PROCEEDINGS OF THE FORTY-EIGHTH WESTERN POULTRY DISEASE CONFERENCE

April 24-27, 1999. Vancouver, Canada

SPECIAL ACKNOWLEDGMENT

The Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions to the Speaker's Fund and the Conference. These contributions provide support for outstanding participants who otherwise might not be able to attend and help pay for some of the costs of this year's meeting in Vancouver, B.C., Canada. Over 50 organizations, companies and individuals have given substantial financial support, and many companies and organizations, including some that also contribute to the Speaker's Fund, send speakers at no expense to the conference. We thank all these people, and acknowledge their support and contributions.

The category of Benefactor is in recognition of extremely generous contributions or sponsorship of special events. To this group, we recognize the Merial Avian Business Unit, American Association of Avian Pathologists, and Elanco Animal Health. Once again, our distinguished Patrons, Donors, Sustaining Members, and Friends of the Conference are listed on the following pages. We greatly appreciate their generosity and say thanks to them and their representatives.

Thanks and appreciation are due to Dr. Keith McMillan, the Program-chair, for putting together an excellent program. Dr. McMillan extends a sincere "thank you" to Drs. Craig Riddell and Detlef Onderka for their help in reviewing title submissions and preparing the program, along with a special "thanks" to Julie McMillan and Denise Zubic for their secretarial support.

Many have provided special services that contribute to the continued success of this conference. We express special thanks this year to Dr. Stew Ritchie, a.k.a. Chicken Stew, who spent the past two years organizing the local arrangements in Vancouver, B.C., Canada; Ms. Nicole Gibson and the Center for Avian Biology for their secretarial support to Dr. Rosenwald; Nancy Edes and the U.C. Veterinary Extension/Public Programs for administering the contributions; Dr. Rosenwald for once again garnering the outstanding contributions; and to all others who contributed to the program and conference.

We acknowledge and appreciate Lina Layiktez and her crew at the University of California, Davis, Conference and Event Services, who handle the registration and administrative duties of this conference.

We thank Dr. Craig Riddell, once again, for producing an outstanding Proceedings, this year published in Canada. We express our gratitude to all authors who submitted manuscripts. We acknowledge and thank Printing Services, University of Saskatchewan, Saskatoon, for handling the printing of the Proceedings. We are especially thankful to Pat Riddell who provided many hours of typing and formatting the manuscripts for the Proceedings. We thank Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design.

We thank Dr. Rich Chin for his invaluable work as an overall co-ordinator and his help to everyone involved in organizing the conference.

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The editor apologizes to any sponsors left off this list. After March 16th, 1999, no additional information could be included in this proceedings. Additional donors will be listed in the meeting program.

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The Proceedings of the 48th 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 discussion and information presented.

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	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
5th WPDC - 1956	D.E. Stover			
6th WPDC - 1957	D.V. Zander			
7th WPDC - 1959	H. E. Adler			
8th WPDC - 1960	R. D. Conrad			
13th WPDC - 1964	W. Hughes			
14th WPDC - 1965	B. Mayeda			
15th WPDC - 1966	R. Yamamoto	D.S. Clark		
16th WPDC - 1967	D.S. Clark	R. Balch		
17th WPDC - 1968	R. Balch	R. McCapes		
18th WPDC - 1969	R. McCapes	D.C. Young		
19th WPDC - 1970[A]	D.C. Young	W.J. Mathey		
20th WPDC - 1971	W.J. Mathey	R. Burdett		
21st WPDC - 1972	R. Burdett	M. Hammarlund		
22nd WPDC - 1973	M. Hammarlund	G.W. Peterson		
23rd WPDC - 1974	G.W. Peterson	C. Riddell		
24th WPDC - 1975	C. Riddell	R. Cooper		
25th WPDC - 1976	R. Cooper	G. Galvan		
26th WPDC - 1977	G. Galvan	D.H. Helfer	H. Bravo	
27th WPDC - 1978	D.H. Helfer	A. Bickford		
28th WPDC - 1979	A.Bickford	J.W. Dunsing		
29th WPDC/ 5th ANECA[B]-1980	J.W. Dunsing (WPDC) A. Mosqueda T.(ANECA)	G.Y. Ghazikhanian	P.P. Levine	
30th WPDC - 1981	G.Y. Ghazikhanian	M. Kumar		
31st WPDC - 1982	M. Kumar	R. Schock		
32nd WPDC - 1983	R. Schock	G.B.E. West		
33rd WPDC - 1984	G.B.E. West	G.J. Cutler		
34th WPDC - 1985	G.J. Cutler	D.W. Waldrip		B. Mayeda
35th WPDC / 11th ANECA-1986	D.W. Waldrip (WPDC) J. Basurto B. (ANECA)	D.A. McMartin (WPDC) M. Padron N. (ANECA)	J.A. Allen (WPDC) A. Tellez-Giron R. (ANECA)	
36th WPDC - 1987	D.A. McMartin	M.M. Jensen		
37th WPDC - 1988	M.M. Jensen	B. Kelly	A.S. Rosenwald	
38th WPDC - 1989	B. Kelly	M. Matsumoto		L. Williams
39th WPDC - 1990	M. Matsumoto	J.M. Smith		D. Young W.J. Mathey
40th WPDC / 16th ANECA-1991	J.M. Smith (WPDC) M. Silva M. (ANECA)	R.P. Chin (WPDC) D. Sarfati M. (ANECA)	A.S. Rosenwald (Joint Dedication)	
41st WPDC - 1992	R.P. Chin	R.J. Terry	M.M. Jensen	H.E. Adler (posthumous) R.A. Bankowski C.E. Whiteman
42nd WPDC - 1993	R.J. Terry	A.S. Dhillon	W.W. Sadler	R.A. Bagley
43rd WPDC - 1994	A.S. Dhillon	H.A. Medina		G.B.E.West

	PRESIDENT	PROGRAM CHAIR	DEDICATION	RECOGNITION
44th WPDC - 1995	H.A. Medina	D. Frame	W.M. Dungan (posthumous)	A.J. DaMassa G. Galvan W.F. Hughes W.D. Woodward R. Yamamoto
45th WPDC / 21st ANECA-1996	D. Frame (WPDC) R. Salado C. (ANECA)	M. Bland (WPDC) G. Tellez I. (ANECA) M. Tomayo S. (ANECA)	D. Zander (WPDC) M.A. Marquez R. (ANECA)	P. Villegas Ben Lucio M. M. Salem V. Mireles C. Riddell
46th WPDC - 1997	M. Bland	J. Andreasen	B. Mayeda	R. Balch P. DeLay J. W. Dunsing D. Helfer D. Stover
47th WPDC - 1998	J. Andreasen	H. L. Shivaprasad	W. J. Mathey	M. Jensen D. McMartin

[A]From 1970 to 1982 the Western Poultry Disease Conference was combined with the Poultry Health Symposium
[B]Asociacion Nacional de Especialistas en Ciencias Avicolas

MINUTES OF THE 1998 47TH WESTERN POULTRY DISEASE CONFERENCE BUSINESS MEETING

President Jim Andreasen called the meeting to order at 4:45 p.m. on Monday, March 9, 1998, at the Holiday Inn Capitol Plaza, Sacramento, California.

APPROVAL OF 46TH WPDC MINUTES

The minutes of the 46th WPDC business meeting were reviewed and a motion for approval was made and seconded. There was unanimous approval of the minutes as written and printed in the proceedings of the 47th WPDC.

ANNOUNCEMENTS

President Andreasen announced that Dr. Donald Zander is not in good health and the Conference would like to send him a copy of the 1998 47th WPDC Proceedings with as many signatures of attendees as possible. The Proceedings was passed around for signing. (Note: it was also circulated at the awards banquet for signatureS and sent to Dr. Zander.)

President Andreasen acknowledged all contributors. Particular thanks were given to those obtaining the level of Benefactors, which included the American Association of Avian Pathologists, the Bayer Poultry Business Unit, Intervet International and Intervet, Inc., and Merial Avian Business Unit.

REPORT OF THE SECRETARY-TREASURER

Dr. R. Chin presented the secretary-treasurer report. For the 1997 meeting, there were $24,550 in contributions, $41,135 income, and expenses of $38,987.50 for a net income of $2,147.50. Dr. Chin noted that there was an incorrect billing by Conference and Event Services of $35/registrant; WPDC should have been billed $30/registrant. The $5/registrant, or $1000 will be credited to the 1998 meeting. For the 1998 meeting, there was $33,100 in contribution support - an increase of 40%. Special acknowledgment was given to Dr. A .S. Rosenwald for obtaining the tremendous support.

REPORT OF THE PROCEEDINGS EDITOR

Dr. C. Riddell presented the proceedings editor report. There were 77 papers this year, which is about average. The production of the Proceedings went fairly well, with most of the papers being submitted via E-mail. There were 17 diskettes sent, though, half of those were also sent via E-mail as back-up. Dr. Riddell gave thanks to Drs. Rosenwald, Shivaprasad and Chin for helping to obtain the late papers. Repro Graphics at U.C. Davis did a good job of printing the Proceedings, though, they did take 4 weeks to produce.

REPORT OF THE LOCAL ARRANGEMENTS COORDINATOR

Dr. L. Woods presented the local arrangements report. Due to personnel changes at the hotel, there was an error in the food order and no muffins were available Sunday morning. Mike Luthi, at UCD, asked for $50 to improve the translator's booth by adding a fan and lighting. Dr. Lucio reports that the conditions were much improved over last year due to the remodeling. Dr. Woods reported that due to complications from last year's computer presentations, we had requested computer presenters to bring their own laptop computer to hook up to the projector. However, the first laptop computer did not have the correct plug-in for the cable. With the newer computers, this situation may correct itself. Finally, Dr. Woods commented that speakers should be informed not to use red or green colors in their presentations as they are hard to see.

OLD BUSINESS

Dr. Y. Ghazikhanian asked about WPDC's relationship with ANECA and the current financial situation following the 1996 meeting in Cancun, Mexico. Dr. Andreasen reported that discussions are still in progress. Dr. Rosenwald commented that we have always had very good relationships with the people from Mexico, especially with Dr. Lucio. Dr. Bland and Dr. Rosenwald are working to resolve the situation with the 1996 meeting.

NEW BUSINESS

President Andreasen stated that the Executive Committee would like to put into nomination the name of Dr. Patricia Wakenell for Program-chair elect. The nomination was seconded by Dr. Mathey. Dr. Mathey then moved that nominations be closed. The motion was seconded by Dr. Ghazikhanian. The vote was unanimous in favor of Dr. Wakenell. President Andreasen nominated the following officers for the 1999 WPDC:

President: Dr. H. L. Shivaprasad
Program Chair: Dr. R. Keith McMillan
Local Arrangements Coordinator: Dr. Stewart Ritchie
Proceedings Editor: Dr. Craig Riddell
Contributions Chair: Dr. A. S. "Rosy" Rosenwald
Secretary-Treasurer: Dr. Rich Chin
Program Chair-elect: Dr. Pat Wakenell

Dr. G. Cutler moved for approval of the listed officers, it was seconded by Dr. Ghazikhanian and unanimously approved by vote.

The 1999 48th WPDC will be in Vancouver, British Columbia, Canada, April 24-27, 1999. A symposium, sponsored by the American College of Poultry Veterinarians, is tentatively scheduled for Saturday, April 24th, on managing rapid growth of today's birds. Dr. S. Ritchie will be program coordinator for the symposium.

The location of the 2000 49th WPDC was discussed. There were no concerns expressed this year with the Holiday Inn Hotel. Dr. D. Frame moved and Dr. Shivaprasad seconded that we return to the Holiday Inn Capitol Plaza in 2000. There was a unanimous vote in favor of the motion. (Note: Tentative dates are March 5-7, 2000.)

President Andreasen passed the presidency to Dr. Shivaprasad. President Shivaprasad thanked outgoing President Andreasen for his excellent work and adjourned the meeting at 5:30 p.m.

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PROCEEDINGS OF THE FORTY-EIGHTH WESTERN POULTRY DISEASE CONFERENCE

ON - FARM HACCP (CALIFORNIA STYLE)

M. C. Bland

3562 Jomar Drive, Napa, California

Most of the food safety issues that are currently discussed are centered around the use of words like Hazard Analysis Critical Control Points [HACCP] and "Mega Regs". Both of these terms primarily involve processing plants. Recently there has been an effort by the poultry industry as well as state and federal government agencies to take food safety issues to the farm level, with a variety of voluntary production food safety programs. Two such programs are the "California Egg Quality Assurance Program" (CEQAP) and the "California Poultry Meat Quality Assurance Program" (CPMQAP). Both programs are industry driven with support from both the university and state agencies. The quality assurance programs utilize management strategies that are implemented at the farm level to help reduce the risk of microbiological contamination at the farm. This presentation will focus on the CPMQAP. Both programs are similar in basic structure and function, however the intent of this presentation is to share with the audience the practical aspects of developing a Poultry Meat Quality Assurance Plan for the poultry producer.

The CPMQAP is based on 13 core components. The first three components involve administration of the program. The last ten cover production practices that have been identified as important points in reducing product contamination. Each plan for each facility is individually designed and written based on how each component will be addressed. What has been found, for the most part, is that the majority of the CPMQAP requirements are already being implemented on the farm in some form or another.

DEVELOPING A QUALITY ASSURANCE PROGRAM

There are a number of steps that can help in undertaking the development of a CPMQAP program. It is important that those who are involved with developing a program take the time to investigate, collect and organize all preexisting documentation and relevant information. This will help avoid "reinventing the wheel". It will also point out what is already available and where deficits lie.

Step 1. Review the core components listed in the CPMQAP. A brief outline of the CPMQAP core components are listed below:

1) Purchase eggs, chicks, poults, ducklings or other birds from breeders and hatcheries participating in the National Poultry Improvement Plan (NPIP) as applicable by species.

2) Decontaminate vehicles prior to transporting live birds.

3) Follow on a regular basis cleaning and disinfecting programs for live production buildings and equipment.

4) Obtain feed from mills that follow good management practices.

5) If used, animal protein ingredients must originate from rendering plants participating in the Animal Protein Producers Industry (APPI) Salmonella Reduction Education Program or equivalent.

6) If used, medications, feed additives and pesticides must be administered according to approved label directions or under veterinary supervision.

7)Maintain an effective flock health program, which can include appropriate vaccinations, monitoring, and periodic necropsy.

8) Maintain a farm rodent monitoring and control program.

9) Maintain a litter management program.

10) Develop and utilize a biosecurity program.

Step 2. Accumulate reference material from the production department, including production management guidelines, appropriate written memos, documents that may be filed with other departments or verbal recommendations that have been implemented on the farm. Most of this material will provide specific information necessary to the plan.

Step 3. Check to see if there are written company policies in regard to on farm safety meetings. Gather any information on pesticide training and safety procedures.

Step 4. Contact the feed mill and hatchery manager to obtain any documents or other information pertaining to the items listed in the plan. Contact the primary breeder company for the necessary documentation [NPIP status] and ask for copies of their breeder or production management guidebooks.

Step 5. Review the production data that is accumulated. Find out how it is recorded and processed.

Step 6. Collect information on flock health monitoring. If applicable, utilize the veterinarian on staff to collect this information. This includes vaccination schedules, mortality and laboratory records. Flock production records can also be referenced as part of the flock health-monitoring program.

Step 7. Gather information on the physical description of each individual facility. This would include, name of facility, address, number of flocks placed annually, flock capacity, type of ranch, number of buildings, and major

equipment systems.

Step 8. Develop an outline by listing the resources that are available. List references and /or provide a short summary that would provide the necessary information next to each core component in the CPMQAP. Include in the outline information that is missing or is needed to complete the program.

The written outline will allow you to see what components are completed and those that are not, as well as where their strengths and weaknesses are in the program. Much of the information needed for the CPMQAP can be found in the company's production guidelines or best management practices. If such a manual is not available maybe it is time to produce one. The information found in the production manual such as cleaning and disinfecting, brooder and grow out management, feed, water and litter management can all be referenced into the facilities CPMQAP.

Records and monitoring requirements are also part of the CPMQAP. Check to see if most of the necessary information required can be found in the production records, especially in the area of rodent control, flock records for medication and vaccination use, as well as records for farm pesticide usage. Where necessary corrective procedures need to be mentioned in the plan or can be referenced from another source [production manual]. For example, if monitoring reveals that rodent activity is increasing and corrective action needs to take place, then you need to state what that action or actions should be. This could mean that more bait stations are needed or that the rodent bait should be changed to one with a different active ingredient. Monitoring such areas as cleaning & disinfection, flock health and rodent control insures that the program goals are being met.

Avoid making the program burdensome. The first page should be used to give a physical description of the facility. The following page or pages are used to describe the plan using the core components. Write the plan in general terms. Reference other sources of material when referring to the core components. This will help avoid having to rewrite the CPMQAP on a frequent basis. Attend CPMQAP training sessions.

What helps validate the CPMQAP process is that the veterinary staff at the California Department of Food and Agriculture [CDFA] reviews each initial farm plan and then on a annual basis. The Quality Assurance Supervisor, an individual designated by the company, also reviews the plan records monthly.

SUMMARY

The California Poultry Meat Quality Assurance Plan (CPMQAP) is a producer orientated production food safety program that is designed to ensure the highest quality and safety of poultry and poultry products. The CPMQAP is voluntary and is based on best management practices. Each plan is individually written to meet the needs of a particular facility. Training, record keeping and research are all part of the plan. Most if not all poultry companies have been operating their facilities under similar conditions. What the CPMQAP has done for the industry is put this information in a logical written document for the government and public to see.

ON-FARM HACCP (A QUALITY ASSURANCE PROGRAM)

R. K. McMillan

Lilydale Foods, 7727 – 127 Ave, Edmonton , AB T5C 1R9, Canada

Poultry meat remains a popular item with consumers but these same consumers continue to demand better quality, more variety and safer food products. In response to consumer concerns and several significant food borne illnesses, regulatory agencies have instituted programs like the "Mega–Regs". Typically, regulatory pressure to control pathogens such as *Salmonella, E.coli, Campylobacter and Listeria*, continues to be focused mainly at the processing plant. This has not achieved significant reductions in food pathogens and neglects the initial exposure of the birds on the farm. It is increasingly evident that a reduction in food borne pathogens will only be achieved through the efforts of everyone in the food production chain.

The purpose of this presentation is to briefly outline the components of a HACCP based quality assurance program developed by the Alberta Chicken Growers in conjunction with processors, academia and government. The objectives in developing the program were to provide the processor with a better quality bird and to address food safety concerns at the farm level through a modification of HACCP principles. Several steps were taken in the development of the program.

1) Poultry House Inventory: Each grower was required to do an inventory of feed and water space and ventilation capacity of each poultry house to determine the placement bird density that the house could support.
2) Define the Product: The processors involved, outlined their requirements on birds coming to slaughter, i.e. specific weight categories, feed withdrawals, processing characteristics, etc.
3) Define the Process: This involved defining the process steps such as cleaning and sanitation, facility preparation, incoming supplies, brooding, growing, pre-harvest preparation and load out.
4) Identification of Hazard: An assessment of potential

microbiological, chemical and physical hazards with respect to facilities, equipment, chicks, feed and water, relative to brooding, growing and harvesting.

5) Determination of Control Measures: To control the majority of identified hazards, seven Good Management Practices (GMP's) were to be written specific to each farm operation. These included a) cleaning and sanitation, b) facility preparation, c) water quality, d) pest control, e) biosecurity, f) culling and mortality disposal, and g) pre-harvest preparation.

6)Determination of Critical Control Points: CCP's included air quality, water and feed, vaccinations and treatments.

7) Establishment of: Monitoring points, monitoring procedures and limits.

8)Establishment of: Corrective actions

9)Establishment of: Record keeping system to record when and how tasks were performed and daily / weekly flock information.

In addition a flock summary report, which originates at the hatchery and comes to the farm when the chicks are placed, is completed by the farm manager and sent to the processing plant three days prior to shipment of the flock to slaughter. The summary report details feed rations, disease problems and medications used along with drug withdrawal times. A live haul report is also submitted.

At processing time, carcasses are tested for *E.coli* (process control) and *Salmonella* sp. (pathogen reduction) according to the Mega–Reg requirements. Each flock is evaluated on grades, size utilization and yield. Condemnations are recorded and blood samples are taken at slaughter time when ever a flock has indications of health problems or particular categories of condemns are found to be increased. The completed flock evaluation is copied to the grower and the field service person as follow up.

After one year of operation, it is difficult to define specific improvements but the indicators are positive. *E. coli* tests show less overall carcass contamination indicating improved feed withdrawal and barn conditions. *Salmonella* isolations appears to be tied closely with specific farms rather than processing sanitation. Better record keeping appears to have increased farm management awareness and interest which is reflected in an improvement in the overall quality of birds at processing.

A PILOT STUDY OF HACCP FOR EGG PRODUCERS IN ONTARIO

R. W. Stevens[A] and P. Hunton[B]

[A]Stevens Associates, Agri-Food Strategies, Guelph, ON, Canada
[B]Ontario Egg Producers, Mississauga, ON, Canada

INTRODUCTION

There is some confusion with regard to HACCP. The confusion starts with the definition of HACCP at the farm. One school of opinion states that classic HACCP cannot be applied on the farm. Being skeptical about true HACCP on the farm, persons in favor of food safety have created pseudo-HACCP programs. They call them HACCP-like and HACCP-based for primary producers. Regardless of the name, they are, in fact, Good Management Practices (GMP's). If farmers will embrace GMP's under a hyphenated HACCP title, that is good for the industry. It is better to be half-HACCP than no HACCP at all! Suffice to say, without GMP's on the farm there is no hope for HACCP. GMP's are frequently called prerequisites for HACCP. HACCP is based on scientific, objective observations. Critical points that can be controlled are those that can be measured quantitatively. Accordingly, this study was conducted to identify those inputs to an egg farm that can be measured objectively and analyzed scientifically.

Three different agencies, each following the Five Preliminary Steps and the Seven Principles of HACCP, have produced three different lists of Critical Control Points (CCP's).

1. In the Pennsylvania Egg Quality Assurance Program, the CCP's are listed as:
   i. Clean and disinfect between flocks
   ii. Control rodents
   iii. Place *Salmonella enteritidis* clean pullet chicks
2. In Canada, the Canadian Egg Marketing Agency (CEMA) initiated its HACCP by identifying five CCP's. Later, those were revised to four points, consisting of:
   i. Pest control
   ii. Facility hygiene
   iii. Sorting and packing
   iv. Refrigerated storage
3. The Ontario Egg Producers' HACCP Pilot Study started with three CCP's:
   i. Pest control
   ii. Facility hygiene
   iii. Egg storage

All other inputs are listed as prerequisites.

In spite of these differences, there appears to be a major interest in the egg cooling storage compartment as a CCP. The temperatures required vary according to national programs. In the U.S.A. the target temperature for egg storage is 7°C (45°F). That is the temperature at which *Salmonella* stop replicating. That is an achievable target in the U.S.A. because most egg farms have on-line washing and

packing without cooling the eggs prior to washing. In Canada, eggs are cooled at the farm before they are transported to a central grading station. Seven-degree eggs would crack when they hit the hot wash water so the target range of temperatures for on-farm storage in Canada is 7°C to 13°C (45°F to 55°F). Compare these standards with those in Great Britain where the new Lion Quality Code of Practice stipulates that all eggs must be stored below 20°C (68°F). The purpose of this study is to determine what temperature profile in an egg room will satisfy HACCP.

MATERIALS AND METHODS

In 1997, 20 egg producers in Ontario volunteered to participate in an HACCP Pilot Study. The study covered 9 input factors, from pullets, feed and egg flats, to pest control and facilities hygiene. The duration of the study was one year or one complete cycle of a laying flock, from empty barn to empty barn. A HACCP coordinator was employed to visit the cooperators regularly, for initial orientation and routine data collection. At each farm visit, she conducted on-site tests and downloaded DataLoggers. The HACCP coordinator was equipped with a laptop computer and portable printer. For the egg storage component of the pilot study, each cooperator received a high/low thermometer and a Hobo DataLogger. The Hobo DataLoggers were set to record temperature and humidity in the egg coolers every 15 minutes. That is 96 data points every day, or 35,040 per year. All data were transferred to a CPU for analysis. The Hobo data were changed to a Lotus format, and innovative macro-programs were created to compress the data and establish tolerance limits. The first analysis was to determine the proportion of time that storage room temperatures fell within the ranges of<7°C, 7° to 10°C, 10° to 13°C, 13° to 20°C, and >20°C. For the second analysis, an arbitrary temperature of 13°C was set as the upper limit for egg storage, and 2 hours was set as the maximum time that a storage room could exceed 13°C. These temperature and time limits could be adjusted as "what if" parameters. With these criteria, a flock profile was created for each of the 20 flocks.

RESULTS AND DISCUSSION

The proportions of time in the five temperature categories are listed in Table 1. An occasional spike of heat above 13°C may have negligible impact on the internal temperature of the egg. It could take from 2 to 5 hours for the internal temperature of the egg to equal the ambient air temperature depending on the initial temperature of the egg, air-flow velocity, differential room temperatures, and types of packing materials. For this study, it was assumed that any period of heat less than 2 hours would not warm the eggs significantly. Therefore, 2 hours was set as the tolerable time period for a storage room to be above 13°C. The data were analyzed to determine the proportion of time that the eggs were held for periods longer than 2 hours above 13°C. When the results from all 20 flocks were combined, the total number of temperature readings was 661,243. For 72% of those readings the temperature conformed with the standards that had been set (≤13°C). Conversely, for 28% of the time the temperatures were not in compliance, exceeding the upper limit of 13°C. For the non-compliant readings, the warm period lasted longer than 2 hours on 2,144 occasions (ave.= 107 per flock). The warm periods lasted from 2 to 6 hours on 986 occasions (ave. = 49 per flock); 6 to 12 hours on 572 occasions (ave = 29 per flock) and >12 hours on 586 occasions (ave. = 29 per flock).

It is proposed that this population of flocks is representative of egg storage compartments in Ontario but since these flock owners volunteered to participate they may represent above-average husbandry practices. With this data as a base, the mission was to "establish critical limits for preventative measures" (HACCP Principle No. 3). The HACCP model must be simple to apply and user-friendly. We propose two criteria for HACCP approval of on-farm egg storage rooms based on DataLogger records. The first is proportion of time above target temperature. In this example, the target temperature was set at 13°C but it could be set at any level as dictated by local circumstance. If the critical limit was set at zero time, none of the flocks in this study would qualify for HACCP. Recognizing that the best managers, with the finest refrigeration equipment, will have

Table 1.

Egg Room Cooler Temperatures
(Percentage of time during one year, 20 flocks)

Temperature range	*Mean ± S.D.*	*Range (min/max)*
>20°C	2.4% ± 4.0	0.0 - 11.4
13° to 20°C	26.1% ± 19.1	2.8 - 84.2
10° to 13°C	47.9% ± 17.6	4.1 - 78.6
7° to 10°C	18.5% ± 13.3	0.3 - 51.3
<7°C	5.1% ± 4.8	0.0 - 14.3

occasional peaks of time above the target temperature, the authors do not recommend zero-time tolerance. A reasonable tolerance is 28% of the DataLogger readings. That is the mean of the pilot study sample. Those egg producers who exceed this level should be advised to take corrective action. If the non-compliant temperature readings exceed 50% of the time, the egg producer should be ineligible for HACCP certification. That level represents one Standard Deviation (1 S.D.) above the mean, of this sample. These levels of tolerance could be adjusted periodically, based on the norms of the population. The second criteria is duration of time above target temperature. The longer the temperature is continuously above the target temperature, the more demerit points the flock would accumulate. Obviously 12 hours of heat is more severe than 2 hours of heat so the duration of time should be weighted to reflect the severity of the potential risk. For this study, the weighting factors are:

2 to 6 continuous hours of heat,
Weighting Factor = 1
6 to 12 continuous hours of heat,
Weighting Factor = 2
more than 12 hours of heat,
Weighting Factor = 4.

With this scoring system, the average flock in the pilot study had a score of 219 demerit points [(49x1) + (27x2) +(29x4) = 219]. Any flock that accumulates over 219 demerit points should be ineligible for HACCP certification. Again, these levels of tolerance could be adjusted periodically, based on the norms of the population.

CONCLUSIONS

It has been demonstrated that critical limits for preventative measures can be established based on statistical data for the egg storage compartment in Ontario. This fulfills Principle No. 3 of HACCP. The monitoring system for gathering objective data and signaling deviations from benchmarks is a Hobo DataLogger. This fulfills Principle No. 4 of HACCP. Also effective record keeping procedures have been established to document the HACCP system (HACCP Principle No.6). Based on this demonstration, egg storage qualifies as a Critical Control Point (CCP). The common belief has been that appropriate science and technology do not exist to support strict HACCP at the farm level. This study was initiated to find appropriate technology and demonstrate its application to HACCP. In the case of egg storage, that mission has been accomplished. Parallel studies are underway to apply new technologies to rodent control, facilities hygiene, barn cleaning and disinfection, pullet health status, and packing materials. In the end, fully legitimate, on-farm HACCP programs may be implemented for table egg producers.

ACKNOWLEDGMENTS

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MICROBIOLOGICAL TESTING - A TWO EDGED SWORD?

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Great Britain has been at the forefront of so called 'food scares' since the 'Salmonella in eggs' saga of the late 1980's. More recent episodes have included Listeria in soft cheese, BSE, Esherichia coli 0157 and Clostridium botulinum in hazelnut yoghurt. Unfortunately the public now has a subconscious link between Salmonella and poultry, but it is also less responsive to, or concerned by, 'food scares' than it was a decade ago. Today the attitude of the average consumer is that they do not want to put the farmer or anyone else associated with the production of food out of business, but they do want safe, wholesome, quality food. Historically, the British Poultry Federation (BPF) appreciated the importance of microbiological testing of products as did the major customers of the industry. The problem was that the latter all had, and demanded the use of, their own preferred microbiological methods. The BPF established a subcommittee which included this paper's author and published "a small booklet of approved microbiological methods for poultry products". The text was a reference for the industry for a decade or so but the 'Salmonella in eggs' scare started in motion a series of events out of which came a modern approach to microbiological testing of poultry products and the supply chain that provides them. This paper will focus on some aspects of this testing.

ACCREDITATION OF LABORATORIES

Major customers now require their suppliers' laboratories to be accredited by a competent accreditation body. The highest accreditation is UKAS (formerly known as NAMAS) which is internationally recognised. Independent testing laboratories need to be NAMAS accredited but in house laboratories can use other, less demanding, schemes. UKAS accreditation requires everything to be operated according to a Standard Operating Procedure (SOP) and for the laboratory to be able to confirm that every stage and aspect of the SOP is complied with. For example in media preparation this includes being able to prove every piece of equipment used

was correctly calibrated, each stage of the process was checked and documented and the media was shown to be able to do what it was intended to do by the use of a challenge test organism.

EXTERNAL CHALLENGE TESTING OF LABORATORIES

A key aspect of UKAS accreditation and most accreditation schemes is external challenge testing. Probably the most popular scheme is the Quality In Microbiology scheme. Under this scheme participating laboratories are sent challenge samples regularly through the year and on completion of a test the laboratory is advised on the correct result and, anonymously, how all participating laboratories performed. The z-score system is used to assess the performance of a laboratory and this will be explained graphically in the presentation. As can be expected, any system of external challenge testing has its weaknesses and for this reason not too much significance should be placed on one result in isolation. The ongoing trend of results is what is important.

RAPID TECHNOLOGIES

This is a subject of much debate. Some 5-10 years ago there was a swing to rapid testing but this has been reversed somewhat. Today the tendency is to use traditional microbiological methods but to then use rapid technologies in their confirmatory stages, for example, the serogrouping of salmonella or the typing of listeria. The new technologies are producing some interesting products for E.coli 0157 testing.

INTERPRETATION OF RESULTS

An area of real concern is the meaning of results. Various restraints mean that in most situations an inadequate number of samples are taken for the generation of meaningful results. This being the case how should results be interpreted and what is their legal significance should a dispute arise? This then means that real significance has to be placed on retrospective trend analysis. How reliable is this? There are various attributes to this debate that are worth reflecting on.

THE CHALLENGES

Microbiological testing is going to have many challenges and this paper concludes by just listing some of these:

* College graduates with virtually no practical experience and totally devoid of practical skills
* Customers with some knowledge who generate unscientific (unrealistic) demands on the laboratory
* Inadequate sample numbers
* 'Continuity of evidence'
* Demands for new tests e.g., E. coli 0157, meat specification, Listeria enumeration and Campylobacter
* Will more sophisticated testing techniques generate tomorrow's 'food scares'
* Handling (abusing) of samples between collection and testing
* Ill-informed inspectors with limited microbiological knowledge who over react to microbiological laboratory results
* Competent laboratories producing more positive results, or, conversely, incompetent laboratories producing more negative results.
* The problems of a positive release system for fresh products
* Different customers requiring different tests and different test methodologies
* The routine use of bioluminescence based technologies to assess effectiveness of cleaning and the consequences of recleaning
* Microbiological monitoring of staff
* Contractual disputes over status of purchased ingredients
* SOPs and calibration checks for every stage of the laboratory's operation
* More testing wanted by customers who want product at lower prices!

Finally, a point for reflection. In Great Britain the external accreditation for laboratories testing end products is now the norm. One view point that is gathering some acceptance is that the veterinary pathology laboratories should now become accredited.

A DECADE OF TRENDS IN BROILER CONDEMNATIONS

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Ten year (1988-1997) national trends in whole bird broiler condemnations were determined with data from the Poultry Slaughter Reports generated by the United States Department of Agriculture National Agricultural Statistics Service. Trends were determined for all categories of whole bird condemnation except tuberculosis because the total number of birds condemned in this category was negligible. Below follows a summary of trends observed for each

category.

Leukosis. Ten year national average condemnation for leukosis was 0.029%, the incidence varied from a high of 0.044% (1988) to a low of 0.017% (1999). Year to year variation in incidence of leukosis appears to correlate well with the emergence of new and more virulent strains of Marek's disease virus and the development of newer and more effective vaccines.

Septicemia/Toxemia. Ten year national average condemnation for septicemia/toxemia was 0.350%. The trend observed was towards a decrease from 0.467% (1988) to 0.326% (1997). A major factor contributing to the decrease observed may be the industry-wide adoption of nipple drinkers.

Airsacculitis. Ten year national average for airsacculitis was 0.238%. The trend observed as in the case of septicemia/toxemia was also towards a decrease from 0.332% (1988) to 0.198% (1997). A major factor contributing to the decrease observed may be the industry-wide adoption of nipple drinkers. In addition, better ventilation of flocks in tunnel ventilated houses and newer and more effective vaccines for the control of infectious bronchitis may have also contributed to the reduced condemnation.

Synovitis. Ten year national average for synovitis was 0.008%. The trend for synovitis has remained unchanged for several years. It is one of the lowest causes of whole bird condemnation.

Tumors. Ten year national average for tumors was 0.038%. There has been a slight decrease in the incidence of whole bird condemnations due to tumors from a high of 0.050% (1992) to a low of 0.028% (1996).

Bruises. Ten year national average for bruises was 0.017%. The trend for bruises has remained fairly constant but an increase to 0.025% was recorded in 1997.

Cadaver. Ten year national average for cadaver was 0.041%. The trend has remained fairly constant for this category of whole bird condemnation.

Contamination. Ten year national average for contamination was 0.068%. The trend has clearly shown an increase in whole bird condemnation due to contamination, from a low of 0.054% in 1988 to a high of 0.091% in 1997. Factors that may be negatively influencing condemnation levels for this category may be reduced antibiotic usage in the final feed and increased surveillance by the USDA.

Overscald. Ten year national average for overscald was 0.009%. The trend for this category was fairly erratic with wide fluctuations from year to year confirming the mechanical nature of this condemnation category.

Other. Ten year national average for other was 0.176%. The trend has been towards an increase from a low of 0.083% in 1988 to a high of 0.239% in 1997. Possible reasons for the trend observed may be the increased incidence of whole bird condemnations due to inflammatory/infectious process and ascites, both of which have been lumped into the "other" category of condemnation.

Total Whole Bird Condemnation. Ten year national average for total whole bird condemnation was 0.974%. The trend has remained fairly stable with a range varying from a low of 0.822% in 1993 to a high of 1.095% in 1988.

REFERENCE

1. Poultry Slaughter Report, National Agricultural Statistics Service (NASS), Agricultural Statistics Board, United States Department of Agriculture (USDA). 1988-1997.

A REVIEW OF BROILER CHICKEN CONDEMNATIONS IN WESTERN CANADA IN 1998

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There are nine conditions that caused significant condemnations in broiler chickens in Western Canada in 1998. This presentation will discuss these conditions relative to different regions of Western Canada defined by the federal government and describe any developing trends. The major conditions are cellulitis, ascites, cyanosis, emaciation, bruising, hepatitis, varus-valgus deformities, air sacculitis and peritonitis. Average incidence of condemnation for these conditions is summarized in Table 1.

Cellulitis is increasing across the country in a linear fashion in broiler chickens during the last decade (1). The incidence appears to increase in the summer, possibly due to increased activity in the birds or proliferation of bacteria in the litter due to warmer temperatures. The incidence of this condition is higher in Ontario and Quebec than in western Canada.

Ascites has also shown a steady increase over the last decade (2). Ascites is higher in Alberta and this is probably due to many farms in Alberta being at relatively high altitudes. Unlike other regions, the condemnations due to this condition do not drop as markedly in warm weather in Alberta. Considerable ascites still occurs in B.C. where

Table 1. National and western Canadian chicken condemnations (percent of chickens slaughtered)in 1998

	Man-Sask	Alberta	B.C.	Canada
Cellulitis	0.5	0.57	0.58	0.73
Ascites	0.34	0.56	0.42	0.42
Cyanosis	0.22	0.38	0.16	0.25
Emaciation	0.17	0.31	0.14	0.19
Bruising	0.04	0.06	0.06	0.08
Hepatitis	0.16	0.05	0.12	0.08
Varus-valgus Deformity	0.02	0.08	0.11	0.06
Air Sacculitis	0.04	0.0002	0.05	0.06
Peritonitis	0.1	0.0003	0.04	0.02

farms are at or near sea level.

Cyanosis is higher in Alberta than in other regions. This is probably related to ascites and farms at higher altitudes. Cyanosis does decrease in summer in most regions, probably due to the fact that cold weather may stress the birds.

Emaciation condemnations are higher in Alberta than other regions and there is no known reason for this. There is no seasonal variation in this condition.

Bruising condemnations are lower in the west than in eastern Canada. It is possible that this is related to differences in transportation.

Hepatitis (including hepatosis) exceeds the national average in Manitoba/Saskatchewan and B.C. It has been shown that this condition may be due to *Clostridium perfringens* (3) and may relate to different farm conditions and medication program.

Varus-Valgus Deformities are very low in incidence in Manitoba/Saskatchewan but high in B.C. This may relate to strain differences, different management practices or different diets.

Air sacculitis is virtually non-existent in Alberta and very low in Manitoba/Saskatchewan. This is probably due to the dry prairie conditions and the fact that most farms on the prairies are isolated. It is a definite problem in B.C. and the east where the climate is different and farms are often much closer together.

Peritonitis is greater in the Manitoba/Saskatchewan region. Incidence in Alberta and B.C. is similar to the national average. Is this difference due to the variable use of this term between regions? Peritonitis may be secondary to an existing condition such as hepatitis or cellulitis.

Some of the variance between regions in incidence of the different conditions is due to different environmental conditions and distribution of some infectious agents. There is, however, some indication that more correlation is required between regions to standardize identification of the different conditions. This is important relative to food safety.

REFERENCES

1. Kumor, L. W., A. A. Olkowski, S. M. Gomis, and B. J. Allan. Cellulitis in broiler chickens: epidemiological trends, meat hygiene, and possible human health implications. Avian Dis. 42:285-291. 1998.

2. Olkowski, A. A., L. Kumor, and H. L. Classen. Changing epidemiology of ascites in broiler chickens. Can. J. An. Sc. 76: 135-140. 1995.

3. Onderka D. K., C. C. Langevin, and J. A. Hanson. Fibrosing cholehepatitis in broiler chickens induced by bile duct ligations or inoculation of Clostridium perfringens. Can. J. Vet. Res. 54:285-290. 1990.

SPLENIC LESIONS AND CONDEMNATIONS IN CHICKENS

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Several diseases affect chicken spleens, and chickens with gross splenic lesions may be condemned at processing. The purposes of the present study were to 1) retrieve, inventory, and compare the type and frequency of light microscopic diagnoses made in chicken spleens that were examined at two laboratories from 1995-98, and 2) determine

if follow-up work is necessary if money were to be made by reducing condemnations attributable to splenic lesions. We found that both laboratories had made similar chicken spleen lesion inventories. Analysis of this data (Fig. 1) showed that diagnoses of inflammation were more common ($P = 0.026$) among chickens that were examined during the growing period, and diagnoses of neoplasia were more common ($P = 0.016$) among chickens that were examined at processing. The frequency of other lesions was about 10% at both laboratories. We know from previous experience that opinions between laboratories on individual cases rarely differs. Therefore, we believe that follow-up work will be necessary if the poultry industry wants to reduce losses that can be attributed to condemation of chickens with splenic lesions at processing.

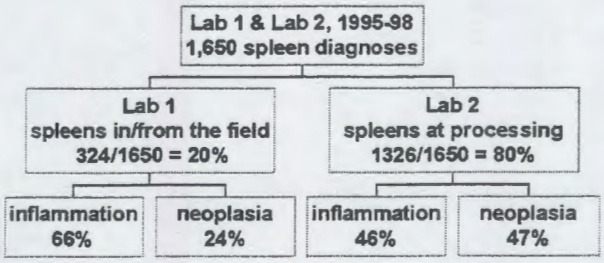

Figure 1. An overview of the type and frequency of light microscopic diagnoses made in chicken spleens that were examined at two laboratories from 1995-98.

(A complete paper will be submitted for review and consideration for publication in *Avian Pathology*.)

EYEDROP VACCINATION OF POULTRY USING PRECOCIOUS AND FIELD EIMERIA STRAINS

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This paper summarizes the investigation of individual eye-drop application of oocysts to birds. Eye-drop application of oocysts can be used where hatchery spray application or peck gels are unavailable and equipment does not permit oocyst vaccination by water or feed. A 4 species *Eimeria* eyedrop vaccine containing 108 oocysts in one dose was prepared. The formulation consisted of 18 oocysts of precocious *E acervulina*, plus 30 oocysts each of precocious *E tenella* and precocious *E maxima* combined with 30 oocysts of a field strain of *E necatrix*. One dose per bird was contained in 25μl of 0.01% formol phosphate buffered saline. The eye dropper bottles used had a 30 mL capacity. One thousand doses were contained in a total of 25 mL and the applicator tips dispensed 40 drops per mL.

The first trial examined the efficiency of the ocular route of vaccination by analysing the percentage of faecal samples containing oocysts 8 days post-vaccination. Two groups of twelve week old caged layers were each given approximately 216 oocysts (x2 dose). A third group of 200 birds was given 324 oocysts (x3 dose) by individual eye-drop. Individual faecal samples (2 g each) were analysed for oocysts using salt flotation. The faeces from the first 2 groups were 98% and 99.5% positive, while the third group was 97.5% positive. There was no pathology in birds or deterioration in the consistency of the faeces. Birds showed no signs of depression. Oocysts produced were of mixed species. The second trial examined the oocyst excretion rate in birds receiving a single eye-drop dose by collecting 2 lots of 200 faecal samples from a group of birds 7 and 8 days post-vaccination. Faeces were 87% positive on day 7 and 56%

positive on day 8 post-vaccination. The third trial examined whether individually vaccinating birds by eye-drop with 108 oocysts (x1 dose) produced an immune response in caged birds aged between 4 and 6 weeks. One group of 200 vaccinated birds was challenged with a x10 dose of vaccine at 21 days post-vaccination. A second group of 200 vaccinated birds was challenged with a x10 dose of vaccine at 41 days post-vaccination. A third group of 200 birds was vaccinated but not challenged. To prevent cross infection each group of birds was located in a separate row of cages. Faeces were collected for oocyst analysis on the day of vaccination, 15, 29 and 49 days post-vaccination. Faeces were negative at the start of the trial. At 15 days post vaccination 2% of faeces were positive. Faeces from vaccinated birds receiving no challenge dose were 1% positive at 29 days post-vaccination and only a few scattered oocysts were detected at 49 days post- vaccination. Faeces collected from vaccinated and challenged birds at the same time were 35% and 22% positive. No coccidiosis pathology, mortality, clinical depression or significant deterioration in faecal consistency was noted during the trial.

The results demonstrate oocysts can be applied by individual eyedrop. Vaccinated birds have a lower faecal oocyst presence in the face of 10x homologous challenge than naïve birds receiving a single dose. The ability to mount this response lasts for up to 7 weeks post-vaccination. This is consistent with an immune response. Field trials have commenced in litter rearing sheds comparing the efficacy and safety of individual eyedrop application with feed and water vaccination.

(A full length article has been submitted to the *Australian Veterinary Journal*.)

NEW SPRAY CABINET TECHNOLOGY: IMMUNITY WITH MINIMAL LESION DEVELOPMENT MAKES VACCINATION A DRUG-FREE ALTERNATIVE FOR COCCIDIOSIS CONTROL IN BROILERS

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Control of coccidiosis in broilers with vaccination has had limited past success due to excessive lesion development and clinical coccidiosis post-vaccination. New spray cabinet application technology has improved the uniformity of vaccine administration. Uniform vaccine application results in minimal lesion development, similar to the minimal lesions that occur when flocks are fed low-level ionophores in the ration. Pen trials comparing chickens vaccinated with a coccidiosis vaccine via spray cabinet to chickens fed salinomycin (60 gm / ton) demonstrated statistically equivalent performance in chickens 35 days of age or older.

COCCIDIOSIS VACCINATION: UNIFORM APPLICATION IS CRITICAL

The coccidia in some coccidiosis vaccines are capable of causing excessive lesions or clinical coccidiosis if susceptible birds are exposed to an uncontrolled dose of oocysts. Likewise, field strains of coccidia may cause clinical coccidiosis in unvaccinated, susceptible birds. When vaccine is applied unevenly, a percentage of the birds will not receive a minimum infective dose. These birds will be susceptible to the field strains that are endemic in the poultry house. Properly vaccinated birds can be expected to shed large numbers of vaccine-strain oocysts at 5 to 7 days post-vaccination, and again at 10 to 14 days post-vaccination. This exposes susceptible birds to an uncontrolled dose of vaccine-strain coccidial oocysts. Susceptible birds may develop excessive lesions or clinical coccidiosis that is due to infection with either field strains or the vaccine strain. Regardless of the origin of the infection, poorly vaccinated flocks may exhibit poor performance, poor flock uniformity or mortality. The difficulty of obtaining consistent vaccination uniformity with either feed application or eye-spray has limited the use of immunization as a viable coccidiosis control method in broiler flocks. A unique spray cabinet was developed for the application of Coccivac-B™ (Schering-Plough/ASL, Union, NJ). The cabinet vaccinates a 100-chick box with a uniform 21-cc shower spray activated by an electric eye. Following vaccination, it is believed that the chicks preen and ingest the vaccine orally. The lack of adverse reactions post-vaccination with this new method indicates that the uniformity of application is improved over previous application methods.

EXPECTED LESIONS FOLLOWING SPRAY-CABINET VACCINE APPLICATION

Low-level coccidial lesions are expected following vaccination with a live oocyst vaccine. Coccivac-B™ contains *Eimeria acervulina, Eimeria maxima, Eimeria mivati* and *Eimeria tenella.* Vaccinated flocks should be

monitored routinely for proper lesion development signaling immunization. The Johnson and Reid (1) scoring system is used to describe the expected lesions below. By this system, a score of 0 to 4 is assigned to a bird where 0 = normal and 4 = most severe case.

No visible lesions: 65% or more of the birds in a flock should have no gross lesions during the peak vaccine cycling period of 14 to 28 days of age. 95 - 100% of the birds should be lesion-free after 35 days of age. Microscopic examination may show oocysts in most birds without gross lesions, indicating a good vaccination take.

Eimeria acervulina and E. mivati: Lesions will peak at 14 to 24 days of age. Up to 30% of the birds in a flock will develop lesion scores of 1, and fewer than 10% may develop lesion scores of 2. By 28 days of age, lesions begin to decline or may be absent.

Eimeria maxima: Lesions will peak at 20 to 28 days of age. Up to 15% of the birds in a flock will develop mild lesion scores of 1. *E. maxima* is very difficult to evaluate with gross lesions. Microscopic evaluation will reveal low numbers of oocysts (1 - 25 oocysts per 100x microscopic field) in 30 - 70% of birds between 14 and 28 days. Fewer than 10% of birds may have as many as 100+ oocysts per 100x microscopic field between 20 and 28 days of age. After 28 days, *E. maxima* oocysts are rarely found.

Eimeria tenella: Lesions will peak at 20 to 24 days of age. Very few birds will exhibit *E. tenella* lesion scores. Fewer than 10% of the vaccinates will develop lesions of 1 or a mild 2. Cecal cores may be found in cull birds or birds that have been off feed.

IMPACT OF LESIONS ON WEIGHT GAIN AND PERFORMANCE

A pen trial conducted by Dr. Greg Mathis at Southern Poultry Research (2) compared weights and feed conversions of birds vaccinated with a coccidiosis vaccine (Coccivac-B™) to birds fed salinomycin (60 g / ton). The vaccinates were vaccinated at day-of-age using a 21-cc spray cabinet. The salinomycin group was fed at the 60 g / ton rate from day 1 through day 35. Both groups were fed bacitracin (BMD™ - Alpharma, Ft. Lee, NJ) at 50 gm / ton in the starter and grower rations. Stafac™ (Pfizer -NY, NY) was used in the withdrawal ration. No amprolium was administered to either group. The trial used Ross X Ross broiler chickens fed a ration designed for maximum growth rate. The design was intended to maximize any weight or performance deficit that might be induced by the coccidiosis vaccine. The birds were weighed individually at 14, 21, 28, 35, 42 and 49 days of age. The results are summarized in Table 1. At 21 days, the vaccinated birds had statistically poorer weight gain and feed conversion than the birds fed salinomycin. Between 28 and 42 days, the vaccinated birds gained more weight with similar feed conversion than the coccidiostat group, making up the deficit seen at 21 days. At 42 and 49 days of age, both groups had equal weight and equal feed conversion.

MANAGEMENT OF A COCCIDIOSIS VACCINATION PROGRAM

Control of lesion development through uniform vaccination is the first step toward making immunization a viable coccidiosis control program. Management of feed access, litter conditions and environmental stress through the peak lesion impact period (16 to 28 days) is also critical to maximum performance. The control of necrotic enteritis during peak lesion development remains an important consideration. Feed additive antibiotics with anti-*Clostridium spp.* activity are recommended at maximum levels during this time period. Where a completely drug-free program is desired, alternative precautions must be taken to control necrotic enteritis.

SUMMARY

Uniform vaccine application via spray cabinet minimizes adverse lesion development and yields equivalent weight gain and performance in vaccinates compared to flocks fed salinomycin. This is the first step toward growing a market-competitive drug-free broiler chicken.

Table 1. Performance: vaccinates vs. salinomycin-fed broilers

	Vaccinates		Salinomycin	
Age (days)	Feed Conversion	Weight (gm)	Feed conversion	Weight (gm)
14	1.19	340.2	1.19	353.8
21*	1.46*	694.0*	1.42*	739.4*
28	1.57	1174.8	1.55	1202
35	1.72	1705.5	1.71	1723.6
42	1.86	2331.5	1.86	2340.5
49	1.94	2812.3	1.94	2807.7

*Denotes statistically significant weight and feed conversion differences between groups.

REFERENCES

1. Johnson, J., and W. M. Reid. Anticoccidial drugs: Lesion scoring techniques in battery and floor-pen experiments with chickens. Exp. Parasitol 28:30-36. 1970.

2. Mathis, G. F. The influence of coccidiosis vaccine Coccivac-B on compensatory weight gain in broiler chickens in comparison with the anticoccidial salinomycin. In: Southern Poultry Science Society Conference, Atlanta, GA. January 18-19. 1999.

PRACTICAL MANAGEMENT OF A DRUG-FREE BROILER PROGRAM

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As a marketing niche, many poultry companies are considering production of natural birds. This may improve profitability for companies because customers are willing to pay a premium for birds produced naturally. There are many definitions of natural or drug-free birds. For the purpose of this presentation, natural or drug-free birds are those that have never been exposed to any type of drug in the feed or water during any phase of life. Producing natural birds may seem like an easy way for broiler companies to improve their bottom line, but it is much more difficult than it may appear at first glance. In the following presentation, programs that have been used to produce drug-free broilers will be discussed.

Necrotic enteritis is the nemesis of a drug-free program. Without the inclusion of growth promotional antibiotics or ionophores in the rations, necrotic enteritis may cause reduced weight gain, reduced livability, and higher feed conversion. The duration of clinical illness may be very short with birds appearing healthy and then found dead in the houses. When birds are posted, the intestines are often friable and enlarged with gas. The internal surface of the gut may be covered by a pseudomembrane. Mortality and reduced performance are caused by *Clostridium perfringens* in combination with dietary factors and damage of the gastrointestinal tract. Outbreaks in broilers may occur between 2-5 weeks. After an episode of necrotic enteritis, *Clostridium perfringens* counts will be very high and a recurrence is likely. A complete house clean-up will result in reduction of bacterial numbers.

Integral in the management of necrotic enteritis and production of drug-free birds is the development of immunity to coccidia. Excessive reactions to vaccination or excessive field leakage in the absence of growth promotional antibiotics may result in clinical necrotic enteritis. Uniform distribution of coccidiosis vaccine will result in the least likelihood of problems. This will ensure that birds develop immunity early and will minimize problems later in the bird's life. Field results from spray vaccination have been outstanding. After spray vaccination the birds preen and ingest the vaccine orally. It is believed that this method of vaccination yields improved coverage over methods used in the past. In addition to even vaccine distribution, the minimization of *C. perfringens* numbers will drastically improve the likelihood of success. Some companies have the luxury of rotating housing so that drug-free birds will be grown in a new house every time. This is only possible in operations where there are two programs. One program would include a standard anticoccidial with a growth promotional antibiotic. The other program would be drug-free. It is believed that *C. perfringens* numbers increase over time on a drug-free program. Constant rotation of housing will minimize problems.

In the last several years, the use of competitive exclusion products has risen to the forefront because of the need to eliminate the pathogens that result in food-borne illness. It is this same principle that may allow the control of necrotic enteritis. It has been demonstrated that the use of some competitive exclusion products may control the disease (1). Practical field experience has yielded mixed results in recent field work. The use of natural competitive exclusion is also an important part of a total program. It was previously believed that placing birds in a perfectly sterile house would eliminate the probability of all diseases. With the current understanding of competitive exclusion, this concept has been challenged. By placing birds on used litter, natural competitive exclusion will occur. In other words, the normal chicken intestinal bacteria will inhabit the gut. The use of old litter will help birds to establish a normal balance, thus minimizing the likelihood of problems with necrotic enteritis. It is logical that if the gut is occupied by the good bacteria, then it is more difficult for other bacteria to set up shop.

In summary, some consumers are willing to pay the price for poultry never exposed to drugs. To cost effectively produce natural birds, the following factors appear to be important in minimizing the chief problem of necrotic enteritis. First, uniform vaccination against coccidia is extremely important. Secondly, the rotation of houses in operations where there are two programs may be helpful. This will minimize the build-up of *C. perfringens*. Finally, the use of competitive exclusion products and natural competitive exclusion may play an increasingly important role in the future.

REFERENCE

1. Hofacre, C. L., R. Froyman, B. Gautrias, B. George, M. A. Goodwin, and J. Brown. Use of Aviguard and other intestinal bioproducts in experimental Clostridium perfringens-associated necrotizing enteritis in broiler chickens. Avian Dis 42:589-596. 1998.

THE ROLE OF LOCAL PUBLIC HEALTH AGENCIES IN AVIAN MEDICINE

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The role that California state agencies (the University of California, Davis and its California Veterinary Diagnostic Laboratory System (CVDLS), and the Animal Health Branch of the California Department of Food and Agriculture) play in avian health and medicine is well-recognized. Additionally, the Veterinary Public Health Section of the California Department of Health Services administers the State's Psittacosis Control Program. What is not generally known are the contributions made by local (county) public health agencies.

There are probably two major reasons for the above lack of perception. The first is that health departments exist primarily and rightly so, to protect the health of the public and second, except for a few counties which employ county/and or public health veterinarians, the involvement of such agencies with avian diseases or avian-related problems other than routine inspection of poultry farms where these exist and the investigation of poultry or poultry product-associated food-borne illnesses, may be considered not significant.

The question then is in what specific areas of avian health and medicine do local health departments play a role? Although mention will be made of other counties where appropriate, for the sake of clarity, this paper will discuss mainly the San Bernardino County Department of Public Health experience.

Cognizant of the significant numbers of domestic animals particularly poultry and dairy cows in San Bernardino County, the County Board of Supervisors found it fit to include provisions for county veterinary services in the County Code. Thus, although the county employs public health veterinarians who are under the Health Department's Division of Preventive Medical Services, they perform a dual function in that with the assistance of both the California Department of Food and Agriculture (CDFA) and the U.S. Department of Agriculture (USDA), they are responsible for the prevention and control of not only the zoonotic diseases but of other infectious diseases of animals (livestock, poultry, companion animals, pet birds, etc.) as well. In zoonotic disease investigations, the public health veterinarians work closely with the Epidemiology Section and Environmental Health Services. In the investigation of all infectious diseases both the Public health Laboratory and the CVDLS are utilized depending upon the problem. Some of these investigations involving avian species have been reported in previous WPDC and other meetings (6,13,14,15,16,17,18,19). An interesting development which may have an impact on psittacosis investigation and control is the repeal in 1998 of the parakeet leg banding law by the California Legislature.

Aside from disease investigations, county veterinary services, officially organized as the Preventive Veterinary Services Section, conduct disease surveillance through the inspection of auction yards, swap meets, pet shops, and other animal establishments. Although poultry are mostly seen in these places, other avian species (game birds, pet birds, and exotic or non-domestic birds) are also encountered. Many of these birds originate from areas outside of San Bernardino County. The continuous importation of exotic birds provides an ever present threat of not only the traditional infectious diseases but emerging infectious diseases as well. (From a historical standpoint, this author, along with the Senior Public Health Veterinarian at the time, may have been one of the first individuals to have seen velogenic, viscerotropic, Newcastle disease (VVND) in parrots but did not know what it was, when we inspected the aviary which eventually was suspected to have been the source of the VVND epidemic in Southern California in 1972. The owner was advised to submit some of the dead birds to the State Laboratory which was located in San Gabriel at that time.)

The Vector Control section of the Department's Division of Environmental Health performs periodic inspections of commercial poultry ranches. Although these inspections are directed mainly to the control of flies and fly-breeding, e.g. manure management, ranches are also checked for the presence of rodents and other vectors/predators. The proper disposal of dead birds and waste eggs is also monitored.

It is interesting to note that in connection with arbovirus surveillance by local health agencies, Culex mosquitoes collected in the Yuma area in Arizona tested positive for Western Equine Encephalitis (WEE) antibodies and about the same time, several young spoonbills at the Phoenix Zoo developed neurologic signs and died. One bird, which tested positive for WEE antibodies survived but had residual blindness. Mosquito control was subsequently carried out by county and zoo officials (1).

Another area of activity by local health agencies which has relevance to poultry health and medicine is the investigation of cases or outbreaks of foodborne illnesses. Such investigations are team efforts involving health department staff from the Epidemiology and Environmental Health sections and the Public Health Laboratory.

THE ROLE OF THE PUBLIC HEALTH LABORATORY

Although the public health laboratory is geared to the examination of human specimens, it also receives environmental and animal specimens. The latter include brains for rabies, droppings for enteric organisms, hair and skin scrapings for fungi, etc. There have been instances of human zoonotic cases in the which the laboratory results were used to trace back to the avian source(s). This is particularly true of psittacosis, *Salmonella* and campylobacter infections. The public health laboratory is also utilized for certain disease studies. For example, a limited study was conducted by the San Diego County public health laboratory in which eight whole raw chickens processed by five different processors and purchased from various supermarkets in San Diego County were examined for *Salmonella.* The following results were obtained: 7 had Campylobacter, 4 had both Campylobacter and *Salmonella* and 1 had neither (Sabet, personal communication, 1999). In another study conducted jointly by the San Bernardino County Vector Control Program and the Public Health Laboratory, from 49 pools of 4 different fly species populating poultry ranches in San Bernardino County tested for *Salmonella*, 7 pools of *Musca domestica* tested positive (10).

SALMONELLA ENTERITIDIS IN SOUTHERN CALIFORNIA

An avian disease of particular importance to the human population of San Bernardino County in recent years has been *Salmonella enteritidis* (SE) associated with consumption of raw or undercooked eggs. This issue has affected all of Southern California (personal communication, Southern California Epidemiology Exchange (SCEE), Spring, 1995).

Since the late 1980's, when SE accounted for less than 10% of all *Salmonella* serotypes in Southern California, this organism has been responsible for an increasing proportion of all isolates reaching 46% in 1995 (personal communication, SCEE, 1995). SE has been implicated in both epidemic illness (11) and in sporadic cases (12). All of Southern California has been affected while there has been until recently, minimal change in *Salmonella* epidemiology in Northern California and in neighboring states. Throughout this remarkable period of increased prevalence an association with eating raw or undercooked shell eggs, often in egg dishes involving other ingredients as well, has been so consistent that the need to re-study SE in chickens in Southern California has become apparent. When appropriately tested, 90% or more of all SE isolates associated with cases in Southern California have been found to be phage type 4 (personal communication, SCEE, 1995; 12).

Europe and the eastern United States have also experienced increases in clinically proven cases of SE. At least in the United States, heavily contaminated farm environments were rather easily identified as the most probable source for many outbreaks (9). Despite tracebacks and other repeated efforts no such "hot" farms have been identified to explain human illness in Southern California. Although the complex channels of the egg distribution system in Southern California have made the traceback process somewhat difficult, there is at present adequate basis for believing the epizootiology of these organisms is different in California (isn't everything?). Feed, water, rodents, insects, layer flocks and farms where chicks mature to layer hens have all been listed as sources of possible sporadic introduction of SE into egg ranches, but proof of any single route remains elusive with the exception of one farm in which infected wild rodents were identified, presumably infected by exposure to sewage treatment effluent (7,8).

In response, the egg industry, working with California State Health and Agriculture officials have attempted to improve egg handling systematically, from farm to table. Farms and distributors now routinely refrigerate eggs, including during transport. Retailers have been required to provide refrigeration as well and restaurants and consumers have been warned not only to keep eggs refrigerated, but also to cook them thoroughly before eating (4). In the past 2-3 years, the number of total *Salmonella* cases reported to local health departments in Southern California has declined. The proportion of SE to all isolates tested remains high, however (personal communication, SCEE, Spring, 1998). The recent changes could be the general impact of the egg quality assurance efforts or, unfortunately, these changes could also reflect a decline in effective case finding and reporting - a hypothesized possible side effect of the increasing number of people whose medical care come from pre-paid health plans, in which non-essential laboratory testing (like culturing for *Salmonella*) can be seen as an inefficient and unnecessary use of resources. In addition to continued efforts to define and control this problem among humans, the need seems obvious for further research of the avian factors which could be controlled. Such efforts have been undertaken and must be continued (2,3,5), but local investigations, in response to ongoing disease occurrence/reporting will remain a critical element of disease control for SE.

REFERENCES

1. Arizona Department of Health Services Vector-Borne and Zoonotic Disease Newsletter. Sept.,1997.

2. Barrow, P. A., and M. A. Lovell. Experimental infection of egg-laying hens with Salmonella enteritidis phage type 4. Avian Path. 20:335-348. 1991.

3. Bichler, L. A.,K. V. Nagaraja, and D. A. Halvorson. Salmonella enteritidis in eggs, cloacal swab specimens, and internal organs of experimentally infected White Leghorn chickens. Am. J. Vet. Res. 57:489-495. 1996.

4. Blumenthal, D. Salmonella enteritidis, from chicken to the egg. FDA Consumer Mag. DHHS Pub. No. (FDA) 91-2238. April, 1990.

5. Corkish, J. D., R. H. Davies, C. Wray, and R. A. J. Nicholas. Observations on a broiler breeder flock naturally infected with Salmonella enteritidis phage type 4. Vet. Rec. 134:591-594. 1994.

6. Franklin, T. E., J. V. Tacal, and B. M. Daft. Feather follicle cysts - a case report. Proc. 32nd West. Poult. Conf.,Davis, California. pp. 115-116. 1983.

7. Kinde, H., D. H. Read, A. Ardans, R. E. Breitmeyer, D. Willoughby, H. E. Little, D. Kerr, R. Giercesh, and K. V. Nagaraja. Sewage effluent: likely source of Salmonella enteritidis phage type 4 infection in a commercial chicken layer flock in Southern California. Avian Dis.40:672-676. 1996.

8. Kinde, H. Salmonella enteritidis phage type 4 infection in a Southern California chicken flock. Calif. Vet. Diag. Lab Notes. 8:1,2-5. 1995.

9. Levy, M. Outbreaks of Salmonella serotype enteritidis infection associated with consumption of raw shell eggs United States 1994-1995. MMWR. 45:737-742. 1996.

10. Mian, L. Vector-borne disease surveillance in San Bernardino County during 1996. SOVE (Soc.for Vector Ecology) Newsletter. 28:10-12. 1997.

11. Mohle-Boetani, J. C., S. Benson Werner, S. Abbott, N. Bendana, R. Bryant, M. Fentersheib, M. Ginsberg, L. Gresham, J. Koehler, and L. Mascola. Salmonella enteritidis infections from shell eggs: outbreaks in California. West. J. Med. 169:299-303. 1998.

12. Passaro, D. J., R. Reporter, L. Mascola, L. Kilman, G. B. Malcolm, H. Rolka, S. Benson Werner, and J. Vugia. Epidemic Salmonella enteritidis infection in Los Angeles County, California - the predominance of phage type 4. West. J. Med. 165:126-130. 1996.

