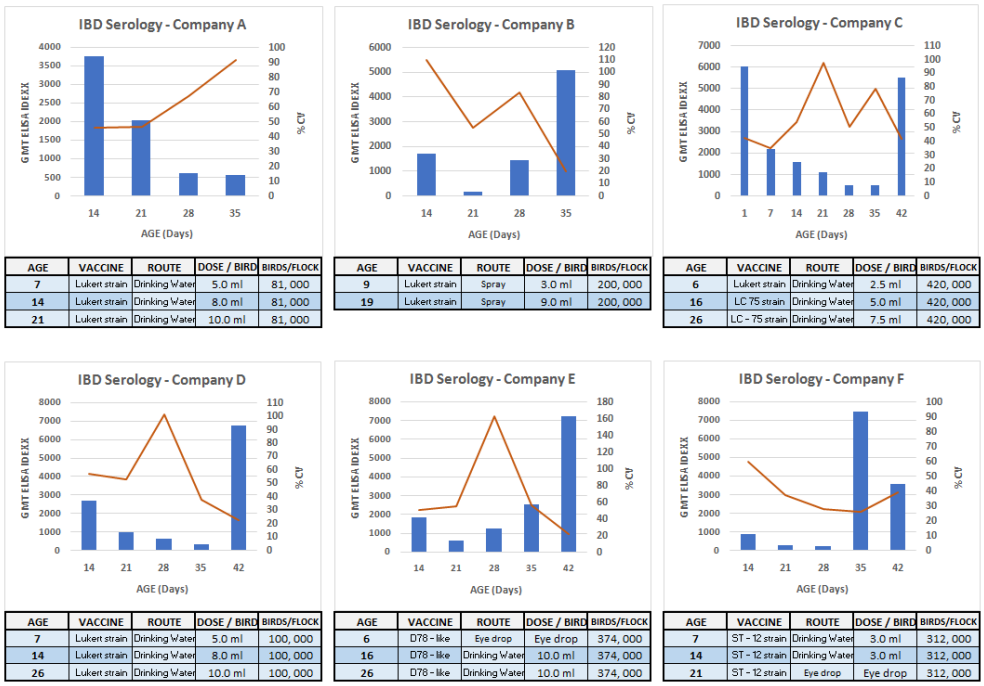


Figure 2. IBD Serological profile by layer company.



AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
7	Lukert strain	Drinking Water	5.0 ml	81, 000
14	Lukert strain	Drinking Water	8.0 ml	81, 000
21	Lukert strain	Drinking Water	10.0 ml	81, 000

AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
9	Lukert strain	Spray	3.0 ml	200, 000
19	Lukert strain	Spray	9.0 ml	200, 000

AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
6	Lukert strain	Drinking Water	2.5 ml	420, 000
16	LC 75 strain	Drinking Water	5.0 ml	420, 000
26	LC - 75 strain	Drinking Water	7.5 ml	420, 000

AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
7	Lukert strain	Drinking Water	5.0 ml	100, 000
14	Lukert strain	Drinking Water	8.0 ml	100, 000
26	Lukert strain	Drinking Water	10.0 ml	100, 000

AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
6	D78 - like	Eye drop	Eye drop	374, 000
16	D78 - like	Drinking Water	10.0 ml	374, 000
26	D78 - like	Drinking Water	10.0 ml	374, 000

AGE	VACCINE	ROUTE	DOSE / BIRD	BIRDS/FLOCK
7	ST - 12 strain	Drinking Water	3.0 ml	312, 000
14	ST - 12 strain	Drinking Water	3.0 ml	312, 000
21	ST - 12 strain	Eye drop	Eye drop	312, 000

Table 1. Summary of IBD serology, histopathology and RT-qPCR results.

Table 1. Summary of IBD serology, histopathology and RT-qPCR results

Layer Farm	Vaccines	IBD Elisa GMT	%CV	Age Days	BURSA HISTOPATHOLOGY SCORING Individual bursas (n=10)										Mean	PCR Results
1 81,000 Pullets	3 live vaccines Lukert strain Drinking water 7, 14, 21 Days	3784	45	14	0	0	0	0	0	0	0	0			0.0	At 28 days Negative (10/10)
		2062	46	21	0	0	0	0	0	0	0	0	0	0	0.0	
		633	67	28	0	5	0	0	0	0	0	0	0	0	0.5	
		576	91	35	0	0	0	0	0	0	0	0	0	0	0.0	
2 200,000 Pullets	2 live vaccines Lukert strain Spray 9, 19 Days	1729	109	14	0	0	0	2	0	0	0	0			0.2	At 28 days IBDV Variant (10/10)
		173	55	21	0	0	0	0	0	0	0	1	0	0	0.1	
		1457	83	28	5	5	5	5	0	5	0	0	3	5	3.3	
		5096	19	35	4	5	4	5	4	4	4	4	4	4	4.2	
3 420,000 Pullets	3 live vaccines Lukert strain Drinking water 6, 16, 26 Days	1619	54	14	0	0	0	0	0	0	0	0	0	0	0.0	At 42 days Lukert strain (10/10)
		1119	97	21	0	0	0	0	0	0	0	0	0	0	0.0	
		529	50	28	0	0	0	0	0	0	0	0	0	0	0.0	
		516	79	35	0	0	0	0	0	0	0	0	0	0	0.0	
		5516	41	42	5	5	5	5	5	5	5	5	5	5	5.0	
4 100,000 Pullets	3 live vaccines Lukert strain Drinking water 7, 14, 26 Days	2742	57	14	0	0	0	0	0	0	0	0	0	0	0.0	At 42 days IBDV Variant (10/10)
		992	53	21	0	0	0	0	0	0	0	0	0	0	0.0	
		642	101	28	0	0	0	0	0	0	0	0	0	0	0.0	
		369	38	35	0	0	0	0	0	0	0	0	0	0	0.0	
		6815	22	42	4	4	4	4	4	5	4	4	5	4	4.2	
5 374,000 Pullets	3 live vaccines Lukert strain First ocular Drinking water 6, 16, 26 Days	1860	51	14	0	0	0	0	0	0	0	0	0	0	0.0	At 35 days D78-like (10/10)
		644	55	21	0	0	0	0	0	0	0	0	0	0	0.0	
		1276	163	28	0	0	0	0	0	0	0	0	0	0	0.0	
		2554	56	35	5	5	3	0	4	4	4	4	0	5	3.4	
		7239	22	42	3	3	3	4	3	3	1	1	2	3	2.6	
6 312,000 Pullets	3 live vaccines ST-12 mild strain (TC) Drinking water 7, 14, 21 Days	883	59	14	0	0	0	0	0	0	0	0	2		0.2	At 35 days IBDV Variant (10/10)
		304	37	21	0	0	0	0	0	0	0	0	0	0	0.0	
		248	27	28	0	0	0	0	0	0	0	0	0	0	0.0	
		7498	25	35	5	5	5	5	5	5	5	5	5	5	5.0	
		3602	39	42	5	3	4	3	5	4	4	4	4	5	4.1	

AUTOMATED, MAGNETIC BEAD-BASED NUCLEIC ACID EXTRACTION FROM 1–48 SAMPLES USING THE NEW INDIMAG 48

EXTRACCION MAGNETICA AUTOMATIZADA DE ACIDO NUCLEICO CON BASE DE PERLAS DE 1 -48 MUESTRAS USANDO EL NUEVO INDIMAG 48

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RESUMEN

Un método de preparación robusto de las muestras que asegure una extracción confiable del ADN y del ARN es la clave para una identificación exitosa de los ácidos nucleicos de los patógenos.

Usando la tecnología de perlas magnéticas, INDICAL BIOSCIENCE desarrollo el Equipo IndiMag de Patógenos (previamente conocido como el kit MagAttract 96 cador de Patógenos) para su uso sobre diferentes plataformas automáticas. El kit puede ser usado para procesamiento en paralelo de diferentes tipos de muestras veterinarias y para la co-extracción de ADN y ARN viral y de ADN bacteriano.

Con el IndiMag 48, INDICAL presenta un nuevo instrumento para la extracción ácidos nucléicos con una base de perlas magnéticas a partir de muestras veterinarias. Diseñado para ser rápido y confiable

como las plataformas actualmente disponibles, pero con una mayor flexibilidad y usos, el IndiMag 48 puede procesar entre 1 y 48 muestras y solo necesita los recipientes plásticos necesarios para el número de muestras deseado.

En este estudio, evaluamos la confiabilidad de los protocolos de extracción del IndiMag 48 para el ARN y ADN de las muestras veterinarias para demostrar la mejor calidad o equivalente de los resultados, mientras se reduce el desperdicio de plástico.

INTRODUCTION

A robust sample preparation method that ensures reliable extraction of DNA and RNA is key to successful identification of pathogen nucleic acids.

Using magnetic-bead technology, INDICAL BIOSCIENCE developed the IndiMag Pathogen Kit (previously known as the MagAttract 96 cador Pathogen Kit) for use on different automated platforms. The kit can be used for the parallel processing of different veterinary sample types and for the co-extraction of viral RNA and DNA and bacterial DNA.

With the IndiMag 48, INDICAL introduces a new instrument for magnetic bead-based extraction of nucleic acids from veterinary samples. Designed to be as fast and reliable as currently available platforms, but with greater flexibility and usability, the IndiMag 48 can process between 1 and 48 samples and only requires plasticware for the desired number of samples.

In this study, we evaluated the reliability of IndiMag 48 extraction protocols for RNA and DNA from veterinary samples to demonstrate improved or equivalent result quality, while reducing plastic waste.

MATERIALS AND METHODS

We evaluated the performance of the IndiMag Pathogen Kit, comparing the 4-step protocol of the IndiMag 48 and the 5-step protocol of the KingFisher Flex System versus results obtained with a well-known column-based method. Nucleic acids were extracted from swabs, allantois fluid, serum, blood, tissue and fecal samples.
The purified nucleic acids were analyzed using real-time bactotype and virotype PCR/RT-PCR kits from INDICAL, detecting different viral pathogens (e.g., Influenza A virus, BVDV, SBV) and bacteria (e.g., *Mycoplasma gallisepticum/synoviae*, *Mycobacterium avium* spp. *paratuberculosis*).

RESULTS

Twelve well-defined swab samples spiked with *Mycoplasma gallisepticum* (ATCC25204, n=3), *Mycoplasma synoviae* (ATCC19610, n=3) and MS-H vaccine strain (Vaxsafe Ms vaccine, n=6) were tested. Comparable results were obtained for the Mg- and Ms-positive samples tested using the bactotype Mycoplasma Mg/Ms PCR Kit with both the IndiMag 48 and the KingFisher Flex System protocols. Mg- and Ms-positive swab samples showed improved results for samples processed using the IndiMag Pathogen Kit on IndiMag 48 versus the column-based method (QIAamp DNA Mini Kit, QIAGEN).

Eight characterized Influenza A-positive allantois fluid samples tested using the virotype Influenza A RT-PCR Kit (INDICAL) also showed comparable results with both the IndiMag 48 and the KingFisher Flex System protocols. Slightly better results were obtained with four samples using the manual extraction method (QIAamp Viral RNA Mini Kit, QIAGEN).

Further data will be shown for different RNA virus-positive serum and blood (BVDV, n=16), tissue samples (SBV, n=14) and fecal samples positive for *Mycobacterium avium* ssp. *Paratuberculosis* (n=13), which indicate equivalent or better performance with IndiMag 48 versus KingFisher instruments.

CONCLUSIONS

Magnetic bead-based nucleic acid extraction is generally faster, easier to automate and better suited to high-throughput testing.

The IndiMag Pathogen Kit demonstrated comparable performance versus a well-known, column-based extraction method, testing poultry samples for avian influenza and *Mycoplasma*. The IndiMag Pathogen Kit, running on the new IndiMag 48 extraction platform, showed equivalent or better results compared with the Kingfisher platform.

IndiMag 48 supports cost-effective nucleic acid extraction with reduced plastic waste, owing to flexible sample input. It is extremely user friendly: it comes with pre-loaded protocols for automation of the IndiMag Pathogen Kit and offers the possibility to add more protocols via a touchscreen. IndiMag 48 is self-contained, requiring no additional software or hardware to create or edit individual protocols, and it has a small footprint, suitable for small labs. The run time and result reliability are comparable to or better than those obtained with the 96-well platform assessed here. Overall, the IndiMag 48 offers veterinary testing facilities a high-quality, reliable option for nucleic acid extraction with high potential for cost savings and plastic waste reduction.

MYCOPLASMA GALLISEPTICUM AND *M. SYNOVIAE* IN CAGE AND FREE-RANGE SYSTEMS LAYER HENS OF GROWING AND EGG PRODUCTION STAGES

MYCOPLASMA GALLISEPTICUM Y *M. SYNOVIAE* EN SISTEMAS DE JAULA Y VIDA LIBRE DE GALLINAS PONEDORAS EN ETAPAS DE CRECIMIENTO Y DE PRODUCCIÓN DE HUEVO

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RESUMEN

La actividad de la producción de huevos emplea un gran número de aves resultando en una alta densidad, lo que favorece la aparición de enfermedades respiratorias en las gallinas ponedoras. La micoplasmosis aviar es una de las más importantes enfermedades respiratorias debido al decremento en el desempeño de la producción y la producción de huevo, causando pérdidas económicas en la industria avícola. Este estudio tiene el objetivo del establecimiento de la prevalencia del *Mycoplasma gallisepticum* (MG) y el *M. synoviae* (MS) en gallinas ponedoras en las etapas de crecimiento y de producción criadas en sistemas de jaula y de vida libre. Se verificó la alta prevalencia del MS en las gallinas ponedoras en el sistema de jaula y baja prevalencia en vida libre, solo en la etapa de crecimiento. La frecuencia del MS en el sistema de jaula fue mayor en la etapa de producción de huevo que en la etapa de crecimiento. Mientras que en el sistema de vida libre no presentaron micoplasmas, especialmente el MS, en la etapa de producción de huevo.

SUMMARY

The egg production activity employs large number of birds resulting in high density, which favors the appearance of respiratory diseases in laying hens. Avian mycoplasmosis is one of the most important respiratory diseases due to decrease in production performance and egg production causing economic losses in the poultry industry. This study aimed to establish the prevalence of *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) in layer hens in growing and production stages reared in cage and free-range systems. It was verified the high prevalence of MS in the laying hens of the cage system and low prevalence in free-range, only in growing stage. The frequency of MS in cage system was higher in the egg production than in the growing stage. While in the free-range system the birds were free from mycoplasmas, specifically MS, in the egg production stage.

INTRODUCTION

Poultry industry increased in importance in Brazilian agribusiness and the egg production in Brazil in 2017 approached 40 billion units with the country remaining among the ten largest egg producers in the world (1). The growing of this industry may be attributed, mostly, to a permanent concern about biosecurity and animal health. In this context, avian mycoplasmosis is one of the diseases with a major economic impact through all levels of poultry activity. The economic losses caused by this disease in layer poultry industry comprised a decline in egg quality and egg production, reduced feed efficiency, high mortality and an elevated cost with antibiotics for treatment (7). Avian mycoplasmosis lead to direct damages in poultry industry and it may be considered an element for suspension of avian products international trading. Considering all these aspects, avian mycoplasmosis are included in the listed diseases of mandatory notification from World Organization for Animal Health (OIE) and among the diseases with priority approach in Brazilian National Program of Avian Health from Brazilian Ministry of Agriculture, Livestock and Food Supply (2, 4, 10). Mycoplasma gallisepticum (MG) and Mycoplasma synoviae (MS) are the main mycoplasma species to affect layer hens and they have been described in Brazilian egg production, with a high prevalence of MS in commercial layer hens (3, 9). The aim of this study was to establish the prevalence of MG and MS

in layer hens in growing and production stages reared in cage and free-range systems.

MATERIALS AND METHODS

Tracheal swabs were collected of six different layer farms with cage system (A, B, C, D, E and F) and of one layer farm of free-range system (G). In farm F and G, 10 tracheal swabs were collected from four flocks, three at growing stage and one at production stage (Table 1). While in the others five farms (A, B, C, D and E)10 tracheal swabs were collected from two flocks, one at growing stage and the other at production stage (Table 1), with a total of 180 samples. All swabs were stored in tubes containing 50% glycerinated modified Frey's broth, which were incubated at 37°C thereafter and had their DNA extracted by the phenol-chloroform method adapted from (8). The PCR reactions used to detect MG and MS were performed according to (6) and (5), respectively. After amplification reaction, each sample was homogenized with loading buffer and GelRed®, applied in 1.5% agarose gel layered in Tris- Borate-EDTA (TBE) 0.5X, and finally submitted to the electrophoresis conditions. After the electrophoresis, the amplicons were visualized under ultraviolet light transilluminator.

RESULTS AND DISCUSSION

All egg farms with cage system presented a high frequency of MS infection, with a positivity of 75% (15/20) at farm A, 95% (19/20) at farm B, 100% (20/20) at farm C, 80% (16/20) at farm D, 55% (11/20) at farm E and 37.5% (15/40) at farm F. The frequency of MG infection was lower: the positive results obtained were of 10% (2/20) at farm A, 5% (1/20) at farm C, 35% (7/20) at farm D and65% (13/20) at farm E, while none of the samples of farms B and F was positive. Only farm E had a MG infection percentage higher than MS. At the free-range system farm (G), no hen was positive for MG and only 5% (2/40) had a positive result for MS (Table 1). Such results show that the percentage of MS infection was significantly higher in the cage system, both at growing and production stages, when compared to free-range system (G-Test of Independence, $p < 0.05$). Observing the growing and production stages, MS infection was higher at production stage in cage system. Such scenario was not found in the free-range system, where no MS was detected at production stage, suggesting that there has been an antimicrobial treatment applied to the hens. MG infection did not present a significant variation between growing and production stages, except for farm E, which had a higher prevalence of MG at production stage.

This study has demonstrated that every cage system farm studied was positive for MS. Such observation endorses the reports of high prevalence of this bacterial agent in cage system layer poultry farms of Southeast region of Brazil. The low prevalence of mycoplasmas in free-range system need more detailed study and the result may have been affected by the use of antimicrobials for treatment of the hens, since MS positivity at PCR was only 5%, but the hens presented respiratory signs.

CONCLUSIONS

The circulation of MG and MS strains among cage system in egg farms and the prevalence of MS in such farms have been confirmed. The MS infection was more frequent at production stage than at growing stage. Studies concerning the prevalence of mycoplasmas and the identification of related species, as well as circulating strains and their phylogeny, are important epidemiological tools for the development of control measures and evaluation of vaccine and sanitary control programs. Free-range system layer farms demand studies with a higher number of samples to ensure the reliability of the results.

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Table 1. Frequency of layer hens PCR-positive for *Mycoplasma gallisepticum* (MG) and *M. synoviae* (MS) at growing and production stages in cage and free-range systems.

SYSTEM TYPE	**FARM**	STAGE				**TOTAL**	
		GROWING*		PRODUCTION*			
		MG	**MS***	**MG**	**MS***	**MG***	**MS***
CAGE SYSTEM	A	1/10 (10%)	6/10 (60%)	1/10 (10%)	9/10 (90%)	2/20 (10%)	15/20 (75%)
	B	0/10 (0%)	9/10 (90%)	0/10 (0%)	10/10 (100%)	0/20 (0%)	19/20 (95%)
	C	1/10 (10%)	10/10 (100%)	0/10 (0%)	10/10 (100%)	1/20 (5%)	20/20 (100%)
	D	6/10 (60%)	8/10 (80%)	1/10 (10%)	8/10 (80%)	7/20 (35%)	16/20 (80%)
	E	5/10 (50%)	4/10 (40%)	8/10 (80%)	7/10 (70%)	13/20 (65%)	11/20 (55%)
	F	0/30 (0%)	10/30 (33.3%)	0/10 (0%)	5/10 (50%)	0/40 (0%)	15/40 (37.5%)
TOTAL*		13/110 (11.81%)	49/110 (44.54%)	10/70 (14.28%)	49/70 (70%)	23/140 (16.43%)	98/140 (70%)
FREE-RANGE SYSTEM	G	0/30 (0%)	2/30 (6.6%)	0/10 (0%)	0/10 (0%)	0/40 (0%)	2/40 (5%)
TOTAL*		0/30 (0%)	2/30 (6.6%)	0/10 (0%)	0/10 (0%)	0/40 (0%)	2/40 (5%)
FINAL TOTAL		13/140 (9.28%)	51/140 (36.43%)	10/80 (12.5%)	49/80 (61.25%)	23/180 (12.77%)	100/180 (55.55%)

*G-Test of Independence, $p < 0.05$

ASSESSING *MYCOPLASMA GALLISEPTICUM* VACCINATION TECHNIQUES IN THE FIELD

EVALUANDO LAS TÉCINAS DE VACUNACIÓN CONTRA *MYCOPLASMA GALLISEPTICUM* EN EL CAMPO

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RESUMEN

El *Mycoplasma gallisepticum* (MG) continúa siendo un patógeno significativo afectando la producción de huevo y las enfermedades respiratorias en el campo. La infección con MG cuanta con una variedad de manifestaciones clínicas, pero incluso en la ausencia de signos clínicos aparentes, el impacto económico puede ser significativo. Los programas de control incluyen vacunaciones y pruebas de muestreo, son las opciones disponibles para proteger y monitorear a las parvadas susceptibles.

Prevenir la presentación de signos clínicos, la pérdida de producción de huevo. Reducción de la transmisión de huevos, reducción del uso de antibióticos e intentar erradicar las cepas virulentas son las razones principales de los productores para implementar programas de vacunación contra el Mycoplasma (Dr. Noala Ferguson-Noel). Por lo tanto, rutas adecuadas de vacunación, y una distribución adecuada de las diferentes vacunas incluidas en el programa son factores críticos para establecer a las parvadas para un buen inicio en el proceso del control del Mycoplasma.

SUMMARY

*Mycoplasma gallisepticum (*MG) continues being a significant pathogen affecting egg production and respiratory diseases in the field. Infection with MG has a variety of clinical manifestations, but even in the absence of apparent clinical signs, the economic impact may be significant. Control programs including vaccination and screening tests are options available to protect and tracking susceptible flocks.

Preventing clinical disease presentation, egg production losses, reduction of egg transmission, reduction of antibiotic usage and attempting virulent field strains eradication are the main reasons for the producers to implement *Mycoplasma* vaccination programs (Dr. Naola Ferguson-Noel). Therefore, adequate vaccination routes, and proper distribution of different vaccines included in the program are critical factors to set the flocks for a good start on the *Mycoplasma* control process.

OBJECTIVE

The objective of this study was to assess three companies' current vaccination techniques and proper immunization in the field. The egg layer companies involved in the study, used the 6/85 MG vaccine strain. These companies vaccinated their pullets at different ages and used different vaccination routes. Bird's tracheae were sampled at 12 days post-vaccination to perform PCR, in addition to serology testing at different points in time. Laboratory results and general performance parameters among the different farms involved in study will be assessed and presented.

A separate study at the University of Georgia (PDRC-UGA) is in progress to evaluate three different vaccination routes, using the 6/85 MG vaccine strain. The objective of this study is to evaluate and to assess fine spray, course spray and eye drop vaccination in SPF leghorn birds at three weeks of age, placed in isolation units. Birds will be screened for *Mycoplasma synoviae* (MS) and MG before vaccination, using ELISA and HI serology and PCR testing methods (choanal swab samples). Birds from the four treatments will be screened for MG and MS throughout five weeks after vaccination. The screening tests will allow to evaluate in a more detailed fashion colonization of the vaccine strain in the trachea and to determine the best vaccination technique for optimal immunization.

MATERIALS AND METHODS

Animals and housing field assessment. Farms assessment was performed in three different companies located in the Midwest and West Coast of the United States (U.S).

Company A, with farms located in the Midwest of the U.S started vaccination 2016, using 6/85 Mg vaccine strain via fine spray (new fine sprayer). A 300,000-white egg layer pullet flock located in cages was evaluated, comparing two sprayers, the standard course drop size and the fine drop size. Tracheal swabs were sampled at 12 days post vaccination and vaccine takes were evaluated using PCR to confirm vaccine strain presence in the trachea. Serology was performed before and every six to eight weeks after vaccination for follow up and surveillance.

Company B, located in the West Coast of the U.S vaccinated the birds at 15-16 weeks of age, using 6/85 Mg vaccine strain via course spray. A 60,000-brown egg layer pullet flock placed in aviary system, was evaluated using PCR at 12 days post-vaccination.

Company C, located in the West Coast of the U.S vaccinated the birds at seven weeks of age, using 6/85 Mg vaccine strain via eye drop. A 60,000-brown egg layer pullet flock placed in aviary system, was evaluated using PCR at 12 days post-vaccination.

EXPERIMENTAL DESIGN

Ninety (90) total birds were assigned to four (4) treatment groups placing fifteen (15) birds per treatment and five (5) birds per isolator. The study will begin when SPF leghorn birds are placed (1 day-of-age), at which time birds will be allocated to isolators. The treatments are identified for the study as follows: Fine spray, Course spray, Eye-Drop and Negative Control (Table 1). Fifteen birds per treatment are designated for the assessment. Birds will be vaccinated at three weeks of age to be examined and screened by PCR (choanal swabs) and ELISA and HI serology testing.

At two weeks of age birds will be screened before vaccination, and subsequent weekly testing will be performed on weekly basis after using with 6/85 MG vaccine strain at three weeks of age.

RESULTS AND DISCUSSION

During the field assessment at company A, vaccination takes and integrity of the vaccine was assessed using PCR and titration and culture of the vaccine sampled during the vaccination process. The titration method was used performing Spearman-Karber calculations, and values were closed to the original titer of the vaccine before application. Vaccine take evaluation of the tracheal swabs taken sampled at 12 days post-vaccination showed 13-30% positive values for 6/85 vaccine strain, suggesting colonization of the trachea after proper vaccination. Companies B and C showed 100% negative vaccine takes after vaccination via standard course drop sprayer and eye drop vaccination.

The field evaluation and assessments lead us to work closely with the producers to work on different vaccination methods and adjustments to improve immunization for *Mycoplasma gallisepticum*, in addition to establish a follow up program throughout the life cycle of the flocks, to determine if additional viral or bacterial challenges and could play a role on complicated respiratory reaction and egg production issues, seen during the last years.

Final results of the study in progress at the University of Georgia will be described and presented.

ACKNOWLEDGEMENTS

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Table 1. Study treatments and description (UGA Study).

Treatment	No. of Birds	Pre-vaccination Screening	PCR Screening
1. Course Spray	15	10	15
2. Fine Spray	15	10	15
3. Eye Drop	15	10	15
4. Negative Control	15	10	15

USING A DUAL RECOMBINANT HVT-ND-IBD VACCINE ALONG AND IN COMBINATION WITH TWO PLAQUE INTERMEDIATE STANDARD STRAIN OF IBDV VACCINE IN THE EVALUATION OF PROTECTION AGAINST AL-2 LIKE VARIANT INFECTIOUS BURSAL DISEASES VIRUS

USANDO UNA VACUNA DUAL RECOMBINANTE DE HVP-ND-IBD JUNTO Y EN COMBINACIÓN CON DOS CEPAS INTERMEDIAS ESTÁNDAR DE PLACA DE UNA VACUNA DE IBDV EN LA EVALUACIÓN DE LA PROTECCIÓN CONTRA LA VARIANTE TIPO AL-2 DEL VIRUS DE LA ENFERMEDAD INFECCIOSA DE LA BOLSA DE FABRICIO

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RESUMEN

La Enfermedad Infecciosa de la Bolsa de Fabricio o Gumboro (IBD, por sus siglas en inglés) es altamente contagiosa en los pollos jóvenes. Es causada por el virus de la Enfermedad Infecciosa de la Bolsa de Fabricio (IBDV, por sus siglas en inglés) y se caracteriza por la inmunosupresión y la mortalidad, generalmente, entre las 3 y las 6 semanas de edad. El IBDV es ubicuo en las operaciones avícolas comerciales. El IBDV causa una inmunodeficiencia de linfocitos B prolongada e incrementa la susceptibilidad a varios virus y parásitos. Tanto las cepas clásicas como las variantes del IBDV han sido aisladas en el sureste de los Estados Unidos. La variante del IBD AL-2 (Al por el Laboratorio Allen) originaria de Delaware en los años recientes ha mostrado en afectas a los pollos de engorda entre las edades de 20 a 30 días o mayores presentando una atrofia de la bolsa de moderada a severa. Debido a que el aislamiento de la variante del IBDV AL-2 en pollos de engorda comerciales se ha incrementado en años recientes en el sureste de los Estados Unidos se han causado problemas de desempeño. Una nueva vacuna, la primera dual recombinante HVP-EN-IBD ha sido lanzada en los Estados Unidos, y será presentada una evaluación de la protección contra la variante AL-2 junto o en combinación con dos cepas vivas de estándar de placa intermedia del IBDV.

SUMMARY

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus, characterized by immunosuppression and mortality generally at 3 to 6

weeks of age. IBDV is ubiquitous in commercial chicken operations. IBDV causes a prolonged B-lymphocyte immunodeficiency and increased susceptibility to various viruses and parasites. Both classic and variant strains of IBDV had been isolated in the southeastern United States. IBDV variant AL-2 (AL for Allen Laboratory) originated from Delaware in recent years has showed to affect broilers between the ages of 20 to 30 days or older showed moderate to severe bursal atrophy. Because IBDV variant AL-2 isolation in commercial broilers has increased in recent years in the southeastern of the United States causing performance issues. A new first dual recombinant HVT-ND-IBD vaccine has been lunch in the United States, an evaluation of the protection against AL-2 variant along or in combination with a live two plaque intermediate standard strain of IBDV vaccine will be presented.

INTRODUCTION

Infectious bursal disease virus (IBDV) is present in poultry producing regions around the world and continues to be a major constraint for poultry producers. IBDV is a member of the genus *Avibirnavirus* in the family *Birnaviridae*, and its genome is composed of two segments of double-stranded RNA. The smaller segment B encodes VP1 and the larger segment A contains two partially overlapping open reading frames. The first, smaller open reading frame encodes a nonstructural protein VP5; whereas the second open reading frame encodes a precursor polyprotein, which is subsequently cleaved into VP2, VP4, and VP3. VP2 and VP3 are the major capsid proteins of IBDV. The VP2 protein has been identified as the major host-protective immunogen of IBDV and contains major epitopes responsible for eliciting neutralizing antibodies. One of the major consequences of IBDV is immunosuppression associated with vaccination failure and susceptibility of chickens to opportunistic pathogens. It was also shown that IBDV-infected birds may become a good propagator for other viral pathogens. Moreover, highly virulent IBDV can cause high mortality in unprotected flocks. IBDV replicates specifically in developing B-lymphoid cells, resulting in the destruction of the precursors of antibody-producing B cells in the bursa of Fabricius, and consequently, the immunosuppression. The first antigenic variant strain of IBDV was isolated from vaccinated flocks on the Delmarva Peninsula in 1985 and since then other variant strains were subsequently isolated in the United States and other countries. Before 1985 most typically isolated were the so-called classic isolates. Mainly in the southeastern of United States the most prevalent strains including classic and especially variants that are gaining grounds resulting in economic losses to poultry producers. In 2009, Dr. Toro (Auburn University) described that a study involving 322 diagnostic cases, broilers aged 20 to 30 days, or older, consistency showed moderate to severe bursal atrophy due to IBDV. IBDV variant AL2 (AL for Allen Laboratory) originated from Delaware in recent years was described. Sequence analysis performed by Dr. D. Jackwood in 2001, showed a close similarity of IBDV variant AL2 with an IBDV variant Tl strain. Besides biosecurity, vaccination is the most important measure to control IBDV in the field.

The apparent inability to control IBDV infection through current vaccination warrants a necessity to develop alternate IBDV vaccine products and strategies that will improve the prophylactic measures vital to control IBDV. Molecular biology techniques have made it possible to use a recombinant vector vaccine to fight against two avian pathogens with a single vaccine. Turkey herpesvirus (HVT) is an excellent candidate for this objective. HVT is nonpathogenic in chicken, it has been widely used in chickens since the 1970s for inducing long-term cross-protection against Marek's disease, it is less sensitive to maternal-derived than traditional attenuated IBDV vaccines and can be securely injected *in ovo* or subcutaneous in day-old chicks and presents no immunosuppressive effects. Bivalent recombinant HVT vaccines have been use commercially to protect against numerous diseases such as NDV, IBDV, ILT, and AI. These recombinant HVT vaccines offer the advantage of inducing an immune response against Marek's disease as well as against a second disease by inserting a foreign gene in the vector that encodes a specific protein to stimulate a protective immune response. Now the first double HVT recombinant vaccine which contain an insertion of the F gene of Newcastle disease virus and the VP2 gene of Infectious bursal disease virus to provide protection against three diseases with early onset of immunity in one single shoot.

OBJECTIVE

It is to evaluate the protection of the first dual recombinant HVT-IBDV dual insert vaccine alone or in combination of a live two plaque intermediate standard strain of IBDV vaccine that will be applied subcutaneous in SPF chickens when challenged at 14 days of age with a contemporary AL-2-like IBDV.

MATERIALS AND METHODS

A small-scale commercial subcutaneous injection system will be used for injection of the chicks for each treatment group (Table 1) will be used. A

minimum of 500 mL of each vaccine at 1x concentration will be needed for vaccination. 250 SPF embryos will be purchased from Charles River SPAFAS and set in PDRC SPF hatchery. At hatch chicks will be injected subcutaneous and placed in units.

The challenge virus used in this study will be AL-2 like IBDV field isolate 124024 case submission to PDRC. The virus will be expanded in three-week-old SPF chickens prior to the start of the study, then titrated in chicken embryos. The virus will be diluted to the target challenge dose in tryptose phosphate broth (TPB) to a target dose of 102.5 – 103.0EID50/dose. Each bird in challenged treatment groups will receive 0.03 mL by the intraocular route of inoculation. Birds will be housed in negative pressure isolation units and have unrestricted access to feed and water. Birds will be fed unmedicated Southern States All Grain Start-N-Grow diet.

On day 18, 15 birds in each group will be bled for IBDV ELISA. Birds in groups 1-5 will be challenge with IBDV AL-2 like 124024. The birds will be observed daily starting at placement on day 1. Bursae will be collected at 25 days of age and placed in formalin for histopathology. The study will be terminated on day 25 and birds and bursae weighed data for B/B ratio will be collected

RESULTS

Although IBDV strains of different antigenic types have been incorporated into vaccines. The efficacy of current live IBDV vaccines decreases in the presence of maternal antibodies, which are essential for the protection of young chickens for the critical first few weeks of life. Live IBDV vaccines also cause various degrees of bursal atrophy and may contribute to the emergence of antigenic variant virus. Previous worked done by Dr. Perozo in 2009 showed that a single HVT-IBD vaccines provide strong, long-term protection against the Delaware variant E strain of IBDV by slowly increasing titers of VP2 antibodies. Previous worked with the first double HVT-ND-IBD recombinant vaccine showed that SPF birds that were vaccinated either by *in ovo* or subcutaneous route and then challenge with NDV Texas GB strain showed 98% protection by the *in ovo* route and 99% protection by subcutaneous route four weeks post-vaccination. In the same study SPF birds were challenge with IBDV STC APHIS showed 97% protection by the *in ovo* route and 100% protection by subcutaneous route four weeks post-vaccination. In another study SPF birds were vaccinated *in ovo* with the double HVT-ND-IBD recombinant vaccine and challenge with IBDV Variant E strain and four weeks and the results showed 90% protection at four weeks post-vaccination.

ENRICHMENT OF NEST ENVIRONMENT

ENRIQUECIMIENTO AMBIENTAL DE NIDOS

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RESUMEN

Las aves de postura en piso seleccionan un sitio de postura (nido) el cual puede verse afectado por varios factores, entre los que destaca la ubicación, la intensidad lumínica, presencia de perchas, tipo de sustrato, la presencia de las cortinas, el tipo de crianza, etc. Se emplearon aves ligeras, donde se colocaron aleatoriamente cortinas de un color distinto en los nidos y algunos nidos sin cortina, como grupo testigo; como parte de un enriquecimiento ambiental para las aves de postura en piso. Con el fin de conocer si este patrón de postura fue o no dependiente de la localización del nido, se realizó una prueba de Xi cuadrada para contrastar la hipótesis de independencia entre color de cortina y localización del nido. Los resultados de la prueba estadística mostraron que ambas variables son independientes (P=0.08). Se observó la preferencia de las aves para realizar la postura en los nidos con cortina de color rojo. En el presente trabajo encontramos que en corto tiempo las aves en reproducción pueden cambiar su preferencia

de nido para realizar la ovoposición. La diversidad de opciones favoreció la producción de huevo limpio.

SUMMARY

The layers on floor will select a site to lay their eggs (nest), which can be affected by several factors, such as the location, light intensity, perch presence, substrate type, curtains presence, rearing type, etc. Light birds were used, randomly with different color curtains in the nests and some of them without curtains, as a control group; as a part of the environmental enrichment for the birds for floor laying. With the aim of knowing whether this laying pattern was or not dependent of the nest location, a square Xi test was performed to contrast the hypothesis of independence between the curtain color and the nest location. The statistical results of the test showed that both variables are independent (P= 0.08). It was observed a preference of the birds to lay in the nests that had a red curtain. In this study we found that in a short period of time the birds in reproduction can change their nest preference to lay. The diversity of options favored the clean egg production.

The presence of the nest motivates the setting in the birds of view, allowing a better production, as well as the animal well-being (3). Some conditions like radiant intensity, the nest's substratum and temperature might they favor outbidding at the aforementioned nests. With the aim of knowing if birds are able to elect the place where they will accomplish the ovoposition and if the aforementioned election is due to his predilection by some color, the following experiment came into question.

MATERIALS AND METHODS

The work was carried out in the Center for Education, Research and Extension in Poultry Production (CEIEPAv), of the Faculty of Veterinary Medicine and Zootechnics, of the National Autonomous University of Mexico, using a booth of natural environment, with pallet of 80 m2 (8 m in width x 10 m in length). The birds had feeding troughs type hopper manual and drinkable of bell semiautomatic.

The nests of plate with three levels utilized each one with 5 spaces themselves, located at different places, within the booth. Himself I assign at random to a color of curtain each nest (blue, red, orange, green) curtain was not placed to the control group.

Animals. They used 117 birds of view of 70 elderly weeks.

Nutrition. The birds fed on a diet the guy practices on the basis of corn and soja bean pasta, the water and the food provided themselves *ad libitum*.

Treatments. Four different color curtains placed themselves in 44 nests at random, leaving 11 nests without curtain that they served as a witness.

Obtaining samples. Once the birds adapted to the curtains, the egg was collected daily every hour, for a period of three days, placing the data in a register.

Statistical analysis. Results were examined with a proof of Xi liked of independence, fixing significancia's level of 0,05. The R utilized the statistical parcel itself (R Core Team 2017. R: To language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria) for the analysis of the data.

RESULTS AND DISCUSSION

The count of eggs for nest and for color of curtain, obtained they show up in the Board 1 during three running days. With the aim of knowing if this employer of view went or no contingent upon the nest's location a proof of Xi liked to contrast the hypothesis of independence between color of curtain and the nest's location came true. The results of the statistical proof evidenced that both variables are independent (P 0,08).

It can be observed than the birds accomplished the ovoposition with bigger frequency at the nests with red curtains (92), whose value represents the obtained view with another colors of curtain practically twice as during the period of experimentation (blue, 46; Orange, 45; Green, 42) or of those nests without curtain (34). Red color is pertinent to emphasize that hens had bigger inclination to the ovoposition at the nests with curtains without importing his location within the booth.

The election of the red color matches with in another authors (1, 2) - Although it is not obvious if animals categorize colors (1), perhaps the chromatic stimuli influence the selection of the nest.

The likes and dislikes of the color change with the age, it would be necessary to establish the differences between the innate behavior or the learning.

We know that the election of the birds to choose a nest can be seen modified by many factors, the curtains painted in colors at the nests would be able to relate with the well-being of the birds, as well as the obtaining of bigger number clean egg (1,2,3).

FINDINGS

It can be concluded that under the experimental conditions exposed in the present work hens presented a marked preference for the nests with red curtains and the location of the nest within booth, although the color of the curtain is a factor that has influence the

predilection of the nest, this predilection is independent to the position of the same. More investigation about the preference of colors at the nests for more periods of time extended, to reinforce these findings is recommended to accomplish.

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Table 1. Egg count by nest and shade color

Shade color \ Location	Nest 1	Nest 2	Nest 3	Nest 4	
Blue	10	11	13	12	46
Red	15	19	25	33	92
Orange	8	19	12	6	45
Green	6	17	10	9	42
Control	6	15	9	4	34
	45	81	69	64	259

A CASE OF HISTOMONIASIS IN A BACKYARD PEACOCK

UN CASO DE HISTOMONIASIS EN PAVO REALES DE TRASPATIO

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RESUMEN

La histomoniasis es una enfermedad causada por protozoarios, la *Histomonas meleagridis,* un parásito unicelular. Este causa lesiones características tipo hígado necrótico junto con cálculos caseosos dentro del lumen del ciego. El nemátodo cecal el *Heterakis gallinarum* es un vector de la histomoniasis. Fue presentado un pavo real adulto, joven en el Laboratorio de Diagnóstico de Enfermedades Animales de la Universidad de Purdue para una necropsia. Los pavos reales, al igual que los pavos comunes, con frecuencia son más severamente afectados que los pollos. La microflora intestinal y las coccidias cecales juegan un papel importante del desarrollo de las lesiones con las infecciones por Histomonas. Las bacterias dentro del ciego requieren la completa patogenicidad de esta enfermedad por protozoarios. En este caso, los hallazgos de *Clostridium perfringens* e *Eimeria* spp. fueron sospechosos de contribuir al desarrollo de esta enfermedad.

INTRODUCTION

Histomoniasis is a protozoal disease incited by the single-celled parasite *Histomonas meleagridis*. It causes characteristic target-like necrotic liver lesions along with caseous cores within the lumen of the ceca. The cecal nematode *Heterakis gallinarum* is a vector for histomoniasis. Although *Histomonas meleagridis* can be shed directly in the feces, it does not survive long in the environment. The protozoa can alternatively be placed within *Heterakis* eggs. Infected eggs pass in feces of the bird and are subsequently ingested directly by other birds or by earthworms who serve as a transport host. Once another bird ingests contaminated feces or infected cecal worms, cecal worm eggs, or earthworms, the protozoal parasite is transported to the ceca where it is released into the lumen, multiplies, and penetrates the cecal wall. From there the parasite gains access to the bloodstream and migrates to the liver where it incites further inflammation and necrosis.

CASE HISTORY

A young, adult male peacock was presented to Purdue University's Animal Disease Diagnostic Laboratory for necropsy. The peacock came from a small backyard flock of free-ranging peafowl and chickens. Multiple of the peafowl in the flock were depressed, had ruffled feathers and were anorectic.

Gross necropsy. There was severe, diffuse muscle atrophy of the pectoral muscles. The keel was prominent and the ceolomic cavity had little to no abdominal or pericardial fat. Air sacs were focally extensive to diffusely thickened, cloudy, and pale, yellow-tan. The lumen of the ceca was markedly expanded by dull, dry, firm, off-white to tan, friable, cecal cores. The center of each core was necrotic and amber to dark brown in color. Cross-sectioning revealed concentric laminations of necrosis and inflammation. The cores were adhered to the mucosa of the ceca. Grey to tan strand-like fibrin or fibrous tissue adhered the ceca to the adjacent air sacs.

Histopathology. The cecal wall was transmurally effaced and by large numbers of macrophages, lymphocytes, plasma cells, and occasional multinucleated giant cells. The cecal lumens were filled with necrotic debris, inflammatory cells, rod-shaped bacteria, and few protozoal trophozoites. Histologically, the liver was also affected. Multifocally there were areas of hepatocellular necrosis with an inflammatory infiltrate similar to that in the cecal wall. Within these foci there are small, round, densely eosinophilic, protozoal trophozoites some are within the cytoplasm of macrophages. These trophozoites are 10 to15 micrometers in diameter. There is an increase in the number of Kupffer cells, which contain brown granular hemosiderin pigment (hemosiderosis).

Further diagnostics. Samples of cecum submitted to bacteriology cultured *Clostridium perfringens*. Many *Eimeria* spp. and rare *Capillaria* spp. were found by parasitology qualitative fecal flotation testing of cecal contents.

DISCUSSION

The comingling of the peafowl with chickens is likely the cause of the clinical outbreak of histomoniasis in this flock. Chickens can be a reservoir of histomoniasis as they are common carriers of the cecal nematode *Heterakis gallinarum*, which is a vector for *Histomonas meleagridis*. Morbidity and mortality are species dependent.

Peafowl, similar to turkeys, are often much more severely affected than chickens. Intestinal microflora and cecal coccidia play an important role in the development of lesions with histomoniasis infections. Bacteria within the ceca are required for full pathogenicity of this protozoal disease. In this case, the findings of Clostridium *perfringens* and *Eimeria* spp. are suspected to have contributed to the development of the disease.

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PREVENTION AND CONTROL OF BIOFILM IN WATER LINE SYSTEMS AS PART OF BIOSECURITY PROTOCOLS

PREVENCIÓN Y CONTROL DE BIO-PELÍCULA EN EL SISTEMA DE LAS LÍNEAS DE AGUA COMO PARTE DE LOS PROTOCOLOS DE BIOSEGURIDAD

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RESUMEN

Las instalaciones de producción animal enfrentan nuevos desafíos de como producir la misma o mayor cantidad de proteína animal sin el uso de protocolos de intervención que estaban disponibles previamente. Uno de los ejemplos es el uso de antibióticos en el alimento o el agua para la prevención o reducción de bacterias patógenas. Con esta imagen en mente, los gerentes y el personal en producción animal están revisando los elementos básicos en sus explotaciones, como el agua y su papel como fuente potencial y/o factor de la persistencia de bacterias. Este trabajo no se dirige a las situaciones en las cuales la calidad microbiológica del agua se ha comprometido desde el principio; al contrario, este trabajo se dirige en como el sistema de líneas de agua para beber (SLAB) podría contribuir en el problema inicial, de prolongación y de persistencia de bacterias debido a ciertos factores favorables que podrían facilitar el desarrollo potencial de bio-película.

El desarrollo progresivo de la bio-película se aparece sobre la superficie interna del SLAB que son usado dentro de las casetas del pollo de engorda. Esta proliferación de sustancias presentará un medio apropiado para bacterias y otra proliferación de contaminantes. Estos nuevos contaminantes se volverán residentes y algunos se podrán multiplicar dentro de dichas superficies y se mantienen protegidas dentro de dichas capas de sustancias poliméricas. Estas nuevas capas y contaminantes en la nueva superficie interna limitaran la acción de químicos, como los desinfectantes.