13. Tacal, J. V. Recent trends in psittacosis. San Bernardino County Dept. Pub. Hlth. Reporting to Physicians 24:3. 1980.

14. Tacal, J. V. Epidemiology of selected cases of psittacosis among pet birds and humans. Proc. 33rd West. Poult. Dis. Conf.,Davis, California. pp 113-115. 1984.

15. Tacal, J. V. Some disease problems of backyard poultry in San Bernardino County. Proc. Jt. Mtg. 35th West. Poult. Dis. Conf. and XI Conv. Annual , ANECA, Puerto Vallarta, Mexico. pp. 190-191. 1986.

16. Tacal, J. V, B. Daft, and J. McClaine. Case report: oleander (Nerium oleander) poisoning in two geese. Proc. 38th West. Poult. Dis. Conf., Tempe, Arizona. pp 167-168. 1989.

17. Tacal, J. V. Case report: heavy infestation of a backyard poultry with the fowl tick, Argas persicus sanchezi. Proc. Jt. Mtg. 40th West. Poult. Dis. Conf. and 16th Conv. Annual, ANECA, Acapulco , Mexico. pp. 266-267. 1991.

18. Tacal, J. V. and R. Morales. Birds, cryptococcosis and HIV infection in humans. Proc. 44th West. Poult. Dis. Conf.,Sacramento, California. pp. 29-31. 1995.

19. Tacal, J. V. Ectoparasites of pets: the public health perspective. Proc. 4th Intl. Symposium on Ectoparasites of Pets.Riverside, California pp. 11-13. 1997.

CHANGES IN THE MICROFLORA OF COMMERCIAL TURKEYS FOLLOWING ANTICOCCIDIAL REMOVAL AND ADDITION OF GROWTH PROMOTANT ANTIBIOTICS

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INTRODUCTION

The gastrointestinal (GI) tract of poultry is densely populated with numerous types of bacteria throughout its length. This microflora has a substantial impact on its host and has both beneficial and detrimental qualities. For instance, although the microflora is essential for competitive exclusion purposes, it comes at the expense of some degree of performance loss as the bacteria compete with the bird for nutrients and may reduce efficiency. This trade-off is acceptable in order to manage and grow turkeys in today's commercial operations.

This same microflora is dynamic and changes in population can occur due to many different factors and/or stresses. For example, it has been documented that heat stress causes a rapid increase of certain normal bacterial populations which make-up the microflora. Other influences such as antibiotic usage, ingredient changes, or certain enteric pathogens can have similar effects. It is quite reasonable to suppose that these same microfloral changes are in part responsible for how field or clinical enteric responses express themselves, however, very little research has been directed into this area. Thus, the rationale for this study was to investigate the microflora changes which occur during the transition period of rotating from an ionophore anticoccidial to different approved growth promotant antibiotics.

MATERIALS AND METHODS

One hundred and fifty, healthy 5-week-old commercial turkey hens were transported from the field and randomly placed into five separate, environmentally controlled floor pens containing 30 birds each. They were then provided feed containing monensin at 72 g/ton for a period of 3 weeks to allow the turkey's microflora to adjust and acclimatize. At 8 weeks of age, each pen was provided a separate treatment diet with the following premixes added as follows:

Treatment 1 – No antibiotic or anticoccidial
Treatment 2 – Monensin at 72 g/ton
Treatment 3 – Virginiamycin at 20 g/ton
Treatment 4 – Bambermycin at 2 g/ton
Treatment 5 – Bacitracin at 50 g/ton

The treatment diets were supplied for a 16 day period during which time 5 hens from each pen were sacrificed at various intervals as follows: 1 day before feed change and 1 day, 3 days, 7 days, and 16 days after feed change. Sections of the crop, jejunum, and cecum from each bird were removed, weighed and placed into Cary-Blair medium. The samples were then homogenized after which serial dilutions were made for plating onto appropriate agar for bacterial enumeration. The oxyrase anaerobic system was used to produce the conditions for incubating the anaerobic plates, and all plates were incubated at 37°C. The number of typical colonies were then counted after a few days incubation and multiplied by the dilution factor to determine the number of colony forming organisms per gram of intestinal material. The groups of bacteria investigated and agar used were:

Lactobacilli: Deman, Rogosa, and Sharpe agar
Enterobacteriacae: Violet Red Bile agar
Clostridia: PRAS-Clostrisel agar
Bacteriodes: PRAS Bacteriodes Bile Esculin agar
Total Anaerobes: PRAS Brain Heart Infusion agar
Total Aerobes: Plate Count agar

RESULTS AND DISCUSSION

After removal of the anticoccidial from the feed and addition of the growth promotant antibiotics, transient changes occurred in bacterial numbers in limited portions of the intestinal tract. Enterobacteriacae counts in the crop and jejunum were reduced the day after removal of monensin and replacement with feed containing no feed additives, but increased to former levels by day 3 after feed change. Lactobacilli, total anaerobic, and clostridia counts in the crop decreased 7 days after removal of the anticoccidial and addition of virginiamycin, bambermycin, and bacitracin, but were similar in all treatments by day 16. Bacitracin also reduced total anaerobic and clostridial counts in the ceca on day 3 after feed change.

In addition, the day after feed change, temporary increases in counts not related to differences in feed treatment occurred for the lactobacilli in the crop, the lactobacilli and total anaerobes in the jejunum, and in the

total anaerobes and clostridia of the ceca. This could imply that the general stress initiated by the feed changes caused a transient rapid increase in the bacterial populations studied. However, the microflora returned to former levels by days 7 and 16.

When counts from different bacterial groups, different times after feed change, and different intestinal portions were grouped and analyzed, several effects were noted. Overall counts increased from the day before feed change to higher levels on days 1 and 3 after feed change but returned to baseline levels by days 7-16. Counts in the ceca were greater than counts in the crop which were greater than those in the jejunum. Counts from highest to lowest when comparing different bacterial groups were as follows: total anaerobes > lactobacilli > clostridia > total aerobes > entero-bacteriacae>bacteriodes.

No differences were noted in counts for any of the bacterial groups enumerated when comparing the three antibiotic treatments. This finding is somewhat surprising in that the different products have varying spectrums of activity. The microflora, under normal circumstances, has the capacity to withstand temporary changes induced when switching from monensin to the growth promotant antibiotics commonly used by the industry. This is only one study conducted under ideal conditions and significant differences could be noted under another set of parameters. There is much more to learn about the turkey microflora.

USE OF A COMMERCIAL COMPETITIVE EXCLUSION PRODUCT IN EXPERIMENTAL CLOSTRIDIUM PERFRINGENS ASSOCIATED NECROTIC ENTERITIS

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SUMMARY

Clostridium perfringens associated necrotic enteritis (NE) is a common problem among rapidly growing broiler chickens that are raised intensively. It was found, throughout 3 studies, that the competitive exclusive (CE) product Aviguard which contains the normal bacterial flora of a chicken ceca was as effective as the antibiotic virginiamycin in preventing NE gross intestinal lesions and mortality. It was also found that Aviguard was more effective reducing NE gross lesion scores than 2 other commercial C.E. products, a probiotic and 3 additional antibiotics flavomycin, avoparcin, and bacitracin.

LITERATURE REVIEW

Necrotic enteritis (NE) in domestic poultry has been well documented since it was first described by Parish in 1961 (8) and that *Clostridium perfringens* is the causative agent (2,4,8). There are many conditions that can predispose the chicken's intestine to an overgrowth of *C. perfringens*. In general, these include diets high in energy and protein from ingredients such as wheat, barley, or high levels of fishmeal (3,4). Damage to the intestinal mucosa is another predisposing factor; for example, injury caused by various strains of coccidia (1,4). Prevention of NE has been accomplished by supplementation of feed with antibacterial compounds such as avoparcin, bacitracin, virginiamycin,and ionophores (7,10,11). Recent European Union Directives indicate a ban on the use of bacitracin zinc, spiramycin, virginiamycin, tylocin, and avoparcin in animal feed beginning July 1, 1999. In those countries that have already limited the use of these drugs, there has been an increase in *C. perfringens* associated NE (9).

Work by Fukata and others has indicated that the intestinal microflora can have an impact on the incidence and/or severity of NE (2,5). The purpose of these 3 studies was to determine whether a commercially available nondefined competitive exclusion (CE) product could reduce NE lesions and mortality.

MATERIALS AND METHODS

Chicken husbandry, groups, and treatment. Study 1 and 2 used one-day-old male broiler chicks, housed in battery cages. Study 3 used one-day-old mixed sex broiler chicks in 72 floor pens covered with soft wood shavings. The experimental design can be found in Table 1. The CE products were given by oral gavage except for one treatment in study 3, where the CE product Aviguard was also administered in the chicken's first 4 hours of drinking water. The antibiotics were included in all diets of the birds in an antibiotic treatment group at the levels indicated in Table 1.

Table 1. Experimental design.

Study No.	Treatment	Level	C. perfringens Challenge	No. of Cages/Pens	No. of Birds/Cage/Pen	Total No. of Birds
	Control	n/a	yes	12	12	144
	Control	n/a	no	12	12	144
1	Aviguard	12.5mg/bird	yes	12	12	144
	Virginiamycin (VM-20)[1]	20 g/t	yes	12	12	144
	Bacitracin MD (BMD)[2]	50g/t	yes	12	12	144
	Control	n/a	yes	12	12	144
	Control	n/a	no	12	12	144
2	Aviguard[3]	12.5 mg/bird	yes	12	12	144
	CE-A[4]	1.0 mg/bird	yes	12	12	144
	CE-B[5]	12.5 mg/bird	yes	12	12	144
	Probiotic[6]	unknown	yes	12	12	144
	Control	n/a	yes	8	56	448
	Control	n/a	no	8	56	448
	Aviguard H_20	12.5 mg/bird	yes	8	56	448
3	Aviguard	12.5 mg/bird	yes	8	56	448
	VM-20	20 g/t	yes	8	56	448
	BMD	50 g/t	yes	8	56	448
	Flavomycin[7]	2 g/t	yes	8	56	448

[1] Virginiamycin, Pfizer Agriculture Division, New York, New York.
[2] Bacitracin MD, A. L. Pharma, Inc., Ft. Lee, N.J.
[3] Aviguard, Bayer Animal Health, Shawnee Mission, Kansas.
[4] CE-A: Broilact, Orion Corporation Animal Health, Medipharm AB, Sweden.
[5] CE-B: Avi-Free, Alltech Biotechnology Center, Nicholasville, Kentucky.
[6] Probiotic, Interbac, Intervet Canada, Whitley, Ontario
[7] Flavomycin, Hoechst-Roussel Animal Health, Somerville, N.J.

Necrotic enteritis challenge model. The challenge model as previously described by George *et al.* uses a standard broiler diet to which fishmeal is added at a 26% level (6). This feed was fed from day 0 to 14 days of age. On day 14, the chicks had gavaged into the crop 50,000 *Eimeria acervulina* oocysts. Then, on days 17, 18, 19, and 20, each challenge chick was given by crop gavage approximately 10^7 colony forming units of a fresh *C. perfringens* culture. On days 21 and 27 or 28, chicks were necropsied and examined grossly for intestinal lesions characteristic of necrotic enteritis (4). Lesion scores were: 0 = none, 1 = mild, 2 = moderate, and 3 = marked (severe).

Biometrics. The mortality data were compared using a chi-square test (12). All other data were analyzed using an analysis of variance with a follow-up Tukey multiple comparison test (12).

RESULTS

Results can be found in Table 2. Chickens that received no treatment did not die and did not develop necrotic enteritis in any of the 3 studies.

In study 1, Aviguard, virginiamycin, and bacitracin were equally effective in preventing NE mortality induced by the challenge model. Chicks that were treated with Aviguard or virginiamycin had significantly lower total intestinal gross lesion scores than their bacitracin counterparts; Aviguard or virginiamycin treated chicks were not significantly different from each other at either day 21 or 27.

In study 2, necrotic enteritis death rates among chicks treated with Aviguard, CE-A, or CE-B were not significantly different from each other; however, deaths among chicks given these treatments were significantly lower than deaths among chicks treated with the probiotic. Chicks treated with Aviguard had significantly lower lesion scores than chicks treated with CE-A, CE-B, or the probiotic; these three microbial treatments were not different from each other at either day 21 or 27.

In the floor pen study 3, there were no significant differences in NE mortality between the treatment groups. Also, there were no differences at day 22 in the NE lesions scores. However, Aviguard administered in the water and virginiamycin were more effective than bacitracin or flavomycin in lowering 28 day NE lesion scores.

Table 2. Mortality and 28 day of age lesion scores from 3 studies on experimental C. perfringens associated with necrotic enteritis (NE).

Study No.	Treatment	Mean Mortality (%)[A]	Lesion Scores (28 day)
1	Challenge Control	4.2[a]	2.4[a]
	Negative Control	0.0[b]	0.0[b]
	Aviguard	0.0[b]	1.3[c]
	Virginiamycin	2.1[b]	1.4[c]
	Bacitracin MD	0.7[b]	2.0[a]
2	Challenge Control	2.1[a]	2.20[a]
	Negative Control	0.7[a]	0.03[b]
	Aviguard	0.7[a]	1.06[c]
	CE-A	0.7[a]	2.11[a]
	CE-B	1.4[a]	1.93[a]
	Probiotic	6.3[b]	2.59[a]
3	Challenge Control	1.34[a]	1.39[a]
	Negative Control	0.45[a]	0.01[b]
	Aviguard H_2O	1.12[a]	0.63[c]
	Aviguard Gavage	0.89[a]	0.63[c]
	Virginiamycin	1.12[a]	0.46[c]
	Bacitracin	1.12[a]	0.91[d]
	Flavomycin	0.67[a]	1.01[b]

[A] Numbers are mean values. Numbers with different letters are significantly different at the 5% level of probability.

DISCUSSION

The NE challenge model used in these studies was effective in inducing the mortality and gross intestinal lesions associated with a *C. perfringens* necrotic enteritis. Equally important, the partially defined CE product, Aviguard, was found to be as effective in reducing NE mortality and intestinal lesion scores as the antibiotic virginiamycin and more effective than bacitracin. In addition, Aviguard was found to be more effective than two other CE products or a probiotic for reducing gross lesions induced by the NE challenge model. When the challenge model was used in a floor pen study, Aviguard administered in the drinking water and virginiamycin were found to be more effective in lowering *C. perfringens* associated necrotic enteritis lesions than either bacitracin or flavomycin.

This work is supported by the findings of Fukata that the intestinal microflora can impact the incidence and severity of the disease necrotic enteritis (5). Results from these studies are important because CE products, such as Aviguard, can be considered a novel and effective non-antibiotic disease preventative in the face of *C. perfringens* associated necrotic enteritis challenge.

REFERENCES

1. Al-Sheikkly, F., and A. Al-Saieg. Role of coccidia in the occurrence of necrotic enteritis of chickens. Avian Dis. 24(2):324-333. 1980.

2. Barnes, E. M., G. C. Mead, and D. A. Barnum. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. Br. Poult. Sci. 13:311-326. 1972.

3. Branton, S. L., and F. N. Reece. Influence of a wheat diet on mortality of broiler chickens associated with necrotic enteritis. Poult. Sci. 66:13326-13330. 1987.

4. Ficken, M. D., and D. P. Wages. Necrotic enteritis. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Iowa State University Press, Ames, Iowa. pp. 261-264. 1997.

5. Fukata, T., Y. Hadate, E. Baba, and A. Arakawa. Influence of bacteria on *Clostridium perfringens* infections in young chickens. Avian Dis. 35:224-227. 1991.

6. George, B. A., C. L. Quarles, and D. J. Fagerberg. Virginiamycin effects on controlling necrotic enteritis infection in chickens. Poult. Sci. 61:447-450. 1982.

7. Kaldhusdal, M., and E. Skjerve. Association between cereal contents in the diet and incidence of necrotic enteritis in broiler chickens in Norway. Preventive Vet. Med. 28:1-16. 1996.

8. Parish, W. E. Necrotic enteritis in the fowl (Gallus gallus domesticus). I. Histopathology of the disease and isolation of Clostridium welchii. J. Comp. Pathol. 71:377-393. 1961.

9. Schaller. National Veterinary Institute, Norway. Personal communication, 1998.

10. Stutz, M. W., S. L. Johnson, and F. R. Judith. Effects of diet and bacitracin on growth, feed efficiency, and populations of Clostridium perfringens in the intestine of broiler chicks. Poult. Sci. 62:1619-1625. 1983.

11. Wicker, D. L., W. N. Isgrigg, J. H. Trammell, and R. B. Davis. The control and prevention of necrotic enteritis in

broilers with zinc bacitracin. Poult. Sci. 56:1229-1231. 1997.
12. Zar, J. H. Biostatistical Analysis, 2nd ed. Prentice Hall, Inc., Englewood Cliffs, NJ. p. 718. 1978.

FACTORS INFLUENCING THE MEASUREMENT OF THE EFFICACY OF COMPETITIVE EXCLUSION PRODUCTS WITH A RECOMMENDED ASSAY

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INTRODUCTION

The principle of competitive exclusion (CE) was first described in 1973 by Nurmi and Rantala (3) and Rantala and Nurmi (5). It is also known as the Nurmi concept (4) and is now widely accepted by poultry scientists, veterinarians and poultry producers as a method of reducing or inhibiting the intestinal colonization of newborn chicks with *Salmonella* organisms. Currently, about 400 million chicks worldwide are treated with CE floras per year. In 1989, scientists working in the field of CE met to discuss the testing requirements for CE products. The result of this meeting was a test method, which is known as the recommended assay of Mead *et al.* (2). As CE preparations are live biological products and the test system uses live animals, numerous factors may influence the efficacy of CE products and test results obtained with them. An overview of general factors, including storage of the CE product, bird susceptibility, stress, coccidiosis, and antimicrobial treatment, was given by Bailey in 1987 (1). The paper presented here, deals with methodological factors, which have an influence on the efficacy results of CE products obtained in the recommended assay of Mead *et al.* (2).

MATERIALS AND METHODS

Thirteen trials were conducted according to the recommended assay of Mead *et al.* (2).

Trial No. 1. Four sachets of the same batch of the CE product Aviguard were compared to investigate the reproducibility of the efficacy results. Three replicates of 12 birds were used per sachet. The chicks were treated with Aviguard at one-day-old. One day later they were challenged with approximately 10^4 *Salmonella kedougou* cells. Seven days after challenge, the chicks were necropsied and the level of caecal *Salmonella* colonization was measured. Based on the colonization levels of the individual birds, the infection factor (IF) and $\log_{10}$ reduction (LR) were calculated for each of the replicates. The formulas used for the calculations are given below:

$$IF = \frac{\log_{10}(\text{bird 1}) + \ldots + \log_{10}(\text{bird 12})}{12}$$

$$LR = IF_{\text{challenge controls}} - IF_{\text{test group}}$$

Trial No. 2. In a second reproducibility study, five replicates of birds were treated with the same Aviguard solution and challenged 24 hours later with approximately 10^4 *Salmonella kedougou*.

Trial No. 3. The reproducibility of the challenge model was investigated using five replicates of 12 birds each. They did not receive an Aviguard treatment, but were challenged with *S. kedougou*.

Trial No. 4. A further reproducibility study, similar to trial no. 3, compared two different *S. kedougou* cultures in five replicates of 12 birds per challenge culture.

Trial No. 5. The effect of *S. kedougou* challenge doses varying between 10^1 and 10^7 per bird was evaluated in untreated birds using 2 replicates of 12 birds per dose level.

Trial No. 6. The kinetics of *Salmonella* colonization was assessed in untreated chicks after a challenge with 10^1, 10^4, and 10^7 *S. kedougou* cells. Twelve birds were challenged with each of the different doses and two birds each were necropsied one, two, three, four, five and six days after challenge.

Trial No.7. Four replicates of 12 birds each were treated with Aviguard and challenged 24 hours later. The protection factor (PF) and the $\log_{10}$ reduction (LR) were compared for the assessment of the results. The formula for the PF is given below.

$$PF = \frac{IF_{\text{challenge controls}}}{IF_{\text{test group}}}$$

Trial No. 8. The influence of three different challenge doses (10^1, 10^4, 10^7) on the *Salmonella* exclusion properties provided by Aviguard was assessed. Three replicates of 12 birds each were allocated to the three treatment groups.

Trial No. 9. The influence of different breeds of birds, SPF, commercial Ross broilers and Ross grandparent birds, on the IF after a challenge with approximately 10^4 *S. kedougou* was investigated in four replicates of 12 birds per breed.

Trial No. 10. Three replicates each of 12 SPF, commercial Ross broilers and Ross grandparents were included in this experiment to assess the influence of the different breeds on the potency results obtained in the complete assay of Mead *et al.* For this purpose, the number of birds in three categories of protection was calculated, i.e. fully protected (FP = not colonized), partially protected (PP = colonized between 10^2 and 10^5) and not protected (NP = colonized above 10^5).

Trial No. 11. The effect of a *S. kedougou* challenge on different days was assessed by inoculating 3 replicates of 12 birds each one day, three replicates two days and another 3 replicates 3 days after Aviguard treatment.

Trial No. 12. The LR of 6 replicates of 12 birds each, which were kept on a wire floor, was compared to 6 replicates kept under standard husbandry conditions on coarse wood shavings.

Trial No. 13. Aviguard, an undefined CE product, was compared to a defined CE product and a probiotic culture. Three replicates of 12 birds each were used per treatment.

RESULTS

Trial No. 1. The LRs obtained after treatment with four sachets of the same Aviguard batch ranged from 6.3 to 6.9. Thus, a good reproducibility of the efficacy results could be shown.

Trial No. 2. The LRs of four replicates ranged between 5.3 and 6.3. However, the LR of one replicate was not higher than 2.9. This is an example for a low efficacy result, which can occasionally be seen in a replicate of birds.

Trial No. 3. The IFs of the five replicates ranged from 6.3 to 6.9 with a coefficient of variation (CV) of 11% indicating a good reproducibility of the *S. kedougou* challenge model.

Trial No. 4. The mean IF of both *S. kedougou* cultures was similar, close to 8 $\log_{10}$. The CV of the five replicates of culture 1 was 9.5%, the CV of culture 2 was 11%. Again a good reproducibility of the challenge model could be shown.

Trial No. 5. Seven days after challenge, the IFs of all replicates of birds regardless of the initial challenge dose were very similar, i.e. around 8.5 $\log_{10}$.

Trial No. 6. The chicks challenged with the highest dose reached maximum colonisation levels already 24 h post challenge. This took two days and three days longer for the birds challenged with a medium and low dose, respectively.

Trial No. 7. Although the PF varied from 8.5 to 57 between the four replicates, the LR varied from 5 to 5.7 only, a difference of less than one log step. This shows the greater reliability of the LR compared to the PF.

Trial No. 8. The birds challenged with the lowest dose showed the highest LR of 6.6. The LR of the chicks challenged with the medium dose was app. 1.5, the LR of the chicks challenged with the highest dose app. 2 log steps lower.

Trial No. 9. The IFs of the Ross grandparent birds were most consistent (CV = 8.7%), followed by the SPF (CV = 9.5%) and the commercial broiler chicks (CV = 15%).

Trial No. 10. All Ross grandparents were FP, whereas 78% and 71% of the commercial broilers and the SPF birds were FP, respectively.

Trial No. 11. The later the chicks were challenged, the higher was the LR. However, the LR only increased by 0.7 log steps from challenge on day 1 after Aviguard treatment to challenge on day 3 after treatment.

Trial No. 12. Birds kept on wire showed a higher LR than birds kept on wood shavings. However, the difference was not higher than 0.5 log steps.

Trial No. 13. The mean LR of the undefined CE product (Aviguard) was 7.5, the mean LR of the defined product 2.5 and the mean LR of the probiotic culture –0.5. The probiotic did not show any efficacy against intestinal *Salmonella* colonisation.

DISCUSSION

Various factors influencing the results of CE products obtained in the recommended assay of Mead *et al.* (2) could be shown. Among these factors are, besides general factors as reviewed by Bailey (1), challenge strain, challenge culture, challenge dose, challenge day, kinetics of infection, bird strain, bird quality, husbandry conditions and type of CE product. These factors should be taken into account when efficacy studies involving CE products are planned. It should be pointed out that results between laboratories can only be compared when the trial procedures are standardized and validated and when the same challenge strain is used, e.g. *S. kedougou*. Other serotypes of *Salmonella* may exhibit different colonisation properties and a higher degree of invasiveness. Furthermore, it was demonstrated that the use of the LR for the assessment of the results in the recommended assay is a more reliable criterion than the PF.

REFERENCES

1. Bailey, J. S. Factors affecting microbial competitive exclusion in poultry. Fd. Technol. 41:88-92. 1987.

2. Mead G. C., P. A. Barrow, M. H. Hinton, F. Humbert, C. S. Impey, C. Lahallec, R. W. A. W. Mulder, S. Stavric, and N. J. Stern. Recommended assay for treatment of chicks to prevent Salmonella colonisation by 'competitive exclusion'. J. Fd. Prot. 52:500-502. 1989.

3. Nurmi, E V., and M. Rantala. New aspects of Salmonella infection in broiler production. Nature 241:210-211. 1973.

4. Pivnick, H., and E. V. Nurmi. The Nurmi Concept and its role in the control of Salmonella in poultry. In: Developments in Food Microbiology, 1st ed. R. Davies, ed. Applied Sci. Publishers Ltd., Barking, UK. pp. 41-70. 1982.

5. Rantala, M., and E. V. Nurmi. Prevention of growth of Salmonella infantis in chicks by the flora of the alimentary tract of chickens. Brit. Poultry Sci. 14:627-630. 1973.

STUDIES ON INTESTINAL STRENGTH OF POULTRY

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ABSTRACT

We have studied several compounds for their efficacy to increase intestinal strength. These compounds included alum, tannic acid, propionic acid, calcium propionate, betaine, and calcium carbonate. Alum was fed to broiler chickens at 0.0, 0.23, 0.47, 0.93, 1.9, and 3.7% with 4 replicate pens of 10 birds per pen to 3 weeks of age. Tannic acid was added to the drinking water of birds at the concentrations of 0.0, 0.05, 1.0, 2.0, and 4.0%, with 4 replicate pens of 10 birds per pen. The tannic acid treatments were initiated when the birds were 3 weeks of age for a 24 hour time period. Two studies were conducted on the effects of propionic acid on intestinal strength. A commercial mold inhibitor (Mycocurb®) was fed at 0.0, 2.27, 4.54, and 9.07 kg/ton, and calcium propionate at 4.54 and 9.07 kg/ton. In the second study 0.0, 2.27, 4.54, 9.07 kg/ton Mycocurb ®, and 4.54 and 9.07 kg/ton propionic acid were fed. These diets were fed to the birds from 1 day of age to 6 weeks of age and there were 4 replicate floor pens of 40 birds per pen. Betaine was either fed at 0.07% for 7 weeks, added to the water at 0.17% from 6 to 7 weeks of age, or fed and added to the drinking water at the stated doses. There were 6 replicate floor pens of 40 birds per pen at each of these treatments. Calcium carbonate was fed at 0.0, 0.2, 0.3, 0.4, 0.5, and 0.6% to 3 weeks of age with 4 replicate pens of 10 birds pen. Consistent to all of these studies a 10 cm length of intestine just posterior to the duodenal loop was removed and its strength determined using elastomeric grips and an instron shear press. These treatments did not increase intestinal strength.

INTRODUCTION

It is important that poultry have strong intestines when processed. Fragile intestines can rupture during processing causing fecal contamination of the carcass, as well as cross contamination of carcasses. Contamination of carcasses with feces is a real food safety concern, and when flocks enter a processing plant with fragile intestines it significantly disrupts the processing of birds, slowing down the lines and requiring reprocessing of the contaminated carcasses. It has been very difficult to measure intestinal strength given the nature of this tissue. We developed a very accurate method to measure intestinal strength (3). This procedure uses elastomeric grips and a shear press. This methodology has allowed us to take a look at various compounds to determine their efficacy to increase intestinal strength.

MATERIAL AND METHODS

A number of studies were conducted using various experimental designs which will be briefly described. Alum was fed to broiler chickens from day of age to 3 weeks of age. The levels of alum were 0.0, 0.23, 0.47, 0.93, 1.9, and 3.7%, and there were 4 replicate pens of 10 birds per pen. The studies on the effect of tannic acid on intestinal strength were conducted by removing the feed from birds when they were 3 weeks of age and adding various levels of tannic acid to the water. The levels of tannic acid in the water were 0.0, 0.05, 1.0, 2.0, and 4.0%, which were provided to the birds for 24 hours with 4 replicate pens of 10 birds per pen. Two separate studies were conducted to determine the efficacy of propionic acid to increase intestinal strength. A commercial mold inhibitor (Mycocurb®), which is a product containing a mixture of organic acids with the primary ingredient being propionic acid, was fed to broiler chickens from 1 day of age to 6 weeks of age at 0.0, 2.27, 4.54, and 9.07 kg/ton, and calcium propionate fed at 4.54 and 9.07 kg/ton. In a second study, Mycocurb® was fed at 0.0, 2.27, 4.54, 9.07 kg/ton, and propionic acid was fed at 4.54 and 9.07 kg/ton to broiler chickens from 1 day of age to 6 weeks of age. In both of these studies there were 4 replicate floor pens of 40 birds per pen at each of the treatments. The effect of betaine on intestinal strength was investigated using a 2X2 factorial arrangement of treatments. Broilers were either not given any betaine, fed 0.07% betaine from 1 day of age to 7 weeks of age, given 0.17% betaine in their drinking water from 6 to 7 weeks of age, or fed 0.07% betaine from 1 day of age to 7 weeks of age and given 0.17% betaine in their water from 6 to 7 weeks of age. There were 6 replicate floor pens of 40 birds pen at each of these treatments. Calcium carbonate was fed to broilers from 1 day of age to 3 weeks of age at the levels of 0.0, 0.2, 0.3, 0.4, 0.5, and 0.6%, and there were 4 replicate pens of 10 birds per pen. At the termination of all of these studies the birds were killed by cervical dislocation, and a 10 cm length of intestine was removed just distal to the duodenal loop. The intestinal strength was determined using elastomeric grips and a shear press.

RESULTS AND DISCUSSION

Observations of fragile intestines have been made by a number of researchers, and suggested causes include deficiencies of fiber (5), reovirus-induced malabsorption syndrome (1), feeding of fish meals contaminated with histamine (4), and the mycotoxin oosporein (6). There is very little data in the literature on the measurement of intestinal strength, probably due to the past difficulty of making these measurements. Warren and Hamilton (7) published data demonstrating that the mycotoxin ochratoxin A decreased intestinal strength, and Dick *et al.* (2) found that the coccidiosis induced by *Eimeria brunetti* decreased intestinal strength. We found that none of the compounds tested increased intestinal strength under the conditions described. Field observations suggest that propionic acid based mold inhibitors can increase intestinal strength. However, we found no evidence that supports the hypothesis that propionic acid increases intestinal strength. The field data might suggest that although propionic acid does not increase intestinal strength it may help to prevent the decrease in intestinal strength caused by mycotoxins. Betaine also has been found to increase intestinal strength, which is thought to be a function of its ability to act as an osmo-regulator of the intestinal epithelium. We did not find that betaine increases intestinal strength, however, it may prevent a decrease of intestinal strength during coccidiosis. It was surprising to us that the astringents, alum and tannic acid, did not increase intestinal strength. These compounds may increase intestinal strength at higher concentrations, but there would be deletarious effects of alum and tannic acid at higher concentrations on both the health and production of poultry. It was thought that calcium carbonate would decrease the insult of the acids secreted during digestion on the intestinal epithelium which would result in stronger intestines. These data indicate that calcium carbonate at the levels and the duration used in these studies did not increase intestinal strength. Although some of the compounds tested in these studies may prevent a decrease in intestinal strength during pathology of the intestinal epithelium, none of the compounds tested increase intestinal strength by themselves.

REFERENCES

1. Apple, R. O., J. K. Skeeles, G. E. Houghten, J. N. Beasley, and K. S. Kim. Investigation of a chronic feed-passage problem on a broiler farm in Northwest Arkansas. Avian Dis. 35:422-425. 1991.

2. Dick, J. W., K. K. Hale, Jr., S. L. Boulton, and V. P. Cheatham. Breaking (tension) strength of broiler intestines. Poult. Sci. 57:1187-1188. 1978.

3. Huff, W. E., J. M. Balog, G. R. Bayyari, and N. C. Rath. The effect of mycocurb®, propionic acid, and calcium propionate on the intestinal strength of broiler chickens. Poult. Sci. 73:1352-1356. 1994.

4. Ito, Y., H. Terao, T. Noguchi, and H. Naito. Gizzerosine raises the intracellular cyclic adenosine-3', 5'-monophosphate levels in isolated chicken proventriculus. Poult. Sci. 67:1290-1294. 1988.

5. Kubena, L. F., J. W. Deaton, J. D. May, and F. N. Reece. A dietary method to correct a gizzard abnormality of broilers. Poult. Sci. 53:407-409. 1974.

6. Pegram, R. A., and R. D. Wyatt. Avian gout caused by oosporein, a mycotoxin produced by Chaetomium trilaterale. Poult. Sci. 60:2429-2440. 1981.

7. Warren, M. F., and P. B. Hamilton. Intestinal fragility during ochratoxicosis and aflatoxicosis in broiler chickens. Appl. Environ. Microbiol. 40(3):641-645. 1980.

THE EFFECTS OF BACITRACIN ON INTESTINAL INTEGRITY IN BROILER CHICKENS

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Numerous studies have shown improvements in rate of growth or in feed utilization when bacitracin (bacitracin methylene disalicylate or zinc bacitracin) is added to broiler diets (1). Bacitracin is approved as a growth promotant in broiler feeds at levels of 4.4 – 55 ppm. Growth promotion appears to be dose related and levels of 27.5 to 55 ppm are commonly used in the industry. Bacitracin methylene disalicylate is also approved at the 110 –220 ppm level as an aid in control of necrotic enteritis caused by *Clostridium* spp., and 55 ppm is used as an aid in the prevention of necrotic enteritis caused or complicated by *Clostridium* spp. or other organisms susceptible to bacitracin (2). The broiler industry has focused on food safety issues recently. A part of ensuring a safe, wholesome, processed broiler is the reduction of fecal contamination to the carcass. Modern broiler eviscerating machines do an excellent job of removing broiler entrails but are subject to error due to differences in bird size, lack of proper adjustments and the integrity of the intestine itself. Therefore, if bird size can remain more uniform and intestines are allowed to be as healthy and strong as possible, would this contribute to less contamination from intestinal breakage? It has been reported for years that under field conditions the addition of bacitracin in a broiler diet results in a thinner and healthier intestinal wall. When the bacitracin is removed, the intestine will become thicker and heavier indicating an inflammatory

response to bacteria in the intestinal tract. *Clostridium perfringens,* the causative organism of necrotic enteritis, causes areas of necrosis that may extend into the submucosa and muscular layers, thereby reducing intestinal strength and consequently making them more susceptible to tearing at processing.

Several studies have been performed to evaluate the intestinal strength of the broiler in both controlled environments and under normal field conditions. When possible, performance, uniformity and intestinal breaking strength were evaluated in these studies.

Trial one. A floor pen trial involved 144 males and 144 female commercial broiler chickens. The birds were fed typical NRC diets and were reared to 59 days of age. Salinomycin was added to the rations at a rate of 44 ppm for the first 28 days. One group of birds received bacitracin methylene disalicylate at 55 ppm in the grower and starter diets and 27.5 ppm in the finisher diet. The control group received no antibiotic in the feed. The birds were challenged with Eimeria oocysts at 10 days of age and *C. perfringens* bacteria at 14 days of age. Birds fed the bacitracin outperformed the birds without bacitracin in the feed. There was a 33% improvement in standard deviation of bird size in the treated birds compared to the controls. There was also an 8.3% improvement in intestinal breaking strength in the treated birds, as compared to controls, at 59 days of age as measured from 15 birds from each treatment group.

Trial two. This trial was similar to trial one and resulted in a 10% improvement in CV% (standard deviation of bird size) and a 7% improvement in gut breaking strength, in treated birds versus controls, when measured at 59 days with a sample size of 16 birds per treatment group. Similar results were obtained in three more controlled studies.

Field study. A large field study was conducted in the southeastern U.S. to determine if bacitracin would affect bird processing. This study was conducted in the autumn, a time when production performance of birds in this area is good. This study showed no differences in gut breaking strength, contamination or line efficiency between antibiotic fed birds and those fed no antibiotic. However, the intestinal health of these birds was excellent at this time period. Contamination was also extremely low and plant efficiency was at its highest during this period. Other operations with processing problems have shown a significant improvement in plant efficiency and a decrease of contamination when bacitracin was added to the feed.

More work is needed to establish the relationship of feed antibiotics to intestinal integrity. However, it seems reasonable that if an intestine is protected from offending bacteria, it might remain healthier and thus less likely to loose integrity and tensile strength. Our findings show that in the presence of an offensive challenge, the addition of bacitracin at 55 ppm in the diet enhances the breaking strength of the intestine. In instances of less challenge, the benefits are less, perhaps indicating an inherent strength upon which no improvement can be made.

REFERENCES

1. Izat, A. L., R. A. Thomas, and M .H. Adams. Effects of dietary antibiotic treatment on yield of commercial broilers. Poult. Sci. 68:651-655. 1989.

2. Feed Additive Compendium. pp. 142, 158. 1998.

WHAT IS YOUR DIAGNOSIS FOR "FOAMING CHICK DISEASE"?

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Five day-old broilers were submitted for diagnostic evaluation due to excessive ongoing mortality reportedly starting within three hours of being placed. Dead birds had litter with saliva pasted to the underside of their heads. Moribund birds had excessive saliva drooling from their beaks and would produce saliva bubbles when their crops were manipulated. Postmortem evaluation of both dead and moribund birds revealed minimal pulmonary congestion, gaseous crop contents and predominantly litter contents in both crops and gizzards. Unaffected pen mates had no gross lesions with their gastrointestinal tracts containing primarily ingested feed.

On a farm visit a few hours later evidence of poor management was found. The farm was unkempt with several large accumulations of garbage in and around large waste containers. The primary source of income for this grower was community garbage collection. There may have been miscommunication between grower and actual bird caretakers. It was a hot day and most of the birds were panting to keep cool. For this integrator complex it was common practice to place starter feed on paper sheets running underneath feeder pens and to overfill feed pans so that food overflowed onto the paper. Service reports indicated minimal feed was placed in feed pans and between feed pans when the day-old broilers were dumped. Feed levels were lower than expected. Dead and affected birds were easy to spot along outside walls and just inside doorways. Previous service reports indicated accumulations of dead birds at the ends of feed lines. These areas also corresponded with outside door openings. Lesions in dead

birds were similar to those found in birds submitted earlier in the day. Affected birds ranged from depressed and somnolent birds to birds obviously in respiratory distress. Some affected birds appeared to have muscle weakness. There were no consistent body postures of dead or dying birds. Diarrhea in some active and lethargic chicks was attributed to over-heating. Later in the visit the farm owner returned to the farm and admitted requesting that diazinon granules be spread outside chicken houses to control rampant fire ant colonies. A preliminary diagnosis of diazinon toxicity was made.

Litter, crop contents and gizzard contents were sampled for diazinon residues. Brains from moribund birds were extracted and held in refrigeration for cholinesterase activity. All specimens were submitted to the toxicology laboratory at Texas A&M College of Veterinary Medicine. Diazinon levels were 2.1 ppm in litter, 9.7 ppm in crop contents and 0.44 ppm in gizzard contents. Brain cholinesterase levels were 0.111 ΔpH *(or μmoles acid/ml/min.)*, normal levels should be greater than 0.15 ΔpH *(or μmoles acid/ml/min.)*. Earlier reports of diazinon toxicity in chickens reported some of the same gross lesions observed in this case (1,2). Organophosphate toxicity is fortunately rare in commercial U.S. poultry production. When confronted with foaming chicks, organophosphate toxicity should be on the list of differential diagnoses.

REFERENCES

1. Hammerlund, M. A., B. Koch, E. B. Howard, and B. McKeen. Diazinon poisoning and rancid fat intoxication in chickens. Proc. 28th West. Poult. Dis. Conf. pp.52-54. 1979.

2. Hill, D. L., C. I. Hall, J. E. Sander, O. J. Fletcher, R. K. Page, and S. W. Davis. Diazinon toxicity in broilers. Avian Dis. 38:393-396. 1994.

STUDIES ON THE PATHOGENESIS OF "FLIP-OVER" SYNDROME IN TURKEY BREEDER CANDIDATE HENS

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INTRODUCTION

Over the past several years there has been an increasing problem in turkey breeding hen candidates during the late grow-out period between 22 - 30 weeks of age. Affected birds are usually found in the early morning flipped onto their backs. Some of these birds are found dead but the majority of them survive for variable periods of time. If they are put back into normal upright position they may appear to recover, but most eventually flip over again. Average mortality is between 1% - 1.5% during a period of 8 weeks. However in some cases, when these birds are on their backs they struggle by flapping their wings and cause panic resulting in pile-ups during the night. In these cases mortality losses are much higher. Affected birds are not able to walk normally and maintain body balance for some period of time. The degree of these signs varies. The incidence is highest during the winter months when the environmental barn temperature drops below 10° C. After the onset of egg production (32-33 wks of age) the incidence of this syndrome drops dramatically. A search of the literature did not reveal any data about this syndrome. Studies were carried out to determine the etiology of this syndrome. First the flip-over hens were examined for gross pathology lesions, then organs were cultured for bacteria, and finally histopathology was performed. In addition, body composition, blood gases, biochemical and hormonal profiles were determined in both normal and flip-over birds.

MATERIALS AND METHODS

Routine necropsy was performed on 36 live and 16 dead flip-over hens. These hens were between 25 - 31 weeks of age. During the growing period all hens were vaccinated with live vaccines against Newcastle disease (ND), hemorrhagic enteritis, coccidiosis, avian encephalomyelitis and with killed vaccines against ND, PMV-3,fowl cholera and erysipelas. Blood samples were collected into both regular vacutainers and vacutainers with heparin or EDTA on the day the hens were found flipped. Live birds were euthanized in a CO_2 chamber. Bacteriological cultures were done from trachea, brain, heart, liver, spleen, and lungs. The following tissues were collected for histopathological examination: brain, thigh and breast muscles, lungs, liver, spleen, trachea and heart. ELISA tests for Mycoplasma gallisepticum (MG), Mycoplasma synoviae (MS), Mycoplasma meleagridis (MM), turkey rhinotracheitis (TRT), Newcastle disease (ND), and Bordetella avium (BA) were performed with all collected sera.

Body composition and blood gases study. From 11 normal and 11 flip-over hens (29-week-old) blood samples were collected using vacutainers with heparin. They were immediately put on ice and tested for pO_2, pCO_2 and pH using a model ABL 330 "Radiometer". All birds were euthanized using a CO_2 chamber. The entire body, as well as livers and abdominal fat were weighed individually.

Biochemical profiles. Biochemical profiles were performed on serum samples taken from 12 flip-overs found in the morning (having been on their backs for an unknown number of hours), 5 recent flip-overs and 12 normal hens at 26 weeks of age. The serum samples from the 5 recent flipped hens were tested in order to eliminate the influence of stress, fasting and dehydration on biochemical profiles. Blood samples were collected into vacutainers and put on ice then centrifuged to separate serum. Sera from both groups were analyzed for Ca, P, Na, K, Na: K, Cl, protein, creatinine, glucose, bilirubin, AST, uric acid and BHBA (betahydroxybutyrate) using the BM-Hitachi 911 system.

Hormonal profiles. For hormonal analysis blood samples were collected from 10 flip-over and 10 normal hens using vacutainers with EDTA. Blood samples were collected at three times: first on the same day as the hens flipped, then 24 hours later and 72 hours later using vacutainers with EDTA. The vacutainers were put on ice and centrifuged to separate plasma. After separation the plasma was stored at -70° C until tested. Double antibody radioimmunoassays were used to determine plasma concentration of the following hormones: insulin (1), triiodothyronine (T3) and thyroxin (T4) (2). Radiolabeled T3 and T4 were purchased from ICN Pharmaceuticals, Costa Mesa, CA. Plasma glucagon was determined utilizing a kit purchased from Linco Research Inc., St Charles, MO. Plasma corticosterone was tested using a kit from ICN Pharmaceuticals Inc.

In order to determine the influence of stress on the levels of thyroid hormones one more experiment was conducted. Five randomly selected hens (26-week-old) were put on their backs with legs tied up for 30 minutes. Blood samples were collected at the start of the experiment and at 1, 3 and 24 hours after the hens were released from the abnormal position. All samples were analyzed for T3, T4 and corticosterone.

Histopathological examinations of thyroid glands. The thyroid glands were collected from 15 flip-over hens and 5 controls. The birds were euthanized in a CO_2 chamber. All glands were fixed in 10% buffered formaline, routinely processed, sectioned at 5 μm and stained with hematoxylin and eosin (H&E).

RESULTS AND DISCUSSION

Gross pathology examinations and histopathology did not reveal any significant lesions. No pathogenic bacteria were isolated. All ELISA tests gave negative results, except normal postvaccinal titers against ND. Mean body weights of flip-over hens were statistically higher than normal hens (12.57 kg vs. 11.02 kg). Also liver weights and abdominal fat weights of flip-overs were statistically heavier than normal birds. In the blood gases study there was no statistical difference in pH, partial pressure of O_2, CO_2, HCO_3, and oxygen saturation of blood between normal and flip-over hens.

Comparisons of each biochemical profile showed that Ca, Na, Na:K, Cl, protein, creatinine, and glucose were significantly lower in flip-over hens than normals. However, potasium, uric acid and betahydroxybutyrate (BHBA) were significantly higher in flip-over hens. These high levels of uric acid and BHBA would indicate uremia and ketosis. Hens with a concentration of uric acid above 800 mmol/L were found dead the next day. In recently flipped hens all biochemical parameters of serum were not statistically different from those of normal hens. Therefore, it can be concluded that the changes in biochemical profiles of hens lying on their backs for longer periods of time were secondary due to stress, fasting and dehydration.

The results of hormonal profiles are presented in the Table 1.

Table 1. Results of hormonal profile of flip-over and normal hens (mean values).

Time of Testing	T3 (pg/ml)		T4 (ng/ml)		Insulin (ng/ml)		Corticosterone (ng/ml)		Glucagon (pg/ml)	
	F[1]	N[2]	F	N	F	N	F	N	F	N
0	277 ~	982	3.2 ~	15.5	2.2	2.9	31 ~	1	276	235
24 hrs	727	875	13.1	12.9	2.5	1.9	3	3.6	313	281
72 hrs	772	1101	13.7	13.5	2.6	2.9	4.9	1.3	249	224

~ Indicates values which are significantly different ($P<0.05$)

[1] Flip-over hens

[2] Normal hens

The concentrations of T3 and T4 hormones were significantly lower in the same-day collection from flip-over hens as opposed to normals. After 24 and 72 hours the T4 levels returned to normal while T3 increased and did not return to normal levels. Corticosterone levels were significantly higher in flip-over hens than normals (as a result of stress) and then returned to normal levels after 24 hours. The concentrations of insulin and glucagon in flip-over hens were not statistically different than those of controls. Low concentrations of T4 and especially T3 would indicate a

problem with secretion of thyroid hormones and their metabolism. This problem seems to be primary since the experiment with hens forced into flipped positions showed that the levels of thyroid hormones were the same at all times; before the stress occurred, 1 hour, 3 hours and 24 hours after flipping. The concentrations of corticosterone were very high after 1 and 3 hours, which indicated a high degree of stress. Therefore one can conclude that the stress of flipping and being in an abnormal position does not have an influence on the concentration of thyroid hormones.

The histological changes of thyroid glands of flip-over hens were characterized by massive infiltrations of mononuclear cells. Many thyroid follicles become misshapen and collapsed with degenerated epithelium. The colloid contained mineral deposits and was discoloured. The flip-over hens had numerous lymphoid germinal centers in the thyroid tissue. There were scattered necrotic foci with infiltration of lymphocytes and plasma cells. Some parts of the thyroid tissue were replaced by fat tissue. Certain hens had atrophic thyroid glands with small misshapen follicles and thickened basement membranes.

The above-described histopathological lesions of lymphocytic thyroiditis together with low levels of thyroid hormones suggest that some turkey hens suffer from hypothyroidism. Low levels of T3 may lead to nervous symptoms resulting in flipping over (personal communication). The histopathology seems to resemble an autoimmune thyroiditis (3). Whether this problem has autoimmune etiology or not will have to be explored in future studies.

REFERENCES

1. McMurtry J. P., R. W. Rosebrough,and N. C. Steele. An homologous radioimmunoassay for chicken insulin. Poult. Sci. 62: 697-701. 1983.

2. McMurtry J, P.,I. Plavnik, R. W. Rosebrough, N. C. Steele, and J. A. Proudman. Effect of early feed restriction in male broiler chicks on plasma metabolic hormones during feed restriction and accelarated growth. Comp. Biochem. Physiol. 91A: 67-70. 1988.

3. Riddell C. Pathology of the endocrine systems. In: Avian Histopathology. 2nd ed. American Association of Avian Pathologists, University of Pennsylvania, Kennett Square, PA. pp 212-213. 1996.

IMPORTANCE OF AVIAN NEONATAL NURTITION: A DIFFERENT CONCEPT, AN ADAPTED PRESTARTER FEED

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Poultry producers expect high performance from their birds. In 1945 it took 24 weeks to produce a 18.4 pound male turkey with a feed conversion of 4.5. By 1985 growth weights of 30 pounds were achieved in 20 weeks with feed conversions of 2.9. Today 20 week old male turkeys are attaining weights of 40 pounds plus with conversions of 3.0 or less. In broilers, over the past 40 years, the time necessary to reach market weight has been reduced one day per year. In 1940, 12 weeks were required for broilers to reach a live body weight of 4 pounds. Today, this period has been reduced by half. This clearly means that the first week posthatch has become an increasingly larger portion of the poult's and chick's total life span. An optimal start is the result of different factors including the quality of the day-old poult/chick, good management, fast growth of the intestinal tract, and an adapted pre-starter feed.

To overcome the difficult posthatch period, Vitamex has developed Galito^{R}, an adapted poult/chick pre-starter feed. Galito^{R} is to be fed as soon as possible after hatch and placement. Fifty grams per poult or chick is fed for the first 3-4 days, followed by regular commercial diets. It has clearly been shown that at initiation of growth and absorption of residual yolk-sac, intake of carbohydrates is absolutely essential for fulfillment of growth potential and rapid gain. Poults/chicks at hatch time have a small digestive tract, which probably does not function during embryonic development. The weight of the supply organs (intestine, liver and spleen) increases ten times during the first week, while the whole body weight increases only three times.

In a pen trial study the effect of pre-starter diet, Galito^{R} was investigated. Body weights of chicks fed Galito^{R} were significantly higher and feed conversion was improved compared with a control group. In a field trial with BUT Big 6 poults, hens slaughtered at 15 weeks and toms slaughtered at 16 weeks had higher weights than controls. Importance of an adapted pre-starter fed during first days after hatch may be essential in order to exploit fully the genetic potential of fast growing turkeys and broilers. This concept is being further evaluated in controlled pen studies.

FIELD EXPERIENCES IN ALTERING BROILER INTESTINAL MICROFLORA WITH THE USE OF FLAVOMYCIN® FEED ADDITIVE

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INTRODUCTION

In 1973, Nurmi and Rantala introduced a technique to increase the resistance of baby chicks to *Salmonella* infection by inoculating them orally with adult fowl intestinal contents. This technique was later termed "competitive exclusion" (6). There have been several proposed mechanisms of action with competitive exclusion. However, most scientists agree that a population of normal protective bacteria in the intestines of chickens do provide a benefit in reducing invading pathogenic bacteria. Several early studies (1,2,5) indicate that *Lactobacillus spp.* is a major component in providing the protective aspect of competitive exclusion. Other protective bacterial species include *Bifidobacterium* and *Bacteroides*. A major component of the protective nature of competitive exclusion is thought to be the production of volatile fatty acids.

Flavomycin® (bambermycins) has been used in broiler diets world-wide for growth promotion and feed conversion improvement since the early 1970's. The spectrum of antimicrobial activity indicates that Flavomycin has little effect against common normal flora bacteria, particularly *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (FOI Summaries, Hoechst Roussel Vet, Warren, NJ). Other commonly used growth promotant antibiotics may have a significant effect in reducing the *Lactobacillus* population of the intestine. Free *et al.* (3) showed that chickens fed Flavomycin at 2 ppm had a significantly greater level of volatile fatty acids in the small intestine than birds fed lincomycin, bacitracin or virginiamycin. Kling and Quarles (4) found that broilers fed with a continuous Flavomycin ration were no more susceptible to necrotic enteritis than birds fed other growth promotants that have a therapeutic claim for the disease. The theory supported by these works, is that Flavomycin does not negatively affect the protective microflora of the chickens intestine. Therefore, by allowing the normal protective bacteria to live, a secondary competitive exclusion is achieved, compared to birds continuously fed bacitracin or virginiamycin

The purpose of these studies was to determine if under field conditions, higher numbers of intestinal *Lactobacillus* could be found compared to other growth promotant programs.

MATERIALS AND METHODS

Trial 1. A U.S. integrated operation was identified as the trial location. The operation normally processes approximately 650,000 broilers per week. The operation has two distinct geographical regions of growers. The north division of growers are supplied feed from one feed mill located in the approximate area of the growers. The south division of growers are supplied feed from two feed mills which are also located in the same geographical region. Approximately 2.5 months before sampling, the north division initiated a growth promotant program of 2g/ton Flavomycin from one day of age until the birds were processed. The south division continued on the program that had been in place of 50g/ton BMD® from one day of age until approximately 19 days of age. The next feed contained 25g/ton BMD from 19 days until approximately 35 days of age. The withdrawal feed contained 10g/ton Stafac® and was fed until approximately 44 days when the birds were processed. Other aspects of broiler management were kept similar between the north and south divisions except for the growth promotant program.

At the time of processing, one farm from each division was randomly selected for intestinal sampling. Intestinal samples were collected sanitarily on the processing line, immediately after evisceration. The samples were placed in sterile Whirl-pak plastic bags containing 100 ml of glycerin-salt solution. The contents of the bag were thoroughly mixed and quick frozen on dry ice. They were immediately shipped via next day courier to the University of Kentucky for microbiological enumeration.

Trial 2. Another US operation that processes over 1 million broilers per week decided to change its growth promotant program on approximately March 23, 1998, to a continuous Flavomycin program at 2g/ton. This operation had been using BMD to approximately 19 days of age, Stafac to approximately 32 days and Stafac in the withdrawal feed. On April 2, 1998, company personnel selected a farm for intestinal sampling as birds from that farm were being processed. Ten birds were randomly selected for intestinal collection. The intestinal sampling procedure was consistent with that noted in Trial 1. On May 26, 1998, the company personnel elected to resample birds from the same farm as the replacement flock returned for processing. Ten birds were selected for sampling.

Trial 3. A third U. S. broiler operation that processes approximately 750,000 birds per week elected to experiment with a continuous Flavomycin program at a level of 2g/ton. Two farms were put on the Flavomycin experiment because they were considered problem clostridial farms. The remaining farms continued on the

pre-selected program of 15g/ton Stafac from day one to approximately day 20, 20g/ton Stafac from day 20 to approximately day 39, and 15g/ton from day 39 until processing at day 58. The treatment farms were cleaned and sanitized before the arrival of the treatment birds. The anticoccidial program for the entire operation was a coccidial vaccine and supplemental amprolium, when needed. One farm was randomly selected by company personnel for intestinal sampling for each growth promotant program. Intestinal samples were sanitarily obtained from 20 randomly selected birds at 21 days of age and 58 days of age. The intestines were collected and shipped to the University of Kentucky in the same manner as noted above.

Enumeration of microbial populations. The contents of each Whirl-pak were weighted and removed from the bags into a blender jar. An appropriate volume of peptone dilution solution was added to the sample and the mixture was blended into a homogenate with as little aeration as possible. A 1:10 dilution sequence was used to prepare dilution representing 10^{-1} to 10^{-6}g of the original sample. Dilutions were plated on selective media to enumerate each of the specific groups of bacteria. Enrichment tubes were inoculated with material from the initial dilution.

Clostridium perfringens were enumerated on Perfringens Selective Medium (O.P.S.P.). These plates were incubated at 35°C in an anaerobic chamber for 72 hr. Coliforms and *E. coli* were enumerated on Violet Red Bile Agar medium containing 4-methylumbelliferyl-beta-D-glucuronide (MUG). Poured plates were overlaid with a second layer of medium to increase the selectivity of the procedure and were incubated at 37°C. *Lactobacilli* were enumerated on Rogosa SL Agar plates. These plates were incubated at 37°C in an anaerobic chamber to allow for the growth of the oxygen-sensitive *Lactobacilli* found in the gastrointestinal tract. *Bifidobacteria* were enumerated on BS-LV agar plates which were overlaid with medium containing neomycin sulfate. These plates were incubated for 72 hours at 37°C in an anaerobic chamber.

RESULTS

Table 1. Trial 1. Bacterial enumeration in number of organisms per gram intestine recovered from whole intestinal samples from 45 day old birds.

	South Division		North Division	
Bacteria	Farm A-1	Farm A-2	Farm B-1	Farm B-2
Lactobacilli[A]	2.24	5.13	22.9	9.33
Bifidobacteria[A]	10.2	12.6	38	28.2
Coliforms[A]	1.13	3.39	1.55	1.35
E. coli[B]	288	725	234	224
C. perfringens[B]	6.46	13.8	1.07	0.11

[A] Bacterial enumeration times 10^6
[B] Bacterial enumeration times 10^3

Table 2. Trial 2. Bacterial enumeration in number of organisms per gram intestine recovered from whole intestinal samples from approximately 40 day old birds.

Bacteria	First sampling (4/2)	Second sampling (5/26)
Lactobacilli[A]	13.2	14.8
Bifidobacteria[A]	9.55	11.7
Coliforms[B]	347	28.8
E. coli[B]	22.4	3.72
C. perfringens[B]	1.23	0.257

[A] Bacterial enumeration times 10^6
[B] Bacterial enumeration times 10^3

Table 3. Trial 3. Bacterial enumeration in number of organisms per gram intestine recovered from whole intestinal samples from 20 and 58 day old birds.

	Flavomycin treated farm		Virginiamycin treated farm	
Bacteria	21 days	58 days	21 days	58 days
Lactobacilli[A]	63.3	8.1	19.7	7.8
Clostridia spp.[A]	2.4	17.8	1.2	1.3
Coliforms[B]	106	27	344	17
E. coli[B]	35.4	4.6	52.8	9.5
C. Perfringens[B]	0.018	0.0007	81.8	0.433

[A] Bacterial enumeration times 10^6
[B] Bacterial enumeration times 10^3

DISCUSSION

In all three field trials, birds that were started on Flavomycin at 2 g/ton and fed Flavomycin until processing have lower numbers of *Clostridium perfringens* per gram of intestine than birds exposed to other growth promotant program. Correspondingly, the birds fed Flavomycin also had higher numbers of *Lactobacillus spp.* and *Bifidobacterium spp.* per gram of intestine than birds fed other growth promotant programs. Additionally, lower numbers of coliforms and *E. coli* were recovered from birds fed Flavomycin. In all three trials, growth promotion and feed conversion rates for the Flavomycin treated birds were as good as or better than birds on other treatments. Based on the results of these trials, it can be stated that the field trials do support the theory that Flavomycin does allow higher numbers of *Lactobacillus spp.* and *Bifidobacterium spp.* to populate the gut. This increased population of beneficial bacteria appear to cause a reduction in the numbers of potentially pathogenic bacteria, compared to other growth promotant programs.

REFERENCES

1. Barnes, E. M., G. C. Mead, D. A. Barnum, and E. G. Harry. The intestinal flora of the chicken in the period 2 to 6 weeks of age, with particular reference to the anaerobic bacteria. British Poult. Sci. 13:311-326. 1972.

2. Barnes, E. M., C. S. Impey and B. J. Stevens. Factors affecting the incidence and anti-salmonella activity of the anaerobic caecal flora of the young chick. J. Hyg., Camb. 82:263-283. 1979.

3. Free, S. M., T. O. Lindsey, and R. D. Hedde. Possible mode of action of antibiotics on energy utilization. Zootecnica Int. pp 48-49. 1986.

4. Kling, H., and C. Quarles. Performance of broilers fed commonly used antibiotics in the presence of necrotic enteritis. Southern Poult. Sci. Abstracts. Atlanta, GA. 1995.

5. Mead, G. C., and B. W. Adams. Some observations on the caecal micro-flora of the chick during the first two weeks of life. British Poult. Sci. 16:169-176. 1975.

6. Nurmi, E., and A. M. Rantala. New aspects of salmonella infection in broiler production. Nature 241:210. 1973.

AN EVALUATION OF IMMUNOHISTOCHEMICAL STAINING OF CHLAMYDIA PSITTACI AND INFECTIOUS BRONCHITIS VIRUS IN LABORATORY AND CLINICAL SAMPLES

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INTRODUCTION

Immunohistochemical (IHC) staining is emerging as a valued procedure to identify antigens in affected tissues (1,2). The advantages of IHC staining are: permanancy of results, ease of interpretation, and the use of a standard light microscope. Previous applications of IHC staining to avian tissues have been limited due to high non-specific background staining which can obscure interpretation. Three commercial IHC kits, namely, peroxidase anti-peroxidase (PAP), Histostain™-SP (streptavidin-biotin labeled antibody [LAB-SA]) and non-biotin amplification (NBA™), were evaluated for their ability to detect and specifically identify microbial pathogens in avian diagnostic specimens. When formalin fixed tissues are available for retrospective studies, IHC methods allow further evaluations of tissue sections for the presence of microorganisms. We present data on the evaluation of the three IHC procedures for two avian pathogens, infectious bronchitis virus (IBV) and *Chlamydia pscittaci,* which affect a plethora of tissues in their hosts and for which diagnostic tests are routinely requested (3,4).

MATERIALS AND METHODS

Tissues. IHC staining for the detection of *Chlamydia psittaci* in formalin fixed paraffin embedded tissue was evaluated using a liver from a confirmed clinical case of avian chlamydiosis and liver sections from other positive clinical cases. Two sources of positive tissues were used for IBV staining: 1) formalin fixed embryonic chorioallantoic membranes (CAMs) harvested at 48 hours postinoculation from SPF chicken eggs inoculated at 9 to 11 days via the allantoic route with 0.2 ml of specific IBV strains (Mass, Ark, Conn) or an untypeable strain and 2) field cases of broiler chickens diagnosed as infected with a nephrotropic strain of IBV.

All IBV suspect tissues were fixed in 10% neutral buffered formalin for 24-48 hours, processed according to standard procedures in histopathology and embedded in paraffin. Blocks were sectioned at 3 μm and tissue sections were adhered to positive charged microscope slides (Fisher Biotech ProbeOn™). In preparation for IHC staining, the tissue sections were deparaffinized, rehydrated, and quenched for endogenous peroxidases in 3% hydrogen peroxide in distilled water for 15 minutes at ambient temperature. Thereafter, sections were rinsed in distilled water, then digested in 0.05% pronase (Protease Type XIV, Sigma Chemical) for 15 min. at 37° C and rinsed again in distilled water prior to immunostaining.

Positive and negative controls consisted of tissues previously shown to contain or be free of the microorganisms in question. Formalin fixed infected and non-infected CAMs served as tissue controls for IBV staining. For *Chlamydia*, positive control tissues originated from birds showing histologic lesions in which *Chlamydia* was isolated by cell culture procedures. Controls for antibody consisted of a duplicate section of test tissue which received non-immune serum in place of primary antibody.

Antibodies. Anti-IBV S_2 group specific monoclonal antibody obtained from Dr. S. Naqi, Cornell University, Ithaca, NY, was used in the evaluations of the IBV staining methods. A cocktail of 6 monoclonal antibodies that were selected for their reactions with formalin inactivated *Chlamydia spp.* was kindly provided by Dr. A. A. Andersen, USDA, ARS, NADC, Ames, Iowa, and used in the evaluations of chlamydial staining. These monoclonals are known to react with the lipopolysaccharide (LPS) component of all chlamydial strains.

IHC Staining. Three immunostaining kits (Zymed Laboratories, San Francisco, CA) were used for comparative staining: 1) Mouse PAP Kit, 2) Histostain™-SP Bulk Kit (LAB-SA) for Mouse Primary and 3) NBA™ Broad Spectrum Bulk Kit. The basic steps for the three IHC staining methods are provided in Tables 1, 2 and 3.

RESULTS

Formalin fixed paraffin embedded IBV-infected CAMs stained with each of the three IHC methods demonstrated specific staining for either Mass, Conn, Ark, and an untypeable strain of IBV. The NBA and LAB-SA methods were approximately equal in their sensitivity. However, the LAB-SA method yielded unacceptably high background staining and non-specific staining in certain tissue areas of the immune serum controls. Of the three methods evaluated, the NBA method was the most sensitive with the greatest specificity and minimal background. Because primary antibody was used at a higher dilution in the NBA method, less antibody was required for an intense stain reaction. The PAP method was shown to be markedly less sensitive and

more time consuming (90 min. longer to perform) than the other 2 methods. Allowing approximately 20 min. of total time for the rinse steps in each of the methods, the PAP method required twice as much time to perform.

Table 1. Basic Steps in the PAP Method using the Mouse Kit.

- Kit blocking serum - 20 min. at room temperature (RT)
- Anti-*chlamydia* Mab cocktail 1:200 dilution or anti-IBV S_2Mab 1:80 in PBS containing 0.075% BRIJ 35 (Sigma) - 60 min. RT
- Kit Bridging antibody - 50 min. RT
- Kit PAP complex - 50 min. RT
- AEC chromogen - 3-4 min. RT
- Mayer's hematoxylin - 1 min. RT
- Automation buffer to intensify nuclear staining - 1 min. RT
- Crystal mount - 2 hr. 60°C
- Permount

Table 2. Basic Steps in the LAB-SA Method using the Histostain™-SP Bulk Kit for Mouse Primary

- Antibody Kit block serum - 10 min. RT
- Anti-*chlamydia* Mab cocktail 1:200 dilution or anti-IBV S_2 Mab 1:300 in PBS containing 0.075% BRIJ 35 (Sigma) - 60 min. RT
- Kit biotinylated secondary antibody - 10 min. RT
- Kit streptavidin peroxidase conjugate - 10 min. RT
- AEC chromogen - 3-4 min. RT
- Mayer's hematoxylin - 1 min. RT
- Automation buffer to intensify nuclear staining - 1 min. RT
- Crystal mount - 2 hr. 60°C
- Permount

Table 3. Basic Steps in the NBA™ Method.