SUMMARY

Production animal facilities are facing new challenges in how to produce the same or greater amounts of animal protein without the use of intervention protocols that were previously available. One of the examples is the use of antibiotics in feed or water for the prevention or reduction of pathogenic bacteria. With this picture in mind, production animal managers and servicemen are reviewing the basic elements in their farms, such as water and its role as a potential source and/or persistence factor for bacteria. This paper does not address the situations in which the microbiological quality of the water has been compromised from the beginning; rather, this paper addresses how drinking water line systems (DWLS) may contribute to the initiation, prolongation and persistence of a bacteria issue due to certain enabling factors that could potentially facilitate biofilm development.

Biofilm progressive development emerges over an inert surface such as the internal sides of the DWLS that are used inside a broiler barn. This substance proliferation will enable an appropriate media for bacteria and another contaminants proliferation. These new contaminants will become residents and sometime could multiply inside those surfaces and maintain protected inside those layers of polymeric substances. These new layers and contaminants on the new inner surface will limit the action of chemicals, such as the disinfectants.

INTRODUCTION

There are many factors that may enable or facilitate biofilm development such as: **corrosiveness**, this is one of the major contributors to biofilm development. Studies have shown that biofilms developed more quickly on iron pipe surfaces than on plastic PVC pipes. Biofilms are difficult and practically impossible to eliminate from the surfaces in drinking water distribution systems due to the protection offered by the microbial self-produced extracellular polymeric substance (EPS) matrix. This matrix protects microorganisms within biofilms from external adverse factors and fluctuations, including chemical disinfection (1).

Chemicals; besides the susceptibility of the material of the water pipe itself, another contributor could be the type of routine/daily products put through the drinking water line systems (DLWS) with the intention of reducing water bacteria contamination.

Some chemistries could prompt higher and faster corrosive effects, such as oxidative chemistries on iron or copper-based pipe surfaces.

Chlorine residues; this includes levels that are insufficient for eliminating bacteria but are sufficient for creating a zone of tolerance that allows the persistence of bacteria such as the coliform. A water line system could experience coliform occurrences even when free chlorine residues average between 2 and 2.5 mg/L. Use of m-T7 medium, a technique that recovers injured bacteria (2), has shown coliform occurrence rates ranging between 10% and 40% — even during months when coliforms were not recovered on the standard m-Endo medium.

Other combined factors; the penetration of free chlorine into a biofilm has been modeled and shown to be limited by its fast reaction rate (2). Essentially, free chlorine is consumed by reaction-diffusion interaction with cellular biomass before it can react with the bacterial components of the film (Chen and Stewart, 1996). Stewart and colleagues demonstrated that free chlorine effectively did not penetrate alginate beads containing bacterial cells. The corrosion of iron pipes can influence the effectiveness of chlorine-based disinfectants for the inactivation of biofilm bacteria (2). Therefore, the choice of pipe material and the accumulation of corrosion products can dramatically impact the ability to control the effects of biofilms in DWLS. This variation in corrosion rates is important because the corrosion products react with residual chlorine, preventing the biocide from penetrating the biofilm and controlling bacterial growth. The use of chlorine-based disinfectants under such conditions are very common in today's animal production facilities.

And finally, certain products added into the water system; some additives and supplements that are used through DWLS may facilitate the development of biofilms because of their adjuvants, vehicles, pH fluctuations or other factors of inherent to its formulations. The frequent administration of these types of products by servicemen and production animal caregivers is another factor that might worsen or contribute to the building up of — or the predisposition towards — biofilms in water lines (3)

Biofilm-associated populations and characteristics: Under field conditions, some of the biofilms reported have been associated with Gram-negative bacilli, *Pseudomonas, Acinetobacter* spp., *Klebsiella* spp., and others. Parasites like *Cryptosporidium* could be trapped in biofilms. Although viruses and *Cryptosporidium* do not grow in biofilm, they can attach to biofilms after a contamination event. Therefore, it is important to thoroughly flush the distribution system to remove these organisms following a contamination event. An intensive monitoring and sampling study was performed in England during the seasons of 2012 and 2013 to accumulate information that may show patterns of seasonality and other potential influential factors over the microbial dynamics inside DWLS under chlorinated treatments. Samples were collected before and during flushing operations during a four months' interval, 5 litters each (1). The detailed microbial analysis was performed using 16S rRNA Illumina MiSeq sequencing. This microbial profile (Graph 1) showed a higher tendency for biofilm formation on cast iron water line surfaces compared to plastic or PVC, as well as some evidence of a potential favorable effect of the warm seasons. It is important to highlight the *Clostridia* findings under this particular study, as *Clostridia* has historically been one of the most critical bacteria to deal with in poultry facilities; because of its difficulty to be removed from poultry farms and its increasing deleterious effect under reduced or none-antibiotic production programs. Chlorine residue levels correlated negatively with Bacteroidetes and Sphingobacteriia and correlated positively with Clostridia. The positive correlation between Clostridia and chlorine can be explained by the known ability of this microorganism to form spores that are very resistant to chlorine and other disinfectants. These results confirm that biofilms provide an environment in which bacteria are protected from chlorine residue. An example of the inefficiency of chlorine in controlling biofilms is the high presence of mycobacteria in the plastic pipe samples.

Biofilm control, management and prevention: These processes could be perform using various approaches, such as: mechanical action, the first line of defense for water lines is to perform systematic flushing of their internal surfaces as performed in other industries, such as clean-in-place (CIP) protocols. Flushing the water lines should be one of the routine protocols performed in order to reduce the slime that may develop between flocks (3), and also in long-living flocks, with modified protocols to reduce the risk of nipple drinkers clogging. Doing so avoids potential water restrictions, potential egg drops and/or negative effects in performance. The temperature of the water used to apply and flush the system could be also a potential beneficial component, because water temperatures between 20°C and 32°C could facilitate surfactant action in the surface to affect the superficial tension and help to expose and break the biofilm community (4) Second through the use of detergents; acid detergents have a powerful descaling ability in the internal surface of the water line. The properties of a lower pH solution could help to reduce the superficial tension of the biofilm and enable the degradation of the bio-slime material, thus making bacteria more susceptible to the environmental conditions and any

chemicals added within the detergent formulations. Third by specific chemicals; some specific chemical principles (5) that work for the immediate and gradual removal of biofilm formations, such as the combination of stabilized hydrogen peroxide and silver nitrate. This chemistry could be used in terminal disinfection conditions at the end of flock removal. Other chemical combinations, such as peracetic acid-based products plus stabilized hydrogen peroxide, have been demonstrated as highly effective in working against the removal of scale and biofilms — at the same time lowering the bacteria log as disinfection properties. These chemical combinations could be used in the removal strategy of biosecurity interventions that address the reduction of the current layers of biofilm that have been developed (6,7). There are other chemicals, such as chlorine dioxide, that could be of use in constant dose controls, with on-demand usage based on a pre-settled dose calibration protocol (8). The precursor of chlorine dioxide (9) is activated with an acid-based product delivered into the DWLS, followed by a change of water pressure when animals are drinking water. So, the activation will occur just when it is needed: on-demand. This final product will help to control and manage biofilm formation. Removing biofilm and reducing biofilm formation helps to manage the negative impact on the taste and odor of the water. Additionally, chlorine dioxide binds mineral elements, such as iron and manganese, that could cause taste and odor issues. These minerals have also been noted as fundamental precursors for bacteria growth (10). We are not going to discuss in this paper the role of chlorine dioxide as a disinfectant, but it has been demonstrated and reported in previous literature

MATERIALS AND METHODS

Chemical products for water intervention: Installation of a hydrogen peroxide/silver nitrate dosage system in the water lines in the farms for study 1 and 2. Installation of a chlorine dioxide (ClO_2) on dosage demand water line system with two pumps to regulate ClO_2 activation by an acid for study 3.

Measuring protocols and metrics: For the three-studies water consumption was evaluated, performance parameters, such as average body weight, feed conversion and mortality, and for study 3 percent of egg production and peak persistence was evaluated. For study 1 a comparison of antibiotic usage was done versus the historical of the same farms with previous flocks. For study 2, a luminometer based equipment that detects adenosine triphosphate (ATP) was used to assess the potential amount of biofilm in 100cc of sampled water before and after water treatment. For study 3 a conductivity tool was used to evaluate the consistence of oxidation reduction potential (ORP) units to try to make sure that the water system was always between 600-800 milivolts (mv). A CHEMetrics instrument was used as well to evaluate the free ClO_2 available in the water line at the end of the drinking line, the goal was to achieve an average concentration between 1 to 2 PPM of free ClO_2. The pH was also measure in a weekly basis, to verify any major variations.

RESULTS

Biofilm experiences

Study 1: Reducing antibiotic usage. The performance records of one poultry complex in the USA were collected between 2013 and 2015 (11). Before installing a hydrogen peroxide/silver nitrate protocol for biofilm and water treatment, the farm was experiencing between 19 and 25 treated flocks per year, mainly due to enteric, respiratory and infectious processes associated with *E. coli* and other opportunistic bacteria. For the year 2015, farms from the same company and same broiler complex had to treat just six flocks from the entire year around. A very different bacteriological picture was achieved after the use of this protocol as part of the program to reduce antibiotic usage in the flocks. (See summary of results in Graph 2.)

Study 2: Reducing biofilm in water lines. Seven different farms were involved in another study (12). Water samples were collected at the end of the water line from each of the seven farms before and after treating the water line with a hydrogen peroxide/silver nitrate protocol. Five of the seven farms showed lower levels of biofilm after the treatment. The levels of biofilm were measured using a luminescent-based technology to detect adenosine triphosphate (ATP). The use of ATP luminescent readers to support sampling of hard surfaces and/or water that may contain biofilm is very reliable, and it had shown high correlation with a lack of hygiene on surfaces (13) The results are expressed in Relative Luminescent Units (RLU) the higher the units, the higher the presence of contaminants, such as biofilm. (See summary of results in Graph 3.)

Study 3: Improving below-average farms. A commercial layer facility in the U.S. that started the adoption of water line treatment protocols between 2016 and 2017 experienced positive changes in different performance index. Some of the below-average company farms that struggled during the peak of production and the weeks of persistence of production (because of the numerous additional stress factors) were included as field treated populations. These farms were the first to show a dramatic change during those approximate 20 weeks of critical egg

production. These farms previously fell 3% to 4% below the average of egg production from overall company-accumulated parameters. During this critical period of 20 weeks, these farms were reporting an increased mortality associated with peritonitis and an overall increase of respiratory issues from their usual figures. Previous records of water consumption were compared between 2016 and 2017 in the same farms for the same time of the year, and it was found that water consumption was higher for those historical below-average farms, demonstrating that the palatability of the water was affected before by the scale and biofilm contents in the water lines and the water itself. The production performance during those 20 weeks became consistent and comparable to the average best-performing farms within the company, including average egg-mass and a reduced prevalence of peritonitis and respiratory issues associated with *E. coli* (personal communication, 2018).

DISCUSSION

Certain management practices currently used for drinking water line systems may lead to the increased risk of developing biofilms, and the problem can persist into the farm and contribute to the growth of resident bacteria. Biofilm formation could help to maintain bacteria associated with this type of community formation, creating a cycle of potential contamination for the entire production site.

Traditional disinfection practices have not demonstrated an efficient performance for the prevention and/or removal of biofilm in drinking water line systems. But new chemistries, like the ones mentioned in this paper, could prove to be part of more effective interventions and protocol practices for animal production facilities.

In addressing these types of challenges from drinking water line systems, there will be a high possibility of a reduced need for the use of antibiotics, because addressing these challenges should lower the level of bacteria within the biological animal production systems.

In commercial layer farms, as well as turkey farms, certain bacteria profiles may serve as indicators of water lines that face tough issues in regard to biofilm. An example of this is the frequency of finding *Ornithobacterium rhinotracheale.* (14)

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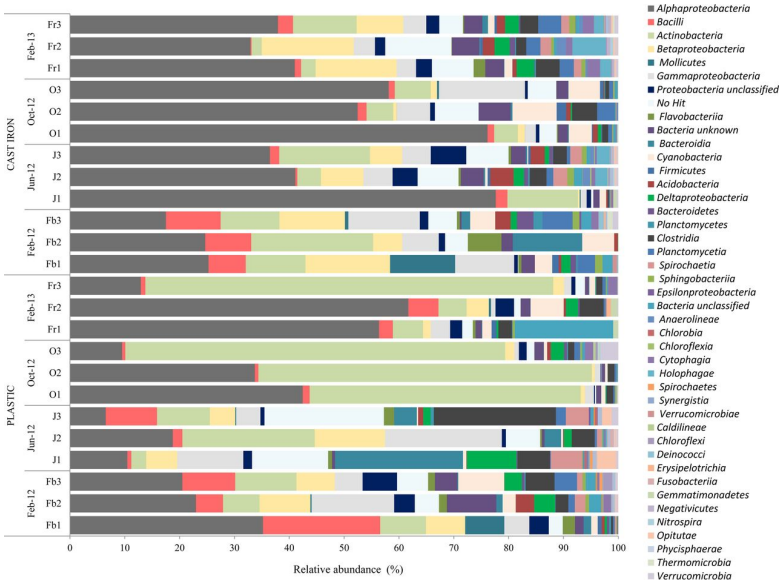
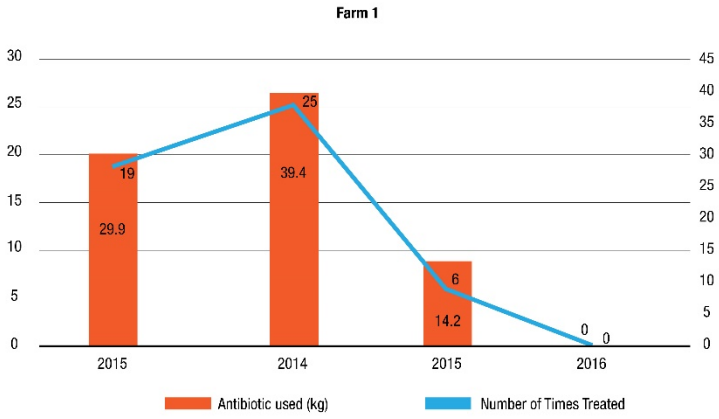
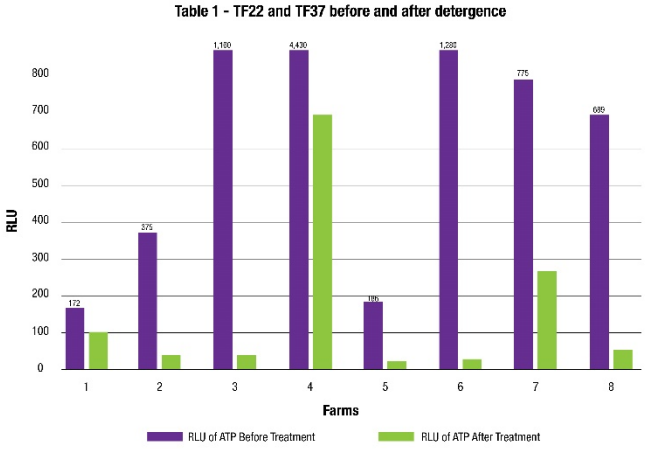
Graph 1



Graph 2



Graph 3



IMPACT OF ENVIRONMENTAL TEMPERATURE ON MIGRATION BEHAVIOR OF THE POULTRY RED MITE

IMPACTO DE LA TEMPERATURA AMBIENTAL SOBRE EL COMPORTAMIENTO MIGRATORIO DEL ÁCARO ROJO AVÍCOLA

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RESUMEN

El control del ácaro rojo avícola (ARA) ha sido un tópico de considerable importancia para la industria de la gallina de postura comercial a nivel mundial. La introducción de nuevos acaricidas sistémicos que requieren que el ARA ingiera sangre han incrementado la importancia de conocer el comportamiento migratorio del ARA para asegurar así una eficacia óptima del producto. En este estudio, se desarrolló una curva de regresión al investigar la tasa de migración del ácaro a temperaturas bajas y altas seleccionadas comparadas con una línea base de 25°C. Para cada temperatura, se trataron 50 adultos y 50 ninfas (proto- o deuto- ninfas) usando un modelo experimental para examinar los efectos de la temperatura sobre la migración del ácaro. Los hallazgos del estudio confirmaron que la temperatura ambiental tuvo un impacto substancial sobre el comportamiento migratorio del ARA. Este descubrimiento, junto con los datos de apoyo del campo, confirman que la importancia de considerar la baja temperatura exterior y la temperatura baja de los microclimas en las casetas de las aves es una fuente viable para que se presente una reinfestación de ARA.

SUMMARY

The control of poultry red mite (PRM) has been a topic of considerable concern for the commercial layer industry worldwide. The introduction of new systemic acaricides that require the PRM to ingest a blood meal have increased the importance of understanding PRM migration behavior to ensure optimal product efficacy. In this study, a regression curve was developed by investigating mite migration rate at selected low and high temperatures compared to a 25°C baseline. For each temperature, 50 adults and 50 nymphs (proto- or deuto-nymphs) were treated using an experimental model to examine temperature effects on mite migration. The findings of this study confirmed that environmental temperature had a substantial impact on PRM migration behavior. This finding, along with supportive field data, confirms the importance of considering low outdoor environmental temperatures and low-temperature microclimates in the poultry house as the source of viable PRM leading to house reinfestation.

INTRODUCTION

Poultry Red Mites (*Dermanyssus gallinae*) and Northern Fowl Mites (*Ornithonyssus sylviarum*) are common ectoparasites of poultry, depending upon blood meals to complete their life cycle. While the NFM is a permanent ectoparasite, remaining on the birds throughout the parasitic life cycle, the PRM hides in the cracks and crevices of the environment, moving at night to feed on the birds, and then returning to sequester themselves during the day (1). Adult mites can live in protected environments for long periods in the absence of hosts (1). These behavioral differences must be considered when developing an effective control strategy.

Fluralaner (Exzolt® - MSD Animal Health) is a systemic poultry acaricide that is applied via drinking water, as opposed to a premise spray or dust. The mites must ingest the acaricide via the blood meal for the product to be effective. For PRM, which migrate between the host and the environment, behavioral differences in migration pattern with different environment temperatures could have a significant impact on the successful and long-lasting control of PRM in a poultry flock. Failure of the entire PRM population in the poultry house to complete migration and ingestion of a blood meal may lead to pockets of untreated PRM or a percentage of untreated PRM that could quickly re-infest the flock.

MATERIALS AND METHODS

A test unit was designed that allows mites to migrate from an "inner-tube" through a funnel to an "outer-tube." Approximately 50 nymphs together with 50 adult mites were exposed to controlled ambient conditions long enough to induce mite migration in search of a blood meal, an indicator for mites' host-seeking behavior. A test-set consisted of 10 test units per ambient test temperature. After placing mites in the inner-tube and putting the test unit together, the unit with mites was adapted to an ambient test temperature for approximately one hour. After the one-hour adaptation time, each test unit was removed from the incubator to replace the tip of the inner-tube with a new specifically cut tip. Then the outer-tube was closed and an attractant was fixed on top of the outer-tube. The test-sets were immediately placed back in an incubator for an incubation time of approximately 24 hours in darkness. After 24 hours of exposing mites to an ambient test temperature, the test-sets were removed from the incubator and each unit was immediately frozen in dry ice to freeze mites in their current location inside the test unit. The numbers of nymphs / adult mites in both, the inner-tube and outer-tube, were counted.

The percentage of reduction in mite migration was calculated for nymphs, adults and nymphs + adults in comparison to the mite migration values (%) demonstrated at 25°C ambient temperature.

RESULTS

An ambient temperature of 25°C in this controlled study resulted in a mite migration rate of 28.7% nymphs, 62.0% adult mites and 34.0% of the total mite population (values served as control values for the calculation of percentage reduction in mite migration). After exposure of poultry red mites to variable ambient temperatures for 24 hours (Figure 1) the reduction in mite migration at

5°C was 95.4% for nymphs, 96.9% for adults, 95.6% for the total mite population.

10°C was 85.0% for nymphs, 91.1% for adults, 86.2% for the total mite population.

15°C was 32.1% for nymphs, 51.8% for adults, 37.1% for the total mite population.

20°C was 3.5% for nymphs, 18.1% for adults, 9.7% for the total mite population.

30°C was 0% for nymphs, 6.2% for adults, 0% for the total mite population.

Although the study was conducted under laboratory conditions, a significant reduction in mite migration rate at $\leq$ 15ºC could also be expected under field conditions.

DISCUSSION

The application of this information is critical to the successful control of PRM in a poultry flock when using a systemic acaricide. Monitoring of a typical European laying facility during the late winter revealed temperature variation within the facility as delineated in Table 1.

Under these circumstances, it would be reasonable to expect that a subpopulation of PRM would fail to ingest a blood meal during the prescribed treatment period. The untreated subpopulation can result in reinfestation of the flock after treatment. Therefore, in colder climates, it is critical to monitor the temperatures at multiple points within a poultry facility prior to the implementation of a systemic acaricide. Environmentally controlled facilities have an option to raise temperatures before and after treatment to improve success. But for many producers, this is not feasible.

When possible, delaying the use of the systemic acaricide until cold weather and resultant low poultry house temperatures no longer limit migration is recommended. If the mite population is intolerable during periods of cold weather and low poultry house temperatures, the systemic acaricide treatment plan should include monitoring of the mite population to alert the producer to the need for re-treatment if re-infestation occurs. Increases in outdoor temperature and resultant increases in poultry house temperature and mite migration activity could provide an opportunity to detect possible reinfestation and aid veterinarians in deciding on appropriate re-treatment intervals for a particular facility and climate.

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Figure 1. Reduction in migration of blood feeding stages of PRM kept 24 hours at various environmental temperatures.

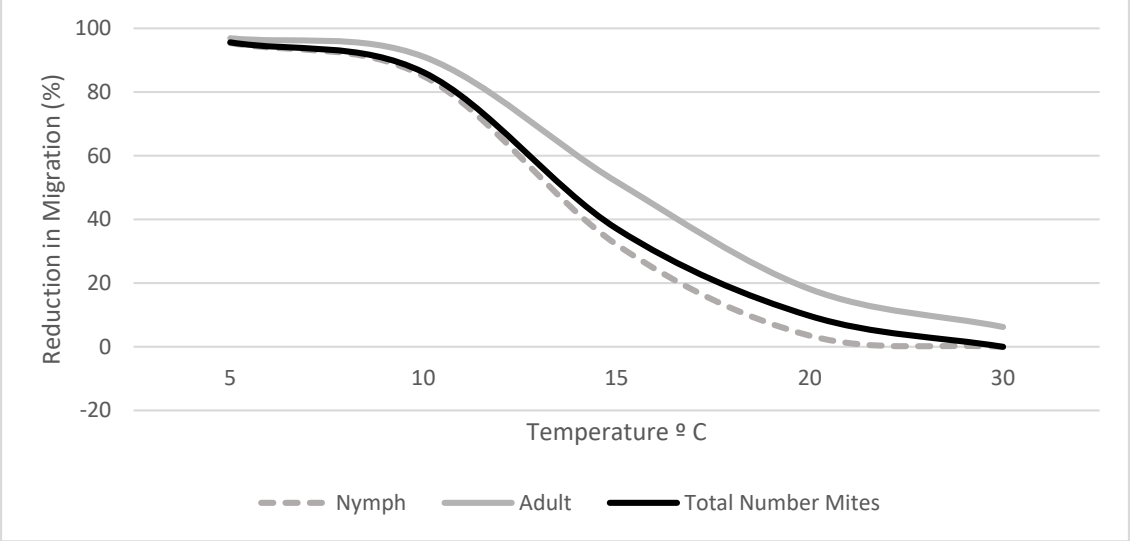

Table 1. Temperatures from a commercial layer facility in March 2018.

Temperatures	Average House 1	Average House 2	Average House 3
Under cages	16.3°C	15.8°C	14.5°C
Floor	13.7°C	15.5°C	12.8°C
Wall	11.2°C	15.2°C	11.6°C
Roof (ceiling)	23.7°C	20.2°C	24.4°C

AMELIORATION OF POULTRY WELFARE FOLLOWING POULTRY RED MITE TREATMENT WITH THE SYSTEMIC ACARICIDE FLURALANER (EXZOLT®)

MEJORAMIENTO DEL BIENESTAR AVÍCOLA SEGUIDO DE UN TRATAMIENTO CONTRA ÁCARO ROJO CON EL ACARICIDA SISTÉMICO FLURALANER (EXZOLT®)

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RESUMEN

La infestación con ácaros rojos avícolas (ARA, *Dermanyssus gallinae*) no solamente causa pérdidas económicas, sino también tiene un impacto sobre el bienestar animal. En este estudio con una duración de 13 semanas, unas instalaciones de gallina de postura comercial infestadas con ARA fueron evaluadas antes y después del tratamiento con fluralaner (Exzolt® - MSD Animal Health). Se midieron los niveles de infestación pre- y post- tratamiento, los biomarcadores de estrés en sangre (corticosterona, adrenalina, ovotransferrina) y los parámetros hematológicos (relación heterófilos: linfocitos), así como mediciones de hemoglobina y la media de hemoglobina corpuscular. Los parámetros de comportamiento también fueron evaluados semanalmente a través de una revisión (actividad) y se muestreo individualmente a las aves (otros comportamientos). La producción de huevo y la mortalidad de las gallinas fue medido a diario por todo el estudio. La reducción de la infestación de ARA seguido del tratamiento resultó en un decremento significativo de rascado de plumas y actividad nocturna así como una reducción significativa de los parámetros de estrés, como los niveles de corticosterona y la relación heterófilos: linfocitos. Desde una perspectiva de producción, la parvada respondió a la reducción del estrés y al mejoramiento de los parámetros sanguíneos con una mejora en la producción de huevo y la reducción de la mortalidad.

SUMMARY

Infestation with poultry red mites (PRM, *Dermanyssus gallinae*) not only causes economic losses, but also has an impact on animal well-being. In this 13-week long study, a commercial layer facility infested with PRM was evaluated before and after treatment with fluralaner (Exzolt®- MSD Animal Health). Pre- and post-treatment infestation level, blood stress biomarkers (corticosterone, adrenaline, ovotransferrin) and hematological parameters (heterophil:lymphocyte ratio), hemoglobin and mean corpuscular hemoglobin were measured. Behavioral parameters were also assessed weekly through scan (activity) and individual bird sampling (other behaviors). Egg production and hen mortality were measured daily throughout the study. The reduction in PRM infestation following treatment resulted in a significant decrease in feather pecking and night time activity as well as a significant reduction in stress parameters such as blood corticosterone levels and heterophil:lymphocyte ratio. From a production perspective, the flock responded to the reduction of stress and improvement of blood parameters with improved egg production and reduced mortality.

INTRODUCTION

Moderate to severe infestation of commercial laying flocks with PRM (*Dermanyssus gallinae*) has been documented to cause economic losses such as reduced egg production and increased second quality eggs (1.) A study measuring corticosterone, adrenaline, noradrenaline, albumin and α-, β- and γ-globulins under controlled conditions concluded that heavy infestation with PRM resulted in increased stimulation of the hypothalamic-pituitary-adrenal cortex axis (somatic stress) and high activity of the sympatho-adrenomedullar system (psychogenic stress) (2), indicating that poultry well-being may be

adversely impacted by infestation. This study further explored the impact of PRM with respect to blood stress biomarkers, hematological parameters and behavioral parameters to determine the impact of PRM on poultry well-being and production performance.

MATERIALS AND METHODS

The study was conducted in a commercial layer facility with enriched cages that housed 12,700, 29-week-old hens infested with PRM, and that had not been treated in the 14 weeks prior to the onset of the study. The hens to be included in the study were randomly selected and individually identified prior to beginning the study. They were required to be healthy, have been in production for at least 4 weeks after housing, be aged over 22 weeks, be available for the 7-week study duration and have a mean PRM infestation score from qualitative trap assessment of at least 2, indicative of a robust mite challenge.

Twenty mite traps (Avivet®) were placed throughout the facility to determine the level of infestation each week for six weeks prior to treatment. At seven weeks, the flock was treated with Exzolt® (fluralaner) solution – two administrations of 0.5 mg/kg body weight separated by seven days. Further traps (20 per assessment) were placed at three days following the first administration, two days following the second administration and then weekly for six more weeks. Traps remained in place for two days prior to assessment. One week before treatment (baseline), blood stress biomarkers (corticosterone, adrenaline, ovotransferrin) and hematological parameters (heterophil:lymphocyte ratio – another stress indicator)(3), hemoglobin and mean corpuscular hemoglobin were measured from the sample of 50 hens identified prior to the start of the study. Care was taken to minimize stress during blood collection. At Week 1 (immediately after the second administration) and Week 6, the same 50 hens were sampled for the blood stress biomarkers and hematological parameters.

Behavioral parameters were assessed weekly through scan observation over 30 minutes, focused on the perch area of each cage and focal sampling (selected behaviors) for one minute at two-minute intervals. Direct visual observation was used during the daytime, and infrared video recording was used for observations after dark, when the mites are most active. Recordings were evaluated at the Universitat Autonoma de Barcelona by scan sampling assessment of defined variables (4.) Defined focal behavior included: body shaking, vertical wing shaking, head scratching, head shaking, preening, social preening, gentle feather pecking, severe feather pecking and aggression. Scan activity levels included resting (sleeping) with head under plumage or wing, resting with eyes open but no movement, or awake, active animals.

Egg production, egg weight and hen mortality were measured daily throughout the study, beginning six weeks before treatment and continuing through treatment and six weeks following treatment.

RESULTS

Mite counts in the traps prior to treatment averaged 1500 to 2200 per trap. From week 3 onward, the mite counts in traps averaged <2 mites /trap, indicating excellent efficacy of the fluralaner treatment, and showing a valid correlation of the welfare parameters with the infestation.

Blood corticosterone level showed a significant reduction from 4.0 ng/mL (baseline) to 1.7 ng/mL ($p < 0.01$) at six weeks post-treatment with fluralaner. The heterophil:lymphocyte ratio also showed a significant reduction from 0.6 (baseline) to 0.1 ($p<0.01$) at six weeks post-treatment. Hemoglobin and mean corpuscular hemoglobin significantly improved from baselines of 7.0 g/dl and 32.9 pg respectively to 7.8 g/dl and 36.9 pg ($p<0.01$.) No significant changes were measured in adrenaline or ovotransferrin.

Egg production significantly increased after the 1st Exzolt® administration compared to pre-Exzolt® levels, with an improvement of 6.4% ($p<0.01$.) Average daily mortality was also significantly improved ($p < 0.05$) post-treatment. Mite counts and hematological parameters are summarized in Table 1 and production parameters in Table 2.

Daytime behavior observations did not show a significant difference in activity between pre- and post-treatment observation periods. Night time activity, however, was clearly reduced following treatment with fluralaner (activity levels are summarized in Figure 1.) The percentage of active hens decreased from 34% before to 11% after treatment ($p<0.01$.) Head scratching (night) and self-preening (day and night) were also significantly reduced after treatment.

DISCUSSION

Infestation with poultry red mites not only causes economical losses to production, it also has an adverse effect on animal well-being. The reduction of the PRM infestation of this flock was accompanied by a significant reduction in head scratching and self-preening and night time activity as well as a significant reduction in stress parameters such as blood corticosterone levels and heterophil:lymphocyte ratio,

both of which continued to drop through the 6-week measurement period after treatment.

The flock responded to the reduction of stress and improvement in blood parameters with improved egg production and reduced mortality.

(D. Temple *et. al*. will submit a full-length article for publication in *Plos One* in 2019.)

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Table 1. Summary of mite infestation level and blood parameters.

Parameter	Baseline (week -1)	Week 1 post-Exzolt®	Week 6 post-Exzolt®
Mite Infestation Level	2228 per trap	10 per trap[a]	1 mite/trap[a]
Blood Corticosterone	4.0 ng/mL	2.8 ng/mL[a]	1.7 ng/mL[a]
Heterophil: lymphocyte ratio	0.6	0.3[a]	0.1[a]
Hemoglobin (Hb)	7.0 g/dL	8.0 g/dL[a]	7.8 g/dL[a]
Mean corpuscular Hb	32.9 pg	33.9 pg	36.9 pg[a]

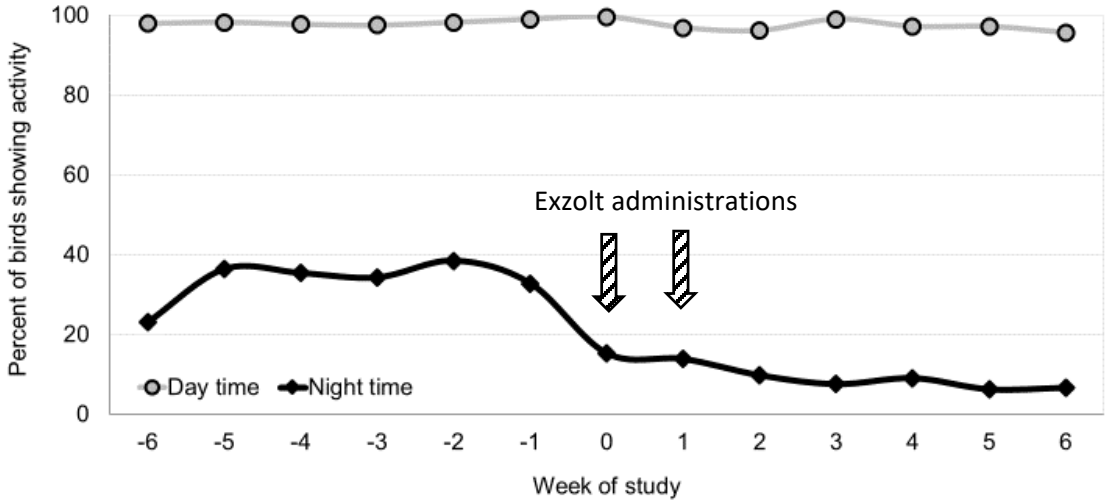
Changes vs baseline:
[a] $p < 0.01$ [b] $p < 0.05$

Table 2. Summary of flock performance parameters.

Parameter	Before treatment (week -7 till -1)	After treatment (week 0 till 6)
Average Daily Mortality	0.012 %	0.007 %[a]
Egg Production	85.2%	91.6%[b]

[a] $p < 0.01$ [b] $p < 0.05$

Figure 1. Activity level observed during the six weeks prior to treatment (weeks – 6 to -1), during and after treatment (weeks 0 to 6) with Exzolt®.



DUAL HVT CONSTRUCT VACCINE (rHVT-ND-IBD) ONSET OF IMMUNITY AND PROTECTION AGAINST VARIANT VIRUSES

VACUNA DUAL DE CONSTRUCCION HVP (rHVP-EN-IBD) AL INICIO DE LA INMUNIDAD Y PROTECCIÓN CONTRA VIRUS VARIANTES

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RESUMEN

El advenimiento de la tecnología de las vacunas de construcción HVP (rHVP) ha cambiado la forma en que vacunamos a los pollos contra varias enfermedades. Hasta hace poco, los productores avícolas estaban limitados por el uso de un solo producto debido a la interferencia entre las diferentes vacunas de rHVP o entre las HVP y las vacunas rHVP. Una vacuna de cepas duales de HVP construida que contiene el gen fusión (F) de la VEN y el gen de la proteína viral 2 (PV2) del VIBD insertado dentro de una región no-esencial del genoma FC 126 HVP ha sido desarrollado para permitirle al productor una protección simultanea contra EN, IBD y enfermedad de Marek. Los estudios fueron conducidos usando tanto rutas de administración subcutáneas como in ovo. Los datos demostraron la eficacia tanto de las cepas clásicas, como de las variantes de IBD con un alto nivel de protección a las dos semanas de edad. La eficacia también fue demostrada contra los diferentes genotipos del VEN, incluyendo las cepas Herts 33/ 56, Texas GB y el Genotipo VII de la Enfermedad de Newcastle.

SUMMARY

The advent of HVT construct (rHVT) vaccine technology has changed the way that we vaccinate chickens against several diseases. Until recently,

poultry producers were limited to the use of a single product due to interference between different rHVT vaccines or between HVT and r-HVT vaccines. A dual HVT construct vaccine strain containing the fusion (F) gene from NDV and the viral protein 2 (VP2) gene from IBDV inserted into a non-essential region of the FC 126 HVT genome has been developed to enable producers to simultaneously protect against ND, IBD and Marek's disease. Studies were conducted using both subcutaneous and *in ovo* routes of administration. Data demonstrates the efficacy of the new vaccine for both classical and variant strains of IBD with a high level of protection at two weeks of age. Efficacy is also demonstrated against different NDV genotypes, including Herts 33/56, Texas GB and Genotype VII Newcastle disease strains.

INTRODUCTION

HVT- construct vaccines providing protection against infectious bursal disease (IBD), Newcastle disease (ND) and infectious laryngotracheitis (ILT) have become widely used and accepted by the poultry industry. Unfortunately, these vaccines cannot be combined with each other or with traditional HVT vaccines without risking interference between the vaccine products (1.) Innovax®-ND-IBD (MSD Animal Health), introduced in 2017, provides protection against both ND and IBD with a single HVT vaccine containing the fusion (F) gene from NDV and the viral protein 2 (VP2) gene from IBDV. Protection is elicited against HVT, ND and IBD without interference. The efficacy of this novel vaccine was explored in several studies examining protection against different ND and IBD challenge strains.

MATERIALS AND METHODS

Studies conducted in the US used specific pathogen free (SPF) white leghorn chickens inoculated with a Innovax ND-IBD according to label instructions, either by subcutaneous inoculation or by the *in ovo* route. Following 9 CFR guidelines, all challenge viruses were titrated in chickens to calculate the virus dilution that causes disease in at least 80% (MDV GA 5 and RB1B strains) or at least 90% (NDV Texas GB strain, classical IBDV STC strain and Variant E IBDV strain) of non-vaccinated chickens. NDV challenge was conducted at 4 weeks of age using the velogenic Texas GB strain by the intramuscular (IM) route with 10^4 embryo lethal dose (ELD_{50}) in 0.2 mL dose. Marek's disease virus challenge was carried out at 5 d post-vaccination, using the GA 5 strain by the intraperitoneal (IP) route (0.2 mL/bird of stock virus diluted 1:8 in Marek's diluent) or with the RB1B strain by the intramuscular route in the leg (0.2 mL/bird of stock virus diluted 1:10 in Marek's diluent). IBDV challenge was conducted by the intraocular route with classical IBDV STC strain using $10^{2.5}$ EID_{50} per dose or with variant E IBDV strain using$10^{2.2}$ EID_{50} per dose. To satisfy registration requirements, IBD studies were conducted at four weeks post-vaccination. An additional US study was conducted to determine onset of immunity of classical IBD (not required by 9CFR for registration.) Again, chickens vaccinated according to label instructions (*in ovo*) were challenged with classical IBDV STC strain using $10^{2.97}$ EID_{50} per dose at 14 days of age and challenged birds were observed at 3 and 10 d post-challenge.

Challenge studies conducted in Europe used SPF white leghorn chickens inoculated according to label instructions with Innovax ND-IBD by subcutaneous route. Following European Pharmacopoeia guidelines, all challenge viruses were used at a dose in chickens that causes disease in at least 70% (RB1B Marek's challenge) or at least 100% (NDV Herts Weybridge 33/56, CS 89 vvIBDV) of non-vaccinated chickens. NDV challenge was conducted in separate groups using the velogenic Herts 33/56 strain by the IM route with $10^{5.0}ELD_{50}$ in 0.2 mL dose (Eu. Ph. 0450) at intervals between two and eight weeks. IBDV challenge (Eu. Ph. 0857) was conducted by the intraocular route with vvIBD strain CS 89 using 300 CID_{50} per dose at intervals between two and eight weeks. Marek's challenge (Eu. Ph. 0589) was conducted using the RB1B vvMDV strain at 9 d post-vaccination. An additional EU study was conducted to determine onset of immunity and protection against an Egyptian genotype VII ND challenge (not required by the European Pharmacopoeia for registration) with and without the addition of live Nobilis® ND Clone 30 or ND C2 given at one day of age via eye-drop following subcutaneous administration of Innovax ND-IBD with a commercial dose. Individual groups of SPF birds were challenged with Egyptian Genotype VII at $10^{5.0}EID_{50}$ per dose IM at 12, 20 and 28 days of age. NDV shedding in this study was determined by RT-PCR of cloacal and choanal samples at 0,3, 7 and 10 days post-challenge.

RESULTS

Marek's disease. The US 9CFR studies demonstrated 95% protection against MDV Ga 5 Strain when challenged at 5 d post-vaccination following *in ovo* vaccination and 85% following subcutaneous vaccination. The EU RB1B vvMDV challenge demonstrated 87.8% relative protection; 87.8% protected vaccinates vs. 100% of the birds

positive in controls (RPP > 80% is required) when challenged at 9 d post-vaccination.

IBD. The requirement for protection in the US or the EU is ≥ 90%. The US 9CFR studies demonstrated 100% protection by subcutaneous and 97% protection by *in ovo* vaccination against STC IBD challenge when challenged at 4 weeks of age. When challenged with Delaware Variant E at four weeks of age, the subcutaneous vaccinates demonstrated 97% protection, while the *in ovo* group demonstrated 91% protection. The 2-week onset of immunity challenge with STC IBD was conducted in two groups of *in ovo* vaccinates and demonstrated 97% and 92% respectively when birds were challenged at two weeks of age.

The European vvIBD CS 89 challenge studies following subcutaneous vaccination demonstrated 90% protection at two weeks, 95% at three weeks, and 100% protection at four, six, and eight weeks (Figure 1.)

ND. The requirement for protection in the US or the EU is ≥ 90% protection against the challenge strain. The US 9CFR studies demonstrated 97% protection by subcutaneous and 100% protection by *in ovo* vaccination against Texas GB challenge when challenged at four weeks of age.

The European registration study using Herts 33/56 challenge following subcutaneous vaccination demonstrated 20% protection at 2 weeks, 68% protection at 3 weeks, 90% protection at four weeks and 100% protection at 6 and 8 weeks (Figure 1.)

The challenge study of Innovax ND-IBD alone with Egyptian ND Genotype VII demonstrated 32% protection against clinical signs when challenged at 12 days of age, but 90% protection at 20 d and 95% at 28 d. When live Nobilis ND Clone 30 was applied by eye-drop at one day of age in addition to Innovax ND-IBD, the protection increased to 100% at all three challenge ages. Similarly, protection increased to 100% at all three challenge ages when Nobilis ND C2 was applied by eye-drop at one day of age in addition to Innovax ND-IBD (Figure 2.)

RT-qPCR of cloacal shedding showed 80 – 100 % of the control birds shedding at 3 d post challenge. At 7 and 10 d post challenge no control birds were left as all died. For the vaccinated groups, the results were in line with the clinical protection observed. After challenge of Innovax ND-IBD alone with Egyptian ND Genotype VII 95% of the birds showed shedding at 3 d post challenge when challenged at 12 days of age, but only 40% shedding at 20 d and no shedding (0%) at 28 d. When live Nobilis ND Clone 30 was applied by eye-drop at one day of age in addition to Innovax ND-IBD, no shedding was seen at all three challenge ages. Shedding (15%) was only seen at the first challenge age when Nobilis ND C2 was applied by eye-drop at one day of age in addition to Innovax ND-IBD and no shedding for the other two challenge ages (Figure 3). For the choanal shedding the results reflected the cloacal challenge results but lower percentages were observed for all groups.

DISCUSSION

This brief recap of studies conducted prior to the registration of Innovax ND-IBD and additional studies to investigate the onset of immunity for IBD and the protection against Delaware Variant E and ND Genotype VII demonstrate the broad efficacy of the first dual construct HVT vaccine introduced to the industry. Additional studies investigating the efficacy in the face of maternal antibody and to gain *in ovo* registration in the EU are on-going.

HVT construct vaccines require a lead time to develop full protection. Maternal antibody can afford early protection for many IBD challenges, but maternal antibody is less effective against ND challenge. The studies conducted with Egyptian Genotype VII challenge demonstrate that the addition of live Nobilis ND Clone 30 or C2 to the dual HVT construct vaccine provided protection against clinical signs as young as 12 days of age. Nobilis ND Clone 30 effectively eliminated the shedding of challenge virus as well already at 12 days of age and Nobilis ND C2 eliminated shedding at 20 and 28 d. Birds vaccinated with Innovax ND-IBD showed increasing level of protection from 12, 20 to 28 days of age which was reflected by reduction in the level of shedding over time.

(A full paper with more detail about the materials and methods and results of the studies will be submitted to *Avian Pathology* by M. van Hulten *et al*. in 2019.)

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Figure 1. Percent protection and onset of immunity after CS 89 IBD challenge or Herts NDV 33/56 challenge by challenge age.

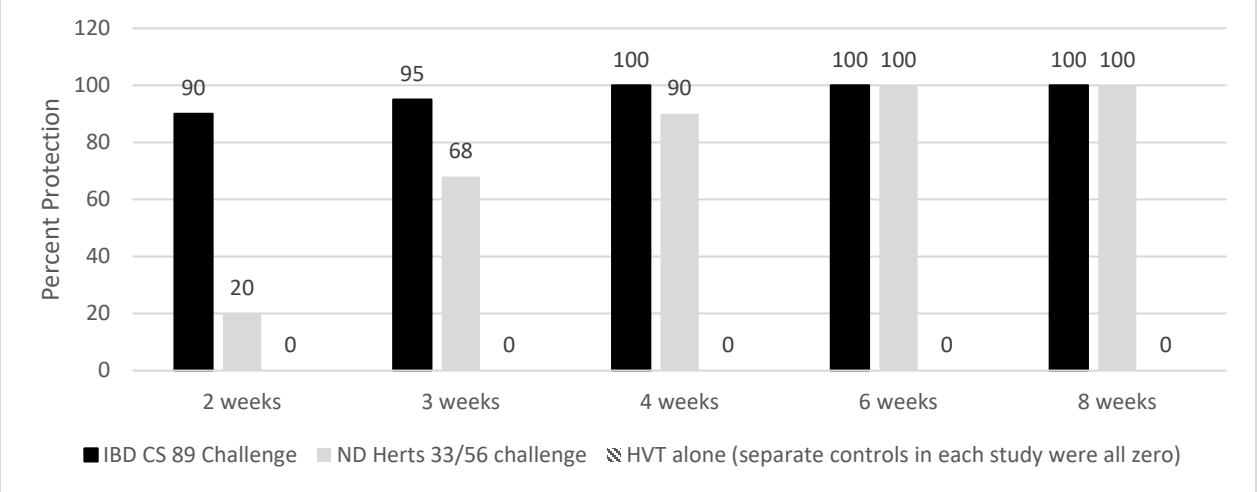


Figure 2. Innovax® ND-IBD percent protection and onset of immunity after NDV Genotype VII challenge alone, and with the addition of Nobilis® ND C2 or Nobilis ND Clone 30 administered at one day of age.