- Kit blocking serum – 10 min. RT
- Anti-*chlamydia* Mab cocktail 1:500 dilution or anti-IBV S_2 Mab 1:600 in PBS containing 0.075% BRIJ 35 (Sigma) – 60 min. RT
- Kit FITC-conjugated secondary antibody – 10 min. RT
- Kit tertiary HRP conjugate – 10 min. RT
- AEC chromogen - 3-4 min. RT
- Mayer's hematoxylin – 1 min. RT
- Automation buffer to intensify nuclear staining – 1 min. RT
- Crystal mount – 2 hr. 60° C
- Permount

The three IHC methods for IBV were further evaluated using formalin fixed paraffin embedded tissue sections of kidneys from twelve clinical cases of suspected or confirmed nephrotropic IBV in chickens (broilers). The results were similar to those observed in CAMs. The NBA method was the most sensitive and specific. The LAB-SA method had a similar sensitivity but had an unacceptable high level of non-specific background staining. The PAP method was the least sensitive of the three methods (Table 4).

Table 4. Comparison of three IHC staining methods in detecting antigens of a nephrotropic IBV in formalin fixed, paraffin embedded kidneys.

	Staining method		
	PAP	LAB-SA	NBA
Specific reaction	+	+++	+++
Non-specific background staining	0	+	0

PAP = Peroxidase anti-peroxidase method
LAB-SA = Streptavidin-biotin labeled antibody method
NBA= Nonbiotin amplification method
+ = Intensity of color reaction
0 = Absence of background staining

The comparative staining for *Chlamydia pscittaci* produced results similar to the IBV tissues. The NBA method was found to be the most sensitive and specific for the identification of chlamydial antigens in avian tissues with little to no background staining. The LAB-SA method was as sensitive but had unacceptable high background stain. The PAP method was the least sensitive.

DISCUSSION

The commercially available NBA staining kit demonstrated a high sensitivity and specificity in the detection of IBV and *Chlamydia psittaci* in formalin fixed paraffin embedded avian tissues. Comparisons of the three staining methods proved that the NBA kit was the most sensitive and specific with no background staining. Although the PAP method produced no background staining, it can produce false negatives due to its lack of sensitivity. The high background associated with LAB-SA could possibly be due to a non-specific reaction with biotin in avian tissue. Other investigators have noted similar background staining in avian tissues using the avidin-biotin complex (ABC) system and were unable to block the binding of the ABC complexes to the tissues with commercial avidin-biotin blocking kits (5). However, in our laboratory, results of previous staining trials with avian liver using LAB-SA staining procedures suggested that when avidin and biotin were blocked and primary antibody was omitted, the protease digestion exposes epitopes in tissues that react with biotinylated secondary antibody to create non-specific staining. Our present findings in this report argue for an IHC (NBA) methodology that circumvents the use of a biotinylated secondary antibody, yet produces an intense specific reaction to provide reproducible and reliable detection of pathogens in avian tissues. Future studies will evaluate the use of available monoclonal antibodies to other avian pathogens for use with the NBA method.

REFERENCES

1. Chen, B. Y., S. Hose, T. Nunoya, and C. Itakura. Histopathology and immunohistochemistry of renal lesions due to infectious bronchitis virus in chicks. Avian Path. 25:269-283. 1996.

2. Vanrompay, D., R. Ducatell, and F. Haesebrouck. Chlamydia psittaci infections: a review with emphasis on avian chlamydiosis. Vet. Micro. 45:93-119. 1995.

3. Cavanagh, D., and S. A. Naqi. Infectious bronchitis. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp.511-526. 1997.

4. Storz, J. Overview of animal diseases induced by chlamydial infections. In: Microbiology of Chlamydia. A. L. Barron, ed. CRC Press, Boca Raton, Florida. pp. 167-192. 1988.

5. Haines, D. M., B. J. Chelack, and K. M. Martin. Immunohistochemistry: "Nuts and Bolts." In: Pathobiology of the skeletal system. Proc. 44th ACVP, San Antonio, Texas. 1993.

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NON-SPECIFIC IMMUNITY INDUCED BY THE ACUTE PHASE RESPONSE IN WHITE LEGHORN CHICKENS

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The acute phase response (APR) is an early and immediate set of reactions which are initiated at the site of inflammation, infection or trauma. During the APR, tissue macrophages or blood monocytes are activated and release alarm cytokines including interleukin 1 (IL-1) and tumor necrosis factor (TNF-α) which can activate the stroma cells to elicit other cytokines and more IL-1, TNF, and IL-6. These cytokines can stimulate the hepatocytes to produce a variety of proteins, acute phase proteins, and initiate the accumulation of inflammatory cells. The objective of our study was to induce the acute phase response by various agents, evaluate and characterize its non-specific immunity against *Escherichia coli* infection in chickens. An *in vivo* model system was established to study the APR. White Leghorn male chicks were raised in isolation to 5 weeks of age when an antigen (formalin-killed *Staphylococcus aureus* or other bacterial suspension) was intravenously injected to induce APR. Sensitized birds were challenged 24 hrs later with 10^7 live *E.coli* O1:K1 strain via an intra-airsac route. Twenty-four hrs after the infection, the spleen was examined for viable bacteria counts. Significant protection against infection was induced by the administration of killed homologous strain, heterologous strains of bacteria, or toxic substance. The viable *E. coli* counts in the spleen were $10^{1.88\pm0.64}$, $10^{2.27\pm1.31}$, $10^{2.68\pm1.37}$, $10^{2.71\pm1.38}$, $10^{3.21\pm1.45}$, and $10^{5.25\pm1.09}$ for formalin-killed *E.coli* O1:K1, *Staphylococcus aureus*, *Salmonella typhimurium*, and *E. coli* strain O78, 3 % silver nitrate solution and a control group, respectively, at 24 hr after challenge.

The questions of how early the anti-bacteria effect of acute phase response appears and how long it lasts were also addressed. Birds were injected with killed *S. aureus* at various time before the *E. coli* O1:K1 challenge. The results showed that the acute phase response against *E.coli* infection appears as soon as 6 hours and lasts less than 6 days after stimulation. The effects of cold stress and corticosterone on the acute phase response were also been studied. Sixty birds were distributed into 5 groups with 12 birds per group. All the birds were injected with killed *S. aureus* suspension IV 24 hr before the *E.coli* challenge except group 5, a non-treatment control, which received an injection of phosphate buffered saline instead. In group 1, birds were given feed containing 40 mg/kg corticosterone for 2 days before *S. aureus* injection. In group 2, birds were injected with ACTH 20 IU/kg body weight every 8 hours at the time of APR-induction. In group 3 (cold response), birds were kept at 10°C between APR induction and *E.coli* challenge. Other birds were kept in a brooder in which they could choose a temperature between 20° to 30° C during the period. In group 4, APR response control, birds received *S.aureus* injection only without any other treatment. The viable *E. coli* counts in spleen reached $10^{6.27\pm0.53}$, $10^{3.62\pm1.62}$, $10^{4.58\pm0.89}$, $10^{2.59\pm0.71}$, and $10^{5.64\pm1.16}$ in group 1 to 5, respectively. This suggests that cold and corticosterone can significantly suppress the protection induced by the acute phase response. These results indicate that the acute phase response induced by various agents nonspecifically protects chickens against *E. coli* systemic infection and this nonspecific protection can be inhibited by corticosterone or cold stress.

(A full paper will be published in *Avian Diseases* or other refereed journal.)

QUALITY ASSURANCE OF MAREK'S DISEASE VACCINE USE IN HATCHERIES

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SUMMARY

New Marek's disease (MD) vaccines were manufactured in Australia from 1997 to overcome the serious outbreaks of MD that had occurred over the past five years. To achieve the full potential of the new vaccines, a quality assurance audit system was designed to improve the administration of vaccines. Audits were based on comparison of existing procedures to a list of standard practices through observation and interview of key hatchery personnel. The more common deficiencies recorded were inadequate vaccine records, incorrect waterbath temperature control, unnecessary removal of unwanted ampoules from liquid nitrogen, slow ampoule thawing time, failure to agitate the vaccine, failure

vaccinate within one hour and limited reconciliation of chicks vaccinated. Repeat visits were made to hatcheries to determine if improvements in handling and administration had been made.

INTRODUCTION

Australia experienced serious outbreaks of MD in imported laying and breeding stock from 1992 to 1997 (1,2). During this period, vaccination programs involved multiple vaccination with lyophilised and cell-associated herpesvirus of turkeys (HVT) vaccines combined with a cell-associated serotype 2 vaccine (strain MDV-19). Cell-associated vaccines were dispensed in plastic ampoules and diluent was held and mixed at refrigerator temperature. A need for broiler vaccination arose and whilst EMBREX INOVOJECT® vaccinating equipment had been imported, none of the available vaccines were suitable for administration by that method. When Bioproperties (Australia) Pty. Limited distributed new MD vaccines in 1997, dramatic improvements in MD control soon were apparent. The new vaccines were manufactured to international standards, dispensed in glass ampoules with diluent suited to room temperature administration and designed for *in ovo* vaccination. To ensure that the full potential of the new vaccines was achieved, a checklist of MD vaccine handling and administration procedures was designed to assist poultry companies in auditing vaccination practices in the hatchery (Table 1). The auditing process was strengthened by regular visits to most hatcheries to ensure that the vaccines were being administered correctly. This paper describes the auditing process and some of the major deficiencies noted in MD vaccine handling and administration in hatcheries.

MATERIALS AND METHODS

Hatcheries. Twenty-three of 26 commercial hatcheries in Australia were audited. The hatcheries included large commercial broiler hatcheries owned by major integrated poultry companies, smaller hatcheries owned by medium-sized broiler companies and hatcheries that specialized in layer chick production. The vaccination equipment in the major hatcheries consisted of either automatic vaccinators or EMBREX INOVOJECT® vaccinating machines. The smaller hatcheries had a mixture of automatic vaccinators and hand vaccination syringes. At the commencement of the audits many of the smaller hatcheries were not equipped to handle cell-associated vaccines and needed to purchase liquid nitrogen storage equipment and to learn the basic procedures for handling cell-associated vaccines. Those hatcheries equipped with EMBREX INOVOJECT ® vaccinating machines were concurrently receiving advice on vaccine administration by EMBREX Inc.

Training Programs. Merial Select Laboratories provided a series of videos and photographic slides illustrating vaccine handling and administration techniques for cell-associated vaccines. These visual aids together with leaflets on the handling of The Mareks Company vaccines were offered to hatcheries in a series of group discussions with hatchery staff. Staff were provided with practical demonstrations of vaccine handling techniques with the equipment that existed in their hatcheries. An initial audit of the MD vaccine handling and administration procedures was usually undertaken following the training program.

Hatchery Audit. Audits were undertaken by prior arrangement but were designed to coincide with the standard vaccination program undertaken at the hatchery. Observations on the methods of vaccine handling and administration were made initially without comment as to the correctness of the procedure. Records of vaccine batch receivals were examined, levels of liquid nitrogen were checked, waterbath and diluent temperatures were measured and the handling steps were timed. The personnel responsible for vaccine handling were interviewed as to why certain procedures were being followed and responses noted on the audit forms. The completed audit form was discussed with the hatchery manager and a covering report was provided to the hatchery manager and the company technical manager.

RESULTS

Training Programs. Training programs focused on the safe handling of liquid nitrogen, removal of only those ampoules required for vaccination, rapid thawing and gentle transfer of all the vaccine to room temperature diluent. It was emphasized to hatchery staff that they were handling living cells that required gentle handling. The need for the correct needle size for transfer of vaccine and for chick vaccination was stressed. Staff were also advised on the need to administer the diluted vaccine within one hour and to frequently agitate the vaccine to maintain the cells in suspension.

Quality Control Audit. The most commonly observed deficiencies are shown in Table 1 and the relative frequency with which they were observed was graded (+ to ++++). The most commonly observed deficiencies in relation to equipment were the lack of a dedicated vaccine preparation room. Where provided, these rooms often lacked air control systems. Due to the larger number of different types of MD vaccines that were required to be held in liquid nitrogen, several hatcheries had not developed adequate recording systems to precisely identify the types and delivery dates of different batches of vaccine. The change to liquid nitrogen handling found several hatcheries deficient in the range of protective clothing.

Table 1. Vaccine quality control audit form for cell-associated Marek's disease vaccines.

Activity	Standard	Comment
Equipment		
1. Location in hatchery	Special area for vaccine preparation Air control systems	++++
2. Vaccine supervisor	Dedicated staff member	++
3. Vaccine storage	Liquid nitrogen storage	+
4. Liquid nitrogen levels	Book for recording level Frequencey of levels check	 +
5. Liquid nitrogen supply	Service contract exists	
6. Vaccine records	Identification of batch and deliveries	++++
7. Protective clothing	Face shield, gloves, long sleeves, ear noise protectors	++
8. Diluent storage	Room temperature at time of use	+
9. Syringes	Changed minimum daily - boiled if reused	
10. Draw off tubes	Changed minimum daily - boiled if reused	
11. Vaccinating equipment	Cleaned daily and flushed with boiling water	+
12. Needle size for vaccinating	19-20 gauge/20 mm length	+
13. Volume of vaccine	Checked daily for volume of 0.2 ml/chick	
14. Function of vaccinating equipment	Checked for faults	
Vaccine Preparation		
15. Vaccine records	Record of batch no. and dilution	
16. Disinfectants used	Use alcohol pads on equipment or diluent ports No contact with vaccine	++
17. Diluent contamination	Pink colour check Yellow bottles not used	
18. Water bath temperature	Check temperature 27-29°C Water bath with adequate heater Temperature maintained during thawing	 +++ +
19. Diluent preparation	Diluent prepared for reconstitution Dye or antibiotics added 15 minutes before use	++ +
20. Syringe and needle size for diluting	Prepare a 5 or 10 ml syringe fitted with an 18 gauge/ 37 mm (1.5") needle containing 2.0 ml of diluent	++
21. Ampoule transfer from liquid nitrogen	Canister not taken higher than container neck Cane lifted to allow ampoule removal Only ampoules required taken out of nitrogen Ampoule placed in water bath immediately after removing from container	+

22. Thawing of ampoules in water bath	Rapid thaw (30-60 sec.) Vaccine gently swirled as thawing No more than two ampoules thawed at once	++ + +
23. Transfer to diluent	Immediately upon thawing Gentle transfer procedure	+
24. Residue in ampoules	Ampoules gently rinsed Check caps, rinse if required	++++
25. All ampoules diluted	All vaccine thawed mixed with diluent	
26. Vaccine mixed	Well mixed and gently rocked Rocked every 10-15 mins during use	+ +++
27. Speed of use	Mixture used within one hour Packs not used within one hour kept cool	++ +
28. Syringe set up	No air bubbles	
Vaccination Procedure		
29. Vaccination technique	Chickens held correctly No hits on spinal column	
30. Vaccination speed	Syringe<1500 hour Accuvac<2500	++
31. Vaccine spillage	Check for misses Use dye	+
32. Missed chickens	Re-vaccinated	
33. Vaccine usage	<1 hour	++
34. Full pack use	All vaccine used before breaks	
35. Cross check on usage	Compare pack number with chick numbers	+++
36. Equipment cleanup	Vaccinators stripped and boiled or preferably autoclaved	++

+ Denotes departure from recommended standard practice (graded + to ++++).

The more commonly observed deficiencies in relation to vaccine preparation included a general lack of appreciation of the need for a high level of hygiene in the handling of MD vaccines. In particular, hatchery staff failed to appreciate that the vaccine should be handled in such a way as to avoid hatchery air and chick dust from contaminating the vaccine. Lifting of ampoules that were not required out of liquid nitrogen for more than 30 seconds was of major concern. Several hatcheries failed to thaw the vaccine within one minute due to inadequate operation of the water bath. Most hatcheries failed to rinse the ampoules thereby losing some 15% of the virus content.

The more commonly observed deficiency in relation to vaccination procedure was failure to administer the vaccine within one hour. This was related to the inappropriate choice of vaccine pack size for the vaccinating equipment. Several hatcheries did not undertake a cross check of the number of vaccine packs utilized with the number of chicks vaccinated and some hatcheries did not undertake a full strip down of vaccinating equipment prior to sterilization.

DISCUSSION

The need for training programs was appreciated by both the hatchery staff and the vaccine manufacturer. Hatcheries that had previously handled cell-free MD vaccines or cell-associated MD vaccines in plastic ampoules had to adopt a new approach to the thawing and the handling of the new vaccines. As it was essential for the glass ampoules to be thawed within one minute, correct waterbath management was needed to achieve this standard. Changes in diluent handling were recommended, as it was required that new

vaccines be mixed with room temperature diluent and be administered at room temperature. The report on the hatchery audit was acted upon quickly by most hatchery managers. Where a repeat visit was undertaken most of the deficiencies had been corrected. However, there was still some concern remaining about the maintenance of high levels of hygiene during vaccine handling and an awareness that living cells require care in vaccine preparation and administration. The techniques of viable cell counting and vaccine virus recovery are being used by some hatcheries to gain further confidence that each chicken is receiving the correct dose of vaccine.

REFERENCES

1. Jackson, C. A. W. Failure of imported genetic strains of layer chickens to respond to Australian-derived Marek's disease vaccines. In: Current Research on Marek's Disease. F. F. Silva, H. H. Cheng, P. M. Coussens, L. F. Lee and L. V. Velicer, eds. American Association of Avian Pathologists, Kennet Square, PA. pp 448-454. 1996.

2. Jackson, C. A. W. Multiple causes of Marek's disease vaccination failure in Australian poultry flocks. Proc. 47th West. Poult. Dis. Conf., Sacramento, CA. pp. 49-51. 1998.

HYPOGLYCEMIA-SPIKING MORTALITY SYNDROME IN BROILER BREEDERS EXPOSED TO A LIGHT-DARK PROGRAM

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During 1998, and early 1999, young broiler breeder chickens were submitted to the Georgia Poultry Laboratory in Oakwood, Georgia, with clinical signs consistent with hypoglycemia-spiking mortality syndrome (HSMS) of broilers (1,2). The affected flocks had been exposed to controlled light-dark cycles in the chicken houses. HSMS has not been previously reported in chickens exposed to light-dark, but only in chickens exposed to 24 hours/day of continuous light. Light-dark exposure has been used very successfully as a tool to prevent HSMS (3,4). We set out to investigate the etiology and pathogenesis of HSMS in these broiler breeder flocks. Experimental inoculations of 1-day-old broiler breeder chicks with filtered fecal homogenates, produced from the feces of the affected field breeder chicks, resulted in a very high percentage (69%) of affected principals. No controls were affected. Previously reported protocols were used in the experiments (2). Electron microscopy, using negative staining, on the feces from both the field and experimental chicks, revealed virus-like particles similar to those we previously described in chicks with HSMS (1,2,3,4,5). In addition, through a combination of electron microscopy and virus isolation, we detected several common chicken viruses (avian encephalomyelitis virus, infectious bronchitis virus, and reovirus), and a small round virus of approximately 32 nm in diameter. We are currently investigating cytopathic effects and positive immunofluorescence in 2 mammalian cell cultures which were inoculated with the feces from the affected field chicks, and tested with convalescent sera from affected experimental chicks.

REFERENCES

1. Davis, J. F. Hypoglycemia-spiking mortality syndrome of broiler chickens. In: Diseases of Poultry, 10th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif, eds. Iowa State Univ. Press, Ames, IA. pp 1023-1025. 1997.

2. Davis, J. F., A. E. Castro, J. C. de la Torre, C. G. Scanes, S. V. Radecki, R. Vasilatos-Younken, J. T. Doman, and M. Teng. Hypoglycemia, enteritis, and spiking mortality in Georgia broiler chickens: experimental reproduction in broiler breeder chicks. Avian Dis. 39:162-174. 1995.

3. Davis, J. F., A. E. Castro, J. C. de la Torre, H. J. Barnes, J. T. Doman, M. Metz, H. Lu, S. Yuen, P. A. Dunn, and M. N. Teng. Experimental reproduction of severe hypoglycemia and spiking mortality syndrome using field-derived and embryo-passaged preparations. Avian Dis. 40:158-172. 1996.

4. Davis, J. F. Remedy for spiking mortality syndrome. Broiler Industry. pp. 32-40. Sept., 1995.

5. Davis, J. F., J. C. de la Torre, A. E. Castro, B. M. Connolly, J. T. Doman, and P. A. Dunn. Experimental reproduction of hypoglycemia-spiking mortality syndrome in broiler chickens with the use of homogenized brains containing arenavirus-like particles. Avian Dis. 41:442-446. 1997.

USE OF INFECTIOUS BURSAL DISEASE PCR, HISTOLOGY AND CHALLENGE TO TROUBLESHOOT POOR BROILER PERFORMANCE

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HISTORY

A multicomplex broiler integrator in the U.S.A. had one complex (A) in which feed conversion and growth in broiler chickens was poorer than other broiler operations in the same region. To determine the importance and effect of infectious bursal disease (IBD) on broiler performance, 10 bursae of Fabricius were collected from 1-5 broiler flocks in each of 9 complexes between 14-28 days of age. The bursae were examined for presence of IBD virus. Viral isolates were typed and some were tested for pathogenicity in chicks from source breeder flocks for complex A. Lesions in the bursae from Complex A were compared with lesions in the bursae from another nearby complex B not experiencing problems.

RESULTS

Histologic lesions in bursae. Dr. Tom Brown of the University of Georgia scored bursal histologic lesions, from 1 (no lesions) to 4 (acute to subacute follicular necrosis) in bursae from complexes A and B. The lesions were very similar in samples between the two complexes (Table 1).

Table 1. Individual bursal histologic scores*

COMPANY	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Complex A	2	1	1	2	2					
	2	3	2	1	2					
	2	3	3	2	3					
	2	2	3	2	2					
	1	2	2	2	1					
	1	2	2	1	2					
	1	1	1	1	1					
	1	2	2	1	3					
Complex B	1	1	1	1	1	1	2	1	1	1
	1	1	1	1	1	1	1	1	1	1
	4	1	1	4	4	1	1	1	4	4
	4	1	4	4	4	4	4	4	4	4
	1	1	1	1	1	1	1	1	1	1

1 = no lesions to 4 = acute/subacute follicular necrosis

Two broiler flocks in each complex had histologic damage. Lesions were actually more severe in complex B where no performance problems were observed.

IBD isolates. Presence and molecular identification of IBD viruses were conducted by Dr. Daryl Jackwood of Ohio State University using molecular assay, RT/PCR-RFLP (reverse transcriptase/polymerase chain reaction-restriction fragment length polymorphism). Eighty-four percent of broiler flocks had an IBD isolate. The only known IBD subtype identified was Del E (37.5%). No "Classic" or Del A was identified. Eight "molecular variants" were found. Complex A had only "molecular variant #6" in 74% of broiler flocks sampled (Table 2).

Importance of "molecular variant" found. Chicks from 5 breeder flocks of complex A were challenged at Auburn University by Dr. Joe Giambrone with IBD "molecular variant #6" from complex A in addition to "Classic" (APHIS challenge strain) and Del E IBD virus (Figure 1). At 7 to 10 days postchallenge, birds were sacrificed and bursa/body weights obtained. Histologic lesion scores of formalin-fixed bursae were determined. Calculations of percent protection scores were made based on bursa/body weight ratio and histologic bursal score determined by Dr. Tom Brown, University of Georgia. Maternal antibody protection of birds challenged with "molecular variant #6" was found to be similar to those challenged with Del E (Table 3). Body weight gains post-challenge were also measured (Table 4). Weight gains analyzed using SAS were found to be the least depressed in birds challenged with "molecular variant #6" as compared to those in birds challenged with standard or Del E.

CONCLUSION

IBD histologic lesions were no more severe in broilers from complex A, which had relatively poorer performance, than in broiler farm complex B. The predominant IBD virus "molecular variant #6" found in complex A was not different clinically (% protection, weight gain depression) from Del E. Use of molecular IBD identification and bursa histology clearly shows that "molecular variant #6" was not a major contributing factor to poorer feed conversion and growth rate in complex A.

Table 2. RT/PCR-RFLP assay of IBD isolates in region around Complex A

	IBD		PCR																												
	New	Known	*Bst*NI*																	*Mbo*I*											
Complex	Subtype	Subtype	424	370	350	210	209	200	172	139	119	480	450	403	362	330	310	280	270	269	260	250	234	229	195	185	120	112	75
A	none																												
	variant #6		X						X		X					X								X					
	variant #6		X						X		X					X								X					
	variant #6		X						X		X					X								X					
1	variant #2		X						X		X			X													X	X	
		Del-E	X						X		X			X										X					
	variant #7		X						X		X						X							X					
	variant #7		X						X		X						X							X					
2	variant #7		X						X		X						X							X					
	variant #7		X						X		X						X							X					
		Del-E	X						X		X			X										X					
	none																												
3		Del-E	X						X		X			X										X					
		Del-E	X						X		X			X										X					
	none																												
		Del-E	X						X		X			X										X					
4	none																												
5	variant #6	Del E	X						X		X					X								X					
	variant #6		X						X		X					X								X					
	variant #3		X						X		X				X									X					
6	variant #4		X						X		X				X						X						X		
		Del-E?	X						X		X			?	X									X					
	variant #9			X					X		X			X										X					
	none																												
7		Del-E	X						X		X			X										X					
		Del-E	X						X		X			X										X					
		Del-E	X						X		X			X										X					
		Del-E	X						X		X			X										X					
8	variant #5		X						X		X				X					X									X
	variant #1		X						X		X			X						X									
	variant #1		X						X		X			X						X									
	variant #1		X						X		X			X						X									

Values are the length in base pairs of the restriction fragments. Shaded boxes designate the presence of a restriction fragment following digestion with *BstN1* and *Mbo1*. Viruses within molecular groups are antigenically similar and some viruses with different molecular groups may be antigenically related.

Figure 1. IBD chick challenge protocol

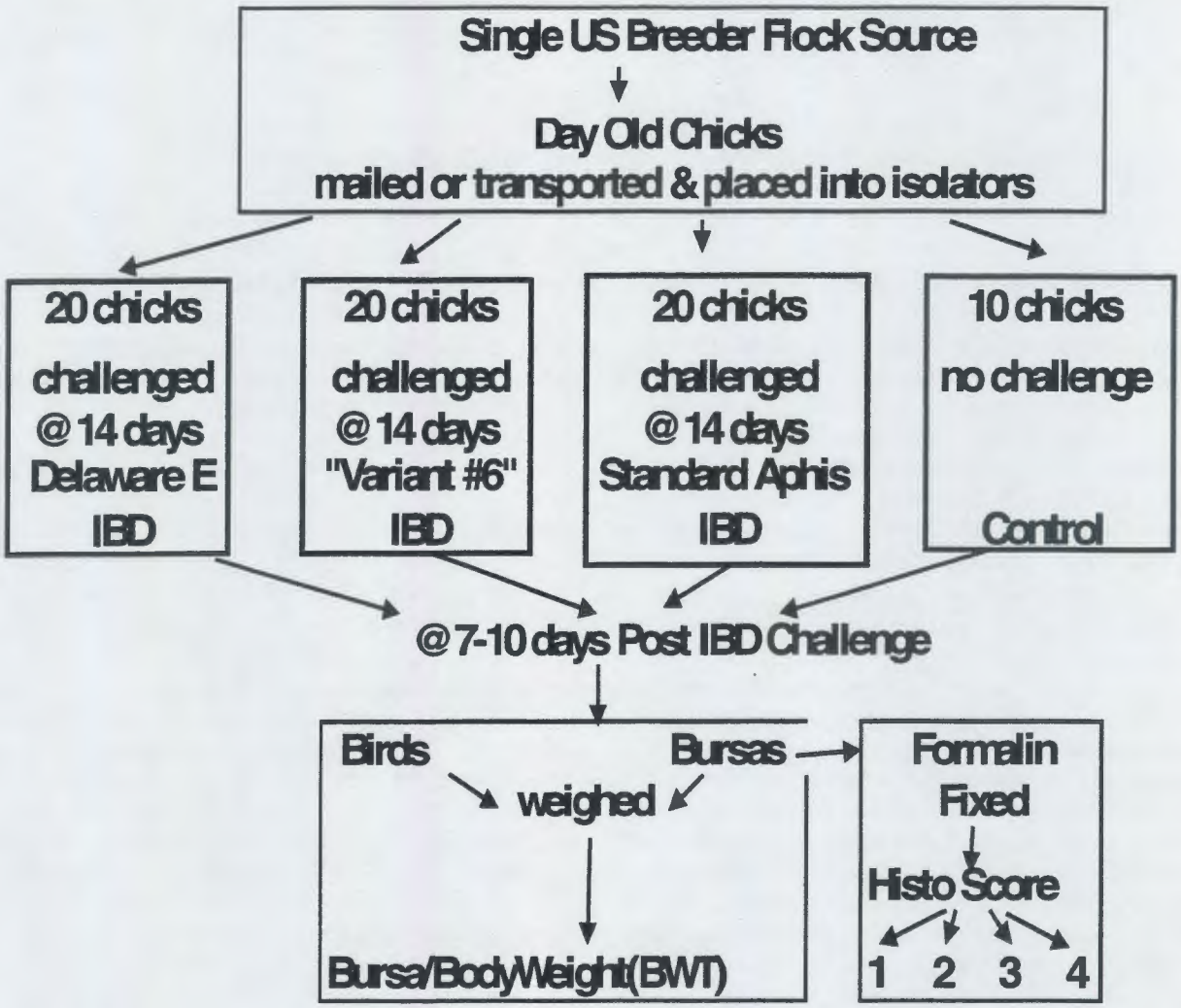

Table 3. Percent IBD protection against different IBD subtypes

Breeder Flock	% Protection					
	Gross			Histo		
	Std	Var E	Var #6	Std	Var E	Var #6
1	55	86	78	13	67	79
2	61	70	48	33	27	27
3	61	77	87	14	100	57
4	22	13	15	13	7	7
5	94	100	94	93	47	60
6	100	86	75.5	80	40	33

Table 4. Effect of different IBD subtypes on body weight

Breeder Flock	Body Weight Gain (gms)*			
	Std	Var E	Var #6	Control
1	319 a	279 b	292 ab	292 ab
2	299 ab	280 b	322 a	315 a
3	301 a	270 b	310 a	320 a
4	255 ab	323 a	291 a	330 a
5	254 b	323 a	291 a	315 a
6	257 b	250 b	265 b	304 a

* means with different letters differ significantly $P<0.05$

PARAMYXOVIRUS INFECTION AND OTHER DISEASE CONDITIONS IN SQUAB OPERATIONS

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PARAMYXOVIRUS INFECTION

History. Four thousand nine hundred and twenty-eight pairs of pigeons are housed on a farm in eastern Washington. Thirty-two pairs are kept in each pen and there are 154 pens in 10 buildings. Each building is 12 X 128 feet in dimension. Approximately 2,000 to 2,500 squabs were raised and about 500 squabs were slaughtered each week in December, 1997. The average live squab weighs 17 ounces; when dressed the birds are sold at $4.50 per pound. At this farm the cost of raising squabs is $2.00 per pound. Other farms in the vicinity include a squab farm located 5 miles away and a commercial chicken farm located approximately 15 miles distant. Not many wild birds reside near or fly over the farm, however a few dead sparrows were found in a feed truck parked in an old barn. This occurred about 3 weeks before illness and mortality was observed in the flock.

Clinical signs and mortality. Five squabs and one adult breeder were submitted to the Avian Health Laboratory with a history of high mortality in squabs and some mortality in the breeders in buildings 3 and 4. Clinical signs were some respiratory problems, head shaking and neck twisting, and watery stools that made the pen floors wet and slippery. Total mortality included approximately 2,500 squabs and 300 adult breeders in a 4-week period.

Gross and microscopic findings. Moderate to marked airsacculitis was present in three birds. A focal granuloma containing caseous material was present in one liver. The livers of four squabs were enlarged. Kidneys and spleens of all birds were enlarged. The intestine walls were edematous and congested; excessive catarrhal exudate was present in the intestine lumen. Lungs were congested and contained frothy contents.

Microscopic changes were moderate to severe, subacute to chronic, airsacculitis with intra-lesional bacteria. Moderate, multifocal bronchopneumonia was present in the lung sections of two birds. Mild, lymphocytic, plasmacytic tracheitis, with possible viral inclusions, was present in tracheas from two birds. Locally extensive, chronic, necrotizing hepatitis with granuloma formation and the presence of fungal hyphae was found in one liver section; liver sections from other birds were normal. Mild splenitis was present in one bird and moderately severe in another bird with the depletion of lymphoid elements. The bursa of Fabricius of two birds had mild to moderate lymphoid atrophy/depletion and heterophilic bursitis. Mild to moderate, or moderately severe, enteritis was present in some birds. Mild to moderate chronic epicarditis was present in the hearts of three birds. The changes in the brains were of mild to moderate multifocal lymphocytic and histiocytic encephalitis with multifocal gliosis. The changes observed in all tissues were consistent with a combination of bacterial and viral infections.

Bacteriology. Low numbers of *E. coli* were isolated from one liver, there was no bacterial growth from a second liver, and *Pasteurella multocida* was isolated in high numbers from a third liver. Three tracheal cultures were negative for *Bordetella*, but *E. coli* and *Staphylococcus* were isolated in moderate to high numbers from all three tracheal specimens. Composite tissues collected from the livers and intestines were negative for *Salmonella*.

Virology. The trachea, lung and liver tissues were macerated and after antibiotic addition were inoculated in SPF embryos via the yolk sac route. Nine embryos on the fourth day and one embryo on the fifth day died postinoculation. All ten embryos died on the second day, post inoculation, on the second passage. Syncytial cell CPE was present in liver cell monolayers. The isolated virus was identified with the help of the electron microscope as Paramyxovirus type 1.

Serology. Four weeks after the initial diagnostic work, sixteen serum samples collected from squabs at slaughter were tested for antibodies against *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Salmonella pullorum*, avian influenza and paramyxovirus. All samples were negative for all the antibodies tested.

Recommendations. The farmer was advised to add chlorine in the drinking water at a rate of 1 part of Chlorox to 1024 parts of drinking water. Chlorox is a household bleach containing 5.25% sodium hypochlorite and is available commercially. The farmer consulted other squab producers in California and found out that those squab producers routinely add Chlorox at a rate of one part of Chlorox with 100 parts of drinking water. Considering this, he administered Chlorox to his flocks at a rate of 1 part of Chlorox with 200 parts of drinking water. The birds did not back off from the water and the pen floors dried up as the watery stools observed in the flock disappeared. Mortality in the squabs and breeders decreased after about 4 weeks of chlorine administration.

CAPILLARIA WORM INFESTATION

Four squab breeders were submitted to the Avian Health Laboratory from northwestern Oregon on July 14, 1998. The case history was listlessness and a daily breeder mortality of 0.5-0.6%. The birds were treated with terramycin but the death loss continued. All four birds were emaciated. The crop mucosa was thickened and whitish in color in 3 birds. The livers and spleens of all four birds were enlarged. The intestines of three birds were distended, thin walled, and contained excessive liquid and gaseous contents. The testicles of three males were inactive. The breeder hen was out of egg production. No bacterial pathogens were isolated from the two liver tissue pool. The livers and intestines were negative for *Salmonella.* Fecal floatation tests disclosed high numbers of capillaria ova and round worms. On histopathology changes observed in sixteen intestine sections included atropy of the mucosa characterized by widely spaced epithelia l crypts and widened and shortened villi. Within the lumen there were numerous cross-sections of nematodes. The nematodes were long and slender and about 25 microns in diameter. Most of these nematodes were free within the lumen, without any obvious embedding in the mucosal wall. A further four squab breeders were submitted on August 19,1998. The case history noted that piperazine had been administered twice to deworm the breeder flock with no improvement. Daily breeder mortality continued to be 0.5-0.6%. Necropsy lesions and fecal floatation tests reconfirmed a severe capillaria infestation.

CANKER OR TRICHOMONIASIS

Mortality in baby squabs was reported from a large squab producer in western Washington. No mortality was observed in the adult breeders. Gross and histopathologic evaluations confirmed trichomoniasis infection. An apparently mild or latent trichomoniasis infection was transmitted to the baby squabs through the crop milk.

AN OUTBREAK OF NEWCASTLE DISEASE IN EXOTIC PHEASANTS AND DOVES

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Newcastle disease virus was isolated from a tissue pool consisting of liver, lung, and kidney from a 3-year-old tragopan pheasant and from the brains of 3 adult cuckoo doves. These birds were from an aviary housing 15 pheasants and 200 doves. Most of the pheasants and 20% of the doves died in a 10 day period after the introduction of approximately 130 exotic doves which had been received following release from a quarantine station. Ten of these 130 birds subsequently died. Clinical signs included sudden death, lethargy, occasional respiratory signs, diarrhea, and neurological signs. Gross lesions in the pheasant included dehydration, congestion of the lungs, pale epicardium, erosions in the esophagus and crop, and mucoid contents in the intestine. One of the cuckoo doves had a few erosions in the crop and esophagus, and two birds had pale epicardium with severe hydropericardium in one of the two. Livers were pale and friable in the cuckoo doves. In three cuckoo doves roundworms were present in the intestine. Microscopic lesions in the tissues from the pheasant included interstitial pneumonia, myocarditis, conjunctivitis, ulcerative esophagitis and ingluvitis, and nonsuppurative encephalomyelitis. Other lesions consisted of enteritis and ulcerative dermatitis associated with vasculitis. Epithelial cells of the esophageal glands and conjunctiva, and neurons of the ganglia subjacent to the adrenal contained eosinophilic intranuclear and intracytoplasmic inclusion bodies. The cuckoo doves had acute necrosis of hepatocytes with fibrin exudation in the sinusoids and eosinophilic intranuclear inclusion bodies in the hepatocytes. *E. coli* was isolated from the livers of the cuckoo doves, but all the birds including the pheasant were negative for *Salmonella* sp. and *Mycoplasma* sp. Serum from the pheasant was positive for avian paramyxovirus type 1 antibodies and had a titer of 1:16 by HI. Transmission electron microscopy on the liver from the cuckoo doves and the esophageal glands from the pheasant revealed nucleocapsids consistent with the morphology of paramyxovirus. The cuckoo doves examined for chlamydia by fluorescent antibody technique were negative. Pathogenicity studies in chickens with the isolated virus identified it as lentogenic. The most probable source of infection with Newcastle disease in this aviary was from the birds added to the aviary from the quarantine station. Lentogenic Newcastle disease virus had been isolated from some of these birds which had died during the quarantine period. Vaccination of the remaining birds in the aviary with Newcastle disease virus vaccine prevented further losses.

PARAMYXOVIRUSES II AND III - CLINICAL AND SEROLOGICAL FINDINGS IN MEAT-TYPE TURKEYS

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Paramyxovirus II (PMV-2, chicken/California /Yucaipa /56) and PMV III (Turkey/Wisconsin/68 and Parakeet /Netherlands/ 449/75) are viruses affecting many species of domestic, pet and wild birds. In 1979-81, during an epizootic of turkey rhinotracheitis (TRT) that swept through Israel, many PMV II viruses were isolated from meat-type and breeding turkey flocks. Subsequently, in 1994, we began to isolate PMV II from many meat-type turkey flocks, at the age of 3 to 8 weeks with respiratory disease. The clinical signs were dyspnea, conjunctivitis, ocular and nasal discharge, and sinusitis. Mortality was 5 to 20% and much higher if bacterial infections were involved. A marked serological response began about 7 days after the first clinical signs. Virus was isolated from tracheal and cloacal swabs and internal organs. PMV III which can cause a severe drop in egg production in turkey breeding flocks, also causes respiratory signs in meat-type turkeys that are not different from those caused by PMV II. More passages in embryonated eggs are needed for isolation of the virus. From October, 1996, to December, 1998, PMV II was isolated from 15 meat-type turkey flocks, PMV III from 5, a dual infection of PMV II/ PMV III from 5 and PMV II/ PMV VII from 2 flocks. In the same period, PMV II was isolated from 26 broiler flocks and a PMV II/ PMV III mixed infection from 3 flocks. Clinical signs with a very high morbidity and mortality were observed in turkey flocks suffering from dual infection with PMV II and PMV III. Inactivated oil emulsion vaccines are being prepared and efficacy trials are under way. Israel is on the main migratory route of wild birds from Europe to Africa and back. Many wild birds overwinter in Israel and we assume that these are involved in the epidemiology of the disease.

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EVIDENCE OF MUSCOVY DUCK PARVOVIRUS IN MUSCOVY DUCKLINGS IN CALIFORNIA

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Muscovy duck parvovirus (MDPV) has been demonstrated in tissue samples from one- to four-week-old commercially-reared Muscovy ducks that presented with increased mortality, weakness, lateral recumbency and an inability to walk. Necropsy findings included paleness of the thigh and leg muscles and of the myocardium, fibrinous exudate on the capsule of the liver, and ascites. Histologically muscle degeneration characterized by severe myositis and mild to moderate myocarditis was observed. The presence of a parvovirus was demonstrated by 1) isolation in embryonated Muscovy duck eggs and detection by negative stain electron microscopy on chorioallantoic fluid from inoculated eggs, 2) polymerase chain reaction (PCR) directly from the suspected organ tissues, and 3) antibody detection by immuno-electron microscopy, ELISA, and immunofluorescence. In addition, the PCR products obtained, that represented 1625 bp (74%) of the capsid VP1 gene (including an hypervariable region between Derzsy's disease virus or goose parvovirus [GPV] and MDPV), were completely sequenced and shown to be 100% homologous with the MDPV 89384 reference strain (1), but only 82.3% homologous with Derzsy's disease virus. To our knowledge this is the first occasion on which MDPV has been detected in the USA.

REFERENCES

1. Jestin, V., M-O. Le Bras, M. Cherbonnel, G. Le Gall, et G. Bennejean. Mise en évidence de parvovirus (virus de la maladie de Derzsy) très pathogènes dans les élevages de canards de Barbarie. Rec. Med. Vet. 167: 849-857. 1991.

A HIGHLY VIRULENT ISOLATE OF INFECTIOUS BURSAL DISEASE VIRUS (IBDV) IN HONG KONG

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ABSTRACT

In this study, we characterized the isolate of infectious bursal disease virus (IBDV) causing outbreaks in poultry farms of Hong Kong in 1995. IBDV was isolated from the infected tissue provided by the Agricultural and Fisheries Department of the Hong Kong Government. Comparing the VP2 sequence of the local isolate to the reported IBDV strains in the genebank, it was shown that the local strain is closely related to the 'very virulent' European strain (UK661, Genebank accession X92760). Analysis of 35 PCR clones of the hypervariable region revealed that a heterogeneous IBDV population was present in the infected tissue. Basically the clones could be divided into 3 groups. Twenty-two clones (group 1) contained exactly the same sequences whereas 11 clones (group 2) had a few mutations, including nucleotides and amino acids, with reference to group 1. The other 2 clones (group 3) were different from the previous 2 groups and also did not show close linkage with any published IBDV sequences in the genebank. At a dose of 200 EID50, this isolate caused 100% infection in both SPF chickens and non-vaccinated Chinese Shek Kei strain chickens and caused 60% mortality in SPF birds.

INTRODUCTION

Infectious bursal disease virus (IBDV) causes an acute and highly contagious infection in young chickens of the age 3 to 6 weeks. Infected chickens either die from acute infection or become severely immunosuppressed and susceptible to other infectious agents (1). During 1994 and 1995, there were outbreaks of infectious bursal disease (IBD) in local poultry farms reported by the Department of Agriculture and Fisheries (AFD) of the Hong Kong Government. Since vaccination against IBDV is a common practice in local poultry farms, the outbreaks were possibly caused by a newly introduced strain or local strains which had undergone mutations, allowing the virus to infect immunized birds.

Three major groups of pathogenic IBDV strains, classical, variant and 'very virulent' (VV) IBDV strains, have been described with varying virulence and antigenicity (1). The variant strains were antigenically different from the classical strains and characterized by their ability to circumvent vaccination with standard type 1 strains (2). VV IBDV strains were isolated in Europe and Japan since 1986. These VV IBDV strains are antigenically similar to the classical European strains but cause high mortality (3). These strains can also infect birds with levels of maternal antibody that are protective against the classical strains. A short hypervariable (HV) region, within the viral gene VP2, was found to be useful for defining the mentioned viral strains by a molecular approach (4). This work was aimed to investigate the IBDV strain causing the local outbreaks. The results would be useful for the design and choice of vaccines for the local poultry industry. The virus was isolated from infected samples provided by the AFD and the viral genes were cloned and studied. The population of IBDV in one of the samples where the highly virulent virus was isolated was further characterized. The virulence of the virus was also tested in specific pathogen free (SPF) chicken and Chinese Shek Kei chicken, a common Chinese poultry strain.

MATERIALS AND METHODS

Cloning of viral gene VP2. Infected bursae which had been confirmed to be IBDV-positive by immuno-diffusion were provided by the AFD. Individual bursa was extensively washed with buffer before extraction of virus to avoid contamination of the tissue from the field. The extraction of virus and cloning of the viral genes by RT-PCR has been described elsewhere (5). The complete VP2 gene was amplified by RT-PCR using the primers VP2-5 (5'- TTTA GCGGCCGCGAGATCAGACAAACGATCGCAG-3') and VP2-3 (5'-TTTAGCGGCCGCTARCACGCAGTCGAGGT TRTG-3') with Expand High Fidelity PCR System (Boerhinger Manheim) and inserted into pBluescript plasmid (Stratagene). The clones were sequenced and compared with existing IBDV sequences (NCBI Genebank). The data were analysed with software, MacDNASIS Pro V2.4 (Hitachi Software Engineering) and GeneWorks (IntelliGenetics Inc., USA).

Cloning of HV region. Since clones of varying sequences in the infected tissue were found, the hypervariable (HV) region was amplified independently from the purified RNA to study this heterogeneous population. The HV region was amplified using *Pfu* polymerase (Stratagene) to minimize mutation due to PCR. Primers

IBDV-HV5 (5'-AAAAGCTTATGTTCTCAGCCAACAT TGATGC-3') and IBDV-HV3 (5'-TTTCTAGAGTTGC CACTCTTTCRTAGGC-3') flanking the HV region were used for the amplification. 35 HV clones were sequenced and compared.

Animal challenge. To determine the 50 % egg infectious dose (EID50) of the inoculating virus, the infected sample was first homogenized with tissue culture medium containing antibiotics and filtered through 0.2 µm membrane. The serially diluted homogenate was inoculated onto the chorioallantoic membrane (CAM) of 10-day-old SPF Leghorn chicken eggs (CSIRO SPF Poultry Isolation Unit, Australia) and the EID50 was calculated by the Reed and Muench formula (6).

SPF Leghorn chicken eggs were hatched and fostered in HEPA (High Efficiency Particle Arresting) filtered air isolators. Day 0 Chinese Shek Kei chicken carrying maternal anti-IBDV antisera were provided by the AFD. The maternal antisera were monitored by a commercial IBDV antisera test kit (IDEXX). Shek Kei chicken were challenged when the maternal antibody dropped to a level that the chicken sera were not considered IBDV positive. SPF chicken and Shek Kei chicken were challenged intranasally with a 200 EID50 at the age of 3-week-old and 26-day-old, respectively. Birds not killed by the virus were sacrificed 72 hr post-infection and the disease was confirmed by examination of the bursae.

RESULTS AND DISCUSSION

Isolates of IBDV in Hong Kong. Two different IBDV clones were isolated from the samples provided by the AFD, namely HK46 and HKL6. The nucleotide and deduced amino acid sequences of the HV region were submitted to NCBI Genebank (Accession AF051838 for HK46 and AF051839 for HKL6). The phylogenetic relationship is shown in Figure 1. HK46 is closely related to the European VV IBDV isolates whereas HKL6 is more similar to the classical strains but contains mutations not found in other strains. Based on the sequence data, it was suggested that HK46 is a VV IBDV strain, however, the identity of HKL6 is unknown. The virulence of HK46 was further confirmed by animal challenge.

Population analysis of IBDV in bursa infected with HK46. During the analysis of the infected samples, clones of minor nucleotide difference were always found from the same sample and the frequency was far higher than expected from PCR mutations. A separate RT-PCR using high fidelity *Pfu* polymerase was done to amplified the HV region from sample NV-13 where HK46 was isolated. 35 clones were sequenced and analysis of these 35 HV clones revealed that a heterogeneous IBDV population was present in the infected tissue. Figure 2 shows the nucleotide alignment of the clones. Basically the clones could be divided into 3 groups. Group 1 has exactly the same nucleotide sequence as the reported HK46 sequence (22 clones). Group 2 contained a few base difference with reference to group 1 (11 clones, 6 different sequences). Group 3 was not similar to HK46 but to HKL6 (one clone same as HKL6 and one with three nucleotides different). It gave the idea that the majority of IBDV (94.2 %) in sample NV-13 was the VV IBDV virus whereas less than 6 % was HKL6-like virus.

The results were unlikely due to contamination of samples because the samples were extensively washed with buffer before virus purification. In addition, all the experimental procedures including virus purification, RNA extraction and RT-PCR were done separately at different times for HK46 and HKL6. Therefore it is highly possible that different IBDV viruses were present in the infected sample.

From the results, it was reasonable to suggest that group 1 and group 2 resulted from the mutation of a common IBDV genome, i.e. the 'very virulent' IBDV. The mutations were possibly accumulated before infecting the chicken or occurred after the infection. On the other hand, group 3 virus was possibly a result of cross-infection of the bird with another field virus. Therefore, the presence of a heterogeneous IBDV population in the infected sample could be explained by (a) the infection with a mixed population of IBDV; (b) virus mutation during the infection; or (c) both.

Interestingly, the variations of nucleotide sequence of group 1 and 2 suggest that IBDV possibly, similar to other RNA viruses (7), takes advantage of the high error rate of the RNA polymerase for replication. RNA-dependent RNA polymerase of plus-strand RNA virus lacks the proof-reading activity of DNA polymerase, resulting in a high error rate in the order of 10^{-4} (8). Mutation can easily occur and accumulate during infection if it is not deleterious to the virus. Consequently, an immune evasive strain or variant results against which current vaccines no longer protect. Since there has been no study on the error rate of the RNA polymerase, VP1, of IBDV, it will be interesting to study the polymerase fidelity, the mutation rate and the divergence of the virus. A high mutation rate of the IBDV genome due to the low fidelity of the replication enzyme might be a mechanism for this virus to escape vaccine protection.

Animal challenge. To confirm that HK46 is a VV IBDV strain, the virus isolate was inoculated into SPF leghorn chickens and non-vaccinated Chinese Shek Kei strain chickens. The chickens were challenged intranasally with 200 EID50 and examined 72 hours post-infection. Any chicken not killed by the virus was sacrificed and infection was confirmed by examining the bursa. In 26 challenged SPF chickens, 12 were already dead or close to dead at the time of examination and 5 of them were severely ill and expected to die within hours. Therefore the mortality caused by HK46 was more than 60 %. Dissection of the chickens revealed that all the chickens were infected and the bursae showed varying lesions, from a covering of yellowish gelatinous oedema to severe haemorrhage. None of the Shek Kei chicken showed signs of illness after challenge, however, dissection of the chickens showed that all of them were infected, with a layer of yellowish gelatinous oedema covering the bursae.

Figure 1. Phylogenetic relation of the nucleotide sequence of the HV region of various IBDV strains. The numbers above the branch are the decimal fraction difference of the nucleotides between the strains. The strains compared include UK661, [accession no. X92760], DV86 (Brown *et al.*, 1994); 90-11 (Lin *et al.*, 1993); KS [accession no. L42284]; OKSMT [accession no. D83985], OKSM [accession no. D49706], TKSMT, TKSM (Yamaguchi *et al.*, 1996c); GLS [accession no. M97346] (Vakharia *et al.*, 1994); Variant A [accession no. M64285] (Lana *et al.*, 1992); Variant E [accession no. D10065] (Heine *et al.*, 1991); Cu-1 [accession no. X16107] (Spies *et al.*, 1989); P2 [accession no. X84034] (Mundt and Muller, 1995); PBG-98 [accession no. D00868], 52/70 [accession no. D00869] (Bayliss *et al.*, 1990); CJ-801BKF [accession no. AF006694] (Cao *et al.*, 1995); STC [accession no. D00499] (Kibenge *et al.*, 1990); Edgar [accession no. A33255]; and 002-73 [accession no. M64738] (Hudson *et al.*, 1986). The phylogenetic tree was calculated by the Unweighted Pair Group with Arithmetic Mean method (UPGMA) using the GeneWorks software (IntelliGenetics Inc, USA).

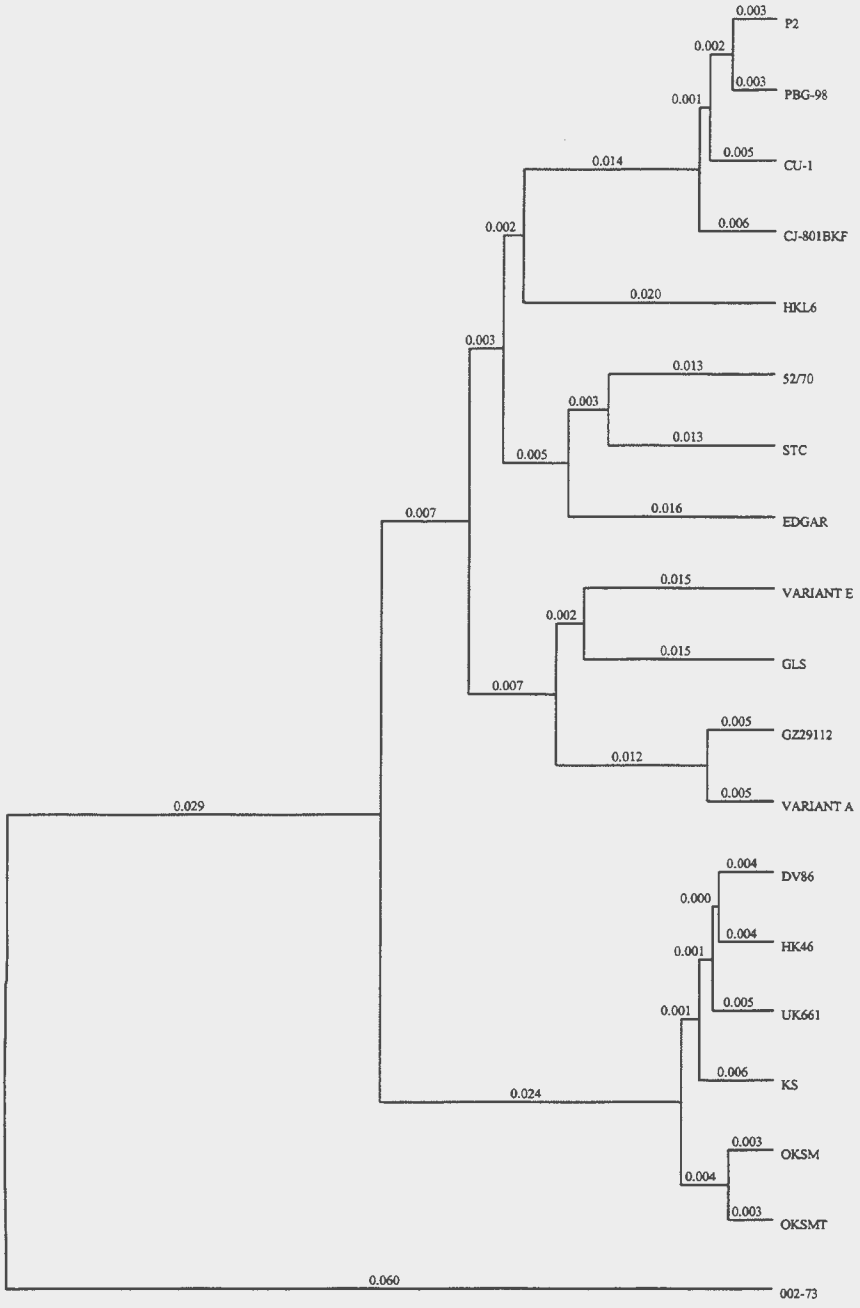

Figure 2. Alignment of nucleotide sequence of HV clones of IBDV infected sample NV-13. Only nucleotide sequence 841 to 1170 is shown here which contained the mutations. The bottom shows the sequence of VV IBDV UK661. The HV clones were categorized into 3 groups. Clones with exact sequences of HK46 and HKL6 strains are marked. Dash (-) represents identical nucleotide to the consensus sequence and dot (.) represents a deletion.

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                          850        860        870        880        890        900        910        920        930        940        950
Group 1 (HK46)      ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
Group 2
Clone 46            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
Clone 59            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
Clone 75            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
6 clones            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 64            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 70            ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------T
Group 3
Clone 2             ----C----- ----GCT--- --G--G---- --C------- -----C---- ---------- ----C--C-- ---------- ---------- -------AA- ----------
Clone 44 (HKL6)     ----C----- ----G-T--- --G--G---- ---------- ---------- ---------- ----C--C-- ---------- ---------- -------AA- ----------
UK661               TCACAAGCCT CAGCATCGGG GGAGAACTCG TGTTTCAAAC AAGCGTCCAA GGCCTTATAC TGGGTGCTAC CATCTACCTT ATAGGCTTTG ATGGGACTGC GGTAATCACC

                          960        970        980        990       1000       1010       1020       1030       1040       1050       1060
Group 1 (HK46)      ---------- ---------- ------A--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Group 2
Clone 46            ---------- ---------- ------A--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 59            ---------- ---------- ------A--- ---------- ------A--- ---------- ---------- ---------- ---------- ---------- ----------
Clone 75            ---------- ---------- ------A--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
6 clones            C--------- ---------- ------A--- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 64            C--------- ---------- ------A--- ---------- ---------- ---------- ---------- -G-------- ---------- ---------- ----------
Clone 70            ---------- ---------- ------A--- ---------- ------.--- ---------- ---------- ---------- ---------- ---------- ----------
Group 3
Clone 2             ---------- -----A---- ---------- --------C- ----T----- ---------- C--------- ------A--- ---------- ---------- ----------
Clone 44 (HKL6)     ---------- -----A---- ---------- --------C- ----T----- ---------- C--------- ------A--- ---------- ---------- ----------
UK661               AGAGCTGTGG CCGCAGACAA TGGGCTGACG GCCGGCACTG ACAACCTTAT GCCATTCAAT ATTGTGATTC CAACCAGCGA GATAACCCAG CCAATCACAT CCATCAAACT

                         1070       1080       1090       1100       1110       1120       1130       1140       1150       1160       1170
Group 1 (HK46)      ---------G ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Group 2
Clone 46            --------CG ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 59            ---------G ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 75            ---------G ---------- ---------- ---------- ---------- ---------- GT-------- ---------- ---------- ---------- ----------
6 clones            ---------G ---------- ---------- ---------- -T-------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 64            ---------G ---------- ---------- ---------- -T-------- ---------- ---------- ---------- ---------- ---------- ----------
Clone 70            ---------G ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Group 3
Clone 2             --------CG ---------- ---------- ---A------ ---------- ----G-T--- ----T----- ---------- ----T----- ------C--- ----------
Clone 44 (HKL6)     --------CG ---------- ---------- ---A------ ---------- ----G-T--- ----T----- ---------- ----T----- ------C--- ----------
UK661               GGAGATAGTA ACCTCCAAAA GTGGTGGTCA GGCGGGGGAT CAGATGTCAT GGTCAGCAAG TGGGAGCCTA GCAGTGACGA TCCACGGTGG CAACTATCCA GGGGCCCTCC
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CONCLUSION

A VV IBDV strain, namely HK46 in this study, was found in local poultry farms, causing outbreaks of IBDV in 1994 and 1995. Analysis of 35 PCR clones of the hypervariable region revealed that a heterogeneous IBDV population was present in the infected tissue. At a dose of 200 EID50, this isolate caused 100% infection in both SPF chicken and non-vaccinated Chinese Shek Kei strain chicken and caused 60% mortality in SPF birds.

REFERENCES

1. Lukert, P. D., and M. Y. Saif. Infectious bursal disease. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard. L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 721-738. 1997.

2. Heine, H. G., M. Haritou, P. Failla, K. Fahey, and A. Azad. Sequence analysis and expression of the host-protective immunogen VP2 of a variant strain of infectious bursal disease virus which can circumvent vaccination with standard type I strains. J. Gen. Virol. 72:1835-1843. 1991.

3. Oppling, V., H. Muller, and H. Becht. Heterogeneity of the antigenic site responsible for the induction of neutralizing antibodies in infectious bursal disease virus. Arch. Virol. 119:211-223. 1991.

4. Bayliss, C. D., D. Y. Spies, K. Shaw, R. W. Peters, A.Papageorgiou, H. Muller, and M. E. G. Boursnell. A comparison of the sequences of segment A of four infectious bursal disease virus strains and identification of a variable region of VP2. J. Gen. Virol. 71:1303-1312. 1990.

5. Kibenge, F. S., D. J. Jackwood, and C. C. Mercado. Nucleotide sequence analysis of genome segment A of infectious bursal disease virus. J. Gen. Virol. 71:569-577. 1990.

6. Reed, L. J., and H. Muench. A simple method of estimating fifty per cent endpoints. Amer. J. Hyg. 27:493-497. 1938.

7. Strauss, E. G., J. H. Strauss, and A. J. Levine. Virus evolution. In: Fields Virology, 3rd ed. B. N. Fields, D. M. Knipe, and P. M. Howley, eds. Lippincott-Raven Publishers. pp. 153-171. 1996.

8. Holland, J., K. Spindler, F. Horodyski, E. Grabau, S. Nichol, and S. VandePol. Rapid evolution of RNA genomes. Science 215:1577-1585. 1982.

PROTECTION OF CHICKENS AGAINST VERY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS WITH A RECOMBINANT MAREK'S DISEASE VIRUS EXPRESSING IBDV VP2 ANTIGEN

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In order to develop a herpesvirus vaccine which can induce immunity against infectious bursal disease (IBDV) for an extended period, a recombinant Marek's Disease virus CVI-988 strain expressing IBDV host-protective antigen VP2 at the *US2* site (rMDV) was developed under the control of an SV40 early promoter. Chickens vaccinated with the rMDV did not show any clinical signs and 55% of the chickens had no or mild bursal lesions after challenge with very virulent IBDV, whereas a conventional IBDV vaccine conferred full protection to chickens. Unvaccinated challenge control chickens and chickens vaccinated with the CVI-988 showed severe clinical signs, severe bursal lesions, and 70-75 % mortality. The rMDV conferred full protection to chickens against very virulent MDV as the CVI-988 strain did, whereas 90 % of the challenge control chickens died of MD. Antibody levels against IBDV and MDV following vaccination increased continuously for at least 10 weeks. No histopathological lesions in the rMDV-vaccinated chickens and no contact transmission of the rMDV to their penmates were found. These results demonstrate that an effective and safe recombinant herpesvirus-based IBD vaccine could be constructed by expressing the VP2 antigen at the *US2* site of the CVI-988 vaccine strain.

(The full length article has been submitted to *Virology*.)

UNCONTROLLABLE INFECTIOUS BURSAL DISEASE ON TWO COMMERCIAL LAYER CHICKEN RANCHES IN SOUTHERN CALIFORNIA

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BACKGROUND

Over the past 3 years, 5 large commercial layer chicken ranches in southern California had repeated episodes of a variety of clinical diseases in 5- to 7-week-old Leghorn pullets. The episodes of disease were associated with atrophy of the cloacal bursa. The lesions of atrophy were compatible with sequelae of preexistent damage caused by infectious bursal disease virus (IBDV) (1,3). The lesions were characterized by interfollicular stromal fibrosis and transformation of lymphoid follicles into small aggregates of histiocytes and spindle cells which surrounded gland-like lumens. In most flocks, the clinical diseases appeared to be related to immunosuppression because they were diverse in nature, for example, nephrogenic infectious bronchitis, poxvirus conjunctivitis, cecal coccidiosis, stunting syndrome, and vaccine-induced infectious laryngotracheitis. Clinical signs attributable to IBDV (vent picking) were observed in only 1 flock. The style of housing varied among the ranches from environmentally controlled buildings to open-sided houses. Biosecurity varied from good to non-existent; management varied from all-in-all-out to coexistence of flocks of different ages in adjacent houses; clean-up between broods was thorough to haphazard; and, IBDV vaccines used varied from low to intermediate virulence. The purpose of this report is to describe results of prospective studies on 2 of the affected ranches in order to monitor maternal immunity, vaccinal uptake and bursal health.

PROSPECTIVE STUDIES

Methods. Five flocks of Leghorn pullets were studied prospectively on 2 of the affected ranches (3 flocks on ranch A and 2 flocks on ranch B). The flocks were vaccinated at 2, 3, 4 and 5 weeks of age with a live attenuated classic strain of IBDV of intermediate virulence according to the manufacturer's instructions. The flocks were randomly sampled by sequential kill at 1 to 3 days of age and thereafter at weekly intervals until 6 to 7 weeks of age. Measurements and examinations were made in order to characterize serum antibody profiles and to determine if, and at what age, IBD lesions occurred in the bursa. If lesions occurred, stored bursa from selected flocks were evaluated for presence of IBDV by virus isolation in embryonating chicken eggs and by reverse transcriptase-polymerase chain reaction (RT/PCR). If IBDV was detected, it was typed by restriction fragment length polymorphism (RFLP) and by antigen capture ELISA (AC-ELISA) using a monoclonal antibody panel (Intervet Inc., Millsboro, DE). The period of time which lapsed between vaccination and sampling for virus isolation/ virus detection was 7 days in ranch A and 10 days in ranch B. Antibody profiles were made utilizing a USDA licensed ELISA serologic test kit containing classic IBDV antigen (Flock Check®, IDEXX, Westbrook, ME). Comparative antibody profiles were made on flocks 1 and 2 in ranch A utilizing a prototype ELISA test kit containing antigen enriched for the key neutralizing and immunodominant epitopes of classic and variant IBDV strains (IDEXX, Westbrook, ME). Experimental transmission of the bursal disease in ranch A was attempted in 5-week-old commercial, seronegative, unvaccinated cockerels reared in isolation. The cockerels were dosed orally with 0.5ml supernatant from pooled bursal homogenate and examined at 3 and 5 days post-administration by histopathology. Control birds were dosed with diluent used to prepare the homogenate; they were housed in separate isolation and examined as per principals.