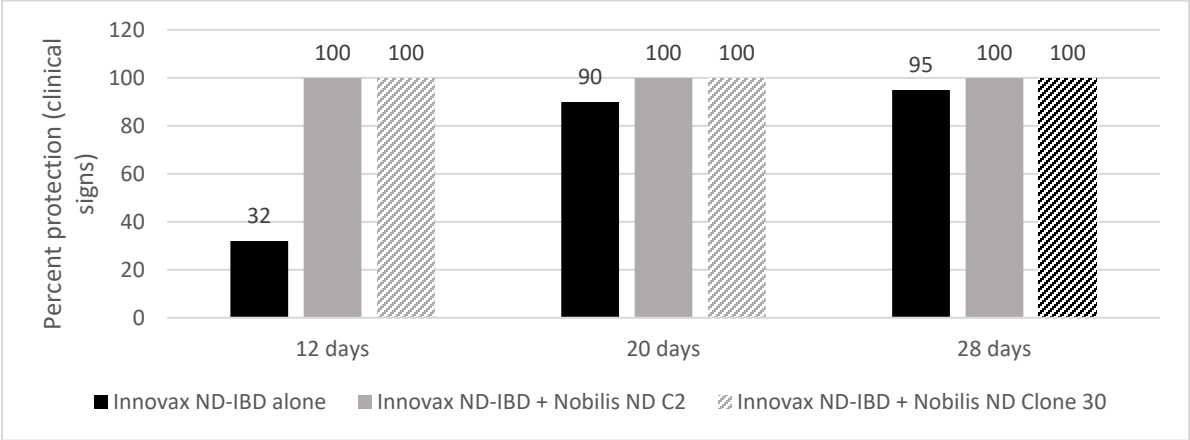
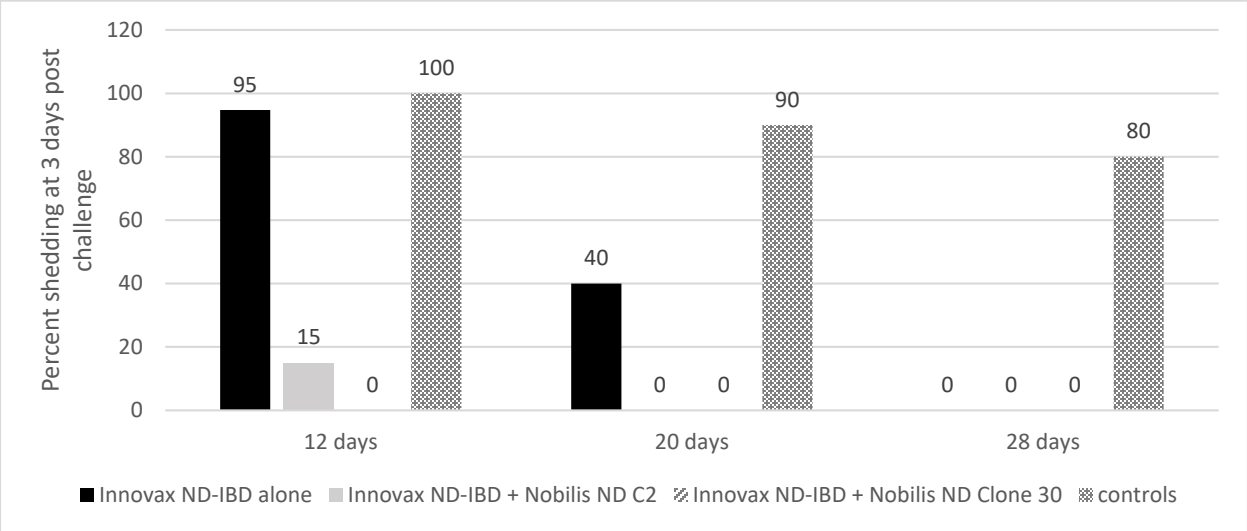


Figure 3. NDV Genotype VII virus shedding (3 d post challenge) for birds vaccinated with Innovax® ND-IBD alone or together with Nobilis® ND Clone 30 or C2 and non-vaccinated control birds (challenges at 12, 20 and 28 d post vaccination).



IMPORTANCE OF THE GIT ALLOMETRY IN NICHOLAS 700 TURKEYS PRODUCTION

IMPORTANCIA DE LA ALOMETRIA DEL TGI EN LA PRODUCCION DE PAVOS NICHOLAS 700

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RESUMEN

En la industria avícola, para lograr el máximo potencial de crecimiento de las aves, son necesarias una adecuada absorción y digestión de los nutrientes, mismos que dependerán del consumo y calidad de los alimentos, así como del desarrollo del Tracto Gastrointestinal (TGI). Con la finalidad de evaluar el desarrollo del TGI, se realizó el siguiente experimento. Se emplearon 120 pavos machos Nicholas 700 de 5 semanas de edad, los cuales se distribuyeron al azar en 2 grupos de 4 réplicas con 15 pavos cada una. Las aves fueron alimentadas en dos etapas: Desarrollo (de 5 a 8 semanas) y Finalización (de 9 a 12 semanas). En la etapa de desarrollo se utilizaron dos dietas: (T1) reducida en proteína y energía (22% PC y 2850 kcal/kg de EM) y (T2) con un mayor perfil nutricional (27% PC y 2875 kcal/kg de EM); en la etapa de finalización solo se ofreció la dieta con mayor perfil nutricional para ambos grupos. Se tomaron muestras para la medición alométrica al final de ambas etapas. Durante la primera etapa, se observó un aumento en los órganos del Tratamiento 1, la proporción con respecto al peso vivo del TGI (11.16 vs 9.85%), proventrículo (0.31 vs 0.27%) y molleja (2.25 vs 1.67%) fue significativamente mayor ($P<0.05$); además, se encontró un aumento ($P<0.05$) en la longitud del TGI (220 vs 207cm), asa duodenal (23 vs 21cm) e intestino delgado (152 vs 142cm). En la etapa de finalización, los pesos y longitudes de todos los órganos medidos fueron estadísticamente similares ($P>0.05$). En conclusión, el uso de dietas de bajo perfil nutricional podría estar asociado con cambios y adaptaciones en el cuerpo del pavo que pueden modificar la alometría de sus órganos del TGI.

SUMMARY

In the poultry industry, to achieve the maximum growth potential of the birds, adequate absorption and digestion of nutrients is necessary, it will depend of the conditions of fed intake and food quality, as well as the development of the gastrointestinal tract (GIT). In order to evaluate the development of the GIT, the following experiment was undertaken. A total of 120 male turkeys (Nicholas 700, 5-wk-old) were randomly distributed in 2 groups of 4 replicates of 15 turkeys each. Birds were raised in an 8-wk feeding trial divided in two stages: Development (5 to 8 week) and Finisher (9 to 12 week). Throughout Development phase two diets were used: (T1) reduced in protein and energy (22% CP and 2850 kcal/kg ME) and (T2) high nutrient profile (27% CP and 2875 kcal/kg ME); in Finisher stage, only high nutrient profile diet was offered in the two groups. Samples were taken for allometric measurements at the end of both stages. During the first stage, an increase of the Treatment 1 organs was observed, the ratio to live weight of the GIT (11.16 vs 9.85%), proventriculus (0.31 vs 0.27%) and gizzard (2.25 vs 1.67%) was significantly higher ($P<0.05$); furthermore, an increase ($P<0.05$) in GIT (220 vs 207cm), duodenum (23 vs 21cm) and small bowel (152 vs 142cm) lengths was found. In the Finisher stage weights and lengths of all organs measured were statistically similar ($P>0.05$). In conclusion, the use of low nutritional profile diets might be associated with changes and adaptations in the turkey's body that may modify the allometry of their gastrointestinal tract organs.

OBJECTIVE

It is important to reach the growth potential maximum expression in meat birds in order to achieve better results in production performance. To make this possible it is necessary an adequate absorption and digestion of nutrients, which will depend on the conditions of the fed intake and the food quality, as well as the development of the GIT, because any damage will have a negative impact, assigning the energy destined to meat production to defense functions.

Organ functions and its weight are correlated with animal live weight, therefore, functions might be established in relation to live weight and organ weight. Any increase in the body or organ size of an animal may modify its form and proportions in order to be more efficient to perform its functions (1). With this motivation, it is necessary to carry out studies that provide information about the changes that may occur in the development of birds GIT, specifically in turkeys, after the use of diets with different nutrient profiles throughout the brooding period.

MATERIALS AND METHODS

This work was accomplished at the Centro de Enseñanza, Investigación y Extensión en Producción Avícola (CEIEPAv), of the FMVZ-UNAM. One hundred and twenty 5-wk-old Nicholas 700 male turkeys were used and randomly distributed in two treatments with four replicates of fifteen turkeys each. The experiment was divided in two stages: the first stage (Development) comprised from week 5 to 8, and the second stage (Finisher) from week 9 to 12. The experimental treatments were administered in the Development stage and consisted in the following diets:

Treatment 1 (T1). Diet with lower protein and energy levels (22% CP and 2850 kcal/kg ME).

Treatment 2 (T2). Diet with higher nutrient profile (27% CP and 2875 kcal/kg ME).

Throughout the finisher stage, treatments were as follows:

T1 and T2. Diet with higher nutrient profile (27% CP and 2875 kcal/kg of energy).

Samples were taken for allometric measurements (weight and length) at the end of both stages (weeks 8 and 12). GIT was dissected from the proventriculus to the cloaca, organs were measured and weighed using a measuring tape and a digital scale respectively. Measurements of GIT organs were obtained as a whole and separately. Once separated from GIT, proventriculus and gizzard were dissected and its content was removed, weight was registered from both empty organs. Finally, small and large intestine were measured and weighed separately, and liver was only weighed.

Means of the data collected were compared by t-test, a significant level of 5% was used for estimating differences between treatments. Database was compiled with Microsoft® Excel® 2013 and JMP® Design of Experiments Software (JMP. 2013. Version 11.0.0. Cary, NC: SAS Institute) was used for statistical analysis.

RESULTS AND DISCUSSION

The first stage, the reduction of protein and energy levels showed an impact ($P<0.05$) in the allometry of T1. Turkeys exhibited an increase in the ratio to live weight of the GIT (11.16 vs. 9.85%), proventriculus (0.31 vs 0.27%) and gizzard (2.25 vs 1.67%); additionally, birds displayed an increment in the length of GIT (220 vs 207cm), duodenal loop (23 vs 21cm) and small intestine (152 vs 142cm). On the other hand, in the Finisher stage no significant differences ($P>0.05$) were found in the organ-live weight ratio, nor in length of the GIT or its segments, so that the adaptive changes manifested in Development stage disappeared, probably as a result of the supplementation of a diet with higher nutrient levels. Similar studies (1, 2), where a restriction of fed intake was used, showed an increase in the relative weight of some gastrointestinal organs (crop, gizzard and small intestine) allowing a greater food storage capacity. The latter suggests that providing a diet with lower nutrient levels to turkeys might increase the relative size and length of some of its organs as an adaptive that may translate to improvements in productive performance related to recovery growth.

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RED MITE IN A BROILER BREEDER FLOCK FROM A (APLENTY) TO Z (ZERO)

ÁCAROS ROJOS EN UNA PARVADA DE REPRODUCTORES DE POLLO DE ENGORDA DE LA A (ABUNDANTES) A LA Z (CERO)

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RESUMEN

Dos parvadas de gallinas en una instalación de producción de de reproductoras pesadas en el oeste de Canadá experimentó un pico severo de mortalidad asociado con septicemia por *E. coli*. Este brote de enfermedad coincidió con cambios regulatorios que prevenían el uso de plaguicidas para el control del diagnóstico de infestación con *Dermanyssus gallinae* (ácaro rojo avícola – ARA). Usando electroforesis en gel de campo pulsante (en inglés PFGE), los aislamientos de *E. coli* de las aves clínicamente enfermas y de los ácaros se determinó que eran indistinguibles, llevando a la conclusión que la septicemia de *E. coli* fue secundaria al parasitismo intenso. Dado que los costos de producción asociados con este brote y la disminución de la lista de productos para el control del ARA, se buscaron otras opciones de tratamiento. Los detalles de este caso, incluyendo el método de usado para cuantificar la infestación de ARA, el tratamiento alternativo y el resultado exitoso serán presentados.

SUMMARY

Two hen flocks on a broiler breeder production facility in western Canada experienced a severe spike in mortality associated with *E. coli* septicemia. The disease outbreak coincided with regulatory changes preventing the use of a pesticide to control a diagnosed *Dermanyssus gallinae* (poultry red mite - PRM) infestation. Using pulsed-field gel-electrophoresis (PFGE), *E. coli* isolates from clinical birds and mites were determined to be indistinguishable, leading to the conclusion *E. coli* septicemia was secondary to heavy parasitism. Given the production costs associated with this outbreak and the diminishing list of products to control PRM, other treatment options were sought. The details of this case, including the method used to quantify the PRM infestation, the alternate treatment and the successful outcome will be presented.

INTRODUCTION

Parasites are a worldwide challenge when rearing poultry flocks (8), with mite infestations threatening overall bird health, wellbeing and production (8, 11). In Canada, there are few products labelled for treatment of mite infestations in poultry, making management of existing infestations extremely difficult. Heavy infestations with PRM can lead to decreased egg production, increased feed costs, anemia, weight loss, and in severe cases, an increase in mortality (11, 12). Mites can also act as reservoirs and vectors for important poultry diseases such as *E. coli* and *Pasteurella multocida*, as well as food borne pathogens like *Salmonella enteritidis* (6, 12). Finally, being indiscriminate feeders, PRM not only irritate poultry operation employees but also pose a human health risk, making employee retention a challenge (1).

CASE REPORT

Case history. In 2013, a PRM (*Dermanyssus gallinae*) infestation was detected in a western Canadian multi-age broiler hatching egg production site. Despite implementing an integrated pest management (IPM) program, complete eradication of PRM was not achieved.

In mid-2017, two separate hen flocks on this facility experienced an increase in mortality. Dead birds from each flock, aged 41 and 31 weeks, were submitted to a diagnostic lab for necropsy. Post mortem findings were consistent with septicemia, primarily manifested as peritonitis and oophoritis. *E.*

coli was repeatedly isolated from sick birds and despite *in vitro* sensitivity to tetracycline, the flocks failed to respond to medication. Treatment with enrofloxacin, while successful during therapy, was met with relapse following completion of drug administration. Mortality during this period continued to increase.

During subsequent farm investigations it was noted that the PRM infestation had worsened, with the increase in population attributed to Health Canada's Pest Management Regulatory Agency prohibiting the use of carbamates in livestock (3). This farm had been using Sevin®, carbaryl (a carbamate), as part of its IPM program. Once the on-farm treatment inventory was depleted, an alternate product was not substituted into the program.

Considering the farm history, IPM program alterations, and treatment failures, hematogenous spread of *E. coli* challenge via PRM was suspected.

Microbiology. To determine whether the mites were playing a role in the dissemination of *E. coli* and development of septicemia, isolates grown from healthy and sick birds, mites and the environment were compared using PFGE. These comparisons revealed isolates from PRM and affected birds were closely related, and importantly, different from the isolates cultured from healthy birds and the environment. This finding supported the final diagnosis of *E. coli* septicemia secondary to PRM (*Dermanyssus gallinae)* parasitism.

PRM reduction. Simultaneously with PFGE analysis, on-farm efforts were directed to PRM reduction. Treatment with tetrachlorvinphos 50% (Debantic® 50WP Insecticide, Bayer), an organophosphate, according to the product label, had no observable effect. In a subsequent attempt to kill mites, permethrin (Ectiban®, Merck Animal Health), a pyrethroid, was applied according to the product label. The following day, additional employees were required to clean dead mites from eggs during collection. Therefore, the treatment was deemed successful at reducing the mite population.

Case results. Following the observable impact on mite populations, the flocks were treated again with enrofloxacin and the subsequent decline in bird mortality persisted.

CASE FOLLOW UP

PRM treatment options. Following the increase in severity of PRM infestation and subsequent *E. Coli* outbreak, it was acknowledged ongoing losses would persist unless the PRM population was eradicated. Most PRM treatment products available in Canada, the US and other parts of the world (pyrethroids and organophosphates) are extremely toxic and can only be applied to the environment in the absence of poultry; not to currently infested poultry flocks. Also, the possibility for residues from these products to occur in meat and/or eggs has been demonstrated in the EU (5). Treatments which can be applied to birds are still extremely toxic compounds and require labor intensive application methods. Finally, efficacious carbaryl products for PRM control, such as Sevin, were banned from use Canada in 2016 (3), as well as the EU and US in 2007 and 2009, respectively (5, 15).

In the EU in 2017, Merck Animal Health launched Exzolt®, a fluralaner product and new treatment option for PRM infestations in poultry species. Oral safety of fluralaner was evaluated in mammals and avian species, and found to be well tolerated with a wide margin of safety in laying hens (7). Additionally, this product was nearly 100% effective for the eradication of PRM infestations in treated laying flocks in the EU (9,10). An Emergency Drug Release (EDR) request for Exzolt was consequently submitted through the Veterinary Drugs Directorate (VDD) of Canada to access this product for use on the premise described in the case report.

PRM monitoring and treatment. Pending approval of the EDR request, the veterinary team attempted to quantify the on-farm mite load with AviVet, a company based in the Netherlands, which provides validated PRM traps and software to monitor mite populations (4). PRM were detected in all 4 production houses, but were not detected in the pullet facilities.

Once the EDR was approved, extensive planning and co-ordination commenced between the local veterinary team, Merck technical service and veterinary team, farm owner, manager and staff. The decision to treat was delayed until three criteria were met: 1. Recommendations from a biosecurity audit were implemented; 2. All six barns on the multi-age premise were populated; and 3. The ambient temperature was warmer (summer).

The biosecurity audit identified fly control as a major risk factor for post-treatment PRM recontamination, as mites carried by flies had been documented on the premise. Additional fly control strategies were implemented following this observation. Requiring all six barns to be populated ensured that all birds and therefore all barns on the site had been treated and that a potential housing reservoir of infection did not remain. Finally, Exzolt treatment was delayed until the summer months because the mite life cycle is temperature dependent. At 15° C (59° F), the total time for PRM development approaches 30 days; however when the ambient temperature is 35°C (95°F), the life cycle is closer to 7 days (14). Historical climate data for the farm location from 1981 to 2010 indicated the warmest temperatures in July, with a

daily maximum of 25°C (77°F) and minimum of 12°C (54°F) (2). Exzolt is labeled for 2 applications, 7 days apart, and it was assumed that therapeutic levels would be maintained in the birds for approximately 2 weeks. Delaying treatment until warmer weather was to ensure the life cycle of all mites on the premise would progress such that at some point during the 14-day therapeutic period, a blood meal would be taken by all PRM on the premise, regardless of their stage in the life cycle.

All six barns received 0.5 mg fluralaner (0.05 mL Exzolt solution)/kg body weight twice, with seven days between treatments as per label instructions. Mite traps were placed 2 days prior to initiating the treatment protocol, and again, 11 days following the last administration for a post-treatment assessment. Traps were subsequently placed 25- and 95-days post-treatment to evaluate for re-contamination.

PRM treatment outcome. Mites were detected in 4/6 barns on the premise prior to treatment with Exzolt. Following treatment, mites were not detected in the post-treatment assessment, nor in any of the re-contamination assessments to date. Mortality due to *E. coli* infection has remained low and for the first time in at least 5 years, the farm is free of PRM.

(A detailed paper will be submitted to *Avian Diseases* by J. Nicholds *et al*. in 2019.)

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EFECTO DE β-MANANASAS EN DIETAS MAÍZ-SOYA EN PARÁMETROS PRODUCTIVOS E INTEGRIDAD INTESTINAL

EFFECT OF B-MANNANASE ON CORN-SOY DIETS ON PRODUCTIVE PARAMETERS AND INTESTINAL INTEGRITY

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SUMMARY

The β-mannans found in the soybean meal are an antinutritional factor for broilers, causing an unproductive immune response, and also increasing the intestinal viscosity and decreasing the amount of available energy. In this current experimental work β-mannanases were evaluated against the non-desired effect of the β-mannans as an alternative to the antibiotics as growth promotors, in broilers Ross 308 strain. 600 chickens were used in 4 treatments and 6 replicates, under a completely randomized design and a factorial 2 x 2 arrange was used for its analysis. The productive parameters were measured: weight gain, feed intake, feed conversion, mortality and carcass yield. Also immunoglobulin A from the jejunum was quantified and the villi length, the cript depth and the villi/cript ratio in the duodenum were evaluated at 21, 35 and 49 days of age in both studies. There were no statistical significant differences in the productive parameters, whereas in the IgA quantification and in the villi histological analysis yes, showing that the β-mannanases stimulate the immune system having more quantity of IgA and that they have a beneficial effect on the villi. As a conclusion, the use of β-mannanases works as an alternative option for growth promotion antibiotics, because the productive parameters can be matched and improve the intestinal health in the chickens. The world trend is focussed in the search for new alternatives for the maximum good use of nutrients included in the broilers diets.

RESUMEN

Los β-mananos que se encuentran en la pasta de soya son un factor antinutricional para los pollos de engorda, que causan una improductiva respuesta inmune, además de incrementar la viscosidad intestinal y disminuir la cantidad de energía disponible. En el presente trabajo experimental se evaluaron las β-mananasas contra el efecto no deseado de los β-mananos y como una alternativa a los antibióticos promotores de crecimiento, en pollo de engorda de la estirpe Ross 308. Se utilizaron 600 pollos en 4 tratamientos y 6 réplicas, bajo un diseño completamente al azar y se usó un arreglo factorial 2 x 2 para su análisis. Se midieron los parámetros productivos: ganancia de peso, consumo de alimento, conversión alimenticia, mortalidad y rendimiento de la canal. También se cuantificaron inmunoglobulinas A de yeyuno y se evaluó la longitud de vellosidades, profundidad de cripta y la relación vellosidad/cripta de duodeno a los 21,35 y 49 días de edad en ambos estudios. No se encontraron diferencias estadísticamente significativas en los parámetros productivos, mientras que en la cuantificación de IgA y el análisis histológico de vellosidades si, mostrando que las β-mananasas estimulan el sistema inmune al haber mayor cantidad de IgA y que tienen un efecto benéfico en las vellosidades. Como conclusión, el uso de β-mananasas funciona como opción alterna a los antibióticos promotores de crecimiento, pues se pueden igualar parámetros productivos y mejorar la salud intestinal de los pollos, ya que, la tendencia

mundial está enfocada en la búsqueda de nuevas alternativas para el aprovechamiento máximo de nutrientes incluidos en las dietas de pollo de engorda.

INTRODUCCIÓN

Las enzimas pueden ser una alternativa a los APC, que son cada vez más utilizadas en la producción de pollo de engorda. El uso de enzimas en los últimos años como aditivos en la dieta se ha expandido rápidamente, numerosos estudios han sido encaminados para estudiar su impacto en el desarrollo del pollo de engorda (14,15). A diferencia de los APC y la resistencia a antibióticos, el uso de enzimas está ampliamente aprobado para su uso en dietas de animales de producción debido a que son productos de la fermentación y por lo tanto, de origen natural, que no representan algún tipo de peligro para el animal y el consumidor (14).

Los β-mananos son un factor antinutricional, que además de aumentar la viscosidad intestinal funcionan como PAMP. Para entender la importancia y la manera en que afectan a los pollos los β-mananos es necesario saber que, en el huésped, los PRR de superficie y citosólicos expresados en el intestino, están involucrados en la detección de motivos moleculares microbianos altamente conservados, llamados PAMP, aunque, dado que hay microorganismos comensales que comparten dichos patrones, el término PAMP ha sido recomendado para cambiarse por MAMP. La interacción PRR-PAMP es importante para desencadenar la señalización, que conlleva a la activación de genes que codifican para moléculas proinflamatorias. (7,21) Los β-mananos que se encuentran en la pasta de soya y otros ingredientes utilizados en las dietas de pollo de engorda se reconocen como PAMP. Los mananos en diferentes configuraciones son parte de numerosos patógenos como hongos, bacterias y virus. La respuesta inmune innata de los animales está altamente especializada para reconocer antígenos de patógenos que incluyen mananos. Se ha demostrado que los β-mananos son capaces de estimular el sistema inmune innato y producir una respuesta no productiva, llamada respuesta inmune inducida por el alimento (FIIR, por sus siglas en inglés), que solo produce un gasto de energía, en la que hay proliferación de monocitos, macrófagos e incremento en la producción de citocinas. (8,12)

Específicamente, las β-mananasas tienen como objetivo compuestos ricos en mananos, como los glucomananos y los β-mananos o también llamados β-galactomananos, que están presentes en ingredientes de la dieta como la pasta de soya, entre otros (19). De acuerdo a la nomenclatura establecida por la Unión Internacional de Bioquímica y Biología Molecular (IUBMB, por sus siglas en inglés), las β-mananasas son referidas como EC 3.2.1.78, clasificación para glucosidasas y endo-hidrolasas de compuestos O- y S-glicosilados (13). La reacción que lleva a cabo es la de hidrólisis aleatoria de enlaces 1,4-β-D-manosídicos de mananos, galactomananos y glucomananos, liberando pequeños fragmentos β-1,4-manano-oligosacáridos (manobiosa y manotriosa), además de pequeñas cantidades de manosa, glucosa y galactosa (3–5,19).

MATERIALES Y MÉTODOS

La presente investigación se realizó en el Centro de Enseñanza, Investigación y Extensión en producción Avícola (C.E.I.E.P.Av) de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Nacional Autónoma de México. La investigación tuvo una duración de 49 días. Los animales permanecieron en el CEIEPAv desde el primer día hasta finalizar el experimento. Las unidades experimentales de esta investigación estuvieron conformadas por 25 pollos de la estirpe Ross 308 de un día de edad, con un peso promedio de 42 g, utilizándose un total de 600 pollos (50% hembras 50% machos), el suministro de agua y alimento durante el trabajo experimental fue realizado *ad libitum*. Los procedimientos de manejo realizados en las aves del presente experimento fueron aprobados por el Comité Institucional para el Cuidado y Uso de los Animales de Experimentación (CICUA-FMVZ-UNAM).

Se evaluó el efecto de la adición de la enzima β-mananasa (0.5 kg/t de alimento) con el efecto del antibiótico promotor de crecimiento Enramicina (0.123 kg/t de alimento). Las unidades experimentales se distribuyeron bajo un diseño completamente al azar y para su análisis se consideró un arreglo factorial 2 x 2, conformado de 4 tratamientos y 6 réplicas. El diseño experimental fue el siguiente:

Tratamiento 1: dieta basal de maíz-soya con 0.123 kg/t de enramicina; tratamiento 2: como 1 + 0.5 kg/t de β-mananasas; tratamiento 3: dieta basal de maíz-soya con 0.5 kg/t de otro aditivo; tratamiento 4: como 2 + 0.5 kg/t de otro aditivo.

Para fines del trabajo a presentar, se mostrará el efecto individual del factor β-mananasas en los tratamientos utilizados.

Se evaluaron semanalmente los parámetros productivos: ganancia de peso, consumo de alimento, conversión alimenticia, consumo de alimento, mortalidad y rendimiento de la canal al final del ciclo. Se evaluó la inmunidad humoral local por medio de la concentración de IgA en yeyuno, por medio de un ensayo inmunoabsorbente ligado a enzimas indirecto (ELISA, por sus siglas en inglés), para lo que se tomaron 12 muestras por tratamiento, a los 21, 35 y 49 días de edad. También se evaluaron al mismo tiempo

las vellosidades intestinales duodenales, con 12 segmentos por tratamiento, se midió la LV, la PC y la RVC. Las aves fueron sacrificadas por dislocación cervical.

Para el análisis estadístico se utilizó un análisis de varianza para un diseño factorial de dos factores y la prueba de Tukey para diferencia de medias entre grupos. Para analizar los resultados de las variables se utilizó el software de computadora JMP®, versión 11. Se utilizó una significancia de $P \leq 0.5$.

RESULTADOS

Parámetros productivos. Para ganancia de peso, consumo de alimento, conversión alimenticia, mortalidad y rendimiento de la canal, no se encontraron diferencias estadísticamente significativas ($p>0.05$) cuando se utilizaron las β-mananasas; cabe mencionar que, en consumo de alimento, con el factor β-mananasas los pollos tuvieron un menor consumo (1.36%) aunque solo fue diferencia numérica.

Inmunidad humoral local. En cuanto a la cantidad de IgA intestinal (ng/mL), se encontró que el factor β-mananasas incrementó la cantidad de IgA en duodeno de manera significativa ($p<0.05$) a los 35 días de edad, en un 23.65% más, que cuando no se utilizaron las enzimas. Para el día 21 de edad solo hubo diferencia numérica mayor por 2.77%, mientras que al día 49 no se encontraron diferencias significativas.

Vellosidades intestinales. En el día 21 de edad, el factor β-mananasas fue significativo en cuanto a la LV y PC, para el LV se tuvieron valores 3% menores, y también para PC (17%), respecto a RVC no hubo diferencias significativas, aunque se obtuvo una diferencia numérica mayor de 2.56% cuando se utilizaron las enzimas.

Para el día 35 de edad, el factor β-mananasas fue mayor numéricamente para la LV (1.56%), mientras que fue significativamente menor para PC (6.94%) y mayor para RVC (5.17%).

Al día 49 de edad, el uso de β-mananasas fue significativo con un valor menor de PC (8.92%) y un valor mayor de RVC (5%), en cuanto a LV no se encontraron diferencias, aunque al usar las enzimas, se obtuvieron vellosidades 2.26% más largas.

DISCUSIÓN

En la reciente década ha sido documentado por diversos investigadores la utilización de β-mananasas como buenas alternativas de los APC, obteniendo mejores conversiones alimenticias, ganancias de peso, entre otros parámetros productivos, así como reducir de manera importante los procesos de inflamación a nivel del intestino, mejorando la salud intestinal del pollo de engorda. (1,2,6,9,10,16,17,20). Entre otros beneficios reportados, están la disminución del desarrollo de lesiones contra desafíos de *Clostridium perfringens* e *Eimeria spp.* y la disminución de viscosidad en dietas con altos niveles de β-mananos. (1). Diferente a los resultados obtenidos por los autores antes mencionados, no se obtuvieron diferencias estadísticamente significativas en los parámetros productivos, aunque cabe señalar que, sin utilizar el APC, la inclusión de las β-mananasas en la dieta puede igualar los parámetros obtenidos utilizando el APC. Por otro lado, se obtuvieron valores mayores de IgA intestinal, lo que muestra que las β-mananasas son inmunoestimulantes y pueden mejorar la salud intestinal de los pollos (11). Para las vellosidades intestinales, utilizando las β-mananasas se pudieron observar mejores valores para PC y para RVC que, aunque las enzimas no sobresalieron en LV, el valor de RVC resulta de más importancia en cuanto a la eficiencia digestiva de los pollos. Esto se explica debido a las moléculas resultantes de la degradación de los β-mananos, que son carbohidratos fermentables, los cuales incrementan los ácidos grasos de cadena corta, que tienen un efecto positivo a nivel de vellosidades (18). Se puede concluir que el uso de β-mananasas puede funcionar como alternativa a los APC, que además con la prohibición de APC en la Unión Europea en el 2006, la tendencia mundial está enfocada en la búsqueda de nuevas alternativas para el aprovechamiento máximo de nutrientes incluidos en las dietas de pollo de engorda.

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IMMUNE CELL SUPEROXIDE DISMUTASE ACTIVITY IS A POTENTIAL MARKER OF MINERAL RELATIVE BIOAVAILABILITY IN LIPOPOLYSACCHARIDE-CHALLENGED LAYING HENS

ACTIVIDAD DE LA SUPERÓXIDO DISMUTASA EN CÉLULAS INMUNES COMO POTENCIAL MARCADOR DE LA DISPONIBILIDAD RELATIVA DE MINERALES EN GALLINAS DE POSTURA DESAFIADAS CON LIPOPOLISACÁRIDOS

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RESUMEN

Ahora existen muchas fuentes nuevas de minerales para cumplir con las necesidades nutricionales que surgen de la producción de carne. Las fuentes de minerales son evaluadas típicamente basándose en su biodisponibilidad relativa. Los minerales traza como el Zn y el Mn juegan un papel crítico como catalizadores de enzimas. Dos fuentes de Zn y de Mn fueron utilizadas para el experimento. Se condujo un experimento para determinar si la actividad de la superóxido dismutasa (SOD) es sensible a las diferentes fuentes alimenticias de minerales de la dieta en un desafío con LPS en gallinas de postura en concentraciones nutricionales y no-nutricionales. Se presentaron interacciones significativas ($P < 0.01$) en la actividad del SOD cuando las fuentes minerales fueron adicionadas ya sea a un 100% o un 50%. Se inyectaron a las gallinas con LPS y los monocitos y heterófilos ($P< 0.05$) tuvieron incrementos de actividad del SOD comparado con células inmunes aisladas de gallinas ponedoras inyectadas con PBS. Sin embargo, las aves adicionadas en su dieta con 50% de SO4 tuvieron menor ($P< 0.05$) actividad del SOD que las otras aves inyectadas con LPS (50% OHCl, 100% SO4, y 100% OHCl). Los monocitos y heterófilos aislados de las aves adicionadas con 50% OHCl en la dieta tuvieron una actividad similar del SOD comparado con las aves adicionadas con 100%, de ya sea fuentes de minerales de SO4 o OHCl. Estos datos nos indican que la actividad del SOD de las células inmunes de las gallinas ponedoras puede ser un parámetro sensible para evaluar los minerales RBV cuando se alimenta con concentraciones sub-nutricionales.

SUMMARY

Many novel mineral sources now exist for meeting the nutrient needs of animals raised for meat production. Mineral sources are typically evaluated based on their relative bioavailability (RBV). Bioavailability is determined by measuring a response parameter that is highly sensitive to the nutrient being tested and then compared to a "benchmark" source. For example, bone ash mineral content is a parameter used to determine RBV for many different sources of minerals including P, Ca, and Zn.

Trace minerals like Zn and Mn play a critical role as enzyme catalysts. The function of these metalloenzymes may prove to be a highly sensitive parameter for the determination of mineral source RBV. Both Zn and Mn are critical for immune system function (5,4) due to their importance in several enzyme systems. For example, superoxide dismutase (SOD) is a metalloenzyme that requires Zn and Mn and could be a sensitive marker for the determination of mineral source RBV in immune challenged laying hens.

The injection of lipopolysaccharide (LPS) derived from gram-negative bacteria stimulates an immune response in poultry (3). During this response, monocytes and heterophils produce SOD to detoxify the by-products of an immune cell mediated super-oxide burst (10). Because of the ability to convert superoxide radicals to hydrogen peroxide and oxygen, SOD is considered a potent antioxidant in poultry (8). Zinc and Mn play a critical role in both stabilizing the enzyme and acting as a catalyst for SOD function. Moreover, deficiencies in dietary supply of Zn and Mn have shown to lower SOD activity (6, 9). Because SOD activity increases in animals exposed to an LPS challenge and is sensitive to dietary trace mineral

status, it may be possible to utilize this challenge model as a method to assess trace mineral RBV.

Two sources of Zn and Mn were utilized in the experiment. The sulfate (SO4) form of these minerals are commodities and used throughout the world. This is why SO4 forms are typically used as the "reference" mineral source when assessing mineral RBV. However, SO4 minerals are highly soluble and may act as nutrient antagonists limiting the benefit they provide the animal. The test source is a novel inorganic source (IntelliBond® Micronutrients, Indianapolis, IN) with a unique crystalline structure consisting of covalent bonds between the mineral, Cl, and OH groups. Because of their OH and Cl groups, these minerals are typically referred to as hydroxychloride (OHCl) trace minerals. The strong covalent bonds bestow beneficial properties like low solubility and reactivity, which may improve RBV (7, 2)

An experiment was conducted to determine whether SOD activity is sensitive to different dietary mineral sources fed to LPS-challenged laying hens at nutritional or sub-nutritional concentrations. Eighty individually caged, 30-week-old laying hens (HyLine W36) were assigned to 1 of 8 treatments. These were arranged as a 2×2×2 factorial: OHCl vs. SO4, nutritional (100%, 100 mg/kg Zn and 90 mg/kg Mn) vs. sub-nutritional (50%, 50 mg/kg Zn and 45 mg/kg Mn) levels, and phosphate-buffered saline (PBS) vs. LPS (500µg/kg bodyweight) injected birds. Experimental diets were fed from 30 to 40 weeks of age. At 41 weeks of age, 5 hens/treatment were injected with LPS or PBS. Approximately 10 ml of blood were sampled per bird, 24 hours after injection for the isolation of monocytes and heterophils (1). The SOD activity of the isolated immune cells was determined using an SOD kit (Cayman chemicals, Ann Arbor, MI). A 3-way ANOVA (SAS, Cary, NC) was used to assess the interaction between mineral level and source in birds injected with LPS or PBS. Significant interactions were further analyzed using Tukey's least square means comparison.

There were significant interactions ($P<0.01$) in SOD activity when mineral sources were fed at either 100% or 50%. Injecting laying hens with LPS increased monocyte and heterophil ($P<0.05$) SOD activity compared with immune cells isolated from layers injected with PBS. However, birds fed the diet with 50% SO4 had lower ($P<0.05$) SOD activity than other LPS injected birds (50% OHCl, 100% SO4, and 100% OHCl). Monocytes and heterophils isolated from birds fed the 50% OHCl diet had similar ($P>0.05$) SOD activity compared with birds fed 100% of either SO4 or OHCl mineral sources.

The experiment was conducted to study the effect of mineral source and supplementation level on the ability of immune cells to produce mineral-dependent SOD as a potential parameter for the determination of mineral source RBV. This was a pilot trial to establish how layer immune cells react to an LPS challenge and then determine if SOD produced by these cells is sensitive to dietary mineral source. The isolated immune cells were sensitive to the immune challenge, as layers injected with PBS either produced fewer immune cells or immune cells with lower SOD activity compared with LPS injected layers. Moreover, immune cells isolated from layers fed the 50% SO4 diet had significantly lower SOD activity than immune cells isolated from layers fed either 50% of the OHCl source or 100% of either mineral source. Because hens fed the 50% OHCl produced immune cells with similar SOD activity to layers fed 100% of either mineral source, this suggests that the OHCl mineral sources have higher RBV than SO4 sources when fed at the 50% level. However, because there were no differences between sources at 100% feeding level, SOD activity may only be sensitive to mineral source when supplemented to the diet at levels below the 100% level used in this experiment. These data indicate that laying hen immune cell SOD activity could be a sensitive parameter to assess mineral RBV when fed at sub-nutritional concentrations.

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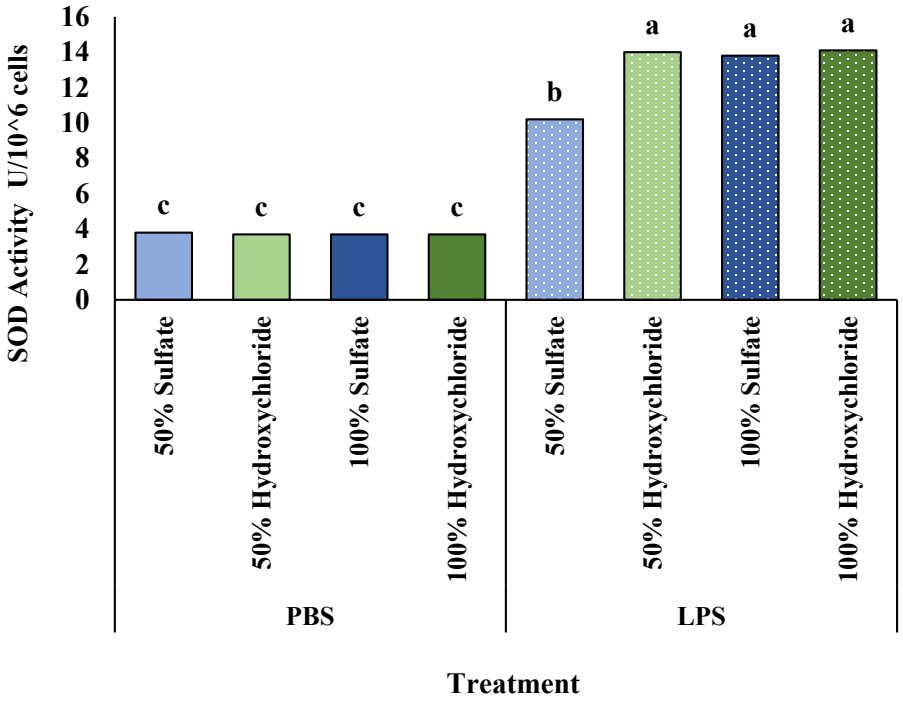
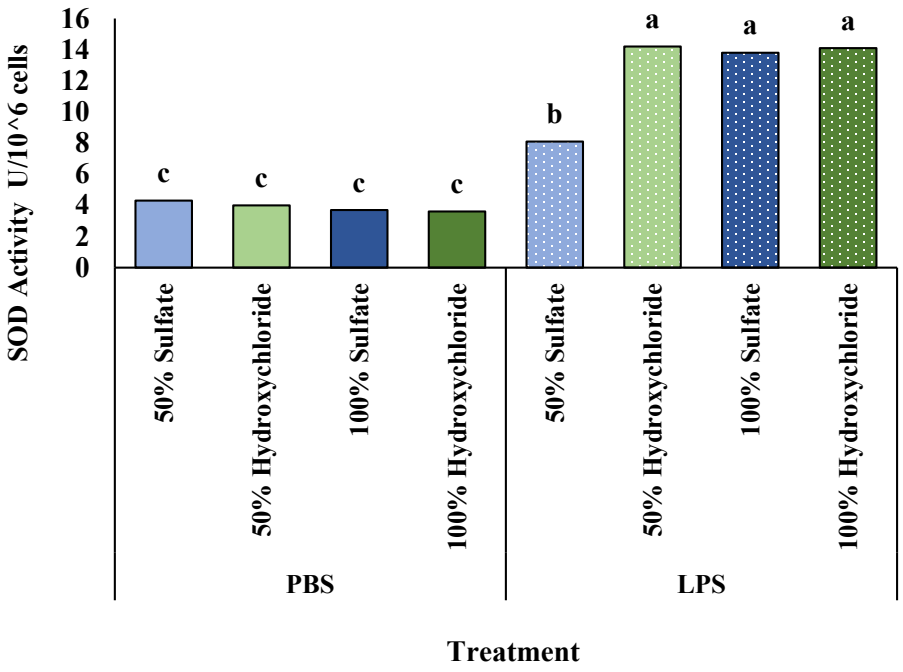
Figure 1. Effects of mineral source (Hydroxychloride vs. Sulfate) on super oxide dismutase (SOD) activity in 41-week old laying hens injected with either phosphate buffered saline (PBS) or lipopolysaccharide (LPS). Activity of SOD was determined in either isolated monocytes (A) or heterophils (B), 24 hours after PBS or LPS injection. Means with no common superscripts differed significantly ($P<0.05$).

A)



B)



ECONOMIC EVALUATION OF THREE SYSTEMS (CONVENTIONAL CAGE, ENRICHED CAGE, AND FLOOR SYSTEM) OF TABLE EGG PRODUCTION IN MEXICO

EVALUACIÓN ECONÓMICA DE TRES SISTEMAS (JAULA CONVENCIONAL, JAULA ENRIQUECIDA, SISTEMA EN PISO) DE PRODUCCIÓN DE HUEVO PARA PLATO EN MÉXICO

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RESUMEN

La producción de huevo para plato en México se lleva a cabo en jaulas convencionales, un asunto que causa controversia con grupos activistas que están a favor del bienestar animal y buscan un mejoramiento en la calidad de vida de las gallinas. Esta situación ha forzado a las compañías de producción de huevo a mitigar con alternativas de sistemas de producción como las jaulas enriquecidas o producción en piso.

El problema con esta mitigación es que no hay información económica para determinar si se

presentará un impacto económico sobre los costos de producción y finalmente habrá un incremento en el precio del huevo. El costo para el consumidor por huevo producido por el sistema convencional es de $1.5 mientras que los huevos producidos en los sistemas alternativos los costos suben a $ 6. Matthews y Summer, en 2015, condujeron un estudio comparativo en el estado de California, EE. UU. donde determinaron el efecto del sistema de alojamiento sobre los costos de producción, obteniendo que la comparación de las jaulas convencionales a los sistemas aviarios se incrementa en un 36%, mientras que las jaulas enriquecidas representan un 13% más. Sin embargo, estos datos no son aplicables a la industria avícola en nuestro país debido a la diferencia en los costos de producción en las entradas, como la mano de obra, la cual es notablemente diferente entre los países desarrollados y aquellos en proceso de desarrollo. Por eso es importante el generar dicha información.

SUMMARY

The production of table eggs in Mexico is carried out in conventional cages, issue that has caused controversy with the activist groups that are in favor of animal welfare and seek an improvement in the quality of life of the hens. This situation has forced egg producing companies to migrate to alternative production systems such as enriched cages and production on the floor.

The problem with this migration is that there is no economic information to determine what will be the impact on production costs and finally what will be the increase in the price of the egg. The cost to the consumer of an egg produced in a conventional system is $ 1.5 while eggs that come from alternative systems cost up to $ 6. Matthews and Summer in 2015 conducted a comparative study in the State of California, USA, where they determined the effect of the accommodation system on production costs, obtaining that compared to the conventional cage the aviary system increases by 36%, while that the enriched cage represents 13% more. However, these data are not applicable to the poultry industry in our country because of the difference in production costs in inputs, such as labor, which there is a notable difference between developed countries and those in the process of development. That is why it is important to generate such information.

INTRODUCTION

Quality is the set of inherent properties of a product that allows to characterize and value it with respect to others. It is therefore important that the products of animal origin are of the highest quality. Among these products is the egg, being the most economical and easy to cook protein. The egg contributes 17% of the protein consumed in Mexico and has an apparent per capita consumption of 22.8 kg. placing our country as the first place in whole egg consumption worldwide. Mexico has a national flock of 156,774,839 laying hens in production, obtaining the fourth place worldwide as a producer of whole egg, Jalisco being the state with the highest production (54%).

The concern in Europe on the issue of animal welfare has led to the banning of the conventional cage since 2012, in this geographical area. Although this movement has reached the American continent and has exerted pressure not only on the egg producing companies, but also on the entire food industry demanding food made with cage-free eggs. Product of these trends, have led to the development of alternative production systems that allow to express the natural behavior of laying hens. Within these systems is the enriched cage, which provides more living space to the hens, in addition to implementing environmental enrichment such as scrapers, hangers, sandpaper to file the nails, etc. Another system that is being used is the aviary which allows the movement of the hen throughout the house; It has several levels to optimize the space and accommodate a greater number of chickens, in addition to an enrichment similar to that of the enriched cage. Finally, another system that is returning is the floor production, which was the first egg production system for dish.

Unfortunately there is no information available in Mexico that at sea is a reference for egg producers in our country that can be a basis for projecting both the advantages and the production as well as the economic impact that the migration of the cage system represents. commercial posture hen.

MATERIALS AND METHODS

The study will be carried out at the Center for Education, Research and Extension in Poultry Production (CEIEPAv) using hens of the Bovans White lineage. It will be taken as a sample the systems that the Center has (conventional cage, enriched cage and floor) and from these design a theoretical model that allows to equalize the populations, to be able to compare them. To achieve this, the same accommodation area will be used (16m wide and 70m long) and then calculate the accommodation capacity of the house in each of the different systems.

The determination of production costs will be made based on the methodology described by A. Aguilar et al. Once all the production costs have been obtained, a comparison between the different

production systems will be made in order to determine what the price would be for each type of egg, as well as to know the profitability of each one.

RESULTS

The lodging capacity of the hens in cage (10,560 hens) was obtained using conventional cages that provide 1380 cm^2 distributed in A or pyramid with two levels per pyramid. Chickens housed in a floor system (10,080 chickens) were calculated using the population density that marks the commercial line (9 birds / m^2). However, the population housed in enriched cage (4000 hens) is smaller because the minimum space required per bird housed is 750 cm^2, for this work he was provided with a living space of 1050 cm^2, of which 250 cm^2 correspond to space of nest and 800 cm2 to free space.

The calculation of production costs was made by inputs covering the following sections: food, depletion of birds, labor, packaging, administrative, depreciation of facilities and medicines.

The production parameters reported for the different production systems are 97.9% in conventional cage, 96.5% in enriched cage and 97.1% in floor system.

With the previously obtained information it is expected that the results obtained in this work are expected to coincide or be similar to those previously reported by Matthews and Summer in 2015.

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ANTIMICROBIAL SENSITIVITY OF *ENTEROCOCCUS* AND *ESCHERICHIA COLI* ISOLATES FROM NON-VIABLE BROILER EMBRYOS AT COMMERCIAL HATCHERIES IN WESTERN CANADA

SENSIBILIDAD A ANTIMICROBIANOS DE AISLAMIENTOS DE *ENTEROCOCCUS* Y *ESCHERICIA COLI* DE EMBRIONES NO VIABLES EN INCUBADORAS COMERCIALES EN EL OESTE DE CANADÁ

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RESUMEN

Previamente encontramos que el 66% de los embriones de pollo de engorda no-viables eran positivos al crecimiento bacteriano, principalmente *Escherichia coli* y *Enterococcus faecalis.* Estos aislamientos bacterianos fueron identificados usando una matriz de espectrometría de masas asistida por desorbción de láser y susceptibilidad realizada por el método de disco de difusión de Kirby-Bauer. Se detectó una resistencia multi-fármaco en el 34.1% de los aislamientos de *E. coli* probados (principalmente a tetraciclina) y un 35.9% de los aislamientos de *E. faecalis.* Todos los aislamientos de *Enterococcus* fueron resistentes al menos a uno de la antimicrobianos probados. Este estudio enfatiza la importancia del monitoreo continuo del uso de antimicrobianos y la emergencia de la resistencia a antimicrobianos en la industria avícola.

ABSTRACT

We previously found that 66% of non-viable broiler embryos were positive for bacterial growth, mainly *Escherichia coli* and *Enterococcus faecalis.* Bacterial isolates were identified using matrix assisted laser desorption mass spectrometry and susceptibility performed by the Kirby-Bauer disk diffusion method. Multidrug resistance was detected in 34.1% of the *E. coli* isolates tested (mainly tetracycline) and in 35.9% of the *E. faecalis* isolates. All *Enterococcus* isolates were resistant to at least one of the antimicrobials tested. This study emphasizes the importance of continuous monitoring of antimicrobial use and emergence of antimicrobial resistance in the poultry industry.

INTRODUCTION

Antimicrobial resistance (AMR) among bacterial species in animals and humans is of a great public health concern due to increased therapeutic failures associated with emergence of multidrug resistant bacteria (4,15). Multidrug resistant *Enterococcus faecium*-associated nosocomial infections in humans is concerning due to limited antimicrobials available against these infections (14). The risk of transferring AMR genes from animals to humans is a serious threat and has led to new regulations on prudent use of antimicrobials in animals in several countries. Selective pressure exerted by the use of sub-therapeutic levels of antibiotics as growth promotors and the prophylactic use of antimicrobials in animals are leading causes of AMR (8). Specifically, *Enterococcus* and *Escherichia coli* have been considered indicators of AMR, and are important pathogens in the poultry industry (1,11). Morbidity and mortality due to amyloid arthropathy (*E. faecalis*) (7), osteomyelitis (*E. cecorum)* (5)*,* yolk sac infection (*Enterococcus* spp. and/or *E. coli*) (5,7), airsacculitis,

pericarditis, peritonitis or salpingitis *(E. coli)* (6) can be economically devastating to poultry producers. Commercial poultry hatcheries may be a significant source of bacteria via egg shell contamination (5) or vertical transmission (13) from breeders to progeny. In a previous study, we found 65.82% of non-viable embryos studied from commercial broiler hatcheries in western Canada which were positive for bacterial growth, with the most prevalent being *Enterococcus* species (29.71%), of which 79.58% were *E. faecalis* and 8.1% *E. faecium,* followed by *E. coli* (19.46%) (12). The objective of our current study was to determine the antimicrobial sensitivity of these isolates.

MATERIALS AND METHODS

Bacteria isolates. *E.* coli (n=170) and *Enterococcus* (n=256) [*E. faecalis* (n=223), *E. faecium* (n=21), *E. avium* (n=5), *E. gallinarum* (n=5) and *E. casseliflavus* (n=2)] isolated from non-viable chicken embryos were used in the current study (12).

Antimicrobial susceptibility testing. Susceptibility testing was performed using the Kirby–Bauer disk diffusion method. The selection of the disk concentration and interpretation of zone diameter was done as recommended by the Clinical Laboratory Standards Institute (10). Antimicrobial agents and disk potencies (µg) were: 1. Amoxicillin-clavulanic acid (30 µg), 2. Ampicillin (10 µg), 3. Apramycin (15 µg), 4. Bacitracin (10 IU), 5. Ceftiofur (30 µg), 6. Chloramphenicol (30 µg), 7. Ciprofloxacin (5 µg), 8. Enrofloxacin (5 µg), 9. Erythromycin (15 µg), 10. Florfenicol (30 µg), 11. Gentamicin (10 µg), 12. Lincomysin (2 µg), 13. Neomycin (30 µg), 14. Penicillin-G (10 units), 15. Spectinomycin (100 µg), 16. Tetracycline (30 µg), 17. Trimethoprim-sulfonamide (1.25 µg), 18. Triple sulfa (0.25 mg) and 19. Tylosin (60 µg).

RESULTS

Antimicrobial sensitivity profiles. All of the *Enterococcus* isolates tested were resistant to at least one of the antimicrobials tested. The descending order of AMR of *E. faecalis* were; tetracycline (73.1%), ceftiofur (47.98%), bacitracin (43.9%), erythromycin (31.4%) and tylosin (30.5%). Multidrug drug resistance was detected in 35.9% of the *E. faecalis* isolates and in 85.7% of the *E. faecium* isolates. The most common resistant phenotype of *E. faecalis* was tetracycline + bacitracin while the most common phenotype of *E. faecium* was ceftiofur + neomycin + tetracycline + trimethoprim-sulfonamide + penicillin.

The *E. coli* isolates tested were resistant to tetracycline (54.39%), ampicillin (50.88%), amoxicillin-clavulanic acid (42.1%), triple sulfa (31.68%), ceftiofur (29.8%), gentamycin (28.6%) and spectinomycin (20.5%) Multidrug drug resistance was observed in 34.1% of the *E. coli* isolates. The most common *E. coli* resistant phenotype was tetracycline resistance (15.3%).

DISCUSSION

We investigated the antimicrobial sensitivity of *Enterococcus* and *E. coli* species isolated from non-viable broiler embryos at commercial hatcheries in Western Canada (12). Resistance to tetracycline (73.44%), bacitracin (42.6%) and tylosin (30.1%) was notable in the *Enterococcus* isolates we tested. Tetracycline resistance is recurrent in intestinal bacteria, likely due to selection pressure from overuse of this antibiotic (15). Commensal microbiota of poultry may be a reservoir of bacitracin resistance which can be easily transferred to *E. faecalis* in humans (3). Since bacitracin is a commonly used growth promoter in poultry in Canada, resistance to bacitracin can be co-selected (3). Additionally, a high incidence of multidrug resistant *Enterococcus* isolates was observed (44.9%), with a higher degree of multidrug resistance in *E. faecium* compared to *E. faecalis.*

E. coli may be pathogenic or non-pathogenic in both humans and animals (9). Livestock are exposed to multiple antimicrobials, thus resistance is increasing (10). In our study, we found 29.82% of the *E. coli* isolates we tested were resistant to ceftiofur (29.82%) and gentamicin (54.39%). Our findings are consistent with Boulianne *et* al, who also found a correlation between ceftiofur resistance and use at the hatchery (2). We also found high resistance to triple sulfa (31.6%) but low resistance to trimethoprim-sulfonamide (3%) in *E. coli*.

There is a high incidence of multidrug resistance in *Enterococcus* and *E. coli* isolated from non-viable broiler embryos at commercial hatcheries. To our knowledge, this is the first study specifically looking at resistance at broiler hatcheries; however, our results are consistent with Boulianne *et* al (2), who studied *Enterococcus* and *E. coli* resistance in broiler and turkey flocks in Quebec, Canada. Resistance to five or more antimicrobials has been previously reported from broiler isolates (16). A relationship between antimicrobial use as growth promoters and resistance in *Enterococcus* species and *E. coli* (16), or environmental bacteria as reservoirs for resistance gene transfer may be possible (2). In May 2014, the Chicken Farmers of Canada voluntarily withdrew the use of category I antibiotics in all broiler production (17). Further investigations are needed to determine if resistance patterns are altered after the withdrawal of

category I antibiotics in Canada. In addition, determining the pathogenesis, virulence and resistance genes are required. However, our present study does highlight the importance of monitoring AMR patterns of bacteria in the poultry industry, in particular, AMR patterns of *Enterococcus* and *E. coli* in non-viable chicken embryos.

(A full-length manuscript has been submitted to *Avian Diseases*.)

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CONSUMO DE ALIMENTO Y PESO DE CODORNIZ JAPONESA EN ENGORDA RELACIONADO CON LA TEMPERATURA AMBIENTE EN TRÓPICO SECO

FEED INTAKE AND BODY WEIGHT OF FATTENING JAPANESE QUAIL RELATED TO DRY TROPICAL AMBIENT TEMPERATURES

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SUMMARY

In order to determine variations in feed consumption and live weight of broiler Japanese quail fed in dry tropical environmental conditions, data from 60 broiler groups from 2014 and 2015 raised in Culiacan Sinaloa, Mexico were analyzed. The birds were fed a flour-based diet, consisting of corn flour and soybean meal with 28.2% CP and 3111 kcal of ME from day 1 to 14 (phase 1) and 24% of CP and 3148 kcal from day 15 to 35(phase 2). During the feeding period average monthly temperature varied from 21.76 to 31.35 ° C., The correlation coefficient of food consumption and the average monthly temperature was 0.07 (P > 0.56), there was no difference (P > 0.05) in feed consumption and live weight. Feed consumption and live weight in phase 1 was 136.32 ± 14.40 g and 83.52 ± 7.22 g, while in phase 2 it was 527 ± 43.7 and 205.32 ± 10.55 g. It is concluded that under these growing conditions feed consumption and live weight did not vary due to the effect of ambient temperatures in the Japanese quail of this study.

RESUMEN

Con el objetivo de determinar la variación en el consumo de alimento y peso vivo de codorniz japonesa en engorda en condición de ambiente de trópico seco, se analizaron los datos de 60 lotes de engorda durante 2014 y 2015 en Culiacán Sinaloa México. Las codornices se les proporcionó alimento en harina a base de maíz y pasta de soya con 28.2% de PC y 3111 kcal de EM de 1 a 14 días (fase 1); y con 24% de PC y 3148 kcal de 15 a 35 d (fase 2). Durante el periodo de engorda la temperatura promedio mensual varió de 21.76 a 31.35 °C. El coeficiente de correlación de consumo de alimento y temperatura mensual promedio es de 0.07 (P > 0.56), y no hubo diferencia (P > 0.05) en consumo de alimento y peso vivo. El consumo y peso vivo en la fase 1 fue de 136.32±14.40 g y 83.52±7.22 g, mientras que en la fase 2 fue fe 527±43.7 y 205.32±10.55 g. Se concluye que bajo las condiciones de crianza de codorniz japonesa en este estudio el consumo de alimento y peso vivo no varía por efecto de la temperatura ambiente.

El estrés por calor es perjudicial para los animales y su efecto es de interés por el efecto en el bienestar animal (3). En este sentido, las aves de corral son muy sensibles y los genotipos modernos producen más calor corporal por su mayor actividad metabólica (1). La producción de carne y huevos disminuye por efecto en el perfil neuroendócrino al disminuir el consumo de alimento y la activación del eje hipotálamo-hipófisis-adrenal (3). En pollos de engorda sometidos a estrés calórico crónico se redujo 16.4% el consumo de alimento y 32.6% la ganancia de peso a los 42 días de edad (6). Sin embargo, Lu *et al*. (4) observaron efecto negativo de la exposición crónica al calor en la deposición de grasa y la calidad de la carne en pollos de engorde, de una manera dependiente de la raza. La codorniz japonesa (*Coturnix coturnix japonica*) es una especie de la avicultura diversificada, criada para la producción de carne y huevos, caracterizada por su rápido crecimiento, madurez sexual temprana, intervalo entre generaciones corto y menos susceptible a enfermedades (2). Prabakaran (5) en la India indica que las codornices se sacrifican a la edad de 4 a 5 semanas con peso de 160 a 180 g, el consumo de alimento es de alrededor de 500 g y la mortalidad de 8 a 10%, sin embargo, menciona que el precio se basa en el número de canales y no en el peso de la canal. El objetivo del estudio fue determinar la variación en el consumo de alimento y peso vivo de codorniz

japonesa en engorda en condición de ambiente de trópico seco.

MATERIALES Y MÉTODOS

Los 60 lotes de codorniz fueron criados durante 2014 y 2015 en la Unidad Avícola de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad Autónoma de Sinaloa, en Culiacán Sinaloa, México (24 46' 13'' LN y 107 21' 14'' LO). El clima de la zona es BS (h') w(w)(e), semiseco muy cálido, con lluvias en verano, con 25.9 ºC de temperatura promedio anual; humedad relativa promedio de 68%, máxima de 81% y mínima 51%; precipitación anual promedio de 688.5 mm. El alimento en forma de harina (Tabla 1) se ofreció *ad libitum*. El programa de iluminación fue de 24 h. La temperatura a la recepción de los lotes fue de 35 a 38 °C, y se proporcionó agua con electrolitos y vitaminas del complejo B.

Las codornices se pesaron una por una a los días 1, 14 y 35 de engorda. La temperatura se registró en cada lote (Termohigrómetro Avaly^{MR}). Para el análisis de los datos se realizó la prueba de t de Student y correlación de Pearson, fijando un valor de alfa máximo para considerar diferencia estadística de 0.05.

RESULTADOS

Aunque hubo diferencia ($P < 0.01$) entre las temperaturas en las temporadas de los años 2014 y 2015 en los que se criaron los 60 lotes de codornices, el peso corporal y el consumo de alimento no fueron afectados ($P > 0.05$) por la variación en las temperaturas (Tabla 2), esto se confirma al observar el coeficiente de correlación entre consumo de alimento y temperatura ambiente ($P > 0.05$) en la caseta donde se criaron las codornices.

DISCUSIÓN

A diferencia del pollo de engorda, en el que el estrés calórico ocasiona disminución de la respuesta productiva debido a la reducción del consumo de alimento y la ganancia de peso (6), en la codorniz japonesa no se observa el mismo efecto, ello puede deberse a la respuesta diferencial de las razas al ambiente, como lo mencionan Lu *et al*. (4). Además de la mayor resistencia, ya que es menos susceptible a enfermedades (2). Se concluye que las condiciones de temperatura ambiente de trópico seco en las que se crían las codornices en engorda no afectan el consumo de alimento y la ganancia de peso corporal.

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Tabla 1. Composición y aporte nutricional del alimento, g por 1000 g.

Ingrediente:	1 a 14 días	15 a 35 días	Ingrediente:	1 a 14 días	15 a 35 días
Pasta de soya	579	460	Metionina	2	3
Maíz	304	460	Treonina	0	2
Aceite de soya	50	40	Vitaminas pollo engorda	2	3
Azúcar	35	0	Minerales	1	1
Piedra caliza	14	14	Adsorbente de micotoxinas	2	4
Monofosfato dicálcico	6	6	Prebiótico	2	2
Sal de mar	3	3	Fitasa	2	2
Composición nutricional calculada					
Proteína cruda, %	28.2	24.0	Metionina, %	0.56	0.56
EM Mcal/kg	3111	3148	Lisina, %	1.99	1.60
Ca, %	0.95	0.91	Materia seca, %	88.3	88.4
P, %	0.38	0.36			

Tabla 2. Temperatura, peso corporal y consumo de alimento de codorniz japonesa en engorda en condición de trópico seco en el Noroeste de México.

Indicador	Época de temperatura [A]		Probabilidad
	Baja	Alta	
Lotes (n)	24	36	
Temperatura media °C	23.8 ± 1.3	29.3 ± 1.5	0.01
Temperatura min, max °C	21.8, 26.0	26.2, 31.4	
Peso corporal, g			
Inicial	10.2 ± 0.6	10.7 ± 0.3	0.01
14 días de edad	82.4 ± 7.9	84.3 ± 6.8	0.34
35 días de edad	216.1 ± 12.8	215.6 ± 9.1	0.85
Alimento consumido, g			
1 a 14 días de edad	134.7 ± 15.4	137.4 ± 13.8	0.47
15 a 35 días de edad	525.0 ± 42.7	528.3 ± 44.8	0.78
1 a 35 días de edad	659.7 ± 53.0	665.8 ± 52.6	0.66
Correlación de temperatura con alimento consumido	0.07	0.08	0.56

[A] Los resultados son medias ± desviación estándar.

IDENTIFICATION OF AVIPOXVIRUS SPECIES FROM CASES RECEIVED IN DMZA

IDENTIFICACIÓN DE ESPECIES DE AVIPOXVIRUS EN CASOS RECIBIDOS EN EL DMZA

N. Prado Ramírez

RESUMEN

La infección por avipoxvirus es una enfermedad común en aves, y es conocido como “viruela aviar”, y puede tener dos presentaciones: una cutánea o seca que puede afectar áreas del cuerpo del ave sin plumas, y una diftérica o húmeda, la última principalmente en membranas mucosas del tracto respiratorio y gastrointestinal. Además de estas dos presentaciones clásicas, la cuales algunas veces se complican por infecciones de otros microorganismos como bacterias y parásitos; también hay una tercera presentación, conocida como sistémica y reportada en canarios, que es similar a la neumonía.

Avipoxvirus infection is a common disease in birds, it is known as "fowl pox", it can have two presentations: cutaneous or dry that mainly affects areas of the body of the bird without feathers, and diphtheric or humid, the latter mainly in mucous membranes of the respiratory and gastrointestinal tract. In addition to these two classic presentations, which can sometimes be complicated by infections of other microorganisms such as bacteria and parasites, there is a third presentation, known as systemic and reported in canaries that is similar to pneumonia.

Avipoxviruses have been identified in approximately 278 species of birds of 23 orders, both domestic and ornamental and companion and even free-living, 10 species of viruses are recognized that affect birds, however, there are reports of 16 species. The Fowlpox, Turkeypox, Pigeonpox, Canarypox, and Psittacinepox, are some of the most common species. In general, the poxviruses are the most complex, they consist of a double strand of DNA and it encodes approximately 300 proteins, unlike other DNA viruses, this replicates in the cytoplasm of cells and 30 to 50% of its genes codes for immunomodulatory proteins, consequently, the great distribution between different species and the presentations of the disease are the product of the co-evolution between the virus and its hosts, these immunomodulatory genes have little conservation among the different poxviruses, unlike the Replication and assembly genes that are highly conserved. In addition, this virus is highly permissive, since genes from other viruses can be inserted into its genome, this has application in the use of the smallpox virus as a vector virus for the development of chimera or recombinant vaccines, both in veterinary medicine and in the human, among the most used viruses are Canarypox and Fowlpox, both are replicated in mammalian cells, but does not result in a productive infection.

Although the diagnosis is simple, in Mexico it is necessary to identify and characterize the virus, not only for scientific interest, but also to know the distribution of Avipoxvirus species in the various species of birds, both production and ornamental, which can be partially help determine if an immunization with commercial vaccines confers protection or even for the future development of vaccines with strains homologous to those found in the affected species. In birds of production such as turkeys, outbreaks of avian pox have been observed that affect the productive parameters despite having been previously vaccinated with Fowlpox. Smallpox in canaries leads to the implementation of long treatments with poor prognosis, due to the systemic presentation of the disease, which can represent a problem that translates into economic losses for those involved in this branch of poultry, hence the importance of the realization of this work.

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IMPACT OF IN WATER TREATMENT WITH A SOLUBLE ZINC AMINO ACID COMPLEX ON ALLEVIATING STRESS IN BROILERS

IMPACTO DEL TRATAMIENTO EN EL AGUA CON UN COMPLEJO DE AMINO ÁCIDO Y ZINC SOLUBLE SOBRE EL ALIVIO DE ESTRÉS EN POLLOS DE ENGORDA

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RESUMEN

El zinc juega un papel importante en la regulación de la respuesta al estrés. Elevar los niveles de zinc en el cuerpo podrían entonces ser de gran utilidad. 96 pollos de engorda machos Ross 308, fueron colocados en 6 jaulas del día 1 al 28 de edad y expuestos a 3 estresantes en un diseño completamente al azar. A la mitad de ellos les fue suministrado un tratamiento en el agua a base de complejo de zinc-amino acido (ProPath®Zn-LQ 18%, *ad libitum,* dilución 1:256) del día 20 al 27 y se comparó con un control no tratado respectivamente. Del día 21 al 28 todas las aves fueron sometidas a estrés calórico moderado continuo (29–34ºC). El día 21 dos grupos recibieron una vacuna inactivada (NDV, Ulster). Otros dos grupos fueron sometidos a 6 horas diarias de restricción de acceso al alimento del día 21-28. El día 28 se tomaron muestras de sangre para evaluar indicadores de estrés, así como respuesta serológica. Se pesaron órganos linfoides. La vacunación tuvo un efecto negativo en el consumo de alimento, peso vivo y peso de la canal ($P < 0.05$). El peso de los timos en el grupo con tratamiento en el agua fue mayor ($P < 0.001$) y su respuesta serológica por HI-NDV mostro una tendencia ($P < 0.07$) sobre el grupo no tratado. El uso de complejos metal amino acido de zinc solubles puede ser útil para aliviar la respuesta al estrés.

SUMMARY

Zinc plays an important role on the regulation of stress. Then increasing zinc status in the body could be useful to manage stress. 96 Ross 308 male broilers were placed in 6 treatments in cages from days 1-28, and subject to 3 stressors in a completely randomized

block design. Half of the birds received a zinc- amino acid complex treatment in the water (ProPath®Zn-LQ 18%, *ad libitum,* 1:256 dilution) from days 20-27 and compared to their respective control per stressor. From days 21-28 all birds were subject to mild continuous heat stress (29–34ºC), on day 21 two treatments received an inactivated vaccine (NDV, Ulster). Two other treatments were subject to 6 hours feed restriction daily from day 21 - 28. On day 28, blood samples were taken to evaluate stress indicators and serology. Lymphoid organs were weighed. Vaccination had a negative impact on feed intake, body weight and carcass weight ($P<0.05$). In water treatment group thymuses weights were significantly higher ($P<0.001$) and HI-NDV serologic response was higher showing a trend ($P<0.07$). Zn amino acid complex could be useful for alleviating stress response.

INTRODUCTION

Stress is one of the most important concerns for the modern poultry industry since birds experience various stressors each day in production[3]. Stress has detrimental impact on performance, health, livability and animal welfare[1]. Zinc plays an important role on the regulation of stress[6]. Previous studies have shown the effect of zinc status in the body on corticosterone and heterophile to lymphocyte ratio response to stress[8]. Zinc – methionine has been used with success on calming down animals subject to stress, being this response attributed more to methionine[8]. However, studies in humans have shown the effect of zinc on regulating excitatory neurological mediators and the increase in neuroplasticity promotors[4,5,6]. In order to evaluate the effect of in-water zinc supplementation using a novel highly water-soluble zinc-amino acid complex. Broilers were subject to different stressors to assess blood indicators, weight gain and immune system response.

MATERIALS AND METHODS

Ninety-six Ross 308 male broilers were placed in 6 cages with 16 birds each from day 1 to 28, in a completely randomized block design. Drinking water treatment was given from day 20 to 27 using a highly soluble zinc- amino acid complex (ProPath®Zn-LQ 18%). Treatment was given *ad libitum* and continuous using a dilution of 7 oz per 2 gallons of stock solution, giving 1 fl oz per gallon 1:128 (7 oz -1:256 final). Treatments: all birds were subject to mild heat stress at 29 – 34ºC from day 21 to 28. At day 21 two treatments were subjected to vaccination stress on day 22 with a subcutaneous injection of an inactivated oil emulsion Newcastle disease virus Vaccine (Ulster 2C strain, Emulvin Plus, Merial) 0.7 mL per bird vaccine. Two other treatments were subjected to 6 h feed restriction daily, at different hours from day 22 to 28. All birds received a live NDV-Las Sota vaccine at day eight and a live IBV-Mass/Con vaccine at day 17 as a standard vaccination protocol.

On day 28 blood samples with citrate were taken to evaluate stress indicators: corticosterone, glucose, insulin, cholesterol, heterophils counts, lymphocyte counts. And serum for hemagglutination inhibition (HI) and ELISA tests. Liver, thymuses, bursae, spleen weights were evaluated on day 28 too. Performance measurements; BWG, FI, and FCR were evaluated from day 21 to 28.

RESULTS & DISCUSSION

Performance showed a reduction in body weight gain in the birds vaccinated with the emulsion vaccine compared to all other groups. Carcass weight showed the same difference as a consequence. No differences between treated and non-treated birds were found.

Significant responses are difficult to establish for serologic titers. However, some consistent trends are observed when comparing zinc water treated groups with controls. HI Log2, GM and positive ELISA show all higher levels for the zinc water treatment groups.

The use of zinc supplementation with the zinc-amino acid complex evaluated during stressful situations could be useful to alleviate stress response and improve its negative impact on performance and immune response**.**

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Treatments	**Vaccination[a]**	**Feed Restriction[b]**	**Heat Stress[c]**	**Water Zinc-AAC[d]**	**# of birds**
Control Heat Stressed	No	No	Yes	No	16
Zinc-AAC Heat Stressed	No	No	Yes	Yes	16
Control Vaccinated	Yes	No	Yes	No	16
Zinc-AAC Vaccinated	Yes	No	Yes	Yes	16
Control Feed Restricted	No	Yes	Yes	No	16
Zinc-AAC Feed Restricted	No	Yes	Yes	Yes	16

[a] Oil emulsion NDV-Ulster 2C (Emulvin, Merial), on d 22

[b] feed was removed for 6 hours daily at different times during light time, d 22 to 28

[c] 29 – 34ºC from day 21 to 28

[d] water treatment with ProPath®Zn-LQ: zinc amino acid complex (Zinpro Corporation, Eden Prairie, MN)

Table 1. Performance of Ross 308 male broilers with zinc water treatment under different stressors.

Water treatment	Stress	21 – 28d BWG, g	28d BW, g	Carcass Wt., g	WOG, %
Ctl.	HS only	405 ^{y}	1174 ^{y}	838 ^{y}	71
Zn-AAC	HS only	402 ^{y}	1229 ^{y}	831 ^{y}	68
Ctl.	HS VACC	337 ^{xy}	1086 ^{x}	783 ^{x}	72
Zn-AAC	HS VACC	331 ^{y}	1016 ^{x}	710 ^{x}	70
Ctl.	HS Feed R.	386 ^{xy}	1210 ^{y}	838 ^{y}	69
Zn-AAC	HS Feed R.	369 ^{xy}	1237 ^{y}	851 ^{y}	69

Within column, means with different literals differ (P<0.05).

Losses in weight gain related to vaccination using emulsion vaccines has been recognized and is expected. This study re-confirms that response.

Table 2. Immune organs weight % of Ross 308 male broilers with zinc water treatment under different stressors.

Water treatment	Thymus, %	Spleen, %	Bursa, %
Ctl.	0.39 ^{xy}	0.08	0.20
Zn-AAC	0.36 ^{xy}	0.09	0.19
Ctl.	0.49 ^{x}	0.10	0.22
Zn-AAC	0.53 ^{w}	0.10	0.23
Ctl.	0.26 ^{y}	0.09	0.25
Zn-AAC	0.33 ^{y}	0.08	0.23

Within column, means with different literals differ (P<0.05).

Thymus weight was lower in feed restricted birds than in vaccinated groups. Water treatment in vaccinated birds had thymuses with higher relative weight than non-treated vaccinated controls. The meaning of this increase might be related to the activity increase created by the vaccine reaction. Birds with a higher status in zinc might be able to further increase T lymphocyte proliferation as it is well documented[9]. No differences were observed for spleen and bursa relative weights.

Table 3. Blood stress indicators of Ross 308 male broilers with zinc treatment under different stressors.

Water treatment	Corticosterone, ng/ml	Glucose, mg/dl	Insulin, g/dl	Heterophils, 10^3/µl	Lymphocytes, 10^3/µl
Ctl.	6.3^{xy}	175^{y}	8.1	2.1	3.1
Zn-AAC	6.8 ^{xy}	161 ^{y}	6.1	2.2	2.9
Ctl.	15.13 ^{x}	136 ^{y}	7.1	2.6	2.6
Zn-AAC	5.9 ^{y}	134 ^{y}	10.3	2.7	2.6
Ctl.	8.9 ^{xy}	177 ^{y}	6.3	2.6	2.4
Zn-AAC	6.11 ^{xy}	168 ^{y}	7.9	2.5	2.9

Within column, means with different literals differ (P<0.05).

Corticosterone showed a significant reduction for the zinc water treated vaccinated group when compared to the control vaccinated group. This increase was not able to be supported by a significant increase in H:L ratio in this study[7]. However other studies have been able to show this response when birds are compared to non-stressed groups[8].

Table 4. Serologic response to NDV of Ross 308 male broilers with zinc treatment under different stressors.

Water treatment	Hi Log_2	HI GM	ELISA	Positive +	n
Ctl.	3.8	13.5	252	2	16
Zn-AAC	5.1	34.8	536	5	16
Ctl.	4.3	19.1	322	5	16
Zn-AAC	5.3	38.3	325	8	16
Ctl.	4.2	18.3	244	2	16
Zn-AAC	5.5	45.9	380	5	16

ENHANCING INNATE IMMUNITY FOR BETTER RESPIRATORY DISEASE PROTECTION

MEJORANDO LA INMUNIDAD INNATA PARA UNA MEJOR PROTECCIÓN CONTRA ENFERMEDADES RESPIRATORIAS

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RESUMEN

El control de las enfermedades infecciosas es el mayor componente de los programas de salud avícola. Una tendencia que va en incremento en la producción moderna de aves es el criar a las aves sin antibióticos. La tendencia de disminuir el uso de antimicrobianos ha incrementado el enfoque en métodos más eficientes, eficaces y con un objetivo para mejorar el sistema inmune de las aves para el control de las enfermedades. El sistema inmune juega un papel esencial en la protección del hospedador contra los desafíos de las enfermedades infecciosas. Aquellos animales que puedan montar una respuesta inmune de lo más inmediata y de lo más exhaustiva son los típicamente mejor protegidos. Nosotros usamos este conocimiento a nuestra ventaja cuando desarrollamos nuevos métodos para proteger al hospedador contra las enfermedades infecciosas. Típicamente, la inmunidad se mejora por el uso de vacunas y adyuvantes para simular el componente adaptativo de la respuesta inmune llevando a una respuesta humoral (p.e. anticuerpos) y / o respuestas mediadas por células. Sin embargo, un componente igual de importante del sistema inmune del ave es con frecuencia no notado es la respuesta inmune innata. La inmunidad innata es característicamente no específica, rápida y la primerísima línea de defensa inmune. Hay varias células inmunes que contribuyen a la inmunidad innata los que incluyen a los leucocitos como los neutrófilos (heterófilos en especies aviares), monocitos, macrófagos y células asesinas naturales. Estas células están involucradas en la fagocitosis, presentación de antígenos, producción de radicales libres y citocinas. (17) Un tipo de célula que ha sido pasada por alto pero ha sido crecientemente reconocida por su papel en la inmunidad innata y adaptativa es el trombocito aviar. (4, 6).

SUMMARY

Controlling infectious diseases is a major component of poultry health programs. An increasing trend in modern poultry production is to raise birds without antibiotics. The trend to decrease the use of antimicrobials has increased the focus on more efficient, efficacious and targeted methods of enhancing the bird's immune system for disease control. The immune system plays a pivotal role in protecting the host against infectious disease challenges. Those animals that can mount the most immediate and most comprehensive immune response are typically best protected. We use this knowledge to our advantage when developing new methods for protecting the host against infectious diseases. Typically, immunity is enhanced by using vaccines and adjuvants to stimulate the adaptive component of the immune response leading to increased humoral (i.e. antibody) and/or cell mediated responses. However, an equally important component of the bird's immune system is the often unnoticed innate immune response. Innate immunity is characteristically nonspecific, rapid and the very first line of immune defense. There are several immune cells that contribute to innate immunity which include leukocytes such as neutrophils (heterophils in avian species), monocytes, macrophages and natural killer cells. These cells are involved in phagocytosis, antigen presentation, free radical and cytokine production.(17) A cell type that has until recently been overlooked but has been increasingly recognized for its role in innate and adaptive immunity is the avian thrombocyte.(4, 6)

Second only to red blood cells, thrombocytes / platelets are the most abundant cells in the circulatory system.(7) Thrombocytes and platelets have long been recognized for their role in hemostasis and wound healing. Thrombocytes differ from platelets in that they have nuclei whereas platelets do not. Thrombocytes are present in lower vertebrates such as the avian species whereas platelets are present in

mammalian species such as humans.(7) The phagocytic ability of avian thrombocytes has been well established.(1) However, more recent studies have demonstrated that avian thrombocytes are involved in the inflammatory response in a variety of ways which encompass both innate and adaptive immune responses to both bacterial and viral pathogens.(3, 4, 18)

There is a plethora of information pertaining to nutraceuticals, botanicals and dietary supplements and their applications for a variety of diseases and disorders.(8, 9, 12, 15) One product that is gaining interest due to its documented effects on the immune system are the beta-glucans. Beta-glucans (β- 1,3 / 1,6 glucans) are derived from extracts of the cell walls of plant and yeast cells (17). Glucans (i.e., β-1,3 glucans) have been used for various health promoting effects in humans and other animals(10, 11, 13, 17). When glucans are consumed orally they have been shown to have immune enhancing effects primarily on the innate immune system including improved macrophage, neutrophil and natural killer cell activity (14, 16, 17). Most of these immune enhancing effects have been attributed to boostering innate immunity by modulating cytokine production in monocytes, macrophages and epithelial cells.(17) Studies in poultry have demonstrated that when glucans were included in the diet certain aspects of the immune system were increased. (2, 19) These effects were attributed to the production of cytokines and support the concept of enhancing the innate immune response. A recent presentation indicated that glucans used as vaccine adjuvants enhanced the immune response and provided improved protection against subsequent challenge (5). This report supports the concept of glucans contributing to both the innate and adaptive immune responses. Interestingly, none of these aforementioned reports includes or discusses the potential role of avian thrombocytes.

We hypothesize that a never before recognized way in which beta glucans enhance the innate and adaptive immune responses is through thrombocytes. We propose to prove this hypothesis by using both *in vitro* and *in vivo* approaches with avian thrombocytes and chickens. For our *in vitro* studies we used flow cytometry techniques to evaluate the innate immune response. Briefly, peripheral blood monocytes (PBMCs) were harvested from chicken blood and after ascertaining their viability to be more than 98%, cells were stained with CD41/61 and mo/macro antibodies. The flow cytometry analysis revealed ~50% of PBMCs to be positive for thrombocytes, and their number was remarkably higher than mo/macrophage cells (74-fold) (Fig 1a). Next, we sought to verify production of reactive oxygen species (ROS) in thrombocytes by using a positive control (tert-butyl hydroperoxide, TBHP) that induces ROS in those exposed. Initially, we treated the cells with TBHP (250 uM) for 30 minutes at 37C and then stained with an ROS indicator dye (2',7' –dichlorofluorescin diacetate, DCFA-DA, uM) and anti-CD41/61 (thrombocyte marker) in serum-free medium. After acquiring the cells by flow cytometry, cells positive for DCF-DA and CD41/61 were analyzed by Flow jo software. As shown in the figure 1b, among the cells exposed to ROS-inducer (TBHP), ~30% of thrombocytes were found positive for ROS as opposed to 0.8% in the untreated control (see upper right quadrant). Based on these data, we conclude that the chicken thrombocytes can be analyzed for their functionalities at a single cell level by flow cytometry.

Next, we repeated the above procedures using lipopolysaccharide (LPS) a known inducer of innate immunity through the pathogen recognition receptor (toll-like receptor ligand) 4 (3) and beta glucan. Those thrombocytes which were exposed to LPS or beta glucan had similar ROS responses as the thrombocytes exposed to TBHP. Thus, we have demonstrated that avian thrombocytes elicit an innate immune response after *in vitro* stimulation with LPS and beta glucan. *In vivo* results are pending and will be presented.

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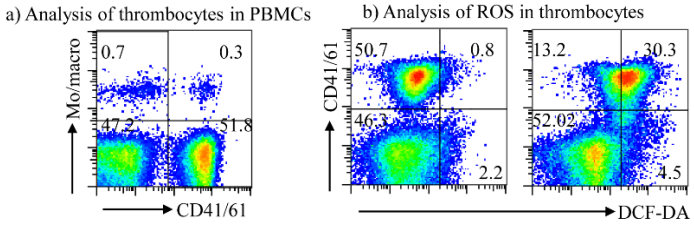
Figure 1. Analysis of ROS production in the chicken thrombocytes. PBMCs obtained from chicken were analyzed for thrombocytes using antibodies for CD41/61 (marker for thrombocytes) and Mo/macro (marker for monocytes) (left panel), and also for ROS production in the thrombocytes by flow cytometry (right panel).



META-ANALYSIS OF THE MOLECULAR CHANGES THAT INCREASE VIRULENCE IN THE HIGH PATHOGENICITY AVIAN INFLUENZA VIRUS

METAANÁLISIS DE LOS CAMBIOS MOLECULARES QUE INCREMENTAN LA VIRULENCIA EN EL VIRUS DE INFLUENZA AVIAR DE ALTA PATOGENICIDAD

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RESUMEN

Los virus de la influenza aviar están entre los más desafiantes que amenazan la salud de la avicultura doméstica debido a su gran habilidad de adaptarse desde las aves acuáticas, para recombinarse y causar gran mortalidad y morbilidad en la industria avícola (16, 28). La Organización Mundial de Salud Animal (OIE) establece que el virus de Influenza Aviar es considerado de ser altamente patogénico si cumple con ciertos criterios: 1) que presente un sitio de división polibásico (CS) (más de dos aminoácidos básicos) localizado en la hemaglutinina (HA); 2) demostrar un índice de patogenicidad intravenosa (IVPI) mayor de 1.2 (14). El propósito de este trabajo es llevar a cabo el estudio de la secuencia de aminoácidos que vienen de virus Influenza Aviar de Alta Patogenicidad sin importar su origen (año, lugar y especies de aves donde fueron aislados) y son identificados a través del análisis en mutaciones en conceso sílico que hayan sido asociados con incremento en la virulencia en aves y también proporcionen el conocimiento en el diagnóstico molecular del HPAI colectado. Los virus que presenten un menor número de mutaciones producen un IVPI similar a los virus que presenten los números más altos de mutaciones.

INTRODUCTION

Influenza A viruses are among the most challenging viruses that threaten the health of domestic poultry due to their great ability to adapt from wild aquatic birds, to recombine and cause great morbidity and mortality in the poultry industry (16, 28). The World Organization for Animal Health (OIE) establishes that an avian influenza virus is considered to be highly pathogenic if it meets two main criteria: 1) present polybasic cleavage site (CS) (more than two basic amino acids) located in hemagglutinin (HA); 2) demonstrate an intravenous pathogenicity index (IVPI) greater than 1.2 (14).

It is internationally accepted that highly pathogenic avian influenza (HPAI) viruses H5 and H7 emerge from low pathogenic avian influenza (LPAI) viruses (3). Noting that the change from LPAI to HPAI has been possible due to 4 mechanisms that occur in HA and described by the OIE: 1) substitutions of non-basic amino acids for basic amino acids (arginine or lysine) in the CS (6,17); 2) inserts of multiple basic amino acids from duplicated or triplicated CS codons, due to a failure of the transcription of the polymerase complex (5,6,29); 3) short inserts of basic and non-basic amino acids of unknown origin in HA; 4) non-homologous recombination which results in the insertion of a "purine" nucleotide sequence at CS of HA (20,29); 5) loss of glycosylation at residue 13 in combination with multiple basic amino acids at the CS of HA (27,29).

JUSTIFICATION

However, in the last three decades, reverse genetics techniques have been developed, which have demonstrated the existence of other molecular changes or mutations different from the accumulation of basic amino acids in HA in different genes of avian influenza viruses and in different viral isolates that cause a high mortality rate in poultry populations. (4,19) Giving rise to the question: does each of the high pathogenicity viruses that affect birds have the same mutations that confer high pathogenicity?

OBJECTIVE

The purpose of the work was to carry out the study of the amino acid sequences coming from the high pathogenicity avian influenza viruses that regardless of their origin (year, place and species of bird where it was isolated) are identified through the

analysis in silico consensus mutations that have been associated with increased virulence in poultry and also provide knowledge in the molecular diagnosis of high pathogenicity virus.

MATERIALS AND METHODS

In order to carry out the meta-analysis, it was necessary to make the literature search of the molecular changes related to high pathogenicity using the Scopus and PubMed database. Subsequently, the obtaining of amino acid sequences of the proteins from high pathogenicity viruses and subsequently the alignment of the sequences using the MEGA v7 program as a bioinformatics tool to verify the presence of each mutation.

RESULTS

During the investigation, 41 mutations that were related to the virulence of HPAI were collected. Where the number of mutations that were obtained by protein was as follows:

PB2: 1
PB1: 3
PA: 1
PA-X: 1
HA: 12
NP: 6
NA: 1
M1: 8
M2: 3
NS1: 5

The nature of the mutation that predominated was of "substitution" type, since of the 41 mutations that were collected, 35 are by substitution, 5 are by elimination and 1 by insertion. Subsequently highlighted 5 for the following reasons: 1) mutation by elimination of amino acids in the carboxyl terminal of NS1 was ruled out due to the lack of accuracy in its role in pathogenicity (1); 2) mutation due to loss of amino acids at sites 191-200 since it refers to attenuation and not to increased virulence (31); 3) mutation by suppression of the PA-X protein since the availability of the protein is uncertain (7); 4) mutation by insertion of arginine at position 338 in HA protein of type H5 because this region is precisely part of the cleavage site and the appearance of basic amino acids in it, are already part of the diagnosis regardless of the type of mutation by the which they originate (substitution, insertion, recombination) (30); 5) substitution mutation S50N in the M1 protein due to the incongruence of the reference strains and that obtained by the authors (8).

The selection of the largest number of viral isolates was determined by: 1) the total availability of their amino acid sequences; 2) the pathogenicity of each strain, which must be reported on an experimental basis and classified as highly pathogenic; 3) cover all subtypes of H5 and H7 with high impact in the poultry industry (H5N1, H5N2, H5N6, H5N8, H7N8, H7N9, etc.). Obtaining a total of 60 strains (32 of subtype H5 and 28 of subtype H7), whose isolates go from 1934 to 2017. Most of these viruses participated in large outbreaks throughout the history of different geographical areas within seven continents (12, 15, 22, 23). While other strains were collected from experimental type articles that made use of the tests established by the OIE.

After the alignment of the viral sequences, most of the mutations that were collected were absent in several amino acid sequences from the HPAI, only 4 were constant. The mutation by elimination of amino acids in the stem of the NA was not constant despite being cited in several articles and its relationship in virulence.

The viruses that presented lower number of mutations produce an IVPI similar to the viruses that presented the highest number of mutations.

DISCUSSION

It was confirmed that the viruses show a great discrepancy in the amount of mutations present in their amino acid sequences, assuring that most of the mutations reported on an experimental basis are characteristic of each viral strain, acting mainly together, and that a virus with more of these mutations does not indicate greater virulence.

Only four substitution mutations were highlighted, since they were found to be constant in all the amino acid sequences and, with the help of a polybasic cleavage site in the HA, they show an even greater virulence of the agent causing the avian influenza: 1. Mutation by substitution C38Y in PB1 produced a more acute death in chickens (21); 2. Substitution mutation E184K in the NP caused the virus to decrease the TMP in chickens by 4.1 days (25,26); 3. Substitution mutation I43M in the M1 protein demonstrated a more pathogenic phenotype in both chickens and ducks (11); 4. Substitution mutation V149A in the NS1 protein was able to convert a virus with IVPI of 0.0 to a virus with IVPI 2.1 and in chicken embryo fibroblasts could antagonize the induction of interferon protein levels (9).

The mutation by elimination of amino acids in NA has been shown to be involved in the adaptation of poultry (10,18), however during the alignment, several amino acid sequences had a long stem, this means that this mutation is not necessary for all HPAI. On the other hand, other studies mention that the relation with respect to the presence or absence of

glycosylation sites with the size of the stem of the NA, can explain the virulence of some subtypes and in turn of some strains (2, 13, 24).

CONCLUSION

The study indicated that it is not possible to proceed to the structuring of a new diagnostic tool of molecular type to identify the level of pathogenicity of a strain, because the virulent quality of high pathogenicity avian influenza viruses is polygenic, where not only a specific gene, protein or amino acid participates, for this fact, these viruses do not share the same virulence markers reported in the experimental literature in a homogeneous way and on the other hand more experimental evidence is needed that encompasses the only substitution mutations that appeared consistently in all viral isolates.

Finally, it is expected that in the coming years there will be more advanced bioinformatics tools, whose data system will analyze more precisely each of the mutations that improve the virulence of avian influenza viruses, evaluating the probability that a LPAI will change to HPAI.