Results. All the flocks were clinically healthy throughout the study period. Four of the 5 flocks studied developed histopathologic lesions compatible with IBDV infection. Apart from cecal coccidiosis in one flock, no co-existent lesions were seen. Peak (80-90%) prevalence of pullets with acute to subacute histologic IBD bursal lesions occurred between 35 to 45 days of age. Lesions were characterized by edema, fibroplasia and mixed-leucocyte infiltration of stroma associated with full-follicular lympholysis attended by edema and heterophil infiltration. This was followed by replacement of follicles by histiocytes. In flocks 1 and 2 on ranch A (that were examined at 7 weeks of age), regenerative follicular lymphoid hyperplasia was prominent.

Antibody profiles made using the licensed kit revealed

marked variation in initial titers among the flocks (average 4500, min.-max. = 1300-8800) and persistent low titer troughs (average 200, min.-max. = 50-500) for periods lasting on average 3.5 weeks (min.-max. = 2-4 weeks) of age. Coefficient of variation (CV) of titers were generally high to very high during the critical decay period: 55% (min.-max. = 40-86%) at 1-3 days, 55% (20-128%) at 1 week, 58% (20-120%) at 2 weeks and 84% (31-124%) at 3 weeks of age. Antibody profiles generated by the prototype kit in flocks 1 and 2 on ranch A were similar to those obtained by the commercial kit during the decay and trough phases. However, at 6 and 7 weeks of age, an extreme spike in antibody titers was measured by the prototype kit in both flocks, whereas, the commercial kit detected only low to moderate rise in titers in the same sera. The difference in magnitude was 3-fold in flock 1 and 6-fold in flock 2 at 7 weeks of age.

Virus isolation yielded birnavirus in 2 of 2 flocks tested on ranch A. A RT/PCR-RFLP pattern consistent with serotype 1 molecular group 4 classic IBDV was detected in 2 of 2 flocks tested on ranch A and in 1 of 1 flock tested on ranch B. AC-ELISA monoclonal antibody reactions consistent with classic IBDV were demonstrated in 2 of 2 flocks tested on ranch A but no IBDV was detected in 1 of 1 flock tested on ranch B.

The experimental administration of bursal extract induced bursal lesions identical to those which were observed to occur spontaneously on both ranches.

DISCUSSION

The observations of the prospective studies reported here appears to vindicate our impression that IBD has become more difficult to control in southern California over the past few years compared to previous years. We were surprised that we did not find a variant IBDV and that our multiple vaccination program, beginning as early as 2 weeks of age, failed to prevent an almost 100% prevalence of bursal damage in 4 of 5 flocks studied; especially since the vaccine we employed has been used by other workers to break through maternal antibody levels comparable to what existed in our pullets (2). The relatively huge antibody spikes detected in the recuperating pullets by the prototype ELISA kit, in contrast to the modest increases measured by the licensed kit, may indicate that the prototype ELISA kit has either increased sensitivity for, or broader recognition of, antibodies to epitopes of classic IBDV not previously recognized by the licensed kit. This possibility is consistent with IBD viruses' known ability to drift antigenically in response to immune pressure (1). It could be reasonably argued that the IBDV detected in our pullets was vaccinal in origin and the antibody "spikes" were caused by a variant IBDV that we somehow failed to detect. We think this is unlikely because the bursal histopathology of both the field and the experimental diseases was more compatible with infection by a classic strain, rather than a variant one, since inflammation was prominent (3), and the flocks were sampled 7-10 days after vaccination by which time the vaccine virus should have been largely cleared from bursal tissue. We do not know why we were unable to prevent the high prevalence of bursal damage in the flocks of this study. However, we agree with others (2) that the most likely explanation might be related to the high variability of maternal antibody levels observed, perhaps working in concert with high field challenge which had the ability to infect significant proportions of the flock by breaking through maternal immunity ahead of the vaccine virus. The possibility that the "classic" virus detected in this study was immunogenically somewhat different from "classic" IBDV remains an unanswered question

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REFERENCES

1. Lukert, P. D., and Y. M. Saif. Infectious bursal disease in Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Said, eds. Iowa State University Press, Ames, Iowa. pp 721-738. 1997.

2. Kouwenhoven, B., and J. van den Bos. Control of very virulent infectious bursal disease (Gumboro disease) in the Netherlands with so called "hot" vaccines. Proc. 42nd West. Poult. Dis. Conf., Sacramento, CA. pp 37-40. 1993.

3. Sharma, J. M., J. E. Dohms, and A. L. Metz. Comparative pathogenesis of serotype 1 and variant serotype 1 isolates of infectious bursal disease virus and the effect of those viruses on humoral and cellular immune competence of specific pathogen free chickens. Avian Dis. 33:112-14. 1989.

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INTERACTION OF INFECTIOUS BURSAL DISEASE VIRUS WITH VACCINE PROTECTION AGAINST INCLUSION BODY HEPATITIS/HYDROPERICARDIUM SYNDROME

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SUMMARY

SPF birds in different groups were challenged at 5, 10 and 15 days of age with a pathogenic strain of infectious bursal disease virus (IBDV) or were not challenged. At 12 days of age all groups except one not challenged with IBDV were given 0.5 ml of a commercial killed inclusion body hepatitis / hydropericardium syndrome (IBH/HPS) vaccine. At 21 days post-vaccination all groups were challenged with a highly pathogenic strain of IBH/HPS. Results showed that when birds are infected with IBDV at an early age there is interference with vaccine protection against IBH/HPS. In order to achieve full protection against IBH/HPS with a killed vaccine, it is important to protect against IBDV before 10 days of age.

INTRODUCTION

IBH/HPS has become a serious problem in several parts of the world since the early 1980s. The disease can cause high mortality and can be transmitted horizontally and vertically. It has been shown for several years that immunosupression can interfere with a good response to vaccines. Many poultry producers, especially in Asia, do not vaccinate against IBDV in broilers. The objective of this study was to demonstrate that early infection with IBD can interfere with the protection provided by commercial vaccines against IBH/HPS.

MATERIALS AND METHODS

One day old SPF birds were raised in isolation units. They were divided into five groups with 15 birds in each. Water and commercial feed were supplied *ad libitum*. Birds were challenged with a virulent strain (Edgar) of IBDV at different ages, with 0.5 ml of a suspension containing $10^{3.5}$ ICD_{50} (titered in 3-week-old SPF birds) by intraocular and intracloacal routes. Group A was inoculated at 5 days, group B at 10 days and group C at 15 days of age. Groups D and E were not inoculated.

A commercial killed IBH/HPS vaccine (0.5 ml) was administered subcutaneously to the birds in groups A to D at 12 days of age. Group E was not vaccinated.

Twenty-one days after the vaccination all groups were challenged intramuscularly with 0.5 ml contining $10^{4.0}$ $CLD_{50\%}$ of a highly pathogenic strain of IBH/HPS (strain DCV-94), per bird. The birds were observed three times a day for 15 days after the challenge. Morbidity and mortality were recorded daily and all dead birds were necropsied. All the livers were examined by histopathology for intranuclear inclusion bodies (IIB), by direct immunofluorescence (DIF) for virus, and virus isolation of adenoviruses in chicken embryos (CE) and chicken embryo hepatocytes (CEH).

RESULTS

Vaccinated birds exposed at an early age to IBDV had lower protection against IBH/HPS challenge in terms of morbidity, mortality, and presence of the challenged virus in livers. Birds in group A, challenged at 5 days of age with IBDV, had 33% morbidity, 27% mortality, 27% livers positive for IIB, 40% livers positive on DIF, and 40% virus isolation in CE and 47% virus isolation in CEH. Birds in group B challenged at 10 days of age with IBDV had 7% morbidity, 0% mortality, 0% livers positive for IIB, 7% livers positive in DIF and 0% virus isolation in CE and 7% virus isolation in CEH. Birds in group C challenged at 15 days of age with IBDV and group D not challenged with IBDV had no morbidity or mortality and all tests to identify virus were negative. Birds in group E not challenged with IBDV but not vaccinated against IBH/HPS had 100% morbidity and mortality and all tests to identify virus were 100% positive.

DISCUSSION

Infection with IBDV at 15 days of age did not reduce the protection in IBH/HPS vaccinated birds according to the measures of morbidity, mortality and presence of the challenge adenovirus. This protection was reduced when the birds were challenged with IBDV at 10 days of age, and was greatly reduced when birds were challenged at 5 days of age. The reisolation of the adenovirus was slightly better in CEH than in CE. There was a good correlation between reisolation and immunofluorescence. The adenovirus used for challenge was highly pathogenic and capable of killing all infected birds and producing classical histological lesion in less than 5 days. This study demonstrated that an infection with IBDV before 10 days of age interfered with the protection of birds by inactivated killed vaccines against IBH/HPS. Avoiding exposure to IBDV for at least the first 10 days of life should be attempted.

CHARACTERIZATION OF FOWL ADENOVIRUSES ASSOCIATED WITH INCLUSION BODY HEPATITIS AND HYDROPERICARDIUM-HEPATITIS SYNDROME IN CHICKENS

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Inclusion body hepatitis (IBH), an economically important disease of commercial chickens, was first reported in the U.S.A. in 1963 (10). Subsequently, the disease has been recognized in numerous countries of the world and continues to be a poultry health problem today (4,7,8,11,12,15,16). Fowl adenoviruses (FAV; group 1 avian adenoviruses; 12 serotypes) are associated with or incriminated in outbreaks of IBH (5,6). The hydropericardium-hepatitis syndrome (HHS) was first recognized in 1985 in Pakistan and resulted in a epornitic leading to the death of over 100 million broilers during the years 1987 and 1988 (3,6,14). HHS is very similar to IBH and has also been associated with FAV (3,6). HHS has also been reported in Iraq, Kuwait and India (1,2,13). A disease syndrome diagnostically similar to HHS has been observed for a number of years in Mexico and several South American countries, but is usually referred to as IBH (14). Mortality, resulting from this disease syndrome, has been reported to be higher than that of classical IBH and a high incidence of hydropericardium has also been observed.

This is a report on the characterization of FAV field isolates or strains which have been associated with or incriminated in IBH and HHS outbreaks.

MATERIALS AND METHODS

Viruses, embryos and cell cultures. FAV isolates and strains associated with or incriminated in IBH and HHS outbreaks in broilers were acquired from national and international sources. Reference strains of FAV were also included as standards in this study. The FAV isolates and strains examined were inoculated into SPF chicken embryos (by AS, YS and DCAM routes) and onto primary CEL cell cultures and the LMH continuous cell line.

FAV antisera preparation and microneutralization procedure. Monospecific FAV antisera were produced in 6-week-old SPF White Leghorns which were inoculated subcutaneously (SC) with 0.5ml of inactivated FAV prototype strains emulsified in oil. A modification of a previously described microneutralization test procedure (9) was used to serotype the acquired field isolates of FAV utilizing the above described antisera. Briefly, the microneutralization test is conducted with equal parts of 200 $TCID_{50}$ of FAV and 8 antibody units of the respective prototype antiserum.

Chickens. SPF White Leghorns, 1 to 35 days of age, were inoculated (0.2-0.5 ml; 10^3-10^5TCID_{50}/0.1 ml) with FAV field isolates and strains by various routes (i.e. OR, SC, IM, and IA) and observed for morbidity, mortality and effect on growth rate (chicks of all treatments were weighed at 7 day intervals). Contact controls were included to evaluate horizontal transmission and blood samples were subsequently collected for antibody assays.

RESULTS

Chicken embryo and cell culture assays. Inoculation of SPF chicken embryos with 10-fold dilutions of selected field isolates and strains of FAV, via the AS, YS and DCAM routes, resulted in little or no replication of these viruses in the AS. However, both viruses replicated well in the YS and on the DCAM producing embryo lesions characteristic of FAV; i.e. small and hemorrhagic embryos with necrotic livers, spleens and kidney urates. The results demonstrated that the YS is the route of choice for the optimal propagation of these FAV isolates 9in chicken embryos. All of the FAV field isolates and strains evaluated replicated to high titers in CEL cell culture and/or the LMH cell line producing a "round-cell" CPE, which is characteristic of FAV.

Serotyping assay. Twenty-four uncloned FAV, isolated from field outbreaks of IBH or HHS in Canada, U.S.A. and South America, were found to share antigens with 7 FAV prototypes by one-way neutralization tests. Two of these field isolates were found to share antigens with 2 prototype viruses and 4 field isolates were untypeable. The South American field isolates of FAV were classified as serotype 4.

Pathogenicity assays of FAV isolates in chickens. Inoculation (i.e. by OR, SC, IM and IA routes) of SPF White Leghorns at 1 to 35 days of age with selected FAV field isolates, resulted in low morbidity, mortality rates ranging from 0 to 100%, and seroconversion. The birds' age and the route of virus inoculation had a direct effect on FAV pathogenicity. Deaths started as early as 3 days post-inoculation (p.i.) and continued through 13 days p.i. and the disease was horizontally transmitted to contact control chickens. Age resistance to disease was noted to develop rapidly. High mortality rates, accompanied by hydropericardium and pulmonary edema, were induced in young chickens infected with the South American isolate by a natural route of exposure. The incidence of hydropercardium was high in experimentally inoculated birds. The gross and microscopic lesions induced by the field isolates were found to be characteristic of IBH and

HHS and the growth rates of infected chickens were significantly reduced.

DISCUSSION

These studies have demonstrated similarities and differences in FAV isolates of differing serotype and origin (i.e. IBH versus HHS outbreaks). The fact that these isolates propagate well in the yolk sac of embryonating SPF chicken eggs and CEL cell cultures was anticipated. However, the high titers obtained with FAV isolates propagated in the LMH cell line were unexpected.

The diversity of FAV antigenic forms found were anticipated, but the extent of antigen sharing with unrelated serotypes encountered with these isolates was unexpected. Perhaps the latter finding can be partially explained by the fact that these field isolates were not clone purified. It is interesting to note that the South American field isolates of FAV were classified as serotype 4; a common finding for FAV associated with HHS on a world-wide basis. The variation in the pathogenicity observed with the FAV field isolates evaluated in this study is characteristic for this group of viruses. The rapidly developing age resistance to disease induction observed in this research, is a common pathologic feature of these viruses. The high mortality rates accompanied by hydropericardium and pulmonary edema induced with the South American field isolates support the suggestion that there are differences in the pathogenic features of classical IBH versus that of HHS.

REFERENCES

1. Abdul-Aziz, T. A., and M. A. Al-Attar., New syndrome in Iraqi chicks. Vet. Rec. 129:272. 1991.

2. Apte, V. H. ,Ventri Biologics, Pune, India, personal communication. 1995.

3. Cheema, A. H., M. A. Afzal, and J. Ahmad., An adenovirus infection of poultry in Pakistan. Rev. Sci. Tech. Int. Epizootics 8:789-798. 1989.

4. Christensen, N. H. and Md. Saifuddin. A primary epidemic of inclusion body hepatitis in broilers. Avian Dis. 33:622-630. 1989.

5. Cowen, B. S., and S. A. Naqi. Classification of avian adenoviruses. J. Am. Vet. Med. Assoc. 181:283. 1982.

6. Cowen, B. S. Inclusion body hepatitis-anaemia and hydropericardium syndromes: aetiology and control. World's Poult. Sci. J. 48:247-254. 1992

7. Cubillos, A., G. Sandoval, J. Ulloa, V. Cubillos, and M. Aguilar. Aislamieto y tepification del virus a cuerpo de inclusion en broilers en Chile. Arch. Med. Vet. (Valdivia) 18:115-122. 1986.

8. Fadly, A. M., and R. W. Winterfield. Isolation and some characteristics of an agent associated with inclusion body hepatitis, hemorrhages and aplastic anemia in chickens. Avian Dis. 17:182-193. 1973.

9. Grimes, T. M., and D. J. King. Serotyping avian adenoviruses by a microneutralization procedure. Am. J. Vet. Res. 38:317-321. 1977.

10. Helmbolt, C. F., and M. N. Frazier. Avian hepatic inclusion bodies of unknown significance. Avian Dis. 7:446-450. 1963.

11. Howell, J., D. W. MacDonald, and R. G. Christian. Inclusion body hepatitis in chickens. Can. Vet. J. 11:99-101. 1970.

12. Rinaldi A., F. Mandelli, D. Cessi, A. Caleri, and G. Gervio. Proprieta di un ceppo di virus CELO isolato dal Pollo in Italia. Clin. Vet. (Milan) 91:382-404. 1968.

13. Saqr M. Sultan Farms, Safat, Kuwait, personal communication. 1995.

14. Shane, S. M. Hydropericardium-hepatitis syndrome, the current world situation. Zootec. Int., pp. 20-27, January. 1996.

15. Wells, R. J. H., and K. Harrigan. A fatal adenovirus infection of broiler chickens: inclusion body hepatitis. Vet. Rec. 94:481-482. 1974.

16. Young, J. A., D. A. Purcell and P. J. Kavanagh. Inclusion body hepatitis outbreak in broiler flocks. Vet. Rec. 90:72. 1972.

EXPERIMENTAL DNA-VACCINATION AGAINST NEWCASTLE DISEASE VIRUS (NDV): TRANSIENT EXPRESSION VECTORS EXPRESSING THE NUCLEOPROTEIN (NP)-, OR HAEMAGGLUTININ NEURAMINIDASE (HN)-GENE

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To better understand the avian immune response against NDV, we studied the immune response against the products of single genes: the nucleoprotein (NP) (1) and the Haemagglutinin Neuraminidase (HN) (2). Both genes were cloned in the transient expression vector pBC/CMV/IL-2 and purified recombinant plasmids pBC-HN and pBC-NP were

produced. In addition empty pBC-vector (pBC-C) was prepared. SPAFAS birds were inoculated with purified plasmid (0.2mg DNA IM and 0.1 mg DNA IV) at day 0 and day 22. Equal numbers of positive control birds received 0.1 ml of live modified La Sota vaccine at day 28. An equal number of negative controls received only saline. All birds were challenged with 0.1 ml 10^2 ELD_{50} of NDV (strain Texas GB). Birds inoculated with pBC-HN did not develop anti-HN antibodies at the day of challenge. However, upon challenge the pBC-HN vaccinated groups had a survival rate of 33% (experiment 1, N = 6) and 50% (experiment 2, N = 10). Mortality was 100% in the negative controls (N = 12). Birds inoculated with pBC-NP showed a high anti-NP ELISA titer; (Experiment 1: 8,310 $\pm$ 3,264, N = 6; Experiment 2: 12,780 $\pm$ 126, N = 10). However, none of the pBC-NP-inoculated birds survived the lethal challenge although the survival time after lethal challenge was 5.15 days compared to 4.40 days for the negative controls. The positive controls (La Sota) survived 100%.

REFERENCES

1. Schaper, U., F. Fuller, M. Ward, Y. Mehrotra, and E. V. De Buysscher. Nucleotide sequences of the envelope protein genes of a highly virulent strain of Newcastle Disease Virus. Virol. 165:291-295. 1988.

2. Ward, M. D., F. Fuller, Y. Mehrotra, and E. V. De Buysscher. Nucleotide sequence and vaccinia expression of the nucleoprotein of a highly virulent strain of Newcastle Disease Virus (to be submitted to Avian Diseases).

(A full-length article is in preparation to be submitted to *Avian Diseases*.)

IMPACT OF VV MAREK'S DISEASE ON MORTALITY AND PRODUCTION IN A MULTIPLE-AGE FARM

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The introduction of a very virulent Marek's disease virus vvMDV into a multiple-age farm in New York State (NYS) and its effects are described.

Background. The farm is a typical medium-size family-operated unit, dedicated to the production of white table eggs. Their market requires that egg production remains uniform throughout the year, and to meet these needs the farm keeps laying hens of different ages, sometimes two flocks in a single house. The farm consists of one 30,000 brooding-rearing house and 4 layer houses: two with capacity for 75,000 layer hens, one for 60,000 and one for 30,000 in cages. Typically 30,000 pullets are raised from one day of age in the farm's brooder house, and 30,000 or 45,000 pullets are bought from pullet growers to fill the layer houses when needed. The pullets are moved into the layer houses when they are 15 to 17 weeks of age. Before the introduction of vvMDV, in October, 1994, cumulative mortality to 68 weeks of age was 5 to 8%. Caged layer fatigue, nephritis and vent lesions accounted for 80% of the daily mortality. MD was observed in about 10% of the layer daily mortality. In growing pullets MD caused up to 1% mortality.

Case history. In October, 1994, 45000 15-week-old pullets were brought to the farm from the Midwest. The farmer considered mortality, observed in the following week after arrival, to be caused by the stress of the trip and leg trauma. When mortality did not diminish in the following weeks, he submitted birds for examination. Lesions characteristic of MD were observed in nerves and viscera. During the first 6 weeks after arrival, neck paralysis was frequently observed, and later one of the most striking lesions was golf ball-size spleens. Mortality reached a peak of 3.6% per week at 22 weeks of age and cumulative mortality at 68 weeks reached 46% in the Midwest-grown flock. Pullets grown at the farm and placed in the same house had clinical MD with similar signs and lesions six weeks after they were exposed to the Midwest flock. Mortality peaked at 1.8% per week at 28 weeks of age and cumulative mortality was 22% at 68 weeks of age.

Virus characterization. Specific-pathogen-free (SPF) chickens placed in the layer house in December, 1994, were severely depressed, emaciated and with paralysis of the neck when collected 10 days after exposure. The MDV isolated from the SPF chickens "was very virulent, significantly more so than the prototype Md5 virus. The isolate ranked with the most virulent that I have thus far isolated" (Witter, personal communication) (2). Our studies confirmed that this is a very virulent virus with a high immunosuppressive potential (1).

Aftermath. The use of trivalent MDV vaccines, and brooding and rearing pullets off the farm helped in reducing MD losses in subsequent flocks. Flocks immediately following the Midwest flock suffered cumulative 15% mortality, and cumulative mortality was under 10% in subsequent flocks. The combined effects of mortality, loss of egg production, partially empty houses and impact on the

market, amounted to $1 million U.S.

REFERENCES

1. Calnek, B. W., R. W. Harris, C. Buscaglia, K. A. Schat, and B. Lucio. Relationship between the immunosuppressive potential and the pathotype of Marek's disease virus isolates. Avian Dis. 42:124-132. 1998.

2. Witter, R. L. Increased virulence of Marek's disease virus field isolates. Avian Dis. 41:149-163. 1997.

SAFETY AND EFFICACY OF FOWL AND PIGEON POX VACCINES ADMINISTERED IN OVO TO SPF AND BROILER EMBRYOS

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SUMMARY

Commercially available tissue culture origin fowl pox (FP) and embryo origin pigeon pox (PP) vaccines labeled for post-hatch administration were tested for safety and efficacy by the *in ovo* route. All five vaccines were shown to be both safe and effective when given *in ovo* using the INOVOJECT® system. It was concluded that the FP and PP vaccines tested are good candidates for obtaining a label claim for the *in ovo* route.

INTRODUCTION

FP is a slow-spreading mechanically transmitted viral disease of chickens and other poultry. Pox infection in broiler chickens can lead to emaciation and poor weight gain while mortality usually remains low (1). FP and PP vaccines given post-hatch are used routinely in some areas of the world to vaccinate broilers and broiler breeders. The objective of the following experiments was to determine if commercial FP and PP vaccines licenced for post-hatch use are good candidates for development of vaccines licensed for *in ovo* administration. Vaccines were tested at several dose levels for safety and efficacy in specific pathogen free (SPF) and broiler chickens.

MATERIALS AND METHODS

Vaccines and Challenge. Three tissue culture origin FP vaccines (FP1, FP2 and FP3) and two embryo origin PP vaccines (PP1 and PP2) were tested. All five vaccines are commercially available and licensed for post-hatch administration in the U.S.A. Vaccine titers were supplied by the manufacturer. The FP challenge was a 1:10 dilution of the USDA (NVSL) FP challenge strain administered in the wing-web. Birds were considered positive for FP lesions if a papule was detected by palpation in the inoculated wing-web as compared to the positive and negative control birds.

Experiment 1. At 18 days of incubation, SPF and Peterson X Arbor Acres eggs were candled to remove non-viable embryos and live embryos were vaccinated manually with either $10^{3.5}$ pox virus content (PVC) of FP2 vaccine or left unvaccinated. Eggs in each group received a full dose of HVT/SB-1 and 0.2 mg gentamicin. Percent hatch of live embryos was determined for each group. Percent hatch of live is recorded on the day of hatch by determining the number of normal chicks hatched and by conducting an egg necropsy to confirm that inoculated embryos were alive and normal on day 18 of incubation.

Experiment 2. FP1 and PP1 were tested for safety in SPF eggs. At 18 days + 0 hrs. of incubation, 232 live embryos/group were vaccinated manually with either $10^{3.5}$ PVC FP1, $10^{3.5}$ PVC PP1 or left non-vaccinated. Eggs in all groups received a full dose of HVT, live infectious bursal disease (IBD) vaccine Bursaplex® and 0.05 mg gentamicin. Percent hatch of live was determined in each group.

Experiment 3. At 18 days of incubation, live SPF embryos were vaccinated manually with $10^{3.5}$ PVC FP2 vaccine or left non-vaccinated. Eggs and chicks received a full dose of HVT/SB-1 and 0.2 mg gentamicin mixed with the FP vaccine. On day 18 of age, 20 chicks in each group were challenged and assessed for wing-web lesions 8 days post challenge.

Experiment 4. Sixty live Cobb X Cobb broiler embryos per group were vaccinated using an INOVOJECT® system at 18 days + 18 hr. of incubation. The eggs were from a breeder flock vaccinated for FP. Three dose levels ($10^{1.0}$, $10^{2.0}$, $10^{3.0}$) of FP1, FP2, FP3, PP1 and P2 vaccines were tested *in ovo* and compared to the release dose given at hatch in the wing web by injection. HVT, Bursaplex® and 0.05 mg of gentamicin were given *in ovo* to all embryos in the study. Positive and negative control groups that did not receive pox vaccines were included. Fourteen birds per group were placed in battery cages, challenged on day 20 and assessed for lesions 8 days post challenge. Percent hatch of live and pre-challenge mortality were measured and used to assess safety.

Experiment 5. Sixty live Cobb X Cobb broiler embryos

per group were vaccinated with either FP1, FP3 or PP1 vaccine using an INOVOJECT® system at either 18 days + 0 hrs of incubation, 18 days + 18 hrs of incubation or left non-vaccinated. The eggs were from a breeder flock vaccinated for FP. Three doses ($10^{1.0}$, $10^{2.0}$, $10^{3.0}$) of each pox vaccine were tested *in ovo* and compared to the release dose given at hatch subcutaneously in the nape. All groups, including positive and negative controls, were given HVT, Bursaplex® and 0.05 mg of gentamicin either *in ovo* or at hatch. All hatched birds were placed in floor pens by group, challenged on day 20 and assessed for lesions 8 days post challenge. Percent hatch of live and pre-challenge mortality were measured and used to assess safety.

RESULTS

Experiment 1. The results of experiment 1 are shown in Table 1.

Table 1. Percent hatch of SPF and broiler embryos vaccinated *in ovo* with FP on day 18 (Expt. 1)

$10^{3.5}$ FP2 *in ovo*[1]	Bird Type	No. hatched/No. live at day 18	% hatch of live
Yes	Broiler	273/281	97.2[A]
No	Broiler	275/284	96.8[A]
Yes	SPF	422/429	98.4[A]
No	SPF	420/428	98.1[A]

[A] Values within column and bird type with different uppercase superscripts are significantly different ($p \leq 0.05$) by ANOVA.
[1] All embryos received HVT/SB-1 and gentamicin.

Experiment 2. The % hatch of live data for SPF embryos given either $10^{3.5}$ FP1, $10^{3.5}$ PP1 or no pox vaccine were 96.11%, 97.4% and 97.8%, respectively. These data were not significantly different ($p \leq 0.05$) by ANOVA.

Experiments 3, 4 and 5. The results of experiments 3, 4 and 5 are shown in Tables 2, 3 and 4, respectively.

Table 2. Percent hatch, mortality and protection of SPF chickens vaccinated either *in ovo* or at hatch with FP vaccine (Expt. 3)

$10^{3.5}$ FP2 vaccine[1]	Route	Challenged	% hatch of live[2]	% mortality[3]	% protected[3]
No	NA	Yes	95.0	0.0	0.0
Yes	*in ovo*	No	96.7	0.0	100.0
Yes	*in ovo*	Yes	96.7	0.0	95.0
Yes	at hatch	Yes	93.8	0.0	100.0

Data not subjected to statistical analysis.
[1] All embryos/chicks received HVT/SB-1 and gentamicin.
[2] The two *in ovo* groups were hatched as one group and segregated after hatch.
[3] n = 20/group; % protected = % of birds without a pox lesion in the challenged wing-web eight days post-challenge.

Table 3. Percent hatch, mortality and protection in broiler chickens vaccinated either *in ovo* using the INOVOJECT® system or at hatch with one of five avian pox vaccines (Expt. 4)

Pox Vaccine Treatment[1]	Pox Vaccine Dose	Pox Vaccine Route	% Hatch of live	Pre-Challenge[2] Mortality (No. placed/No. dead)	% Protected[3]
FP1	$10^{1.0}$	*in ovo*	96.7	0/14	71.4^{BCD}
	$10^{2.0}$	*in ovo*	96.7	0/14	64.3^{BC}
	$10^{3.0}$	*in ovo*	91.7	0/14	78.6^{BCD}
	$10^{3.2}$	at hatch	98.3	0/14	85.7^{BCD}
FP2	$10^{1.0}$	*in ovo*	95.0	1/14	69.3^{BCD}
	$10^{2.0}$	*in ovo*	90.0	0/14	78.6^{BCD}
	$10^{3.0}$	*in ovo*	93.3	0/14	85.7^{BCD}
	$10^{3.4}$	at hatch	96.7	0/13	100.0^{D}
FP3	$10^{1.0}$	*in ovo*	91.7	2/13	45.4^{AB}
	$10^{2.0}$	*in ovo*	88.3	1/13	58.3^{BC}
	$10^{3.0}$	*in ovo*	96.7	1/14	76.9^{BCD}
	$10^{3.2}$	at hatch	91.7	2/14	90.9^{BCD}
PP1	$10^{1.0}$	*in ovo*	91.7	0/14	50.0^{B}
	$10^{2.0}$	*in ovo*	91.7	2/14	83.3^{BCD}
	$10^{3.0}$	*in ovo*	96.7	1/14	92.3^{CD}
	$10^{2.9}$	at hatch	98.3	1/13	100.0^{D}
PP2	$10^{1.0}$	*in ovo*	95.0	0/14	57.1^{BC}
	$10^{2.0}$	*in ovo*	85.0	0/13	76.9^{BCD}
	$10^{3.0}$	*in ovo*	90.0	0/14	92.9^{CD}
	$10^{3.0}$	at hatch	98.3	2/14	100.0^{D}
Positive[4]	None	---	98.3	1/14	7.7^{A}
Negative[4]	None	---	95.0	1/14	100.0^{D}

^{A,B,C,D} Values within columns with different uppercase superscripts are significantly different ($p \leq 0.05$) by Fisher's exact test (chi-square statistic).

[1] FP = fowl pox tissue culture origin; PP = pigeon pox embryo origin; All embryos received Marek's HVT, Bursaplex® IBD vaccine and gentamicin *in ovo*.

[2] Birds culled due to leg problems were not included in the mortality.

[3] % of birds without a pox lesion in the challenged wing-web eight days post-challenge; n = 12-14 birds/group

[4] Positive and negative controls not vaccinated; positive control challenged

Table 4. Percent hatch, mortality and protection in broiler chickens vaccinated either *in ovo* using the INOVOJECT® system or at hatch with one of three avian pox vaccines (Expt. 5)

Pox Vaccine Treatment[1]	Egg Age (Day+hr)	Vaccine Dose (PVC)	Vaccine Route	% Hatch of live	% Pre-challenge mortality	% Protected[3]
FP1	18+0	10^1	*in ovo*	94.9	0.0	25.4^{AB}
	18+0	10^2	*in ovo*	95.0	0.0	31.6^{AB}
	18+0	10^3	*in ovo*	96.6	0.0	91.2^{DE}
	18+18	10^3	*in ovo*	96.7	1.7	91.2^{DE}
	---	$10^{3.2}$	at hatch[2]	90.0	1.7	100.0^{E}
FP3	18+0	10^1	*in ovo*	93.0	1.9	34.0^{B}
	18+0	10^2	*in ovo*	95.0	5.7	41.8^{B}
	18+0	10^3	*in ovo*	96.6	1.8	78.2^{CD}
	18+18	10^3	*in ovo*	98.3	1.8	80.4^{CD}
	---	$10^{3.2}$	at hatch[2]	89.8	3.4	96.2^{E}
PP1	18+0	10^1	*in ovo*	93.3	0.0	33.9^{B}
	18+0	10^2	*in ovo*	91.7	1.8	32.1^{B}
	18+0	10^3	*in ovo*	96.5	1.8	69.8^{C}
	18+18	10^3	*in ovo*	100	1.7	96.5^{E}
	---	$10^{3.1}$	at hatch[2]	96.7	0.0	98.2^{E}
Positive[4]	---	None	---	91.7	1.8	14.8^{A}
Negative[4]	---	None	---	94.9	0.0	100.0^{E}

[A,B,C,D,E] Values within columns with different uppercase superscripts are significantly different ($p \leq 0.05$) using Fisher's exact test (chi-square statistic).

[1] FP = fowl pox tissue culture origin; PP = pigeon pox embryo origin; All embryos received Marek's HVT, Bursaplex® IBD vaccine and gentamicin *in ovo*.

[2] Eggs were not injected. Chicks were vaccinated on day of hatch with full dose as provided by the manufacturer

[3] 50-58/group were challenged on day 18; % protected = % of birds without a pox lesion in the challenged wing-web eight days post-challenge.

[4] Positive and negative controls not vaccinated; positive control challenged

DISCUSSION

The results of these studies show that all five vaccines tested appear to be safe for % hatch in SPF and broiler chickens (Tables 1 - 4). There was no increase in after hatch mortality in SPF chickens vaccinated with FP2 (Table 2) and in broilers vaccinated with either FP or PP (Tables 3 and 4). In experiments 3 and 4, both Marek's disease vaccine and IBD vaccine were mixed with one of the pox vaccines and administered *in ovo* using the INOVOJECT® system, indicating that combining these three vaccines was safe in these laboratory studies. In experiment 2, SPF birds were well protected at eight days post challenge in the *in ovo* and at hatch vaccinated groups (Table 2). Protection in broilers showed a dose dependent response for each of the vaccines tested. In all but one case, percent of broilers protected given a pox vaccine *in ovo* at 10^3 PVC was not significantly different ($p \leq 0.05$) from broilers given a full dose of pox vaccine at hatch (Tables 3 and 4). The full dose given at hatch was a higher dose than the 10^3 PVC given *in ovo* in most cases. Furthermore, the near perfect conditions of laboratory administration of the vaccines at hatch resulted in 100% of the birds being vaccinated properly. It is doubtful if day of hatch vaccination in commercial hatcheries is as accurate as that in the laboratory. Thus, one would expect *in ovo* vaccination and at hatch vaccination to be equally

effective in commercial settings. *In ovo* administration of 10^3 PVC at 18 days + 0 hours using the INOVOJECT® system was as efficacious as that delivered at 18 days + 18 hours when using FP1 and FP3, but was significantly ($p \leq 0.05$) less effective using PP1 (Table 4). These data suggest that commercial tissue culture origin FP vaccines and embryo origin PP vaccines licensed for post-hatch administration are good candidates for obtaining an *in ovo* route of delivery license claim.

REFERENCES

1. Tripathy, D. N., and W. M. Reed. Pox. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. eds. Iowa State University Press, Ames, IA. pp. 643-659. 1997.

PERSISTENCE OF INFECTIOUS BRONCHITIS VIRUS IN VACCINATED CHICKENS

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Persistence of IBV in experimentally infected chickens has been studied before. However, those studies were on group-housed chickens in which true persistence of IBV versus reinfection from penmates could not be distinguished. Experiments conducted in our laboratory with individually housed chickens have demonstrated for the first time that IBV persists in the absence of reinfection from other birds. In an experiment in which 5-week-old (maternal antibody-free) chickens were vaccinated with Massachusetts-type vaccine, virus shedding in tracheal secretions and feces was observed until 63 days post-vaccination. On the other hand, IBV was isolated from the tissues of those chickens until 154 days post-vaccination (i.e., when the experiment was terminated). In a similar experiment in which 1-day-old maternal antibody-positive chickens were vaccinated, virus shedding from trachea and cloaca was observed until 77 days post-vaccination, and at least one of the seven birds vaccinated was found to carry IBV in the lungs and kidney tissues until 175 post-vaccination (end of the experiment). These findings confirm that IBV vaccine viruses may have long-term persistence in vaccinated chickens. Studies are underway to compare biological properties and molecular characteristics of persistent viruses with those of the parent vaccine virus.

PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF ESCHERICHIA COLI FROM BROILERS WITH CELLULITIS AND OTHER COLIBACILLOSIS LESIONS

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We have observed an association between cellulitis and serositis (airsacculitis, pericarditis, hepatitis and peritionitis) in slaughter broilers. Because birds that are condemned for cellulitis are not examined for internal lesions, the extent of this association was not known. It had also been observed that *Escherichia coli* isolated from cellulitis lesions had the same general characteristics as other *E. coli* pathogenic for poultry.

This study had two objectives: (1) to investigate the association of cellulitis with other lesions that were due to *E. coli,* and (2) to determine whether one type or more than one type of *E. coli* was the cause of infection in those birds with both cellulitis and other lesions. We examined 237 birds with cellulitis from 30 flocks in Saskatchewan, Canada. Of the birds examined 35% had dual lesions that consisted of gross lesions in the heart, air sacs, bones or liver in addition to cellulitis. *E. coli* was isolated from each cellulitis lesion and from 71% of the other lesions. The occurrence of dual lesions was not evenly distributed among the flocks. In some flocks no birds had dual lesions while in other flocks most birds had dual lesions. The reason for this difference is not known.

We examined the biotype, aerobactin production, serumsensitivity, antibiotic susceptibility and production of K1 capsule of the *E. coli* isolates. *E. coli* with the same genotypic and phenotypic profiles were isolated from cellulitis and other lesions in 19% of the birds with dual lesions. In these birds it appeared that one strain of *E. coli* had caused both types of lesions. Analysis of these strains by repetitive-sequence based polymerase chain reaction failed to reveal any differences. Further work will be necessary to determine which disease was the primary infection or if they occurred independently. In the other 81% of the birds different *E. coli* were isolated from the various lesions. In these cases it appeared that two independent infections had occurred.

This study confirmed that two types of disease caused by *E. coli* occur concurrently, not only at the flock level, but at the level of the individual bird. It also confirmed that in some, but not all cases, identical isolates of *E. coli* were capable of causing cellulitis and serositis in an individual bird.

(A full report describing this work will be submitted to *Infection and Immunity* for publication.)

ASSESSING SAMPLING BIAS IN MOLECULAR EPIDEMIOLOGIC STUDIES USING ISOLATES OF ESCHERICHIA COLI FROM AVIAN CELLULITIS LESIONS AS AN EXAMPLE

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Avian cellulitis in broilers is primarily caused by *Escherichia coli*, and we are using DNA fingerprinting techniques to study the distribution and persistence of cellulitis-associated *E. coli*. We collect birds at the

processing plant that we suspect will have lesions and then culture these lesions in the laboratory. We prefer to collect our sample birds before the carcasses are eviscerated so that the skin of the bird is still intact and the lesion is not contaminated. However, we are using visual cues, such as skin discoloration, to select birds that we suspect have lesions. If we are cueing on specific lesion attributes that are not representative of all cellulitis lesions, we may not be sampling from the entire population of birds affected with cellulitis in the flock. Consequently, we may not be isolating and fingerprinting all of the different *E. coli* that have caused cellulitis in a particular flock.

In order to assess this potential sampling bias, we developed the following validation experiment. Two different observers selected birds that they suspected would have cellulitis lesions. They sampled birds off the processing line for a fixed period of time. All of the cellulitis-affected broilers that were missed by the observers but identified by the USDA inspectors during the same time period were also collected. We performed two trials on different flocks, one in which 12 birds were collected by the observers and 16 were missed, and the other in which 10 birds were collected by the observers and 14 were missed. We DNA fingerprinted all isolates of *E. coli* that were cultured from the lesions using pulsed-field gel electrophoresis (PFGE) and the enzymes *Not*I and *Sfi*I. We were then able to determine whether the lesions and the *E. coli* isolates collected by the observers were representative of the entire sample of lesions and isolates from the flock. We concluded that our sampling design was not introducing bias to the inferences that we make concerning *E. coli* distribution and persistence. However, we feel that a validation experiment such as this is warranted for many molecular epidemiologic studies in which sampling is based on clinical or pathologic manifestations.

(A full length article will be submitted for publication to *Journal of Veterinary Diagnostic Investigation.*)

SURVEILLANCE FOR VEROTOXIN PRODUCING ESCHERICHIA COLI IN MEAT TURKEY FLOCKS DURING REARING AND PROCESSING

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The present investigations were carried out to estimate the prevalence of verotoxin producing Escherichia coli (VTEC) infections in turkey meat flocks and to determine means of further contamination in a processing plant. Eleven commercial turkey flocks with about 94,000 birds were monitored for verotoxin producing E. coli (between 1996-1997). Faecal samples were collected at 4 week intervals from the 1st week of age through the 16th and 20th/21st week of age (age of slaughter of females and males respectively) and tested. During slaughter and processing samples were collected from the following sites: scalding water, liver swabs, swabs from the skin surface over thigh, lumbosacral and around the cloacae of turkey carcasses after evisceration, skin swabs after cooling, skin swabs after further processing and samples from breast muscle after cutting. Samples were inoculated first at 37°C for 5 to 6 hours in a pre-enrichment modified tryplicase soy broth (MTSB) supplemented with novobiocin. The MTSB was shaken at 100 r.p.m. The samples were then incubated at 37°C for 16 to 18 hours in an enriched MTSB with mitomycin C. This broth was shaken at 180 r.p.m. The broth was then examined for the presence of verotoxin using a commercial ELISA (Premier EHEC®"Hiss, HiSS Diagnostics, Freiburg) and tissue culture using a VERO-cell line. Positives and suspected positives were then confirmed using PCR. The presence of EHEC of serogroup O157 was carried out using a visual immunoprecipitate assay (VIP EHEC®).

During the rearing period VTEC was detected in 3 out of 11 flocks. In two flocks O157 and in one flock non-O157-VTEC was detected. The first positive results were obtained at 12 weeks of age. Four out of 153 tested samples (2.6 %) were positive. Shedding of the organisms in the turkey flocks was intermittent. During processing VTEC was detected in all the monitored flocks (3 male and 8 female) including those which were negative during the rearing period. This suggests that cross contamination in the processing plant is very common. The isolation rates at processing were significantly higher in flocks which were positive during the rearing periods in comparison to flocks which were negative. Samples from scalding water, liver, skin after evisceration, and skin after cutting had the highest contamination rates.

THE EFFECTS OF DEXAMETHASONE IN AN EXPERIMENTAL MODEL OF TURKEY OSTEOMYELITIS COMPLEX

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SUMMARY

We have demonstrated that the synthetic glucocorticoid, dexamethasone (DEX), when inoculated into the thigh muscle of turkeys at a dosage of 2mg/kg, will induce all of the lesions of turkey osteomyelitis complex (TOC), including green liver, arthritis/synovitis/tendonitis, and muscle and bone lesions. Dexamethasone treatment results in TOC lesions with or without concurrent airsac inoculation with *Escherichia coli.* In experiment 1, DEX treatment alone produced an 8% incidence of TOC in 5-week-old birds, however, DEX treatment combined with airsac inoculation with 1 x 10^2 cfu of *E. coli* produced the highest TOC incidence (27%). In experiment 2, birds which survived experiment 1 were treated with a second series of DEX injections at 13 weeks of age, resulting in multiple TOC lesions in 60% of the birds and green liver in 44%. In experiment 3, birds were treated with DEX at 5 weeks of age and challenged with air sac inoculation of *E. coli.* Survivors were again treated with DEX at 13 weeks of age. The two DEX treatments resulted in 64% TOC and 64% green liver in birds never challenged with *E.coli* while the incidence was significantly lower in birds challenged with 50 cfu *E.coli.* In experiment 4, birds were treated with DEX at 2, 5, and 10 weeks of age. The incidence of TOC increased after the second and third DEX treatments, resulting in 67% TOC and 35% green liver after the third DEX treatment. Seven healthy control birds from experiment 4 were treated with a single series of DEX injections at 12 weeks of age. Green liver was present in 2/7 and TOC lesions in 6/7 of these birds within 9 days post-treatment. These results indicate that treatment of turkeys with 2mg/kg DEX induces the development of TOC, especially in older birds, and suggests that immuno-suppression due to a high stress response may be involved in the etiology of TOC. Treatment with DEX is being used to test nutritional and physiological immunomodulators, and may be useful in the genetic selection of turkeys with a lower stress response and better disease resistance.

INTRODUCTION

Turkey osteomyelitis complex (TOC) is a syndrome defined by the U. S. Food Safety Inspection Service (FSIS) to describe normal-appearing processed turkey carcasses which contain lesions including arthritis/synovitis, soft-tissue abscesses , and osteomyelitis of the proximal tibia (8). The presence of a green liver is used as an indicator to remove suspect turkeys from the processing line. These turkeys are then subjected to a standard 10-cut procedure to detect the suspected bone, joint, and soft-tissue lesions. While it has been assumed that most turkeys with TOC lesions also have green livers, lesions have been detected in birds without green livers both experimentally (10) and in commercially grown birds (3, 6, 7,). Also, at least half of the birds which are examined due to the presence of a green liver have no other lesions, and are thus downgraded unnecessarily (1, 2, 7, 12, 13, 15). Because of the deficiencies of the present inspection system, we have endeavored to determine the etiology of this condition in order to enable its prevention and control. Previous research has indicated that the lesions are caused by various opportunistic organisms, mainly *Staphylococcus aureus* and *Escherichia coli* (3, 7, 9, 14) suggesting that TOC incidence may be influenced more by deficiencies in the host immune response rather than by the virulence of any one organism. This syndrome is primarily a disease of adolescent male turkeys (7, 13, 14) indicating that sex hormone effects on immunity may be involved (11). Birds with TOC lesions have been shown to have higher levels of cell-wall deficient bacteria in their livers (3), and decreased indices of cell-mediated immunity (4) leading to the hypothesis that defects in the immune response of individuals within flocks of male turkeys , possibly owing to divergent selection for fast growth (5), may be responsible for the occurence of these opportunistic infections. In the following studies we demonstrate that these immune defects may also be directly influenced by individual differences in the stress response.

MATERIALS AND METHODS

Experiment 1. Six-hundred five-wk-old male turkeys were given 3 intramuscular injections of 2 mg/kg BW of dexamethasone (DEX) on alternating days or were uninoculated. On the day of the third DEX injection, the left thoracic air sac of each bird was injected with sterile tryptose phosphate broth (TPB) or with TPB containing $1x10^2$, $1x10^3$, $1x10^4$, or $1x10^5$ cfu of *Escherichia coli.* All birds dying during the experiment and birds necropsied at 2 weeks post inoculation were examined for lesions of TOC following the standard 10-cut procedure used by the FSIS.

Experiment 2. One hundred and fifty healthy turkeys which survived the challenge in Experiment 1 were allowed to grow to 13 weeks of age, at which time they were treated with a second set of DEX injections. Birds dying during the experiment and birds necropsied at 8 and 15 days after

DEX injection were examined for TOC lesions.

Experiment 3. Three hundred and forty-eight 5-week-old male turkeys were treated with DEX injections followed by air sac inoculation with 0, 25 or 50 cfu of *Escherichia coli.* Survivors of this challenge were maintained until 13 weeks of age, at which time they were inoculated with a second series of DEX injections. Birds dying during the experiment and birds necropsied at 3 weeks after DEX injection were examined for TOC lesions.

Experiment 4. Seven-hundred and twenty male turkeys were given 3 intramuscular injections of 2 mg/kg BW DEX at 2 weeks of age, or were left uninoculated. At 5 weeks of age, DEX-treated birds were again treated with 2 mg/kg DEX. All mortalities were examined for TOC lesions. Two weeks later, 30 control and 50 DEX-treated birds were necropsied and examined for TOC lesions. Surviving birds were raised until 10 weeks of age, at which time all DEX-treated birds were again treated with 2 mg/kg DEX. All birds dying during the experiment were examined for TOC lesions and 2 weeks after the last DEX injection, 40 control and 11 DEX-treated survivors were necropsied and examined for TOC lesions. Seven non-DEX-treated, unchallenged control birds were saved and injected with 3 intramuscular injections of 2mg/kg DEX on alternating days. Surviving birds were necropsied and examined for TOC lesions 9 days after the last DEX injection.

RESULTS

Experiment 1. Dexamethasone treatment, by itself, increased TOC incidence from 0 to 8%, and there was a synergistic interaction between DEX treatment and *E. coli* challenge (Table 1). While TOC incidence was significantly increased by the lowest level of *E. coli* inoculated (1×10^2 cfu), increasing the number of bacteria did not further increase TOC incidence. There was no significant increase in TOC incidence in non-DEX-treated birds treated with less than 1×10^5 cfu of *E. coli.*

Experiment 2. There were no TOC lesions observed in dead birds until day 6, after which all dead birds had TOC lesions including osteomyelitis of the proximal tibia, purulent and caseous synovitis/tendonitis, and abscesses in the soft tissues of the leg. Before day 6 most livers were described as pale with grainy-appearing plaques, however green-fringed livers appeared after day 6. Between days 8 and 15 post-DEX injection, 67% of all dead birds had green or green-fringed livers and also had TOC lesions. Fifty-five percent of all birds with TOC lesions had green livers and forty-five percent of those birds with one or more TOC lesion did not have a green liver. Green livers were not seen in any birds necropsied at 8 or 15 days post-treatment (Table 2). The most frequent lesion seen was purulent synovitis/tendonitis which was generally accompanied by abscesses in the soft tissue of the leg and thigh. About half of the mortalities with these lesions also had osteomyelitis of the proximal tibia (Table 2).

Experiment 3. A second series of DEX injections resulted in 64% incidence of both TOC and green liver (Table 3), however birds previously inoculated with *E. coli* had a significantly lower incidence of green liver and TOC compared to birds only treated with DEX.

Experiment 4. There were no mortalities due to DEX treatment in birds treated at 2 weeks of age. Mortality increased with age and number of DEX treatments with 32 % mortality at 5 weeks of age (after 2 DEX treatments) and 79% mortality at 10 weeks of age (after 3 DEX treatments). There was no evidence of lameness in birds treated with DEX at 2 weeks of age, therefore no birds were necropsied. There was no incidence of either TOC or green liver in non-DEX-treated birds at 5 weeks of age, however 5-week-old birds had 27% TOC and 8.3% green liver after the second DEX treatment (Table 4). There was 4.9% TOC and 2.4% green liver in 10-week-old non-DEX-treated birds and 67% TOC and 35% green liver in 10-week-old birds after the third DEX treatment (Table 4). When control birds which had never been treated with either DEX or *E. coli* were injected with 2mg/kg DEX, 6/7 (86%) had TOC lesions and 2/7 (28.6%) had green livers (Table 4).

Table 1. Experiment 1. Effect of dexamethasone (DEX) injection and Escherichia coli respiratory challenge on the incidence of turkey osteomyelitis complex (TOC).

E. coli Titer[1]	TOC Incidence[2]	
	With DEX	No DEX
0	8.33±5.76[b]	0.00±0.00[b]
2	27.08±6.48[a]	7.41±5.10[ab]
3	23.64±5.78[a]	3.23±3.23[b]
4	20.37±5.53[a]	0.00±0.00[b]
5	10.71±4.17[b]	22.22±8.15[a]

[a-b] Main effect means with no common superscript are significantly different ($P \leq 0.05$).

[1] Inoculum was diluted in tryptose phosphate broth so that a 200 μL injection contained either 0, or approximately 10^2, 10^3, 10^4, or 10^5 colony forming units of a pathogenic strain of E. coli.

[2] Values indicate the mean±SE of all mortality and either 10 necropsied birds from each pen or all of the surviving birds from each pen having less than 10 survivors.

Table 2. Experiment 2. Incidence of turkey osteomyelitis complex (TOC) lesions in dead birds and in turkeys necropsied 8 and 15 days after a second treatment with dexamethasone.

Time	Osteomyelitis[1]	Synovitis/ Tendonitis	Abscess in Soft Tissue	Multiple Lesions[2]	Green Liver[3]
8 days	5/69 (7%)	11/69 (16%)	10/69 (14%)	10/69 (14%)	0/42 (0%)
15 days	14/43 (33%)	21/43 (49%)	1/43 (2%)	17/43 (39%)	0/42 (0%)
Dead birds	13/43 (30%)	27/43 (63%)	14/43 (33%)	26/43 (60%)	19/43 (44%)

[1] Both proximal tibias of each bird were examined by a straight cut along the topof the tibia and by slicing multiple transverse cuts on the medial condyle of each tibia. Fifty percent of birds with bone lesions had bilateral bone lesions.
[2] Data represents the number of birds that had more than one TOC lesion, including osteomyelitis, tendonitis/synovitis, and soft-tissue abscesses.
[3] Ninety-four percent of birds with green livers had one or more TOC lesions. Fifty-five percent of birds with one or more TOC lesions had green livers, whereas forty-five percent had one or more TOC lesions without the presence of a green liver.

Table 3. Experiment 3. Effect of a second dexamethasone (DEX) injection on turkeys previously challenged in a DEX-E.coli respiratory infection on the incidence of turkey osteomyelitis complex (TOC) and green liver.

E. coli cfu[1]	TOC Lesions[2] %		Green Liver[2] %	
	No DEX	DEX	No DEX	DEX
0	6.25±6.25	63.64±15.21[b]	0.00±0.00	63.64±15.21[a]
25	0.00±0.00	42.86±13.73[ab]	0.00±0.00	21.43±11.38[b]
50	10.00±6.88	20.00±20.00[a]	0.00±0.00	20.00±20.00[b]
	Probability Values		Probability Values	
	DEX	0.0001	DEX	0.0001
	E. coli	0.1516	E. coli	0.0061

[1]Inoculum was diluted in tryptose phosphate broth so that a 200μL injectioncontained either 0.25, or 50 colony-forming units of a pathogenic strain of E. coli.
[2]Values indicate the mean ± SE of all dead birds and necropsied birds. Means within a column or row with no common superscript are significantly different ($P \leq 0.05$).

Table 4. Experiment 4. Effect of sequential dexamethasone (DEX) treatments on the incidence of green liver (GL) and turkey osteomyelitis complex (TOC) lesions in dead birds and necropsied turkeys never inoculated with Escherichia coli and the effect of a single DEX treatment on 12-week-old turkeys never previously treated with DEX.

Time of treatments	No DEX		DEX	
	TOC	GL	TOC	GL
2 weeks of age	nd[1]	nd	nd	nd
2 and 5 weeks of age[2]	0/30	0/30	26/96 (27%)	8/96 (8.3%)
2, 5 and 10 weeks of age[3]	2/41 (4.9%)	1/41 (2.4%)	33/49 (67%)	17/49 (35%)
12 weeks of age	---	---	6/7 (86%)	2/7 (28.6%)

[1] nd=not done. There was no mortality or lameness after the single treatment.
[2] There was no mortality in 30 non-DEX-treated birds. There were 46 dead birds and 50 additional birds were necropsied in the DEX-treated group. Percentages include all dead and necropsied birds.
[3] There was one dead bird and 40 necropsied birds in non-DEX-treated birds. There were 38 dead birds and 11 additional birds were necropsied in the DEX-treated group. Percentages include all dead and neropsied birds.

DISCUSSION

In these studies glucocorticoid treatment, either with or without air sac inoculation of *Escherichia coli,* produces green liver, synovitis/tendonitis, abscesses in the soft tissue of the leg and under the sternum, and osteomyelitis of the proximal tibia . These results support our hypothesis that the TOC problem is not due to the pathogenicity of any one organism, but is related to stress-induced immunosuppression in a sub-population of male turkeys which respond to the stressors of modern poultry production in an inappropriate and detrimental manner (5, 10, 11).

The timing of DEX injections in this model is thought to mimic the stress experienced when poults are moved from a brooder house to a grow-out house, generally around 5 weeks of age, as well as the stresses associated with catching and transportion of mature birds. It seems clear from these data that the deleterious effects of glucocorticoid treatment increase with both the age of the bird and with multiple treatments. Other effects of DEX treatment either with air sac inoculation of *Escherichia coli* or multiple DEX treatments alone include severe airsacculitis/pericarditis, increased heterophil/lymphocyte ratio, and isolation of *Staphylococcus aureus* from blood, air sacs and TOC lesions. Body weights and relative weights of the bursa of Fabricius of these birds are decreased and liver, heart, and spleen weights are increased (10, 11).

The treatment of seven 12-week-old male turkeys, which had never been challenged with either *E. coli* or previously with DEX, with a single series of DEX injections resulted in TOC lesions in 6/7 of the birds and green-fringed livers in 2/7 birds. These birds did not lose weight as do younger birds treated with DEX, and the 3 dead birds, as well as the 4 necropsied birds appeared well-fleshed and hydrated. These effects in older male birds need to be studied for their value as a means of genetic selection for male turkeys with a lower stress response. Within these studies there is always a population of survivors which appear relatively impervious to the effects of DEX. These birds may represent a population which have a moderated response to the stressors of poultry production and are less likely to lose weight or become immunosuppressed. Lameness appeared in these 12-week-old male turkeys within 2 days of DEX treatment, which suggests that while TOC may be endemic within a population, stressors occuring at the end of the grow-out, especially catching and transportation, may be responsible for a significant proportion of TOC incidence.

In summary, we have established an experimental model for the reproduction of all of the lesions described in the TOC syndrome, including green liver, arthritis/synovitis extending into the tendons and producing purulent and caseous lesions in the muscles of the leg and thigh, and osteomyelitis of the proximal tibia. These lesions are identical to those seen in commercial processed turkey carcasses, and in older birds they occur without external signs of septicemia/toxemia. In this model they are always associated with repiratory infection, whether or not the birds are inoculated with bacteria. While the *E.coli* inoculum is cultured from the majority of lesions of young birds, and those treated with one DEX injection, *S. aureus* is also isolated from these birds, and is the predominant isolate in the blood, air sac and TOC lesions of birds given only DEX treatment. We hope this model will be of value in the identification of nutritional and physiological immunomodulators that can decrease TOC incidence, as well as a means of selection for birds more resistant to the stressors of turkey production.

REFERENCES

1. Barnes, H. J., S. C. Clark, and B. J. Tilley. Green liver/osteomyelitis complex in turkeys: an overview. Proc. Avian Skeletal Disease Symposium, AAAP/AVMA, San Antonio, TX. pp. 73-77. 1990.

2. Barnes, H. J., M. T. Correa, C. Woodlief, L. Shaw, C. J. Diaz, and J. W. Coleman. Green liver discoloration and osteomyelitis in turkeys at processing. Proc. 130th Annual Meeting of the Am. Vet. Med. Assoc., Minneapolis. 1993.

3. Bayyari, G. R., W. E. Huff, R. A. Norton, J. K. Skeeles, J. N. Beasley, N. C. Rath, and J. M. Balog. A longitudinal study of green-liver osteomyelitis complex in commercial turkeys. Avian Dis. 38:744-754. 1994.

4. Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, and J.K. Skeeles. Immune and physiological responses of turkeys with green-liver osteomyelitis complex. Poult. Sci. 76:280-288. 1997.

5. Bayyari, G. R., W. E. Huff, N. C. Rath, J. M. Balog, L. A. Newberry, J. D. Villines, J. K. Skeeles, N. B. Anthony, and K. E. Nestor. Effect of the genetic selection of turkeys for increased body weight and egg production on immune and physiological responses. Poult. Sci. 76:289-296. 1997.

6. Bickford, A. A., R. P. Chin, R. Droual, and G. L. Cooper. Evaluation of bone and muscle lesions in market-age tom turkeys with green discoloration of livers. Proc. West. Poult. Dis. Conf., Davis, California. pp. 46-47. 1990.

7. Clark, S. R., H. J. Barnes, A. A. Bickford, R. P. Chin, and R. Droual. Relationship of osteomyelitis and associated soft-tissue lesions with green liver discoloration in tom turkeys. Avian Dis. 35:139-146. 1991.

8. Cook, R. E. Turkey osteomyelitis - synovitis complex. Inspection standards and procedures. Technical Services, Food Safety and Inspection Service. U.S.D.A., National Agricultural Library, Washington, D.C. 1988.

9. Droual, R., R. P. Chin, and M. Rezvani. Synovitis, osteomyelitis, and green liver in turkeys associated with Escherichia coli. Avian Dis. 40:417-424. 1996.

10. Huff, G. R., W. E. Huff, J. M. Balog, and N. C. Rath. Effects of dexamethasone immunosuppression on turkey osteomyelitis complex in an experimental Escherichia coli respiratory infection. Poult. Sci. 77:654-661. 1998.

11. Huff, G.R., W. E. Huff, J. M.Balog, and N. C. Rath. Sex differences in the resistance of turkeys to *Escherichia coli* challenge after immunosuppression with dexamethasone. Poultry Sci. In press. 1999.

12. McCaskey, P. C. Incidence and pathogenesis of the osteomyelitis-synovitis complex in turkeys and its association with abnormally green livers. J. Am. Vet. Med. Assoc. 194:1798. 1989.

13. Mutalib, A., B. Miguel, T. Brown, and W. Maslin. Distribution of arthritis and osteomyelitis in turkeys with green liver discoloration. Avian Dis. 40:661-664. 1996.

14. Nairn, M. E. Bacterial osteomyelitis and synovitis of the turkey. Avian Dis. 17:504-517. 1973.

15. Tilley, B. J., and H. J. Barnes. Factors associated with green livers in turkeys. Proc. West. Poult. Dis. Conf., Sacramento, California. p. 46. 1990.

ANTIBODY RESPONSES IN BROILER CHICKENS FOLLOWING SUBCUTANEOUS INOCULATION WITH CELLULITIS-DERIVED ESCHERICHIA COLI

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An ELISA test was developed to measure the IgG antibody titer of broiler chickens that were challenged with *Escherichia coli* originating from cellulitis lesions. Hyperimmune serum was prepared in SPF chickens to 4 cellulitis-derived *E. coli* isolates to serve as positive control serum. Eight broilers per isolate received a subcutaneous injection of approximately 10^7 cfu/ml bacteria in sterile phosphate buffered saline. The broilers were bled every 3 to 4 days following challenge for a total of 30 days. The resulting cellulitis lesions were scored by palpation and body weights were also recorded on bleeding days. Necropsy and examination for gross lesions was performed at the conclusion of the study. We were able to measure an antibody response to challenge in all of the broilers. We also evaluated the effect of the challenge on average daily weight gain. We tested the relationship between serologic titer and severity of cellulitis lesion scores. We found a measurable titer in sera collected on and after 4 days post-inoculation. Titer varied with isolate. In one group, the mean IgG titer continued to increase over the 30 day duration of the trial. In 3 groups the IgG titer gradually peaked and then dropped to a plateau which continued throughout the trial. Palpable lesion size peaked at approximately 8 days post-inoculation in most birds, then steadily decreased, but lesions did not completely resolve. Changes in lesion severity did not correlate with changes in antibody titer. There was no effect of challenge on average daily weight gain. This information is critical for evaluating the potential efficacy of vaccination for cellulitis in broiler chickens.

(A full length article will be submitted for publication in *Avian Diseases*.)

EXPERIMENTAL INFECTION OF BROILER CHICKS WITH SALMONELLA ENTERITIDIS ISOLATES FROM THE ENVIRONMENT

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Paratyphoid infections (PT) often have very different consequences for newly hatched poultry than for more mature birds. In susceptible young chicks and poults, experimental or natural PT can sometimes lead to illness and high death rates. Older birds are far less susceptible to the lethal effects of PT but may experience intestinal colonization and even systemic dissemination without significant morbidity or mortality. In younger birds high mortality has been primarily associated with *Salmonella enteritidis* (SE) phage type 4 infection. Other SE phage types have also been occasionally associated with field reports of morbidity or mortality in chickens. In experimental studies other SE phage types have caused mortality when administered in high doses to susceptible young chicks.

Nine groups of 24 one-day-old broiler chicks were inoculated into their crops with 0.5 ml saline containing 1 X 10^6 colony forming units of *Salmonella*. Six groups of

broiler chicks were inoculated with SE isolates belonging to phage types 4, 5A, and 8 obtained using drag swab techniques from poultry houses on Washington and California farms. Other groups were inoculated with either SE phage type 4 of poultry origin, SE phage type 4 of human origin, or *Salmonella pullorum*. One group of broiler chicks was kept as an uninoculated control. The chicks were observed daily for clinical signs and mortality. Two chicks were randomly selected from each group at 7 and 14 days postinoculation for necropsy and histopathology. Body weights were obtained at 1, 2 and 3 weeks of age. The remaining chicks from each group were euthanized and necropsied at 3 weeks of age.