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EFFECT OF 1,25(OH)2D3 ON GENE MRNA EXPRESSION OF INNATE IMMUNE TOLL-LIKE RECEPTORS, PROTEINS SIGNAL ADAPTERS, RIG-I-LIKE RECEPTOR, IFN TYPE I, IFN-INDUCED PROTEINS, AND PROINFLAMMATORY CYTOKINES ON CHICKEN FIBROBLAST INFECTED WITH IBDV

EFECTO DEL 1,25(OH)2D3 SOBRE LA EXPRESIÓN DEL GEN MRNA DE LOS RECEPTORES TIPO TOLL DE LA INMUNIDAD INNATA, ADAPTADORES DE PROTEÍNAS DE SEÑALIZACION, RECEPTORES TIPO RIG-I, IFN TIPO I, PROTEÍNAS IFN-INDUCIDAS, Y CITOCINAS PROINFLAMATORIAS SOBRE FIBROBLASTOS DE POLLOS INFECTADOS CON IBDV

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RESUMEN

La nutrición apropiada de las aves influencia la respuesta del sistema inmune. Deficiencias o excesos de proteínas, amino ácidos específicos y vitaminas pueden influenciar esta respuesta. Ha sido reportado que la Vit D es un inmunomodulador que puede contribuir a mejorar la respuesta immune de las aves contra diferentes tipos de patógenos. El objetivo de este estudio fue el de evaluar los efectos de la suplementación de la vitamin D y el de caracterizar la respuesta inmune innata en fibroblastos de aves infectados con el virus de la enfermedad infecciosa de la bolsa (VEIB). Fibroblastos de aves (DF-1) fueron cultivados (37 C, 5% CO2) en placas de 24 pozos por triplicado a una concentración de 5x105 células / pozo. Después de 24 h de cultivo el medio de cultivo fue suplementado con 500nM of 1, 25(OH)2D3 (Metabolito activo). 16 h después de la suplementación del medio con Vit D, las células fueron inoculadas con una cepa vacunal modificada tipo intermedia viva del VEIB (UNIVAX-BD®) a dos diferentes concentraciones (MOIs: 0.1 y 1). Los resultados evidencian la presencia de ARN viral en las primeras 3 h post inoculación (pi), alcanzado niveles máximos de expresión a las 6 pi. Resultados: La

suplementación de la vit D no fue correlacionada con una disminución de la capacidad infectiva del virus o el de proteger los fibroblastos contra la infección de VEIB; sin embargo la vit D si modifico genes asociados a la inmunidad innata tales como Vitamin D receptor (VDR), TLR-3 and 21, MDA5, TRIF, MyD88, IRF-7, IFN-alpha, IFN-beta, OAS, PKR and Viperin. Conclusión: La suplementación de vit D no influyo en la disminución de la capacidad infectiva del virus o de proteger las células de una eventual infección; sin embargo la vit D modifico la expresión de genes relacionados con la respuesta inmune innata indicando un efecto inmunomodulatorio importante.

SUMMARY

Proper nutrition in chickens positively influences the response of the immune system. Deficiencies or excesses of proteins, specific amino acids and vitamins can influence that response. Vitamin D has been reported as an immunomodulator that can contribute to improve the immune response in chickens against different pathogens. The aim of this study was to evaluate the effects of vitamin D supplementation and to characterize the immune innate response in chicken fibroblast infected with infectious bursal disease virus (IBDV). Fibroblasts (DF-1) were cultured in triplicate at a concentration of 5x105 cells / well in plates of 24 wells. After 12 h of culture, the cells were supplemented with 500 nM of 1,25(OH)2D3. Sixteen hours after supplementation, IBDV was inoculated as a modified live vaccine (UNIVAX-BD®) with two different MOIs (0.1 and 1). The presence of viral RNA was detected at 3 h.p.i., reaching its maximum peak at 12 h.p.i. Vitamin D supplementation was not found to decrease the infectious capacity of the virus or to protect the cells from infection with IBDV, however Vit D modified genes associated with the innate immune response such as Vitamin D receptor (VDR), TLR-3 and 21, MDA5, TRIF, MyD88, IRF-7, IFN-alpha, IFN-beta, OAS, PKR and Viperin. In conclusion Vitamin D supplementation favored the innate immune response in fibroblast infected with IBDV.

INTRODUCTION

Innate immunity constitutes the primary barrier of recognition and effector action against viruses. Among the most important elements are the Toll-like receptors (TLRs) that act as mediators in the early inflammatory response to viral infections. The mechanism by which vitamin D synergizes with other factors such as dsRNA and alters gene expression is not known. However, it is believed that there is an interaction together with vitamin D receptor (VDR) and other transcription factors that intervene against the viral infection. Different responses of the TLRs have been found in the presence of 1,25 (OH) 2D (1, 2, 3).

In chickens, the immunomodulatory effect of vitamin D has been studied from different approaches. The experiments are generally aimed at evaluating the effect of vitamin supplementation on chickens' diet. Thus, it has been possible to establish its beneficial effect in young broiler chicks determining blood parameters and the weight of organs associated with immunity (4). It has also been demonstrated that dietary supplementation with vitamin D improves small intestinal morphology and protective humoral immunity to infection, demonstrated by improvement of the length of the duodenum and of the jejunum and the total serum IgG values, all without effect on weight gain and feed efficiency (5). Likewise, trials have been conducted evaluating vitamin D supplementation on the innate immune response in broiler chickens with an optimal level in calcium and deficient in phosphorus finding that in that case, supplementation with vitamin D considerably augmented transcription of TLR2b, TLR4, CATH1, and CATHB1 and predominantly Th2 cytokines in spleen and robust immunomodulatory property with a more favorable Th2 response (6). The effect of vitamin D on cellular populations associated with the immune system has been determined in chickens in macrophages treated with 1,25 (OH) 2D3 in the presence and absence of TLRs ligands, finding that this treatment increased the ability of macrophages to respond to stimuli and produces nitric oxide (NO), but vitamin D3 alone did not activate macrophages and resulted in the down-regulation of CD86, MHC-II, CXCL8 and IL-1β (7). On functional abilities of chicken T lymphocytes, it was determined that vitamin D inhibited the abilities of T lymphocytes to produce IFN-γ and proliferate in vitro but retained their ability for cytotoxicity (8). Another important aspect is the effect of vitamin D in hens and its immunomodulatory effect on progeny. In this case, vitamin D metabolites such as 25-hydroxy vitamin D3 (25-OHD) have been used, finding that maternal 25-OHD increased hatchability and in vitro chick innate immunity towards E. coli; also that can improve the hen age effect as the greatest factor influencing early chick innate immunity (9).

Keeping in mind that there are very few studies that address the effect of vitamin D on IBDV infection, particularly determining the immunomodulatory capacity on the innate immune response at the cellular level, this response was evaluated by determining the expression of genes associated with immunity innate in chicken fibroblast susceptible to the IBDV.

MATERIALS AND METHODS

Cell culture. Chicken fibroblast (DF1) cell line was grown in RPMI medium supplemented with 10% fetal bovine serum heat inactivated, 5% chicken serum heat inactivated in a 5% CO_2 incubator at 37° C. Cells were cultured, harvested and diluted to get a final concentration of 2x106 cells/mL in a final volume of 48 mL.

Treatments. After 12 h of culture, the cells were supplemented with 500 nM of vitamin D. Sixteen hours after supplementation, IBDV was inoculated as a modified live vaccine (UNIVAX-BD®) with two different MOIs (1 and 0.1).

***In vitro* test.** The cells were recovered in three different times (0, 3, 6, 12, 24, and 36 h.p.i.). Subsequently, extraction of RNA and cDNA synthesis was done. All samples were processed by real-time PCR to determine the relative expression of 12 genes (VP2 IBDV, vitamin D receptor, TLR-3, TLR-21, MDA5, MyD88, TRIF, IRF-7, INFα, INFβ, OAS, PKR, Viperin, IL- 12, IL-6, IL-1β,). Levels of expression for all genes were calculated relative to β-actin and gene expression. For the statistical analysis, the Proc Mixed Procedure of SAS was used to analyze CT values for all genes based on one level of vitamin D, two levels of MOI and six times (0, 3, 6, 12, 24, 36 h). Gene expression fold change, standard error and statistical significance were calculated using REST 2009 and data were considered significantly different at $P < 0.05$.

Gene expression. RNA was isolated using Invitrogen Trizol® Reagent (Life Technologies Inc. Burlington, ON, Canada) according manufacturer's protocol. cDNA was produce using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Mississauga, ON, Canada) according manufacturer`s protocol. Gene expression was measured using CFX Connect Real-Time System Bio-Rad (Bio-Rad Laboratories, Mississauga, ON, Canada) in a 96-well plate using a 10µL total reaction volume. Primer concentrations for the target and housekeeping genes were adjusted according to the results obtained in a standard curve for each specific case. SsoAdvancedTM SYBR® Green Supermix was used according Bio-Rad instruction manual (Mississauga, ON, Canada).

Statistical analysis. Statistical analysis of the relative gene expression in present study was done using REST-2009 Software (Technical University Munich. Qiagen, Germantown, MD, USA) which compares each treatment individually with the control group and expresses the relative gene expression as fold change of the target gene taking into consideration a reference gene (housekeeping gene, βactin in the present work) and the efficiency of reference and target genes. Results from this analysis are given as fold change with a respective p value. For the present work statistical difference is considered when p value is lower than 0.05. Values of fold change higher or lower than 1 with $p<0.05$ are considered statistical significant in both up-regulated and down-regulated respectively

RESULTS

The earliest viral RNA was detected at 3 h p.i. and its maximum peak was at 24 h p.i. at MOI 1 and Vit D supplementation ($p<0.05$). Vitamin D receptor (VDR) gene expression was significantly upregulated ($p<0.05$) in either Vit D supplemented or not supplemented, however it was p.i and MOI dependent. The intracellular endosomal membrane receptor (TLR-3) and the signal adapter TRIF were significantly upregulated ($p<0.05$) at any MOI and with or without Vit D supplementation during the first 24 h p.i; conversely the interferon regulatory factor 7 (IRF 7) was upregulated ($p<0.05$) only in vit D supplemented macrophages. Expression of interferon type I alpha (IFN-α and β) were significantly upregulated ($p<0.05$), and also it was Vit D dependent during the first 24 h p.i and 36 h p.i respectively. IFN-inducible virus inhibitory proteins OAS, PKR and viperin were significantly upregulated ($p<0.05$) only at 24 and 36 h p.i. in cells supplemented with Vit D. There was a significant upregulation ($p<0.05$) of the pro-inflammatory cytokines IL-1β and IL-6 following the same patterns of IFN-inducible antiviral proteins.

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SEROLOGICAL AND HISTOPATHOLOGICAL MONITORING OF THE IMMUNE RESPONSE IN FUTURE LIGHT LAYERS, AFTER THE APPLICATION OF A VECTORIZED VACCINE AGAINST MAREK'S DISEASE AND GUMBORO

MONITOREO SEROLÓGICO E HISTOPSTOLÓGICO DE LA RESPUESTA INMUNE EN PONEDORAS LIGERAS FUTURAS, DESPUÉS DE LA APLICACIÓN DE UNA VACUNA VECTORIZADA CONTRA LA ENFERMEDAD DE MAREK Y GUMBORO

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RESUMEN

El uso de cepas leves o intermedias para la vacunación contra la enfermedad de Gumboro son seguras pues estas causan menos daño en la bolsa de Fabricio que las cepas agresivas, pero estas no son muy efectivas en la presencia de anticuerpos maternos y contra las cepas muy virulentas del virus de Gumboro. Las capas menos atenuadas o agresivas pueden exceder los anticuerpos maternos pero dañan severamente los folículos de la bolsa de Fabricio causando inmunosupresión.

Este dilema se ha solucionado a través de la aplicación de vacunas vectorizadas de la enfermedad infecciosa de la bolsa (IBD), la cual usa el virus de Marek HVP como vector, al cual se le ha insertado el gen de la proteína VP2 del virus de Gumboro. La vacuna vectorizada HVP-IBD es segura y efectiva, ya que no tiene interferencia con los anticuerpos maternos, la cepa Rispens de Marek y / o SB1, proporcionando protección contra ambas enfermedades.

INTRODUCTION

The use of mild or intermediate strains for vaccination against Gumboro disease are safe because they cause less damage to the bursa of Fabricius than hot strains, but they are not very effective in the presence of maternal antibodies and against very virulent strains of Gumboro virus. The less attenuated or hot strains may exceed the maternal antibodies but with severe damage to the follicles of the Fabricius pouch causing immunosuppression.

This dilemma has been solved through the application of the vectorized infectious bursal disease (IBD) vaccine, which uses the Marek's HVT virus as a vector, which has inserted the VP2 protein gene of the Gumboro virus. The vectorized HVT-IBD vaccine is safe and effective, since it has no interference with maternal antibodies, does not cause damage to the bursa of Fabricius and can be applied at day of age in the incubator, along with other strains of Marek Rispens and / or SB1, providing protection against both diseases.

MATERIALS AND METHODS

Study carried out in 23 flocks of commercial chicken, vaccinated in the subcutaneous incubator plant with a recombinant vaccine of MAREK HVT with the VP2 protein insert of the Gumboro virus and the serotypes SB1 and Rispens, in comparison with a usual program used in flocks of commercial chicks under the control system of Marek's disease with serotypes HVT-SB1-Rispens applied in the subcutaneous incubator and two vaccines in drinking water in the breeding stage for the prevention of Gumboro disease.

Analysis of information at 3, 5, 7, 9, 12 and 15 weeks of age

INTERPRETATION TECHNIQUES AND CONSIDERATIONS OF *MYCOPLASMA* SEROLOGY TESTING IN DAY OLD CHICKS FROM VERIFIED CLEAN BREEDER STOCK BASED ON EXPERIMENTAL TESTING OF BIRDS PRE- AND POST-SHIPMENT CONDITIONS

TÉCNICAS DE INTERPRETACIÓN Y CONSIDERACIÓN DE LAS PRUEBAS SEROLÓGICAS PARA *MYCOPLASMA* EN POLLOS DE UN DÍA DE EDAD DE REPRODUCTORAS VERIFICADAS COMO LIMPIAS BASANDOSE EN PRUEBAS EXPERIMENTALES DE AVES EN CONDICIONES PRE Y POST EMBARQUE

I. Rubinoff

Hyline

RESUMEN

Un grupo de pollos de un día de edad fueron evaluados por contenido serológico de antibióticos en condiciones típicas replicando en ambientes pre y post embarque de pollos. Los resultados de esta prueba son comparados con el desempeño promedio de la prueba de ELISA para *Mycoplasma* para una mejor comprensión de como el equipo de ELISA y técnicas de laboratorio pueden afectar los resultados de la prueba. La discusión sobre mejores métodos para evaluar la salud de esta población seguirá después de la presentación de los datos.

ABSTRACT

A group of one-day old chickens were evaluated for serologic antibody content in conditions replicating typical pre and post shipment environments of chicks. The results of this testing are compared with the average performance of Mycoplasma ELISA testing to better understand how ELISA kit and lab techniques may affect test results. Discussion on best methods to evaluate the health of this population will follow presentation of the data.

SUMMARY

Day-old chick serology is a common practice on receipt of high value birds or flocks that are at high risk of vertical disease transmission. In the USA, primary breeders are under NPIP Mycoplasma control programs and must supply chicks free from all avian Mycoplasmas. In other parts of the world, certified poultry health schemes follow similar control measures. Grandparent and parent breeder flocks must be tested every two to three weeks to ensure the freedom of their progeny. Despite the high levels of testing and focus on origin source flock freedom of disease, many customers still test day old chicks upon arrival to the farm. For GP and PS birds sent to a country with a poultry health scheme mandating the freedom of avian mycoplasmosis, if testing up on arrival demonstrates an elevated number of false positive results this can raise concerns about bird health from the recipients of the chicks and the ability to utilize the flock in a breeder or commercial capacity. Direct comparison of the values has been difficult until now, as the labs running the pre and post shipment serology were typically different, and no standard of Lab QC was measurable between the testing events.

The purpose of this work was to simulate the conditions of shipment on a group of chicks from placement and collect samples pre- and post-shipping conditions to compare the incidence of positive results and gain a better understanding of how to interpret these results.

ELISA is a common diagnostic test used for screening birds to assure freedom of infection in poultry for avian mycoplasmosis. The sensitivity of these tests is typically 100% and the specificity in general populations is greater than 98%. In this project, results from field testing of day-old chicks both pre and post shipment were retrospectively reviewed. Following this, USDA approved commercial ELISA test kits were used to measure incidence of positive results on 250 known antibody negative chicks. The testing was performed on samples collected at hatch and again after the chicks were subjected to stressors simulating transport typical of shipment and placement of a flock. The birds were kept in a bio-secure location between testing events. Testing was done by trained technical service persons. All positive ELISA results were confirmed negative by HI and plate agglutination testing, submitted to the PRDC, in Athens GA (n=20).

RESULTS

Retrospective results from field testing showed incidences in which up to 25/52 birds tested with ELISA A were positive, which were not confirmed by subsequent testing. In the testing events simulating pre-shipment chick conditions, ELISA B yielded 9/250 and 17/250 positive results, in pre and post shipment groups, respectively. This lot of ELISA kits was later subjected to a field action indicating less specificity then normal and removed from commercial use proactively by the vendor. Subsequent testing on individual ELISAs for MG and MS from this vendor provided 1/250 pre-shipment positives and 1/250 post shipment positives. This equates to a 99.6% specificity and is in line with expected performance. Testing of the MSMG combination ELISA from Company A on the same samples yielded 100% negative results when done in the company's facility by trained technical service people.

DISCUSSION

The data from this study showed that the number of false positive reactions before and after shipment conditions was the same. However, several factors may vary between testing events such as the lab conditions, technicians running the assay, and potentially kit lot being used. ELISA remains a suitable screening test, and positive samples resulting from arrival testing of day-old birds should be immediately repeated on ELISA to rule out laboratory error, and/ or confirmed on alternate confirmatory methods, such as HI or PCR - before the flock is treated as positive.

PEPTIDOGLYCANS, GASTROINTESTINAL FUNCTIONALITY, AND POULTRY PERFORMANCE

PEPTIDOGLICANOS, FUNCIONALIDAD GASTROINTESTINAL, Y DESEMPEÑO AVÍCOLA

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RESUMEN

Mantener un tracto digestivo sano, y funcional ayuda a cumplir sus funciones como la digestión y la absorción de nutrientes, así como, eliminar los desechos y mantener una microbiota equilibrada. Las actividades de reconocimiento de patrones moleculares como los lipopolisacáridos (LPS) y los peptidoglicanos (PGN) ayudan a mantener este equilibrio (1, 2, 4). Los PGN son reconocidos actualmente como componentes naturales de la pared celular de bacterias presentes en el lumen intestinal., cuando los PGN son expuestos, pueden desarrollar respuestas inmunes indeseables, afectando la funcionalidad gastrointestinal y el uso de nutrientes. (3) Esta condición puede ser contrarrestada con la adición de muramidasas en el alimento. El presente estudio fue desarrollado con pollos de engorda machos Ross 380 alimentados hasta los 49 días de edad con dietas suplementadas con Muramidasa 007* (MUR), los resultados mostraron un mejoramiento significativo ($P \leq 0.0001$) en la ganancia peso corporal (93gr/ pollo de engorda) y en la tasa de conversión alimenticia (-0.16).

ABSTRACT

Maintaining a healthy, and functional digestive tract helps fulfill functions such as digestion and absorption of nutrients; as well as, eliminating waste and keeping the microbiota in balance. Activities as recognition of the molecular patterns in lipopolysaccharides (LPS) and peptidoglycans (PGNs) help to maintain this balance (1,2,4). PGNs are currently recognized as natural components of the wall cell bacteria present in the intestinal lumen, when PGNs are exposed, can develop undesirable immune responses affecting gastrointestinal functionality and the use of nutrients. (3) This condition can be counteracted by the addition of muramidases in feed. The present study was developed with male broilers Ross 380 fed until 49 days old with diets supplemented with Muramidase 007* (MUR), results showed significantly improving ($P \leq 0.0001$) in body weight gain (93 g / broiler chicken) and in feed conversion rate (-0.16).

INTRODUCTION

The worldwide increasing demand for safe and affordable food has done high pressure on poultry production systems looking to make it more efficiently; The right functionality of the digestive tract has become a concept that supports productivity greatly (1). The natural and constant cellular turnover in the intestinal lumen causes the high presence of PGNs, these are exposed by replication and/or bacterial death and can develop undesirable immune responses affecting gastrointestinal functionality and the optimal use of nutrients (2,3,5,7).

To counteract this effect, a nutritional additive based on MUR was developed. This enzyme, also known as N-acetyl muramidase, and belongs to the family of glycosyl-hydrolytic enzymes, where glucanases and hemicellulases also belong. MUR is a new microbial enzyme that hydrolyze PGNs at the B-1,4-hydrolytic site, that it is located between N-acetyl muramic acid and N-acetyl glucosamine. Due to the MUR activity on PGNs, these are divided in peptides and glucans counteracting proinflammatory processes and contributing to the optimal gastrointestinal tract (GIT) functionality, without modifying the intestinal microbiota (6,8), and achieving optimal growth rate and feed efficiencie. The aim of this study was to evaluate the addition of muramidase 007 * at dose of 25,000 LSU (F) / kg of feed in diets of male Ross 380 broilers, fed up from 1 to 49 days old to improve performance parameters.

MATERIALS AND METHODS

The study was conducted at the Center for Education, Research and Extension in Poultry Production (CEIEPAv) of the Faculty of Veterinary Medicine and Zootechnics (FMVZ) of the UNAM, CEIEPAv is located in Mexico City. The average annual temperature is 18° C and 60% RH. A total of 518 male broiler chickens belonging to the Ross 308 strain were randomized divided into two treatments with 7 replicates of 37 birds per experimental unit, broilers were housed in a density of 16 birds per m^2. The treatments were Control Diet (CT) without any antibiotic growth promoter and CT plus the addition of MUR * (CT+MUR) at dose of 25,000 LSU (F) / kg. Diets used were based on sorghum, soybean meal, canola and DDGs, with phytase (1,000 FYT/Kg) and without coccidiostats. Four feed phases were used: pre-starter (0 - 7d), starter (8 - 21d), grower (22 - 35d) and finisher (35 - 49d). Broilers consumed water and feed ad libitum and were housed in accordance with the standards of the strain. Broiler's management was approved by the Internal Committee for the Care and Use of Experimental Animals of FMVZ-UNAM. Weekly productive parameters as body weight gain, feed consumption, percentage of mortality and feed conversion were evaluated.

The data obtained were analyzed by an ANOVA with significance of 0.05 (Statistix 10.0).

RESULTS AND DISCUSSION

During a period of 49-days, broilers fed with CT+MUR, showed significantly greater body weight gain (3,285g vs. 3,177g; $P \leq 0.001$), better daily weight gain (65.4g vs 63.4g; $P \leq 0.0001$), with a significant decrease in feed intake (6,621g vs 6,931g; $P \leq 0.0001$); as well as, better feed conversion rate (2.06 vs 2.22; $P \leq 0.0001$), compared with broiler fed with CT. Similar studies with different doses of MUR showed a linear increase for body weight gain in broiler chickens; as well as, an improvement in feed conversion rate (4). Results in this study indicates that the addition of MUR in the diets contributed to improve gastrointestinal functionality, decreasing the energy expenditure of maintenance, and improving the productive efficiency of broilers.

(The article will be published in its entirety in *Poultry Science*.)

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NECROTIC ENTERITIS PREVENTION AND CONTROL IN BROILERS: THE EFFECTS OF AN ANIMAL-USE ONLY ANTIBIOTIC COMPARED TO SHARED-CLASS ANTIBIOTICS

PREVENCIÓN DE LA ENTERÍTIS NECRÓTICA EN POLLOS DE ENGORDA: LOS EFECTOS DE LOS ANTIBIÓTICOS DE USO EXCLUSIVO-ANIMAL COMPARADO A LOS ANTIBIÓTICOS DE CLASE-COMPARTIDA

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RESUMEN

La Avilamicina es un antibiótico primero en su clase, de uso exclusivo en animales, para el alimento desarrollado para la prevención de la mortalidad causada por la enteritis necrótica asociada con el *Clostridium perfringens* en los pollos de engorda. Bajo la supervisión de un veterinario, la Avilamicina apoya la salud del ave y su bienestar, y demostró tener un enfoque responsable para el uso de antibióticos.

SUMMARY

Avilamycin is a first-in-class, animal-use only, in-feed antibiotic developed for the prevention of mortality caused by necrotic enteritis associated with *Clostridium* perfringens in broiler chickens. Under supervision of a veterinarian, Avilamycin supports bird health and well-being, and demonstrates a responsible approach to antibiotic use.

STUDY DETAILS

Necrotic enteritis is a serious *C. perfringens* disease and a constant threat to the health and welfare of poultry flocks. Due to recent reduction of antibiotic use in poultry, the incidence of necrotic enteritis (NE) is on the rise in some regions and productions systems, with daily mortality rates as high as one percent. *C. perfringens* is a gram-positive, rod-shaped anaerobic bacteria that can be found in the intestinal tract of healthy birds and contaminated environments (e.g., litter, feed, soil). The bacteria multiplies very rapidly, and can survive in the litter under extreme conditions as dormant spores. The bacteria is transmitted both horizontally (e.g., fecal-oral, insects) and vertically. The risk of NE caused by *C. perfringens* is increased by any disturbance that damages the GI epithelium, including coccidiosis, mycotoxins and common environmental stressors. *C. perfringens* overgrows and produces potent toxins. Necrotizing alpha toxins cause damage, and toxins from the GI tract can enter blood and cause toxemia/death. These *C. perfringens* toxins easily lead to necrosis of the intestinal lining.

Avilamycin has a bacteriostatic effect. It attaches at two bacterial sites (one on 30s ribosome and one on 50s ribosome) to stop initiation and interferes with protein assembly. Without assembly, *C. perfringens* cannot produce the toxins that cause necrotic enteritis. It helps promote healthy birds, and it also fits within most recent antibiotic policies looking to reduce shared-class antibiotic use. It is the first animal-use only antibiotic for the prevention of mortality caused by necrotic enteritis associated with *Clostridium perfringens* in commercial poultry. In recent studies, Inteprity® (avilamycin) reduced mortality due to necrotic enteritis between 76 to 93 percent versus the control group

Avilamycin, an oligosaccharide antibiotic of the orthosomysin group, was recently introduced to the US broiler industry as an animal-use only antibiotic for prevention of mortality associated with Necrotic enteritis (NE) and the first to be introduced requiring veterinary oversight with the Veterinary Feed Directive (VFD). Studies assessing the impacts of avilamycin followed by an ionophore (animal-use only program) compared to antibiotics (shared-class) programs on NE prevention are described below.

Avilamycin field comparison with an integrated broiler producer. This study was conducted to evaluate the effect of Inteprity® when followed by an ionophore (animal-use only program) to control mortality associated with necrotic enteritis exposure compared to other antibiotics (shared-class program). An integrated broiler production facility conducted a field head-to-head study to compare the effect of (Avilamycin - Inteprity®), BMD® and Stafac® on mortality caused by necrotic enteritis (NE) and performance in broilers during summer and fall growing periods.

Table 1 describes the four different treatment groups in this study:

Table 2 shows necrotic enteritis mortality, lesions and coccidiosis lesions per group.

The Inteprity® Avilamycin treatment group had the lowest feed conversion rates at processing.

Key findings and conclusions**:**

This model using litter from previous NE challenge study represented typical field exposure resulting in low mortality and lesions

Animal-use only programs when fed in starter or starter-grower feeds provided significantly better FCR than the shared-class program

Feeding Inteprity/Monteban significantly reduce d *E. maxima* lesions

Feeding Inteprity/Monteban improved performa nce as measured by gain, feed intake and feed conversion versus a shared-class program.

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Table 1.

Treatment Group	Program	Starter Day 0-14	Grower 1 Day 15-21	Grower 2 Day 22-28	Finisher Day 29-42
1	Non-medicated	—	—	—	—
2	Inteprity/ Monteban®	Inteprity 22.7 g/ton	Inteprity 22.7 g/ton	Monteban 54 g/ton	Monteban 54 g/ton
3	Inteprity/ Coban®	Inteprity 22.7 g/ton	Inteprity 22.7 g/ton	Coban 90 g/ton	Coban 90 g/ton
4	BMD®/ Stafac®	BMD 50 g/ton	BMD 50 g/ton	BMD 50 g/ton	Stafac 20 g/ton

Table 2

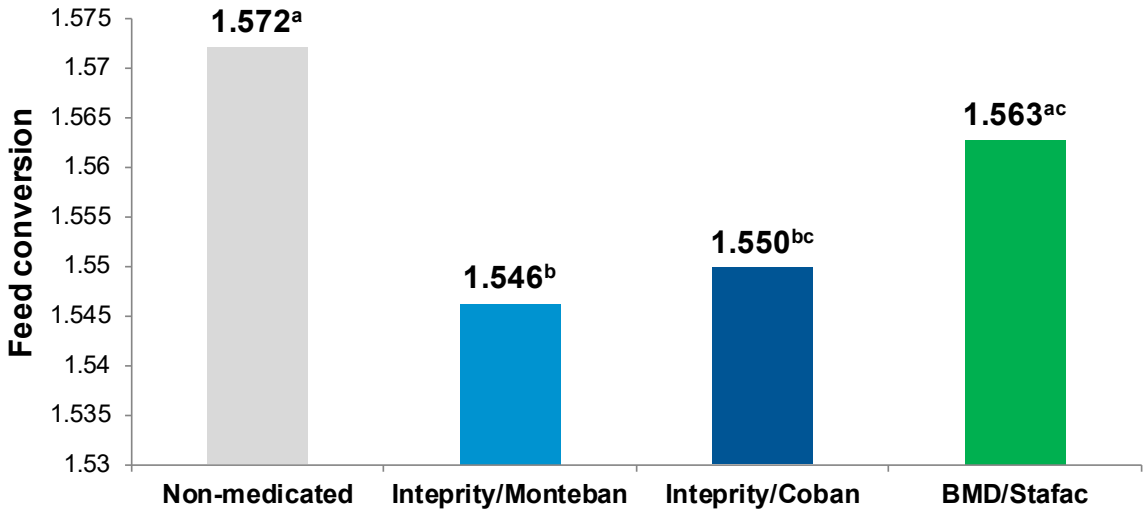
Treatment Group	1	2	3	4	
Treatment	Non-medicated	Inteprity/ Monteban	Inteprity/ Coban	BMD/ Stafac	*P*-value
NE mortality, %	4.17	4.91	4.17	5.83	0.8049
NE Lesion score, day 21	0.20	0.21	0.16	0.22	0.9413
NE Lesion score, day 28	0.20	0.22	0.49	0.33	0.0560

Table 3.

Treatment Group		1	2	3	4	
Treatment		Non-medicated	Inteprity/ Monteban	Inteprity/ Coban	BMD/ Stafac	*P*-value
E. acervulina	Day 21	2.04[a]	1.40[b]	1.22[b]	1.87[a]	< 0.0001
	Day 28	1.24	1.36	1.07	1.40	0.1573
E. maxima	Day 21	0.78[a]	0.79[a]	0.38[b]	0.82[a]	0.0004
	Day 28	1.22[a]	0.64[b]	1.29[a]	1.11[a]	0.0016
E. tenella	Day 21	0.11	0.19	0.11	0.16	0.6937
	Day 28	0.44	0.19	0.24	0.31	0.1313

[ab]Means within the same row without a common superscript are different ($P \leq 0.05$).

Table 4.



[abc]Means without a common superscript are different ($P \leq 0.05$).

TRANSMISSION PROFILE OF *MYCOPLASMA GALLISEPTICUM* VACCINE STRAIN MG-70 IN FREE RANGE CHICKENS

PERFIL DE TRANSMISION DE LA CEPA VACUNAL MG-70 DE *MYCOPLASMA GALLISEPTICUM* EN POLLOS LIBRES DE JAULA

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RESUMEN

El *Mycoplasma gallisepticum* (MG) es conocido por causar enfermedad respiratoria aguda o subclínica en pollos, causando una disminución en el desempeño en la producción y en la producción de huevo. Se ha demostrado que el uso de vacunas atenuadas es eficiente para reducir las pérdidas económicas y controlar la diseminación de este patógeno. Una de las vías de vacunación más ampliamente utilizada es el método por gota ocular, que puede ser responsable de una falla en la vacunación debida a un gran número de aves para inmunizar a la vez. La capacidad de transmisión de la cepa vacunal MG-70, una ampliamente utilizada en Brasil, se logró experimentalmente por medio de la seroconversión y la recuperación con PCR en la traquea de aves vacunadas a las 8 semanas de edad, vía ocular y con sentinelas. Fue posible observar que las aves sentinelas presentaron una respuesta immune humoral y la transmisión de la cepa vacunal se confirmó en esas aves por PCR.

SUMMARY

Mycoplasma gallisepticum (MG) is notable for causing acute or subclinical respiratory disease in chickens, causing a decrease in production performance and in egg production. The use of attenuated vaccines has been shown to be efficient in

reducing economic losses and controlling the spread of this pathogen. One of the mostly used vaccination route is the eye drop method, which may be responsible for vaccination failure due to the large numbers of birds to be immunized at once. Transmissibility of the MG-70 vaccine strain, one of mostly used in Brazil, was accomplished experimentally by seroconversion and PCR recovery in trachea of birds vaccinated at the 8th week of age, via ocular and sentinels ones. It was possible to observe that the sentinel birds presented humoral immune response and the transmission of the vaccine strain was confirmed in those birds by PCR.

INTRODUCTION

The poultry sector is increasingly important in the context of the worldwide agribusiness and the concern of sanitary issues have accompanied and favored this evolution. The economic losses attributed to *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) in commercial laying hens are due to drop in egg production, feed efficiency decrease and high cost of medication with antimicrobials and vaccination (4; 10).

Among the *Mycoplasma* species, MG have a greatest impact due to the losses caused by chronic respiratory disease (CRD). Economic losses associated with MG infection are the result of the interaction of the pathogen with other bacteria, poor health control and inadequate environmental conditions (10). The attenuated vaccine is recommended for commercial laying hens in order to reduce production losses and prevent the transmission of this infection (5; 12).

The effectiveness of attenuated vaccine is based on the stimulation of cellular and humoral immune responses, also acting as an instrument of competitive exclusion in relation to field strains in poultry farms (10). MG-70 strain, Conn F (MG-F), ts-11 and 6/85, are four types of attenuated vaccines all acting in reducing the transovarian transmission and losses on egg production (13). Even being attenuated, MG-F can be pathogenic for poultry, being transmissible among unvaccinated flocks (7, 8). On the other hand, 6/85, ts-11 and MG-70 strains considered non-pathogenic are able to compete with the pathogenic field strains in the upper respiratory tract receptors preventing diseases (4; 1; 9).

The diagnosis of mycoplasma infection can be made by polymerase chain reaction (PCR) and serological methods as serum agglutination reaction (SAR), and enzyme-linked immuno-sorbent assay (ELISA) (11; 15; 12). The objective of this study was to evaluate the profile of horizontal transmission, seroconversion and recovery of MG-70 in chicken's trachea.

MATERIAL AND METHODS

This experiment was conducted in Cachoeiras of Macacu-RJ, Brazil, beginning with 300 1-day-old chicks from Lohmann Brown Lite. The experiment was authorized by the permission number 1004 from the Animal Care Ethic Committee of the Fluminense Federal University, Niterói/RJ, Brazil. A total of 300 laying hens were used, being 250 vaccinated by eye-drop method at 8 weeks of age with MG - 70 strain (Myco-Galli MG-70, Biovet, São Paulo, SP, Brazil) (Group 1) and 50 non-vaccinated sentinels birds immunized by contact transmission (Group 2). Blood samples for serum tests were collected at 8th, 9th, 10th, 12th, 15th, and 18th of age for SAR and ELISA serology. Approximately 3.0 mL of blood was collected from the brachial vein of each bird. The sera obtained were immediately tested for SAR against MG and MS antigens according to the manufacturer's instructions (INATA, Uberlândia, MG, Brazil). Undiluted positive sera by SAR were considered suspicious and were, therefore, diluted further and retested. Serum samples were considered positive if the reaction occurred at $\geq$ 1:10, according to the guidelines of the National Poultry Health Program, Brazil, 1994. MG ELISA were analyzed by MG Antibody Test Kit (IDEXX, SP, Brazil). Positive reactions were considered when the optical density (OD) was $\geq$ 0.2. Tracheal swabs were collected at the 8th, 9th, 10th, 12th, 15th, and 18th weeks of age layered in 1 ml of Frey`s medium and frozen at -20º C. Swab samples were submitted to DNA extraction by the phenol-chloroform method (14), without previous incubation. The quantification of DNA was performed using the spectrophotometer Biodrop Touch® (Biochrom, UK). The pair of "primers" and amplification conditions for Mollicutes PCR (16). The reaction contained 1X TE buffer; 2mM MgCl2; 0,2mM dNTP; 0,6µM of each primer; 1U Taq DNA and 100ng of DNA to a final volume of 25µL. After amplification reaction, 5µL of each sample was homogenized with 1µL of loading buffer and GelRed®, applied in 1.5% agarose gel layered in Tris-Borate- EDTA (TBE) 0.5X , and finally submitted to the electrophoresis conditions (14). After the electrophoresis, the amplicons were visualized under ultraviolet light transilluminator.

RESULTS AND DISCUSSION

At the 8th week of age, all groups were negative by serologic methods (ELISA and SPA) and PCR for Mollicutes. The birds didn't have clinical signs during

the experiment, this absence was also reported when used 6/85, ts/11 and MG-F vaccine strains (6, 8).

PCR started the detection of *Mycoplasma* species at the first week after the exposure on the 8th week of age, being the earliest test in this MG diagnosis.In this study, the transmission of the MG-70 strain from vaccinated birds to the sentinels occurred at 9, 10, 12, and 15 weeks after vaccination (G-test of independence, $p < 0.05$) as demonstrated by PCR, while for ELISA positive reaction appeared at the 10th week for both vaccinated and sentinel group. Transmission measured by positive reaction to SAR only appeared four weeks post-vaccination. Seroconversion between vaccinated and sentinel birds was significantly difference by ELISA at the 18th week (Binomial test, $p < 0.05$) (Table 1). The weeks with the highest frequencies for PCR detection were the 10th with 80% in vaccinated birds and 60% in sentinels and the 12th with 50% in vaccinated birds and 70% in sentinel ones. At 18th week of age both groups were negative by PCR demonstrating that trachea recovery is transitory, while seroconversion persist, as in a study with ts-11 strain (2).

In the comparison among tests, SAR issued the highest positivity rates followed by ELISA and PCR (G-test of independence, $p<0,05$), proving that SAR was more sensitive than ELISA after three weeks post infection.

CONCLUSION

The transmission of the MG-70 vaccine to sentinel birds was confirmed by serology and PCR. PCR was more efficient in early diagnosis and transmission detection as compared to SAR and ELISA. Positivity rates for MG in birds were detected at higher frequency by SAR than ELISA.

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Table 1 Serological response in Optical Density (OD) of MG, vaccineted chickens (group 1) and sentinel chickens (group 2).

Group	9th	10th	12th	15th	18th
1	0,117 ±0,043[a]	0,227 ± 0,095 [a]	0,208 ± 0,131 [a]	0,314 ± 0,127 [a]	0,496 ± 0,285[b]
2	0,118 ± 0,039[a]	0,151 ± 0,088 [a]	0,131 ± 0,101 [a]	0,379 ± 0,334 [a]	0,225 ± 0,163 [a]

APLICACIÓN COLORIMETRICA DE LA LINEA BASE EN LA INTERPRETACIÓN DE RESULTADOS SEROLOGICOS DE ELISA EN AVES

BASELINE COLORIMETRIC APPLICATION IN SEROLOGIC RESULTS INTERPRETATION FROM ELISA IN POULTRY

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SUMMARY

The use of serology in poultry is a commonly used tool to follow up the flock´s health, one of the referenced serological techniques is indirect ELISA (Enzyme-linked immunosorbent assay)which provides readings based in related titers with the behavior present in birds. Taking into account the above, the baseline will help us to estimate the minimum and maximum titers considered as normal to determine the vaccination program, the geographic zone or the season. With the help of the xChekPus software, we can perform the baseline calculation taking into account 300 serum of the same age minimum, the same vaccination program and the zone in conditions for the productive parameters considered as normal. It is important considering that is very difficult to have a universal baseline because not all the geographic zones behave in the same manner, and not all the producers use the same vaccination program. Therefore, it is recommended to make a specific baseline for each enterprise, in this way we can assure that it will be the same measurement. Once the baseline calculation is done, this can be referenced at the right moment with the reports and making use of the colorimetry we can obtain graphics in yellow, green and red color, which will help us to interpret the results easily. The yellow color indicates that the obtained titers are found within the minimum and maximum range considered as normal and the red color means very high titters. The objective of the actual work is to facilitate the reading of the results and interpretation for the veterinarian in charge of the poultry health for the decision making.

RESUMEN

El uso de la serología en la avicultura es una herramienta común mente usada para dar seguimiento a la salud de las parvadas, una de las técnicas de referencia serológica es la ELISA indirecta (Ensayo Inmuno-absorbente ligado a enzima) la cual proporciona lecturas basadas en títulos relacionados con el comportamiento de los anticuerpos presentes en las aves, tomando en cuenta lo anterior, la línea base nos ayuda a poder estimar los títulos mínimos y máximos considerados como normales para un determinado calendario de vacunación, zona geográfica o época del año; con la ayuda del software xChekPlus, podemos hacer el cálculo de la línea base tomando como mínimo 300 sueros de la misma edad, mismo calendario de vacunación y zona en condiciones de parámetros productivos considerados como normales. Es importante tomar en cuenta que es muy difícil contar con una línea base universal ya que no todas las zonas geográficas se comportan de igual manera, ni todos usan el mismo calendario de vacunación, para esto se recomienda hacer la línea base específicamente de cada empresa de esta manera aseguramos que la misma sea a la medida. Una vez que se hizo el cálculo de la línea base está puede ser referenciada al momento de hacer los reportes y haciendo uso de la colorimetría podemos obtener gráficos de color amarillo, verde y rojo, los cuales nos ayudan a interpretar con más facilidad los resultados. El color amarillo indica que los títulos obtenidos se encuentran por debajo del límite mínimo considerado como normal, ósea títulos bajos, el color verde indica títulos que se encuentran dentro del rango mínimo y máximo considerados como normales y el color rojo refiere a títulos altos. El objetivo del presente trabajo es facilitar la lectura e interpretación de resultados al médico encargado de la sanidad de las aves para la toma de decisiones.

APLICACIÓN Y VENTAJAS DE UTILIZAR LÍNEAS BASE

Toma de decisiones rápidas y oportunas. Al identificar títulos fuera de la línea base, es posible implementar acciones en el campo que limiten la pérdida de productividad y económica dando entrada al tratamiento profiláctico oportuno que permite el mejor control de las enfermedades aminorando la pérdida económica.

Ajustes en los calendarios de vacunación. En correlación con la línea base se pueden ajustar los calendarios para alcanzar los títulos determinados como protectivos de cada enfermedad. Esta determinación debe hacerse evaluando la presencia o ausencia de signos clínicos correlacionada con los títulos. De este modo, la serología se convierte en una herramienta de monitoreo de la salud, dando el tiempo necesario al médico de campo en la toma de decisiones enfocadas en el nivel primario y secundario de prevención como se muestra en la (Figura 1).

Requerimientos para elaborar una línea base. Tener en cuenta que los datos obtenidos para calcular la línea base deben de ser tomados de una base de datos propia de cada empresa, derivado de un monitoreo serológico pensado en el calendario de vacunación actual, población, zona, región y granja; de una edad con parámetros considerados normales.

Número de muestras. Para obtener el tamaño de muestra mínimo en una población infinita, se utilizó el programa Epidat versión 3.1 para calcular la proporción de la población a muestrear, con un intervalo de confianza del 95% de manera aleatoria, arrojando un total de 275 muestras ajustado a 300, de tal manera que si queremos hacer una línea base es necesario contar de 275 - 300 lecturas de diferentes aves de una misma enfermedad en condiciones normales.

Interpretación de la línea base. Los títulos obtenidos deben ser comparados con la línea base establecida de acuerdo a la edad, estatus de desafío, calendario de vacunación, etc.

El software XchekPlus de IDEXX, refiere la línea base de los resultados y los analiza en forma de gráfica utilizando un código de colores: Amarillo: títulos por debajo la línea, Verde: Dentro de la línea. Rojo: Por encima de la línea. (Grafica 1.)

Títulos considerados como normales. Las barras verdes indican que los títulos obtenidos se encuentran dentro de los títulos considerados como normales o esperados como se muestra en la (Gráfica 2).

Títulos bajos. Las barras amarillas indican que los títulos se encuentran por debajo del mínimo considerado como normal, de tal manera que si estamos haciendo un seguimiento post vacunación y encontramos este tipo de títulos aunado a un coeficiente de variación (CV%) mayor 40%, podríamos pensar que sucedió algo inadecuado en el proceso de vacunación.

Por otro lado, si estamos realizando un monitoreo de cualquier enfermedad y encontramos que todos los resultados presentan líneas amarillas, podríamos pensar en un problema de inmunosupresión. (Gráfica 3).

Títulos altos. Las barras rojas muestran títulos altos o de posible desafío como se muestra en la (Gráfica 4).

Gráficos con múltiples barras de colores. En el caso que se presenten gráficas con barras de los tres colores, podrían ser interpretadas de la siguiente manera (Gráfica 5):

Barras amarillas: posibles animales en viremia.

Barras verdes: animales que no han sido desafiados.

Barras rojas: posibles animales desafiados.

CONCLUSIÓN

La interpretación de resultados basados en la colorimetría referenciados a la línea base facilitan la lectura y el entendimiento de los títulos presentes en los animales.

La línea base nos ayuda a comparar datos nuevos con información histórica.

Provee información para anticiparse a brotes, evaluar tendencias y comparar programas de vacunación.

Excelente forma de monitorear cambios en el manejo y establecer tendencias epidemiológicas.

Las herramientas informáticas como el xChekPlus® facilitan el establecimiento y uso de las líneas base.

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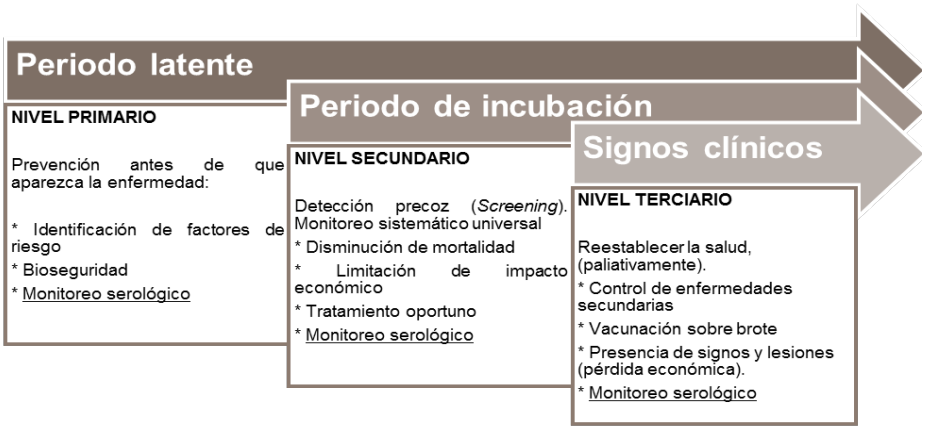
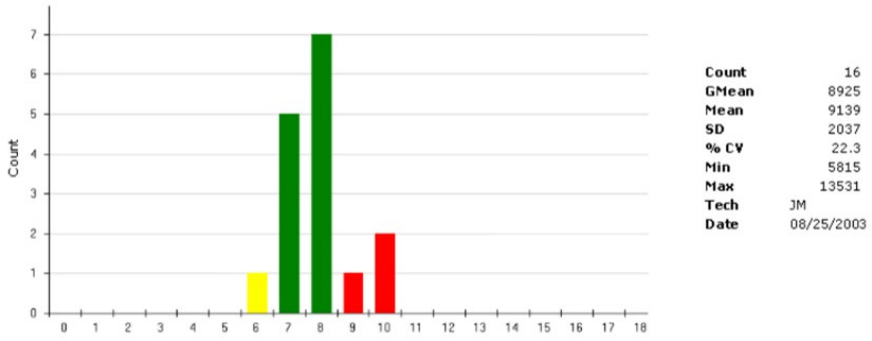
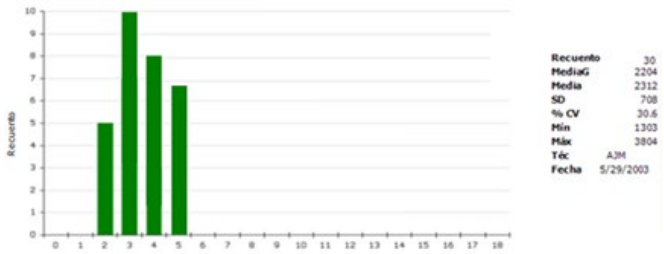
Figura 1.



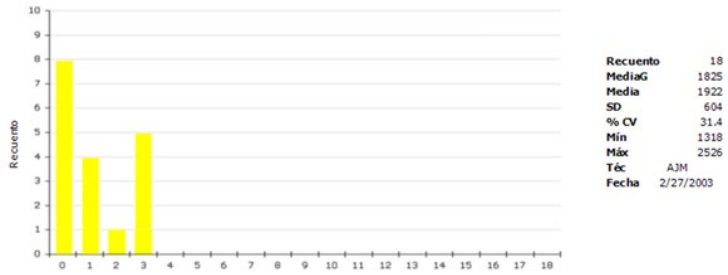
Graph 1. Muestra resultados con colorimetría referenciados a una línea base.



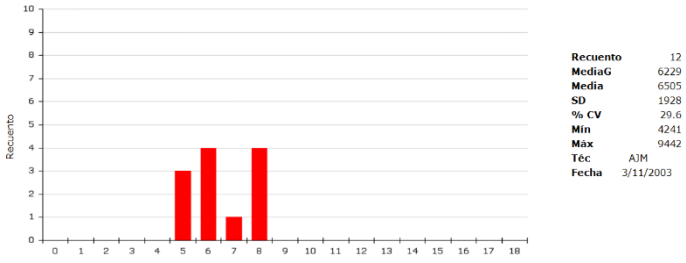
Graph 2. Muestra títulos considerados como normales.



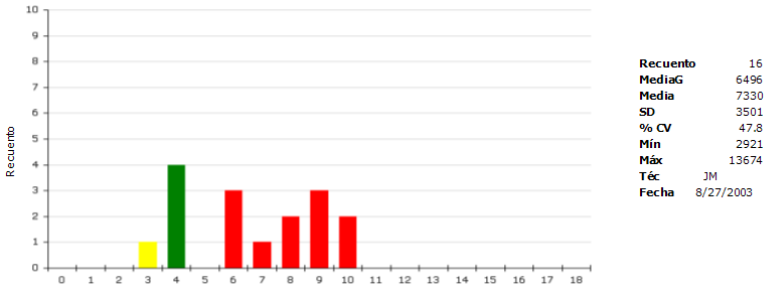
Graph **3.** Muestra títulos considerados como bajos.



Graph **4.** Muestra títulos considerados como altos.



Graph **5.** Muestra animales en diferentes estadios serológicos.



ANÁLISIS DE LOS RESULTADOS DE SENSIBILIDAD ANTIMICROBIANA REALIZADOS EN AISLAMIENTOS DE *ESCHERICHIA COLI* PROVENIENTES DE AVES DE MÉXICO DURANTE UN PERIODO DE CATORCE AÑOS COMPRENDIDOS ENTRE 2005 Y 2018

ANALYSIS OF THE ANTIMICROBIAL SUSCEPTIBILITY TEST RESULTS PERFORMED IN *ESCHERICHIA COLI* ISOLATES FROM MEXICAN COMMERCIAL FLOCKS IN A PERIOD OF FOURTEEN YEARS BETWEEN 2005 AND 2018

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SUMMARY

Antimicrobial susceptibility tests were performed on a total of 617 purified isolates of *Escherichia coli* (*E. coli*). These isolates were obtained from clinical cases of Mexican birds in a period of fourteen years between 2005 and 2018. The antimicrobial susceptibility tests were performed by using a diffusion disc method (sensidisc), following the standard operating procedures approved by the Clinical and Laboratory Standards Institute (CLSI). Among the antimicrobial products tested were: amoxicillin, enrofloxacin, florfenicol, fosfomycin, gentamycin, combined gentamicin with nalidixic acid, neomycin, norfloxacin, and sulfachlorpyridazine sodium with trimethoprim. The results showed that there is a reduced susceptibility by *E. coli* towards amoxicillin, and the combination of sulfachlorpyridazine sodium with trimethoprim in a period of fourteen years. In addition, in the past nine years, the results indicated that *E. coli* has a reduced susceptibility to florfenicol as well.

RESUMEN

Se realizaron pruebas de sensibilidad antimicrobiana en un total de 617 aislamientos de *Escherichia coli* (*E. coli*) purificados, obtenidos de casos clínicos de aves de México en un período de catorce años comprendidos entre el año 2005 al año 2018 utilizando el método de difusión con disco (sensidiscos) y siguiendo los lineamientos estandarizados y aprobados por el Clinical and Laboratory Standards Institute (CLSI). Los antimicrobianos probados incluyen amoxicilina, enrofloxacina, florfenicol, fosfomicina, gentamicina, gentamicina combinada con ácido nalidíxico, neomicina, norfloxacina y sulfacloropiridazina sódica con trimetoprim. Los resultados indican que en el período de catorce años ha existido una baja sensibilidad de *E. coli* hacia amoxicilina y hacia la combinación de sulfacloropiridazina sódica con trimetoprim. Así mismo, los resultados de los últimos nueve años indican que se ha disminuido la sensibilidad de *E. coli* hacia el florfenicol.

INTRODUCCIÓN

La realización de pruebas de sensibilidad antimicrobiana a los aislamientos bacterianos de casos clínicos de los animales resulta en una excelente herramienta para conocer la resistencia que desarrollan los microorganismos hacia los diferentes antimicrobianos utilizados en el campo, por lo que el objetivo de este trabajo es presentar los resultados de sensibilidad antimicrobiana de *E. coli* realizados en el laboratorio de Diagnósticos Clínicos Veterinarios, durante un período de catorce años comprendidos del 2005 al 2018.

MATERIALES Y MÉTODOS

La prueba de susceptibilidad antimicrobiana se realizó por el método de difusión con disco, bajo los lineamientos y directrices del CLSI que incluyen la concentración del fármaco que deberán tener cada uno de los sensidiscos de acuerdo al antimicrobiano seleccionado.

Los 617 aislamientos utilizados en este estudio corresponden a aislamientos obtenidos a partir de aves enfermas que llegan de toda la República Mexicana al Laboratorio de Diagnósticos Clínicos Veterinarios S.A. de C.V. Las bacterias fueron aisladas, purificadas e identificadas por pruebas bioquímicas, reacciones enzimáticas o pruebas de PCR.

Los antimicrobianos utilizados y su concentración fueron amoxicilina (20 µg), enrofloxacina (5 µg), florfenicol (30 µg), fosfomicina (200 µg), gentamicina (10 µg), gentamicina combinada con ácido nalidíxico (10 µg + 30 µg), neomicina (30 µg), norfloxacina (10 µg) y sulfacloropiridazina sódica con trimetoprim (23.75 µg + 1.25 µg).

Se consideró como sensible al antimicrobiano a aquellas cepas que mostraron un diámetro del halo inhibitorio de 8 mm en adelante.

El porcentaje de sensibilidad anual se consideró como bajo con menos de 54% de cepas sensibles, como medio entre 55% y 68%, como alto con más de 69%.

CONCLUSIONES

Los antimicrobianos hacia los cuales se presentó menor sensibilidad a las cepas evaluadas durante el período de catorce años fueron amoxicilina y la combinación de sulfacloropiridazina + trimetoprim.

Los fármacos en los cuales se presentó mayor sensibilidad fueron neomicina, la combinación de gentamicina + ácido nalidíxico, gentamicina, norfloxacina y fosfomicina.

A partir del año 2010 se observan cambios importantes en la sensibilidad de *E. coli* hacia enrofloxacina, ya que de un 79% obtenido en el período 2005 - 2009 bajó a un 45% en 2010, en el período 2011 – 2017 se obtuvo un 60.8% y en el 2018 tuvo un incremento en el porcentaje de la sensibilidad del 93% dejando como promedio en un 68%. En cuanto a la sensibilidad de *E. coli* hacia el florfenicol, se observa que ésta ha disminuido en los últimos ocho años, ya que de un 65.6% obtenido en el período de 2005 - 2009 disminuyó hasta un 38.3% en el período 2010 – 2018. Por lo que respecta a la sensibilidad de *E. coli* hacia fosfomicina, se observa que ésta también ha disminuido fuertemente en el período que comprende 2010-2014 y en este último período del 2015 - 2017 ha tenido una recuperación de la sensibilidad hasta alcanzar un 81% en el 2017 pero en el 2018 se observa una caída de la sensibilidad en un 19.7%.

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Tabla 1. Resultados de las pruebas de sensibilidad antimicrobiana.

Resultados: Porcentaje Anual de Sensibilidad Antimicroniana de *Escherichia coli*

Antimicrobiano	Concentración en µg	*2005* 41	*2006* 38	*2007* 26	*2008* 17	*2009* 53	*2010* 33	*2011* 61	*2012* 72	*2013* 30	*2014* 54	*2015* 76	*2016* 34	*2017* 42	*2018* 40	Promedio
Amoxicilina	20 µg	29%	42%	27%	0%	36%	4%	15%	22%	ND	ND	ND	ND	ND	ND	**22%**
Enrofloxacina	5 µg	71%	79%	77%	75%	79%	45%	61%	54%	53%	65%	76%	50%	67%	93%	**68%**
Florfenicol	30 µg	78%	68%	78%	27%	77%	48%	47%	ND	30%	33%	36%	35%	48%	30%	**49%**
Fosfomicina	200 µg	85%	88%	69%	100%	83%	55%	54%	55%	53%	61%	70%	75%	81%	65%	**71%**
Furaltadona	300 µg	90%	100%	ND	100%	84%	85%	95%	92%	ND	ND	ND	ND	ND	ND	**92%**
Gentamicina	10 µg	ND	ND	ND	ND	ND	ND	ND	83%	77%	83%	88%	68%	88%	55%	**77%**
Gentamicina + Ac nalixidico	10 µg + 30 µg	100%	87%	96%	86%	94%	73%	59%	61%	59%	77%	81%	59%	93%	68%	**78%**
Neomicina	30 µg	100%	97%	100%	100%	100%	94%	98%	97%	100%	98%	100%	100%	100%	100%	**99%**
Norfloxacina	10 µg	67%	65%	78%	87%	91%	67%	79%	74%	50%	81%	80%	65%	79%	68%	**74%**
Sulfacloropiridazina + Trimetoprim	23.75 µg + 1.25 µg	13%	40%	19%	21%	58%	27%	17%	19%	20%	13%	24%	29%	29%	18%	**25%**

* ND: No disponible

- Bajo: menos de 54% de cepas sensibles.
- Medio: entre 55 y 68% de cepas sensibles.
- Alto: más del 69% de cepas sensibles.

TOLL-LIKE RECEPTOR (TLR)7 MEDIATED ANTIVIRAL RESPONSE AGAINST AVIAN INFLUENZA VIRUS INFECTION IS ATTRIBUTABLE TO INTERLEUKIN (IL)-1β PRODUCTION

LA RESPUESTA ANTIVIRAL MEDIADA POR RECEPTORES TIPO TOLL CONTRA INFECIONES DEL VIRUS DE INFLUENZA AVIAR ES ATRIBUIBLE A LA PRODUCCIÓN DE INTERLEUCINA (IL)-1β TOLL-LIKE RECEPTOR (TLR)7 MEDIATED ANTIVIRAL RESPONSE AGAINST AVIAN INFLUENZA VIRUS INFECTION IS ATTRIBUTABLE TO INTERLEUKIN (IL)-1β PRODUCTION

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RESUMEN

El objetivo de este estudio fue el determinar a los mediadores proinflamatorios que son activados más adelante de la ruta señalada del TLR7 en macrófagos aviares y sus papeles en la respuesta antiviral contra la infección del virus de la influenza aviar (AIV, por sus siglas en inglés). En este estudio, primero, estimulamos a los macrófagos aviares con un análogo del ssARN, resiquimo, y se encontró que el ssARN fue capaz de incrementar el ON y la producción del IL-1β en los macrófagos aviares. Segundo, hemos observado cuando los macrófagos aviares fueron estimulados con el ssARN, esto provoca una respuesta antiviral contra el AIV. Finalmente, demostramos que cuando se bloquea la respuesta del IL-1β usando el antagonista del receptor IL-1 (IL-IRa) y la producción de ON usando un inhibidor selectivo de la sintetasa de óxido nítrico inducible (iNOS), N-([3-(Amino-metil) fenil] metil) etanimidamida dihidrocloro (1400W), la respuesta antiviral contra el AIV es atribuible a la producción de IL-1β y no a la producción del ON. Este estudio proporciona una visión del mecanismo de la respuesta antiviral mediada por el ssARN, particularmente contra la infección del AIV.

SUMMARY

The objectives of this study were to determine the pro-inflammatory mediators that are activated downstream of TLR7 signaling pathway in avian macrophages and their roles in antiviral response against avian influenza virus (AIV) infection. In this study, first, we stimulated avian macrophages with the analog of ssRNA, resiquimod, and found that the ssRNA was capable of increasing NO and IL-1β production in avian macrophages. Second, we observed when the avian macrophages were stimulated with ssRNA, it elicits an antiviral response against AIV. Finally, we demonstrated that when we blocked the IL-1β response using IL-1 receptor antagonist (IL-1Ra) and the NO production using a selective inhibitor of inducible nitric oxide synthase (iNOS), N-([3-(Aminomethyl) phenyl] methyl) ethanimidamide dihydrochloride (1400W), the antiviral response against AIV is attributable to IL-1β production and not to the NO production. This study provides insights into the mechanisms of antiviral response mediated by ssRNA, particularly against AIV infection.

INTRODUCTION

Macrophages are one of the major immune cell types involved in the innate immune system that recognize and eliminate various microbes. The microbial recognition by macrophages is mediated by the receptors expressed on macrophages referred to as pattern recognition receptors (PRRs) including various types of toll-like receptors (TLRs) (1-3). In response to a virus infection, the TLRs recruit downstream adaptor molecules activating intracellular signaling cascades (4) with a consequence of upregulation of gene transcription for the production of pro-inflammatory molecules. The activated pro-inflammatory mediators includes antiviral cytokines such as interleukin (IL)-1β and inducible nitric oxide synthase (iNOS) (5-7). The iNOS will facilitate production of a potent highly reactive antiviral free radical molecule, nitric oxide (NO), as a part of innate host defense against invading infectious agents (8, 9).

Of the many types of TLRs in birds, TLR7 is the only identified receptor that binds with viral single-stranded ribonucleic acid (ssRNA) or its synthetic analogs (i.e. resiquimod, imiquimod, gardiquimod and ioxoribine) (7, 10). In chickens, ssRNA can induce antibacterial effects against *Salmonella Enteritidis* (11) and antiviral effects against infectious bursal disease virus infection (12, 13). Recently, a study demonstrated that ssRNA upregulates mRNA expression of pro-inflammatory mediators including IL-1β and iNOS in chicken *in vivo* (14). However, the antiviral response of TLR7 activation against avian influenza virus (AIV) infection is not known. AIV infections are prevalent globally causing severe diseases in birds, mammals as well as in humans (15). Therefore, our objectives of this study were to determine whether 1) activation of the TLR7 signaling pathway in avian macrophages produces pro-inflammatory molecules involved in antiviral activity and 2) these pro-inflammatory mediators are attributable to antiviral response against AIV infection in avian macrophages.

MATERIALS AND METHODS

In this study, first, we stimulated avian macrophages with the analog of ssRNA, resiquimod (Selleckchem, Houston, TX, USA) (10µg/ml) or only growth medium (control). The macrophage culture supernatants were collected at 24 hours post-treatment and NO production was determined using Griess assay reagent system as described previously (9). For the quantification of IL-1β production following ssRNA treatment of avian macrophages, Protein transport inhibitor cocktail (2µl/ml) (cocktail of brefeldin A and monensin, eBioscience, San Diego, CA, USA) was added to culture medium following 6 hours of incubation in order to prevent release of IL-1β to the extracellular space. After 24 h of stimulation, the cells were fixed with 4% paraformaldehyde and subsequently immunofluorescent staining for IL-1β was performed and analyzed the data as described previously (16).

Second, we stimulated avian macrophages with either ssRNA (10µg/ml), ssRNA (10µg/mL) combined with N-([3-(Aminomethyl) phenyl] methyl) ethanimidamide dihydrochloride **(**1400W) (Sigma-Aldrich, St. Louis MO, USA) (100µM), 1400W (100µM), or only growth medium as a control. We used 1400W, a selective inhibitor of iNOS (17, 18), to block NO production. Then, the macrophage culture supernatants were collected at 24 h post-treatment and 250µL of the collected cell culture supernatants were transferred on to Madin-Darby canine kidney epithelial (MDCK) cell monolayers before infecting with H4N6 low pathogenic AIV (LPAIV) (50 PFUs/well). The remaining culture supernatants were used to determine NO production from macrophages.

Finally, we stimulated avian macrophages with either ssRNA (10µg/mL) or only growth medium (control). The macrophage culture supernatants were collected at 24 h post-treatment and 250µL of the cell culture supernatants were transferred to Douglas Foster (DF)-1 cell monolayers. The receiving DF-1 cells were pre-incubated (30 minutes) with 1.2 µg/mL IL-1 receptor antagonist (IL-1Ra) (Kingfisher Biotech, Inc., CITY MN, USA). After 24 h of transferring macrophage culture supernatant, the DF-1 cells were infected with H4N6 LPAIV (0.1 MOI). Twenty-four hours post-infection, the infected DF-1 cell culture supernatants were collected from each well and titrated in MDCK cell monolayers in 10 fold serial dilution.

RESULTS

In this study, first, we found that the ssRNA was capable of increasing NO and IL-1β production in avian macrophages. Second, we observed when the avian macrophages were stimulated with ssRNA, it elicits an antiviral response against AIV. Finally, we demonstrated that when we blocked the IL-1β response and the NO production, the antiviral response against AIV is attributable to IL-1β production and not to the NO production. This study provides insights into the mechanisms of antiviral response mediated by ssRNA, particularly against AIV infection.

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ANTEMORTEM DETECTION OF WOODEN BREAST AND ASSOCIATION WITH "TURTLE BIRDS"

DETECCION ANTEMORTEM DE PECHUGA DE MADERA Y LA ASOCIACIÓN CON "AVES TORTUGA"

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RESUMEN

La enfermedad muscular de pechuga de madera de los pollos de engorda causa pérdidas económicas significativas debido al rechazo de las canales. Las "Aves Tortuga", o aves que accidentalmente se caigan de espaldas y no se pueden reincorporar, muestran una alta incidencia de pechuga de madera en una parvada de enseñanza de pollos de engorda. En un estudio de seguimiento, los músculos de la pechuga (*Pectoralis major*) de los pollos de 56 días de edad fueron calificados en macro para pechuga de madera. También fue determinada la habilidad de las aves de tocar sus alas sobre su espalda y la habilidad de reincorporarse cuando son colocadas sobre sus espaldas. La habilidad de tocar sus alas está altamente correlacionada con una baja calificación macro de pechuga de madera ($P< 0.05$). La prueba antemortem de toque de alas puede servir como una herramienta para la identificación de aves vivas con pechuga de madera. El análisis histopatológico del musculo de la pechuga es actualmente un progreso y será presentado. Aunque la pechuga de madera ha sido considerada como un problema de la calidad de la carne, también afecta adversamente en la vida del ave y contribuye a la mortalidad.

SUMMARY

Wooden breast is a muscle disease of broiler chickens causing significant economic losses due to carcass rejection. "Turtle birds," or birds that accidentally fall on their backs and cannot right themselves, displayed a high incidence of wooden breast in a broiler teaching flock. In a follow-up study, breast muscles (*Pectoralis major*) of 56-day-old broilers were grossly scored for wooden breast. Ability of birds to touch the wings over the back and right themselves when placed on their backs was also determined. Wing-touch ability was highly correlated with lower gross wooden breast scores ($P<0.05$). Antemortem wing-touch testing may serve as a tool to identify wooden breast in live birds. Histopathologic analysis of breast muscle is currently in progress and will be presented. Although wooden breast has previously been considered primarily a meat quality issue, it may also adversely affect live birds and contribute to mortality.

AN EVALUATION OF THE COMPATIBILITY OF THE SIMULTANEOUS USE OF A FEED ADDITIVE PROBIOTIC AND LIVE *SALMONELLA* TYPHIMURIM VACCINE IN BROILERS

UNA EVALUACIÓN DE COMPATIBILIDAD DEL USO SIMULTANEO DE UN PROBIÓTICO ADITIVO DE ALIMENTO Y UNA VACUNA VIVA DE *SALMONELLA* TYPHIMURIUM EN POLLOS DE ENGORDA

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RESUMEN

El incremento del uso de probióticos aditivos de alimento en la industria avícola de Norte América ha resultado en una preocupación de los profesionales de la producción avícola en cuanto a la compatibilidad de estos productos con la administración de vacunas vivas contra *Salmonella* Typhimurium (ST). La preocupación se centra en una posible interferencia de la inmunidad conferida por la vacuna de ST debido a los efectos de competitividad exclusión de los probióticos. Ha surgido un interés adicional en cuanto a los efectos aditivos o sinergias que puedan existir entre los probióticos y la vacuna viva de ST. Se condujo un estudio con pollos en corrales de piso para evaluar las posibles interacciones de un probiótico aditivo de alimento de multi-cepas y la administración al día de edad de una vacuna viva de ST comúnmente usada. Se investigaron tres tratamientos. (1) CON (sin vacuna viva de ST o probiótico). (2) ST (solo la vacuna viva de ST). (3) ST + PRO (vacuna viva de ST + probiótico aditivo de alimento). El probiótico aditivo de alimento usado es un probiótico recientemente introducido de multi-cepas que contiene dos cepas de *Bacillus subtilis* y una cepa de *Bacillus amyloliquefaciens.* El probiótico fue incluido en todas las dietas a una tasa de inclusión de 453 gr por tonelada de alimento, iniciando al día de edad. La prueba fue conducida a los 39 días de edad. Para evaluar los efectos aditivos potenciales o sinergias, todos los animales tratados fueron desafiados individualmente con *Salmonella* Heidelberg resistente al ácido nalidíxico (Nal^R SH) por sonda oral a los tres días de edad. La invasividad de la vacuna viva de ST fue determinada al cultivar el hígado/ bazo de un número representativo de aves de cada tratamiento antes del desafío con la Nal^R SH. Los efectos aditivos potenciales y sinergias del probiótico con la vacuna viva de ST fe medido por la prevalencia o enumeraciones de Nal^R SH de hisopos cloacales al día 26 de edad y de todo el ciego al día 39 de edad. Los resultados de la prueba no indican una interferencia de la vacuna viva de ST por el probiótico.

SUMMARY

Increased use of feed additive probiotics in the North American poultry industry has resulted in concern among poultry production professionals regarding the compatibility of these products with the administration of live *Salmonella* Typhimurium (ST) vaccines. The concern centers on the possible interference of immunity conferred by the ST vaccine due to the competitive exclusion effects of probiotics. Additional interest has surfaced regarding additive effects or synergies that may exist between probiotics and live ST vaccines. A broiler floor-pen study was conducted to assess possible interactions of a multi-strain feed additive probiotic and the day-of-age administration of a commonly-used live ST vaccine. Three treatments were investigated. (1) CON (no live ST vaccine or probiotic). (2) ST (live ST vaccine only). (3) ST+PRO (live ST vaccine + feed additive probiotic). The feed additive probiotic used is a recently-introduced multi-strain probiotic containing two *Bacillus subtilis* strains and one *Bacillus amyloliquefaciens* strain. The probiotic was included in all diets at an inclusion rate of 1lb. per ton feed, starting at 1 day-of-age. The trial was concluded at 39 days-of-age. To assess potential additive or synergistic effects, all treatment animals were individually challenged with 3.0 x 10^7 nalidixic acid resistant *Salmonella* Heidelberg (Nal^R SH) by oral gavage at 3 days-of-age. The invasiveness of the live ST vaccine was determined by culturing the liver/spleen of a representative number of birds from each treatment immediately prior to the Nal^R SH challenge. Potential additive or synergistic effects of the probiotic with the

live ST vaccine were measured by the prevalence or enumerations of Nal^R SH from cloacal swabs at 26 days-of-age and whole ceca at 39 days-of-age.

RESULTS

Results are shown in Table 1 below.

DISCUSSION

Results of this trial indicate no evidence of interference of the live ST vaccine by the probiotic.

A higher prevalence of *Salmonella* spp. of liver/spleen samples indicates invasiveness by the live ST vaccination. Boot-sock samples of bedding material at 26 days resulted in a 100% infection environmental rate for *Salmonella* spp. All samples were positive for the bacteria for all treatment groups. These positive environmental samples indicate colonization and cohort spread of the 3 day-of-age Nal^R SH challenge. The ST+PRO treatment showed a reduction in Nal^R SH cecal enumerations as well as a numerical improvement in animal performance in regard to mortality adjusted feed conversion rates and average body weight gains compared to the other treatment groups at 39 days-of-age. These data indicate possible synergistic effects of the live ST vaccine with the use of a feed additive Bacillus-based probiotic. Additional research needs to be conducted to better define the mode of action of each of these interventions to determine if the effects are synergistic or additive.

Table 1. Results.

	CON	**ST**	**ST+PRO**
Prevalence of *Salmonella* spp. liver/spleen cultures – 3 days (%)	**0**	**75**	**100**
Mortality Adj. FCR – 39 days	**1.47**	**1.50**	**1.37**
Avg. Body Wt. Gain (lbs.) – 39 days	**4.09**	**4.75**	**4.73**
% Prevalence Nal^R Salmonella -39 days	**70**	**50**	**60**
Mean MPN enumeration 39-day cecae	**0.85**	**0.05**	**-0.01**

THE RELIABILITY OF DIFFERENT HISTOLOGIC FEATURES IN THE EVALUATION OF THE INTESTINAL HEALTH

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RESUMEN

La evaluación de la salud intestinal en aves de corral requiere estimar cambios celulares en el intestino. La histología es una buena herramienta en la evaluación de la salud intestinal. Sin embargo, es subjetiva dando resultados erróneos especialmente en aves sanas. Este trabajo evaluó un sistema objetivo para categorizar lesiones histológicas comparando la salud intestinal de aves sanas sometidas a distintos manejos. Se establecieron tres criterios a evaluar: razón vellosidad / cripta, índice vellosidad e índice intestinal basado en características celulares histológicas. Los resultados del calculo de estos criterios fueron usados como estimados de performance intestinal. Pavos en distintos grupos sometidos a diferentes manejos fueron usados para evaluar el sistema. Adicionalmente se utilizaron grupos de pollas de reposición de postura con diferente peso corporal. Los resultados fueron comparados con resultados productivos y clínicos. Este sistema es una potencial herramienta para evaluar salud intestinal en aves sometidas a distintas vacunas y/o tratamientos. Su validación esta pendiente.

SUMMARY

Evaluation of gut health requires paying attention to cellular changes within the intestine. Histologic examination is a good tool to evaluate gut health. However, it can be subjective and may give inaccurate results especially in the case of healthy birds. In this work, we develop an objective histologic lesion scoring system that can assess the intestinal performance, even in healthy birds. The challenge is the degree of the sensitivity of the scoring system in comparing the intestinal health of different healthy groups of birds subjected to different management systems. The developed scoring system established three standards for the evaluation; villous: crypt ration, villous index and intestinal index relying on multiple histologic cellular features. The resultant numerical scores can help with assessing intestinal performance. A trial of healthy turkey groups, subjected to different management systems, were used to validate the developed histological scoring system. Additionally, we used the system with different groups of layer pullets that displayed varying body weights. The resultant scoring data was correlated with the clinical results to determine the reliability of this histologic scoring system. This histologic system can be a potential tool to assess the intestinal health in birds treated with different vaccines and drugs. Further validation is pending.

MATERIALS AND METHODS

Trials. Intestinal histologic sections from two trials were used to validate this histologic scoring scheme. The first trial section came from 1-and 4-week-old turkeys. Different groups were normal healthy birds that were subjected to different lighting and feed systems. The second trial sections came from five layer pullet houses at 2-,3- and 4-week-old birds that had variable body weights and sizes. From each house, intestinal sections were collected from 5 large birds and 5 small birds and were sent fixed in formalin.

Histologic Scoring System. The scoring system used three standards for evaluation; Villous: Crypt Ratio which evaluates intestinal villi shortening and crypt hyperplasia; Villous Index that considers the villous thickening in addition to the Villous: Crypt ratio; and Intestinal Index that considers other histologic inflammatory features. This scheme produced numerical scores that enabled comparison of intestinal health between groups in each trial. The resultant scoring data were correlated with clinical data including body weights.

RESULTS

The resultant data of the scoring system showed a reliable correlation with clinical data (Body weight) with some exceptions. Some of the scoring data did not show statistically significant differences between small and large birds. Further validation is pending.

Significance. This histologic system can be a potential tool to assess the intestinal health in birds treated with different vaccines and drugs. It will

support establishing the experimental bird model that will save time and effort by establishing different endpoints.

THE SCIENTIFIC BASIS FOR THE CAGE-FREE EGG MOVEMENT

LAS BASES CIENTIFICAS PARA EL MOVIMIENTO DE HUEVO LIBRE DE JAULAS

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RESUMEN

Los pollos son aves inquisitivas, sociales, activas, con características complejas cognitivas y de comportamiento. Décadas de estudios publicados en la literatura de etología han mostrado que, al igual que otras especies domésticas, los pollos retienen gran parte de su comportamiento básico de sus ancestros silvestres, los gallos selváticos de la India y el Sureste de Asia. Convencionalmente, los sistemas intensivos de alojamiento no fueron diseñados para mostrar su comportamiento natural, y esto reduce el bienestar de la gallina en la producción de huevos.

SUMMARY

Chickens are inquisitive, social, active birds, with complex cognitive and behavioral characteristics. Decades of published studies in the ethology literature have shown that, like other domesticated species, chickens largely retain the basic behavioral biology of their wild ancestors, the Junglefowl of India and Southeast Asia. Conventional, intensive housing systems were not designed to accommodate natural behavior, and this reduces the welfare of hens in egg production.

Chickens spend approximately half of their waking time budget in foraging-related behavior (3, 18). They ground scratch and ground peck to find edible vegetation, grains, insects and grubs, and to explore their environment. Chickens exhibit "contra-freeloading", foraging for feed on the ground even when it is freely available in a feeder (6), suggesting that not only the feed, but the appetitive, food-gathering behavior itself is rewarding.

Egg-laying in a protected, hidden nesting site has been a key behavior ensuring survival of the species in the wild. The motivation of a hen to leave the flock and seek out a secluded nesting location in which to lay her clutch of eggs is strong and highly conserved. It is controlled hormonally by a pulse in progesterone levels associated with ovulation, triggering nesting behavior approximately 24 hours later (29). Domesticated hens show the same tendency to seek a darkened, enclosed nesting site, and in behavioral assessment trials, hens will push through heavily weighted doors to access a nest box. In fact, 20 minutes prior to oviposition, hens will push with more force for access to a nest box than they will push for feed, after several hours of feed deprivation (2, 7).

Roosting in trees is also an anti-predator behavior, another highly conserved trait with motivation that persists even in the commercial laying environment. In behavioral assessments, hens prefer the highest perches for resting at night (19, 22). The foot of a hen is anatomically adapted to close around a tree-branch. Multiple studies have shown that perching increases bone strength (5, 12, 17). Free-range hens have greater cortical bone thickness than those reared in conventional cages (21), demonstrating that natural behavior, movement and exercise improve skeletal health.

Light, heat and the presence of a dusty substrate trigger dustbathing, and the behavior is socially facilitated (4). Dustbathing in certain substrates, such as kaolin clay, can aid in preventing lice and mite infestation (14), and working a dusty substrate through the plumage balances lipid levels (16, 25). However, even featherless strains of chickens will dustbathe (26), therefore it is thought to be motivated by positive affect, i.e. that it is a pleasurable activity (28).

Even in the commercial production environment, the need to express species-typical natural behavior remains strong. Until recently, the importance of accommodating behavioral needs was underappreciated in some veterinary, and animal science programs, and in consideration of hen housing

designs. A more encompassing view of animal health includes the behavioral well-being of the animals. The World Organization for Animal Health (OIE) recognizes that "An animal experiences good welfare if the animal is healthy, comfortable, well nourished, safe, is not suffering from unpleasant states such as pain, fear and distress, and is able to express behaviours that are important for its physical and mental state" (30). There is increasing recognition that animal welfare is not only about minimizing negative experiences but also providing the animals "with opportunities to have positive experiences" such as "comfort, pleasure, interest, confidence and a sense of control" (15).

While a benefit of conventional cage housing is to separate hens from their manure, reducing the likelihood of parasitic and bacterial disease, the cost of such systems is suppressing nearly all natural behavior. Society perceives cage confinement negatively, and this intuitive conclusion is well supported by the animal behavior research. The egg industry is experiencing a modernization of hen housing to address the growing scientific and social concern, and this is the basis of the global, cage-free egg movement.

Throughout Europe, battery cages have been banned by law (Directive 1999/74/EC), and nearly half of all hens are now reared in cage-free facilities. In Germany, The Netherlands and Sweden, over 80% of hens are kept cage-free (8), and Switzerland banned battery cages in 1992 (13).

In the United States, seven states have enacted legislation to ban or restrict the use of cages for commercial egg production, and over 200 major brands have public commitments to purchase only cage-free eggs by 2025 or sooner. The projected supply needed to meet all of the demand is over 50 billion eggs annually (20).

The movement is international, with major egg buyers extending their policies and seeking additional cage-free eggs to meet their corporate social responsibility commitments. For example, in 2016 Sodexo, a food service company with operations in 80 countries, announced that it would source only cage-free, for both its liquid and shell eggs. Sodexo uses approximately a quarter of a billion shell eggs worldwide on an annual basis. More recently in India, the High Court of Delhi banned establishment of any new battery cages facilities, a decree that affects the entire nation.

Cage-free housing systems have higher welfare potential, but the management of hen health in these open systems is more complex. Pullets must be reared in specialized facilities to develop the proper musculoskeletal growth and spatial navigation skills to prepare them to jump between levels in an aviary system (9, 24, 1, 17), and they must be given access to loose litter or other substrate to prevent the development and spread of abnormal feather pecking behavior (23). Perches and ramps must be carefully placed to prevent keel bone fractures and other injuries (10). Litter depth and moisture levels must be monitored and controlled to reduce air contamination and prevent the proliferation of pathogenic microorganisms. Cage-free hens may require more vaccinations, and strict biosecurity. In addition to careful management of the housing environment, hen genetics must also be suited to cage-free production.

Early adopters are reporting success, and there is much to learn from countries that have been producing cage-free eggs in aviary systems for over 20 years. Kaufmann-Bart and Hoop (2009) report that "Vaccination and hygiene were the most effective precautions against infections, and control strategies brought about a marked decline in notifiable diseases, especially for Salmonella Enteritidis. Fifteen years after the ban on battery cages in Switzerland, the health and egg production of laying hens is good" (13).

A survey published in 2015 of 47 aviary flocks in Belgium reported that mortality in aviaries has now declined to a level comparable to cage systems (4.1% at 60 weeks of age) and concluded that the "…comparatively low mean cumulative mortality found in the present study most likely has multi-factorial causes, such as better disease control, adjusted rearing conditions, reduced feather pecking and cannibalism, improved feeds and improved management by farmers due to increasing experience with the system" (11).

The veterinary community will increasingly be called upon to help address health challenges and reduce mortality in cage-free systems as the trend continues and an increasing number of hens are kept in cage-free systems.

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OVERVIEW ON RESPIRATORY DISEASES AFFECTING POULTRY, WITH EMPHASIS ON MULTIFACTORIAL UPPER RESPIRATORY DISEASES AND THEIR OUTCOMES

VISIÓN GENERAL DE LAS ENFERMEDADES RESPIRATORIAS QUE AFECTAN A LA AVICULTURA, CON ÉNFASIS EN LAS ENFERMEDADES MULTIFACTORIALES RESPIRATORIAS SUPERIORES Y SUS RESULTADOS

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RESUMEN

Las enfermedades que afectan los sistemas respiratorios y digestivos en la avicultura comercial (pollos y pavos) son las enfermedades más comunes que se ven en la avicultura. Las enfermedades de estos dos sistemas pueden contar cerca del 70 a 80% de todas las enfermedades avícolas o casos diagnosticados en el laboratorio. Las enfermedades del sistema respiratorio en general no solo son complejas, pero también son los factores económicamente más importantes en el campo donde los pollos, pavos y patos son criados intensivamente

OVERVIEW

Diseases affecting the respiratory and digestive systems in commercial poultry (chickens and turkeys) are some of the most common diseases seen in poultry. Diseases of these two systems may account for nearly 70 % to 80 % of all the poultry diseases or cases diagnosed in a diagnostic laboratory. Diseases of the respiratory system in general, are not only complex, but also the most economically important factors in the field where chickens, turkeys and ducks are raised intensively.

The diseases of the respiratory system are greatly influenced by the environment in which the poultry are raised as well as various management factors. These include flock and farm density; housing systems including indoor or outdoor systems; antibiotic free or organically raised; season; geographic region; vaccinations for breeders, layers, broilers and turkeys; lack of quality feed and/or water; chilling; overheating; poor litter quality and ventilation; ammonia and other gases. Others factors such as breeder age and health; egg handling; hatchery and brooder quality; quality of chicks and poults; contaminated equipment and people; transportation; cleaning and disinfection; length of down time; wild birds; synergistic mixed infections and disease interactions; emerging and reemerging diseases and agents; immunosuppression; and importantly biosecurity also influence the occurrence and severity of respiratory diseases.

Regardless of whatever management practices are used, genetics and nutrition also play a significant role in the initiation and outcome of a disease. Therefore, it is important that one take into consideration careful evaluation of detailed history of the clinical problems and management factors and provide accurate and prompt results on various tests done such as serology, fluorescent antibody (FA), bacteriology, mycology, parasitology, virus isolation, biotechnology (PCR and sequencing), toxicology, nutritional analysis, histopathology, immunohistochemistry and electron microscopy. Other tests such as hematology, cytology and serum chemistry should also be considered, but are not practical and are not used commonly in poultry diagnostic medicine.

Causes of the respiratory disease in commercial poultry are multifactorial and include viruses, bacteria, fungi, parasites, neoplasia, nutrition, toxicities and metabolic diseases. In the field it is very rare that chickens or turkeys are affected by one single etiology, such as, a virus or bacterium or parasite or fungus*, etc*., but a combination of various disease agents. For example, colibacillosis in broilers or pullets can be secondary to Infectious Bronchitis virus (IBV) and often with Infectious Bursal Disease virus. It is not unusual to find multiple infectious agents such as IBV, *Aviabacterium paragallinarum*, *Ornithobacterium rhinotracheale*(ORT), *E. coli* and/or MG and MS as causes of respiratory disease in pullets, layers and broiler chickens. In one outbreak of LPAI H7N3 outbreak in turkeys, respiratory signs and increased mortality in a flock was attributed to NDV, HEV, *E.*

coli, ORT, *B. avium*, *Riemerella anatipestifer* and *Pseudomonas* spp.

The outcomes of the respiratory diseases in general are poor weight gain, decreased egg production, poor egg quality (external and internal), decreased livability, increased morbidity and mortality, increased condemnations at the processing plant and zoonotic diseases such as salmonella, chlamydia, flu, etc. In reportable diseases such as virulent Newcastle disease or highly pathogenic avian influenza the consequences are depopulation of entire flocks, loss of genetic stock, increased price for consumers and loss of international trade.

Below is a list of various etiologic agents/diseases that can cause respiratory disease in chickens and turkeys:

Viruses: Infectious bronchitis – Coronavirus, Avan Paramyxovirus -1 and Newcastle disease virus (END), Avan Paramyxoviruses- 2 and 3, Avian Influenza – Orthomyxovirus, Infectious Laryngotracheitis (ILT) – Gallid herpersvirus 1, Swollen Head Syndrome/Turkey Rhinotracheitis – Avina Metapneumovirus, Fowl Pox – Poxvirrus, Adenovirus (Group II in turkeys), Reovirus, Marek's Disease – Herpersvirus (lymphoma in lungs), *, etc,* and Retrovirus (J virus)

Bacteria: *E.* coli (colibacillosis), Mycoplasma *gallisepticum,* Mycoplasma *synoviae,* Mycoplasma *meleagridis* (turkeys), Pasteurella *multocida,* Ornithobacterium *rhinotracheale,* Avibacterium *paragallinarum,* Bordetella *avium* (*B. hinzii* occasionally), Staphylococcus *aureus* (turkey poults), Riemerella *anatipestifer* (ducks and turkeys), Salmonella spp. (*S.* Pullorum/ *S.* Gallinarum), Chlamydia *psittaci* (turkeys, chickens and ducks), Streptococcus spp., Pseudomonas *aeruginosa,* and Gallibacterium *anatis*

Fungi: Aspergillosis (*A. fumigatus, A. flavus*) and Ochroconis (Previously Dactylaria)

Parasites: Cryptosporidiosis Leucocytozoon spp.

Neoplasia: Lymphoma (Marek's and retroviruses), Metastatic adenocarcinoma from ovary, etc*.,* Hemangioma/sarcoma, Sarcomas, adenomas, and others

Nutritional: Vitamin A deficency

Toxic: Ammonia, CO_2, CO, dust, Teflon (polytetrafluoroethylene - PTFE), Cotton defoliants, Ionophores (degeneration of tracheal muscles), and Formaldehyde,

Metabolic/genetics: Pulmonary hypertension syndrome (ascites) and Dilated cardiomyopathy

Others: Hypersensitivity (anaphylactic shock due to vaccine), Faulty and spray vaccination, and Others.

Briefly the anatomy of the respiratory system in poultry consists of external nares, nasal passages (turbinates and sinuses), choana (palatine cleft), larynx, syrinx, bronchi (primary,), lungs which are covered by pleura and contains secondary and tertiary or parabronchi (with atria and air capillaries) and eight air sacs. Other organs that are either directly or indirectly connected with the respiratory system include pharynx leading to the eustachian tubes and middle ears, conjunctiva including *membrana nictitans* (3rd eyelid), gland of Harder, lacrimal gland and nasal or salt gland. Most of organs are lined by cuboidal or ciliated columnar epithelium and contain lamina propria and goblet cells. Gland of Harder located behind the eye and it is an important gland containing plasma cells that secretes antibody (IgA) which along with the mucociliary system in the nasal cavity, trachea and bronchi including BALT, CALT provides a major defense mechanism to the respiratory system. A few cartilaginous nodules in the lungs of chickens are considered normal.

Clinical signs due to respiratory diseases range from nasal and ocular discharge, gasping or open mouth breathing, wheezing, snick with various mortalities in a flock. Rarely the birds may not have any clinical signs and die acutely such as in Bird flu and Newcastle disease. Similarly, gross and histopathologic or microscopic lesions due to respiratory diseases range from catarrhal to fibrinous to lymphoplasmacytic airsacculitis, pleuritis, pneumonia, sinusitis/rhinitis, laryngitis, tracheitis, and conjunctivitis. Granulomatous inflammation is more common in lungs and air sacs due to bacterial and fungal infections. Collapse or flattening of the trachea is common in turkeys and rare in chickens due to *Bordetella avium*.

It is important that a thorough necropsy and testing of representative 6 to 10 live and dead birds should be performed in order to provide accurate and timely results and diagnoses to the clients. A tentative diagnosis of diseases of the respiratory system can be made based on history, clinical signs and gross and histopathology. Confirmatory diagnosis can be made based on virus, bacterial and fungal isolation, serology, fluorescent antibody test, immunohistochemistry, polymerase chain reaction (PCR), and sequencing, such as in IBV.

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OUTBREAKS OF LOW PATHOGENIC AVIAN INFLUENZA H7N3 IN TURKEYS IN CALIFORNIA

BROTES DE INFLUENZA AVIAR DE BAJA PATOGENICIDAD H7N3 EN PAVOS EN CALIFORNIA

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RESUMEN

La influenza aviar (IA) es una enfermedad viral de varias especies de aves que puede causar signos respiratorios, baja en la producción de huevo y mortalidad variable en pavos, pollos, codornices, etc., dependiendo de la patogenicidad del virus, baja (IABP) o alta patogenicidad (IAAP). Se presentó un brote de IA durante septiembre del 2018 en pavos de 15 semanas de edad en una parvada de 7000 en el valle central de California. Los signos clínicos incluían signos respiratorios con senos paranasales inflamados y mortalidad incrementada. La patología de seis pavos reveló conjuntivitis, sinusitis, traqueítis y neumonía. El PCR de hisopos orofaríngeos y la inmunohistoquímica fue positiva para IA. El virus de IA fue secuenciado y se encontró que era IABP H7N3. Varias semanas después se presentó otro brote similar en pavos de 10 semanas de edad y se detectó por vigilancia la enfermedad en otras dos locaciones. En el 2015 se dio otro brote de IABP H7N3 en pavos. La información de la vigilancia, epidemiología, virología y el desecho de las aves será presentado y discutido.

SUMMARY

Avian influenza (AI) is a viral disease of various species of birds that can cause respiratory signs, drop in egg production and variable mortality in turkeys, chickens, quail, etc., depending on the pathogenicity of the virus, low (LPAI) or highly pathogenic (HPAI) virus. An outbreak of AI occurred during September 2018 in 15-week-old turkeys in a flock of 7000 in central valley of California. Clinical signs included respiratory signs with swollen sinuses and increased mortality. Pathology of six turkeys revealed conjunctivitis, sinusitis, tracheitis, and pneumonia. PCR of oropharyngeal swab and immunohistochemistry were positive for AI. AI virus was sequenced and found to be LPAI H7N3. Several weeks later another similar outbreak occurred in 10-week-old turkeys and the disease was detected in two other ranches by surveillance. In 2015 there was also an outbreak of LPAI H7N3 in turkeys. Information on surveillance, epidemiology, virology and disposal of birds will be presented and discussed.