The mean body weights were lower in broilers inoculated with SE phage type 4 (human origin), in broilers inoculated with SE phage type 5A and in broilers inoculated with *S. pullorum* than in uninoculated controls at 7, 14 and 21 days post-inoculation. The differences between the first two groups and the uninoculatd controls at 7 and 14 days were significantly different. High mortality was observed in chicks inoculated with *S. pullorum*, SE phage type 4 (human origin) and SE phage type 5A. Lesser mortality was observed in chicks inoculated with SE phage type 8 and SE phage type 4 (isolate from California). No signs of illness or mortality were observed in the uninoculated controls. Pericarditis, perihepatitis and peritonitis with congestion of mesentery were found in all treated groups. A thickened pericardial sac, irregular epicardial surfaces and a retained yolk sac attached to the posterior abdominal wall were present in broilers that died between 14 and 21 days from the *S. pullorum* inoculated groups. Histopathological changes from all dead birds from all treatment groups were fibrinosuppurative inflammation of the peritoneal surface of the intestine and pancreas, yolk sac and epicardium. In chronic stages, these birds also had severe extensive myocarditis characterized by infiltration of macrophages and a few lymphocytes.

(A full length article will be submitted for publication in *Avian Diseases*.)

RESULTS OF A SALMONELLA ENTERITIDIS VACCINATION FIELD TRIAL IN BROILER BREEDER FLOCKS IN THE NETHERLANDS

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The present *Salmomella enteritidis* control program in The Netherlands consists of compulsory monitoring of reproduction flocks, the elimination or treatment of *S. enteritidis* infected flocks, and implementation of preventive measures following a standardized biosecurity program (SBP) based on a case-control study of risk-factors (1). Recently, vaccination was proposed as a new tool in S. *enteritidis* control in poultry. The additional effect of vaccination to SBP on reduction of *S. enteritidis* infections in broiler breeder flocks has been evaluated in a field trial in The Netherlands from August, 1995, until December, 1997. The effect was studied in a situation of increased infection risk, while its influence on the official serological *Salmonella* monitoring programme was analysed as well.

In this field trial 64 flocks, with an increased risk of *S. enteritidis* infection on farms with the SBP, were vaccinated. Fifteen flocks were vaccinated on the first day of life and at 7 weeks of age with VAC-T®, a vaccine based on an attenuated *S. typhimurium* strain, followed by a vaccination at 16 weeks of age with TALOVAC® (Orffa, The Netherlands), a vaccine based on an inactivated *S. enteritidis* strain. Another 49 flocks were vaccinated at 12 and 16 weeks of age with SALENVAC® (Hoechst, Benelux), a vaccine based on an inactivated *S. enteritidis* strain. The control group consisted of 608 non-vaccinated flocks with an average risk of infection, hatched in the same period as the vaccinated groups, and also on farms with the SBP. The occurrence of *S. enteritidis* in the vaccinated and non-vaccinated groups was assessed by means of serological and bacteriological examination and the proportion of *S. enteritidis* infected flocks in the different groups was compared.

Both vaccination programs induced a long lasting antibody-response in the LPS-BD-ELISA and gm-DAS-ELISA which are tests for detecting antibodies against *Salmonella* group B/D antigen and *S. enteritidis* flagellar antigen respectively (2, 3, 4). Incidentally, titres of 1:16 were seen in the Spg-RPA-test which is a test for detecting *S. pullorum/gallinarum* antibodies. The proportion of *S. enteritidis* reinfection (infection in flocks placed on a previously contaminated farm) was significantly lower (P=0.02) in the vaccinated groups compared with the control group. There was an indication (P=0.12) that the proportion of *S. enteritidis* infected flocks was lower in the vaccinated groups than in the control group.

From this field trial it can be concluded that vaccination against *S. enteritidis* on farms with the SBP resulted in a statistically significant reduction of *S. enteritidis* reinfections. The applied vaccination programs exclude serological monitoring of *S. enteritidis* infection and incidentally of *S. pullorum /gallinarum* infection. Based on the results of this field trial, we advise vaccination of broiler breeder flocks in The Netherlands when there is a risk of *S. enteritidis* reinfection on farms with the SBP. In view of the

interference with serology by vaccination, these flocks are to be monitored by bacteriology.

REFERENCES

1. Fris, C., J. van den Bosch and A. Van Ditmarsch. A retrospective case-control study of risk factors associated with Salmonella enterica subsp. Enterica serovar Enteritidis infections on dutch broiler-breeder farms. Avian Pathol. 24 : 255-272. 1993.

2. Heijden van der, H. M. J. F., J. C. van den Wijngaard, J. Bongers, and A. C. A. van Exsel. ELISA voor serologisch onderzoek naar Salmonellae bij pluimvee. Stichting Gezondheidsdienst voor Dieren in Noord Brabant. 1990.

3. Zijderveld van, F. G., A. M. van Zijderveld-van Bemmel, R. A. M. Brouwers, T. S. de Vries, W. J. Landman, and W. A. de Jong. Serological detection of chicken flocks naturally infected with Salmonella enteritidis, using an enzyme-linked immunosorbent assay based on monoclonal antibodies against the flagellar antigen. Vet. Quart. 15(4):135-137. 1993.

4. Zijderveld van F. G., A. M. van Zijderveld-van Bemmel, and J. Anakotta. Comparison of four different enzyme–linked immunosorbent assays for serological diagnosis of Salmonella enteritidis infections in experimentally infected chickens. J. Clin. Microbiol. 30:2560-2566. 1992.

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USE OF NEGATIVE AIR IONIZATION FOR REDUCING AIRBORNE LEVELS OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS IN A ROOM CONTAINING INFECTED CAGED LAYERS

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INTRODUCTION

Salmonella infections in poultry may be initiated at a number of different sites. While oral ingestion appears to be the most common route, infections via the eye, cloaca, and, in newly hatched chicks, the navel have been observed (3,4). Invasion via the respiratory tract has also received an increasing amount of attention in recent years (1) and airborne transmission of salmonellae has been documented in several cases (2,5,8,14). Removal of airborne organisms therefore becomes an important step in reducing *Salmonella* problems but there are few methods available to achieve this removal. Negative air ionization provides promising new technology for dust and pathogen elimination from the air. Such a device imparts a negative charge on airborne particles which subsequently will be attracted to grounded surfaces such as the wall and floors, effectively removing them from the air environment. Previous studies demonstrated that substantial dust and dander reduction was observed in hatching cabinets subjected to negative air ionization (9,10) and this significantly reduced airborne bacterial levels in these cabinets (11). Ionization devices recently were shown to effectively reduce airborne transmission of viral (12) and bacterial (6) pathogens in enclosed isolation cabinets. As horizontal transmission of *S. enteritidis* via particles floating in the air can also occur in caged hens (8,14), the current study was undertaken to determine if negative air ionization could also be applied to a large room environment as a means to reduce airborne *S. enteritidis* levels.

MATERIALS AND METHODS

Room Setup. Two trials were conducted in adjacent rooms of a climate-controlled biocontainment building. In one room, a custom-built (Patent application number 09/122,850 filed July 28, 1998) negative air ionizer was suspended from the ceiling 2.2 m from the concrete floor in the approximate room center. Power was administered at -30 kV and was constantly applied except during periodic ionizer cleaning. Cleaning of the ionizer was achieved by carefully brushing dust, dander, and feathers from each bar and the ground plane.

Experimental Design. One hundred and twelve Single Comb White Leghorn hens (>55 weeks old), retired from the Southeast Poultry Research Laboratory specific-pathogen-free flock, were divided equally into two groups and placed into individual laying cages in the two rooms. Hens were molted via feed removal as previously described (8) and on day 4 following cessation of feed, each hen received an oral dose of 1 ml of 1 X 10^7 rifampicin resistant *S. enteritidis* phage type 13a. Relative airborne levels of *S. enteritidis* in each room were determined over the next 10 days by exposing to the room air for 24 hours, with the lids removed, petri plates containing brilliant green agar containing 200 µg/ml rifampicin. The plates were placed in 12 different locations on top of the cages and on the sidewalls within the

rooms and were replaced daily. Exposed plates were incubated for 24 hours at 37°C and the number of *S. enteritidis* were determined.

RESULTS AND DISCUSSION

Previous studies showed that hens infected during a molt shed large numbers of *S. enteritidis,* rapidly contaminating the floors underneath and increasing the organism levels in the air (8). In the current trials, airborne levels similarly increased over time and air samples approached 100% culture positive by 72 hr post challenge (Fig. 1). In trial 1 (Fig. 1A), 58% of the air samples from the nonionizer room were culture positive at day 2 post challenge compared with 17% of ionizer room samples . A 90% *S. enteritidis* reduction efficiency was observed at this time (Fig. 2A). However, the number of positive samples from the ionizer room continued to increase and reached levels similar to those in the room lacking the instrument. A subsequent examination of the ionizer revealed that the bars and ground plane were covered with a thick coating of dust and dander collected during the 7 days prior to the bird challenge. Removal of this debris on day 7 resulted in a dramatic drop in the number of positive plates and the efficiency of *S. enteritidis* removal for days 8-10 averaged 82% (Fig. 2A).

The experiment was repeated with the exception that the ionizer was routinely cleaned and significant reductions in airborne *S. enteritidis* levels were rapidly observed (Fig. 2B). A power supply failure occurred between day 2 and 3 which affected the efficiency of *S. enteritidis* removal from the air but subsequent replacement of the power supply on day 3 reduced the levels of circulating *S. enteritidis* on day 5. While the percentage of culture-positive samples were not diminished at this time (Fig. 1B), the over-all numbers of organisms/sample were reduced from an average 25 *S. enteritidis*/plate to 1-2 organisms per plate (Fig. 2B). *Salmonella enteritidis* reduction efficiency for the remainder of the trial averaged 96.5%.

Negative air ionization could therefore serve as an important tool to reduce the spread of *S. enteritidis*, as well as other salmonellae, in a poultry operation. This could be especially important in those situations where the likelihood of transmission is high such as with newly hatched chicks (2, 4) or with adult hens during stress situations (7,8,13). The economic feasibility of using this technology in a commercial poultry setting remains to be determined but the effectiveness of negative air ionization under experimental conditions indicates that it could have significant applications in reducing *Salmonella* problems on the farm.

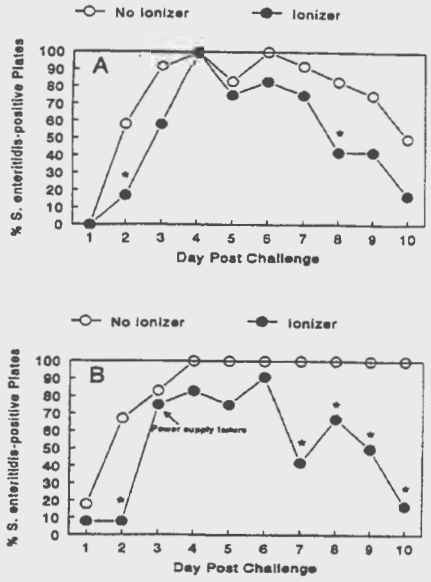

Figure 1. Percentage of *S. enteritidis* culture-positive plates from the room containing the ionizer (filled circles) vs the room lacking the device (open circles) on days 1-10 post challenge. A = experiment 1 and B = experiment 2. * = significantly different percentage of positive plates in the ionizer vs nonionizer room ($P < 0.05$).

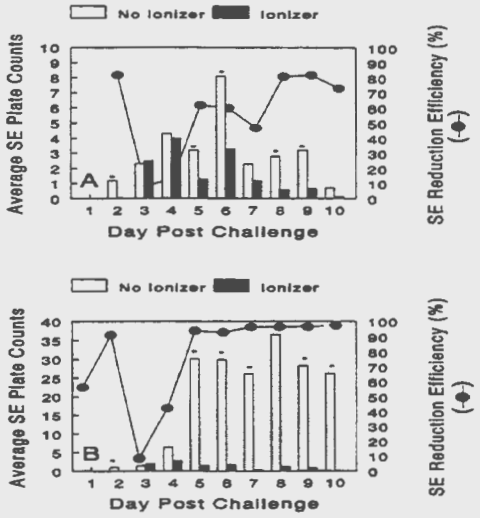

Figure 2. Mean counts of *S. enteritidis* on plates from the room containing the ionizer (filled bars) vs the room lacking the device (open bars) on days 1-10 post challenge. A = experiment 1 and B = experiment 2. * = significantly different *S. enteritidis* numbers in the ionizer vs nonionizer room ($P < 0.05$). The filled circle represents the daily reduction efficiency of *S. enteritidis* by the ionizer.

REFERENCES

1. Baskerville, A., T. J. Humphrey, R. B. Fitzgeorge, R. W. Cook, H. Chart, B. Rowe, and A. Whitehead. Airborne infection of laying hens with Salmonella enteritidis phage type 4. Vet. Rec. 130:395-398. 1992.

2. Cason, J. A., N. A. Cox, and J. S. Bailey. Trans-

mission of Salmonella typhimurium during hatching of broiler chicks. Avian Dis. 38:583-588. 1994.

3. Cox, N. A., J. S. Bailey, and M. E. Berrang. Alternative routes for Salmonella intestinal tract colonization of chicks. J. Appl. Poult. Sci. 5: 282-288. 1996.

4. Cox, N. A., J. S. Bailey, L. C. Blankenship, R. J. Meinersmann, N. J. Stern, and F. McHan. Fifty percent colonization dose for Salmonella typhimurium administered orally and intracloacally to young broiler chicks. Poult. Sci. 69:1809-1812. 1990.

5. Gast, R. K., B. W. Mitchell, and P. S. Holt. Airborne transmission of Salmonella enteritidis infection between groups of chicks in controlled-environment isolation cabinets. Avian Dis. 42:315-320. 1998.

6. Gast, R. K., B. W. Mitchell, and P. S. Holt. Application of negative air ionization for reducing experimental airborne transmission of Salmonella enteritidis to chicks. Poult. Sci. *in press*.

7. Holt, P. S. Horizontal transmission of Salmonella enteritidis in molted and unmolted laying chickens. Avian Dis. 39:239-249. 1995.

8. Holt, P. S., B. W. Mitchell, and R. K. Gast. Airborne horizontal transmission of Salmonella enteritidis in molted laying chickens. Avian Dis. 42:45-52. 1998.

9. Mitchell, B. W. Effect of space charging on dust reduction in a poultry hatching cabinet. ASAE Paper 964069, Phoenix, AZ. 1996.

10. Mitchell, B. W., R. J. Buhr, M. E. Berang, N. A. Cox, and J. S. Bailey. Characterization and reduction of airborne particulates in the hatching cabinet with an electrostatic charge. Poult. Sci. 77 (S1): 146. 1998.

11. Mitchell, B. W., R .J. Buhr, M. E. Berang, J .S. Bailey, and N. A. Cox. Reduction of airborne bacteria in the hatching cabinet with an electrostatic space charger. Poult. Sci. 77 (S1): 151. 1998.

12. Mitchell, B. W., and D.J. King. Effect of negative air ionization on airborne transmission of Newcastle disease virus. Avian Dis. 38:725-732. 1994.

13. Nakamura, M., N. Nagamine, T. Takahashi, S. Suzuki, M. Kijima, Y. Tamura, and S. Sato. Horizontal transmission of Salmonella enteritidis and effect of stress on shedding in laying hens. Avian Dis. 38:282-288. 1994.

14. Nakamura, M., M. Takagi, T. Takahashi, S. Suzuki, S. Sato, and K. Takehara. The effect of the flow of air on horizontal transmission of Salmonella enteritidis in chickens. Avian Dis. 41:354-360. 1997.

HIGHLY FIMBRIATED STRAINS OF SALMONELLA AS POTENTIAL VACCINE CANDIDATES FOR USE IN POULTRY

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ABSTRACT

Fimbriae are bacterial proteinaceous structures that have been shown to mediate the adhesion of bacteria to the host tissue. Studies were conducted to investigate the use of a highly fimbriated isolate of *Salmonella typhimurium* (ST) as a potential vaccine strain to control ST infection in turkeys. Sixty seven isolates of ST from turkeys were examined for hemagglutination and by electron microscope for quantitative and qualitative expression of fimbriae. A vaccine was prepared with an isolate that expressed a high degree of fimbriae. Commercial turkeys were immunized and challenged either orally or by contact exposure to infected birds. Protection was demonstrated by reduced numbers of ST isolated from vaccinated birds challenged by contact exposure as compared to control birds. The results suggested that vaccinating turkeys twice with a bacterin made of a highly fimbriated strain of ST will reduce the infection rate of ST in a field situation.

INTRODUCTION

Fimbriae are proteinaceous non-flagellar filamentous structures on the surface of bacteria. They have the ability to agglutinate erythrocytes. This ability may or may not be inhibited by mannose and mannose derivatives (4, 6, 11). The immunogenic nature of the fimbriae have been investigated for the potential use of fimbriae in vaccines to prevent the attachment of bacteria. Fimbrial based vaccines have been used for a decade to prevent neonatal diarrhea caused by *Escherichia coli* in calves and in newborn pigs (8). A purified fimbrial antigen (K88ab, K88ac, K99 and 987P), along with a purified B subunit of heat labile (LT) *E. coli* enterotoxin, was shown to be efficient in preventing colibacillosis in pigs when compared with non-fimbrial vaccines (10). Pathogenic *E.coli* (O1a, O2a and O78) isolated from turkeys has been shown to express fimbriae and there is evidence to indicate that the fimbriae are involved in attachment to the mucosa of the intestinal tract

(9). The great majority of the enterotoxigenic *E. coli* (more than 90%) that are associated with disease in animals is part of a small family of fimbriated and enterotoxin producing types (2, 8). Three types of bacterial fimbriae are described (11). It has been demonstrated that type 1 fimbriae are involved in the attachment of *Salmonella* to the intestinal enterocytes (7). Type 1 fimbriae of *Salmonella* exhibit mannose sensitive agglutination of erythrocytes unlike type 2 fimbriae (3, 5). We hypothesize that selecting a *Salmonella* strain that expresses a high amount of type 1 fimbriae, and using it in a vaccine, will provide protection against *Salmonella* colonization by blocking the binding of the bacteria with anti-fimbrial antibodies.

MATERIALS AND METHODS

Sixty seven isolates of ST of poultry origin were examined for the quantitative and qualitative expression of fimbriae. Because the type 1 fimbriae of *Salmonella* agglutinates red blood cells (RBC), all 67 isolates of ST were screened for their ability to hemagglutinate RBC of chicken, rabbit, sheep and turkeys by the procedure described by Back (1). Isolates that showed high hemagglutination and isolates that showed the lowest hemagglutination were incubated at 37°C for 24, 48, 72 hr to further evaluate their quantitative expression of fimbriae by electron microscopy (EM) (1). An isolate that had the highest degree of expression of fimbriae was used to prepare an oil bacterin. For comparison, an isolate that had a low expression of fimbriae was also prepared as a bacterin.

To evaluate the bacterins 8-week-old commercial turkeys tested free of *Salmonella* were divided into 6 groups. Eighteen birds in group 1 and 17 birds in group 2 were vaccinated with a bacterin made of the ST that expressed the highest amount of fimbriae (HF). Seventeen and 20 birds in groups 3 and 4, respectively, were vaccinated with a bacterin made of ST that expressed the lowest amount of fimbriae (LF). Eighteen birds in group 5 and 73 birds in group 6 were sham vaccinated with PBS. Birds in groups 1, 2, 3 and 4 were revaccinated at 3 weeks after the first vaccination with the same vaccine as before. The vaccine had a titer of 1 x 10^{10} CFU / ml and was administered by the intramuscular route (0.5 cc per bird). Two weeks after the second vaccination birds in groups 1, 3 and 6 were challenged by the oral route while birds in groups 2, 4 and 5 were challenged by contact exposure with known infected birds. The oral challenge dose was $2x10^9$ CFU/ml of ST which was nalidixic acid resistant. Two weeks after the challenge, all birds were euthanized and the cecal junction, liver and spleen were cultured for Salmonella. The reduction of *Salmonella* isolations from internal organs in the vaccinated birds was used to assess the degree of protection.

RESULTS

The results of ST hemagglutination of RBC of chickens, rabbits, sheep and turkeys are shown in Table 1. There was a significant variation in the degree of hemagglutination with RBC from different sources. When D-mannose was added to the RBC suspension, agglutination was inhibited completely, confirming that fimbriae type 1 were involved.

Table 1. Hemagglutination of red blood cells by Salmonella typhimurium.

RBC Source	% of Isolates that hemagglutinated RBC
Chicken	93
Rabbit	51
Sheep	9
Turkey	100

The presence of fimbriae was observed in all isolates of ST examined by EM but the amount of fimbriae was variable between isolates. In any given sample there were bacteria expressing fimbriae as well as bacteria not expressing fimbriae. Isolates incubated for 24 hours expressed fewer amounts of fimbriae than those incubated for 48 or 72 hours.

Table 2 shows the recovery of ST from vaccinated and unvaccinated birds challenged by the oral route. Birds that were vaccinated with the bacterins made of the HF strain or the LF strain of ST showed no difference in the clearance of *Salmonella* from the liver, spleen and cecal junction when compared with the control birds. In contrast, birds that were vaccinated with a bacterin made of the HF strain of ST and challenged by contact exposure were able to maintain spleen and liver free of *Salmonella* invasion after challenge (Table 3). Birds unvaccinated but exposed by contact with infected birds developed *Salmonella* infection in the cecum as well as in the liver and spleen.

Table 2. Percent isolation of Salmonella in turkeys challenged orally.

		Percent Isolation		
Groups	Vaccine	Cecal Junction	Liver	Spleen
1	HF	11.1	6	0
3	LF	35	0	5.8
5	PBS	28.5	4	8.2

HF: Highly Fimbriated
LF: Low Fimbriated

Table 3. Percent isolation of Salmonella in turkeys challenged by contact exposure.

		Percent Isolation		
Groups	Vaccine	Cecal Junction	Liver	Spleen
2	HF	17.7	0	0
4	LF	15	0	5
6	PBS	26.3	5.3	15.8

HF: Highly Fimbriated
LF: Low Fimbriated

DISCUSSION

In the present study we selected a ST isolate that expressed a high degree of fimbriae and evaluated the selected strain in a killed vaccine for the control of salmonellosis in turkeys. The expression of fimbriae was observed in all isolates of ST analyzed but the degree varied. The difference in hemagglutination of chicken, rabbit, sheep and turkeys RBC by the ST examined indicated differences in the surface receptors for the type 1 fimbriae. It is also possible that there may be more than one type 1 fimbria expressed by the different isolates. In the birds vaccinated with bacterins made of HF and LF ST strains, and challenged orally with ST, there was a slight or no reduction in the clearance of *Salmonella*. One possible reason that may explain the recovery of *Salmonella* in the vaccinated birds was the high challenge dose used in this experiment. A high challenge dose is required to establish a persistent infection, up to two weeks, with ST in turkeys of 13 to 15 weeks of age. In contrast, birds that were vaccinated with HF and LF bacterin and challenged by contact exposure had a lower rate of *Salmonella* infection than birds not vaccinated . This contact exposure challenge simulates the field situation. Very rarely under natural conditions will birds be exposed to a massive dose of *Salmonella* at one time unlike the oral challenge in this situation. These findings may be useful in designing a program to control ST infection in a turkey population at risk of infection. Further work is needed to better understand the role of fimbriae in the prevention of ST infection in poultry.

REFERENCES

1. Back, A. New strategies for the development and delivery of Salmonella vaccines. Ph.D. thesis. College of Veterinary Medicine, University of Minnesota, Saint Paul, MN 55108. 1998.

2. Blake, M. S., C. M. MacDonald, and K. P. Klugman. Colony morphology of piliated Neisseria meningitidis. J. Exp. Med. 170:1727-1736. 1989.

3. Crichton, P. B., D. E. Yakubu, D. C. Old, and S. Clegg. Immunological and genetical relatedness of type 1 and type 2 fimbriae in Salmonella of serotypes Gallinarum, Pullorum and Typhimurium. J. App. Bacteriol. 67:283-291. 1989.

4. Duguid, J. P., E. S. Anderson ,and I. Campbell. Fimbriae and adhesive properties in Salmonella. J. Pathol. Bacteriol. 92:107-138. 1966.

5. Even, S. W. B. et al.. Salmonella enterica var. Typhimurium and S. enterica var. Enteritidis express type 1 fimbriae in rat in vivo. FEMS Immunol. Med. Microbiol. 18:185-192. 1997.

6. Josiane, F., W. W. Kay, and T. J. Trust. Purification and characterization of fimbriae from S. enteritidis. J. Bacteriol. 168:221-227. 1986.

7. Lindquist, B. L., E. Lebenthal, P. C. Lee, M. W. Stinson, and J. M. Merrick. Adherence of Salmonella typhimurium to small-intestinal enterocytes of the rat. Infect. Immun. 55:3044-3050. 1987.

8. Moon, H. W., and T. O. Bunn. Vaccines for preventing enterotoxigenic E. coli infections in farm animals. Vaccine 11: 213-220. 1993.

9. Nagaraja, K. V., D. A. Emery, J. A. Newman, and B. Pomeroy. Identification and isolation of somatic pili from pathogenic E. coli of turkeys. Am. J. Vet. Res. 44: 248-287. 1983.

10. Osek, J. M., M. Truszynski, K. Tarasiuk, and Z. Pejsak. Evaluation of different vaccines to control of pig colibacillosis under large-scale farm conditions. Comp. Immunol. Microbiol. Infect. Dis. 18:1-8. 1995.

11. Thorns, C. J., M. G. Sojka, and D. Chasey. Detection of a novel fimbrial structure on the surface of Salmonella enteritidis by using a monoclonal antibody. J. Clin. Microbiol. 28:2409-2414. 1990.

MULTIPLE DRUG-RESISTANT SALMONELLA TYPHIMURIUM IN QUAIL

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The multiple drug-resistant strain of *Salmonella typhimurium* definitive type 104 (DT104) has been an important cause of human and cattle salmonellosis in the United Kingdom (UK) since the early 1990's (3, 4). These

isolates have shown a genetically encoded antimicrobial resistance pattern to ampicillin, chloramphenicol, streptomycin, sulfonamides and tetracycline (3, 4). In animals, multiresistant DT104 is primarily a pathogen of cattle, but also has been isolated from a wide range of a mammals and birds (1, 3, 4). Multiresistant DT104 cases in poultry have been documented in chickens, turkeys (UK, U.S. and Canada) and quail (Canada) (2, 3, 4, C. Poppe, personal communication).

Young bobwhite quail from four different hunting preserve growers were submitted to the Clemson University Animal Diagnostic Laboratory in August and October of 1997 (3 cases), and October of 1998 (1 case). The cases involved 4-day-old and 3-wk-old quail with a complaint of increased mortality ranging from 1.6 to 8.6%. Postmortem lesions included emaciation, dehydration, distended abdomens, and dark colon contents, which were gaseous and fluid or pasty in consistency. Group B *Salmonella* isolated from the intestines and/or livers from the first 3 cases was resistant to ampicillin, chloramphenicol, triple sulfa and tetracycline (streptomycin was not included in the antimicrobial panel at that time). The Group B *Salmonella* isolated from the intestines and livers from the last case was resistant to the same antimicrobials and streptomycin. The isolate involving the one case of 3-wk-old quail was phage typed as *S. typhimurium* DT104. Isolates from the other case of 3-wk-old quail and the two cases of 4-day-old quail were phage typed as *S. typhimurium* DT104b.

REFERENCES

1. Besser, T. E., C. C. Gay, J. M. Gay, D. D. Hancock, D. Rice, L. C. Pritchett, and E. D. Erickson. Salmonellosis associated with S. typhimurium DT104 in the USA. Vet. Rec. 140:75. 1997.

2. Miller, M. A. Widespread emergence in the US of multiple drug-resistant type of Salmonella typhimurium. FDA Veterinarian. 12(4):5-6. 1997.

3. Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe. Epidemic in cattle and humans of Salmonella typhimurium DT 104 with chromosomally integrated multiple drug resistance. Vet. Rec. 134:577. 1994.

4. Threlfall, E. J., J. A. Frost, L. R. Ward, and B. Rowe. Increasing spectrum of resistance in multiresistant Salmonella typhimurium. Lancet. 347:1053-1054. 1996.

(Part of this case report has been submitted to *Avian Diseases* for publication.)

EVALUATION OF A MODIFIED LIVE SALMONELLA TYPHIMURIUM VACCINE IN U.S. COMMERCIAL BROILER FIELD TRIALS

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A modified live vaccine has been recently approved in the U.S.A. for use in young growing chickens to provide protection from multiple strains of *Salmonella*. Upon completion of clinical laboratory efficacy trials and safety tests in non-target animal species, field safety trials commenced on three different companies' broiler farms in three different geographical sites. Paired houses allowed for separation of vaccinated and control birds. The vaccine, Megan™ Vac 1, was administered to treated birds by coarse spray and again as a field boost at 14 days of age. Environmental samples were collected to assess degree of *Salmonella* contamination in the houses. Meconia samples collected from incoming chicks at all three trial sites were culture positive for Groups B and C_3 *Salmonella*. Megan™ Vac 1 was not isolated from litter in buildings where birds were vaccinated. At processing, 100 whole carcass post-chiller rinsates were collected from each trial site and assessed for *Salmonella* sp. In the first trial eight percent of the carcass rinsates from the control group were positive for Group C_2 and C_3 *Salmonella* while no *Salmonella* was detected or cultured from samples from the vaccine treated group. In the second trial *Salmonella* was not cultured from rinsate samples from either the control or vaccine treated groups. In the third trial 12 percent of the carcass rinsates from the control group were culture positive for Groups B and C_2 *Salmonella* while one hundred percent of the carcass rinsates from the vaccine treated group were *Salmonella*-free. These data show a practical new tool to address *Salmonella* control in commercial broiler operations.

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

IDENTIFICATION OF SALMONELLA MULTIPLICATION "HOT SPOTS" IN POULTRY LITTER

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INTRODUCTION

Numerous studies have revealed that *Salmonella*-positive broiler farms are often the source of carcass contamination at processing plants. During production or grow-out, poultry litter is probably the most significant source of *Salmonella*. As an economic and environmental necessity, industry reuses litter from previous flocks, does not fully disinfect between flocks, and generally relies on compacted earth rather than wood or concrete for house floors. As a consequence, industry is always vulnerable to the recurrence and amplification of *Salmonella* and *Escherichia coli* problems.

The main objectives of this study are 1) to confirm equilibrium relative humidity (a measure of water activity or Aw) as an indicator of the *Salmonella* status of the house; 2) use the drag swab method and quantitative litter cultures to determine if there are *Salmonella* "hot spots" in the poultry house; 3) to ascertain the possibility of relating numbers of *Salmonella* and *E. coli* from litter samples to Aw levels; 4) to determine Aw levels in relation to moisture content (MC), pH, air flow, house temperature, house humidity, and *Salmonella/E. coli* counts; 5) to assess house management strategies to see if poor management affects the moisture in the litter subsequently leading to higher bacterial counts.

Farm contamination is recognized as a "critical control point" in emerging mandated HACCP programs for the production, processing, and marketing of broiler meat. It is therefore imperative to promptly identify practical, dependable on-farm *Salmonella* risk reduction procedures. Identification of *Salmonella* multiplication "hot spots" in poultry litter is a vital step towards the attainment of this objective.

MATERIALS AND METHODS

Broiler houses on farms with a history of carcass contamination with *Salmonella* during processing were visited. Litter samples were collected from areas with high and low volume airflow (dead spots) as well as from dry (away from drinkers) and caked litter (under nipple drinkers). These samples were promptly evaluated for Aw, MC , and pH, then analyzed for *Salmonella* and *E. coli* counts. Four litter surface drag swabs per house were also taken during the time of litter sampling to confirm the presence of *Salmonella*. *Salmonella* counts were determined using the 5-tube most probable number technique. Standard analytical methods were employed for coliform counts, MC, and pH. The Decagon II Water Activity System (Pullman, WA) was used to measure Aw. Precision instrumentation was used to simultaneously measure airflow, ambient temperature, and relative humidity. Data obtained were tested for correlations between ventilation, *Salmonella* and *E. coli* counts, and the physical/chemical characteristics of litter such as caking.

RESULTS AND DISCUSSION

To date, five broiler houses have been visited, four of which have been surveyed more than once.

Objective 1. Results so far confirm our earlier findings that high Aw levels, especially when accompanied by relatively higher MC, are associated with a higher prevalence of *Salmonella*.

Objective 2. Generally, drag swabs were more frequently positive than individual samples. This suggests that *Salmonellla* are unevenly distributed throughout the house. Thus, lengthwise swabbing provides a better indication of the presence of *Salmonella*.

Objective 3. While drag swabs are useful for detecting *Salmonella*, individual litter samples provide a better indication of the impact of Aw and MC on contamination levels. To date, we have observed higher *Salmonella* counts at Aw values between 0.90 to 0.95 and lower *Salmonella* counts at Aw values above and below this range.

Objective 4. Data accumulated to date suggest that Aw and MC are the best indicators of *Salmonella* and *E. coli* contamination.

Objective 5. In the course of our surveys, it was noted that in some houses, certain management practices may have prompted the presence of a dangerous interrelated trio of high Aw, MC, and high levels of *Salmonella/E. coli* (e.g. leaking nipple drinkers and faucets, excessive water pressures, fans removed or completely absent from certain locations). Also, fans were sometimes improperly used at ground level. Some houses appeared to be overcrowded.

We are attempting to codify discrepancies in house management and quantify their effect on the contamination levels detected in the houses.

Based on data accumulated thus far, it appears that proper ventilation can substantially reduce actual litter/manure humidity levels and concomitantly, *Salmonella*

and *E. coli* counts. We have begun to identify houses that will be used to measure proper drinker maintenance/management and ventilation as a significant intervention against the Aw-MC-bacterial load triad. Various changes in drinkers and fans have been considered and will continue to be evaluated within the next six months.

(A complete report on this study will be published in a refereed journal.).

INCREASED MORTALITY IN LAYER FLOCKS DUE TO EGG PERITONITIS

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Egg peritonitis was responsible for increased mortality in three flocks of caged layers on three separate, single-age farms. The flocks were sourced from three different hatcheries. In flocks 1 (30,000 birds) and 2 (24,000 birds), mortality started to increase on day 4 and day 6 respectively after placement in the laying barn. Both flocks were moved from the pullet barn to the laying barn at 19 weeks of age. Mortality in flock 1 was restricted to one end of the house where the first load of pullets was placed. In flock 3 (33000 birds), mortality increased suddenly at 26 weeks of age, six weeks after housing. In all three flocks, high mortality continued for 9-11 days before returning to an acceptable level. The percentage mortality within the high-mortality period was 0.79 in flock 1, 1.066 in flock 2, and 1.63 in flock 3. Postmortem examination of dead birds from the three flocks revealed various degrees of egg peritonitis. The abdominal cavity contained yellowish exudate, which in some cases had offensive odour. Most of the examined birds had a regressed ovary. In some birds, the oviducts were congested and contained fibrinous exudate. Fibrinous pericarditis was present in many birds; a few birds had perihepatitis. The peritoneal exudate in birds from all flocks, and affected oviducts in birds from flock 1 were cultured for bacteria. Hearts and livers with pericarditis and perihepatitis were also cultured. In flocks 1 and 2, lungs, tracheas and/or cecal tonsils were collected for virus isolation. Blood samples were collected from 10 birds in each of flocks 1 and 3. Sera were tested for antibody titers to infectious bronchitis (IB) and Newcastle disease (ND) by ELISA, and for antibodies to *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) by the serum plate agglutination test. All laboratory tests were conducted at the Animal Health Laboratory, University of Guelph. Flocks 1 and 3 were treated with neomycin in drinking water; flock 2 was not treated.

In all flocks *Eschericia coli* was isolated from the peritoneal exudate. Also, numerous colonies of *Pasteurella haemolytica* were isolated from the peritoneal exudate and oviducts of some birds from flock 1. No virus was isolated from lungs, trachea, or cecal tonsils. All sera were negative for antibodies to MG and MS. Sera from flocks 1 and 2 had elevated titers to IB virus or ND virus, respectively, suggesting field challenge with these two viruses. The possible pathogenesis of this egg peritonitis will be discussed.

UNUSUAL BACTERIA APPARENTLY ASSOCIATED WITH RESPIRATORY DISEASE IN OSTRICHES AND A TURKEY

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We have encountered unusual, non-fermentative bacteria from submissions associated with respiratory disease in ostriches and a turkey.

A total of four isolates were obtained from four different ostriches sourced from four different properties. The isolates were from eye abscess (two isolates), throat abscess and air sac. In all four cases, no other bacterial pathogen was isolated and there was histopathological evidence of an infective process occurring at the site of isolation. By conventional phenotypic methods, the isolates were all gram-

negative rods that were non-fermentative, non-motile and which were oxidase and catalase positive. The isolates were haemolytic on sheep blood agar. As the identification of non-fermentative bacteria is a difficult task, we used 16S rRNA sequencing to assist in the identification of these ostrich isolates. Near complete 16S rRNA sequences were obtained from two of the ostrich organisms. Comparison with the known sequences deposited in the recognised international databanks indicated that the nearest relatives of the ostrich organisms were all members of the *Haemophilus-Actinobacillus-Pasteurella* complex. However, the isolates were deep branching and only distantly related to the *Haemophilus-Actinobacillus-Pasteurella* group.

The turkey isolate was obtained as part of a mixed flora from the lung of a turkey suffering from a complicated respiratory disease complex. The isolate was a gram-negative rod that was non-fermentative, non-motile and which was oxidase and catalase positive. The turkey isolate was similar to the ostrich isolates in that the isolate was haemolytic on sheep blood agar but differed from the ostrich isolates in that it was urease positive. As with the ostrich isolates, we used 16S rRNA sequencing to assist in the identification of this isolate. A nearly complete 16S rRNA sequence was obtained from the turkey isolate. Comparison with the known sequences deposited in the recognised international databanks indicated that the nearest relative of the turkey isolate was *Taylorella equigenitalis*.

This study illustrates the power of the new molecular approaches to assist in the identification of new suspect pathogens. The conventional phenotypic identification yielded little useful information and indicated that possibly the ostrich and turkey isolates were similar. In contrast, the molecular approach yielded more substantial information. We were able to establish that the ostrich isolates were quite distinct from the turkey isolate. Further, we have been able to establish the nearest known relatives of the isolates. This allows us to identify additional characterization tests that may provide useful information. As well, we have been able to confirm that the isolates represent apparently new species and possibly even new genera. Further detailed characterization, such as DNA-DNA hybridization and more extensive phenotypic characterization (the latter guided by our knowledge of the nearest relatives), is required before these isolates can be definitely assigned to a species and genus.

SEROLOGICAL RESPONSE TO LABORATORY AND FIELD VACCINATION OF CHICKENS WITH TWO LIVE MYCOPLASMA VACCINES, TS-11 (MYCOPLASMA GALLISEPTICUM) AND MS-H (MYCOPLASMA SYNOVIAE)

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INTRODUCTION

Serological monitoring of mycoplasma infections in poultry flocks can be undertaken using rapid slide agglutination (RSA), hemagglutination inhibition (HI) or more recently enzyme linked immunosorbent assay (ELISA). The RSA test with stained antigen (Intervet, Boxmeer, Holland) has proved a reliable and simple test that can be undertaken in the laboratory and field, but cross reactions between *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS) can be a problem. The HI test is more specific but less sensitive than RSA. ELISAs, while compatible with laboratory automation have proved to be variable in their sensitivity and specificity.

Unlike the serological findings of field challenged flocks or bacterin and live F strain vaccinated flocks, the serological response to the attenuated mycoplasma vaccine strains ts-11, 6/85 and MS-H has been more difficult to interpret (5,10,11). Whithear (9) has reviewed the development of these vaccines. Veterinarians ideally need to be able to use the serological response to vaccination as a marker of its efficacious application. Consistent and typical serological responses may indicate protection from field challenge and even possibly allow differentiation between vaccine and field challenge. This paper will present both laboratory and field serological data that has been obtained during vaccine development and field application of the two live mycoplasma vaccines ts-11 and MS-H. The correlation between serum antibody level and protection was also investigated.

RESULTS

Live attenuated MG vaccine ts-11. In one laboratory study commercial vaccine (Vaxsafe MG, Bioproperties) was used in specific pathogen free (SPF) chickens over four separate experiments during an 18-month period (10). The aim of this study was to correlate level of serum antibody with protection against challenge. In some of these experiments up to 50% of birds gave a 0 RSA score but were still protected from challenge. An ELISA developed in this laboratory, using an affinity purified major surface protein

known as pMGA (6), was more sensitive than the RSA test but again there was no correlation of serum antibody level with protection.

All flocks included in a field survey were derived from commercial layer pullets or broiler breeder flocks that had been tested to be negative by RSA to MG immediately prior to vaccination. The ts-11 vaccine (dose approximately 10^7 colour changing units [CCU]) was administered by eye drop at 3 to 16 weeks of age. The first post-vaccination serological test was 3 to 4 weeks after administration. Approximately 10 to 20 bloods were tested from each "flock" and although this is not statistically representative it is typical of what is undertaken in the field. While results were variable, usually 20 to 40% of birds were positive by RSA and these were scores of trace or 1 with an occasional 2 on a RSA scale of 1 to 4. The ELISA proved to be more sensitive but titer levels were variable and not necessarily correlated with the RSA results. Some flocks that were entirely negative on RSA had a high number of ELISA positives. A small number of flocks had RSA scores of 1 to 4. These flocks were free of any evidence of clinical signs referable to MG and there was no evidence of vertical transmission to progeny. Only the ts-11 vaccine strain was recovered from tracheal cultures.

Repeat testing of flocks for up to 60 weeks post-vaccination demonstrated a reduction in the level of serum antibody and the number of positives with both the RSA and ELISA. While it was common to find flocks with no reactors some flocks persisted with strong positive reactors despite no evidence of any MG related disease. Once again only ts-11 could be recovered from these birds. There were also periods when a series of flocks from different commercial organizations would have negative RSA scores. Subsequent testing by ELISA revealed positive results. This would indicate that the ELISA is more sensitive than the RSA at detecting ts-11 vaccinated birds but may also be due to variability in RSA antigen batches.

Live attenuated MS vaccine MS-H. Serological responses elicited to the temperature sensitive clone MS-H during the development of this vaccine (7) were 9 out of 10 at 3 weeks and 10 out of 10 at 5 weeks post-vaccination. RSA scores ranged from 1 to 4 with a mean of 2.5 at 3 weeks and 3 at 5 weeks post vaccination. A positive correlation was demonstrated between vaccine dose and RSA score and this was in turn positively correlated with the level of protection against experimental challenge (4). This finding is not consistent with the studies with MG where serum antibody levels were not correlated with protection (10). For MS-H at least 2.5 X 10^6 CCU are required to elicit a protective immunity under experimental conditions.

Field evaluation of MS-H (5) vaccination in commercial broiler breeders revealed that antibodies in the RSA test were not detected until at least 6 weeks post-vaccination, but by 12 weeks post vaccination 100% of birds were positive to the RSA test with scores of 1 to 4. Serial monitoring of these flocks detected serum antibodies in 100% of samples with an RSA score of 1 to 3 for up to 55 weeks post-vaccination. Extensive serological data obtained from diagnostic samples as part of routine flock monitoring have consistently confirmed similar findings with seroconversion being delayed until 6 weeks post-vaccination after which RSA scores are high for all birds and remain this way for the life of the flock. Where serum antibodies have not been detected by 12 weeks post-vaccination tracheal cultures have been negative for MS-H and there has been a concern with vaccine handling or the dose of vaccine administered.

In a recent study (8) an ELISA was developed using a major membrane component of MS called MSPB, as the diagnostic antigen to attempt to improve the specificity for MS. After inoculation of SPF chickens with a field strain of MS designated 7NS, MSPB antibodies were detected within 7 days and sharply rose at 10 to 17 days post-inoculation. Using the same ELISA with commercial flocks vaccinated with MS-H, MSPB antibodies were not detected until 22 days post-vaccination and complete seroconversion was not seen until 75 days post-vaccination. In another study using frozen stored serum samples from vaccinated flocks the ELISA serum antibody levels were compared to those obtained with the RSA test. Generally there was a good correlation between the two tests with these field samples.

DISCUSSION

The mechanisms of immunity to mycoplasma infections have not been fully characterised (9). While studies of bursectomised chickens demonstrate the importance of immunoglobulin for resistance to infection with MG and MS (2,3) there is lack of correlation between levels of specific circulating antibody and protection for MG (9). Dosage is important for the attenuated vaccine strains ts-11, 6/85 and MS-H. Application of these vaccines at below the recommended doses either as a consequence of deliberate dose reduction or inappropriate vaccine handling, or administration will increase the susceptibility of the vaccinates to challenge with MG and MS (4,9).

Using the RSA test or ELISA for monitoring the effectiveness of vaccination with ts-11 is unreliable. As an indicator, field veterinarians could expect to see at least 20% of sera with a low RSA score 4 weeks after vaccination in the majority of flocks. A proportionally higher number would be positive by ELISA. Flocks negative by RSA should be tested by ELISA where in most situations a number of positives will be detected. Flocks with high RSA scores or ELISA readings should be examined for any clinical signs and cultured for field strains of MG. The laboratory would need to be able to differentiate ts-11 using standard restriction endonuclease or random amplified polymorphic DNA testing (1).

For MS-H, interpretations of RSA test results appear to be more straightforward. Approximately 6 weeks post-vaccination seroconversion is usually evident in some birds and by 12 weeks all birds will have moderate to high RSA scores. These scores will persist for the life of the flock. Negative results are usually indicative of at least partial

vaccination failure.

REFERENCES

1. Fan, H. H., S. H. Kleven, and M. W. Jackwood. Application of polymerase chain reaction with arbitrary primers to strain identification of Mycoplasma gallisepticum. Avian Dis. 39:729-735. 1995.

2. Kume, K., Y. Kawakubo., C. Morita., E. Hayatsu, and M. Yoshioka. Experimentally induced synovitis of chickens with Mycoplasma synoviae: effects of bursectomy and thymectomy on course of the infection for the first four weeks. Am. J. Vet. Res. 38:1595-1600. 1977.

3. Lam, K. M., and W. Lin. Resistance of chickens immunised against Mycoplasma gallisepticum is mediated by bursal dependent lymphoid cells. Vet. Microbiol. 9:509-514. 1984.

4. Markham, J. F., C. J. Morrow, and K. G. Whithear. Efficacy of a temperature-sensitive Mycoplasma synoviae live vaccine. Avian Dis. 42:671-676. 1998.

5. Markham, J. F., P. C. Scott, and K. G. Whithear. Field evaluation of the safety and efficacy of a temperature-sensitive Mycoplasma synoviae live vaccine. Avian Dis. 42:682-689. 1998.

6. Markham, P. F., M. D. Glew., M. R. Brandon., I. D. Walker, and K. G. Whithear. Characterization of a major hemagglutinin protein from Mycoplasma gallisepticum. Infect. Immun. 60:3885-3891. 1992.

7. Morrow, C. J., J. F. Markham, and K. G. Whithear. Production of temperature-sensitive clones of Mycoplasma synoviae for evaluation as live vaccines. Avian Dis. 42:667-670. 1998.

8. Noormohammadi, A. H., K. G. Whithear, and G. F. Browning. Seroconversion of farm chickens after vaccination with Mycoplasma synoviae MS-H vaccine. In: Proc. 4th Asia Pacific Poultry Health Conference, Melbourne, Australia. p.142. 1998.

9. Whithear, K. G. Control of avian mycoplasmoses by vaccination. Rev. Sci. Tech. Off. Int. Epiz. 15:1527-1553. 1996.

10. Whithear, K. G., K. E. Harrigan, M. Benham, and J. Markham. Serum antibodies in chickens following vaccination with Mycoplasma gallisepticum ts-11 and their relationship to protection. In: Proc. XI International Congress of the World Veterinary Poultry Association, Budapest, Hungary. p. 89. 1997.

11. Whithear, K. G., Soeripto, K. E. Harrigan, and E. Ghiocas. Immunogenicity of a temperature sensitive mutant Mycoplasma gallisepticum vaccine. Aust. Vet. J. 67:168-174. 1990.

IMMUNO-HISTOCHEMICAL AND BACTERIOLOGICAL INVESTIGATION OF THE PATHOGENESIS OF ORNITHOBACTERIUM RHINOTRACHEALE INFECTION IN CHICKENS WITH OSTEITIS AND ENCEPHALITIS SYNDROME

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Since 1991 *Ornithobacterium rhinotracheale* has been associated with respiratory disease in broiler chickens and turkeys. The clinical signs of this infection include weakness, growth retardation and increased mortality. The pathology consists of airsacculitis, fibrino-purulent pneumonia, tracheitis and occasionally encephalitis and arthritis. This disease has been reported all over the world and in several species of birds. After birds were primed with respiratory viruses, airsacculitis and pneumonia could be reproduced experimentally by aerosol challenge with *O. rhinotracheale*.

In the last few years, *O. rhinotracheale* has also been associated with high mortality in 28-day-old and older broiler chickens. In these cases a subcutaneous edema around the skull with a severe bacterial osteitis and osteomyelitis, particularly of the aerated skull bones, together with encephalitis was found but without important involvement of the respiratory tract. Bacterial, histological and immuno-histochemical examination was performed and *O. rhinotracheale* was isolated from affected materials and demonstrated in fixed samples of affected tissues. Recently several outbreaks of lameness problems in turkeys have been associated with *O. rhinotracheale* in different areas of the world.

An intravenous challenge model was developed for experimental reproduction of the disease in chickens and to study the pathogenesis. In this model increased mortality and clinical disease with osteitis and arthritis was reproduced. *O. rhinotracheale* could be reisolated from affected organs and could be designated as the cause by immuno-histochemistry. These results indicate that under certain conditions *O. rhinotracheale* can cause mortality with severe osteitis and secondary encephalitis or arthritis in broiler chickens or lameness in turkeys. This can occur with or without respiratory infection.

BORDETELLA AVIUM AND ORNITHOBACTERIUM RHINOTRACHEALE FROM CALIFORNIA POULTRY SUBMISSIONS

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Bordetella avium and *Ornitobacterium rhinotracheale* are bacterial species frequently isolated from the upper respiratory trat of poultry. *B. avium* is a recognized etiologic agent of an acute upper respiratory disease in turkeys (bordetellosis or turkey coryza) which has a high morbidity but a low mortality (3). A significant aspect of bordetellosis is that it predisposes the host to secondary infection by other viral or bacterial agents. The role of *B. avium* in chickens is less well defined but it is generally viewed as an opportunistic pathogen (2). *O rhinotracheale* has been associated with respiratory disease in chickens and turkeys (1). Although infection of either chickens or turkeys can occur at an early age, disease appears more significant in older (breeder) birds. A retrospective analysis was done on poultry submissions to the California Veterinary Diagnostic Laboratory System (CVDLS) from 1955 to 1998. Submissions in which *B. avium* or *O. rhinotracheale* were isolated were examined with respect to isolate source, monthly distribution of submissions, and age of submitted birds.

A total of 656 isolations of *B. avium* were made during 1995 to 1997 while 589 isolations of *O. rhinotracheale* were made during this same time. Respiratory tissue was the predominate source of both *B. avium* and *O. rhinotracheale* isolations. *O. rhinotracheale* was also isolated from more non-respiratory tissue than was *B. avium*. Non-respiratory tissues included liver, heart sac, abdomen, joint and oviduct. The trachea was the most frequent site of isolation for both *B. avium* and *O. rhinotracheale*, 85% and 52%, respectively. *O. rhinotracheale* was isolated from the lung, air sac and sinus (18%, 18%, and 7%, respectively) more frequently than *B. avium* (7%, 4% and 4%, respectively). The age distribution of the chicken and turkey cases for *B. avium* and *O. rhinotracheale* were similar and most likely represents the distribution of cases submitted to CVDLS. For turkeys, *B. avium* infection occurred at an earlier age (about 2 weeks of age), had a bimodal frequency at 6 and 8 weeks of age and rapidly declined at 11 weeks of age. *O. rhinotracheale* infection generally occurred at 5 weeks of age, had a bimodal frequency at 8 and 10 weeks of age and then gradually declined at 17 weeks of age. For chickens, *B. avium* infection had a bimodal frequency at 4 and 7 weeks of age while *O. rhinotracheale* infection was seen primarily at 7 weeks of age. The age distribution for chickens primarily represents broiler birds. The monthly distribution of turkey cases was similar for both *B. avium* and *O. rhinotracheale*. Infection with either organism occurred throughout the year but increased in May and remained at an elevated level until November. The monthly distribution of chicken cases did not show any significant seasonality.

REFERENCES

1. Chin, R., and R. Droual. Ornithobacterium rhinotracheale. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa pp. 1012-1015. 1997.

2. Jackwood, M. W., S. M. McCarter, and T. P. Brown. Bordetella avium: an opportunistic pathogen in Leghorn chickens. Avian Dis. 39:360-367. 1995.

3. Skeeles, J., and L. Arp. Bordetellosis (Turkey Coryza). In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 275-287. 1997.

DEVELOPMENT OF AN EXPERIMENTAL MODEL OF BACTERIAL CHONDRONECROSIS WITH OSTEOMYELITIS IN BROILER CHICKENS USING STAPHYLOCOCCUS AUREUS ADMINISTERED BY AEROSOL

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In the present study, experiments were designed in an attempt to reproduce bacterial chondronecrosis with osteomyelitis in broiler chickens using the aerosol route of infection. Broiler chicks were exposed to a suspension of *Staphylococcus aureus* by aerosol, or exposed to *S. aureus* and subsequently inoculated with chicken anaemia virus (CAV) alone, or with CAV and infectious bursal disease virus (IBDV). *S. aureus* was recovered and bacterial chondronecrosis with osteomyelitis was diagnosed, by histology, in the proximal end of the femur and/or tibiotarsus of lame birds exposed to *S. aureus* with and without CAV and IBDV infections. Birds fed 60% of the recommended feed intake for the breed developed a lower incidence of *S. aureus* infection and/or bacterial chondronecrosis ($P<0.05$) than birds fed 100% of the recommended intake. A significantly lower incidence of *S. aureus* was recovered ($P<0.05$) in birds simultaneously exposed to *S. aureus* and inoculated with CAV and IBDV at 21 days of age, than in birds exposed to *S. aureus* at 10 days and inoculated with CAV and IBDV at 21 days of age. The incidence of bacterial chondronecrosis in birds, given no CAV or IBDV, was greater following exposure to *S. aureus* at 1-day-old than at 10 days of age. The incidence of lesions was similar in birds exposed to *S. aureus* at one or at 10 days of age when birds were also inoculated with CAV and IBDV at 21 days of age. However, the majority of lesions occurring in birds inoculated with CAV and IBDV at 21 days of age, were diagnosed approximately 10 days earlier in birds exposed to *S. aureus* at 1-day-old compared to birds exposed at 10 days of age. The findings suggest that broilers may be at greater risk of developing bacterial chondronecrosis with osteomyelitis at an earlier age, if colonized by pathogenic *S. aureus* at 1-day-old compared to those colonized at a later stage of the production cycle. With the exception of birds exposed to *S. aureus* at 1-day-old, a higher incidence of bacterial chondronecrosis was diagnosed in birds exposed to *S. aureus* and inoculated with CAV and IBDV than in birds exposed to *S. aureus* alone. The findings of the present study suggest that inoculation with CAV and IBDV at day 21 enhanced the development of bacterial chondronecrosis in birds, exposed to *S. aureus* at day 10 and fed 100% of the recommended feed intake or *ad libitum*.

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PERSISTENCE OF HAEMOPHILUS PARAGALLINARUM: FIELD OBSERVATION AND LABORATORY FINDINGS

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A field outbreak of infectious coryza suggested that *Haemophilus paragallinarum* may persist in chickens after clinical signs of infectious coryza have subsided. Three groups of 20 adult hens each were infected with serotype A, B or C strain of *H. paragallinarum* via the infraorbital sinus. They were bled and isolation attempts were made at necropsy at 28 days post-infection. While *H. paragallinarum* was isolated from 50% of the birds inoculated with type C strain,

0% and 20% of the birds infected with type A and B strain, respectively, yielded the organism. The results indicate that *H. paragallinarum* may persist as long as 4 weeks in infected chickens without signs of coryza.

(A full paper will be published in *Avian Diseases*.)

EGG COOLING - PITFALLS AND PROBLEMS IN BACTERIOLOGICAL EVALUATION

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Experimental contamination of the surface of shell eggs by dipping in a culture of *Salmonella enteritidis* (SE) resulted in the presence of SE in/on the shells as well as the shell membranes but not in the egg content. Disinfection with Lugol's solution, chlorhexidine, ethanol, quarternary ammonium solutions or flaming after dipping in ethanol failed to achieve complete decontamination of the shell and membranes with resulting false positives when eggs were broken for culturing of the content. Dipping eggs for three seconds in boiling water resulted in complete destruction of SE in shells and membranes but sometimes caused the eggs to crack. A method of aseptically opening eggs without risk of contaminating the content from the shell or membrane was developed. SE deposited in/on the shell and membranes did not multiply during storage of the eggs at 20°C for four weeks and counts actually decreased. No SE was detected in the contents of any contaminated eggs.

(A full length article will be published in the *International Journal of Food Microbiology*.)

A REVIEW OF EGGSHELL ABNORMALITIES IN TODAY'S LEGHORN INDUSTRY

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Production of "grade A" eggs is a major goal for any egg producer. Genetic selection of laying hens for a high rate of egg production, together with improvements in nutrition, environment and management, have resulted in a tremendous increase in the productivity of layer flocks but inferior eggshell quality continues to be a major problem for egg producers throughout the world. Eggs of poor shell quality are downgraded or lost and may cause significant economic loss. The value of this loss is estimated at about $US300-500 million in the United State and $Cdn32 million in Canada. Although some eggshell defects are unavoidable in any commercial layer operation, there are acceptable numbers which if exceeded warrant an investigation to determine the cause of the problem. It is extremely important for an egg producer to keep records of downgraded and rejected eggs.

Eggshell abnormalities/defects seen in commercial cage operations include: (1) misshapen eggs; (2) thin-shelled (weak shell), soft-shelled, and shell-less eggs; (3) body-checked (ridged) eggs; (4) rough textured shells (extra calcium deposition); (5) flat-sided eggs; (6) cracked and broken eggs; (7) dirty and yolk- and blood-stained eggs. With the exception of cracked and dirty and stained eggs, all other eggshell defects arise in the oviduct before the egg is laid. Cracked and broken shells are the most common problem that cause significant economic losses. In a flock of 50,000 hens with an average hen housed flock production of 80%, each 1% incidence of broken eggs will cause a financial loss of $14,600 a year, assuming a price of $1.20 per dozen eggs. Iindividual farms vary widely in the incidence of cracked eggs. In Canada, generally 5-7% of eggs processed at egg grading station (packing plant) are classified as cracked. Egg cracking and breakage can be attributed to on-farm or off-farm causes.

Poor shell quality and eggshell abnormalities can be caused by many factors and determining the cause of the problem is not always an easy task. A thorough investigation may be required. A knowledge of the physiology of eggshell formation and eggshell structure is needed to understand the development of eggshell abnormalities. An egg traverses the oviduct in about 23 to 25 hours. The shell formation occurs in the shell gland in which the egg remains for about 20 hours. The most intensive shell formation occurs during the dark period prior to laying. The eggshell is made up of organic matrix and calcium carbonate and trace minerals. The shell of an egg contains about 2.2 grams calcium present

in almost pure calcium carbonate. Shell quality is under the influence of many factors which include age of the flock, time of oviposition, nutrition and water quality, environmental temperature, lighting program, stress and diseases, cage design and cage density, and egg collection equipment and egg handling system.

PATHOGENESIS OF ARTHROPATHIC AND AMYLOIDOGENIC ENTEROCOCCUS FAECALIS INFECTIONS IN BROWN LAYERS

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To study the pathogenesis of arthropathic and amyloidogenic *Enterococcus faecalis* infections in brown layers, *E. faecalis* was applied via different routes to six-week and day-old brown replacement pullets and embryonated eggs. Intravenous, intra-articular and intraperitoneal inoculation of six-week-old brown layer pullets with *E. faecalis* resulted in amyloid arthropathy, while intramuscular, oral and intratracheal inoculation did not. Oral inoculation of day-old chickens did not cause any pathology either. However, intramuscular inoculation of day-old chickens with 10^6 cfu resulted in severe growth retardation and arthritis in half of the birds, and joint amyloidosis in approximately 40%. In egg transmission studies neither egg dipping in *E. faecalis* broth, nor inoculation of the air chamber with *E. faecalis* reproduced the condition, although a few chicks became septicaemic. Yolk sac inoculation of six-day-old embryos caused embryonic death within 2 days. In contrast, egg albumen inoculation with the mentioned isolate led to arthritis in 1/6 of the progeny, indicating the possibility of vertical transmission by the oviductal route. Anti-*E. faecalis* antibodies were confirmed by ELISA in 14/15 of experimental birds with induced arthritis. Samples of hatchery air (hatcher and processing room), Marek's disease vaccine suspensions and injection needles collected during chick processing, revealed variable levels of *E. faecalis* contamination. This observation suggests a possible infection route in day-old chickens through intramuscular vaccination of Marek's disease vaccine if arthropathic and amyloidogenic *E. faecalis* were present.

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FOWL CHOLERA IN PEN-RAISED RING-NECK PHEASANTS

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Fowl cholera (avian pasteurellosis) is caused by *Pasteurella multocida* and has been reported in numerous species of birds. Most reported outbreaks are in turkeys, chickens, ducks and geese. The disease is highly contagious and can cause high morbidity and mortality. Clinical signs in acute cases may not be observed, but can include dyspnea, ruffled feathers, and diarrhea. In chronic cases, there can be swelling of the wattles, sinuses, joints, foot pads and sternal bursae. This swelling is due to exudate that can be mucoid to caseous. This report describes a case of fowl cholera in pen-raised pheasants.

Seven 8-week-old pheasants were submitted to the California Veterinary Diagnostic Laboratory System (CVDLS), Fresno branch laboratory , with clinical signs of severe swelling of the infraorbital sinuses, but low mortality (two birds). These birds were grown in pens vegetated with lambs quarter and eventually were released onto public lands for hunting. The flock consisted of approximately 700 birds. The birds were brooded at a different ranch, approximately 150 miles away, and shipped to this ranch one week prior to submission to the lab. Birds first developed swollen sinuses 3 days after arrival on the grow-out ranch. All seven birds

had severe sinusitis, either unilateral or bilateral, with mucoid to caseous exudate. There was mild, excess tracheal mucus in two birds and a small number of coccidial oocysts were seen in a direct smear of the intestinal mucosa. *P. multocida*, serotype 6, was isolated from the sinuses and trachea in large numbers. In addition, *Escherichia coli*, *Pasteurella haemolytica*-like bacteria and *Mycoplasma* sp. (not *M. galliseptcium*, *M. synoviae*, *M. meleagridis* nor *M. iowae*) were isolated from the sinuses. The sera contained antibodies against paramyxovirus 1 and adenovirus type 2, however, no viruses were isolated from pooled tissues. Seven weeks later, three 15-week-old pheasants were submitted to the CVDLS, Fresno branch laboratory, again, with similar lesions and necropsy findings. Again, *P. multocida* and *Mycoplasma* sp. were isolated from the sinuses. The sera from these birds did not contain antibodies to adenovirus type 2, and contained very low titers (1:4 and 1:8) to paramyxovirus 1. The owner reported that the flock was treated with terramycin, lincomycin-spectinomycin and erythromcyin, but without much improvement. He reported that only about 20 birds were found dead over the past seven weeks. In addition, the birds did not appear to be putting on the proper weight nor feathers.

A field investigation of the ranch revealed very few birds with swollen sinuses. In discussing treatment, it was found that the owner improperly medicated by sprinkling the medicine on top of the water in the water troughs once-a-day. After designing a system to properly medicate the birds, the birds were treated with tetracyclines over a period of 3 weeks. The owner reported that there was a noticeable improvement in the birds which responded by putting on weight and proper feathering. The birds were released onto public land for hunting over a period of four weeks starting at 24 weeks of age. Birds with sinusitis and any that appeared sick were destroyed. The source of the *P. multocida* infection was not determined. However, because the birds were reported to have severe swollen sinuses three days after arriving on the grow-out ranch, it is probable that the birds were infected at the brood ranch. Vaccination history and treatments, if any, at the brood ranch, were not available. Another possible source was a nearby lake, approximately 2 miles away, from which numerous coots died due to fowl cholera earlier in the year. However, this source seems unlikely in that the *P. multocida* isolated from the coots was a serotype 1, as is commonly found in waterfowl, and not the one found in these pheasants.

The role of the *Mycoplasma* spp. in this disease outbreak also needs to be considered. Though *Mycoplasma* spp. have been isolated from about 90% of tracheas of clinically normal pheasants (A. DaMassa, personal communication), *M. imitans* has been reported to cause sinusitis in ducks, geese and partridges in Europe. Additional work is needed to determine if the mycoplasma isolated from these pheasants is *M. imitans* and whether or not it is the primary cause of the sinusitis.

The release of pen-raised pheasants is a common practice for many hunting clubs. This can be a major concern to the commercial poultry industry if diseased birds are being released into the wild. Thus, strict biosecurity practices need to be emphasized on all poultry ranches.

THE POULTRY SCIENCE ASSOCIATION'S ELECTRONIC JOURNAL AND OTHER OUTREACH PROGRAMS

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Professional societies, such as the American Association of Avian Pathologists (AAAP) and conferences, such as the Western Poultry Disease Conference (WPDC), have distinguished themselves through the years by providing service to their members. They have provided information on new technology, a better understanding of members' areas of interest, a focus on common problems, and a means for affecting change and progress within the industries they serve. Those of us working with the poultry industry must dedicate ourselves to keeping abreast of the latest technology, maintaining a background of science-based information, and be willing to change with the times, when justified. This paper provides information about the Poultry Science Association which often has interests in common with the AAAP and WPDC.

OBJECTIVES OF THE POULTRY SCIENCE ASSOCIATION

The Poultry Science Association (PSA) was founded in 1908 with an initial membership of 66 poultry scientists. It was founded for professional scientists with an interest in poultry science and an active interest in research, teaching, extension and the poultry industry. In its constitution, the objective of the association was stated: "for the advancement of knowledge and technology in poultry science, especially as it relates to resident and extension teaching, research and technical service". Subsequent definitions of the association's objectives added:

To stimulate the discovery, application, and dissemination of knowledge.