AN OUTBREAK OF INFECTIOUS LARYNGOTRACHEITIS IN A FLOCK OF PEAFOWL, BUT CHICKENS COMINGLED-WITH PEAFOWL WERE NOT AFFECTED

UN BROTE DE LARINGOTRAQUEITIS INFECCIOSA EN UNA PARVADA DE PAVO REALES, PERO LOS POLLOS CON LOS QUE COHABITABAN NO FUERON AFECTADOS

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RESUMEN

La laringotraqueitis infecciosa (LTI)es una enfermedad respiratoria primaria de los pollos de todas las edades y rara vez en faisanes, pavo reales y pavos. Esta enfermedad por el Herpesvirus Gallid I y se caracteriza por signos respiratorios y conjuntivitis, la laringitis y traqueítis necro-hemorrágica es asociada con sincitios conteniendo cuerpos de inclusión intranucleares.

Un brote de LTI se presentó en cuatro pavos reales de ocho semanas de edad en una parvada de 30 pavos reales. Los signos clínicos incluían problemas respiratorios y lagrimeo y la patología de las cuatro aves reveló conjuntivitis, sinusitis, y laringitis así como traqueítis con sincitios y cuerpos de inclusión intranucleares. El PCR de los hisopos traqueales y la inmunohistoquímica fueron positivos a LTI. El virus de LTI fue aislado y secuenciado de 996 bp del gen ICP4 revelando secuencias similares a otros virus aislados de seis pollos de traspatio. Setenta y cinco pollos de aves de caza criados con los pavo reales nunca se enfermaron. Los pavo reales ni los otros pollos criados en cohabitación nunca fueron vacunados contra LTI.

SUMMARY

Infectious laryngotracheitis (ILT) is a respiratory disease primarily of chickens of all ages and rarely of pheasants, peafowl and turkeys. The disease is caused by Gallid Herpesvirus I and it is characterized by respiratory signs and conjunctivitis, necrohemorrhagic laryngitis and tracheitis associated with syncytia containing intranuclear inclusion bodies.

An outbreak of ILT occurred in four to eight-week-old peafowl in a flock of 30 peafowl. Clinical signs included respiratory signs and lacrimation and pathology of four chicks revealed conjunctivitis, sinusitis and laryngitis and tracheitis with syncytia and intranuclear inclusion bodies. PCR of tracheal swabs and immunohistochemistry were positive for ILT. ILT virus was isolated and sequencing of 996 bp of the ICP4 gene revealed sequences similar to other viruses isolated from six backyard chickens. Seventy-five game chickens raised with peafowl were never sick. Neither the peafowl nor the chickens raised co-mingled had ever been vaccinated for ILT.

VACCINATION MONITORING OF RECOMBINANT HVT VECTORED VACCINES FOR NDV USING A NDV-F ELISA

MONITOREO DE LA VACUNACIÓN CON PRODUCTOS RECOMBINANTES DE VECTORIZADOS EN HVP PARA EL VEN USANDO UN ELISA VEN-F

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RESUMEN

La enfermedad de Newcastle (EN) continúa siendo una considerable amenaza económica en la industria avícola mundial. La vacunación contra el virus de la EN (VEN) junto con una buena bioseguridad son importantes para una exitosa prevención y control de la enfermedad. Hay muchas vacunas apropiadas contra la EN para su uso en la avicultura comercial. La generación más nueva de vacunas contra EN incluyen el uso del Herpesvirus de Pavo (HVP) para expresar el gen de fusión (F) del VEN en vacunas recombinantes (r)HVP-F. Por lo tanto, cuando se vacuna con estos productos, la inmunidad depende en la replicación del virus del HVP y consecuentemente en la expresión del gen F del VEN. Serológicamente el monitorear los anticuerpos contra la EN con frecuencia aplica para confirmar el éxito de la vacunación. Con el ensayo inmunoabsorbente ligado a enzimas (ELISA) clásico para VEN, es ampliamente sabido que hay una sensibilidad temporal baja a las vacunas rHVP-F. Para un mejor monitoreo de la vacuna contra EN cuando se utilizan la vacuna rHVP-F, BioChek ha desarrollado un kit de prueba de ELISA de Anticuerpo de Proteína de Enfermedad de Newcastle-Fusión para medir la cantidad de anticuerpos inducido por la vacuna rHVP-F. Las muestras colectadas en varios momentos después de la vacunación de pollos libres de patógenos específicos (SPF) y pollos de engorda con diferentes vacunas r-HVP-EN, los anticuerpos fueron detectados iniciando a la semana 1 de edad en los pollos SPF y al inicio de la semana 2 de edad en los pollos de engorda, los cuales eran positivos a anticuerpos maternos, usando la prueba de ELISA VEN-F. Además, la prueba de ELISA VEN-F fue capaz de detectar los anticuerpos de 7-14 días antes que la prueba clásica de ELISA para el VEN. Además, cuando se usó la prueba de ELISA VEN-F, tanto los títulos como el porcentaje de aves positivas mostraron ser mayores después de la vacunación en comparación con la prueba de ELISA para VEN.

SUMMARY

Newcastle disease (ND) remains a considerable economic threat on the world poultry industry. Vaccination against ND virus (NDV) along with good biosecurity are important to successfully prevent and control the disease. There are many ND vaccines suitable for use in commercial poultry. The newest generation of ND vaccines includes using Turkey Herpesvirus (HVT) to express the fusion (F) gene of NDV, recombinant (r)HVT-F vaccines. Thus, when vaccinating with these vaccines, immunity depends on the replication of the HVT virus and consequently on the expression of the F gene of NDV. Serologically monitoring of antibodies against ND is often applied to confirm the success of vaccination. With the current classical NDV Enzyme-linked immunosorbent assays (ELISA), it is widely known that there is a low temporal sensitivity to the rHVT-F vaccines. To better monitor the ND vaccine application when rHVT-F vaccines are utilized, BioChek has developed a Newcastle Disease-Fusion Protein Antibody ELISA test kit to measure the amount of rHVT-F vaccine induced antibodies. In samples collected at various times after vaccination of specific-pathogen-free (SPF) chickens and broilers with different r-HVT-ND vaccines, antibodies were detected starting at 1 week of age in SPF chickens and starting at 2 weeks of age in broilers, which were maternal antibody positive, using the NDV-F ELISA. Furthermore, the NDV-F ELISA was capable of detecting antibodies 7-14 days earlier than the classical NDV ELISA. In addition, when using the NDV-F ELISA, both titers and percentage of positive birds were shown to be greater after vaccination in comparison with the NDV ELISA.

CASE REPORT: RUNTING AND STUNTING SYNDROME IN A FLOCK OF COMMERCIAL BROWN LAYERS

REPORTE DE CASO: SINDROME DE MALA ABSORCION EN UNA PARVADA COMERCIAL DE PONEDORAS MARRON

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RESUMEN

El Síndrome de Mala Absorción ha sido descrito en ponedoras comerciales marrón y es caracterizado por un severo retraso, mortalidad aumentada, inmunosupresión, y enteritis cística severa. Se evaluó una parvada de ponedoras comerciales marrón desde los 3 hasta los 10 días de edad que fueron alojadas en una caseta con historial de retraso temprano. Se condujeron observaciones diarias y diagnósticos incluyendo histopatología, aislamiento viral, y un PCR tanto en tejidos intestinales como linfáticos. Se condujo un EM en tejidos intestinales cada día junto con una evaluación con microscopio de luz de raspados intestinales. Todos los virus aislados después fueron caracterizados por similitud. Fue notado un patrón de patógenos similares, y estos serán usados para desafiar aves jóvenes en estudios posteriores.

SUMMARY

Runting and Stunting Syndrome has been described in commercial brown laying chickens and characterized by severe stunting, increased mortality, immunosuppression, and severe cystic enteritis. A flock of commercial brown layers was evaluated from three until 10 days of age and housed in a building with a history of early stunting. Daily observations and diagnostics were conducted including histopathology, virus isolation, and PCR of both intestinal and lymphatic tissues. EM was conducted on intestinal tissues each day along with light microscope evaluation of intestinal scrapings. All viruses isolated were further characterized for similarity.

A pattern of similar pathogens was noted, and these will be used to challenge young birds in future studies.

INTRODUCTION

Runting and Stunting Syndrome (RSS) has been described in commercial brown laying chickens and characterized by severe stunting, increased mortality, immunosuppression, and depressed egg production. Histologic examination of intestinal lymphoid tissues has suggested severe cystic enteritis and lymphoid depletion of possible viral etiology. This study examined brown layer chickens housed in a commercial aviary housing system with a history of RSS in previous placements of brown layers.

MATERIALS AND METHODS

Pullets of a commercial brown layer strain from a single breeder flock and hatch date, hatched by a commercial pullet hatchery were observed. All pullets were vaccinated with a commercial coccidiosis vaccine at one day of age. They also received routine day-of-age vaccinations of AE/FP/PP, HVT-ILT, IBD, Rispens, and SB1. On each day, from three days of age through 10 days of age, four "stunted" birds and four "normal-sized" (non-stunted) birds were randomly selected for observation and necropsy. The following observations and samples were collected:

Body weights

Intestine, thymus, and bursa for histopathology – fixed tissues from each group (stunted and normal-size) were evaluated for intestinal villi length, intestinal crypt depth, thymus and bursal lymphoid depletion, and general pathology.

Intestine, thymus, and bursa for virus isolation/PCR – PCR and virus isolation were conducted on fresh tissues from each group to determine the presence of rotavirus, astrovirus, coronavirus, and reovirus. All reoviruses identified were characterized for genotype

Wet saline scraping analysis on mid gut and ceca – light microscopic observations were recorded for wet saline scrapings of the midgut and ceca, and included intestinal epithelium integrity, presence of coccidia, presence of other protozoa, presence of motile rod and coccoid bacteria, and other miscellaneous findings.

Electron microscopy – EM was conducted on intestinal, thymus, and bursal tissues.

RESULTS

During the 10-day observation period, the flock continued to eat and drink normally. There was no evidence that the size differences were related to feeding or drinking behavior. Body weight data revealed that the stunted birds weighed 32% less than the normal birds at day 3, and this variance increased daily until day 10 when it reached 51%.

The thymus and bursa were small in the stunted birds compared to the non-stunted birds from day three to day 10. Histopathology revealed thymus and bursal lymphocytic depletion, almost exclusively in the stunted birds from day three to day 10 and peaking at day nine. Intestinal lesions were minimal to moderate and included shortening of the villi and crypt hyperplasia, which trended higher in the stunted birds. The villus-to-crypt ratio trended lower in the stunted birds compared to non-stunted birds. Cystic crypts in the duodenum were observed at day four in the stunted birds, but not in the non-stunted birds. Mild, sporadic dysbacteriosis was observed in the stunted birds. Mild cycling of *Eimeria* spp. was observed beginning at day seven in both groups.

Wet saline intestinal scrapings revealed increasing intestinal villus damage beginning at day seven in the stunted birds while intestinal integrity remained normal in the non-stunted birds. Coccidia vaccine oocysts appeared to cycle well beginning at five days in the non-stunted birds while little cycling was observed in the stunted birds. No other protozoa were present in either group. Both groups demonstrated motile rod and coccoid bacteria beginning at day five. Observations for LSFO's and other bacteria were unremarkable.

No rotavirus or coronavirus were detected on PCR, however all birds in both groups were positive for astrovirus isolation from day three until day 10. Also, reovirus was isolated from both groups on days nine and 10. The reoviruses are all from Genotype 3 and are all identical to each other.

Electron microscopy detected a coronavirus in the group of stunted birds at day six.

DISCUSSION

Lesions are consistent with stress to primary immune organs and to the intestinal mucosa, with the effects trending more severe in the stunted birds. This is consistent with gross findings and mucosal scrapings. The stunting was not associated with the cycling of the coccidiosis vaccine, as the growth of *Eimeria* spp. was much more prominent in the non-stunted birds. The abnormal villi in the stunted birds may not support *Eimeria* spp. growth to the same degree as the normal tissue. All birds were positive for astrovirus and a Genotype 3 reovirus was isolated from birds at days nine and 10.

Follow-up studies will infect day-old brown layer pullets with a combination of the two isolated viruses in an attempt to repeat the described lesions.

LATEST NEWCASTLE DISEASE OUTBREAKS IN POULTRY, DIAGNOSTICS, SURVEILLANCE, AND LESSONS LEARNED

ÚLTIMOS BROTES DE LA ENFERMEDAD DE NEWCASTLE EN LA AVICULTURA, DIAGNÓSTICO, VIGILANCIA, LECCIONES APRENDIDAS

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RESUMEN

El virus virulento de la enfermedad de Newcastle (vVEN) amenaza a la avicultura mundial y causa infecciones severas y con frecuencia mortales en aves susceptibles. La nomenclatura del virus ha cambiado en los años recientes y es importante el definir claramente al virus. La enfermedad de Newcastle (EN) es definida por la Organización Mundial de Salud Animal (OIE) como un paramixovirus tipo 1 (APMV-1) que tiene un índice de patogenicidad intracerebral (ICPI) de 0.7 o mayor en pollos de un día de edad o tiene múltiples bases amino en el término C de la proteína F2 (posición 113-116) y fenilalanina en el término N (posición 117) de la proteína F1 en aves. Cuando la EN es detectada en aves, el país está obligado a reportar la infección.

La enfermedad de Newcastle virulenta es endémica en muchos países en las Américas, África y Asia, permanece siendo una amenaza de introducción a otros países debido a que es altamente infecciosa y transmisible.

El control del vVEN continúa siendo dificil en el campo, a pesar de la disponibilidad de vacunas efectivas. Como con muchas enfermedades, pueden ser efectivas en reducir los signos clínicos de la enfermedad, pero actualmente se han visto inefectivas por si solas para erradicar al virus de un país. La naturaleza altamente infecciosa y transmisible del virus y la infección de aves de traspatio, donde frecuentemente faltan la vacunación y la bioseguridad, se ha probado como un nido de la infección que ha complicado el esfuerzo del control. Es importante invertir en el desarrollo de la investigación de mejores herramientas para la detección y prevención de la enfermedad y tratar de erradicar este patógeno.

INTRODUCTION

Virulent Newcastle disease virus (vNDV) is a threat to poultry worldwide and causes a severe and often fatal infection in naïve birds. The nomenclature for the virus has changed in recent years and it is important to clearly define the virus. Newcastle disease (ND) as defined by the World Organization for Animal Health (OIE) is an avian paramyxovirus type 1 (APMV-1) virus that has an intracerebral pathogenicity index (ICPI) of 0.7 or greater in day old chickens or has multiple basic amino at the C terminus of the F2 protein (position 113-116) and phenylalanine at the N terminus (position 117) of the F1 protein in poultry. When ND is detected in poultry, a country is obligated to immediately report the infection. It is important to understand that the definition of poultry is defined, and some backyard birds, including chickens, may not meet the definition of poultry. Additionally, a virus that meets the definition of Newcastle Disease that was isolated from wild birds is not immediately notifiable. It is recommended by O.I.E. that Newcastle disease virus in birds other than poultry should not result in the ban on trade of poultry (1).

The virus that causes Newcastle disease must be differentiated from other closely related viruses that are not pathogenic and are also avian paramyxovirus type 1. Low virulent avian paramyxoviruses often co-circulate with the virulent viruses, and are often used as live vaccines (LaSota and B1 vaccines). Because APMV-1 exists as a spectrum of disease from apathogenic to highly virulent, 5 different pathotypes have been defined. This is apathogenic, lentogenic, mesogenic, neurotropic velogenic and viscerotropic velogenic, from least to most virulent. In general, the apathogenic and lentogenic viruses are not considered to be reportable NDV. Some mesogenic and most neurotropic velogenic and viscerotropic velogenic meet the definition of reportable NDV because they have an ICPI above 0.7 and/or have a cleavage compatible with virulent NDV (2).

To add to the confusion, the International Committee on the Taxonomy of Viruses (ICTV), the official organization for naming viruses has renamed the avian paramyxovirus genus and species to be avian avulavirus 1. The ICTV does not attempt to

differentiate the virulent from non-virulent viruses. The ICTV 2018 list of avulavirus (avian paramyxoviruses) serotypes have now defined 19 different species (serotypes) of virus, most having been found in wild birds <https://talk.ictvonline.org/taxonomy/p/taxonomy-history?taxnode_id=20181590>. For purposes of this review, Newcastle disease viruses that meet the O.I.E. definition of reportable will be described as virulent Newcastle disease virus (vNDV).

Virulent Newcastle disease is endemic in many countries in the Americas, Africa, and Asia, and it remains a threat for introduction in all other countries because the virus is highly infectious and transmissible. Most vNDV are found in chickens and other poultry species, but two exceptions are known where the virus is endemic in wild birds, pigeons and cormorants. The pigeon adapted viruses, which meet the OIE reportable definition of NDV, are commonly found in both wild and domestic pigeons and is highly virulent in these species (7). The pigeon adapted virus is also commonly referred to as pigeon paramyxovirus, but genetically it is closely related to chicken adapted vNDV. The cormorant virus has become endemic in North America in cormorant populations in Mexico, the United States and Canada and is associated with periodic mortality events (4). Both the pigeon and cormorant viruses have the potential for infection of other avian species, but the viruses do appear to be highly adapted to pigeons and cormorants respectively, and rarely are they implicated with infections in poultry.

Recent outbreak in California. Many countries have worked to remain free of vNDV in poultry, but they have to vigilant to identify and hopefully rapidly respond to outbreaks if they occur in their country. A recent outbreak of vNDV was identified in May 2018 in southern California in the same area that vNDV was found and eradicated in 2002-2003. The outbreak in California has continued until at least February of 2019. The virus has been almost exclusively found in backyard poultry in California, but recently the virus has been detected in commercial layer farms in southern California and in one epidemiologically linked backyard flock in Utah. This outbreak is similar to the 2002-2003 vNDV outbreak because most cases are in backyard flocks in urban areas. This has required the contacting of tens of thousands of home owners to find who has non-commercial poultry and testing large numbers of birds. Currently infected birds have been found on over 300 premises. Infected birds have been found when owners have reported sick or dead poultry or by routine surveillance of areas with known cases. Owners are compensated for birds that need to be euthanized to control the outbreak and they must agree to remain without birds for an extended period of time.

Diagnostic testing. In the United States the primary diagnostic tool for vNDV is with real-time RT-PCR. The National Animal Health Laboratory Network (NAHLN) has become well established with approved laboratories in most U.S. states. Each laboratory has to be approved for individual diseases, which requires passing a proficiency panel testing on a yearly basis that is administered by the USDA's National Veterinary Services Laboratories (NVSL). The Newcastle disease test is typically performed by screening with the matrix test that is designed to identify all Class II avian paramyxoviruses. If the matrix test is positive, then reflexively the samples are also tested with the fusion test that targets the fusion cleavage site and can differentiate virulent and non-virulent NDV (11). After the initial detection of virus in California and the subsequent virus isolation and sequencing, it was determined that the California 2018 virus was detected with high sensitivity with the virulent fusion test, and it was decided that the fusion test could be used for surveillance and confirmation in California. Samples are still periodically sent for virus isolation and sequencing to detect if viral variants are present that might affect routine testing.

Sequence analysis. The full genome sequence was determined within a few days after virus isolation, and the California 2018 (CA18) was determined to be a Class II genotype Vb virus that was most closely related to viruses from Central America and also related to the California 2002 (CA02) virus. However, the genetic distance of the CA18 with the other sequences available in GenBank were not close enough to provide a definitive source of the CA18 virus. Unfortunately, there has been relatively few viral sequences deposited in GenBank from Mexico or Central American countries in the last 10 years, and the closest match was to a Honduras virus from 2007. The few Mexican viral sequences available were also genotype V, but from a different sublineage, Vc. Until more information becomes available, the source of the CA18 outbreak can't be determined accurately, and although related to the CA02 virus, molecular clock analysis supports that the CA02 and CA18 outbreaks are not directly related.

Pathogenesis of the California 2018 virus. Viral characterization of the California 2018 virus was compared with the California 2002 virus and a Belize 2008 virus, all three were Class II genotype Vb viruses. All three viruses were used to inoculate chickens at a low, medium, and high doses in three week old and adult chickens. Not surprisingly all three viruses were extremely virulent, killing all the birds in the medium and high doses in four to six days. The higher challenge dose killed birds on average a day earlier than the medium dose. The low challenge dose either did not infect the birds or only infected some of

the birds, which resulted in the infected birds eventually infected the originally non-infected birds. Naïve contact controls were also added to cages two days after the original challenge, and if the directly infected birds became infected they were able to infect all the contact control birds. This demonstrated that all three viruses were highly transmissible and all infected birds shed large amounts of virus in oropharyngeal and cloacal swabs. The clinical disease for all three viruses were similar with conjunctivitis seen in most birds. Often the birds would become lethargic and their condition would quickly deteriorate. Many birds were euthanized because of inability to walk or difficulty in standing. Some birds also had neurologic lesions. Gross lesions often included pinpoint hemorrhages in the proventriculus as well as other organs and enlargement of cecal tonsils. Other lesions likely related to dehydration were also observed. Microscopic lesions were found in many organs including the brain. Overall, all three viruses were highly virulent and caused many of the classic lesions associated with vNDV.

Control efforts. Vaccination is commonly practiced in countries with large commercial poultry industries. Only a few countries, like Sweden, have tried to prevent infection without the use of live vaccines. Three types of vaccines are commonly used, including live attenuated vaccine virus, inactivated adjuvanted vaccines, and more recently viral vectored vaccines. Each vaccine has advantages and disadvantages, but before that is discussed antigenic variation has to be considered. Avian paramyxovirus 1 viruses, both virulent and non-virulent are all considered a single serotype, and vaccines made from any virus in the group experimentally will protect (if antibody titers are high enough) any other virus of the same serotype. However, it is widely understood that there is a large genetic differences in avian paramyxoviruses. We can phylogenetically separate APMV-1 viruses into two genetic groups or Classes, I and II. Again viruses from one Class will protect against viruses in the class, but antigenic differences can be detected and challenge studies showed improved protection (decreased virus shedding) occur when matched vaccines are used compared to mismatched vaccines (9). Almost all vNDV are in the Class II group, and within this group there is extensive viral variation referred to as viral genotypes. Currently there are at least 18 defined viral genotypes, each 10% or more different at the nucleotide level from other genotypes. Again matching genotype to challenge strain appears to provide better protection compared to a heterologous challenge (5). Currently most vaccine seed strains are genotype 1 or 2, while endemic vNDV are other genotypes. So although vaccines, at least experimentally, are efficacious, in the field were exposure to vNDV is high, vaccinated flocks can be infected and may show some clinical disease and drops in egg production. Matching vaccines to field strains is recommended.

Live attenuated vaccines. It was recognized as early as the 1940s that attenuated APMV-1 viruses could be used as vaccines to protect against vNDV. These viruses were naturally occurring and did not require additional attenuation in the laboratory. These live vaccines can be extremely valuable tools in the immunization of poultry because they mimic the natural route of exposure and stimulate a humoral, cell mediated, and mucosal immune response. When given to chickens with no previous exposure to the vaccine or maternal antibody, a protective immune response can be elicited in as little as seven days (3). However, since most commercial poultry have maternal antibody or some previous exposure to vaccine or field virus, the immune response is less than in naïve animals. Maternal antibody remains the biggest confounding factor in getting reliable protection from Newcastle disease vaccination. The other confounding factor is the vaccine reaction that may occur after vaccination. Using a live APMV-1 vaccine always presents a risk of a vaccine reaction because the virus is replicating in the respiratory tract. The severity of the vaccine reaction is often related to co-infection (mycoplasma, infectious bronchitis, etc.), poor environmental conditions, or administration method (particle size for aerosolized vaccine). Because the vaccine reaction can affect both livability and weight gain, it currently remains a balance of when and how to vaccinate to have the least negative effects. One critical determinant is whether vaccination is occurring in a country with endemic infection with high likelihood of flock exposure or countries that are normally free of vNDV but still vaccinate. For countries like the United States, which are normally free of vNDV, the vaccination programs are designed to limit the risk of adverse reactions by using less aggressive vaccines like B1 or C2. These vaccines are less aggressive than LaSota vaccines, but they also stimulate less of an immune response, and often vaccination of commercial broilers may not provide acceptable levels of protection when challenge does occur (8). In countries with high exposure, they are more likely to use more aggressive vaccines and more often. Some countries are still using mesogenic vaccines because of the exposure risk and the willingness to accept the reactions related to the vaccines.

Killed adjuvanted vaccines. Killed adjuvanted vaccines are used to provide a strong humoral immune response that persists longer than the live vaccines antibody response. These vaccines are also safer, but the major detractor is that they have to be administered

by injection. Some vaccination of killed vaccines may occur in the hatchery because administration can be more streamlined, but the immune response is compromised by the presence of maternal antibody and the immaturity of the chick's immune response. The combination of live vaccines early and killed vaccines two or three weeks later, a prime –boost response, remains a viable approach to establishing a good immune response, but the expense of handling individual birds, particular broilers, makes it too costly for many poultry operations.

Live-vectored vaccines. Live vectored vaccines, using fowlpox or herpesvirus of turkeys, to express Newcastle disease virus antigens has become an increasingly useful tool for vNDV control. Both vectors can be administered *in ovo* or at day of hatch, which allows cost effective administration, and both vectors are generally not affected by maternal antibody because they spread cell to cell. These vaccines will establish a persistent infection that continues to produce NDV antigen and can stimulate an immune response. Although vectored vaccines can't completely overcome the vaccine suppression of maternal antibodies, they can eventually induce a protective immune response(6). This delay from vaccination and protection is likely three to four weeks (10). The other advantage of the vectored vaccines is that they can provide both a cellular and humoral immune response and can be used as the prime response in a prime boost strategy. The vectored vaccines in general don't provide a strong humoral immune response, and because only the NDV fusion gene is expressed, antibody tests other than the hemagglutination inhibition test must be used.

CONCLUSION

The control of vNDV continues to be difficult in the field, despite the availability of effective vaccines. As with many diseases, vaccines can be effective at reducing clinical disease, but currently has been ineffective by itself in eradicating virus from a country. The highly infectious and transmissible nature of the virus and the infection of backyard poultry where vaccination and biosecurity are often lacking, has provided a nidus of infection that has complicated control efforts. In the United States, the recent vNDV outbreak is being contained through aggressive surveillance and testing programs, but the urban nature of the outbreak has to this point delayed complete eradication. It is important to invest in research to develop better tools for the detection and prevention of disease to try to eradicate this pathogen.

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DETERMINATION OF METABOLIZABLE ENERGY AND AMINO ACID APPARENT ILEAL DIGESTIBILITY OF THE JUMBO SQUID MEAL (*DOSIDICUS GIGAS*) AND ITS USAGE IN BROILER DIETS

DETERMINACIÓN DE LA ENERGÍA METABOLIZABLE Y AMINOÁCIDOS EN LA DIGESTIBILIDAD APARENTE ILEAL DE LA HARINA DE CALAMAR GIGANTE (*DOSIDICUS GIGAS*) Y SU USO EN DIETAS DE POLLO DE ENGORDA

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RESUMEN

Los objetivos de estos estudios fueron el determinar, primero, el contenido de energía metabolizable (AMEn) y de aminoácidos en la digestibilidad aparente ileal (DAI) de la harina de calamar gigante (HCG), y segundo, sus efectos sobre el desempeño del pollo de engorda (peso corporal, consumo de alimento y relación alimento a ganancia), rendimiento en canal, color amarillo de la piel en vivo y sabor de la carne cuando se usa en un 16 y 20% de sustitución parcial de la pasta de soya. Para esto, fueron usados noventa y seis pollos de engorda Ross 308 de un día de edad por 21 días y, subsecuentemente, setenta y dos pollos de engorda por seis semanas para alcanzar el primero y segundo objetivo respectivamente. El AMEn fue de 3376.15 Kcal/ Kg y la DAI de aminoácidos fue en promedio del 73.7%. la inclusión al 20% de HCG no tuvo efecto sobre las mediciones del objetivo secundario. Se concluye que la HCG puede ser usada en las dietas avícolas en ciclos de seis semanas.

ABSTRACT

The objectives of these studies were to determine, first, the metabolizable energy content (AMEn) and amino acids apparent ileal digestibility (AID) of jumbo squid meal (JSM), and second, its effect on broiler performance (body weight gain, feed consumption and feed: gain ratio), carcass yield, in vivo skin yellowness and meat flavor when use it in 16 and 20% of soybean meal partial substitution. To this, ninety-six one-day-old mixed Ross 308 broiler chickens were used for 21 days and, subsequently, seventy-two broiler chickens for six weeks to achieve the first and second objectives respectively. The AMEn was 3376.15 kcal/kg and the amino acid AID average was 73.7%. The 20% JSQ inclusion had no effect over the second objective measures. It is concluded that JSM can be used in poultry diets in a six-week cycle.

INTRODUCTION

In Mexico, 5.54 kg of every 10 kg of available protein to feed population are provided for poultry products. Given that, feed costs are at least the 60% of total productions costs, find alternative non-conventional ingredients to satisfy the protein requirements of birds without giving quality and flock performance up, are needed. That is the reason why this study suggests the JSM usage as a protein source in soybean meal partial substitution.

MATERIALS AND METHODS

JSM was elaborated from entire squids (mantle, pen, tentacles, and viscera) trapped in Baja California Sur, Mexico. Crude protein (CP), true protein, non-protein nitrogen, and phosphorus were determined to it.

Bioassay 1. Ninety-six, one-day-old Ross 308 mixed broiler chicks were divided in a completely randomized design into three treatments with four replicates of eight birds each one (four males and four females). They were housed in stacked electric bird cages, under temperature conditions and nutritional requirements suggested for the strain (3, 4). Three diets were formulated in a sorghum + soybean meal basis, partially replacing the last one for 8 and 16% of JSM; Titanium dioxide (TiO2) was included as the indigestible dietary marker. Feed and water were provided ad libitum for the 21 days that the experiment lasts. EMAn was determined by excreta collection from 19 to 21 days and, it was calculated according to the Leeson and Summers (7) procedure. At the end of the trial, chickens were slaughtered and, digesta samples from the ileum were collected in order to determine its amino acid profile, and to calculate AID coefficients according to Lemme *et al*. (8) procedure. Analysis of TiO2 marker was made in excreta, ileal digesta, and diets.

Bioassay 2. Seventy-two, one-day-old Ross 308 mixed broiler chicks were divided in a completely randomized design into three treatments with four replicates of six birds each one (three males and three females). They were housed in stacked electric bird cages, under temperature conditions and nutritional requirements suggested for the strain (3, 4). Three diets were formulated in a corn + soybean meal basis, partially replacing the last one, with 16 and 20% of JSM employing the results obtained in the bioassay 1. The growth performance parameters were measured at 10, 24 and 42 chick's days old and, at the end of the essay, in vivo skin yellowness in the right apterilic rib area, carcass yield and meat flavor were also measured.

RESULTS AND DISCUSSION

The AMEn value of the JSM was 3376.15 kcal/kg (Table 1), it is 6.6% higher than the one reported for Remigio (2006) who used JSM made of only viscera. In comparison with another ingredient (1) JSM was 11 and 44% higher than a fish (3037 kcal/kg y 60.3% PC) or a soybean meal (2346 kcal/kg y 48% CP) respectively, but similar to a sorghum (3263 kcal/kg) or a yellow corn (3340 kcal/kg).

On the other hand, the JSM essential amino acids profile (except tryptophan and phenylalanine which were lower) compared with those of the soybean meal (47% CP) (1), the first ones were in higher amounts. However, the digestibility of the JSM ones (excluding tryptophan), was 12.6% lower than the soybean meal ones (5).

In the second essay, there was no significant differences ($p<0.05$) between treatments neither for productive parameters nor carcass yield. The 16% of JQM inclusion had lower skin yellowness than the control treatment. In this regard, many authors have reported different results about the JSM pigment content, however, the skin yellowness obtained values were according to the normal ranges (18-20 b* *in vivo*, (9) accepted by the Mexican market. Non-unpleasant flavors were detected on the broiler meat ($p>0.05$).

It is concluded that either 16 and 20% JSM inclusions can partially replace soybean meal in diets for broiler chickens through all the live phases (starter, grower, finisher) without impairing growth performance, carcass yield, *in vivo* skin yellowness and meat flavor.

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Table 1. Chemical composition, amino acid profile and AID coefficient of jumbo squid meal (*Dosidicus gigas*).

	g/100g	
Moisture	6.11	
Crude protein (Nx6.25)	73.5	
True protein	55.14	
Non-protein nitrogen	18.36	
Total phosphorus	1.32	
Available phosphorus	1.19	
Ash	9.65	
Total energy (kcal/kg)	4875.00	
AMEn (kcal/kg)	3376.15	
Amino acid	Total g aa/100g	Digestibility coefficient (%)
Methionine	1.423	89.6
Methionine + Cysteine	2.015	69.7
Lysine	3.872	80.4
Threonine	2.736	74.5
Tryptophan	0.400	ND
Arginine	4.312	83.2
Isoleucine	2.398	77.3
Leucine	4.503	77.3
Valine	2.862	77.8
Histidine	1.581	24.8
Phenylalanine	2.246	78.9

ND= Not determined

Table 2. Growth performance of a 6-weeks cycle in broiler chickens.

	Control diet	Diet + 16% JSM	Diet + 20% JSM
Body weight gain (g/bird)[1]	1865 ± 53.10	1792 ± 53.10	1663 ± 53.10
Feed consumption (g/bird)[1]	3362 ± 71.63	3240 ± 71.62	3121 ± 71.62
Feed: gain ratio (kg:kg)[1]	1.807 ± 0.041	1.809 ± 0.041	1.878 ± 0.041
Carcass yield (%)[2]	73.49 ± 0.44	72.88 ± 0.47	72.08 ± 0.42
Skin yellowness b*[2]	22.35 ± 0.83[a]	19.42 ± 0.83[b]	20.58 ± 0.80[ab]

[1] Mean ± Standard Error of the Mean; n=12 (ANOVA One-way).
[2] Mean ± Standard Error of the Mean; n=72 (ANOVA Two-way).
[a, b] Values within a column with different superscript letter differ $p<0.05$ (Tukey-HSD).

VIRULENT NEWCASTLE DISEASE IN SOUTHERN CALIFORNIA, 2018-2019

ENFERMEDAD DE NEWCASTLE VIRULENTA EN CALIFORNIA, 2018-2019

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RESUMEN

La enfermedad de Newcastle virulenta (vND) es una enfermedad viral contagiosa y casi siempre fatal que afecta el sistema respiratorio, nervioso y digestivo de pollos y otras aves, causada por cepas altamente virulentas del paramyxovirus aviar tipo 1. Los signos clínicos incluyen muerte súbita, aumento de la mortalidad, depresión, dificultad respiratoria, descarga nasal, diarrea, disturbios neurológicos, e hinchazón alrededor de los ojos y el cuello. En aves no vacunadas la letalidad es casi del 100%, pero la enfermedad puede también causar muerte en aves vacunadas. Un diagnóstico de vND fue confirmado en California el 17 de mayo del 2018. Desde entonces, y hasta el 30 de enero del 2019 se han confirmado 362 casos of vND en California, principalmente en aves de exhibición de traspatio, pero también en 4 ranchos comerciales. Un caso de vND fue además detectado en Utah. Este es el primer brote de vND en los Estados Unidos desde el 2003.

ABSTRACT

Virulent Newcastle disease (vND) is a contagious and almost always fatal viral disease affecting the respiratory, nervous and digestive systems of poultry and other birds, caused by highly virulent strains of avian paramyxovirus type 1. Clinical signs include sudden death, increased mortality in the flock, depression, respiratory difficulty, nasal discharge, diarrhea, neurological disturbances, and/or swelling around the eyes and neck. A death rate of almost 100 percent can occur in unvaccinated poultry flocks, but vND can also infect and cause death in vaccinated poultry. A diagnosis of vND in California was confirmed on May 17, 2018. Since then, and as of January 30, 2019, 362 cases of vND have been confirmed in California, primarily in backyard exhibition birds, but also in 4 commercial flocks. One case of vND was also detected in Utah. This is the first outbreak of vND in the U.S. since 2003.

INTRODUCTION

On May 16 2018, 2 dead chickens from a small flock of backyard exhibition chickens in Los Angeles County, California, were submitted to the California Animal Health and Food Safety Laboratory, with a clinical history of high mortality in a period of a week. Gross lesions included conjunctivitis, edema of the head and neck, cutaneous hemorrhages of the head, hemorrhages of pharynx, proventriculus, trachea, intestine and cloaca, and cecal tonsil necrosis. Real-time PCR of oropharyngeal swabs collected from these birds was positive for virulent Newcastle disease (vND) virus. This presumptive diagnosis was confirmed by the United States Department of Agriculture's Animal and Plant Health Inspection Service on May 17, 2018.[1]

MATERIALS AND METHODS

During the current outbreak, all field cases are being tested by CAHFS with real-time PCR on oropharyngeal and/or cloacal swabs, according to the National Animal Health Laboratory Network protocols, to establish a presumptive diagnosis. This diagnosis is later confirmed by the United States Department of Agriculture's Animal and Plant Health Inspection Service. Necropsies are performed in some cases, such as when urgent preliminary results are required. Gross lesions are considered compatible or not compatible with vND. Routine surveillance via PCR on oropharyngeal swabs of all commercial poultry ranches has been performed regularly in Southern California during most of the outbreak.

RESULTS

From May 16, 2018 through January 30, 2019, 362 cases of virulent Newcastle disease (vND) have been diagnosed in California, primarily in backyard exhibition birds. These cases were diagnosed in San Bernardino, Riverside, Los Angeles and Ventura Counties. vND was also recently confirmed in a flock of backyard exhibition chickens in Spanish Fork, Utah. Between December 14, 2018 and January 31, 2019, vND was also diagnosed in four commercial chicken flocks and in a backyard/non-commercial layer flock in Riverside County, via routine surveillance testing.

Gross lesions identified so far in backyard birds submitted for necropsy, which later tested positive for vND by PCR, included conjunctival, tracheal, proventricular, intestinal and cloacal hemorrhages; and/or fibrino-necrotizing stomatitis, pharyngitis, esophagitis and laryngotracheitis, and cecal tonsil necrosis. Occasionally, no gross lesions were observed in a few cases with positive PCR results. Lesions observed in commercial chickens were much more subtle or completely absent.

DISCUSSION

vND is a highly fatal disease of birds, including domestic and wild species (1). VND has significant economic impact to the poultry industry because of the high morbidity and mortality in chickens (1).

The clinical history, gross examination, and rapid reliable molecular testing are key for the rapid diagnosis of vND.[1] In the current outbreak, a reliable PCR technique is available to confirm the diagnosis of vND, and most field diagnoses are confirmed by this technique. This has the advantage of a speedy and accurate diagnosis, in addition to reducing the number of birds that are submitted to the laboratory for necropsy, which reduces the risk of contamination and disease transmission. Gross diagnosis remains however, fundamental for early detection. This was demonstrated in the current outbreak, in which initial recognition of compatible gross lesions prompted molecular testing and confirmation of the disease within a few hours.[1] Necropsies are also essential when immediate presumptive results are required.

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Figure 1. Multifocal-to-coalescing fibrino-necrotizing stomatitis, pharyngitis, and esophagitis.

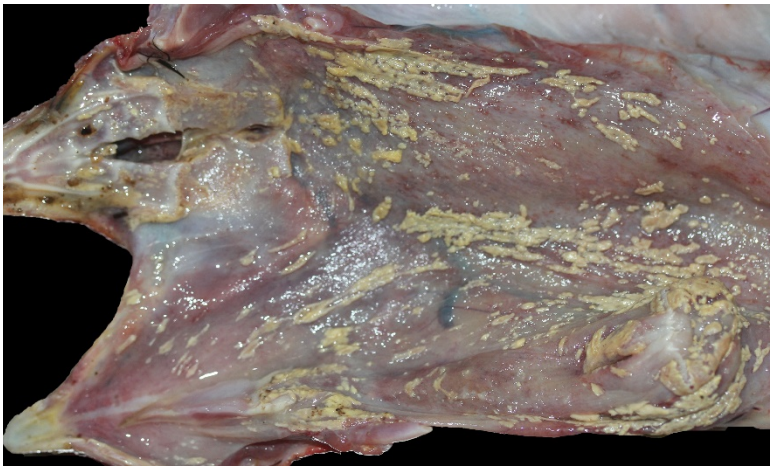
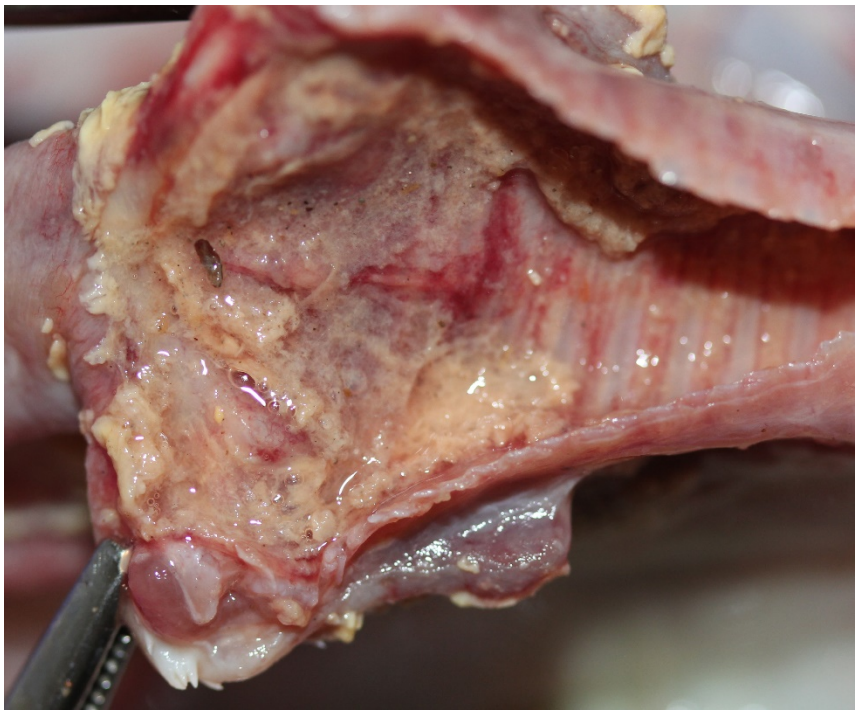


Figure 2. Diffuse fibrino-necrotizing tracheitis



ESTABILIDAD DE LAS VACUNAS EN TABLETAS EFERVESCENTES CON DIFERENTES DILUYENTES DURANTE 60 Y 120 MINUTOS

STABILITY OF EFFERVESCENT TABLET VACCINES WITH DIFFERENT DILUENTS THROUGH 60 AND 120 MINUTES

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SUMMARY

The titer was determined for three vaccines: Newcastle disease, infectious bronchitis, and infectious bursal disease, using effervescent tablet vaccines suspended in distilled water, PBS, hard water (400 ppm CaCo and 5 ppm Cl) with and without addition of protector of commercial vaccine. The dilution of the vaccines in "hard water" decreased the titration of the vaccines after 60 minutes of contact. The titration of vaccines remained higher than the guaranteed minimum titers when they were diluted in distilled water, PBS, distilled water with vaccine protector, PBS with vaccine protector and "hard water" with vaccine protector throughout 60 and 120 minutes of contact. The presentation of vaccines to active virus in effervescent tablets is stable up to 120 minutes when it is diluted in distilled water and PBS. The vaccine protector reduces the diminishing effect of the "hard water" of the titration of the vaccines when it is added during its preparation.

RESUMEN

Se determinó el título de tres vacunas de virus activo de Enfermedad de Newcastle, Bronquitis Infecciosa e Enfermedad Infecciosa Bursal en presentación de tabletas efervescentes, resuspendidas en agua destilada, PBS, agua "dura" (400 ppm CaCo y 5 ppm Cl), con y sin la adición de un protector de vacuna comercial. La dilución de las vacunas en "agua dura" disminuyó los títulos de las vacunas después de 60 minutos de contacto. Los títulos de las vacunas permanecieron superiores a los títulos mínimos garantizados cuando se diluyeron en agua destilada, PBS, agua destilada con protector de vacuna, PBS con protector de vacuna y "agua dura" con protector de vacuna durante 60 y 120 minutos de contacto. La presentación de vacunas a virus activo en tabletas efervescentes es estable hasta por 120 minutos cuando es diluida en agua destilada y PBS. El protector de vacuna reduce el efecto del agua "dura" de disminución del título de las vacunas cuando se adiciona en la preparación.

INTRODUCCIÓN

TAbic® es una tecnología patentada por Phibro para la formulación y presentación de antígenos de vacunas en tabletas efervescentes (5). El título del virus vacunal debe mantenerse durante el tiempo que dura el proceso de aplicación[1]. La calidad del agua es importante para mantener la eficacia de la vacuna (3,4). Las condiciones fisicoquímicas del agua en algunas granjas, como pH extremos o altas concentraciones de cloro y sales minerales pueden degradar a los virus vacunales y reducir la eficacia del proceso de vacunación (1,3,4). Es frecuente que se usen productos para disminuir los efectos de una calidad inadecuada de agua sobre los virus vacunales (2). Es importante verificar que dichos productos no afecten la viabilidad de las vacunas a virus activo.

En el presente estudio se determinó el título de las vacunas TAbic V.H., TAbic H120 y TAbic M.B. diluidas en agua destilada, PBS y agua dura y clorada, inmediatamente después de su preparación, a los 60 minutos y a los 120 minutos después de su preparación, sin y con la adición de un producto comercial utilizado como estabilizador de la solución vacunal.

MATERIALES Y MÉTODOS

Vacunas (5): TAbic V.H. Vacuna para la Enfermedad de Newcastle, TAbic M.B. Vacuna para la Enfermedad Infecciosa Bursal y TAbic H120 Vacuna para la Bronquitis Infecciosa.

Estabilizador de vacunas (2): Custovac D®, 1.5 g/L

Diluyentes: Agua destilada estéril, Solución Salina Amortiguada de Fosfatos (PBS), Agua dura y clorada (5 ppm de cloro en forma de hipoclorito de

sodio, 400 ppm de dureza en forma de Carbonato de Calcio., pH 9).

Diseño experimental: dilución de la vacuna en agua destilada estéril, PBS estéril, agua dura y clorada, agua destilada estéril con estabilizador de vacuna, PBS con estabilizador de vacuna y agua dura con estabilizador de vacuna.

Tiempo de contacto con el diluyente: 0 minutos, 60 minutos, 120 minutos.

Titulación de las vacunas (6): Enfermedad de Newcastle y Bronquitis Infecciosa: inoculación en embrión de pollo. Enfermedad Infecciosa Bursal: inoculación en fibroblastos de embrión de pollo.