To create a forum for the exchange of information among various segments of the poultry industry.
To publish original research, reviews, and timely information in the PSA journal.
To recognize outstanding personal achievements.

MEMBERSHIP

During the 90 years since it was formed, the association grew steadily until it reached its present level of about 3000 members. This includes 1860 members from the United States, 172 members from Canada, and 1017 members from other foreign countries. Typical membership consists of about 1500 individual professional members, 1000 institutional members (governmental agencies, universities, and libraries) and about 500 students, retirees and other classifications. Requirements for membership include: having an interest in poultry, a letter of recommendation from an existing member, payment of annual dues (1998: $55.00), and a willingness to participate in association activities including service on one of the association's many committees. Institutions pay an annual subscription fee of $125.00.

BENEFITS OF MEMBERSHIP

Scientific journals. A primary benefit of being a member of the PSA is publishing in and having access to the association's journal - Poultry Science. The journal is published monthly and in 1998 consisted of some 291 full-length scientific papers in almost 2000 pages of text. In addition, a separate publication includes about 600 short abstracts of papers presented at the association's annual meeting and the annual meeting of the Southern Poultry Science Society (SPSS). Currently the journal is published in both hard copy and electronic formats.

The PSA journal is divided into eight subject matter sections. These include: breeding and genetics (23 papers, 1998), education and production (19), environment and health (43), immunology (10), metabolism and nutrition (54), molecular biology (4), physiology and reproduction (34), and processing and products (31).

Annual meetings. A second major benefit of membership is to attend and participate in the PSA annual meeting. Each year PSA holds their summer meetings in different regions of the country. Last year the meeting was held at Pennsylvania State University, this year the meeting will be held at the University of Arkansas, and next year a joint meeting will be held with the World's Poultry Science Association in Montreal, Canada.

The 1999 annual meeting will feature 5 symposia, 250 presented papers, 150 poster presentations, 25 exhibits, a barbecue and a gala awards banquet. At the awards banquet, a dozen or more of our members will receive almost $25,000 in awards for outstanding programs in areas of egg science, nutrition, broiler research, poultry products, leadership, turkey research, extension, teaching, and student manuscripts.

Other benefits. Members receive quarterly newsletters, access to a job opportunities program, an annual "resources" directory listing all poultry and allied faculty of each of the university and government departments in the U.S. and Canada, and a tri-annual professional directory for the American Dairy Science Association, the American Society of Animal Science, and the Poultry Science Association with some 10,000 names, addresses, phone/FAX/e-mail numbers. In addition, the association maintains representation with numerous other societies including: CAST, AIBS, FASS, WPSA, AAAS, ARPAS, and others. Through the Federation of Animal Science Societies (FASS), we maintain a representative in Washington to keep us abreast of legislative issues affecting our association and our industries.

ELECTRONIC PUBLISHING

An electronic publishing committee was appointed several years ago to review our existing methods of publishing our journal and handling manuscripts. Much progress has been made and much more is needed. Some 300 manuscripts are submitted annually by diskette and editor/author communications are still largely carried out by sending hard copies of the manuscript back and forth in the mail. Abstracts for the annual meeting are usually submitted through the PSA web-site. This represents approximately 600 submissions annually.

Today, the monthly journal, Poultry Science, is now available either electronically or by the traditional mailed hard copy system. The electronic version is accessed using a member-only password to the PSA web-site. This version has many features which has made it a more desirable option for many of our members:

It can be accessed within a day of its completion. The electronic version is available about 2 weeks earlier to U.S. and Canadian members.
It can be 4 to 6 weeks earlier for overseas members using normal mail.
It can save a $200 charge for overseas airmail deliveries.
It is completely searchable by title, author, key-words, or by sub-sets of phrases.

The future. A new dues structure will probably be created to allow a lower fee if the electronic version is selected instead of the hard copy version. In reality, this will result in a higher dues structure than at present if one wishes to continue having both options. A current consideration is to place at least 5-10 years of back issues of Poultry Science on CD-ROM disks. This technology will allow the combination of 3 years of journals (6000 pages) on one disk at a relatively small cost. In addition, the data will be fully searchable.

PROTOZOAL SINUSITIS IN VARIOUS AVIAN SPECIES

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Avian sinus infections are a significant health consideration. The etiologic agents of sinusitis and their pathogenicity can vary greatly. The same can be said of various protozoal infections. Pigeons commonly demonstrate mild, innocuous *Trichomonas sp.* infections of the oral cavity. If these remain limited, they are of little significance. However, pigeons can also succumb to a a systemic *Trichomonas* sp. infection which is highly pathogenic (1). Passerine species commonly suffer from protozoal infections of the crop and esophagus. However recently, passerine infections associated with severe protozoal sinusitis which can progress to encephalitis were examined. Examination of these infections, in comparison to those of other avian species demonstrates the significance of protozoal sinusitis in various avian species.

Two instances of sinusitis were investigated. The first involved a canary breeding facility experiencing 33% (200/600 birds) mortality over a one-month period. The second involved a wild bird rehabilitation facility with a group of ten mockingbirds (*Mimus polyglottus*) all with refractive sinusitis. Clinical signs in both instances included cough, sneezing, fluffing and marked unilateral or bilateral distention of the ventral aspect of the infraorbital sinus. Neurologic dysfunction characterized by depression and an inability to stand was noted in one bird presented for examination.

Diagnostic tests included histopathology, aerobic and Mycoplasma culture of sinus exudate, *Mycoplasma gallisepticum* serum plate agglutination, serum hemagglutination inhibition for paramyxovirus 1, 2 and 3, *Chlamydia* fluorescent antibody evaluations on multiple tissues and both scanning and transmission electron microscopy on sinus exudate and cultured organisms. Histology demonstrated a severe caseonecrotic sinusitis. Inflammation often extended to surrounding tissue with local osteitis. In the bird with neurologic signs, marked invasion of the central nervous system was demonstrated with an extensive focal area of malacia. In sinuses with minimal bacterial invasion, protozoal organisms could be easily detected arranged in rows along the epithelium. Many birds demonstrated protozoal organisms at the leading edge of necrosis and within inflammatory exudate. Bacterial cultures demonstrated mixed organisms. No *Mycoplasma* was isolated from any sinuses. However, *Klebsiella pneumonia, Escherichia coli*, and coagulase positive *Staphylococcus sp.* were isolated from the exudate. Bacterial organisms could often be seen on histology. Tests for conditions such as vitamin A deficiency, *Chlamydia* and paramyxoviruses all proved negative.

Electron microscopic examination of protozoal organisms in the sinuses and of organisms grown on diamond media demonstrated organisms with 4 anterior flagella, a costa and a marginal flagellum of the undulating membrane. This marginal flagellum is the differentiating feature when comparing *Trichomonas gallinae* and *Tetratrichomas gallinarum*. Scanning electron microscopy did not clearly demonstrate extension of the marginal flagellum beyond the undulating membrane. For this reason, in both cases, the organisms are presumed to be *T. gallinae* but definitive identification is still not complete.

The significance of these infections lies not with the species effected, but rather with the potential for similar infections in domestic avian species. Trichomonads have been identified as aggressive pathogens of the oropharynx in pigeons and birds of prey - especially owls (2,3). In owls, they have been described as causing encephalitis via extension as was noted in one of the birds examined in this study. Recent publications have identified trichomonad systemic infections in pigeons (1) and related *Tetratrichomonas sp.* invasive infections in a mocking bird (4). These cases all demonstrate that *Trichomonas sp.* and *Tetratrichomonas sp.* infections can be very invasive and pathogenic. Trichomonad infections have also recently been described involving the upper respiratory tract as well as digestive tracts of mule ducks (5). The mule duck infection was associated with high mortality and gross histologic findings similar to this case in passerines.

REFERENCES

1. Charlton, B. R., A. A. Bickford, G. L. Cooper, and Hung-Woon Chia. Systemic Trichomoniasis in a squab operation. Avian Dis. 35:426-432. 1991.

2. Poleras, M. A, E. B. Wheeldon, and C. J. Sedwick. Trichomoniasis in owls : report on a number of clinical cases and a survey of the literature. In: Raptor Biomedicine , P. I. Redig University of Minnesota Press, Minneapolis, MN pp. 88-92. 1993.

3. Jessup, D. A. Trichomoniasis in great horned owls. Mod. Vet. Practice 61:601-604. 1980.

4. Patton, C. S., and S. Patton. Tetratrichomonas gallinarium encephalitis in a mocking bird (Mimus

polyglottos). J. Vet. Diag. Invest. 8:133-137. 1996.

5. Tsai, S. S., T. C. Chang, M. Kuo, and C. Itakura. Respiratory and intestinal trichomoniasis in mule ducks. Avian Pathol. 26:651-656. 1997.

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CAUSES OF MORTALITY IN MALE TURKEYS DURING THE LAST PART OF GROW-OUT

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INTRODUCTION

Increased mortality in turkey toms at the end of the grow-out is a source of important losses (up to 1% per week), and the causes of the mortality are not well known. Many people in the poultry industry believe that breed is a potential predisposing factor. Specific strains of turkey are considered to be more predisposed to leg problems. A cohort type study was conducted at North Carolina State University in collaboration with an integrated turkey company to determine the causes of death in turkey toms in the last part of the grow-out period and to compare the causes of mortality based on breeds.

MATERIALS AND METHODS

Mortality occurring during the winter of 1997 was collected according to the following protocol. All farms under contract with the participating integrator and whose flocks were divided between houses with birds from the same breed (Hybrid, Nicholas or BUTA) and reaching 16 weeks of age were identified. Four houses (each from a different flock) were monitored for each breed (total of 12 houses). For each house, all birds found dead on Tuesdays, Wednesdays and Thursdays were collected. Collection started when the birds reached 16 weeks of age and stopped when the flock was shipped for processing. Each bird was tagged, weighed, and assessed for cannibalism/persecution (data not reported) prior to necropsy. The necropsy included an evaluation of the metatarsus, tibiotarsus and coxofemoral joints. Visceral organs were also examined. Of a total of 641 birds examined, 216 were BUTA, 210 were Hybrid, and 215 were Nicholas. Categorical information (e.g., fractures versus no fractures by breed) was analyzed using the chi-square test and weight differences were assessed using ANOVA or student T- tests depending on the comparison.

RESULTS

Leg problems were the most prevalent conditions. Fractures were observed in 21.6% of the birds; 16.2% had ruptured tendon; osteomyelitis affected 15.1% of them; and tibial dyschondroplasia was noted in 23.1%. Septicemic, respiratory and cardiovascular lesions were also observed, but these were less prevalent, each one found in less than 10% of the birds (except for aspergillosis on some farms).

Fractures. Most of the fractures appeared to be spontaneous. The most frequent was a spiral fracture of the femur. The proportion of fractures did not differ depending on breed ($p = 0.87$) or farms ($p = 0.42$). Fractures were directly related to weight and age. Toms with fractures were on average 1.71kg heavier than toms without fractures ($p=0.0001$), and mortality due to fractures peaked at 18-19 weeks of age. A marked decrease in fractures was recorded for 20-week-old birds (9%). This suggests that birds with a weaker skeletal structure were likely to experience fractures before reaching 20 weeks of age.

Ruptured tendons. The tendon of the gastrocnemius muscle was, by far, the most affected. In many instances, the ruptures were bilateral. The prevalence increased with age, but was not breed or farm dependent ($p>0.05$).

Osteomyelitis. 17.4% of the birds were affected with osteomyelitis (OM). There was no breed predisposition ($p= 0.79$). However, some farms were more affected than others (range of 2.3% to 36.5%, $p>0.0001$). Birds with OM were significantly lighter. The prevalence of OM decreased with age.

Tibial dyschondroplasia. Tibial dyschondroplasia (TD) was a prevalent lesion found in 23.1% of the birds (28% of BUTA, 19.0% of Hybrid and 21.9% of Nicholas). The difference by breed was statistically marginal ($p=0.07$). A significant difference was observed at the farm level (9.6% to 33.3%, $p=0.0003$). However, the vast majority of lesions were mild (i.e., on a scale of 1 [very mild lesions] to 4 [severe], 57% scored a 1, 41% scored 2, and 2% scored 3 or 4). Severity of lesions was not breed or farm dependent.

CONCLUSION

This study could not demonstrate a breed predisposition

for leg problems among birds dying between 16 and 20 weeks of age. It is possible that a breed difference exists in younger birds. Morbidity could also vary depending on breed, but these hypotheses could not be tested with this project. Some conditions (OM, aspergillosis) seemed to be house/farm dependent. This could be attributed to differences in management and environmental conditions depending on the farm or house (e.g., litter, disease challenge, ventilation, sanitation). The high prevalence of fractures and ruptured tendons without any breed or farm predisposition suggests a common determinant factor such as feed. For example, a diet marginal in amino acids could lead to both problems. Further investigations will be conducted to determine the cause(s) of these non-infectious problems.

MODULATION OF HETEROPHIL FUNCTION BY POULTRY FEED ADDITIVES

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ABSTRACT

Poultry diets often are supplemented with various antibiotics and anticoccidial drugs as prophylactic measures against infection and to improve growth. To understand whether continuous exposure of birds to antibiotics, anti-coccidial drugs, and growth promoters would affect innate immunity, the *in vitro* effects of some commonly used supplements on heterophil functions were examined. The supplements tested were bacitracin, chlortetracycline, roxarsone, monensin, salinomycin, and tylosin. The effects of these supplements on viability, respiratory oxidative burst, interleukin-6 (IL-6), and gelatinase production were examined using heterophils that were activated with bacterial lipopolysaccharide (LPS) and killed *Staphylococcus aureus* (SA) cells. Both monensin and salinomycin reduced heterophil viability. Some drugs partially inhibited LPS/SA induced respiratory oxidative burst activities whereas others stimulated basal levels of this function. The LPS/SA-stimulated IL-6 production was similarly affected. The gelatinase production was constitutive and was only marginally increased due to LPS/SA and was minimally affected by different drugs. In conclusion, it appears that heterophil function may be affected by antibiotics and anticoccidial drugs.

MATERIALS AND METHODS

Commercial broilers were fed National Research Council recommended diets and *ad libitum* water without antibiotics or growth promoters. EDTA-anticoagulated blood was collected when the chickens were 6-8 weeks old. Heterophils were isolated using density gradient centrifugation over polymorphoprep ™ medium (6). Heterophils purified from at least 3-4 chickens were pooled and used for various experiments. Using phenol red free RPMI-1640 medium containing 2% heat-inactivated fetal bovine serum but no antibiotics, the heterophils were diluted to a concentration of 10 X 10^6 cells /ml. The medium alone without heterophils was treated identically and used as respective blanks to eliminate the effects due to drugs alone. Bacitracin, chlortetracycline, monensin, roxarsone, salinomycin, and tylosin were diluted in medium and added to heterophil cultures to achieve a final concentration of 10 µg/ml. Heterophils were pre-incubated with these drugs for one hour at 37^0 C, after which one set of culture was exposed to activating agents LPS or killed and dried *S. aureus* (SA) at a concentration of 10 µg/ml for 24 hrs. Because both activators elicited similar responses with respect to interleukin-6 production and respiratory oxidative burst, a 1:1 mixture of LPS and *S. aureus* was used in subsequent experiments. The plates were incubated for an additional 22 hrs. at 37^0 C, centrifuged at 400 X g for 15 min, and the conditioned media harvested for interleukin-6 bioassay and zymographic assay of gelatinase as described previously (3, 4). A quantitative assay of gelatinase was done at 37^0 C using an assay kit obtained from Molecular Probe (Eugene, OR). Dichlorofluorescein diacetate (DCF-DA) oxidation was used to determine respiratory oxidative burst index as described earlier (6). The viability of cells following exposure to different drugs in the presence or absence of LPS/SA was determined at 24 hrs. using a MTT reduction assay (4). The assays were done in duplicate and each set of experiments was repeated 3 times. The results were expressed as percent of control.

RESULTS AND DISCUSSION

The purity of heterophils as determined using fluorescein isothiocyanate (FITC) staining, was >98%. The quality and viability of cells were also checked after 24 hrs. of incubation using control cultures and FITC/ propidium iodide staining (6). The heterophils had bilobed nuclei and fluorescent green cytoplasmic granules. The rod type granules, characteristic of heterophils, had an intensely fluorescent mid point. LPS/SA caused cytoplasmic rarefaction and appeared to increase membrane fluidity at 24 hrs. Except for monensin and salinomycin, none of the other

drugs affected viability of cells. Monensin and salinomycin at 24 hrs. incubation decreased the viability of heterophils as indicated by a decrease in MTT reduction and increased cytolysis. The cytolytic effects of these ionophores on heterophils is perhaps similar to that responsible for anticoccidial properties. Treatment with monensin and salinomycin also resulted in lower basal and stimulated levels of IL-6 and gelatinase activities. Monensin has been reported to be cytotoxic to other cell types. Salinomycin has been shown to cause a decrease in the blood levels of heterophils (7). The respiratory oxidative burst was stimulated by LPS/SA within 15 minutes and continued to increase linearly. Monensin and salinomycin increased basal levels of respiratory oxidative burst whereas chlortetracycline and tylosin partially inhibited both basal and activated levels of respiratory oxidative burst. Likewise heterophil IL-6 production was affected differently by different drugs. LPS/SA caused several fold increase in interleukin-6 production by 24 hrs. Chlortetracycline increased the basal level of IL-6 production which was not further affected by LPS/SA. Roxarsone increased the basal respiratory burst whereas IL-6 production was not affected. There is little information on the effects of many of these drugs on avian heterophils or avian immune function. Roxarsone is a commonly used growth promoter in poultry feed but little is known of its effect on the immune systems. The data reported in the literature suggests that it may not impair antibody response to Eimeria oocyst challenge (1). The effect of these drugs on immune functions needs further investigation.

In conclusion, these results show that at least some antibiotics and feed additives depress heterophil functions although none of the drugs tested were able to cause a complete inhibition of LPS/SA-induced activation of heterophils. These results raise the possibility that continuous presence of these drugs and their metabolites *in vivo* may subdue certain immune functions. The extent and magnitude of such suppression may be transitional and would probably depend upon the type and combination of drugs used.

REFERENCES

1. Danforth, H. D., K. Watkins, A. Martin, and M. Dekich. Evaluation of the efficacy of Eimeria maxima oocyst immunization with different strains of day-old broiler and roaster chickens. Avian Dis. 41:792-801. 1997.

2. Erf, G. Immune system function and development in broilers. 46th Annual Nat. Breeders Roundtable Proc. pp. 109-123. 1997.

3. Rath, N. C., W. E. Huff, G. R. Bayyari, and J. M. Balog. Identification of transforming growth factor-β and interleukin-6 in chicken ascites fluid. Avian Dis. 39:382-389. 1995.

4. Rath, N. C., W. E. Huff, J. M. Balog, G. R. Bayyari, and R. P. Reddy. Matrix metalloproteinase activities in avian tibial dyschondroplasia. Poult. Sci. 76:501-505. 1997.

5. Rath, N. C., W. E. Huff, J. M. Balog, and G. R. Bayyari. Cell death in avian tibial dyschondroplasia. Avian Dis. 42:72-79. 1998.

6 . Rath, N. C., G. R. Huff, J. M. Balog, and W. E. Huff. Fluorescein isothiocyanate staining and characterization of avian heterophils. Vet. Immunol. Immunopathol. 64:83-95. 1998.

7. Shalaby, M. A., A. A. el-Sanousi, M. M. Yehia, A. Naser, and I. M. Reda. The effect of salinomycin on the immune response of chicks. Dtsch. Tierarztl. Wochenschr. 100:182-185. 1993.

(Complete results from this study will be published in a refereed journal.)

UPDATE ON INFLUENZA

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In the new century we can expect to have occasional infections with low path avian influenza viruses in domestic poultry as a result of spread from feral birds and from live bird markets. Many of these infections will only be recognized because of surveillance programs, not because of serious clinical disease. We will always have to be alert for the rare disastrous highly pathogenic (HP) avian influenza. Fortunately, it has not occurred in the United States since its eradication after the 1983-84 outbreak in Pennsylvania. Over the past few years, HP avian influenza has occurred in Italy (H5N2), Australia (H7N3) and Hong Kong (H5N1). The Hong Kong virus demonstrated for the first time that an avian flu virus could transmit directly to humans, in this case with high mortality in both the infected chickens and humans. It means that we can no longer claim that avian flu viruses are never a threat to human health, and it means that highly mobile people from far away could transmit this virus back into poultry populations as carriers and shedders of the virus.

In the past 15 years the poultry veterinary community has learned a lot about this virus infection in poultry. It is now clear that the influenza problem in turkeys is most often the result of infection from feral birds to range-reared birds, which is then transmitted to other turkey farms to produce an outbreak. These outbreaks occur each year and involve multiple serotypes. In chickens, the outbreaks are much less frequent, involve only a single serotype and we know that the virus source most often is the live bird market system, at least in the northeast.

In only the past year has there been any real change in the potential for reducing these outbreaks for the long term. After eradicating influenza every year, the Minnesota turkey industry made a major change in its production practices by no longer range rearing on most farms. No cases of influenza were reported in the U.S. Animal Health Association report this year. In the northeast, major changes have been made in the control of avian influenza (AI) in the New York live bird market. The State of New York has enacted regulations, which directly control the live bird market system. Two inspectors who work full time in the market enforce these regulations. The regulations require; that trucks and crates must be cleaned and disinfected, that live poultry more than seven days old must originate from an AI-monitored source flock or that the source flock is serologically negative on a test conducted within the past ten days, that AI positive farm premises or dealer facilities must be depopulated, cleaned and disinfected, that all qualified poultry be kept separate from other poultry, and that no live poultry can be moved from a New York City market to any area outside of the city. The New York regulations have stimulated other states to provide for Avian Influenza Monitored Flock Programs to qualify poultry from their states for movement into the live bird markets.

Another major effort by the USDA, APHIS, Veterinary Services has also occurred recently. A final report on a survey of the live bird market system conducted by them was published in November, 1998. The survey of this complex system provides new information on the geographical distribution of participants, the numbers and types of participants, the numbers and types of birds, the biosecurity methods used and the regulatory and surveillance programs involved. In addition, USDA has formed a Live Bird Market Working Group, which meets in March, 1999. I believe that these new efforts by USDA supports the premise that avian influenza in not a state or regional problem. Outbreaks of avian influenza affect not only the poultry producer involved, but also the entire poultry industry. It is clear now that the presence of low path flu has and can be used as a trade barrier. All of these efforts may well contribute to a reduced risk of introducing this virus into commercial poultry operations in the future.

In the future, it remains important for the poultry industry and its government agriculture partners to be prepared to respond to outbreaks of both highly pathogenic and low pathogenic avian influenza virus infections. It is sometimes more difficult to deal with non H7 or H5 flu viruses because of the mild clinical nature of the infection and the reduced risk of the virus changing to high path. So it remains important to have a response team and a written plan of dealing with outbreaks. The industry and state governments must be able to respond quickly with quarantine and programmed control of the outbreak. If they wait for Federal Government intervention it may be too late to control the disease. The issue of the use of killed or vectored vaccines remains unsolved. Their use in the control of low pathogenic avian influenza outbreaks as part of a control program continues to be denied. The Hong Kong H5N1 virus stimulated concern about the introduction of this agent which could destroy our poultry industry and the need for a supply of vaccine. The concern was soon forgotten. The

uneasy threat of bio-terrorism involving highly pathogenic avian influenza or other agents to shut down a country's food supply is real. Perhaps we need to reconsider the need for a readily available vaccine supply.

I am encouraged by the recent progress made in efforts to control the source of most avian influenza virus infections in commercial poultry and an enlarged leadership role of USDA in the control of avian influenza.

RETROSPECTIVE STUDY OF A TURKEY CORONAVIRUS OUTBREAK

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Enteric diseases are an economically significant health problem affecting the turkey industry. Over the past 5 years, enteric diseases of poults have been responsible for losses in excess of 100 million dollars in the southeastern United States. Poult enteritis is a multifactorial syndrome and all the components have not been adequately elucidated. However, turkey coronavirus (TCV) has been implicated in numerous cases. This disease has been costly to the industry. One integrator reported a 3.86% livability disadvantage, 0.21 feed conversion disadvantage, 3.35 pounds loss of weight gain, and 2 cents per pound added cost for TCV-affected flocks in 1997.

TCV has plagued the NC turkey industry for several years. Outbreaks typically occur in the spring and summer months and companies experience an "off season" in the winter. Companies have worked independently on eradication efforts focused largely on biosecurity and farm clean-out procedures. The key to success in other regions of the country has apparently involved early detection of TCV flocks followed by aggressive regional "down time." In response to industry requests, a monitoring program was put in place to keep integrators aware of when and where TCV-positive flocks existed in western North Carolina and adjacent South Carolina. The goal of this program was to provide the industry with the information they needed to take aggressive regional action.

A retrospective study was conducted, using a tempero-spatial approach, to map out incidence and prevalence of TCV-positive flocks in western North Carolina and South Carolina. Four major turkey-producing companies as well as one smaller company and one independent research facility made their records available and agreed to participate in a prospective monitoring program administered as a continuation of this effort. Two smaller companies made their records available but did not participate in the monitoring program. The results show that TCV-positive flocks existed in the region during the winter "off season" and could have served as a reservoir of TCV for a subsequent outbreak in the spring.

(These findings will be submitted for publication as a full-length article.)

EXPERIMENTAL INFECTION OF SPECIFIC-PATHOGEN-FREE CHICKENS WITH TURKEY CORONAVIRUS

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Three-day-old specific-pathogen-free (SPF) chickens were inoculated by combined oral and intracheal routes with turkey coronavirus (TCV) NC95 strain. Chickens were examined on sequential days postexposure (PE) for development of clinical signs, pathologic lesions and presence of virus in tissues. TCV antigens were detected in tissues of infected chickens using a monoclonal antibody (MAb)-based immunoperoxidase procedure. No clinical signs were observed in chickens inoculated with TCV and weight gain was comparable to sham-inoculated controls.

Pale, mildly distended intestines were observed only in infected chickens necropsied on days 2-4 PE. Viral antigens were detected in apical villus enterocytes in the duodenum, jejunum and ileum on days 2-8 PE, and in epithelium of bursa of Fabricius on days 2-4 PE. No TCV antigens were detected in other tissues. TCV was isolated from pooled cecal contents of infected chickens on days 2-8 PE; virus was not isolated from respiratory tissues. These findings indicate that chickens are susceptible to TCV infection, and suggest the possibility that chickens may be inapparent reservoirs of the virus.

(Submitted for publication in *Avian Diseases*).

TURKEY CORONAVIRUS OUTBREAKS IN VIRGINIA AND WEST VIRGINIA IN 1996, 1997 AND 1998

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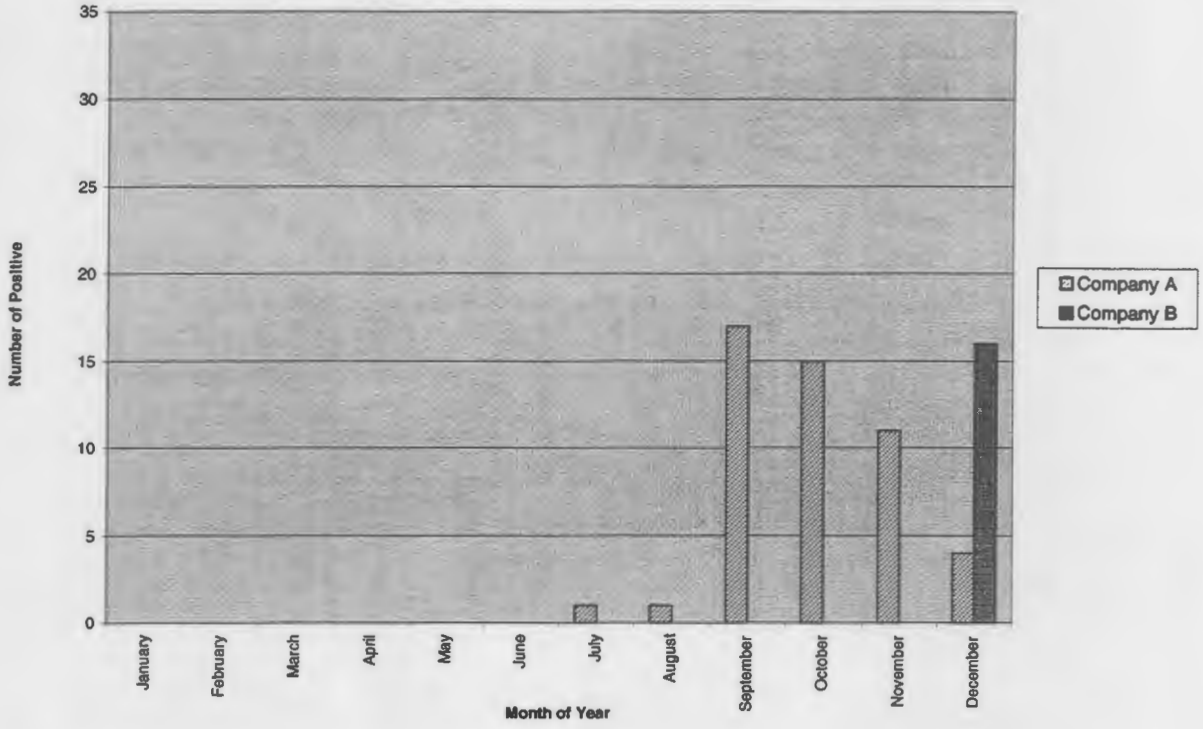
Turkey coronavirus (TCV) has been described elsewhere. This disease is also known as mud fever, blue comb disease, transmissible enteritis and infectious enteritis. TCV had been determined to cause big losses to the Minnesota turkey industry between 1951 and 1971, but has not been reported there since 1977. Although other agents (vibrio, reovirus, enterovirus, papovavirus and others) have been implicated in turkey enteritis, TCV has been shown to cause some typical but not specific signs, symptoms and side effects.

During the spring of 1991 turkey flocks in North Carolina started experiencing a "turkey enteritis" designated as "Spiking Mortality in Turkeys". This syndrome has been commonly present in turkey flocks from late spring to the end of fall and early winter. This syndrome has caused multi-million dollar losses in North Carolina. Large amounts of human and economic resources were committed to find the possible cause, methods of control, adequate biosecurity, treatments and a vaccine. In 1996 some North Carolina turkey companies started to test their flocks for TCV by Indirect Fluorescent Antigen (IFA) test. Several flocks were positive for TCV by serology. This syndrome has been a major problem and/or concern not only in North Carolina but also for other neighboring turkey growing states (South Carolina, Virginia, West Virginia, Indiana, Ohio and Pennsylvania). TCV has not been reported by growers in Ohio; about one outbreak a year has been reported in Pennsylvania in the last 3 years; outbreaks were reported in Indiana in 1991 and 1992 but none since then; yearly outbreaks have occurred in South Carolina especially in farms closer to western North Carolina; annual positive cases have been diagnosed by serology in Virginia and West Virginia since 1996. Turkeys grown in North Carolina have been processed on a regular basis in Virginia.

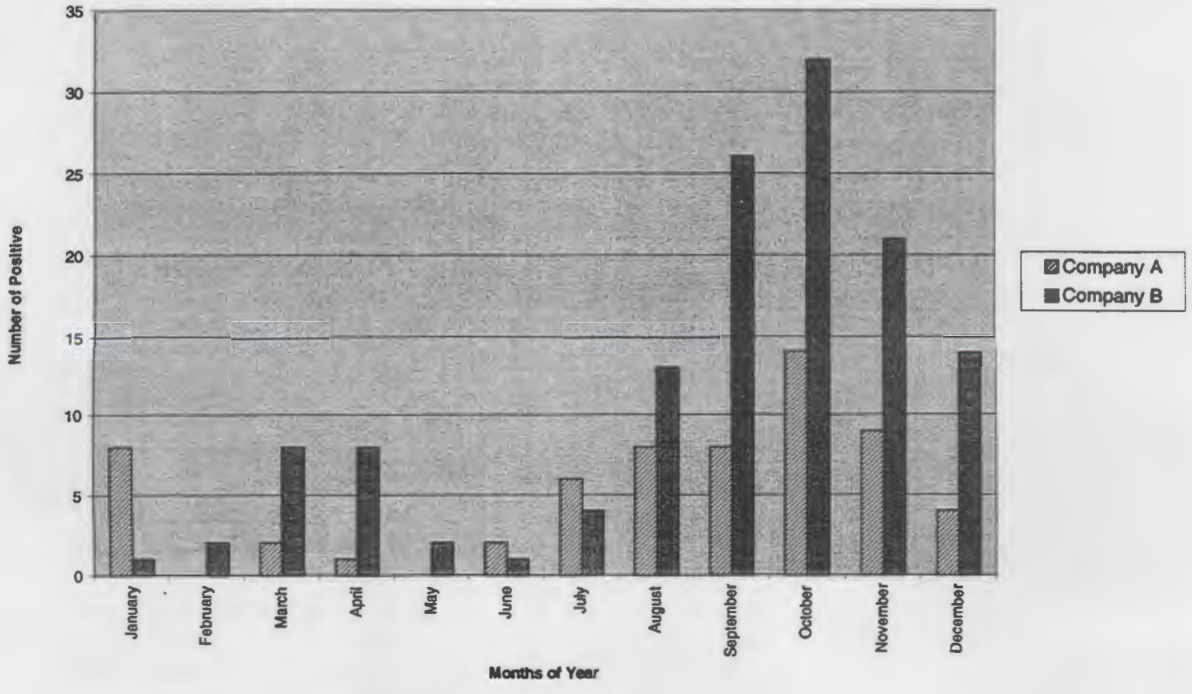
Both Virginia and W. Virginia produce turkeys year-round. Many turkey and chicken farms are located very close to each other. The growing units are closely interconnected by their growing contracts to either of the two major integrators in the area. The following factors are considered important in the epidemiology of TCV: close proximity of farms, poultry concentration, common access roads, farm visitors, equipment sharing, traffic in and out turkey farms, independent manure-litter brokers-haulers, and litter application. Some of these factors have played a role and contributed to the spread of disease agent(s) in the area. Any breach of biosecurity increases the potential for an outbreak. The Virginia and West Virginia turkey growing area produces about 30 million turkeys a year. Turkey farms can have 1 to 8 houses. Most of the turkey growers have two age groups at a time. There are also growers with one age and some with 3 ages. Most turkeys are marketed at 14-20 weeks of age.

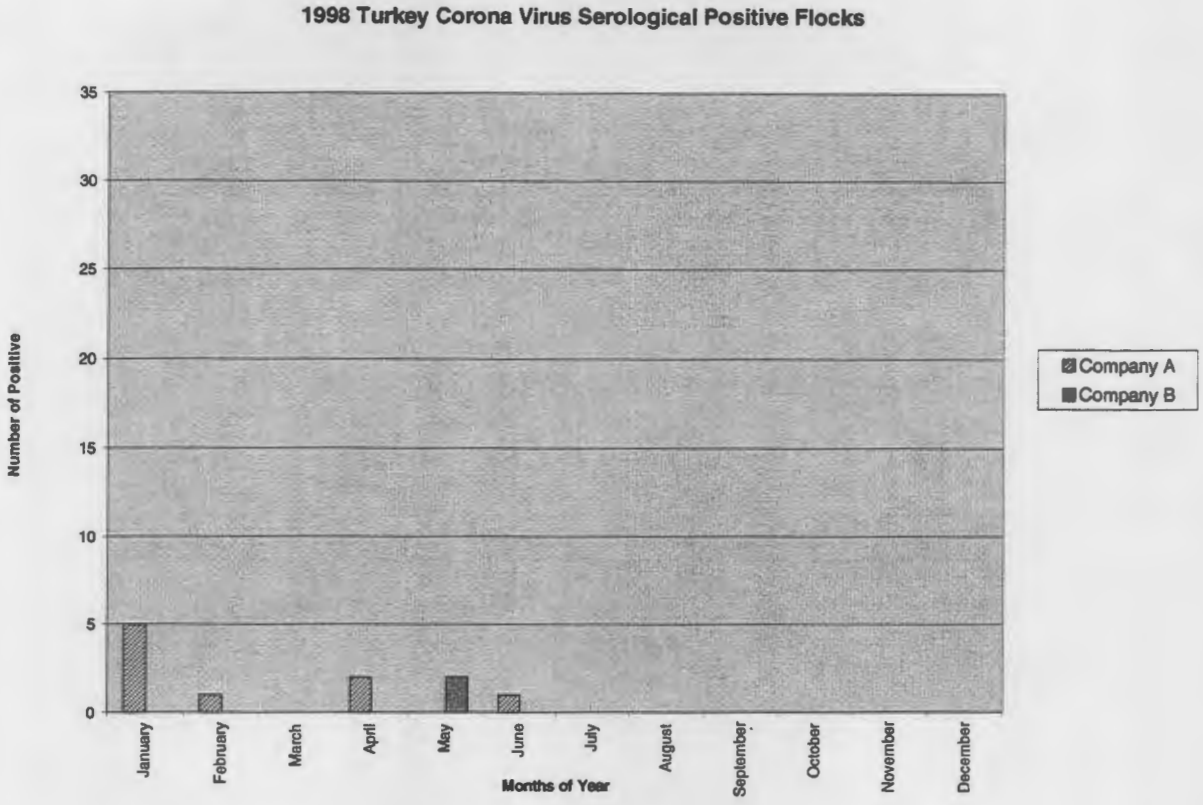
TCV diagnosis can be performed by the IFA test, which detects the presence of antibodies in serum samples and by the Direct Fluorescent Antibody (DFA) test, which detects the presence of virus in intestinal epithelium samples from field cases. Virginia and West Virginia turkey flocks have been tested serologically (IFA) since 1996 by Purdue University, North Carolina State University, Virginia Polytechnic Institute & State University and the State of Virginia Laboratory System, Harrisonburg Branch. Sixty-five flocks were positive for TCV in1996, 210 flockswere positive in 1997 and 1[illegible] flocks were positive in 1998. There is no information available on serology for TCVor isolation of TCVprior to 1996. The distribution of the positive flocks is outlined in the following 3 graphs.

1996 Turkey Corona Virus Serological Positive Flocks



1997 Turkey Corona Virus Selogical Positive Flocks





Significant reduction in TCV positive flocks and complete eradication in 1999 have resulted from the following efforts: farm depopulation, cleaning, sanitation and disinfection of premises, grower education on biosecurity procedures, improved manure handling and distribution, grower involvement, increased monitoring; faster action on flocks showing signs and symptoms of the disease, improvement in communication, and prompt and appropriate actions by people involved. Without the cooperative efforts of both integrators and allied industry, eradication would have been impossible

REVIEW OF NEWCASTLE DISEASE VIRUS IN AUSTRALIA

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INTRODUCTION

Australia has been free of virulent Newcastle disease since two outbreaks were successfully eradicated in 1930 and 1932. The later outbreak was believed to be due to contaminated material from the original outbreak. Since 1966, when an avirulent form of Newcastle disease virus (NDV) called V4 was identified, a large number of additional isolates have been recovered from domestic poultry and wild birds in Australia. These endemic Australian viruses are all similar resembling the V4 isolate (2). Further studies have revealed that some of these endemic strains are capable of causing a mild conjunctivitis and tracheitis (1). These viruses are usually recovered from cases of late respiratory disease in broilers. This condition is observed around 4 to 7 weeks of age and invariably in chickens that are infected with other poultry respiratory pathogens or immunosuppressive agents. Factors such as stocking density, hot weather and husbandry practises can also affect the course of the disease. Chorioallantoic immunofluorescence pathogenicity tests using endemic strains are consistent with those using avirulent or lentogenic

strains of the virus (3). These low virulence strains are more readily transmissible by the aerosol route when compared to the original V4 isolate, and for that reason may be more suitable as vaccine candidates.

VIRULENT NDV OUTBREAK

In late 1998 a poultry farm comprised of multiage caged and free-range layers, broilers, and a number of other avian species including waterfowl, ostriches and wild pigeons experienced increased mortality in one of the layer flocks. Clinical signs included depression, green diarrhoea, blue combs, peritonitis and shelless eggs. This mortality was initially diagnosed as Marek's disease (MDV) which had been a prevalent and severe problem for the entire layer industry at the time. Fowl cholera involvement was also considered. Histopathology revealed a non-suppurative meningoencephalitis and nerve degeneration that was interpreted as MDV but *Pasteurella multocida* was not recovered. Mortality then commenced in the broilers and neurological signs were observed in a 5-week-old pullet flock. It was only after the disease had been present on the property for approximately one month in the originally affected birds that clinical signs of head tremors, torticollis and incoordination became obvious. Subsequent and retrospective serology from the clinically infected flocks revealed haemagglutination inhibition (HI) titers of up to 2^{10}. Virulent NDV (vNDV) was isolated from tracheal, cloacal swabs and brains of the clinically affected birds. Two other properties that had contact with this property either by egg movements or the receipt of young broilers for a free-range growout became infected. On the second layer farm vNDV was isolated from pooled cloacal and tracheal swabs. It was identified in fixed tissues from the free-range broiler farm where PCR revealed the nucleotide sequence of the cleavage site to be consistent with vNDV. Based on nucleotide analysis the virus isolated from the second commercial layer property was identical to the original isolate. There was no evidence to suggest that the vNDV originated from the other avian species on the original property.

The intracerebral pathogenicity index (ICPI) for these two viruses was 1.6 and 1.9 (on a scale of 0 to 2.0). The endemic Australian strains usually have an ICPI of around 0.5. These vNDV were invasive into the full thickness of the mesoderm of the chorianoallantoic membrane of inoculated embryonated eggs.

DISCUSSION

Molecular evidence indicated that the vNDV in this outbreak was not an exotic strain, but a mutation from a pre-existing Australian strain of the virus. This was based on three observations – cleavage site nucleotide sequence, overall genetic relationships, and amino acid sequence of the HN protein. The HN sequence on the virulent virus was identical to that of other Australian strains and different to that seen in other countries. This was particularly so in the area of the HN protein beyond the cleavage site for activation of the protein. F gene sequence analysis of a 400 base pair fragment also showed that the three virulent viruses were the same, and the same as that of a low virulence strain of NDV (Peat's Ridge) that had been isolated in Australia from broilers with late respiratory disease complex.

Amino acid sequences at the cleavage site of the various NDV strains are:

V4	GKQGRL
Endemic Australian Isolates	RKQGRL
Low virulence 1998	RRQGRL
vNDV 1998	RRQRRF
Exotic Virulent NDV	RRQRRF

The transmissibility of this virulent Australian NDV appeared to be limited as evidenced by the failure of certain layer and broiler flocks on two of the properties to seroconvert to NDV despite close contact. Also despite extensive interaction between egg movements, feed trucks, dead bird disposal vehicles, personnel and equipment there was no evidence of spread to any regional properties and the outbreak was contained. The farm from which the Peat's Ridge virus was isolated was approximately 40 miles away and had no contact or association with the outbreak property. Clinically the presence of vNDV on the infected layer property preceded the placement of the broiler flock from which the Peat's Ridge or low virulence NDV was isolated.

Serological monitoring found that greater than 50% of birds infected with the virulent virus had titers greater than 2^5. On neighbouring farms infected with endemic NDV less than 25% of flocks have titres greater than $2^3$and very few greater than or equal to 2^5. Until future studies are undertaken to determine the distribution of the low virulence virus and its epidemiological role, if any in the occurrence of vNDV virus, titer levels will be used as an indicator of the possible presence of vNDV. The concern here is that chickens previously exposed to endemic strains of NDV may on exposure and possible infection with vNDV not show clinical signs but allow the persistence of the virus until susceptible chickens are introduced.

REFERENCES

1. Hansson, E., J. G. Young, P. T. Hooper, and A. J. Della-Porta. Virulence and transmissibility of some Australian and exotic strains of Newcastle disease virus used in some vaccines. Aust. Vet. J. 77:51-53. 1999.

2. Hooper, P. T., E. Hansson, J. G. Young, G. M. Russell, and A. J. Della-Porta. Lesions in the upper respiratory tract in chickens experimentally infected with Newcastle disease viruses isolated in Australia. Aust. Vet. J. 77:50-51. 1999.

3. Hooper, P. T., G. M. Russell, C. J. Morrow, and Y. Segal. Lentogenic Newcastle disease virus and respiratory disease in Australian broiler chickens. Aust. Vet. J. 77:53-54. 1999.

BROILERS FROM PARENTS THAT HAVE SUBGROUP-J AVIAN LEUKOSIS/SARCOMA VIRUS TUMORS ARE NOT AS ECONOMICAL TO PRODUCE AS BROILERS FROM PARENTS THAT DO NOT HAVE TUMORS

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Poultry health professionals and producers know that avian leukosis/sarcoma virus (ALSV or ALV) infections will adversely affect breeder flock performance (1). Recently, concern about the possible effects of subgroup J ALSV (ALV-J) on broilers has increased. Specifically, there is concern that broilers from parents with ALV-J tumors might be less economical to produce than broilers from parents without tumors.

In the present study that encompassed 3.5 million broilers, the production performance of broilers from breeders with ALV-J tumors was markedly worse than the performance of broilers from breeders without tumors. Specifically, broilers from parents with ALV-J tumors had significantly lower average body weights ($P = 0.000006$), lower livability ($P = 0.00003$), lower flock-performance-rank ($P = 0.05$), higher feed conversion ratios ($P = 0.00007$), higher adjusted feed conversion ratios ($P = 0.01$), and higher 2-week mortality ($P = 0.008$). No differences in average daily gain ($P = 0.34$) or condemnations at processing ($P = 0.25$) were seen.

REFERENCE

1. Payne, L. N., and A. M. Fadly. Leukosis/sarcoma group. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, Y. M. Saif eds. Iowa State University Press, Ames, Iowa. pp. 414-466. 1997.

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UNUSUAL EYE LESIONS ASSOCIATED WITH ALV-J VIRUS

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During a 3-week period in April, 1998, selected 56- to 59-week-old male and female CobbxCobb broiler breeders from an 18,000 bird flock were submitted to the University of Delaware Lasher Diagnostic and Research Laboratory in Georgetown, Delaware. Poultry servicemen's complaints were that the broiler breeders had been sick for 8 weeks and they had eye lesions, and that production was off.

Composite necropsy findings included blepharitis with and without caseous plaques, ophthalmitis, eye abscesses, facial swelling, periorbital sinusitis, brown/red foul smelling ear lobe and wattle edema, poor fleshing, airsacculitis, ovarian regression, peritonitis, keel abscesses, enlarged cecal tonsils, capillariasis, heterakiasis, and bumble foot. The most severely affected chickens had a putrefactive odor. Histologically the primary eye lesions were 2 tumors associated with ALV-J infection; myelocytomas containing variable numbers of promyelocytes, and a histiocytic sarcoma (4). The eye with the histiocytic sarcoma also had a promyelocyte containing myelocytoma. ALV-J has a tropism for cells of the myelomonocytic series (5). It is of interest that in this ALV-J initiated disease outbreak, one eye contained neoplasia involving both the myelocytic system (myelocytoma) and the monocytic system (histiocytic sarcoma).

Prior to this submission, ocular myelocytic myeloid leukosis (myelocytomatosis) lesions in Delmarva broiler breeders were associated with grossly visible systemic tumors containing large numbers of myelocytes with prominent eosinophilic cytoplasmic granules. Ocular lesions also contained myelocytes with prominent eosinophilic granules. The ocular neoplasms that are the subject of this report were not associated with grossly visible systemic tumors at necropsy. Histologically the myelocytomas were relatively immature often containing an abundant number of promyelocytes and lesser numbers of smaller cells that were probably myeloblasts. This finding is not unlike that reported by Payne (3) that the expression of ALV-J in the field in the U.K. usually involves myelocytes, but in some cases more immature promyelocytes, stem cell tumors, and lesser numbers of histiocytic sarcoma.

Ocular tissues from this Delmarva broiler breeder flock were collected at various stages of lesion development. The earliest lesion recognized was a lymphoplasmacytosis within the lumina of uveal blood vessels including the choroid, ciliary body, ciliary processes and iris. This was followed by the arrival of myelocytes, promyelocytes and possibly myeloblasts. Accumulations of these cells could become quite extensive resulting in congestion with possible progression to ischemic necrosis. In severe lesions tumor cell spread and growth might be found in the posterior and anterior chamber, vitreous body, periorbital tissues and eyelids. One eye that lacked an infectious process was in the mid-development phase of ophthalmacrosis. The early eye lesions consisting primarily of a lymphoplasmacytosis of the uvea resembles at low power the non-neoplastic eye lesions of Marek's disease. On closer examination the early ALV-J eye lesions have a much higher concentration of plasma cells than Marek's disease lesions and may contain small concentrations of immature heterophils. When heterophils are associated with Marek's disease lesions they are typically mature (2) and not myelocytes, promyelocytes or myeloblasts.

Some eyes with tumors developed a severe bacterial panophthalmitis and bacterial blepharoconjunctivitis. Eyelids from these cases had a large build-up of bacterial laden keratinaceous debris in exaggerated conjunctival folds. It is thought that the bacterial process probably relates to a neoplasia-related loss of tear cleansing action. Tears are sterile and protect the eye and conjunctiva through their bactericidal properties and their function of washing foreign material from the surface of the conjunctiva and cornea (6). The lack of tears leads to drying of the corneal and conjunctival epithelium which becomes inflamed, eroded, and ulcerated (1), enhancing the entry of opportunistic bacteria. The eye infections may also simply relate to trauma to a blind or partially blind eye. In one case submission, pure cultures of *Proteus mirabilis* were isolated from abscesses, and lesions of the keel, wattle, and eye. In other submissions from this same breeder flock, a variety of bacteria including *Pasteurella multicida* and *Pasteurella haemolytica* were isolated from lesions. The debility present in this flock of broiler breeders might not only relate to ALV-J initiated neoplasia and immunosuppression, but also to a bacteremia/septicemia.

REFERENCES

1. Kumar, V., R. S. Cotran, and S. L. Robbins. Basic Pathology, 5th edition. W. B. Saunders, Philadelphia, Pennsylvania. pp. 150. 1992.

2. Payne, L. N. Pathology. In: Marek's Disease Scientific Basis and Methods of Control. L. N. Payne, ed. Martinus Nijhoff Publishing, Boston, Massachusetts. pp. 63. 1985.

3. Payne, L. N. HPRS-103: a retrovirus strikes back. The emergence of subgroup J avian leukosis virus. Avian Pathol. 27:S36-S45. 1998

4. Payne, L. N., A. M. Gillepsie, and K. Howes. Myeloid leukaemogenicity and transmission of the HPRS-103 strain of avian leukosis virus. Leukemia 6:1167-1176. 1992.

5. Payne, L. N., K. Howes, L. M. Smith, and K. Venugopal. Current Status and Diagnosis, Epidemiology and Control of ALV-J. In: Avian Tumor Viruses Symposium. A. M. Fadly, K. A. Schat, and J. L. Spencer, eds. American Association of Avian Pathologists, 40th Annual Meeting, Reno, Nevada. pp. 58-62. 1997.

6. Ross, M. H., L. J. Romrell, and G. I. Kaye. In: Histology, a text and atlas, 3rd ed. Williams and Wilkins, Baltimore, Maryland. pp. 757. 1995.

DEVELOPMENT OF AN AVIAN LEUKOSIS J VIRUS SPECIFIC ANTIBODY ELISA

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Subgroup J avian leukosis virus (ALV-J) is a recently discovered member of the avian leukosis -sarcoma group of retroviruses (1). The virus has a broad host range and all lines of chickens tested are susceptible to infection (2). It induces myelocytic myeloid leukosis (ML) and has a tropism for the cells of myeloid rather than lymphoid lineage (3). The virus has unique envelope properties and nucleotide sequence analysis has shown that the gp85 domain of the J subgroup virus has about 40% identity with the corresponding regions of the other ALV subgroups (4). Among the ALV-encoded proteins, the gp85 protein contains targets for neutralizing antibodies as well as regions that may be important in interactions with host cell receptors. A baculovirus recombinant gp85 protein of strain ADOL-Hc1 of the ALV-J virus was developed and used for the development of an ELISA for the detection of specific antibodies to ALV-J. The recombinant gp85 protein had an approximate molecular weight of 80 to 90 kD as seen on SDS gels and immunoblot analysis. Mono-specific chicken sera raised against ADOL-Hc1 ALV-J virus and a monoclonal antibody raised against HPRS-103 virus reacted specifically to the gp85 protein and no reaction was noticed with ALV subgroup viruses A-, B-, C-, D-, and E-specific sera in western blot and ELISA. This suggests that the recombinant gp85 protein is highly specific to ALV-J.

Serum samples used for the evaluation of the ALV-J antibody ELISA were collected from flocks experimentally infected with prototype ADOL-Hc1, and naturally infected flocks from the field. The ALV-J ELISA detected antibodies 4 weeks post- infection wit h the Hc1 strain. Sera raised against new field isolates (ADOL-5701, ADOL-6803, ADOL-6827) and Hc1 of ALV-J strains showed little or no cross reaction in the neutralization assay, suggesting a high degree of antigenic diversity between the ALV-J isolates. Despite the high degree of antigenic diversity, the gp85 protein based ELISA detects the subgroup J specific antibodies with a high degree of specificity, sensitivity and demonstrates broad serological cross reactivity to the ALV-J isolates.

REFERENCES

1. Payne, L. N., S. R. Brown, N. Bumstead, K. Howes, J. A. Frazier, and M. E. Thouless. A novel subgroup of exogenous avian leukosis virus in chickens. J. Gen. Virol. 73:801-807. 1991.

2. Payne, L. N., K. Howes, A. M. Gillespie, and L. M. Smith. Host range of Rous sarcoma virus pseudotype RSV (HPRS-103) in 12 avian species: support for a new avian retrovirus envelope subgroup, designated J. J. Gen. Virol. 73:2995-2997. 1992.

3. Payne, L .N., A. M. Gillespie, and K. Howes. Myeloid leukaemogenicity and transmission of HPRS-103 strain of avian leukosis virus. Leukaemia 6:1167-1176. 1992.

4. Bai, J., L. N. Payne, and M. A. Skinner. HPRS-103 (exogenous avian leukosis virus, subgroup J) has an env gene related to those of endogenous elements EAV-O and E51 and an E element found previously only in sarcoma virus. J. Gen. Virol. 69:779-784. 1995.

DEVELOPMENT OF AN AVIAN LEUKOSIS VIRUS SUBGROUP J SPECIFIC ANTIBODY TEST

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Avian leukosis virus subgroup J (ALV-J) has become a serious problem for the broiler industry. Diagnostics used in eradication programs have focused on measurement of the virus by testing virus isolation extracts, cloacal swabs, albumen and meconium for group specific antigen (p27). Because ALV-J has a high rate of horizontal transmission, an antibody test provides an additional level of flock status information. Virus neutralization tests for ALV-J antibody are limited because of the antigenic variation in neutralizing epitopes. An ALV-J antibody ELISA based on recombinant gp85 glycoprotein has been developed in which the measurement of antibody is not limited to neutralizing titers, but also can detect antibody against other epitopes, which may be more highly conserved. The format of the test includes recombinant gp85 antigen coated on the solid phase and an anti-chicken IgG:HRPO for the detection of specimen

antibody.

Immunoblot testing of the recombinant gp85 using subgroup specific antisera (A, B, C, D, E, J) and an ALV-J gp85 monoclonal demonstrated the J specificity of the antigen. ALV-J specificity for the ELISA was confirmed by testing antisera against other common avian pathogens and ALV subgroup antisera. Test results for all non-J monospecific antisera were negative. The ALV-J antibody ELISA has a specificity of 99.7% for a virus isolation negative population (n=1159) representing multiple broiler breeder sources. Sensitivity of the assay was evaluated by monitoring seroconversion of virus isolation positive broiler-breeder flocks followed over time. The percentage of birds that seroconvert within field infected flocks ranged from <10% up to 95% and may depend upon the history of vertical and horizontal transmission occurring within the flock.

ALV-J antibody ELISA test results for individual birds do not indicate ALV-J status. A seronegative bird could be immune tolerant and actively shedding, whereas a seropositive bird has been exposed to ALV-J, but may not shed the virus. Therefore ALV-J ELISA antibody test results should be interpreted only on a flock basis. The ALV-J specific antibody test described provides another tool for monitoring the ALV-J status of flocks.

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AVIAN LEUKOSIS VIRUS SUBGROUP J - PROSPECTS FOR CONTROL

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INTRODUCTION

Avian leukosis virus of subgroup J (ALV-J) has recently emerged as an economically important pathogen in broiler breeder operations in many countries (12). This new problem has come at a time when the majority of commercial layers in North America and other parts of the world are from breeding stock that has been freed of leukosis viruses. Many breeding companies started to test for leukosis viruses in the late 1970's and have invested large sums of money to produce virus-free stock (2). While ALV-J presents new challenges, methods that are already familiar to the industry have formed the basis of the control programs (11,18,22).

AVIAN LEUKOSIS VIRUSES

Avian leukosis viruses (ALVs) are divided into subgroups based on envelope antigens that are products of the *env* gene. Exogenous leukosis viruses, meaning those that are transmitted from bird to bird, cause a variety of tumors. Until the emergence of ALV-J, subgroups A and B were the only exogenous leukosis viruses of concern to the poultry industry. Subgroups C and D have been considered oncogenic laboratory strains of virus. Subgroup E viruses are found in commercial poultry but are not known to be oncogenic and are of endogenous origin. ALVs share a common group specific (gs) antigen that is part of the nucleocapsid and is produced by the gag gene. Antiserum against the p27 component of the gs antigen is used as a reagent in serological tests. The *env* gene of ALV-J differed substantially from that of subgroups A-E but was closely related to that in certain endogenous retroviral elements that are part of the normal chicken genome (11).

TESTS FOR INFECTION WITH ALVs

Virus neutralization test procedures have been used to detect envelope antigens and are subgroup specific. However, antigenic variation has been found among isolates of ALV-J and this must be considered when interpreting test results (5, 11). Enzyme linked immunosorbent assays (ELISA) to detect antibody to subgroups A and B have proven useful in programs to eradicate subgroup A and B viruses from egg stocks. It is anticipated that an ELISA, which uses improved reagents such as the env-gp85 recombinant protein, should prove useful in large scale testing for antibody to ALV-J (24). The discovery that egg albumen from ALV-infected hens contained high levels of gs antigen led to the development of rapid tests to detect infected chickens (23). Initially the complement fixation test was used to detect antigen but this was soon replaced by the more efficient ELISA (17). Both polyclonal and monoclonal antibodies against gs antigen (p27 component) of the virus have been successfully used as test reagents.

A complicating factor in tests based on detection of gs antigen is that chickens may carry endogenous viral (*ev*) genes that produce the same antigen as do the exogenous viruses. The problem is greatest in meat stocks since they usually carry more ev genes than egg stocks (10,15,19). In White Leghorn stocks, infection with exogenous virus usually results in higher levels of gs antigen than that produced by *ev* genes. To distinguish between exogenous

and endogenous infections it may be necessary to inoculate specimens onto cell cultures. Isolation and identification of viruses in cell cultures has been greatly facilitated by the development of lines of chickens that are selectively susceptible or resistant to known subgroups of ALV. For example, cells from ALV6 chickens are resistant to subgroup A and E but are susceptible to other subgroups including subgroup J (4, 16). Specimens, such as blood, that are inoculated onto cell cultures may contain endogenous gs antigen that could not be distinguished by ELISA from antigen produced by cell cultures infected with exogenous virus. To avoid this problem, the cells could be fixed and tested by the indirect immunofluorescence assay (Spencer, unpublished) for gs antigen produced by exogenous virus.

Polymerase chain reaction (PCR) technology has been used to detect infection with ALV-J but none of the primers developed to date have detected all of the isolates of subgroup J (11).

TRANSMISSION OF ALVs

Exogenous ALVs are transmitted congenitally and horizontally (14). Rubin (13) showed that congenital transmission caused a state of immunological tolerance. Chickens infected in this manner are permanently viremic, do not develop antibody and shed virus into the environment throughout their lives. A concern with ALV-J is that there is rapid horizontal spread of virus and many chickens infected soon after hatch develop infections comparable to those in congenitally infected birds. When they mature, these chickens will congenitally transmit infection to a high percentage of their progeny. Other chickens infected post-hatch develop antibody but a low level of infection may persist. Chickens in this category may congenitally transmit infection to a low percentage of their progeny.

Allantoic fluid from congenitally-infected embryos has been found to contain ALV and thus it is likely that the feathers and skin of the chicks would be covered with virus (Spencer and Chan, unpublished). Virus on skin and feathers would be expected to spread horizontally as chicks rub together. The importance of skin as a portal for entry of ALV was demonstrated by Weyl and Dougherty (25). They showed that infection could readily be established by inoculating ALV onto an area of skin where a few feathers had been removed. Other portals for entry of virus were the mouth, nares and eyes.

Subgroup E viruses differ from exogenous viruses in that they are usually transmitted as ev genes that are part of the hosts genome. Virtually all chickens carry ev genes but there are usually more of these genes in meat breeds than in White Leghorns (15). Complete endogenous viruses may be transmitted as are the exogenous viruses (10).

INFECTION AND DISEASE

Studies have shown that ALVs can replicate in virtually all tissues and organs of susceptible chickens but certain sites favor intense virus replication. It is of interest that there can be extensive virus replication in heart and other organs (3, 7, 21), without evidence of overt disease. However, chickens that are subclinically infected with the virus are less productive than uninfected stock (6). The capacity of subgroup A virus to induce lymphoid leukosis has been associated with its propensity to multiply in the bursa of Fabricius (8, 1). However, breed of chickens may influence capacity of the virus to multiply in bursal tissue since the RAV-1 strain of subgroup A virus induced lymphoid leukosis in White Leghorns but caused nephroblastomas and myelocytomas (one bird) in meat chickens (9). Payne (11), reported that ALV-J had a low tropism for cells of the bursa but a high tropism for cells of the myelomonocytic series. He noted that the virus caused myeloid leukosis but not lymphoid leukosis in meat stocks. ALV-J also induced myeloid leukosis in White Leghorns, but there were differences among strains of birds in the degree of genetic susceptibility to the disease. Histologically, the presence of immature myelocytes with eosinophilic cytoplasmic granules has been considered pathognomonic for myeloid leukosis (4).

ERADICATION PROCEDURES

Programs to eradicate ALV-J should include management practices to limit the horizontal spread of infection (20) and testing procedures to identifiy and eliminate hens that are likely to congenitally transmit virus. This is difficult because chickens infected in the post-hatching period may have varying degrees of infection and no single test procedure has been found to identify all chickens that can congenitally transmit virus. Furthermore, the antigenic and molecular variation among isolates of ALV-J has complicated detection of infection. Thus, it has not been possible to eradicate the virus in one generation. Payne (11) outlined a program for eradication of ALV-J from meat stocks and noted that after 6 generations of testing, the prevalence of infection had been markedly reduced but not eliminated. It is anticipated that improved reagents (24) and application of highly sensitive test procedures, such as PCR, should facilitate control programs.

REFERENCES

1. Arshad, S. S., K. Howes, G. S. Barron, L. M. Smith, P. H. Russell, and L. N. Payne. Tissue tropism of the HPRS-103 strain of J subgroup avian leukosis virus and of a derivative acutely transforming virus. Vet. Pathol. 34:127-137. 1997.

2. Chase, W. B. Eradication of avian leukosis virus by breeding companies: results, pitfalls and cost benefit analysis. Proc. Avian Tumor Virus Symposium, Seattle, WA. pp. 5-8. 1991.

3. Dougherty, R. M., and H. S. Di Stefano. Sites of avian leukosis virus multiplication in congenitally infected

chickens. Cancer Res. 27:322-332. 1967.

4. Fadly, A. M. Infeccion y tumores por el subgroupo J del virus de la leucosis aviar en pollos de carne en los estados unidos: una actualizacion. Proc. Expoaviga 98, XXXV Symposium of the WPSA, Barcelona, Spain. pp. 23-27. 1998.

5. Fadly, A. M., and E. J. Smith. An overview of subgroup J-like avian leukosis virus infection in broiler breeder flocks in the United States. Avian Tumor Virus Symposium, American Association of Avian Pathologists, Reno, Nevada. pp. 54-57. 1997.

6. Gavora, J. S., J. L. Spencer, R. S. Gowe, and D. L. Harris. Lymphoid leukosis virus infection: effects on production and mortality and consequences in selection for egg production. Poult. Sci. 59:2165-2178. 1980.

7. Gilka, F., and J. L. Spencer. Viral matrix inclusion bodies in myocardium of lymphoid leukosis-infected chickens. Am. J. Vet. Res. 46:1953-1960. 1985.

8. Gilka, F., and J. L. Spencer. Importance of the medullary macrophage in the replication of lymphoid leukosis virus in the bursa of Fabricius of chickens. Am. J. Vet. Res. 48:631-620. 1987.

9. Gilka, F., J. L. Spencer, and J. R. Chambers. Response of meat-type chickens to infection with RAV-1 avian leukosis virus. Avian Pathol. 20:637-647. 1991.

10. Ignjatovic, J. Replication competent endogenous avian leukosis virus in commercial lines of meat chickens. Avian Dis. 30:264-271. 1986.

11. Payne, L. N. HPRS-103: A retrovirus strikes back. The emergence of subgroup J avian leukosis virus. Avian Pathol. 27:S36-S45. 1998.

12. Payne, L. N., S. R. Brown, N. Bumstead, K. Howes, J. A. Frazier, and Margaret E. Thouless. A novel subgroup of avian leukosis virus in chickens. J. Gen. Virol. 72:801-807. 1991.

13. Rubin, H. A virus in chick embryos which induces resistance in vitro to infection with Rous sarcoma virus. Proc. Natl. Acad. Sci. U.S.A. 46:1105-1119. 1960.

14. Rubin, H., A. Cornelius, and L. Fanchier. The pattern of congenital transmission of an avian leukosis virus. Proc. Natl. Acad. Sci. U.S.A. 47:1058-1069. 1961.

15. Sabour, M. P, J. R. Chambers, A. A. Grunder, U. Kuhnlein, and J. S. Gavora. Endogenous viral gene distribution in populations of meat-type chickens. Poultry Sci. 71:1259-1270. 1992.