RESULTADOS

El título mínimo garantizado de la vacuna TAbic V.H. de Enfermedad de Newcastle es de 7.52 $DIEP_{50\%}/mL^5$. El título de la vacuna diluía en agua destilada fué de 9.0 $DIEP_{50\%}$/mL al iniciar la prueba, 8.46 $DIEP_{50\%}$/mL a los 60 minutos y 8.77 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS fué de 8.52 $DIEP_{50\%}$/mL al iniciar la prueba, 7,75 $DIEP_{50\%}$/mL a los 60 minutos y 8.36 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua destilada y adicionada con el protector de vacuna fue de 9.43 $DIEP_{50\%}$/mL al iniciar la prueba, 9.02 $DIEP_{50\%}$/mL a los 60 minutos y 8.40 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS y adicionada con el protector de vacuna fue de 8.25 al iniciar la prueba, 8.3 a los 60 minutos y8.30 a los 120 minutos. El título de la vacuna diluida en agua dura fué de 7.87 $DIEP_{50\%}$/mL al iniciar la prueba, 7.69 $DIEP_{50\%}$/mL a los 60 minutos y 7,25 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua dura más el protector fuè de 8.0 $DIEP_{50\%}$/mL al iniciar la prueba, 8.07 $DIEP_{50\%}$/mL a los 60 minutos y 7.88 $DIEP_{50\%}$/mL a los 120 minutos. (Figura 1)

El título mínimo garantizado de la vacuna TAbic H120 de Bronquitis Infecciosa es de 5.0 $DIEP_{50\%}$/mL (5). El título de la vacuna diluía en agua destilada fue de 6.29 $DIEP_{50\%}$/mL al iniciar la prueba, 5.78 $DIEP_{50\%}$/mL a los 60 minutos y 5.25 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS fué de 6.20 $DIEP_{50\%}$/mL al iniciar la prueba, 5.80 $DIEP_{50\%}$/mL a los 60 minutos y 5.30 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua destilada y adicionada con el protector de vacuna fue de 6.17 $DIEP_{50\%}$/mL al iniciar la prueba, 5.47 $DIEP_{50\%}$/mL a los 60 minutos y 5.70 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS y adicionada con el protector de vacuna fue de 6.20 $DIEP_{50\%}$/mL al iniciar la prueba, 6.05 $DIEP_{50\%}$/mL a los 60 minutos y 6.05 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua dura fué de 5.95 al iniciar la prueba, 6.05 $DIEP_{50\%}$/mL a los 60 minutos y 5.70 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua dura más el protector fuè de 5.95 $DIEP_{50\%}$/mL al iniciar la prueba, 5.94 $DIEP_{50\%}$/mL a los 60 minutos y 5.53 $DIEP_{50\%}$/mL a los 120 minutos. (Figura 2).

El título mínimo garantizado de la vacuna TAbic M.B. de la Enfermedad Infecciosa Bursal es de 4.0 $DIEP_{50\%}/mL^5$. El título de la vacuna diluída en agua destilada fue de 4.35 $DIEP_{50\%}$/mL al iniciar la prueba, 5.26 $DIEP_{50\%}$/mL a los 60 minutos y 4.53 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS fué de 4.14 al iniciar la prueba, 4.75 a los 60 minutos y 4.47 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluída en agua destilada y adicionada con el protector de vacuna fue de 4.86 $DIEP_{50\%}$/mL al iniciar la prueba, 5.02 $DIEP_{50\%}$/mL a los 60 minutos y 4.60 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en PBS y adicionada con el protector de vacuna fue de 6.0 $DIEP_{50\%}$/mL al iniciar la prueba, 4.91a los 60 minutos, a los 120 minutos el título no fue determinado. El título de la vacuna diluida en agua dura fué de 4.60 $DIEP_{50\%}$/mL al iniciar la prueba, 4.04 $DIEP_{50\%}$/mL a los 60 minutos y 3.60 $DIEP_{50\%}$/mL a los 120 minutos. El título de la vacuna diluida en agua dura más el protector fué de 4.58 $DIEP_{50\%}$/mL al iniciar la prueba, 4.63 $DIEP_{50\%}$/mL a los 60 minutos y 4.50 $DIEP_{50\%}$/mL a los 120 minutos.

DISCUSIÓN

Las vacunas TAbic V.H., TAbic H120 y TAbic M.B. mantienen un título superior al título mínimo garantizado hasta por 120 minutos, cuando son diluidas en agua destilada estéril y en PBS. La dilución de la vacuna en agua clorada y dura disminuye significativamente el título de las vacunas TAbic V.H. y TAbic M.B. después de 60 minutos de contacto. El estabilizador de vacunas Custovac no afectó negativamente el título de las vacunas TAbic V.H., TAbic H120 y TAbic M.B. al ser utilizado con agua destilada ó PBS como diluyente para las vacunas. El estabilizador de vacunas Custovac mantuvo la estabilidad de las vacunas TAbic V.H., TAbic H120 y TAbic M.B. durante 120 minutos aún en presencia de agua clorada y dura, con títulos superiores a los títulos mínimos garantizados para cada vacuna.

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Figura 1. Titulación de la vacuna TAbic® V.H. con diferentes diluyentes.

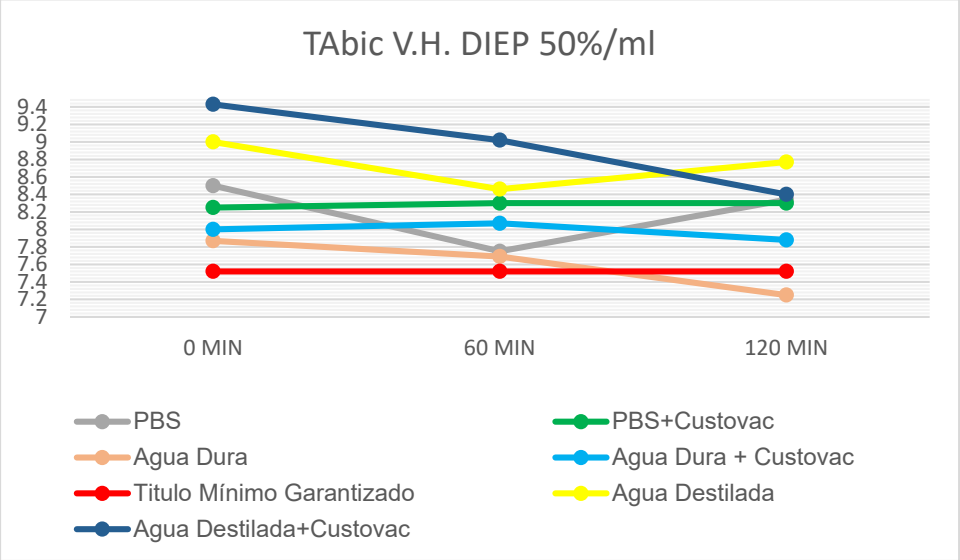
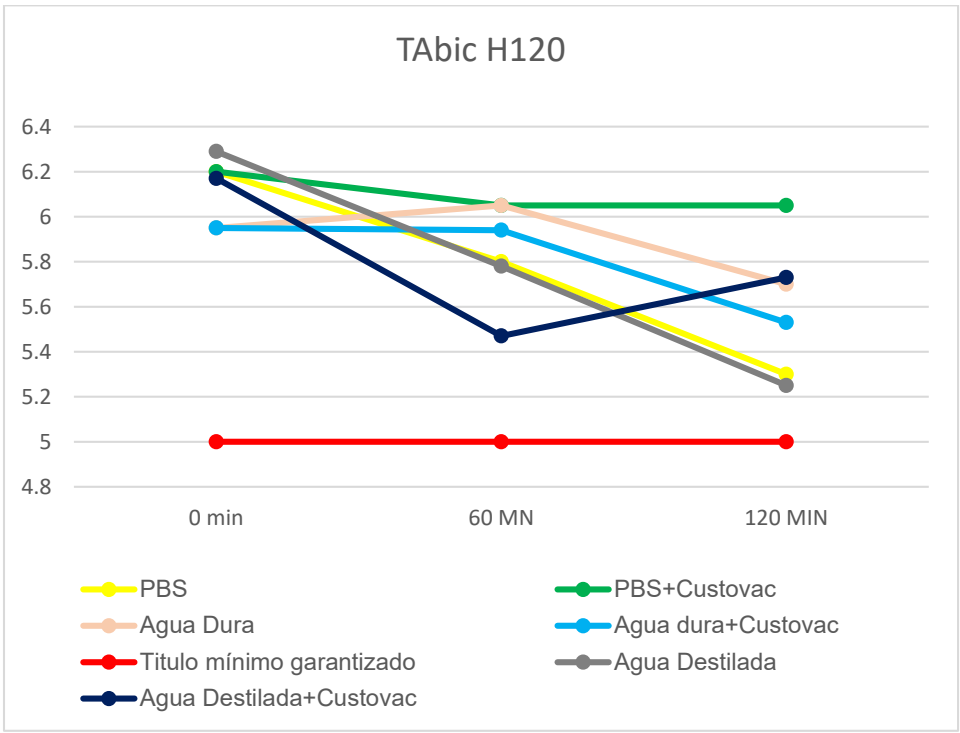


Figura 2. Titulación de la vacuna TAbic® H120. con diferentes diluyentes.



COMBINATION OF SODIUM BUTYRATE WITH ANTIBIOTIC GROWTH PROMOTERS IN BROILER CHICKENS

COMBINACIÓN DE BUTIRATO DE SODIO CON ANTBIÓTICOS PROMOTORES DE CRECIMIENTO EN POLLOS DE ENGORDA

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RESUMEN

El objetivo de este estudio de campo fue el evaluar el efecto del uso combinado de butirato de sodio protegido y antibióticos como promotores de crecimiento sobre el desempeño de pollos de engorda. Fueron usados un total de 603,000 pollos Ross 308 de un día de edad alojados en 16 casetas, y distribuidos aleatoriamente a dos tratamientos: Control (T1, n=9), dieta estándar con BMD® y Colistina; Butirato (T2, n= 7) dieta estándar + butirato de sodio protegido (Gustor® N´RGY) a una dosis de 1.5 Kg/ t, en la fase de pre-inicio y 1 Kg/ t en las fases de inicio y crecimiento. El periodo de alimentación fue de 42 días. El grupo T2 tendió (P = 0.1345) al incremento del peso corporal final (2642.43 contra 2553.56), sin embargo, no se detectaron diferencias (P> 0.05) en el consumo de alimento, la relación de la conversión alimenticia (1.60 contra 1.65), el índice de crecimiento y de productividad. Se concluye que la inclusión de butirato puede mejorar el desempeño productivo del pollo de engorda incluso si se usan antibióticos como promotores de crecimiento.

SUMMARY

The aim of this field study was to evaluate the effect of the combined use of protected sodium butyrate and antibiotics growth promoters on the performance of broiler chickens. A total of 603,000 one-day-old Ross 308 chicks housed in 16 sheds were used, randomly distributed in two treatments: Control (T1, n = 9), standard diet with BMD® and Colistin; Butyrate (T2, n = 7), standard diet + protected sodium butyrate (Gustor® N'RGY) at dose of 1.5 kg / t, in pre-starter phase and 1 kg / t in starter and growth phases. The feeding period was 42 days. The T2 tended (P = 0.1345) to increase the final body weight (2642.43 vs 2553.56), however, no differences were detected (P> 0.05) in feed intake, feed conversion ratio (1.60 vs 1.65), growth rate and productivity index. It is concluded that the inclusion of Butyrate can improve the broilers productive performance even if antibiotics are used as growth promoters.

INTRODUCTION

Enteric diseases are one of the most important problems in the poultry industry because of high economic losses due to decreased weight gain, increased mortality rates, worse feed conversion ratio, greater medication costs, and increased risk of contamination of poultry products for human consumption (3). Several pathogens including viruses, bacteria, macroparasites and other infectious and non-infectious agents are reported as possible causes of enteric diseases either alone or in synergy (3). Many conditions have been associated with gastrointestinal problems such as diarrhea, wet droppings, dysbacteriosis, intestinal colibacillosis, malabsorption syndrome, coccidiosis and NE. Enteric disorders are frequently associated with an overgrowth of *Clostridium perfringens* (4). Infections with this bacterium in poultry can cause NE, necrotic dermatitis, cholangiohepatitis, as well as gizzard erosion (4).

Antibiotics have been used as an effective tool to improve animal performance, by selectively modifying the gut microflora, decreasing bacterial fermentation, reducing thickness of the intestinal wall and suppressing bacterial catabolism (3). However, antibiotics have come under increasing scrutiny by government regulators, scientists and consumers because of the emergence of antibiotic-resistant "superbugs". European and now American countries have prohibited o limited the use of in-feed antibiotics in poultry feed (2).

As a consequence, the development of alternatives to antibiotics receives considerable attention. Among these alternatives, organic acids (acetic, propionic and butyric) are considered to be popular and suitable for in-feed use. In poultry

production, SB has been used as a gut health booster, sometimes as an unprotected salt or in the form of protected derivatives such as butyrate glycerides or butyrate-loaded matrices, because it the release location may affect the observed responses (1, 2). Dietary SB supplementation has been shown to improve growth performance and resilience of broiler chickens through distinct mechanisms: it is an agonist of free-fatty acid receptors, an inhibitor of proinflammatory pathways, an epigenetic modulating agent and acts as an energy source (2). Secondly, SB influences the microbiota residing in the avian gastrointestinal tract (GIT) as a result of its bacteriostatic properties (1, 2).

Due to the multiple biological effects, the SB can be used strategically in the programs of intestinal health for broiler chicken, in specific stages where their effects complement or substitute the AGP. Therefore, the objective of this field study was to evaluate the effect of concomitant use of protected SB, with BMD and Colistin in broilers during the first 18 days of feeding.

MATERIALS AND METHODS

This study was conducted on a commercial farm in southeastern Mexico. A total of 603,000 one-day-old Ross 308 chicks in mixed flock housed in 16 sheds were used, randomly distributed in two treatments: Control (T1, n = 9), standard diet with BMD® (22 ppm) and Colistin (20 ppm) (1 to 42 d); Butyrate (T2, n = 7), standard diet with the same AGP + protected sodium butyrate (Gustor® N'RGY) at dose of 1.5 kg / t, in pre-starter phase (1 to 7 d) and 1 kg / t in starter (8 to13 d) and growth (14 to 18 d) phases. The base ingredients of the diet were corn, soy meal, meat meal, soybean oil and bird oil, corn gluten feed. Enzymes as phytases, β-glucanases and xylanases were used as well as coccidiostats in rotation, nicarbazine-narasin and lasolasid. The sheds were equipped with automatic feeders and drinkers and controlled climate. The density was 18 birds / m^2. The feeding period was 42 days.

The data that were recorded weekly were feed intake (FI), body weight gain (BWG) and accumulated mortality (M), with these data was calculated, growth rate (GR), feed conversion ratio (FCR) and productivity index (PI). The data were analyzed in a design of measures repeated over time, using PROC MIXED of SAS 9.2 (SAS Institud Inc. Cary, NC, USA). When differences were detected, the separation of means was performed using the Tukey test. The level of significance was set at 0.05.

RESULTS

The effects of dietary SB supplementation on the growth performance of broilers are presented in Table 1. The results indicated that dietary SB supplementation had no significant effects (P>0.05) on BWG, FI, FCR, GR or PI of chicks during the overall period (1 to 42 d). However, the final body weight tended (P=0.1345) to be numerically (88.9 g) higher in T2. On the contrary, accumulated mortality was 1.4% lower (P <0.05) in T1 vs T2.

DISCUSSION

In the present study, SB addition did not influence the BWG, FI, FCR, GR or PI of broilers under commercial feeding management, although numerically, the BWG and FCR were improved with the inclusion of protected SB, which economically could be attractive. These results differ from previous reports had indicated that protected sodium butyrate mirrors the beneficial effects on growth performance of broilers in terms of increased feed intake coupled with body weight gain and significantly improved FCR even when AGP were used in the diet (5). These variable results may be attributed to the kind of protection of SB used and the type of microbial environment to which the chicks were exposed and the type, combination and dose of AGP used. It is concluded that the inclusion of protected SB could improve the broilers productive performance even if antibiotics are used as growth promoters.

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Table 2. Performance of broiler chickens from 1 to 42 days of age, fed diets supplemented or not with sodium butyrate combined with BMD and Colistin[A].

Variable[B]	T1(CONTROL)	T2 (BUTIRATO)	P-value
Final body weight (1 to 42 d), g	2553.56±38.45	2642.43±43.59	0.1345
Feed intake (1 to 42 d), g	4199.77±46.18	4198.60±52.37	0.9867
ADG, g	60.79±1.55	62.92±1.76	0.3823
Feed conversion ratio (1 to 42 d)	1.65±0.04	1.60±0.04	0.4138
Mortality, %	3.81±0.22b	4.95±0.25a	0.0025
Growth rate	2.26±0.01	2.27±0.01	0.5163
Productivity index	366.77±25.53	382.68±28.96	0.6832

[A]BMD = bacitracin methylene disalicylate.
[B]ADG = average daily gain.

EFFECT OF AN ORGANIC ACIDS BASED FEED ADDITIVE AND ENROFLOXACIN ON THE PREVALENCE OF ANTIBIOTIC-RESISTANT *E. COLI* IN CECUM OF BROILERS

EFECTO DE UN ADITIVO DE ALIMENTO A BASE DE ÁCIDOS ORGÁNICOS Y ENROFLOXACINA SOBRE LA PREVALENCIA DE *E. COLI* RESISTENTE A ANTIBIÓTICOS EN EL CIEGO DE POLLOS DE ENGORDA

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RESUMEN

El uso de antibióticos es monitoreado en algunos países para evitar la resistencia. La resistencia a las fluoroquinolonas es alta en países donde este antibiótico es usado. El objetivo de este estudio fue investigar el efecto de la adición en dietas con un aditivo a base de ácidos, así como el antibiótico fluoroquinolona, sobre la prevalencia de *E. coli* resistente a antibióticos. Se dividieron aleatoriamente cuatrocientos pollos de engorda Ross 308, en tres tratamientos: el primer grupo, control negativo, no fue suplementado con ningún aditivo; el segundo grupo, recibió un aditivo en el alimento a base de ácido fórmico, acético y propiónico (FA) y el tercer grupo recibió enrofloxacina en el agua de bebida (AB). Se colectaron muestras fecales en pollos de un día de edad. En el día 17 y 38 de la prueba, se tomaron

muestras cecales de cada uno de los 8 corrales, y se determinó el conteo de *E. coli* y la *E. coli* resistente a antibióticos. Se demostró la presencia de *E. coli* resistente a antibióticos en los pollos de engorda de un día de edad. Los pollos de engorda suplementados con FA y los tratados con AB no presentaron una influencia significativa sobre el número total de *E. coli* en el contenido cecal en los días 17 y 38 de la prueba. La suplementación con FA mejoró el desempeño en el crecimiento y significativamente bajo (P≤ 0.05) la resistencia de la *E. coli* a la ampicilina y tetraciclina comparado con el control y el grupo AB, así como bajo (P≤ 0.05) la resistencia de la *E. coli* al sulfametoxazol y ciprofloxacina comparado con el grupo AB. El tratamiento AB incrementó (P ≤ 0.05) la ganancia diaria de peso promedio en comparación con el grupo control e incrementó (P≤ 0.05) el número de *E. coli* resistente a la ciprofloxacina, estreptomicina, sulfametoxazol y tetraciclina.

ABSTRACT

Antibiotic use is monitored in some countries in order to avoid resistance. Resistance against fluoroquinolones is high in countries where this antibiotic is used. The objective of this study was to investigate the effect of feeding diets with an acids-based additive, as well as fluoroquinolone antibiotics, on the prevalence of antibiotic resistant *E. coli.* Four hundred Ross 308 broilers were randomly divided into 3 treatments: the first group, negative control, was not supplemented with any feed additive; the second group has received feed additive based on formic, acetic, and propionic acid (FA) and the third group has received enrofloxacin (AB) in water. Fecal samples of one-day old chicks were collected. On d 17 and d 38 of the trial, cecal samples from each of the eight pens were taken, and the count of *E. coli* and antibiotic-resistant *E. coli* was determined. Antibiotic-resistant *E. coli* in one-day-old chicks was shown. Broilers supplemented with FA and treated with AB did not have a significant influence on the total number of *E. coli* in the cecal content on d 17 and d 38 of the trial. FA supplementation improved growth performance and significantly decreased ($P \leq 0.05$) *E. coli* resistance to ampicillin and tetracycline compared to the control and AB groups, as well as decreased ($P \leq 0.05$) sulfamethoxazole and ciprofloxacin-resistant *E. coli* compared to the AB group. AB treatment increased ($P \leq 0.05$) the average daily weight compared to the control group and increased ($P \leq 0.05$) the number of *E. coli* resistant to ciprofloxacin, streptomycin, sulfamethoxazole and tetracycline.

INTRODUCTION

The application of antibiotics for the treatment of disease, disease prevention, and growth promotion in food-producing animals provides favorable conditions for the selection, persistence and spread of antibiotic-resistant bacteria and their resistance determinants at the farm level (2, 3, 4, 5 7; Miranda *et al.*). Thus, resistance to antibiotics has become a global concern not only in human but also in animal health. Furthermore, antibiotic-resistant (AR) bacteria and determinants generated at the farm may spread to humans through direct contact, contamination of meat, or environmental pathways (1).

To study the emergence of antibiotic resistance (AR) in gram-negative bacteria, *E. coli* are widely accepted as indicator bacteria (9). They are commensal members of the normal gastrointestinal microbiota in humans and animals, can be rapidly altered by exposure to antibiotics, according to Francino (6), and act as an important pool of resistance determinants (10). Possible contamination of poultry meat with AR *E. coli* may also occur during slaughtering. Moreover, *E. coli* is also of widespread importance, as it is a major pathogen in commercially produced poultry that contributes to significant economic losses (8). The present trial shows that FA can improve growth performance. Furthermore, the number of *E. coli* resistant to ampicillin, ciprofloxacin, sulfamethoxazole, and tetracycline was higher in the AB group compared to the FA group. Whether AB can be replaced for disease prevention and reduction of mortality with FA should be clarified with further trials.

MATERIALS AND METHODS

The animal experiment was conducted at the Center of Applied Animal Nutrition in Mank, Austria. All procedures involving animal handling and treatment were approved by the local state office "Amt der Niedero¨sterreichischen Landesregierung Abteilung Agrarrecht," which is the authority for animal care in Lower Austria. The official number of the trial approval is LF1-TVG-39/030–2016. A total of 480 mixed-sex, one-day-old broiler chickens (Ross 308) were randomly assigned to 3 treatments, with 8 pens per treatment and 20 birds per pen. All groups received a common basal diet without coccidiostats from hatch until 38 d of age. The composition of the starter and grower diets mets or exceeded the requirements of the National Research Council (1994) and is presented in Table 1. Chicks had free access to feed and water supplied through nipple drinkers.

The first group of chickens was a negative control group fed a basal diet. The feed additive (FA) group

also received the control group diet supplemented "on top" with a feed additive based on 20% formic, 10% acetic, and 5% propionic acids, as well as 2.5% cinnamaldehyde (Biotronic® Top3; BIOMIN Holding GmbH, Getzersdorf, Austria) at a dosage of 2 kg/t of feed. The antibiotic (AB) treatment group received the same diet as the control group, but 10 mg enrofloxacin per kg body weight (Baytril, 10% oral solution, Bayer, Leverkusen, Germany) was provided via drinking water from d 14 to d 16 of the trial, before the change to the grower diet.

All groups were subjected to the same rearing, environmental and sanitary conditions. Ventilation and temperature control, light intensity and day-length were applied according to the management handbook's guidelines. Wood shavings were used for bedding. Clinical observations were done twice a day, and all incidents were recorded. The study was supervised by an independent, licensed local veterinarian.

CONCLUSION

A high prevalence of AR *E. coli* in all experimental groups was observed throughout the study. Dietary supplementation with FA and treatment of broilers with AB did not have a significant influence on the total number of E. coli on d 17 and d 38 of the trial. Supple- mentation with FA contributed to better growth performance and a decrease in ampicillin- and tetracycline- resistant *E. coli* in the cecum of broilers compared to control and AB group. The decrease ($P \leq 0.05$) in sulfamethoxazole and ciprofloxacin-resistant E. coli com- pared to the AB group was observed in the FA group. Treatment of broilers with AB increased ($P \leq 0.05$) the number of *E. coli* resistant to ciprofloxacin, streptomycin, sulfamethoxazole, and tetracycline in the ce- cum. However, fewer ($P \leq 0.05$) *E. coli* were resistant to cefotaxime, and ESBL-producing E. coli was observed in the group treated with enrofloxacin.

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DIAGNOSTIC STRATEGIES IN THE ONGOING BATTLE AGAINST AVIAN INFLUENZA

ESTRATEGIAS DE DIAGNÓSTICO EN LA CONTINUA BATALLA CONTRA LA INFLUENZA AVIAR

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RESUMEN

Las pruebas para los virus de influenza se han realizado usando kits de pruebas para múltiples especies del (virus de Influenza Aviar (AI) tipo A ARN con un panel de 83 aislamientos aviares y 8 porcinos. Se pusieron a prueba cincuenta muestras derivadas de un brote de baja patogenicidad de AI H7N7 y 33 aislamientos adicionales cubriendo todos los subtipos de hemaglutinina y neuraminidasa. En resumen, el PCR de influenza A de BioChek se desempeñó significativamente (P< 0.0001) mejor que el PCR Nagy, así como el Spackman del virus de Influenza A aviar gen M. El PCR H7 de BioChek (como parte del kit múltiple) también se desempeñó mejor que el PCR H7 en casa y el desempeño equivalente fue notado para el H5 y H9 entre el juego múltiple H5/ H7/ H9 de BioChek y los ensayos en casa de H5 y H9.

SUMMARY

Testing for influenza viruses was performed using a multi-species Avian Influenza Type A RNA test kit with a panel of 83 avian and eight swine isolates. Fifty samples derived from a low pathogenicity AI H7N7 outbreak and 33 additional isolates covering all hemagglutinin and neuraminidase subtypes were tested. In summary, the BioChek Influenza A PCR performs significantly (P<0.0001) better than both the Nagy and the Spackman avian Influenza A virus M gene PCRs. The BioChek H7 PCR (as part of the multiplex kit) also performed better than the in house H7 PCR and equivalent performance was noted for H5 and H9 detection between the BioChek H5/H7/H9 multiplex kit and the in house H5 and H9 assays.

INTRODUCTION

Over the last decades, the number of avian influenza outbreaks has increased drastically. The incidence and spread seem to have become so severe that endemic infections across the world have become a reality. One of the most effective ways to identify and monitor AI viruses in poultry is by use of a continuous global surveillance program (1). Recently, BioChek has developed two new real time PCR kits, an Influenza type A and an Influenza H5/H7/H9 multiplex qPCR kit, which can be used as diagnostic tools in large-scale AI surveillance programs. Both kits have been validated against the Nagy (3) and Spackman (5) generic Influenza A detection assays and in house H5, H7 and H9 specific PCRs respectively by a UK reference laboratory.

MATERIALS AND METHODS

Samples. A total of 83 avian samples comprising a variety of subtypes and 8 swine isolates were tested. Of the samples, 50 consisted of cloacal and oropharyngeal swabs from a low pathogenicity AIV H7N7 outbreak. In addition, isolates consisting of 11 H5 strains, 6 H7 strains, 2 H9 strains, and 14 other subtypes were tested.

Molecular testing of samples. Following extraction carried out using the QIAmp viral RNA BioRobot kit as customized for the reference laboratory in conjunction with a Universal BioRobot (Qiagen, Manchester, United Kingdom (UK)) as described (4), samples were tested against the in-house assays of the reference laboratory using a commercial AI Type A RNA test kit (BioChek B.V.) and internal assays, Nagy (3) and Spackman (5) generic influenza A detection assays, as well as the 'perfect match' [PM] PCR for swine influenza. In addition, known H5, H7, and H9 strains were tested using a commercial AI H5/H7/H9 multiplex RNA test kit (BioChek B.V.) as

well as internal assays (single H5, H7, and H9 specific PCRs).

Statistical analysis. Mean Ct values were compared using analysis of variance (ANOVA) with Minitab software. P values <0.05 were considered statistically significant.

RESULTS

As illustrated in Figure 1 and Table 1, lower mean Ct values were reported with the commercial multispecies AI Type A RNA kit for both the avian and swine samples compared with the internal assays used by the reference laboratory.

Results of the comparison between the commercial AI H5/H7/H9 multiplex RNA test kit and the singleplex assays as used by the reference lab are shown in Table 2. The Ct values of the H5, H7, and H9 subtypes on the commercial AI H5/H7/H9 RNA test kit were comparable to with the internal single H5, H7, and H9 specific PCRs ($p>0.05$).

DISCUSSION

These results clearly show that the commercial multispecies AI type A RNA test kit is a very sensitive method for the detection of AI type A. Furthermore, both avian and swine samples can be screened using the kit. While comparable mean Ct values were observed for both H5 and H7 subtypes, the internal assay reported a lower mean Ct for H9, but this was based on only two available samples. A relative decrease in sensitivity has been described as one of the limitations of multiplex assays (2). Yet, the H5/H7/H9 multiplex PCR RNA assay offers an alternative test method for fast and cost-effective screening of AI subtypes.

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Figure 1. Comparison of mean Ct values on different assays for different subtypes of AI.

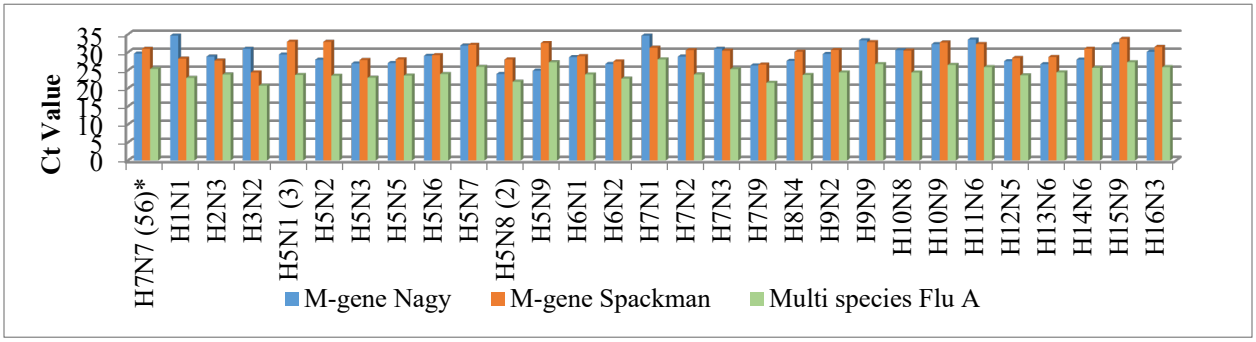

Table 1. Comparison of mean Ct values of internal assay and the commercial multispecies AI Type A RNA test for swine isolates.

Isolate	Subtype	M-gene PM	Multi species Flu A (BC)
A/Swine/England/SP15001249/2011	avH1N1/H1N2	27.00	24.90
A/swine/England/4202/2013	pH1N1	26.81	26.17
A/swine/England/33780/06	H1N2	25.33	23.21
A/swine/England/267/2007	avH1N1	30.66	28.44
A/Swine/Italy/41350-3/11	H1N2	30.20	27.58
A/Swine/England/041118/2013	H1N2	26.26	24.91
A/Swine/USA/Iowa-A01203121/2012	H3N2	27.04	27.65
A/Swine/Spain/23885/2011	H3N2	26.68	24.76

Table 2. Mean Ct values of different subtypes with the commercial AI H5/H7/H9 multiplex RNA test and internal tests.

AI Subtype	H5 (BC multi)	H5HA2	H7 (BC multi)	H7HA2	H9 (BC multi)	H9
H5N1	23.70	30.34	no ct	no ct	no ct	no ct
H5N2	23.51	27.96	no ct	no ct	no ct	no ct
H5N3	22.95	28.02	no ct	no ct	no ct	no ct
H5N5	23.54	28.56	no ct	no ct	no ct	no ct
H5N6	23.96	30.63	no ct	no ct	no ct	no ct
H5N7	25.96	31.66	no ct	no ct	no ct	no ct
H5N8	21.83	27.39	no ct	no ct	no ct	no ct
H5N9	27.23	33.97	no ct	no ct	no ct	no ct
H7N1	no ct	no ct	33.40	30.66	no ct	no ct
H7N2	no ct	no ct	27.50	27.90	no ct	no ct
H7N3	no ct	no ct	30.07	28.70	no ct	no ct
H7N7	no ct	no ct	28.98	29.78	no ct	no ct
H7N9	no ct	no ct	25.98	34.78	no ct	no ct
H9N2	no ct	no ct	no ct	no ct	33.99	29.14
H9N9	no ct	no ct	no ct	no ct	36.33	31.15

A BACKYARD POULTRY FORENSIC FILES: THE WEIRD, UNUSUAL, AND CRAZY UNTOLD CASES

ARCHIVOS FORENSES EN UNA OPERACION AVICOLA DE TRASPATIO: LO RARO, LO INUSUAL Y LOS CASOS LOCOS NO CONTADOS

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RESUMEN

Pequeñas parvadas de aves domésticas de especialidad están explotando en popularidad, impulsadas por el deseo del público de tener huevos y carne frescos. Desafortunadamente, muchos productores son nuevos en la avicultura, y no tienen el entrenamiento en la cría básica de aves. Además, la demanda del público para productos orgánicos limita los tipos de intervenciones que pueden ser usadas cuando se presentan problemas con enfermedades en sus granjas. Nosotros presentaremos cuatro casos de clientes del Servicio de Diagnóstico Aviar de la Universidad de Purdue.

INTRODUCTION

Small specialty poultry flocks are exploding in popularity driven by public desire to have local fresh eggs and meat. Unfortunately, many of the farmers are new to poultry farming, and do not have training in the basics of poultry rearing. In addition, public demands for organic products limit the types of intervention that can be used when disease issues do occur on the farms. We will be presenting four unusual cases from clients of the Purdue University Poultry Diagnostic Service.

CASE REPORTS

Case Report 1 (multidrug toxicoses). A chicken was presented to the necropsy service with a history of not eating or drinking, force feeding, skin abnormalities and subsequent death. The chicken had numerous open, weeping skin lesions and the entire body was greasy. A visit to the farm identified one moribund chicken with a large fungal mat on the larynx and 7 additional chickens with variable size fungal mats in the oral cavity. Case details including clinical exam, farm conditions, necropsy and histopathology will be presented.

Case Report 2 (suspect arsenic poisoning). Two ducks were presented to the necropsy service with a history of anorexia, feather loss, weight loss and subsequent death. The birds were cachexic, poorly feathered and had serous atrophy of fat. The owner reported that he had increased arsenic levels in his urine and suspected intentional poisoning of his ducks, geese and himself. A farm visit was requested by the Indiana Board of Animal Health. Case details including clinical exam, farm conditions, necropsy and histopathology will be presented.

Case Report 3 (lead toxicity). A family reported that their toddler children had sustained permanent neurologic damage due to lead toxicity. The State Chemists office and the family requested a farm and flock evaluation to determine if eggs were the source of the lead. Case details including clinical exam, farm conditions, and toxicology will be presented.

Case Report 4 (tiny eggs). A client requested a farm visit to evaluate 2 Polish hens that were purchased at auction 3 months previously. The hens laid eggs that measured 3 cm in length and contained no yolk. No normal eggs had been laid in the previous 3 months. During the farm visit, one hen was euthanized and a gross examimation was performed. Case details including clinical exam, farm conditions, and necropsy and will be presented.

SUMMARY

After 11 years of small farm visits conducted by the Purdue Poultry Diagnostic Medical Service, we have encountered numerous unusual and often rare cases. I have never encountered cases such as the four above in 40 years of commercial poultry practice. Pet poultry are subjected to many situations that are atypical for poultry, and remind us to continue to pursue even unusual avenues of medicine when caring for small flocks.

COMPARATIVE PERFORMANCE OF EXTRACTION TECHNIQUES WHEN USING RT PCR FOR IDENTIFICATION OF *MYCOPLASMA* AND S*ALMONELLA* DNA

DESEMPEÑO COMPARATIVO DE TÉCNICAS DE EXTRACCIÓN CUANDO SE USA EL RT PCR PARA LA IDENTIFICACIÓN DEL ADN DE *MYCOPLASMA* Y *SALMONELLA*

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RESUMEN

El estudio fue completado para comparar el método de ebullición para la extracción de perlas magnéticas de muestras aviares cuando se probaron subsecuentemente en PCR en tiempo real para la identificación de *Mycoplasma* y *Salmonella.* Los datos producidos apoyan un incremento en la sensibilidad del PCR cuando fue usada la extracción con perlas magnética, así como menor evidencia de la amplificación de la inhibición.

SUMMARY

The study was completed to compare boiling method to magnetic bead extraction of avian samples when subsequently tested on real-time PCR for *Mycoplasma* and Salmonella identification. The data produced support an increase in sensitivity of PCR when magnetic bead extraction was used as well as less evidence of amplification inhibition.

OVERVIEW

It is a common practice for laboratories to use a basic boiling method for the extraction of *Salmonella* and *Mycoplasma* DNA. Boiling disrupts the cell envelope and allows the DNA to be released. However, many inhibitors can remain in the sample, causing reduced polymerase chain reaction (PCR) efficiencies during testing, potentially resulting in false negative results. There are additional extraction options outside of boiling such as magnetic bead purification that can both disrupt the cell envelope while also removing inhibitors. Using these alternative methods will decrease the risk of false negative results.

The most typical sample type used for *Mycoplasma* PCR testing is tracheal swabs collected from poultry houses. These samples can often be contaminated with dust and blood, both of which are known inhibitors of DNA amplification. *Salmonella* environmental testing commonly used drag swabs and boot covers which are pre-enriched before lysis. Environmental samples are inherently contaminated with organic material due to the nature of the sample.

To assess the impact of extraction method on PCR results, a direct comparison was performed at the University of Pennsylvania using either a common boiling lysis method, or the IDEXX RealPCR® Magnetic Bead extraction kit. Both lysed and extracted samples were then tested with the IDEXX RealPCR *Salmonella* DNA mix and IDEXX RealPCR MS/MG multiplex DNA mix. Both DNA mixes contain an internal sample control (ISC) or an internal amplification control (IAC) for confirmation that lysis and/or extractions were successful.

When evaluating more than 100 avian samples for *Mycoplasma* DNA and 140 environmental samples for *Salmonell*a DNA we identified an increase in accurate detection of *Mycoplasma* DNA when using IDEXX RealPCR Magnetic Bead extraction over the boiling lysis method. Additionally, the magnetic bead process stream lines laboratory efficiencies for testing.

Detailed results will be shared in full during the poster session.

GUMBORO: THE SILENT DISEASE TOOLS TO DISCOVER AND FIGHT THE DISEASE

GUMBORO: LA ENFERMEDAD SILENCIOSA HERRAMIENTAS PARA DESCUBRIR Y COMBATIR LA ENFERMEDAD

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RESUMEN

En años recientes la precepción general en la industria avícola de que la enfermedad de Gumboro está bajo control ya que los casos de la forma aguda de la enfermedad con mortalidades y grandes pérdidas son más esporádicos y usualmente causado por algunas fallas o equivocaciones durante el proceso de vacunación. Sin embargo, la enfermedad de Gumboro sigue siendo un problema actualmente como una forma subclínica más comúnmente, pero esta forma de infección se caracteriza por la ausencia de signos clínicos, y en muchos casos no es reportada por los productores.

Como el Gumboro puede estar silenciosamente presente e indirectamente afectar negativamente la producción avícola, es crucial hacer la mayor diferencia con las herramientas de diagnóstico disponibles para instalar un programa de seguimiento para detectar la presencia de la cepa silvestre que podría causar inmunosupresión en nuestras aves. Por estas razones Hipra ha implementado el Programa Hipragumboro® en diferentes países de Latino-América para promover un mejor control de la enfermedad, dando seguimiento a las parvadas problemáticas e identificar los puntos débiles en los programas de control de Gumboro. El programa toma en consideración todos los aspectos críticos y puntos a ser verificados para la implementación de un buen programa de vacunación y ayudar a identificar el agente causal, usando todas las herramientas de diagnóstico actualmente disponibles.

SUMMARY

Infectious bursal disease (IBD), also known as Gumboro disease, is a highly contagious viral infection of worldwide distribution, occurring in all major poultry production areas. The incidence of the infection is high, while the severity of the disease will depend on the age and breed of the chickens, and the virulence of the virus. Signs of the disease can include a rapid drop in feed and water consumption, mucoid diarrhoea with soiled vent feathers, ruffled feathers, listless chicks with unsteady gait or sitting in hunched position, picking at own vent and sleeping with beak touching the floor.

The causal agent of Gumboro disease is the infectious bursal disease virus (IBDV), which belongs to the genus Avibirnavirus within the family Birnaviridae (3). The virus targets the developing B lymphocytes of the bursa of Fabricius and other lymphoid and non-lymphoid tissues causing the apoptosis of these cells (1, 2) with a final atrophy of the Bursa and consequent immunosuppression. Of the two known serotypes, only the viruses belonging to the serotype 1 can cause disease in the chickens (5). Traditionally the IBD viruses are classified into classical, variant and very virulent strains, taking into account the antigenic and pathogenic characteristics. At present it is demonstrated that the most precise way of classifying the IBDV strains is by phylogenetic analysis of the sequences corresponding to the hyper-variable region of VP2. Most recently, Michel and Jackwood (2017) proposed a new classification scheme of IBDV strains into genogroups, based on this analysis, including new lineages isolated in the latest years. The new classification divides the IBDV strains into 7 genogroups, so that the classic, variant and very virulent strains correspond to Genogroup 1, Genogroup 2 and Genogroup 3, respectively. The most recent lineage described as “distinct strain”, dIBDV, is clearly highly genetically divergent from the other three traditional genogroups. This lineage includes strains that have been isolated from several countries of South and North America, Asia and Europe (4) and by the new classification are included in the Genogroup 4 (6). The dIBDV strains cause a bursal atrophy with consequent immunosuppression but with absence of clinical signs (7), similar to the subclinical form of the disease caused by variant strains.

In the classical form of the disease, chickens are most susceptible to clinical disease at three to six weeks of age, while the infections before three weeks of age are usually subclinical with no detectable symptoms. At present, these early subclinical

infections are the most common and most economically important as the disease can cause a severe, long-lasting suppression of the immune system. The chickens that are immunosuppressed by an early IBD infection do not respond well to vaccination and are more susceptible to other diseases, including those that do not normally affect healthy chickens. The indirect economic impact of the disease is due to secondary infections, growth retardation and carcasses condemnation at the slaughterhouse. Moreover, the increased use in antibiotics to control the secondary infections constitutes a growing public health concern and an economic loss.

In recent years there is a general perception in the poultry industry that Gumboro disease is under control as the cases of acute forms of the disease with mortalities and big losses are more sporadic and usually caused by some failure or mistake during the vaccination process. However, Gumboro disease is still a problem now days as the subclinical form is the most common, but this form of infection is characterized by the absence of clinical signs, and in many cases it is unnoticed by the producers. These are usually worried to solve other health problems that are actually caused by the primal infection with IBD and consequent immunosuppression.

As IBD can be silently present causing and indirect economic impact in the poultry production, it is crucial to make the most of the different diagnostic tools available and to install a follow up program to detect the presence of a wild strain that could be causing immunosuppression in our birds. For these reasons in recent years Hipra has implemented the Hipragumboro® Program in different countries of Latin America to promote a better control of the disease, following up problematic flocks and identifying weak points in Gumboro control programs. The program takes into consideration all the critical aspects and points to be check for the implementation of a good vaccination program and to help to identify the causing agent, using all the diagnostic tools currently available and that in many cases are not used. The points that are important to obtain a good and continuous control of the disease are: levels of maternal derived antibodies (MDA) for IBD (that are really important for the vaccination in the farm), vaccination audits, macroscopic evaluation of the bursa (size and appearance), seroprofiles (at different stage depending on the type of birds) and molecular diagnosis. In many cases the molecular diagnostic has been the starting point in the program, as it is the only analysis that can show you exactly the IBDV strain that is circulating in the farm and that colonized the bursa of the birds.

As we show in a previous work (8), we have found that in the Latin America countries the strains most commonly present in problematic flocks are the variant ones (Fig.1), and in recent years we also start to isolate some dIBDV strains. The use of the molecular diagnosis as a double aim, as diagnostic tool in case of suspected infection but also as follow up to check that the virus implemented in the farm is the vaccine strain we are using in our program, to confirm that Gumboro is under control. However, another critical point in defining the right vaccination program, that should be regularly checked, is the level of MDA of our chicks/pullets, as there could be big differences between the flocks coming from mothers of different ages.

Before deciding the vaccination scheme it is also important to know the characteristics of the different vaccines present in the market, as changing the vaccine in use without changing the date of application (in case of live attenuated vaccines applied in the farm) could lead to a failure in the protection. The regular control of the MDA is important because any change in the vaccination program of the breeders could have an effect on the progeny that could also cause a failure in their protection. Vaccination audits should be regularly conducted, in both cases of applications in the hatchery or in the farm, with a special attention to the second one that is more critical to obtain a uniform result between different farms, as many elements are in involved.

For this reason, follow a checklist during the vaccination day can help to reduce the risk of mistakes. By conducting necropsies routinely, we have a rough but quick idea of what is happening in our farms, but most of the time we do not standardize or record the results of those necropsies. We can use this routine praxis to evaluate Gumboro disease situation in the farms, recording also with pictures the situation to have the possibility of future comparisons and anticipate possible problem. The post mortem analysis should be conducted in a significant number of birds between 28 and 35 days of age to evaluate the bursa of Fabricius condition. By visual inspection we can assess: size of the bursa and appearance of the bursa. Excluding an IBD infection, disorders in the bursa of Fabricius can also be caused, among other reasons, by: immunosuppressive conditions, too virulent vaccine strain and vaccination mistakes. In case of suspicion of bad bursa condition and for further investigation histopathology study can also be recommended.

Serology is a really important tool but if we do not keep a base line of seroprofiles as a reference we do not know the real situation in the farms. We can explain a single serologic result but we will never have an accurate interpretation or at least the whole understanding of the situation. Taking blood samples at marketing age or at specific points of the life of the birds is essential to know the situation of the flocks

and to be able to compare with the mean of previous flocks. This way we can detect seasons of high or low field virus pressure as well as vaccination failures and make the right corrections if needed.

During the latest year thanks to follow up program, especially in Mexico, we have been able to solve many cases in which the sanitary problem of some farms did not look related to the presence of IBDV and improve the zootechnical results adjusting the vaccination scheme in use and with a continuous follow up of the situation.

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Figure 1. Phylogenetic tree of field and reference IBDV strains based on partial nucleotide sequences of the VP2 gene (from nucleotide positions 620 to 1180).