16. Salter, D. W., and L. B. Crittenden. Artificial insertion of a dominant gene for resistance to infection with avian leukosis virus into the germline of the chicken. Theor. Appl. Genetics 77:457-461. 1989.

17. Smith, E. J., A. Fadly, and W. Okazaki. An enzyme linked immunosorbent assay for detecting avian leukosis-sarcoma viruses. Avian Dis. 23:698-707. 1979.

18. Spencer, J. L. Progress towards eradication of lymphoid leukosis viruses -- A Review. Avian Pathol. 13:599-619. 1984.

19. Spencer, J. L., and J. R. Chambers. Endogenous leukosis viral antigen in eggs from meat-type chickens on an avian leukosis virus eradication programme. Avian Pathol. 21: 251-259. 1992.

20. Spencer, J. L., and R. W. Fairfull. Study to eradicate avian leukosis virus from a caged White Leghorn population. Proc. 42nd West. Poult. Dis. Conf., Sacramento, CA. pp. 65-68. 1993.

21. Spencer, J. L., F. Gilka, J. S. Gavora, and P. F. Wright. Distribution of group specific antigen of lymphoid leukosis virus in tissues of laying hens. Avian Dis. 28:358-373. 1984.

22. Spencer, J. L., L. B. Crittenden, B. R. Burmester, W. Okazaki, and R. L. Witter. Lymphoid leukosis: interrelations among virus infections in hens, eggs, embryos and chicks. Avian Dis. 21:331-345. 1977.

23. Spencer, J. L., L. B. Crittenden, B. R. Burmester, C. Romero, and R. L. Witter. Lymphoid leukosis virus and gs antigen in unincubated chicken eggs. Avian Pathol. 5:221-226. 1976.

24. Venugopal, K., K. Howes, G. S. Barron, and L. N. Payne. Recombinant env-gp85 of HPRS-103 (subgroup J) avian leukosis virus: Antigenic characteristics and usefulness as a diagnostic reagent. Avian Dis. 41:283-288. 1997.

25. Weyl, K. G., and R. M. Dougherty. Contact transmission of avian leukosis virus. J. Natl. Cancer Inst. 58:1019-1025. 1977.

INCIDENCE OF ASCITES SYNDROME AND RELATED PATHOLOGY IN FEED RESTRICTED BROILERS RAISED AT SIMULATED HIGH ALTITUDE

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SUMMARY

It has been demonstrated that the incidence of ascites syndrome can be significantly reduced through feed restriction. Unfortunately, the restriction programs practiced also have a detrimental effect on growth characteristics. This experiment was conducted to determine if feed restriction could be used to reduce the incidence of ascites in broilers raised in a hypobaric chamber without adversely affecting weight gain. A total of 600 commercial broiler males were utilized for this study. Three hundred and sixty birds were placed in the hypobaric chamber at a simulated 9,500 ft above sea level, and 240 birds were placed at local elevation (1,260 ft above sea level). At each altitude, there were 4 treatments: 1) Full fed controls; 2) Feed available for 8 hours each day for 6 weeks, the duration of the study; 3) Feed available for 8 hours a day during the first 3 weeks, then full feed for the remaining 3 weeks; and 4) Full feed for the first week, then 3 weeks of 8 hour feed availability, then 2 weeks of full feed. Birds and feed were weighed weekly and birds which died were necropsied to determine the cause of death. At the end of the study, blood samples were taken and birds were necropsied, scored for ascites and organ weights were recorded. Birds grown in the hypobaric chamber with full feed had a significantly higher incidence of ascites syndrome and had hematological profiles typical of ascites syndrome. Birds raised in the hypobaric chamber and subjected to partial restricted feeding (Groups 3 & 4) had weight gains comparable to full fed controls and had blood profiles typical of ascites syndrome yet did not have a high incidence of mortality due to ascites. Clinical chemistry reflected the feeding status of the birds.

INTRODUCTION

Feed restriction is becoming a more common commercial treatment employed to reduce the incidence of ascites in broilers and roasters (1,2). This method is thought to have an effect by slowing the growth of the birds. One important observation to note is that slowing growth does not always reduce ascites incidence. When birds are grown in a hypobaric chamber, ascites incidence increases while the overall growth rate of the birds decreases. Birds grown at simulated high altitude are always much lighter than their counterparts grown in an environmentally matched chamber at a local altitude (1,260 ft above sea level). Another aspect of using feed restriction to prevent ascites is the timing and duration of the restriction. It would be advantageous to be able to restrict feed intake for only a short time and still have ascites mortality reduced. As birds develop ascites they begin to exhibit specific hematological changes (3,4). These changes occur prior to gross physical changes and can be used as markers that birds or groups are in the process of developing ascites syndrome. This experiment was designed to identify a feed restriction program that could be used to reduce the incidence of ascites syndrome in broilers raised in a hypobaric chamber, while allowing for compensatory growth.

MATERIALS AND METHODS

Six hundred vaccinated commercial broiler males (Cobb) were placed at one day of age. Three hundred sixty birds were placed at a simulated 9,500 ft above sea level. These birds were randomly assigned to one of four treatment groups (5 replicates/treatment group). Group 1 (Control) received full *ad lib* feed. Group 2 had feed available for eight hours daily for the duration of the experiment (six weeks). Group 3 had feed available for eight hours daily for the first three weeks then received full *ad lib* feed for the remaining three weeks. Group 4 had full *ad lib* feed for the first week, eight hour feed availability for the next three weeks, then full *ad lib* feed for the remaining two weeks. The remaining 240 chicks were placed at 1,260 ft above sea level. They were randomly assigned to the same feed treatments as the birds at simulated high altitude and there were five replicates/treatment group. Pen weights and feed intakes were obtained weekly with the birds at high altitude remaining at high altitude during the weighing. The feed was a complete starter and grower. Water was provided *ad lib* consumption, even during periods of feed restriction. Temperature and ventilation between the two chambers were

matched and set to maintain standard conditions. All birds which died were weighed and necropsied to determine the cause of death. At six weeks of age, blood samples were taken via heart puncture and clinical chemistry (550 Express Plus, Ciba Corning, Oberlin, Ohio) and automated blood cell counts (CELL-DYN 3500, Abbott Diagnostics, Abbott Park, Illinois) were conducted. Birds were necropsied, scored for ascites on a scale of 0-3 (0 having no sign of ascites, 3 having severe ascites), and weights of ventricles and total heart, liver, and spleen were obtained. The experimental design was a 2 x 4 factorial. Data were subjected to an ANOVA using SAS and, when necessary, means were separated using Duncan's multiple range test.

RESULTS AND DISCUSSION

At six weeks, under both simulated high altitude and local elevation, birds that had been restricted and then allowed full feed (Groups 3 and 4) weighed the same as full fed controls. Only birds that were restricted for the entire six weeks were significantly lighter. Birds in all treatments grown at simulated high altitude weighed significantly less than birds grown at the local elevation. The effect of feed restriction treatments on ascites incidence was the same at both altitudes. Ascites incidence was defined as the percentage of birds that either died from ascites or were scored at necropsy as being ascitic. Full fed controls had the highest ascites incidence (high altitude 65%, local elevation 27%) with all feed restriction groups having significantly less. At high altitude, Group 2 birds that were restricted the entire time had an ascites incidence that was even significantly lower than the other restricted groups (Group 2-4%, Group 3-39%, Group 4-26%). At low altitude the incidence of ascites was very low and not significantly different among restricted groups (Group 2-5%, Group 3-2%, Group 4-7%). The feed restriction programs used in this experiment were effective in decreasing ascites incidence and the timing was such that birds were able to exhibit compensatory gain after release from restriction. This indicates that further decreases in growth rate at simulated high altitude reversed the trend of increasing ascites incidence. While the partial restriction feed treatments (Groups 3 and 4) were adequate to reduce ascites incidence to the level of the completely restricted birds at local elevation, this was not the case at simulated high elevation. Resuming full feed at high altitude allowed for compensatory gain and resulted in increasing ascites incidence. It is probable that if these birds had been allowed to continue full feed for several more weeks, their ascites incidence would approach that of the full fed controls.

Organ weights and hematological parameters are frequently used to determine the development of ascites. Before a bird develops ascites right ventricular hypertrophy can be detected, along with an increase in RBC, hematocrit and hemoglobin levels. At necropsy, partially restricted birds (Groups 3 and 4) raised at simulated high altitude had as much right ventricular hypertrophy as the full fed controls. This was measured as right ventricle weight as a percent of total heart weight. They also had significantly higher RBC, hematocrit and hemoglobin levels than the birds restricted the entire time (Group 2). This is an indication that though these feed restriction programs allowed a decrease in ascites incidence at six weeks, the birds released from restriction were beginning to develop ascites. This indication of development of ascites was not seen in the birds grown at local elevation. At local elevation, all restricted groups (Groups 2, 3 and 4) had significantly less right ventricular hypertrophy, lower RBC, hematocrit and hemoglobin levels when compared with the full fed controls. Since the incidence of ascites syndrome at a low elevation is very low, we would not expect to see the development of ascites after removal of restriction. The timing of feed restriction may be important. Birds returned to full feed exhibited rapid growth and began to develop ascites. While not statistically significant, there was a numerical increase in the ascites incidence in birds that had been returned to full feed for longer periods. At high altitude, birds that had been on full feed for three weeks prior to the end of the study had a 39% incidence of ascites while birds that had only been on full feed for two weeks prior had an ascites incidence of 26%.

Blood samples taken at six weeks were analyzed to determine serum Ca, P, albumin, glucose, triglycerides, and total protein levels. There were no significant differences in Ca, albumin or total protein levels. Differences in glucose and triglycerides were observed at simulated high altitude. Blood samples were taken 1-2 hours after birds on feed restriction had their feed returned to them. This was reflected in increased levels of glucose and triglycerides in the Group 2 restricted birds. The other groups had not been deprived of feed and thus did not consume large quantities immediately prior to being bled. This effect was not seen at local elevation, perhaps because blood samples were drawn 3-4 hours after feed was returned. Thus, the difference in clinical chemistry appeared to be influenced by the feeding status of the birds.

REFERENCES

1. Acar, N., F. G. Sizemore, G. R. Leach, R. F. Wideman, Jr., R. L. Owen, and G. F. Barbato. Growth of broiler chickens in response to feed restriction regimes to reduce ascites. Poult. Sci. 74:833-843. 1995.

2. Arce, J., M. Berger, C. L. Coello. Control of ascites syndrome by feed restriction techniques. J. Appl. Poult. Res. 1:1-5. 1992.

3. Maxwell, M. H., G. W. Robertson, and S. Spence. Studies on an ascitic syndrome in young broilers 1. Haematology and pathology. Avian Pathol. 15:511-524. 1986.

4. Maxwell, M. H., S. G. Tullett, and F. G. Burton. Haematology and morphological changes in young broiler chicks with experimentally induced hypoxia. Res. Vet. Sci. 43:331-338. 1987.

OCCURRENCE OF SALMONELLA ENTERITIDIS IN COMMERCIAL LAYERS IN MOROCCO

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SUMMARY

The studies were conducted to investigate the status of *Salmonella enteritidis* (SE) contamination in 18 commercial layer operations in Morocco. Thirteen hundred and thirty-five samples including eggs, feed, dust, cloacal swabs and litter were collected. In total 5.24% of the samples were contaminated by *Salmonella* spp. Half of the farms were contaminated. The main source of *Salmonella* contamination was feed and no *Salmonella* were isolated from the eggs. *S. enteritidis* was the most frequent serotype (57.14%) and 88.9% of the 50% of the farms contaminated with *Salmonella* were infected with SE.

INTRODUCTION

Salmonella infection is considered to be common in poultry which is considered to be one of the largest reservoirs for the organism. *Salmonella* from poultry can be incriminated in large numbers of human infections. For a long time *Salmonella typhimurium* has been considered the most frequent non host-adapted *Salmonella* serotype in poultry. However, since the nineteen eighties, an upsurge of *Salmonella enteritidis* (SE) has been reported in many countries (1,2,3). In Morocco the incidence of SE infection in humans increased in the nineteen nineties. Eggs and the egg products were always incriminated (4,5,6). The main objective of the current study was to define the occurrence of SE in commercial layers in Morocco.

MATERIALS AND METHODS

Eighteen layer operations producing table eggs were involved in the study. The total capacity of the 18 farms was over 1,000,000 layers (between 6,000 and 240,000 layers per farm). The study was conducted between February and July, 1998. Each farm was visited 2 times for the collection of the samples. A total of 1335 samples including dust samples, cloacal swabs, litter, feed and eggs (fresh and stored) was collected. Conventional methods as described by Williams *et al.* (7) were used for the isolation and identification of *Salmonella* from the collected samples. All *Salmonella* isolates were serotyped according to the White and Kauffman scheme.

RESULTS AND DISCUSSION

The percentage of contamination by *Salmonella* in 1335 samples was 5.24% and *Salmonella* was isolated from 50% of the farms. No *Salmonella* was isolated from fresh and stored eggs. This result may be due to the number of eggs sampled. Many authors report low egg contamination with SE, even with large number of samples (8,9,10). The main sources of *Salmonella* isolation were feed (50%), dust (43%), litter (14%) and cloacal swabs (14%). The serotyping of isolates (Table 1) demonstrated the predominance of SE. SE was isolated in 88.9% of the contaminated farms. Its high occurrence in commercial layers in Morocco might explain the upsurge of human SE food-poisoning in

Table 1. Serotypes of Salmonella isolates from commercial layers in Morocco.

Serotype	Source					
	Eggs	Dust	Cloaca	Feed	Litter	%
S. enteritidis	-	+	+	+	+	57.14
S. pullorum/ gallinarum	-	+	+	+	-	21.43
S. infantis	-	-	-	+	-	7.14
S. typhimurium	-	-	-	-	-	7.14
S. virshow	-	-	-	+	-	7.14

Morocco. Studies are underway using plasmid profiling and finger- printing to compare the human and avian SE isolates.

REFERENCES

1. Humphrey, T. J., J. G. Grvickhank, H. Chart, and B. Rowe. Salmonella enteritidis PT4 : Chicken and eggs. The Lancet pp.720-722. 1988.

2. Poppe, C., K .A. Mc Fadden, A. M. Brouwer, and W. Demczuk. Characterisation of Salmonella enteritidis strains. Can. J. Vet. Res. 57:176-184. 1993.

3. Williams, M., B. Engström, and H. Wahlström. Control of Salmonella enteritidis in Sweden. Proc., International Course on Salmonella Control, Animal Production and Products, Sweden pp 29-34. 1993.

4. Zouhdi, M., A. Benouda, et M. A. Alaoui. Etat actuel de la résistance des salmonelles aux antibiotiques au CHU Ibn Sina-Rabat Biologie-Infectiologie, Tome II, N° spécial:24-28. 1996.

5. Benchekroun, T. S., A. Karim, M. J. Benkhraba, L. El Harim-Roudies, M. A. Alaoui, et A. El Malki Tazi. Salmonelloses non thyphoïdiques chez l'enfant. Biologie-Infectiologie, Tome II, 1:38:44. 1996.

6. Benouda, A., M. Zouhdi, Z. Hajjam, O. Sayrth, and M. A. Alaoui. Epidémiologie des salmonelloses non typhoïdiques. Biologie-infectiologie, Tome III, N° 3:42-46. 1997.

7. Williams, J. E., E. T. Mallinson, and H. G. Snoeynbos. Salmonellosis and Arizonosis. In: Isolation and Identification of Avian Pathogens, 2nd ed. S. B. Hitchner, C. H. Domermuth, H. G. Purchase, and J. E. Williams, eds. American Association of Avian Pathologists, New Bolton Center, Kennett Square, PA. pp. 1-8. 1980.

8. Hunton, P. Salmonella : perception versus reality. Poult. Int. 30:32-35. 1991.

9. Humphrey, T. J., H. Chart, A. Baskerville, and B. Rowe. The influence of age on the response of SPF hens to infection with Salmonella enteritidis PT4. Epidemiol. Infect. 106:33-43. 1991.

10. Henzler, D. J., E. Ebel, J. Sanders, D. Krodet, and J. Mason. Salmonella enteritidis in eggs from commercial chicken layer flocks implicated in human outbreak. Avian Dis. 38:37-43. 1994.

BEHAVIOUR OF A VERY VIRULENT MAREK'S DISEASE VIRUS IN TWO VACCINATED COMMERCIAL CHICKEN FLOCKS IN ARGENTINA

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INTRODUCTION

Marek's disease (MD) exists in virtually all commercial chicken flocks throughout the world and it has been considered the most serious of the many infectious diseases which afflict poultry (3). Exposure to the etiologic agent can take place soon after hatching, and mortality from the disease occurs after a few weeks to a few months. It may be sporadic throughout the lifespan of the flock or, in acute outbreaks, over 50% of the flock may succumb within a few weeks.

After the application of first generation MD vaccines in the early 1970s, problems with the occurrence of MD in vaccinated flocks were thought to be due primarily to mismanagement of the vaccines or to early heavy exposure with the virus. At that time, vaccination of 1-day-old chicks became a common practice, drastically reducing economic losses associated with MD. However, in the late 1970s vaccine breakdowns began occurring in flocks in the United States even though they had been properly vaccinated with herpesvirus of turkeys (HVT). Since then a number of very virulent or variant pathotypes of MD virus (MDV) have been isolated from various parts of the world. (2, 4, 5, 8, 9, 11, 12, 14, 17).

Increased incidence of MD in vaccinated flocks has also been observed in Argentina. Problems with the occurrence of MD in vaccinated flocks were thought to be due primarily to mismanagement of the vaccines (1), but the association of these problems with variant pathotypes of MDV has been reported later (2). One of those isolates, characterized as a very virulent (vv) field strain of MDV was chosen to challenge experimental chickens. Three vaccine viruses from each of the three MD viral serotypes were evaluated alone and in various combinations for protection against challenge with this vv MD virus. In this study the pathogenicity of the virus, efficacy of vaccination, the relative bursa weight, and the behaviour of the virus in two commercial chicken lines were evaluated. The preliminary results are presented here.

MATERIALS AND METHODS

Experimental chickens. Chickens were obtained from the specific-pathogen-free (SPF) flocks and two commercial chicken lines (Coms and Comw). Commercial and SPF birds of genetically MD-susceptible P-2a (B19 B19) and MD resistant N-2a (B21 B21) were housed in filtered-air isolators for the entire experimental period.

Virus source. A Marek's disease isolate previously typed as vvMDV NULP-4 was inoculated into SPF birds and spleens were collected aseptically and gently forced through a 60 μm autoclavable screen (Tetco, Inc., Elmsford, N.Y.). Cells were washed in phosphate-buffered saline, and separated by centrifugation over Ficoll-Hypaque (Pharmacia, Piscataway, N.J.). Lymphocytes at the interface were collected, washed, counted and stored at -196°C with 7% dimethylsulfoxide. NULP-4 was titred on chicken kidney cell cultures and herpesvirus plaques were serologically identified as serotype 1 by staining with specific monoclonal antibodies kindly provided by Dr. L.F. Lee and Dr. R.L. Witter (6).

Vaccines. The FC-126 strain of HVT (serotype 3) (16), 301-B1 (serotype2) (15), and CVI988 (Rispens) strain of MDV (serotype 1) (10) from commercial sources were used.

Pathology. Birds dying during the experiments or killed at the end of the experiments were necropsied and gross lesions were recorded. Questionable tissues were examined histologically. Tissues were embedded in paraffin and cut and the sections stained with hematoxylin and eosin.

Experimental design. One experiment with ten different treatments was carried out. Groups of 8 to 20 chickens from two commercial lines, Coms and Comw, and SPF P-2a (susceptible) and N-2a (resistant), were vaccinated subcutaneously with 2000 plaque forming units (PFU) of HVT, HVT+301-B1, Rispens (CVI988), CVI988+ HVT, at one day of age. Similar groups were left unvaccinated. All five groups were challenged intra-abdominally at 7 days of age with 2X106 spleen cells with NULP-4 per chicken. Uninoculated and unvaccinated birds served as negative controls. Chickens were observed for 70 days.

Statistical analysis. The chi-square test was used to determine differences between groups in MD incidence. Relative weights of the bursa of Fabricius were analyzed using the Fisher test (7, 13).

RESULTS

Results are shown in Table 1.

CONCLUSIONS

NULP-4 did not produce 100% mortality in vaccinated and non-vaccinated P-2a and non-vaccinated N-2a as reported before (2). Rispens vaccine alone or with HVT gave 100% protection except in one of the two commercial lines. Both commercial lines, chosen to represent results of MD in the field, showed resistance to MD.

In general the relative weights of bursa of Fabricius in survivor birds increased from the non-vaccinated groups, to groups vaccinated with HVT, to groups vaccinated with 301-B1+ HVT, to groups vaccinated with CVI988, and to groups vaccinated with CV1988 + HVT. They were higher in non-vaccinated and non-challenged groups.

REFERENCES

1. Buscaglia, C., and C. Crosetti. Studies on Marek's disease vaccination failures in Argentina. Proc. 2nd Meeting on the Cooperative Research Project in the Area of Veterinary Sciences between National University of La Plata and Japan International Cooperation Agency pp.19-21, 1993.

2. Buscaglia, C., P. Nervi, J. L. Garbi, and M. Píscopo. Isolation of very virulent strains of Marek's disease virus from vaccinated chickens in Argentina. Proc.44th Western Poultry Disease Conference, 5-7 March 1995, Sacramento, CA. pp 53-57. 1995.

3. Calnek, B. W., and R. L. Witter. Marek's disease. In: Diseases of Poultry, 10th ed. B. W. Calnek, J. Barnes, C.W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 369-413. 1997.

4. Edison , C. S., R .K. Page, and S. H. Kleven. Effectivness of cell-free and cell-associated turkey herpesvirus vaccine against Marek's disease in chickens as influenced by maternal antibody, vaccine dose, and time of exposure to Marek's disease virus. Avian Dis. 22:583-597. 1978.

5. Imai, K., N. Yuasa, H. Iwakiri, K. Nakamura, H. Hihara, T. Ishita, A. Inamoto, I. Okamoto, K. Ohta, and M. Maeda. Characterization of very virulent Marek's disease virus isolated in Japan. Avian Path. 21:119-126. 1992.

6. Lee, L. F., X. Liu, and R. L. Witter. Monoclonal antibodies with specificity for three different serotypes of Marek's disease viruses in chickens. J. Immunol. 130:1003-1006. 1983.

7. Lison, L. Estadistica Aplicada a la Biologia Experimental. Bs As: Editorial Universitaria de Buenos Aires. 1-357.

8. McKim-Breschkim, J. T. Faragher, J. Withell and W. M. Forsyth. Isolation of very virulent Marek's disease viruses from vaccinated chickens in Australia. Aust. Vet. J. 67:205-209. 1990.

9. Powell, P.C., and F. Lombardini. Isolation of very virulent pathotypes of Marek's disease virus from vaccinated chickens in Europe. Vet. Rec. 118:688-691. 1986.

10. Rispens, B. H., H. v. Vloten, N. Mastenbroek, H. J. L. Maas, and K. A. Schat. Control of Marek's disease in the Netherlands. I. Isolation of an avirulent Marek's disease virus (strain CV1 988) and its use in laboratory vaccination trials. Avian Dis. 16:108-125. 1972.

11. Schat, K. A., B. W. Calnek, J. Fabricant, and H. Abplanalp. Influence of oncogenicity of Marek's disease virus on evaluation of genetic resistant. Poult. Sci. 60:2559-2566. 1981.

Table 1. Protective efficacy of day-old vaccination and relative bursal weight in survivors in SPF P-2a and N-2a chickens and commercial lines coms and comw challenged with NULP-4 at 8 days of age.

Chicken strain	Vaccine	Challenge	Birds with MD/ No. In Group (%)[A]		Relative weight of bursa of Fabricius[B]
P-2a	None	Yes	16/18	(88.9)**	2.14^{c}
P-2a	HVT	Yes	6/8	(75.0)	1.81^{b}
P-2a	301-B1+HVT	Yes	2/22	(9.1)	1.66^{b}
P-2a	CV1988	Yes	0/18	(0.0)	1.62^{b}
P-2a	CV1988+HVT	Yes	0/8	(0.0)	1.70^{b}
N-2a	None	Yes	4/10	(40.0)**	1.29^{b}
N-2a	HVT	Yes	8/22	(36.4)	1.20^{a}
N-2a	301-B1+HVT	Yes	0/20	(0.0)	1.73^{b}
N-2a	CV1988	Yes	0/18	(0.0)	2.71^{d}
N-2a	CV1988+HVT	Yes	0/8	(0.0)	0.96^{a}
Coms	None	Yes	4/24	(16.7)**	2.23^{c}
Coms	HVT	Yes	12/20	(60.0)	2.92^{d}
Coms	301-B1+HVT	Yes	0/16	(0.0)	4.13^{g}
Coms	CV1988	Yes	2/18	(11.1)	1.43^{b}
Coms	CV1988+HVT	Yes	0/16	(0.0)	4.75^{h}
Coms	None	No			5.53^{i}
Coms	HVT	No			1.91^{b}
Coms	301-B1+HVT	No			4.19^{g}
Coms	CV1988	No			3.45
Coms	CV1988+HVT	No			4.87^{h}
Comw	None	Yes	2/12	(16.7)*	1.78^{b}
Comw	HVT	Yes	0/12	(0.0)	2.55^{c}
Comw	301-B1+HVT	Yes	0/20	(0.0)	3.80^{f}
Comw	CV1988	Yes	0/18	(0.0)	2.92^{d}
Comw	CV1988+HVT	Yes	2/12	(16.7)	3.91^{f}
Comw	None	No			4.40^{h}
Comw	HVT	No			2.17^{c}
Comw	CV1988	No			3.31
Comw	CV1988+HVT	No			3.67^{f}

[A] Data from birds in a given genetic strain were compared by the chi-square test; ** indicates significant differences ($P<0.01$); * indicates significant differences ($P<0.05$)

[B] Values with different superscripts were significantly different ($P<0.05$)

12. Schat, K. A., B. W. Calnek, and J. Fabricant. Characterization of two higly oncogenic strains of Marek's disease virus. Avian Path. 11:593-605. 1982.

13. Snedecor, G. W., and W. G. Cochran. In:Statistical Methods, 7th ed. Iowa State University Press, Ames, Iowa. 1980.

14. Witter, R. L. Characteristics of Marek's disease viruses isolated from vaccinated commercial chicken flocks: association of viral pathotype with lymphoma frequency. Avian Dis. 27:113-132. 1983.

15. Witter, R. L. New Serotype 2 and attenuated serotype 1 Marek's disease viruses: comparative efficacy. Avian Dis. 31:752-765. 1987.

16. Witter, R. L., K. Nazerian, H. G. Purchase, and G. H. Burgoyne. Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. Am. J. Vet. Res. 31:525-528. 1970.

17. Witter, R. L., J. M. Sharma, and A. M. Fadly. Pathogenicity of variant Marek's disease virus isolants in vaccinated and unvaccinated chickens. Avian Dis. 24:210-231. 1980.

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MACROSCOPIC AND MICROSCOPIC PATHOLOGY OF AN EXOTIC NEWCASTLE DISEASE OUTBREAK

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Newcastle disease is a viral disease that affects many species of birds (1). It is caused by avian paramyxovirus type 1. Disease severity may vary from inapparent infection to severe respiratory signs, diarrhea, paralysis and death. The less pathogenic (lentogenic and mesogenic) strains are commonly found in poultry in the United States. However, velogenic Newcastle disease is considered a foreign animal disease in the U.S.A., and currently is defined as exotic Newcastle Disease (END), regardless of the tissue tropism (3). This paper describes the macroscopic and microscopic lesions observed in a back yard flock of game chickens infected with END.

Case history. An isolated case of END occurred in a back yard flock of Aseel, Shamo, and Malay game chickens in the Central Valley of California. In 1998, between the last week of May and first week of June, 33 chickens (19 dead and 14 live) from a flock of 48 chickens were submitted for diagnosis to the California Veterinary Diagnostic Laboratory System (CVDLS), Fresno branch. Hens and immature chickens kept in a common pen showed sudden signs of listlessness, respiratory distress, diarrhea, and death. All male adult chickens were kept, in individual pens, at the opposite side of the yard from where the outbreak started. None of these male birds became sick.

The flock had been vaccinated with B1 Newcastle disease vaccine in the water during June, 1997. The last bird introduced to the flock was a male, purchased from a swap meet approximately 1 month before the outbreak.

Gross pathology. Complete necropsies were performed on all the submitted game chickens. The most significant gross findings were dehydration, cyanosis of comb and head, and multifocal linear hemorrhages and/or ulcers in the larynx, trachea and throughout the digestive tract, including the oral cavity, esophagus, proventriculus, and intestines. Hemorrhages were most severe in lymphoid tissue, such as the lymphoid tissue at the esophageal-proventricular junction, cecal tonsils and thymus. Most chickens also had congestion and hemorrhage in the conjunctiva. Gross changes were not observed in the nervous system and lower respiratory tract.

Histopathology. Tissues were collected for histopathology, fixed in 10% buffered neutral formalin, processed and examined by light microscopy. Necrosis, hemorrhage, and inflammation were most severe in association with lymphoid tissue, including cecal tonsils, bone marrow, bursa of Fabricius, thymus and spleen. Similar changes were also found in the mucosa of the conjunctiva, trachea, esophagus, proventriculus, and intestines. There was extensive vasculitis in many organs and occasional thrombosis of vessels in the lung and liver. Mild perivascular cuffing was associated with occasional blood vessels of the brain.

Portions of esophagus from 3 chickens were prepared for transmission electron microscopy (TEM) and examined. Occasional endothelial cells contained parallel linear structures, consistent with the morphology of nucleocapsids of paramyxoviruses, in their cytoplasm. The average nucleocapsid diameter was ± 13.8 nm. Collins and Gough (2) reported a similar nucleocapsid diameter for avian paramyxovirus.

Hemorrhages in the conjunctiva and trachea, and linear hemorrhagic ulcers in the gastrointestinal tract, especially involving the esophagus, proventriculus and cecal tonsils,

and necrosis of lymphoid tissue are characteristic, but not pathognomonic, for END (1). Isolation and identification of the virus are essential for confirmation of END. Avian paramyxovirus type I was isolated from trachea and lung, cecal tonsils and conjunctiva separately. The isolate was classified as highly pathogenic (MDT = 49 hours, IVPI = 2.86) and produced early death with severe gross and histologic changes in experimentally inoculated chickens.

This END outbreak could have had a large impact on commercial poultry in California, as well as for the rest of the United States. Its rapid diagnosis was extremely important in limiting this disease to the original back yard flock. The flock was depopulated as soon as the virus was classified END. The outbreak was resolved within 14 days of the on-set of clinical signs, and since then no other clinical cases have been seen. Preventing the introduction of NDV by strict biosecurity, rapid diagnosis, and depopulation is essential if END is to be controlled.

REFERENCES

1. Alexander, D. J. Newcastle disease and other avian Paramyxoviridae infections. In: Diseases of Poultry, 10th ed. B. W. Calnek, H. J. Barnes, W. M. Beard, L. R. McDougald and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp. 541-569. 1997.

2. Collins, M. S., and R. E. Gough. Characterization of a virus associated with turkey rhinotracheitis. J. Gen. Vir. 69:909-916. 1988.

3. Kleven, S. H., and R. J. Eckroade. Report of the committee on transmissible diseases of poultry and other avian species. Proc. 101st Annual Meeting of the United States Animal Health Association. Louisville, Kentucky. pp. 473-519. 1997.

RESULTS OF ANTICOCCIDIAL SENSITIVITY TRIALS DONE ON AVIAN COCCIDIA COLLECTED FROM ABOVE, AVERAGE AND BELOW AVERAGE PRODUCTION FARMS

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Battery cage sensitivity trials were run with up to 10 different anticoccidial compounds using mixed coccidial species inocula obtained from pooled litter samples collected from above, average, and below average production farms located in 5 geographically different broiler growout complexes. Results were analyzed to determine the extent of anticoccidial resistance of the coccidia from the different production farms by determining the degree of efficacy for each anticoccidial in chickens at 6 days post-challenge (PI) with the pooled inocula. Lasalocid (100 ppm), salinomycin (60 ppm) and semiduramicin (25 ppm) were found to have an 80 to 100% efficacy rating, as defined by a 45% or greater protection of weight gain coupled with lowered intestinal lesion scores and feed conversions measured at 6 days PI, against the coccidial species and strains found in the above average farms from each of the 5 different geographical areas. Monensin (110 ppm), narasin (80 ppm), nicarbazin (125 ppm) and robenidine (33 ppm) were 40 to 60% effective against coccidia from these same farms. All anticoccidials tested showed a lower efficacy rating, varying from complete resistance (0%) to 80% effectiveness, for coccidial species and strains in the pooled inocula from average and below average production farms. Lasalocid consistently showed the greatest efficacy against coccidia in all farms tested. Addition of roxarsone (50 ppm) to lasalocid did not increase the efficacy rating for this anticoccidial with any of the challenge inocula. Results of these studies showed that considerable variability in anticoccidial resistance does occur between farms with different production rankings.

RESULTS OF A SALMONELLA ENTERITIDIS VACCINATION FIELD TRIAL IN COMMERCIAL LAYER FLOCKS IN THE NETHERLANDS

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The effect of vaccination added to a Standardized Biosecurity Programme (SBP) on reduction of *Salmonella enteritidis* infections in commercial layer flocks was evaluated in a field trial in The Netherlands. The effect of vaccination was studied in situations of increased infection risk and its effect on serological *Salmonella* tests (gm-DAS-ELISA and indirect LPS-BD-ELISA) was evaluated. An attenuated *S. gallinarum* 9R live vaccine (Nobilis SG®, Intervet, The Netherlands) was used. Flocks were vaccinated subcutaneously twice at 6 and at 14-16 weeks of age. A total of 81 commercial layer flocks (2,256,725 chickens) with *S. enteritidis* infection in a previous flock or in a current flock on the same farm were vaccinated. These 81 flocks were housed on 30 different farms with a SBP (or an application for the same), 38 flocks were housed in free range houses (four with outside runs), 34 in battery houses and 9 flocks in aviary houses (2 with outside runs).

The occurrence of *S. enteritidis* in vaccinated flocks was assessed by serological examination at 10, 16, 24, 32, 40, 48, 56, 64 and 72 weeks of age. At each sampling 60 bloods were collected from each flock and sera were pooled into 10 pools. For *Salmonella* diagnosis the indirect LPS-BD-ELISA and the gm-DAS-ELISA, which are tests for detecting antibodies against Salmonella group B/D antigen and *S. enteritidis* flagellar antigen, respectively, were conducted. The vaccine induced an antibody response in the LPS-BD-ELISA as expected and as a result vaccinated flocks were eventually only tested with gm-DAS-ELISA. Detection of a *S. enteritidis* field infection is still possible with serological monitoring.

This trial has yet to be completed. In January, 1999, one vaccinated flock had become infected with *S. enteritidis* at 72 weeks of age (1.2%). The vaccinated group has been compared with a non-vaccinated group that was monitored in accordance with a compulsory monitoring programme for *S. enteritidis* (once, six weeks before slaughter). This group was hatched in the same period as the vaccinated flocks. On this non-vaccinated group 11.6% (192/1660) *S. enteritidis* infections have been detected todate. The results of vaccination look promising but have yet to be statistically analyzed.

BIOTYPING OF HAEMOPHILUS PARAGALLINARUM FROM AVIAN CORYZA CASES IN MEXICO

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In Mexico, infectious coryza is an enzootic disease found mainly in densely populated poultry areas. The purpose of the present work was to determine the carbohydrate fermentation and antimicrobial patterns of isolates, to serotype the isolates by hemagglutination-inhibition test, and to determine the frequency of serotypes of *Haemophilus paragallinarum* in clinical cases in central Mexico. Forty isolates were tested by techniques proposed by Blackall and Yamaguchi (1, 4). All isolates acidified glucose and mannose and were negative in galactose, lactose, trehalose and xylose. There were differences between isolates in maltose, mannitol and sucrose. The isolates were grouped in four biochemical biovariants. All isolates were susceptible to penicillin, ampicillin and erythromycin. There were differences between isolates in susceptibility to streptomycin, neomycin and tetracycline. The isolates were placed in five groups according to susceptibility. The hemagglutination-inhibition test was used to serotype the isolates according to Page's scheme (3). Twenty-one isolates (52.5%) were serotype A, 5 (12.5%) were B, and 14(35%) were C. The discriminatory ability of the three typing techniques was analyzed using the Simpson's diversity index described by Hunter and Gaston (2). The highest index (0.960) was obtained combining the three techniques.

REFERENCES

1. Blackall, P. J., L.E. Eaves, and D.G. Rogers. Serotyping of Haemophilus paragallinarum by the Kume hemagglutinin scheme-proposal of a new serovar and altered nomenclature. J. Clin. Microbiol. 28:1185-1187. 1990.

2. Hunter, P. R., and M. A. Gaston. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. 26:2465-2466. 1988.

3. Page, A. L. Haemophilus infections in chickens. 1. Characteristics of 12 Haemophilus isolates recovered from diseased chickens. Am. J. Vet. Res. 23:85-95. 1962.

4. Yamaguchi, T., P J. Blackall, S. Takigami, Y. Iritani, and Y. Hayashi. Pathogenicity and serovar-specific hemagglutinating antigens of Haemophilus paragallinarum serovar B strains. Avian Dis. 34:964-968. 1990.

ADHERENCE OF HAEMOPHILUS PARAGALLINARUM TO CHICKEN TRACHEAL EPITHELIAL CELLS

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INTRODUCTION

The microbial adherence to host cells is an essential step in infection (1). It is unknown if there are any differences between serotypes or isolates of *Haemophilus paragallinarum* in adherence to chicken respiratory cells. The purpose of the present research was to determine any variation in adherence of forty serotyped isolates of *Haemophilus paragallinarum* to chicken tracheal epithelial cells.

MATERIALS AND METHODS

Bacteria and cultures. Strain 0083, 0222 and Modesto (Page's serotypes A, B and C) provided by Escuela Nacional de Ciencias Biológicas, Instituto Politécnico Nacional were used as *Haemophilus paragallinarum* reference strains. Forty field-isolates were studied. A *Pasteurella avium* field isolate was employed as control. All bacteria were cultured in fermentation broth (DIFCO) with 1% yeast extract and 1% heat-inactivated horse serum. The cultures were incubated at 37°C for 20 hr. The bacterial cells were precipitated by centrifugation at 5000 rpm for 15 minutes at 4°C. Bacterial cells were resuspended in phosphate buffer solution (pH 7.4) containing 1.2×10^9 bacteria/ml to an optical density equivalent to a McFarland number 4 tube (2).

Cells. Chicken tracheal epithelial cells were obtained from 7-week-old broiler chickens raised in our laboratory. Cells were obtained in erythrocyte lysis buffer with 1% bromhexine (3). After three hours storage at 4 °C, cells were resuspended in Hank-gelatin solution.

Adherence test. Equal volumes of each isolate and tracheal epithelial cells were mixed at 37° C for 1 hr. After a double wash of mixed cells in Hank-gelatin solution, cells were fixed to slides and stained with Giemsa-May-Grünwald and basic fuchsin. All bacteria adhered to twenty-five tracheal epithelial cells were counted. Tukey test was used for statistical analysis (2).

RESULTS AND DISCUSSION

Table 1. Adherence of Haemophilus paragallinarum and Pasteurella avium isolates to chicken epithelial tracheal cells.

Serotype	Isolates	Mean	Standard Deviation
A	21	36.182	6.785
B	5	32.200	4.404
C	14	33.957	5.965
Pasteurella avium	1	0.0000	0.000

Table 2. Comparison between adherence means of Haemophilus paragallinarum isolates to chicken epithelial tracheal cells by Tukey test.

Serotype	Mean Differences	Confidence Range	Significance ($p<0.01$)
A vs B	0.1545	0.0578 - 0.2508	***
A vs C	0.0862	0.0193 - 0.1531	***
B vs C	-0.0681	-01691 - 0.0330	

Other authors have proposed that *Haemophilus paragallinarum* hemagglutinins are main pathogenic and virulence factors, and it is accepted that hemagglutinins are lipopolysaccharides of the bacterial outer membrane (4,5,6). In the present work, serotype A isolates adhered to epithelial cells significantly more than serotypes B and C, and bacterial adherence was higher to cilia than the remaining body cell (data not showed). Some isolates showed adherence to the entire whole cell. Yamaguchi *et al.* (7) identified other hemagglutinins in *Haemophilus paragallinarum* field-isolates serotyped as B. The variation in the ability of the bacteria to adhere to epithelial tracheal cells seemed to be a characteristic of an isolate rather than a characteristic of a serotype.

REFERENCES

1. Bonilla-Ruz, L. F., and G. A. García-Delgado. Adherence of Pasteurella multocida to rabbit respiratory epithelial cells *in vitro*. Rev. Lat. Amer. Microbiol. 35:361-369. 1993.

2. Glorioso, J. C. Adhesion of type A Pasteurella multocida to rabbit pharyngeal cells and its possible role in rabbit respiratory tract infections. Infec. Immun. 35:1103-1109. 1982.

3. González, A. C. Patogenicidad de Escherichia coli aisladas de casos clínicos de septicemia aviar en México. Demostración de producción de la toxina letal para pollos por inoculación en pollitos, y adherencia a células epiteliales traqueales. M.V.Z. thesis. Universidad Nacional Autónoma de México, México. 1993.

4. Iritani Y., T. Yamaguchi, K. Katagari, and H. Arita. Hemagglutination inhibition Haemophilus paragallinarum type 1 hemagglutinin by lipopolysaccharide. Am. J. Vet. Res. 42:689-690. 1981.

5. Ueda, S., Y. Nagasawa, T. Suzuki, and M. Tajima. Adhesion of Haemophilus paragallinarum to cultured chickens cells. Microbiol. Immunol. 61:251-253. 1982.

6. Yamaguchi T., M. Kobayashi, S. Masaki, and Y. Iritani. Isolation and characterization of a Haemophilus paragallinarum mutant that lacks a hemagglutinating antigen. Avian Dis. 37:970-976. 1993.

7. Yamaguchi T., P. J. Blackall, S. Takigami, Y. Iritani, and Y. Hayashi. Pathogenicity and serovar-specific hemagglutinating antigens of Haemophilus paragallinarum serovar B strains. Avian Dis. 34:964-968. 1990.

DETERMINATION OF HEMAGGLUTINATION-INHIBITION ANTIBODIES AGAINST INFECTIOUS CORYZA IN LAYER HENS VACCINATED IN THE FIELD

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A sample of commercial layer hens vaccinated in the field were tested by the hemagglutination-inhibition test and challenged to determine the protective titer against respiratory signs of infectious coryza. A modified method was used for production of hemagglutinin antigens; a fermentation broth with 1% horse serum was used. There was neither enzymatic, physical or chemical treatment. Isolates from outbreaks of disease in the field were used for challenge to measure the protective effect of bi- and trivalent commercial vaccines and to relate this to hemagglutination-inhibition antibodies as described by others. A titer of 40 was observed to be protective against respiratory signs of the disease, similar to other reported studies. It was concluded that the method used for hemagglutinin antigen production is suitable for determination of hemagglutination-inhibition antibodies in routine serology to predict the protective effect produced by bi- and trivalent commercial vaccines under field conditions.

DETERMINATION OF EFFECTIVE ROUTES OF ADMINISTRATION FOR INCLUSION BODY HEPATITIS / HYDROPERICARDIUM SYNDROME KILLED VACCINE

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SUMMARY

One-day-old commercial birds were used to compare 3 different routes of administration for a commercial killed inclusion body hepatitis (IBH) / hydropericardium syndrome (HPS) oil emulsion vaccine. At 12 days of age the birds received a 0.5 ml / dose of vaccine. The first group was vaccinated subcutaneously in the wing fold (WF). A second group was vaccinated intramuscularly deep in the upper portion of the breast (IM). A third group was vaccinated subcutaneously in the middle of the back of the neck (SQ). One non-vaccinated group was used as a control (CTRL). At 21 days post-vaccination, all the birds were challenged with a highly pathogenic fowl group 1 adenovirus (FAV 1). The birds were observed for 7 days after challenge. Based on morbidity, mortality, liver and cardiac lesions, microscopic lesions and direct immunofluorescence, it can be concluded that the SQ route is the most effective route of administration, and that the WF application can be a viable alternative.

INTRODUCTION

IBH/HPS can kill up to 80% of infected broilers in a 2 to 3 week outbreak. Killed oil emulsion vaccines have proven their efficacy against mortality due to IBH/HPS. Poultry producers use different routes to inject killed vaccines. In Asia it is very common to inject subcutaneously in the wing fold, while in Latin America it is common to inject killed vaccines subcutaneously in the back and midportion of the neck in broilers and to use the deep intramuscular route in the upper portion of the breast in breeders and layers. The objective of this study was to compare and determine the most effective route of administration for IBH/HPS killed oil emulsion vaccines to protect against a very virulent IBH/HPS virus.

MATERIALS AND METHODS

One-day-old commercial birds were raised in isolation units. They were divided in four groups each with 10 birds. Water and commercial feed were supplied *ad libitum*. 0.5 ml of a commercial killed IBH/HPS vaccine was administered to the birds at 12 days of age. Group A was vaccinated subcutaneously in the wing fold (WF). Group B was vaccinated intramuscularly deep in the upper portion of the breast (IM). Group C was vaccinated subcutaneously in the middle of the back of the neck (SQ). Group D was not vaccinated. Twenty one days after vaccination all groups were challenged with a highly pathogenic strain of IBH/HPS (strain DCV-94), $10^{4.0}$ $CLD_{50\%}$ in 0.5 ml per bird by the intramuscular route. The birds were observed three times a day for 7 days after the challenge. Morbidity and mortality were recorded daily. All dead birds were necropsied and livers were collected for histopathology, virology and immunofluorescence (DIF) tests. On histopathology livers were examined for intranuclear inclusion bodies.

RESULTS

Birds in group A (WF) had no morbidity, mortality or hepatic lesions, but were 30% positive to FAV-1 by DIF. Birds in group B (IM) had 30% morbidity, mortality and hepatic lesions, and were 30% positive to FAV-1 by DIF. Birds in group C (SQ) had no morbidity, mortality or hepatic lesions and were negative to FAV-1 by DIF. Birds in group D (control) had 100% morbidity and mortality after 72 hours, with 100% hepatic lesions, and were 100% positive to FAV-1 by DIF.

DISCUSSION

The SQ route fully protected the birds against challenge virus. The WF route can be a viable alternative but the technique is not very convenient. The IM route did not provide full protection as was expected. The SQ route did not present any difficulties for those who injected the vaccine, and no abnormal reactions were seen at site of injection. Strain DCV-94 (challenge strain) proved its virulence by killing all the birds in a control group in a 72 hours period with signs and lesions typical of IBH/HPS.

TWENTY YEARS EXPERIENCE IN CONTROLLING FOWL CHOLERA IN CHICKENS, DUCKS AND TURKEYS IN EGYPT

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Economically, perhaps the most important enzootic bacterial disease that currently threatens poultry breeding in Egypt is fowl cholera (FC) (Avian Pasteurellosis) (4). This disease is caused by *Pasteurella multocida* organisms of various serotypes. Several epornitics of FC have been recorded in Egypt for twenty years (1). According to the annual reports of the General Organization of Veterinary Services about 30 to 40 outbreaks of the disease occur annually on private and government farms.

The first step in control was the recognition of the most predominant serotypes responsible for these outbreaks. Isolates of *P.multocida* are classified into five capsular types designated A, B, D, E and F, and 16 somatic types on the basis of antigenic differences in their lipopolysaccharides. A standardized system for designating serotypes has been recommended (2). Using this system the four important avian *P.multocida* serotypes encountered in Eqypt were A:1, A:3, A:4 and D:11. This serotyping scheme has facilitated the understanding of the epidemiological features and immunoprophylaxis of pasteurellosis in chicken, ducks and turkeys (1).

Further characterization of *P.multocida* field isolates recovered from major outbreaks of FC was done by using protein profile and DNA fingerprinting pattern analysis (7). Comparison was made with reference standard strains of the same serotype. It was concluded that Egyptian field isolates of *P.multocida* had their own characteristic protein profiles that justified using some of them as seed materials for preparation of vaccines. This finding was utilized in the selection of local isolates for preparation of an Egyptian polyvalent vaccine. Only one isolate was similar to the CU vaccinal strain as it had a specific 48 KDa protein band. This isolate was obtained from a farm that was using CU vaccine. The protein analysis conducted differentiates between the vaccinal CU strain belonging to A:3x4 serotype (3) and field isolates of the same serotype. Computer analysis of restriction endonuclease Hpa II patterns using genomic DNA from 4 *P.multocida* standard strains and 8 local field strains also revealed the existence of genotypic differences among strains of the same serotype. The plurality of *P.multocida* serotypes recognized among different avian species made it imperative to rely on either autogenous or polyvalent bacterins.

In a five year investigation of duck farms plagued with epornities of avian pasteurellosis, nine of 58 isolates of *P. multocida* showed variation in xylose fermentation activity. Seven A:3 serotypes revealed cross reaction with the A:4 serotype and accordingly were designated as A:3x4 (5). The same serotypes were repeatedly isolated from duck farms during the five year investigation in spite of autogenous vaccinations. This was explained by carrier birds, vaccination failure due to immune suppression and management errors (multi-age rearing, over crowding, bad hygiene and poor environment control) encouraging spread and perpetuation of infection. The study also revealed the importance of checking any variation in serotypes of field isolates serotype to ensure effective vaccination with autogenous bacterins. Higher protection against avian pasteurellosis was provided by a polyvalent oil adjuvant vaccine prepared from the most frequent *P.multocida* field isolates in comparison to imported vaccines. In layer chickens the best protection is obtained by inoculation of either three doses of locally prepared oil adjuvant polyvalent vaccine at 8, 14 and 20 weeks of age, or by primary vaccination of chicken with one dose of CU vaccine followed by two doses of polyvalent vaccine or by vaccination with two doses of CU vaccine followed by one dose of polyvalent vaccine. An outer membrane protein (OMP) vaccine with an alhydragel adjuvant experimentally inoculated induced a significant increase in anti- *P.multocida* titers and high protection against challenge infection.

REFERENCES

1. Azzam, A. H., S. M. Gergis, and A. M. W. Kheir Eldin. Comparative efficacy and safety of polyvalent bacterins and avirulent live CU fowl cholera vaccines in protecting chicken and turkeys against fowl cholera. Proc. 2nd Cong. Fac. Vet. Med. Cairo Univ. pp. 75-77. 1992.

2. Carter, G. R., and M. M. Chengappa. Recommendations for standard system of designating serotypes of Pasteurella multocida. Proc. Ann. Meet. Am. Assoc. Vet. Lab. Diagn. 24:37-41. 1981.

3. Derieux, W. T. Serotype results of Pasteurella multocida isolated from poultry in South Carolina. Avian Dis. 18:265-266. 1974.

4. Gergis, S. M. Fowl cholera vaccine production in Egypt. Im. Symp. Canada Egypt McGill Agric. Response Program (CEMARP). pp. 83-85. 1987.

5. Gergis, S. M., I. A. Ghanem, E. Y. El-Naemaeey ,and M. M. Aly. Studies on Pasteurella multocida in duck farms and merits of autogenous vaccination. Proc. Egyptian-German Vet. Med. Conference. pp. 503-510. 1992.

6. Rhoades, K. R., and R. B. Rimler. Somatic serotypes of Pasteurella multocida isolated from avian hosts (1976-1988). Avian Dis. 34:193-195. 1990.

7. Seham, A. E., W. A. Ghoniemy, H. A. Fadl, and S. M. Gergis. Protein profile and DNA fingerprinting for comparison of some Pasteurella multocida serotypes locally isolated from cases of fowl cholera with standard serovars. Proc. 4th Vet. Med. Zag. Conference. pp. 161-171. 1998.

STUDIES ON THE EFFECTS OF RECOMBINANT CHICKEN INTERFERON ON THE INFECTION, REPLICATION AND SPREAD OF ALV-J IN VITRO

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Avian leukosis virus-J subgroup (ALV-J) causes a variety of neoplastic manifestations, most typically in broiler strains of chickens. The virus has been isolated from both mature broiler breeders and also broilers as young as four weeks of age. Due to the widespread problems this virus has caused the poultry industry, coupled with the fact that vaccines against avian retroviruses have not been completely successful in the past, it was decided to determine if the virus could be controlled by means other than vaccination.

In this initial work, a recombinant chicken interferon (ChIFN) was used to affect changes *in vitro* on the growth of ALV-J in a chick embryo fibroblast (CEF) culture system. In the first experiments, graded doses of ChIFN, from 10 units per ml to 10,000 units per ml, were added to CEF cultures immediately after infection with 10-fold serial dilutions of ALV-J (Hc1 strain). At 4, 7, and 10 days post-initiation (dpi) supernatants were sampled and the cultures trypsinized and the medium replaced to promote cell replication. A final harvest of both the supernatant and cells was done at 16 dpi. Samples were tested by antigen-capture ELISA for the presence of p27, a major group-specific antigen of avian leukosis/sarcoma viruses. The results showed a clear dose-response with the ChIFN. Complete suppression of p27 expression at 4 dpi was achieved with doses as low as 10 units per ml. It was also apparent that once the blocking effect of ChIFN was removed, virus replication and expression of antigen commenced, thus indicating that production of infectious virus was not affected. However, levels of expression of antigen following removal of the ChIFN were reduced in a dose-response manner.

In a second series of experiments, CEFs were infected with ALV-J at a single constant concentration of approximately 10^3 $TCID_{50}$. The ChIFN was administered in a series of concentrations from 10 units per ml to 5000 units per ml using different increments than those used in the first experiment. Cultures were treated in a similar manner to those previously, but both supernatants and cells were harvested at each time point and tested by ELISA. The purpose of this was to determine if the virus was accumulating within the cells, but not elaborated into the supernatant. Additionally, supernatants taken at 4 dpi were reinoculated into fresh, untreated CEFs to determine if the p27 detected was indicative of infectious virus. In these experiments, the dose-response effect of the ChIFN was reproduced. The results again showed a reduction in p27 antigen at doses as low as 10 units per ml at 4 dpi and inhibition of p27 expression to levels below the positive/negative cutoff value were seen at a dose of 1000 units or higher. The p27 antigen levels from within cells was consistently higher than that found in the supernatants until termination of the experiment, at which time the antigen levels between the two fractions were equivalent. The results of subculturing of the supernatants after 5 days in culture showed that the p27 antigen detected was indicative of infectious virus as the p27 levels of the harvested subcultures paralleled the levels in the transferred supernatants. Once again, the levels of virus expression climbed rapidly upon removal of the block on day 4 dpi.

Further studies, including demonstration of the effects of administration of ChIFN either prior to or subsequent to ALV-J infection are underway.

TREATMENT OF FERTILE HATCHING EGGS WITH A MIXTURE OF GLUTARALDEHYDE AND QUATERNARY AMMONIA COMPOUNDS, THE EFFECT ON MICROBIAL COUNTS

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INTRODUCTION

The hygiene of fertile hatching egg is important in obtaining maximum hatchability and good quality chicks (4). The demand for chicks and poults and the movement of people, vehicles and material between farms and hatcheries has been increasing. Strict sanitary standards that permit the collection of only clean fertile hatching eggs are needed (1). A great source of microbial contamination within the hatchery is contaminated hatching eggs collected on the breeder farms. Fussell showed that eggs may be contaminated by the passage of bacteria through the shell which contains 7000 - 17000 pores of which 1% are open (2). The temperature and humidity conditions inside incubators and hatching machines allow proliferation of bacteria and molds. Some eggs may explode providing a culture medium for bacterial growth. The most common ways to produce hatching eggs are to keep nests clean and dry, to collect only clean eggs and only from the nest and to spray eggs with a disinfectant solution. In this study a mixture of glutaraldehyde and cocobensildimetilamonium was evaluated as a disinfectant for hatching eggs.

MATERIALS AND METHODS

Three groups of twenty fertile hatching eggs were collected at a breeder farm and were either untreated or treated by spray with either glutaraldehyde and cocobensildimetilamonium or only glutaraldehyde. Once the shells were dry they were swabbed. The technique for measuring shell contamination developed by Gentry (3) was used. Assay tubes with spiral plugs containing a single swab in 10 ml of phosphate buffered saline (PBS) were sterilized. Excess PBS was removed from the swab before a square inch area of egg shell was rubbed with rotary movements. The swab was than replaced in the tube. The assay tubes were kept under refrigeration until cultured in the laboratory. From each tube a 1 ml aliquot was placed in a sterile plastic petri dish and agar at 45-50°C added. The dishes were rotated and allowed to solidify at room temperature. After 48 hrs incubation at 37°C bacterial colony counts were conducted and expressed as colony-forming units/square inch. A similar procedure using potato dextrose agar and incubation at 26°C was used to determine mold and yeast counts. Some petri dishes showed very heavy growth. Serial tenfold dilutions were used to obtain counts with these samples. The data were subjected to analysis of variance and Duncan's multiple range test.

RESULTS

Results are presented in Table 1.

The treatments combined gluteraldehyde and cocobenzildimetilamonium and gluteraldehyde alone significantly reduced bacterial counts. The combined treatment reduced bacterial counts to a lower level than the gluteraldehyde alone but this difference was not significant. The treatments did not significantly reduce mold counts.

REFERENCES

1. Samberg. Y., and M. Meroz. Application of disinfectants in poultry hatchery. Rev. Sci. Tech. 14:365-380. 1995.

2. Cox, N. A., J. S. Bailey, M. E. Bercang. Automated spray sanitizing of broiler hatching eggs. Total bacteria and coliform recovery after using an egg spraying machine. J. Appl. Poult. Res. 3:234-247. 1994.

3. Gentry, R. F., and C. L. Quarles. The measurement of bacterial contamination on egg shell. Poult. Sci 51:930-933. 1972.

4. Buhr, R. J., and J. M. Mauldin. Automated spray sanitizing of broiler hatching eggs hatchablility of nest clean and dirty eggs. J. Appl. Poult. Res. 3:226-233. 1994.

Table 1. Bacterial and mold counts from egg shells

Treatment	Mean bacterial count	Mean mold count
Untreated	9505.7[A]	21.95[A]
Glutaraldehyde + cocobenzildimetilamonium	1022.0[B]	10.15[A]
Glutaraldehyde	2554.2[B]	13.45[A]

[A] Values with different superscripts in a row are significantly different

IN OVO VACCINATION WITH A NOVEL NEWCASTLE DISEASE VACCINE IN SPF AND BROILER EMBRYOS; EVALUATION OF SAFETY AND EFFICACY

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With the introduction of the automated egg injection system, the *in ovo* route of mass vaccination has become widely used in various parts of the world. Currently, vaccines licenced for the *in ovo* route of administration include Marek's and infectious bursal disease vaccines. There have been several attempts at developing other *in ovo* viral vaccines such as vaccines against Newcastle disease virus (NDV), infectious bronchitis virus (IBV), and others. In this paper we report on the development of a novel ND vaccine that was shown to be safe and effective in both SPF and broiler type chickens. The novel ND vaccine was formulated by mixing the appropriate amount of NDV type B1, La Sota strain with several concentrations of NDV neutralizing antibodies (NDA). The vaccine virus is a commercially available NDV vaccine and was used at 10^5 EID_{50}. Anti NDV activity in the NDA was determined using a viral neutralization assay and the 50% Neutralization Dose (ND_{50}) was calculated. Several NDA doses ranging from 0 to 216 ND_{50} were mixed with the above virus dose prior to administration. In separate experiments, the different formulations of the ND vaccine were administered *in ovo* on day 18 of embryonic development to either SPF or broiler embryos. Safety and efficacy parameters were evaluated. Safety parameters included hatchability and post-hatch mortality, while efficacy parameters included protection against a standard Texas GB challenge administered at an appropriate time. Thus, the objectives of these experiments were to establish one or more formulations that would be safe and efficacious in both SPF and broiler chickens. Results of these experiments suggested that several of these formulations were both safe and efficacious in both SPF and broiler chickens and that ND vaccine can be made safe and efficacious for *in ovo* administration.

THE USE OF WESTERN BLOTTING IN EPIDEMIOLOGIC STUDIES OF COMMON VIRUS DISEASES

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Western blotting has the unique ability to show recognition of distinct protein components of a virus with a polyclonal serum. This procedure allows for monitoring of specific antibodies directed at a viral agent by a population of immunized chickens. The western blot shows which viral proteins invoke an immune response, in what order, and the persistence of the antibodies over time. By studying the results of experimentally infected SPF chickens it may be possible to use this information as a diagnostic tool to determine when infection has occurred in a naturally occurring outbreak.

This study observes the immune response to avian influenza virus (AIV) H9 N2 TW66, Newcastle disease virus (NDV) La Sota, and infectious bronchitis virus (IBV) Mass. After immunization the first blood samples are taken 3 to 5 days later, then at regular intervals for the one month duration of the project. Semi-purified virus preparations are run on 10% tris-glycine acrylamide gels and transferred to a PVDF membrane. Membranes are blocked with 5% non-fat dry milk in PBS and serum is diluted for use in the same solution. A rabbit anti-chicken IgG alkaline-phosphatase labeled secondary antibody is used to detect the presence of chicken IgG. Serum samples are tested with blot strips and antibodies to specific viral proteins are identified.

Western blots of AIV temporal serum samples show a change in antibody directed at the viral proteins over time. Initial antibody detection was found at day 11 in 2 out of 8 samples with a single antibody to, what is suspected to be, a multi-meric form of an influenza antigen. The bloods at day 15 revealed antibodies to AIV nucleoprotein in 5 out of 9 birds, antibodies to neuraminidase in 2 of 9, and antibodies to either matrix protein or hemagglutinin-2 (HA-2) in 2 of 9 birds. Due to the closeness in size, matrix protein could not be distinguished from HA-2. Subsequent blood samples show an increased number of birds reacting to neuraminidase and nucleoprotein but not to matrix protein and/or HA-2.

IBV infected chickens first display antibodies to IBV at day 7 postinoculation. The first antibody response is to nucleocapsid protein in 8 of 8 serum samples and antibodies to membrane protein in 3 of 8 samples. The following blood samples at day 10, shows 8 of 8 birds positive for antibodies for both nucleocapsid and membrane protein. Antibodies to the S1(spike) protein are present in later samples but not in all birds.

Blots of serum samples of NDV immunized birds at day 5 reveals 1 of 10 birds with antibodies to several viral proteins. By day 8 all birds were responding to the immunization with antibodies to numerous NDV antigens. The antibodies to the many NDV proteins persist throughout the duration of the project.

It is hoped that characterization of the immune response to these viruses by western blotting will lead to a better understanding of the progression and antigen specificity of antibody production after infection. This information would be useful for diagnosis or confirmation of an outbreak and also can help to establish the time period the infection occurred.

COMPARISON OF A CHICKEN ORIGIN COMPETITIVE EXCLUSION CULTURE AND A LYOPHILIZED PROBIOTIC TO FRESH TURKEY CECAL MATERIAL FOR EFFICACY AGAINST SALMONELLA COLONIZATION

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To reduce the level of *Salmonella* on processed product from turkeys may require the level of *Salmonella* coming from the farm to be reduced. For *Salmonella* reduction to be successful in commercial turkeys, it may be necessary to reduce *Salmonella* colonization of breeders. It has been well documented that one tool of the poultry industry used to reduce *Salmonella* colonization is to rapidly establish an adult intestinal flora in day old chicks or poults. This process has been termed competitive exclusion (C.E.). There have been many studies with chicken origin C.E. products used in chickens, but less has been done to show that chicken origin C.E. can be as effective in turkeys as fresh turkey cecal material. The breeding industry is concerned that fresh cecal material can not be as readily controlled for freedom from pathogens as a commercially available lyophilized C.E. product. Fresh caecal material is also not as stable.

Four studies were performed to compare the effectiveness of a commercial lyophilized C.E. product of chicken origin (Aviguard), fresh cecal material, and a lyophilized *Lactobacillus acidophilus* probiotic. The challenge model, as described by Mead, *et al.* (1), involved administering the C.E. product at 1 day of age, challenging in 24 hours with a naladixic acid resistant *Salmonella kedougou* and then culturing ceca in 5 days. In 3 of the 4 studies, it was shown that the chicken origin C.E. culture, Aviguard, was as efficacious in preventing *Salmonella* colonization as fresh cecal material of turkey origin. It was also found that the coarse spray application of Aviguard was as effective in reducing the level of *Salmonella* in the ceca as direct gavage into the crop. A commercial probiotic given multiple times to the same poults prior to and after *Salmonella* challenge could not prevent cecal colonization to the same extent as the undefined commercial chicken origin culture, Aviguard, or as the undefined fresh turkey origin cecal material.

REFERENCE

1. Mead, G. C., P. A. Barrow, M. H. Hinton, F. Humbert, C. S. Impey, C. Lahallec, R. W. A. W. Mulder, S. Stavric, and N. J. Stern. Recommended assay for treatment of chicks to prevent Salmonella colonisation by "competitive exclusion". J. Fd. Prot. 52:500-502. 1989.

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ISOLATION, IDENTIFICATION AND PATHOGENICITY OF MOROCCAN FIELD ISOLATES OF INFECTIOUS BURSAL DISEASE VIRUS

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INTRODUCTION

Infectious bursal disease (IBD) is a highly contagious immunosuppressive disease of young chickens caused by a double-stranded birnavirus. Two serotypes of infectious bursal disease virus (IBDV) are known; serotype 1 is responsible for classic IBD with severe damage to the bursa of Fabricius and serotype 2 is not pathogenic (5). Protection against IBD is achieved by vaccination of breeders to confer protective maternal antibodies to progeny chicks (5). Failure of vaccination in commercial chickens in spite of high levels of maternal antibodies has been reported in the USA, Europe and Japan (6, 7, 8). This failure has been explained by the presence of variant strains of IBDV, which differ in antigenicity and pathogenicity (4, 6).

In Morocco IBD has become endemic since outbreaks that were associated with high mortality and severe bursal lesions in 1991 (1). Thereafter, different vaccination programs have been implemented. Despite vaccination, IBD is still occurring in classic or sub-clinical forms in vaccinated flocks in the field. The course of this low efficiency of vaccination is unknown. The objectives of this work were to isolate, identify and characterize the pathogenicity of field IBDV in Morocco.

MATERIALS AND METHODS

Collection of samples. Bursae of Fabricius (BF) were collected from chickens originating from 22 poultry farms (17 broiler farms and 5 layer farms) with a clinical history of IBD. Bursae were pooled by farm, homogenized in PBS (20% suspension), frozen and thawed three times, and clarified by centrifugation first at 3000 g for 30 min and then again at 8000 g for 15 min. Supernatants were then filtered through 450 nm Milipore filters, and stored at -80°C.

Isolation of IBDV. Six 10-day-old specific pathogen free (SPF) chicken embryos (Biopharma, Rabat, Morocco) were inoculated via the chorioallontoic membrane (CAM) using 0.2 ml from each specimen. Three negative control embryos were inoculated with 0.2 ml of sterile PBS and 3 positive controls were inoculated with 0.2 ml of a pathogenic IBDV strain suspension (VGP 9112, Rhône Mérieux, France). Embryos were incubated for 7 days and mortality and lesions were recorded. The CAMs were collected and stored at -80°C for a second passage. Chicken embryo fibroblasts (CEF) were prepared from 10-day-old SPF chicken embryos (2), cultured in 24 well plates and inoculated with 200 μl of specimen per well. They were incubated at 37°C and 5% CO_2, and examined for the presence of cythopathic effect (CPE) at 3 and 6 days postinoculation. Two passages were performed.

IBDV isolates were identified by the agar gel precipitation (AGP) test on supernatants from original bursal homogenates and CEF suspensions. The antiserum was prepared in guinea pigs immunized with inactivated IBDV in adjuvent at days 0, 14, and 28. IBDV strain VGP 9112 was used. In order to rule out the presence of Newcastle disease (NCD) virus the hemagglutination inhibition test (HI) was carried out on fluid from eggs with dead embryos from all specimens. Confirmation of the presence of IBDV was performed by a second passage in SPF chicken embryos followed by AGP test.

Determination of pathogenicity of field IBDV isolates. Two trials were conducted. In the 1st trial, twenty 4-week-old SPF chickens were assigned into 2 challenge groups of 8 birds each (G1 & G2) and 1 unchallenged control group of 4 birds (G3). In the 2nd trial, thirty-six 3-week-old SPF chickens were assigned into 3 groups of 12 birds each, 2 challenge groups (G4 & G5) and 1 control group (G6). Birds were tested for the presence of IBDV antibodies before and after challenge using the AGP test. Groups G1, G2, G4 and G5 were challenged separately with 4 different field IBDV isolates. Each bird was inoculated with an original bursal homogenate by the occulo-nasal route. Birds were observed daily for clinical signs and mortality. After 7 days post-inoculation birds were weighed, humanely killed and necropsied. The BF were examined, weighed and fixed in 10% neutral buffered formalin for histopathological examination. The spleen, thymus and cecal tonsils were also collected for histopathology. Mortality, BF : body weight ratios, number of birds with lesions, and microscopic lesion scores in lymphoid organs (3) were used to compare challenged and unchallenged groups. BF/body weight ratios were subjected to analysis of variance within each trial.

RESULTS AND DISCUSSION

Results of the isolation and identification of IBDV from 22 field specimens are summarized in Table 1. Specimens 4, 5, 7, 15, 17, 20 and 21 did not induce characteristic CPE in

Table 1 : Results of isolation of IBDV on chicken embryos and fibroblast cultures inoculated with 22 specimens, and their identification with agar gel precipitation

N° of specimen		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	C+	C-
Cytopathic effect in C.E.F. cultures (2^{nd} Ps)		+	+	+	-	-	+	-	+	+	+	-	-	+	+	-	+	-	+	+	-	-	+	+	-
% embryo specific mortality		67	67	67	67	67	67	50	67	67	67	83	50	50	83	50	67	50	33	17	0	67	17	100	0
AGP test	O.B.H	+	+	+	-	-	+	-	+	+	+	-	-	+	+	+	+	-	+	-	-	-	-	+	-
	CEF (2^{nd} Ps)	+	+	+	-	NT	+	NT	+	+	+	-	NT	+	+	+	+	-	+	+	+	-	+	+	-

C.E.F : chicken embryo fibroblastes, AGP : agar gel precipitation, NT : not tested, OBH : original bursal homogenates. Ps : passage. C+ : positive IBDV control, C- : negative IBDV control.

Table 2 : Pathogenicity of 4 field IBDV isolates inoculated to SPF chickens

Groups of birds		Mortality at days post inoculation							% total mortality	% birds with gross bursal Lesions		Mean microscopic lesion scores at 7 days postinoculation				Mean bursa/body weight ratio
		1	2	3	4	5	6	7		hemorrage	Caseous exudate	BF	Thymus	Spleen	Cecal tonsils	
Trial 1	Group 1 (specimen 10)	0/8	0/8	1/8	1/8	0/8	0/8	0/8	25 %	25 (2/8)	0	2.7	0.7	0	0.7	1.63
	Group 2 (specimen 14)	0/8	0/8	3/8	0/8	0/8	0/8	0/8	37.5 %	25 (2/8)	25	3.2	1	0.2	0.6	1.93
	Group 3 control	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0 %	0	0	0	0	0	0	4.09
Trial 2	Group 4 (specimen 3)	0/12	0/12	6/12	3/12	0/12	0/12	0/12	75 %	75 (9/12)	0	4.3	1	3	1.3	2.67
	Group 5 (specimen 9)	0/12	0/12	3/12	3/12	3/12	0/12	0/12	75 %	33.3 (4/12)	0	3.3	2	3	0.7	2.26
	Group 6 control	0/12	0/12	0/12	0/12	0/12	0/12	0/12	0 %	0	0	0	0	0	0	6.05

CEF cultures. The effect of specimens 11 and 12 was not recognizable until the second passage. Specimens 1, 13 and 16 induced very obvious CPE whereas the degree of CPE was variable in cultures inoculated with specimens 2, 3, 8, 9, 10, 14, 18, 19 and 22. All specimens induced high (67-100%) specific embryo mortality (SEM) except specimen 20, which did not induce any mortality, and specimen 22, which caused low (17%) SEM. The AGP test indicated the presence of IBDV antigens in both original specimens and cell suspensions from 12 specimens (1, 2, 3, 8, 9, 10, 13, 14, 15, 16 and 18). Specimens 4, 11, 17 and 21 were negative for IBDV antigens in both original homogenates and cell suspensions. Specimens 19 and 22 were negative when tested as original bursal homogenates but were positive when cell cultures were tested. The HI test was negative for the presence of NCD virus in for all specimens except specimen 7, which was positive to NCD. On the basis of characteristic CPE on CEF cultures, chicken embryo mortality above 50% and positive AGP tests, specimens 1, 2, 3, 8, 9, 10, 14 and 16 were selected for confirmation of the presence of IBDV antigens using a 2nd passage in 10-day-old chicken embryos followed by the AGP test. Specimens 1, 2, 8, and 16 induced 67% embryo mortality whereas, specimens 3, 9, 10, and 14 resulted in 100% SEM. Homogenates of CAM and dead embryos from all specimens were positive for IBDV. These last 4 specimens were selected for pathogenicity studies.

Results of the pathogenicity tests of the above four specimens are summarized in Table 2. Mortality of SPF chickens was observed at day 3 post-inoculation for all challenged groups. At 7 days postinoculation, mortality rate reached 25%, 37.5%, and 75% for groups 1, 2, and 4 and 5, respectively. No mortality was observed in control groups. Major gross lesions observed in challenged birds were bursal hemorrhage which occurred in 25 % of birds in G1 and G2, 75% in G4 and 33% in G5. Mean bursa/body weight ratios at 7 days postinoculation were significantly decreased in challenged groups G1, G2, G4 and G5 relative to control groups. There was no significant difference between challenge groups within each trial. Microscopic lesion scores showed evidence of bursal damage in all treated groups. This was relatively higher in group 4.

The results of this study confirms the presence of IBDV in poultry farms with a clinical history of IBD in Morocco. Four isolates studied caused high mortality in young SPF chickens associated with severe gross and microscopic lesions in the BF consistent with IBD. Isolates tested in trial 2, particularly isolate 3 (G4), seemed to be more pathogenic than those tested in trial 1. This may be explained by the age difference between the birds used in trial 1 and trial 2. These isolates will be further characterized and used in the evaluation of vaccination programs used in the field.

REFERENCES

1. Bouzoubaa, K., T. Jaouzi, A. Amara, M. El Houadfi, F. Kichou, M. Mouahid, and J.G. Bell. Severe outbreaks of infectious bursal disease in Morocco. Proc. 41st West. Poultry Dis. Conf., Sacramento, California. pp. 3-4. 1992.

2. Freshney, R. I. Culture of Animal Cells – A Manual of Basic Techniques, 2nd ed. Wiley-Liss, New York. 1987.

3. Henry C. W., R. N. Brewer, and S. A. Edgar. Studies on infectious bursal disease in chickens. 2. Scoring microscopic lesions in the bursa of Fabricius, thymus, spleen, and kidney in gnotobiotic and battery reared white leghorns experimentally infected with infectious bursal disease virus. Poult. Sci. 59:1006-1017. 1980.

4. Jackwood, D. H. and Y. M. Saif. Antigenic diversity of infectious bursal disease viruses. Avian Dis. 31:766-770. 1987.

5. Luckert, P. D. and Y. M. Saif. Infectious bursal disease. In: Diseases of Poultry, 9th ed. B.W. Calnek, H.J. Barnes, C.W. Beard, W.M. Reid, and H.W. Yoder, eds. Iowa State University Press, Ames, Iowa. pp. 648-663. 1991.

6. Snyder D. B. Changes in the field status of infectious bursal disease virus. Avian pathol. 19:419-423. 1990.

7. Tsukamoto, K., N. Tanimura, H. Hihara, J. Shirai, K. Imai, K. Nakamura, and M. Maeda. Isolation of virulent infectious bursal disease virus from field outbreaks with high mortality in Japan. J. Vet. Med. Sci. 54:153-155. 1992.

8. Van Den Berg, T. P., M. Gonze, and G. Meulemans. Acute infectious bursal disease in poultry: isolation and characterization of highly virulent strain. Avian Pathol. 20:133-143. 1991.

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COMPARISON OF SEROLOGICAL METHODS FOR THE DETECTION OF ANTIBODIES TO INFECTIOUS BURSAL DISEASE VIRUS

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In recent years, variant strains of infectious bursal disease virus (IBDV) have been isolated from vaccinated flocks in the United States. These variant strains are antigenically different from the so-called classic strains of IBDV most typically isolated before 1985. The variant IBDV strains lack epitope (s) defined by neutralizing monoclonal antibodies. Since these variant strains appeared in the field, many commercially available live and killed vaccines for IBDV have been reformulated. This was done in order to better match the greater antigenic spectrum of viruses recognized to be circulating in the field. These antigenic differences have demonstrated a need for improved detection of antibodies to the broader antigenic spectrum of IBDV. Efforts were made to improve the ELISA in this regard. Three antigen preparations were investigated. IBDV antigens of tissue culture origin and of bursal origin, and a recombinant antigen expressed in baculovirus were investigated. The bursal origin antigen provides excellent specificity and superior sensitivity for the detection of antibody to a greater antigenic spectrum of bursal disease viruses. This improved ELISA has a low coefficient of variation within a flock following vaccination when compared to other commercially available ELISA kits. It also has an excellent correlation with the virus neutralization assay.

BACTERIAL ETIOLOGY OF AA-AMYLOID-ARTHROPATHY IN CHICKENS

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Data from the routine postmortems performed at the Animal Health Service over a period of two years (1996 and 1997) were analyzed for the occurrence of amyloid arthropathy. Only submissions of commercial chickens with breed specification and older than a week were included in the analysis. The average number of birds per submission sent for postmortem analysis was five. Amyloid arthropathy was found in 12.9% (249/1930) of brown layers necropsied during both years, while in broiler breeders it was only seen in 2.9% (53/1815). The condition was never recorded in white layers and broiler chickens. When these data were analyzed per year, an increase in prevalence of amyloid arthropathy was found in both brown layers and broiler parent birds. In 1996, of necropsied brown layers, 11% (118/1070) was suffering from amyloid arthropathy and in 1997 it was 15.2% (131/860). For broiler parent birds the percentages were 0.9% (11/1190) and 6.5% (42/625) respectively. Amyloid arthopathy was encountered once in an Indian peafowl (1).

In submissions in which amyloid arthropathy was diagnosed and in which bacteria were isolated from joints, *Enterococcus faecalis* was the major pathogen isolated from joints in brown layers 46/60 (76.6%), while in broiler breeders it was *Staphylococcus aureus* 47/64 (73.4%). A detailed review of 13 field cases from which birds were submitted for extensive postmortem analysis was performed. In these cases flock data were noted, serological, virological, bacteriological and histopathological analysis were performed as described (2,3). Brown replacement pullets were most frequently affected (10/13), with 2 flocks affected during production. The condition was also found in 3 broiler parent flocks. Amyloid arthropathy was found in flocks kept in different housing systems without season variability. The percentages of lame birds varied from 0.5 to 14%. Amyloid was found in 33% of organs and 54% of examined joints (knee and hock joint). *E. faecalis* and chicken anemia virus were isolated in 12% of affected birds. No correlation was found with *Mycoplasma synoviae* and reovirus serology.

Studies were also performed to evaluate by intravenous inoculation the amyloidogenic potential of arthrotropic bacterial species (*S. aureus*, *Escherichia coli* and *Salmonella enteritidis*) isolated from chickens as well as several *E.*

faecalis isolates compared to the amyloidogenic *E. faecalis* strain, which was previously isolated from amyloidotic joints. As chicken anemia virus was also isolated from amyloidotic joints of field cases, it was also screened for its amyloidogenic potential. In a second experiment, *M. synoviae*, inactivated *E. faecalis* isolate 6085.94, Freund's adjuvant, *E. faecalis* from brain, *E. coli*, *S. aureus*, *S. enteritidis*, chicken anemia virus and an arthrotropic reovirus field isolate were also screened for amyloidogenicity by intra-articular injection. These studies showed that the ability to elicit extensive amyloid arthropathy is reserved primarily to *E. faecalis*, but that this property is not common to every *E. faecalis* isolate. Intra-articular application of complete Freund's adjuvant also led to the formation of extensive joint amyloid deposits. Of the other micro-organisms studied, *S. aureus*, *S. enteritidis* and *E. coli* were also able to cause joint amyloidosis, but in very small amounts. *M. synoviae*, inactivated *E. faecalis*, chicken anemia virus and reovirus did not cause amyloid arthropathy after intra-articular inoculation.

These findings are consistent with results of the analyses of previous field cases (2) and of the induction of amyloid arthropathy (3) in chickens, suggesting an important role for *E. faecalis* in AA-amyloid-arthropathy in chicks..

Amyloidogenic, non-amyloidogenic, amyloid-associated and other *E. faecalis* isolates were typed by analysis of chromosomal DNA restriction endonuclease digests by pulsed-field gel electrophoresis (PFGE) following a modification of the method described by Smith and Cantor (4) and Murray *et al.* (5). All amyloidogenic and amyloid-associated *E. faecalis* isolates collected over a period of four years and from several European countries had similar restriction endonuclease digestion patterns, suggesting clonal spread.

REFERENCES

1. Landman, W. J. M., and E. Gruys. Amyloid arthropathy in an Indian Peafowl (Pavo cristatus). Vet. Rec. 142:90-91. 1998.

2. Landman, W. J. M., E. Gruys, and R. M. Dwars. A syndrome associated with growth depression and amyloid arthropathy in layers: a preliminary report. Avian Pathol. 23:461-470. 1994.

3. Landman, W. J. M., N. H. M. T. Peperkamp, C. A. M. Koch, P. C. J. Tooten, P. A. P. Crauwels, and E. Gruys. Induction of amyloid arthropathy in chickens. Amyloid: Int. J. Exp. Clin. Invest. 4:87-97. 1997.

4. Smith, C. L., and C. R. Cantor. Purification, specific fragmentation, and separation of large DNA molecules. Methods Enzymol. 155:499-567. 1987.

5. Murray, B. E., K.V. Singh, J. D. Heath, B. R. Sharma, and G. M. Weinstock. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J. Clin. Microbiol. 28:2059-2063. 1990.

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EVALUATION OF INFECTIOUS BURSAL DISEASE IN MEXICO BY SEROLOGY, HISTOPATHOLOGY AND IMAGE PROCESSING

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A field trial was conducted to determine the effect of infectious bursal disease virus infection in the field and the effect of vaccination in a broiler flock. Four production units, each with 15 000 broilers, were vaccinated with a commercial vaccine at day 1 and 10. Evaluation was done by the seroneutralisation test, histopathology and image processing. In image processing histology slides of bursa tissue are analyzed by means of a color differentiation that localizes active lymphocytes using a computer program. Bursae were sampled weekly. A field challenge was detected at 28-35 days of age. A direct relation was observed between the serology and the lesions detected by histopathology. Seven days after the challenge total recovery was observed. This indicated that the vaccine provided protection..

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A LONGITUDINAL STUDY OF LEG WEAKNESS IN FIVE COMMERCIAL BROILER FLOCKS

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Bacterial chondronecrosis with osteomyelitis of the proximal end of the femur and/or tibiotarsus (BCF/T) was diagnosed by histology as the predominant cause of lameness in a recent study of broiler flocks (2). In order to confirm and expand the latter findings, the present study was extended to include lame birds, birds found dead in the broiler house and birds culled for reasons other than lameness from five flocks. Several contemporary studies have examined the incidence of bacterial chondronecrosis with osteomyelitis of the proximal end of the femur and/or tibiotarsus (BCF/T) in lame broilers (3, 4). The present study attempted to evaluate the contribution of BCF/T to overall flock losses and the relative contribution of BCF/T and other lesions of the skeleton to the total incidence of birds culled due to lameness as this was not determined in previous studies (2, 3, 4).

MATERIALS AND METHODS

Four broiler flocks (Flocks 2, 3, 4 and 5), from two genotypes (Ross and Cobb) were selected at random from approximately 250 broiler flocks. These were being reared for commercial purposes in Northern Ireland within two commercial broiler production companies. Flocks 2, 3 and 4 were male, Flock 5 was female. One of the farm managers (Flock 2) was considered to be a poor record keeper by his company advisory personnel. Therefore an additional male flock (Flock 1) was included in the study. Flock 1 was selected since Flock 1 and Flock 2 were derived from the same parent flocks, hatched in the same hatchers and delivered to their respective rearing farms from one lorry on the same day.

The stockmen were instructed to record the daily incidence of (a) dead birds, (b) total birds culled excluding lame culls, designated " other culls" and (c) birds culled only due to lameness on a recording sheet, from day-old until processing. The five site managers were asked to exclude immobile or moribund birds from their count of lame birds. Immobile and moribund birds were included in the count of other culls in the present study. On each Thursday morning from week 1 until slaughter, all dead and culled birds collected were submitted for necropsy.

Necropsy and skeletal examination were carried out. Lesions suggestive of tibial dyschondroplasia (TD) were graded as previously described (2). The proximal end of the femur or tibiotarsus was processed for histology as were all bone samples with no lesions suggestive of BCF/T on macroscopic examination.

RESULTS

Incidence of overall flock losses and lame culls. The incidence of overall flock losses recorded in four male and one female broiler flock was 6.03%, 4.59%, 5.9%, 4.1% and 4.51% respectively. The incidence of lame culls recorded in the four male and one female broiler flock was 1.06%, 0.09%, 0.39%, 0.37% and 0.79% respectively.

Necropsy findings. A total of 526 birds were submitted for examination. Of these, 110 birds were discarded due to carcass decomposition. Of the 416 birds examined, 191 had been categorized as lame culls, 87 as other culls (birds culled for reasons other than lameness) and 138 birds had been found dead. The predominant findings are presented in Tables 1, 2 and 3.

Table 1. Incidence of predominant macroscopic skeletal lesions found at necropsy of 191 lame broilers from four male and one female flock.

	Flock				
Lesion (%)	1	2	3	4	5
Spondylolisthesis	8.8	10.0	5.7	0.0	11.8
Scoliosis	2.5	0.0	0.0	0.0	5.9
Exudate in hip or hock joint	0.0	20.0	13.2	0.0	11.8
Mild TD	13.9	10.0	11.3	26.6	20.6
Moderate TD	8.8	20.0	0.0	6.6	0.0
Severe TD	5.1	40.0	5.7	6.6	11.8
Angular or torsional limb deformity	26.6	10.0	5.7	6.6	0.0
Osteomyelitis in one or more bones	0.0	10.0	20.7	20.0	8.8
Number of birds examined	79	10	53	15	34

Table 2. Incidence of predominant macroscopic skeletal lesions found at necropsy of 138 broilers found dead in four male and one female flock.

	Flock				
Lesion (%)	1	2	3	4	5
Spondylolisthesis	6.2	0.0	2.8	0.0	10.5
Scoliosis	6.2	2.2	0.0	0.0	5.2
Exudate in hip or hock joint	15.6	6.6	11.1	66.6	5.3
Mild TD	21.8	4.4	16.6	33.3	26.3
Moderate TD	12.5	0.0	0.0	0.0	15.7
Severe TD	0.0	2.2	2.8	0.0	0.0
Angular or torsional limb deformity	9.4	4.4	2.8	0.0	0.0
Osteomyelitis in one or more bones	0.0	11.1	13.8	33.3	10.5
Number of birds examined	32	45	36	3	22

Table 3. Incidence of most common macroscopic skeletal lesions at necropsy of 87 broilers culled for reasons other than lameness from four male and one female flock.

	Flock				
Lesion (%)	1	2	3	4	5
Spondylolisthesis	0.0	0.0	14.2	0.0	10.5
Scoliosis	6.9	0.0	0.0	0.0	0.0
Exudate in hip or hock joint	6.9	0.0	14.2	0.0	5.2
Mild TD	17.2	20.0	0.0	23.5	26.3
Moderate TD	13.7	20.0	28.4	23.5	0.0
Severe TD	3.4	0.0	0.0	0.0	0.0
Angular or torsional limb deformity	6.9	0.0	0.0	5.9	0.0
Osteomyelitis in one or more bones	3.4	6.6	28.4	5.9	0.0
Number of birds	29	15	7	17	19

Histological findings and age of diagnosis of lesions of BCF/T in lame birds. The incidence of BCF/T in lame birds ranged from a minimum of 3.8% (Flock 1) to a maximum of 33.9% (Flock 3). The incidence of BCF/T in dead birds ranged from a minimum of 4.5% (Flock 5) to a maximum of 33.3% (Flock 4). The incidence of BCF/T in birds culled for reasons other than lameness ranged from a minimum of 5.3% (Flock 5) to a maximum of 28.5% (Flock 3). The overall incidence of BCF/T in all birds examined from Flock 1, Flock 2, Flock 3, Flock 4 and Flock 5 was 5.0%, 12.8%, 27.0%, 20.0% and 10.0% respectively.

Of the 191 lame birds, BCF/T was diagnosed in 33 birds (17.3%). Of the 138 birds found dead in the broiler house, BCF/T was diagnosed in 17 birds (12.3%) and in 7 of the 87 other culls (8.0%).

At necropsy, BCF/T was suspected in 38 cases macroscopically; histological examination confirmed these cases and detected a further 19 lesions of BCF/T.

No lesions of BCF/T were diagnosed in week one. The peak incidence of BCF/T occurred most frequently at five weeks of age.

DISCUSSION

TD was the most common lesion of the skeleton detected in the present study. In a previously published study (2) only lesions of severe TD were present in lame birds. Mild and moderate lesions of TD can be detected in birds considered "normal" on the basis of their gait scores (1). In this former study, BCF/T (20.4%), angular or torsional limb deformities (13.6%), spondylolisthesis (11.4%) and severe TD (4.5%) were the predominant lesions identified as causes of lameness by macroscopic and microsopic examinations. In the present work, BCF/T (17.3%), limb deformities (13.6%), spondylolisthesis (7.8%) and severe TD (8.4%) were the most common causes of lameness detected in 191 birds culled because of lameness. The present study supports the previous findings (2) and confirms that BCF/T is the most common cause of lameness in commercial broilers in N. Ireland. It was also demonstrated during the work of this study that the incidence of BCF/T and of non-infectious causes of lameness may vary from flock to flock, regardless of their common parentage.

REFERENCES

1. Kestin, S. C., T. G. Knowles, A. E. Tinch, and N. G. Gregory. Prevalence of leg weakness in broiler chickens and its relationship with genotype. Vet. Rec. 131: 190-194. 1992.

2. McNamee, P. T., J. J. McCullagh, B. H. Thorp, H. J.Ball, D. A. Graham, D. A. McConaghy, and J. A. Smyth. A longitudinal investigation of leg weakness in two commercial broiler flocks. Vet. Rec. 143: 131-135. 1998.

3. Thorp, B. H., C. C. Whitehead, L. Dick, J. M. Bradbury, R. C. Jones, and A. Wood. Proximal femoral degeneration in growing broiler fowl. Avian Path. 22: 325-342. 1993.

4. Thorp, B. H., and D. Waddington. Relationships between the bone pathologies, ash and mineral content of long bones in 35 day-old broiler chickens. Res. Vet. Sci. 62:67-73. 1997.

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HISTOLOGY, IMMUNOHISTOCHEMISTRY AND ULTRASTRUCTURE OF HYDROPERICARDIUM SYNDROME IN ADULT BROILER BREEDERS AND BROILER CHICKS

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Hydropericardium syndrome (HPS) was first reported in growing broiler chicks in Pakistan (7). It was mainly seen in commercial broiler chicks aged 3 to 5 weeks and was characterized by hydropericardium with no clinical signs except sudden death (60-70% mortality) (3). The most characteristic histological lesion was found in the liver; intranuclear inclusion bodies in hepatocytes associated with multifocal necrosis of hepatocytes, hemorrhages and mononuclear cell infiltration (4). The syndrome was reported in Iraq in 1991 (1) and India in 1993 (10). Adenovirus was isolated from the livers of chickens affected with HPS (7). The serotype of the adenovirus isolated from the chickens with HPS was type 4 (10). The disease was reproduced in broiler chicks by inoculation of liver homogenate from the HPS-affected chickens (2,3,10). From histological features, viral isolation, and experimental reproduction of the disease, group I adenovirus was considered to be the most likely causative agent of HPS. In Pakistan, Iraq and India, HPS was seen mainly in commercial young broiler chicks. There have been no detailed reports of outbreaks of HPS in adult chickens. This paper describes detailed pathological investigations of HPS in adult broiler breeders and broiler chicks in Japan.

In 1996, HPS occurred in an adult broiler breeder flock (5,000 birds) on a farm in Tokushima, Japan. The mortality rate was 6.4%. Then HPS appeared in a 2- to 4-week-old commercial broiler flock (11,220 birds) on the same farm. The mortality rate was 20.2%. HPS was also seen in a 3- to 4-week-old commercial broiler flock (34,145 birds) on a different farm. The mortality rate was 26.1%. There were no apparent clinical signs except sudden death. Ten 250-day-old broiler breeders and ten 2- to 4-week-old broiler chicks were examined pathologically and microbiologically.

Gross and histological lesions in the broiler breeder chickens were similar to those in the broiler chicks. The common characteristic gross lesion was hydropericardium. The liver showed enlargement with occasional petechial hemorrhages. In the liver, multifocal necrosis of hepatocytes and hemorrhages associated with intranuclear inclusion bodies in hepatocytes were seen in histology. The intranuclear inclusions were positive for group I adenovirus by immunohistochemistry and consisted of numerous viral particles. Large aggregates of hexagonal virus particles 65-70 nm in diameter were observed. In the spleen, there were many macrophages engulfing fragmented erythrocytes and/or yellow pigments were seen in the sinuses. In the lung, the interlobular interstitial connective tissues showed edema and there was a marked increase of macrophages in the parenchyma. There were no significant lesions in the bone marrow nor in the bursa of Fabricius or thymus. Serotype 4 group I adenovirus was isolated from liver samples in CK cells. No other viral agents were isolated from liver samples.

The necrosis of hepatocytes with intranuclear inclusion bodies and hemorrhages in the present cases were similar to the lesions described in field cases of inclusion body hepatitis (IBH) (6) but the lymphocyte depletion in the bursa of Fabricius and thymus, and hypoplasia of bone marrow and the subsequent aplastic anemia often described in IBH were not described in HPS. The lymphoid and bone marrow lesions of IBH field cases may be associated with IBDV and CAV (9). There was neither pathological nor virological evidences of IBDV or CAV infection. A high mortality rate is characteristic of HPS: 60-70% in Pakistan, 10-30% in Iraq, and 10-60% in India, but the mortality rates in the present cases were not so high. The reason for the chickens' deaths in HPS is unknown. Hydropericardium is prominent in dead birds. Cardiac lesions may, therefore, be associated with death. Adenoviral antigens could not be found by immunohistochemistry in the hearts in the present cases. Ahmad *et al.* (3) reported that chickens inoculated with liver homogenate from HPS chickens had hydropericardium and died but the chickens inoculated with pericardial fluid from HPS had no lesions and did not die. Hydropericardium may be caused indirectly by HPS adenovirus infection. HPS is seen predominantly in 3 to 5-week-old broilers (4) and rarely in egg-type birds. IBH is seen in 5- to 8-week-old young

broilers (8) and also rarely in egg-type birds (5). There have been no previous reports of HPS or IBH in adult chickens. From histology, immunohistochemistry, ultrastructure and virology, the chickens with HPS were infected with group I adenovirus. The virus appears to be the pathogenic agent of HPS. No agents, such as IBDV and CAV, were associated with HPS in the present cases, so it is possible that the present group I adenovirus strain (serotype 4) may be very virulent. Its pathogenicity needs to be studied further using SPF chickens.

REFERENCES

1. Abdul-Aziz, T. A., and M. A. Al-Attar. New syndrome in Iraqi chicks. Vet. Rec. 129: 272. 1991

2. Abdul-Aziz, T. A., and S. Y. Hasan. Hydropericardium syndrome in broiler chickens: its contagious nature and pathology. Res. Vet. Sci. 59:219-221. 1995.

3. Ahmad, I., M. Afzal, M. I. Malik, Z. Hussain, and W. Hanif. Disease pattern and etiology of hydropericardium syndrome (Angara disease) in broiler chickens in Pakistan. Pakistan J. Agri. Res. 10:195-199. 1989.

4. Akhtar, S. Hydropericardium syndrome in broiler chickens in Pakistan. World's Poult. Sci. J. 50:177-182. 1994.

5. Fadly, A. M., and R. W. Winterfield. Isolation and some characteristics of an agent associated with inclusion body hepatitis, hemorrhages, and aplastic anemia in chickens. Avian Dis. 17:182-193. 1973.

6. Gallina, A. M., R. W. Winterfield, and A. M. Fadly. Adenovirus infection and disease. II. Histopathology of natural and experimental disease. Avian Dis. 17: 343-353. 1973.

7. Khawaraja, D. A., S. Ahmad, A. M. Rauf, M. Zulfiqar, S. M. Mahmood, and M. Hassan. Isolation of an adenovirus from hydropericardium syndrome in broiler chicks. Pakistan J. Vet. Res. 1: 2-17. 1988.

8. McFerran, J. B. Adenovirus (group 1) infections of chickens. In: Diseases of poultry, 9th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr. eds., Iowa State University Press, Ames, Iowa. pp.553-563. 1991.

9. McFerran, J. B., and T. J.Connor. Further studies on the classification of fowl adenoviruses. Avian Dis. 21:585-595. 1977.

10. Naeem, K., T. Niazi, S. A. Malik, and A. H. Cheema. Immunosuppressive potential and pathogenicity of an avian adenovirus isolate involved in hydropericardium syndrome in broilers. Avian Dis.39:723-728. 1995.

11. Survashe, B. D., S. V. Pandit, G. R. Ghalsasi, and S. Mallikarjun. Inclusion body hepatitis/hydropericardium syndrome (Leechi heart disease) in India - an overview. In Proceedings of XXth World's Poultry Congress, New Delhi, India. pp.375-380. 1996.

MONITORING MEAT BIRD POPULATIONS IN PREPARATION FOR PROCESSING

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This is a technical outline covering the procedure used to predict "processability" of broilers. In modern plants, line speed or line efficiency is most often decreased by bird disposition (pathology) and/or carcass quality: trimming of inspection directed portions or meat product quality standards. When such conditions occur, plants may respond in several ways: add trimmers, activate more salvage stations or slow line speeds. The cost-effective response may vary from company to company. The process of producing a marketable meat bird is affected by many factors, the more dominant of these factors can be used to predict the end result. The methodology is based on:

1. Regression analysis correlating production parameters with some 400 independent variables assigned to each grow-out flock.
2. Field observations of individual houses. Service personnel perform individual house observation using a check-off list, which can also be used as a poultry technician's report to accredited veterinarians for export certification.

Regression analyses suggest trends while individual house data can be used for specific flock prediction. Specific factors predicted at processing, and assumptions and correlation used in these predictions are as follows:

1. **Septicemia - Toxemia condemns:**
   a. Higher hatchery bacterial counts (fluff and environmental) at time of hatch increase condemns.
   b. Higher second week mortality (and first week to a lesser extent) increases condemns.
   c. Higher rating farm management will decrease condemns (rating scale 1 to 4 based on ranch conditions during grow-out).
   d. Higher average daily weight gains increases condemns.
2. **IP (Cellulitis):**
   a. Higher average daily weight gains increase IP.

Thirty-five days test weights can be used for predictions.

b. Placement density (birds per square foot) affects IP Higher density increases IP; lowering density will decrease IP.

c. Temporary increases in placement density (over-hatch) will be especially notable in following the above correlations.

d. Individual house total mortality is inversely proportional to IP. High first or second week mortality coupled with continued (higher than expected) weekly mortality decreases IP; increase in last week mortality alone suggests higher IP.

3. **Air Sac involvement**:

a. The most dominant "predictor" is evaluation for respiratory signs (visual, noise) with necropsies done in the field when signs are detected.

b. Average daily weight gain is associated with lower air sac involvement (healthy birds have less disease).

c. Higher second week mortality (above 50% of first week) increases late air sac involvement.

d. Uniform temperatures during brooding tend to reduce air sac involvement.

e. Increasing mortality during the last 7 days is associated with increasing air sac involvement.

4. **Diseased legs:**

a. Supervisors' flock condition check list is the primary "tool" in predicting leg problems; down bird incidence in flock approaching market will correlate with incidence of diseased legs.

b. IP will be increased by higher incidences of diseased legs.

c. Similarly contamination will be increased by higher levels of leg problems. Birds with limited access to feed have disturbed intestinal problems.

5. **Skin quality (breast "buttons", thigh scratches, flap tears):**

a. Supervisors visual observation check-off list is the primary "tool" in predicting skin quality as it records litter conditions, visible scratches, abnormally placed equipment or "junk piles".

b. Higher average daily weight gains increase trim needed to meet quality standards.

c. High predicted incidence of IP is correlated with higher trim levels and consequently higher placing densities result in higher trim.

6. **Contamination (feces or ingesta):**

a. Variations in feeding program (wrong type of feed for a specific stage being delivered after 3 weeks of age) will increase contamination.

b. Feed lack increases contamination. Lack of feed can be due to obvious factors (no feed, broken feeder) or may be related to less obvious factors (birds too cool during feed withdrawal period or temperature changes interrupting eating patterns).

c. Signs of clinical disease indicate increased contamination. Signs of respiratory, enteric or systemic disease are all correlated with increased contamination. The correlation is with birds disposed to "wash" procedure and the offending material may be ingesta, fecal or other.

SUMMARY

The above outlines our procedure for predicting processing plant disease and trim results. Correlations given are obtained from regression analysis and higher correlations are listed first for each factor. Often visual examination provides the highest correlation. Correlations themselves do not provide an exact prediction. When taken together, however, they assist in predicting flock-processing results.

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We would like to extend our appreciation to Sharon Cox for data collection and regression analysis.

TWO BREEDS OF CHICKENS WITH STRIKING DIFFERENCE IN SUSCEPTIBILITY TO DEVELOP AA-AMYLOIDOSIS APPEAR TO HAVE IDENTICAL SAAs

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Reactive amyloid arthropathy has been described mainly in brown layer pullets suggesting the occurrence of breed susceptibility. Data from 14,000 necropsies of commercial chickens over two years showed that amyloid arthropathy occurred regularly in brown layers, whereas their white counterparts were free of this disease (1). The susceptibility

of brown layers was confirmed experimentally by intravenous injection of amyloid-inducing *Enterococcus faecalis* isolated from a spontaneous case resulting in a significant higher incidence of amyloid arthropathy in brown layers. Enhanced susceptibility of organisms to develop systemic AA type amyloidosis has often been attributed to the expression of an amyloidogenic serum amyloid A (SAA) protein. Therefore, the brown chicken SAA gene was characterized and SAA cDNA from white and brown layers were compared for the first time. The brown chicken SAA gene was found to be a single copy gene, as in the duck (2), in contrast to mammals which possess multiple SAA genes. The chicken gene has 4 exons and 3 introns. The first exon occupies a conserved position and is not translated. Sequence analysis of SAA cDNA from white and brown layers showed identical predicted amyloid precursor proteins in both breeds. Therefore, differences in susceptibility for amyloid arthropathy must be caused by other factors than SAA protein structure. Additionally, investigation of SAA gene expression using *in situ* hybridization revealed the presence of SAA mRNA in liver and also in the synovial membranes of amyloid affected joints. Although local SAA synthesis by synovial fibroblasts has been reported in rabbits (3), it has never been mentioned in association to local amyloid deposition. Our findings indicate that the likelihood of developing *E. faecalis*-induced amyloid arthropathy in chickens is breed dependent but is not a consequence of a more amyloidogenic SAA. It is hypothesized that in brown layers intravenous injection of *E. faecalis* induces a systemic acute phase response and colonizes the joints. Subsequently the inflamed synovium proliferates and produces SAA, which is locally processed into AA fibrils.

REFERENCES

1. Landman, W. J. M., A. E. J. M. V.d. Bogaard,, P. Doornenbal, P. C. J. Tooten, A. R. W. Elbers, and E. Gruys. The role of various agents in chicken amyloid arthropathy. Amyloid: Int. J. Exp. Clin. Invest. In press.

2 . Guo, J. T., C. E. Aldrich, W. S.Mason, and J. C. Pugh. Characterization of serum amyloid A protein mRNA expression and secondary amyloidosis in the domestic duck. Proc. Natl. Acad. Sci. U.S.A. 93:14548-14553. 1996.

3. Mitchell, T. I., C. I. Coon, and C.E. Brinckerhoff. Serum amyloid A (SAA3) produced by rabbit synovial fibroblasts treated with phorbol esters or interleukin 1 induces synthesis of collagenase and is neutralized with specific antiserum. J. Clin. Invest. 87:1177-1185. 1991.

IMMUNE RESPONSE ASSESSMENT IN TURKEY BREEDERS VACCINATED AGAINST NEWCASTLE DISEASE USING MATHEMATICAL MODELS

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The interpretation of the immunological response after vaccination is a difficult problem faced by veterinarians. Several factors such as vaccine strain, titer and route of administration, concurrent immunosuppressive diseases, environment and farm management can influence the immune response. We investigated a simplified mathematical model using age and antibody titers to evaluate the immunological response in turkey breeders from one farm. The birds were divided into 11 different age groups and 880 blood samples were analyzed for antibodies against Newcastle disease after vaccination using the hem-agglutination inhibition test. Age and antibody titers were tabulated in a dispersion diagram. Three mathematical models were created utilizing regression analysis. A high correlation was found between these mathematical models and the expected immunological response for each age group of animals ($p<0.05$). We concluded that mathematical models can be created for each individual farm and offer an objective interpretation of the serological results of the immune response against Newcastle disease virus vaccine in turkey breeders.

CORRELATION BETWEEN AFLATOXIN AND OCHRATOXIN LEVELS WITH PRODUCTION PARAMETERS IN A POULTRY COMPANY

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Quantitative analyses for aflatoxin and ochratoxin were performed on 24 feed samples that were collected from the feed mill and poultry farms, and on liver and kidneys of underdeveloped birds that had been fed the analyzed feed. Samples were divided into four groups on a basis of time corresponding to summer, fall, winter and spring (Groups 1, 2, 3 and 4). Toxin detection was performed by the use of a commercial ELISA testing system, and the results were studied by correlation and regression analyses. Aflatoxin and ochratoxin levels, together and separately, were compared to the flocks' productive rate, feed conversion, percentage mortality, weight gain rate, percentage of condemnation and the producer's efficiency index. From the results obtained, it was concluded that it is possible to correlate feed and broiler visceral levels of aflatoxin and ochratoxin with a flock's productive parameters.

USE OF STATISTICAL TECHNIQUES ON THE INTERPRETATION OF ROUTINE SEROLOGICAL DATA PRODUCED BY A POULTRY INDUSTRY

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A search for mathematical models which could explain the antibody curve produced by vaccination against infectious bursal disease, Newcastle disease and infectious bronchitis was the main objective of this work. The data originated from samples collected from 20 breeder flocks. Sera were tested by hemagglutination-inhibition (HI) for antibody against Newcastle disease and by virus-neutralisation (VN) against infectious bursal disease and infectious bronchitis. Sera were collected from 5- to 65-week-old birds at 5 week intervals. Geometrical Mean Titres (GMT) were transformed into base 10 logarithms and a dispersion diagram was plotted, where the X independent variable was the birds' age (5 to 65 weeks) and the Y dependent variable was the antibodies titre against the specific vaccine. Variance and Linear and Non-Linear Regression Analyses were performed and, from the results obtained, we concluded that it was possible to draw mathematical models that explain the relation between antibody levels after vaccination and the birds' age.

FIELD EXPERIENCES IN CHANGING BROILER-HOUSE SALMONELLA POPULATIONS WITH THE USE OF FLAVOMYCIN® FEED ADDITIVE

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INTRODUCTION

Flavomycin® (bambermycins) has been used in broiler diets world-wide for growth promotion and feed conversion improvement since the early 1970's. The spectrum of antimicrobial activity indicates that Flavomycin has little effect against common normal flora bacteria, particularly *Lactobacillus acidophilus* and *Bifidobacterium bifidum* (FOI Summaries, Hoechst Roussel Vet, Warren, NJ). It has been discussed that these normal flora bacteria may offer a natural protection against other bacteria that may be more pathogenic in nature, particularly *Escherichia coli*, *Clostridium* spp. and *Salmonella* spp. Recent field work conducted by Hoechst Roussel Vet indicates that higher numbers of *Lactobacillus* spp., *Bifidobacterium* spp. and other aerotolerant anaerobes are recovered from the intestinal contents from broilers fed Flavomycin from one day of age until processing compated to birds fed other growth promotant programs. In contrast, lower numbers of *Clostridium* spp., coliforms and *Salmonella* have been recovered from these birds, compared to birds fed other growth promotant programs. The purpose of these studies was to determine if lower levels of *Salmonella* could be detected in the growout houses from birds fed Flavomycin compared to other growth promotant programs.

An integrated operation in the western U.S. was identified as the trial location. The operation normally processes approximately 650,000 broilers per week. The operation has two distinct geographical regions of growers. The north division of growers are supplied feed from one feed mill located in approximately the same area as the growers. The south division of growers are supplied feed from two feed mills which are also located in the same geographical region. Approximately 2.5 months before sampling, the north division initiated a growth promotant program of 2g/ton Flavomycin from one day of age until the birds were processed. The south division continued on the program that had been in place which consisted of 50g/ton BMD® from one day of age until approximately 19 days of age. The next feed contained 25g/ton BMD from 19 days until approximately 35 days of age. The withdrawal feed contained 10g/ton Stafac® and was fed until approximately 44 days when the birds were processed. Other aspects of broiler management were kept similar between the north and south divisions except for the growth promotant program. Six farms were selected for testing at each division. The selection included two of the better producing farms, two average producing farms and two underperforming farms from each division. Two houses per farm were sampled. All farms and houses were selected by the company personnel. Litter drag swabs were prepared by Dr. W. D. Waltman, Georgia Poultry Laboratory, and stored frozen in double strength skim milk transport media. The sampling of broiler houses was conducted according to NPIP protocol (2) with four swabs obtained for each identified house. All houses were sampled when the chickens were 35 days of age. With the completion of the sampling, all swabs were shipped via next day courier to Dr. W.D. Waltman. Isolation and identification and confirmation of *Salmonella* spp. were performed by the Georgia Poultry Laboratory according to previously described sampling techniques (3).

RESULTS

Results are summarized in Table 1.

Table 1. Number of positive isolations of Salmonella spp. By farm from drag swab samples.

North Division Farms (Flavomycin)			South Division Farms (BMD/BMD/Stafac)		
Farm ID	No. Samples	No. Salmonella positive	Farm ID	No. Samples	No. Salmonella positive
A	8	0	A	8	0
B	8	0	B	8	6
C	8	0	C	8	8
D	8	1	D	8	8
E	8	0	E	8	2
F	8	6	F	8	5

DISCUSSION

Forty-one of the 48 samples (85%) obtained from the north division were negative for *Salmonella* spp. Nineteen of the 48 samples (40%) obtained from the south division were negative for *Salmonella* spp. Serological grouping of the *Salmonella* isolates by the Georgia Poultry Laboratory resulted in only serogroup C2 isolated from the north division and both serogroup B and C2 from the south division. There is a considerable difference in *Salmonella* recoveries between the two divisions. The significance of broilers arriving at the processing plant free of *Salmonella* is well understood. Numerous studies have been summarized to show that a significant step in reducing *Salmonella* contaminated carcasses is to reduce the level of *Salmonella* arriving in broilers at the plant (1). The results of one trial does not indicate a significant trend. However, these findings are significant enough to warrant additional field research.

REFERENCES

1. Hargis, B. M., D. J. Caldwell, J. A. Byrd, D. E. Corrier, and L. H. Stanker. Preharvest crop contamination and salmonella recovery from broiler carcasses at processing. International Symposium on Food-Borne Salmonella in Poultry. Baltimore, MD. July 1998.

2. National Poultry Improvement Plan and Auxiliary Provisions. USDA, Animal and Plant Health Inspection Service, Veterinary Services, Publication APHIS 91-55-021. U.S. Government Printing Office. June 1994.

3. Waltman, W. D., A. M. Horne, C. Pirkle, and T. G. Dickson. Use of delayed secondary enrichment for the isolation of salmonella in poultry and poultry environments. Avian Dis. 35:88-92. 1991.

REDUCTION OF SALMONELLA TYPHIMURIUM COLONIZATION IN BROILER CHICKENS BY A NEWLY DEVELOPED COMPETITIVE EXCLUSION CULTURE (PHLEX)

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Human outbreaks of *Salmonella* infection are often associated with consumption of poultry. The beneficial effects of probiotics in reducing human pathogens in poultry products has been demonstrated by many investigators. The phenomenon, known as 'competitive exclusion' (CE), is the basis for the development of defined or undefined mixed bacterial cultures which are used to reduce *Salmonella* colonization in chickens. The spectrum of microflora used in such commercial products differs widely. In addition, due to different methods of propagation, storage and delivery, the efficacy of CE preparations also varies. We are currently in the process of defining a probiotic mixture of indigenous bacteria of normal chicken gut origin. A final mixed culture ('Phlex') of these bacteria was achieved by a defined method and was used in laboratory trials. The ongoing trials were conducted with one-day-old broiler chicks raised in a heated brooder house and provided with feed and water *ad libitum*. The chicks were given orally approximately 10^8-10^9 cfu/chick of the mixed culture. The chicks were then challenged orally (~10^2-10^6 cfu/chick) with a nalidixic acid resistant strain of *Salmonella typhimurium* 3 days after the Phlex treatment. Control chicks without the Phlex treatment were raised and challenged similarly in the same brooding facility. Five to seven days after the challenge, the chickens were killed and the cecal contents were cultured on selective brilliant green agar plates after enrichment in selenite broth. Preliminary results showed moderate to significant reduction of cecal colonization by *S. typhimurium* in the Phlex treated chicks. Phlex does not contain any known avian pathogen and was found to be safe to use on day old broiler chicks. Laboratory analysis for further characterization of the preparation and more trials for the reduction of *Salmonella* by competitive exclusion are currently underway.

COMPARATIVE EFFECTS OF A MANNAN OLIGOSACCHARIDE AND AN ANTIBIOTIC GROWTH PROMOTER ON PERFORMANCE OF COMMERCIAL BROILERS

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ABSTRACT

Alternatives to growth promoting antibiotics are being sought by the industry, government and consumers. The following study was undertaken to evaluate the comparative effects of Bio-Mos, a mannan oligosaccharide from yeast cell wall material, and Bacitracin Methylene Disalycylate (BMD) on the performance of broiler chickens. A negative (no growth promoter) control was included in the experimental design. There were no differences among the three treatments in average liveweight and feed conversion at 22 days of age. Birds receiving both Bio-Mos and BMD had significantly heavier average liveweights and better feed conversion at 49 days than did the negative controls. The Bio-Mos and BMD fed birds did not differ significantly in either body weight or feed conversion. There were no treatment differences in mortality. Based on these data Bio-Mos is an alternative to the use of BMD as a growth promoter in commercial broiler diets.

INTRODUCTION

The current study was undertaken to compare the effects of Bio-Mos, a mannan oligosaccharide from yeast cell wall material, and Bacitracin Methylene Disalycylate (BMD) on broiler performance and production economics.

MATERIALS AND METHODS

Birds and housing. The study involved 720 straight run Ross X Ross broilers (sourced from Wampler Foods, Inc., Harrisonburg, VA) housed in 24 (1.2m x 1.5m) concrete floor pens, with 7.5 cm of new wood shavings. Stocking density was 16.7 chick / sq. m. (30 birds/pen), 8 pens per treatment.

Diets and treatments. All treatments received a three phase feed program scheduled on a feed per bird basis. The three dietary treatments consisted of a negative control (no additive), a positive control (BMD) and Bio-Mos. BMD was added at 50 g/t in the starter and grower and 25 g/t in the finisher. Bio-Mos was added at 1 kg/T in the starter and 0.5 kg/T in the grower and finisher. All birds received Avatec as a coccidiostat at 90 g/ton. All broilers received starter feed in crumble form from day 0 to 21, grower feed in pellet form from day 22 to 35, and finisher feed in pellet form from day 35 to 49. Feed and water were provided *ad. libitum*. All deaths that occurred during the first 7 days were replaced with a hatchmate. Visual health inspection of all birds within the facility were performed at least twice daily. All birds that died during the study were examined post-mortem to determine probable cause of death. All birds were weighed by pen on days 22 and 49. Liveweights, weight gain, feed consumption, mortality and production economics were used to evaluated the effects of the additives. Data were subjected to ANOVA and Fisher's Protected LSD model was used to compare the means.

RESULTS

Performance. There were no differences ($p>0.05$) found between the day 22 average liveweights and feed conversion of all groups in this trial. Broilers receiving Bio-Mos and BMD feeds were heavier ($p<0.05$) and had lower feed conversion ($P<0.05$) at day 49 than the broilers receiving negative control feed. There were no statistical differences ($p>0.05$) in mortality found due to treatment. Through day 22, mortality was extremely low (3/treatment group) with seven deaths due to air sac disease and two from cardiac disorders. Through day 49 mortality was lowest for the Bio-Mos group and highest for the negative control group (numerically). In addition, high morbidity was observed in the negative control group between days 22 and 49. There were 8, 14 and 10 deaths between 22 and 49 days in the Bio-Mos, negative control and BMD groups, respectively (Table 1).

Table 1. Effects of Bio-Mos, BMD and a negative control on broiler performance.

	Liveweight (kg)		FCR		Mortality (%)	
	Day 22	Day 49	Day 22	Day 49	Day 22	Day 49
Bio-Mos	0.696[a]	2.507[a]	1.657[a]	1.830[a]	1.25	4.58
Negative Control	0.727[a]	2.392[b]	1.665[a]	2.014[b]	1.25	7.08
BMD	0.692[a]	2.581[a]	1.669[a]	1.815[a]	1.25	5.42

[ab]Means in a column with different superscripts dieffer ($P<0.05$)

Cost analysis. Cost analyses (US$) were conducted to determine feed cost per kg of meat and market value of whole carcass. Actual feed costs per pen were used to determine the feed cost per kg of meat gained. A 73% carcass yield was used to determine market value of whole carcass. The following prices and formulas were used to determine cost analyses:

Whole bird	$1.08/kg
Bacitracin MD	$0.04/g
Bio-Mos	$3.30/kg
Starter Feed	$0.2423/kg
Grower Feed	$0.2313/kg
Finisher Feed	$0.2202/kg

The following calculations were used for cost analyses
Feed cost/lb of meat = Total feed cost per pen ÷ Total final pen weight
Feed cost/bird = Feed cost/lb of meat x Final weight/bird
Market value of carcass = Final weight/bird x 73% x Whole bird price/lb
Return/bird less feed costs = Market value of carcass - Feed cost/bird

The cost of feed per kg of meat gained for the broilers receiving Bio-Mos and BMD was lower than that of the broilers receiving control feed while cost of feed per broiler receiving Bio-Mos was lower than the cost of feed per control broiler. The estimated average market value of each carcass from the broilers receiving Bio-Mos and BMD was higher than that of the controls. The estimated average return per bird after feed costs of broilers receiving Bio-Mos and BMD was higher than that of the broilers receiving control feed. A $0.03 greater return per bird after feed costs was provided by the broilers receiving BMD than the broilers receiving Bio-Mos. A 14 cent and 17 cent greater return per bird after feed costs was provided by the Bio-Mos and BMD feed groups, respectively, over the negative control group (Table 2).

Table 2. Cost analysis.

	Feed cost/kg meat	Feed cost/bird	Carcass market value	Return/bird less feed cost
Bio-Mos	0.4267[a]	1.0687[a]	1.9751[a]	0.9063[a]
Negative-control	0.4656[b]	1.1138[b]	1.8848[b]	0.7710[b]
BMD	0.4227[a]	1.0893[ab]	2.0332[a]	0.9439[a]

[ab]Means differ, P< 0.05.

CONCLUSIONS

Owing to inclement weather in the Shenandoah Valley at the time of the study (January-February, 1998), the environment resulted in an added stress placed on the birds. Under these conditions Bio-Mos and BMD had comparable effects on broiler performance. Both treatments improved performance compared to the negative control. It is concluded that Bio-Mos is a viable alternative to BMD for broiler production.

COMPARATIVE EFFECTS OF A MANNAN OLIGOSACCHARIDE AND AN ANTIBIOTIC GROWTH PROMOTER ON PERFORMANCE OF COMMERCIAL TURKEYS

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ABSTRACT

Alternatives to growth promoting antibiotics are being sought by industry, governments and consumers. The following study compared effects of a commonly used antibiotic growth promoter, BMD with Bio-Mos, a mannan oligosaccharide derived from yeast cell wall material, on commercial turkey performance. The treatments consisted of: 1) negative control (no growth promoter), 2) Bio-Mos, 3) BMD, 4) Bio-Mos + BMD. To assure a disease challenge, used litter was topdressed on fresh pine shavings. Body weights of the control birds were significantly lower than all other treatments. Weights for the Bio-Mos and BMD birds did not differ and were intermediate to the negative control and the Bio-Mos + BMD treatments. The combined treatment differed from, and was superior to, the negative control and BMD, but was not significantly different from Bio-Mos fed birds. There were no significant differences in feed conversion before adjusting for mortality. After adjusting for mortality there were differences. The negative control had the worst feed conversion, the combined

treatment the best feed conversion, and the two growth promoters by themselves intermediate feed conversions.

INTRODUCTION

The use of growth promoting antibiotics is routine in livestock and poultry production. Alternatives are being sought. The current study was undertaken to compare the effects of Bio-Mos, a mannan oligosaccharide from yeast cell wall material, and Bacitracin Methylene Disalycylate (BMD) in turkey production.

MATERIALS AND METHODS

Birds and housing. The study involved 720 Hybrid toms housed in 40 (1.2m X 3.7m) cement floor pens. Stocking density was 4 toms / sq. m. (18 birds/ pen), 10 pens per treatment. In addition to fresh pine shavings litter, used litter was placed in each pen.

Diets and treatments. All treatments received a six phase feed program scheduled on a feed per bird basis. The four dietary treatments consisted of a negative control (no additive), BMD, Bio-Mos and BMD + Bio-Mos. BMD was added to the first ration at 55g/tonne, and at 27.5g/tonne to the remaining 5 rations. Bio-Mos was added at 1 kg/tonne to the first ration and 0.5 kg/tonne to the remaining 5 rations. Feed was in crumble form for the first 3 weeks and pelleted thereafter. Feed and water were provided *ad libitum*. Birds that died during the first 7 days were replaced with a hatch mate. Visual health inspection of all birds within the facility was performed at least twice daily. All birds that died during the study were examined postmortem to determine probable cause of death. Birds were weighed by pen at 6, 12, 15 and 18 weeks of age. Liveweights, weight gain, feed consumption, mortality, feed cost / kg of meat, feed cost/bird, market value/bird and return/bird less feed costs were analyzed by analysis of variance. Fisher's Protected LSD model was used to compare the means of each treatment.

RESULTS AND DISCUSSION

Performance. Average weights were unaffected by treatment at 6 and 12 weeks, however birds given the combination treatment were significantly heavier at 15 and 18 weeks. Control birds were lighter than all other birds throughout the study, however the difference was significant only at week 18. Weights of birds on the Bio-Mos and BMD treatment diets were intermediate to control and combination treatment means (Table 1).

Table 1. Effects of Bio-Mos and BMD on average liveweight of turkeys at 6, 12, 15 and 18 weeks.

	Week 6	Week 12	Week 15	Week 18
Control	1.993[a]	7.281[a]	9.348[b]	11.868[c]
Bio-Mos	2.031[a]	7.518[a]	9.698[ab]	12.563[b]
BMD	2.127[a]	7.545[a]	9.716[ab]	12.455[b]
Bio-Mos + BMD	2.116[a]	7.535[a]	9.804[a]	12.787[a]

[ab]Means in a column with different superscript(s) differ ($p \leq 0.05$).

Feed conversion was unaffected by treatment at week 6, however the feed conversion of the control birds was greater than that of treated birds thereafter. Turkeys receiving Bio-Mos had significantly better feed conversion than control birds at weeks 12 and 15 while those given either BMD or the combination had intermediate feed conversions. At week 18 feed conversion of birds on the combination treatment was lower than all other treatments but only significantly different from the control (Table 2). When feed conversions were adjusted for mortality (calculated by dividing total feed consumed by total liveweight plus weight of all mortality) feed conversions were lower than the control for birds receiving either Bio-Mos or BMD during week 15 and for the combination treatment at week 18 (Figure 1).

Table 2. Effects of Bio-Mos and BMD on average feed conversion of turkeys at 6, 12, 15 and 18 weeks.

	Week 6	Week 12	Week 15	Week 18
Control	1.591[a]	2.436[b]	2.805[b]	3.370[b]
Bio-Mos	1.616[a]	2.302[a]	2.662[a]	3.122[ab]
BMD	1.556[a]	2.341[ab]	2.692[ab]	3.154[ab]
Bio-Mos + BMD	1.519[a]	2.391[ab]	2.739[ab]	2.974[a]

[ab]Means in a column with different superscript(s) differ ($p \leq 0.05$).

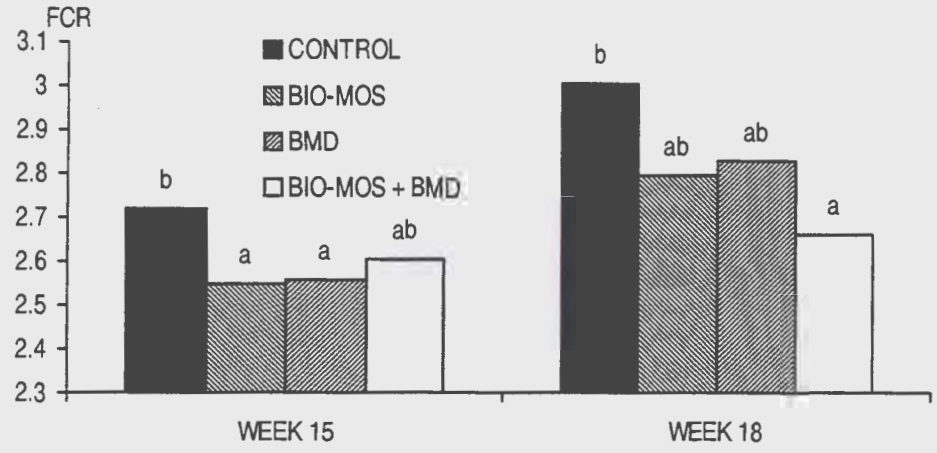

Figure 1. Effect of Bio-Mos and BMD on feed conversion of turkeys adjusted for mortality at weeks 15 and 18. Columns at a single age with different superscripts differ ($P \leq 0.05$).

The majority of the mortality was due to cardiac disorders. Other causes of death were air sacculitis and peritonitis. Mortality (calculated after day 7) did not differ significantly among treatments and was 17.22, 15.63, 14.40 and 14.95% for the control, Bio-Mos, BMD and combination treatments, respectively.

Cost analysis. Cost analyses were conducted to determine feed cost per kg of meat and market value of whole carcass. Actual feed cost per pen was used to determine feed costs per kg of meat gain. A 70% carcass yield was used to determine the market value of a whole carcass. The following prices were used to determine cost analysis: Whole bird \$0.66/kg; BMD, \$0.04/g (\$2.20/tonne); Bio-Mos, \$3.30/ kg. Starter 1, Starter 2, Grower 1, Grower 2, Finisher 1, and Finisher 2 feeds were \$190.59, \$182.02, \$175.40, \$171.30, \$166.96, \$162.92 per tonne, respectively. Average feed cost per bird did not differ with treatment; however cost of feed per kg of meat for turkeys receiving Bio-Mos alone or with BMD was significantly lower than that of the control. Carcass value of turkeys receiving Bio-Mos or BMD was significantly higher than the control while highest carcass value was obtained from combining the additives ($P<0.05$). Returns per bird were higher than for Bio-Mos, BMD and Bio-Mos + BMD than for the control ($P<0.05$) (Table 3).

Table 3. Effect of Bio-Mos and BMD on economic performance (values are in USD).

	Control	Bio-Mos	BMD	Bio-Mos+BMD
Feed cost/kg of meat	0.503 [b]	0.470 [a]	0.481 [ab]	0.465 [a]
Feed cost/bird	5.964 [a]	5.896 [a]	5.988 [a]	6.126 [a]
Market value of carcass	5.730 [c]	6.066 [b]	6.013 [b]	6.359 [a]
Return/bird less feed cost	-0.234[b]	0.169 [a]	0.025 [a]	0.233 [a]

[ab]Means in a row with different superscript(s) differ ($p \leq 0.05$).

CONCLUSIONS

Bio-Mos and BMD provided similar responses in turkey performance in this trial. Both treated groups performed better than unsupplemented birds in a stressed environment (inclusion of used litter). It is concluded that Bio-Mos is a viable alternative to antibiotic growth promoters where insurance against stress related opportunistic organisms is needed.

NATIONAL U.S. SURVEY OF PATHOGENS IN POULTRY LITTER

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INTRODUCTION

Poultry litter is a combination of poultry waste, various bedding types, and other materials that accumulate during the broiler production process. It is useful as an alternative feed source for cattle and also as a fertilizer. One of the concerns regarding poultry litter is its microbiological composition. This is important for a number of reasons including the possibility of passing on pathogens to food-producing cattle, the spread of bacteria onto land and into the environment, and the health and performance of broilers which are grown on used litter. Poultry litter is a beneficial and economical by-product of the poultry industry, but it is necessary to further investigate its microbiological makeup to ensure its safety and to search for its best uses. The objective of the present study was to collect samples of poultry litter throughout the U.S. and determine the presence of pathogenic bacteria.

MATERIALS AND METHODS

Litter collected for this study was taken from broiler houses throughout the U.S. Twelve different regions including ten farms per region were sampled. The litter collection procedure involved scraping with the heel of the hand, while wearing a sterile latex glove, along the surface gathering the top few centimeters of litter. Poultry litter samples were taken from five different locations within each house and combined into one 100 gram sample per house. Collected litter was analyzed for total bacteria, gram negative bacteria, gram positive bacteria, *Staphylococcus, Escherichia coli,* and coliforms. Percentage litter moisture and pH were also taken and recorded.

RESULTS AND DISCUSSION

The results of this trial indicate that an abundance of bacteria are found in fresh poultry litter. Among the analyzed pathogens, gram positive bacteria were the most prevalent when comparing mean CFU/g, while coliforms were identified least often. It is also worth noting that the presence of pathogens may be related to pH. It appears, according to preliminary results, that the presence of bacteria is greater with increasing pH.

Table 1. Mean CFU/g for all regions.

Region	Total Bacteria	Gram Neg.	Gram Pos.	*Staphylococcus*	*E.coli*	Coliforms
Kentucky	1.26×10^{10}	2.88×10^{6}	-	1.56×10^{8}	3.67×10^{7}	1.07×10^{7}
Carolinas	2.43×10^{10}	3.02×10^{9}	-	1.73×10^{10}	1.75×10^{5}	1.02×10^{8}
Pennsylvania	9.32×10^{9}	4.76×10^{9}	2.52×10^{9}	3.18×10^{9}	1.99×10^{9}	-
Delmarva	4.90×10^{10}	4.52×10^{10}	2.16×10^{10}	2.89×10^{9}	5.05×10^{7}	1.03×10^{8}
Arkansas	1.93×10^{11}	5.58×10^{10}	2.61×10^{11}	3.31×10^{11}	3.11×10^{8}	1.12×10^{8}
Oklahoma	4.06×10^{11}	7.68×10^{9}	2.29×10^{11}	3.08×10^{11}	1.69×10^{5}	3.02×10^{7}
Georgia	2.36×10^{10}	1.21×10^{9}	1.66×10^{10}	1.72×10^{10}	7.29×10^{7}	5.90×10^{8}
Alabama	1.28×10^{11}	2.01×10^{9}	1.16×10^{11}	7.93×10^{10}	4.16×10^{6}	5.02×10^{6}
Mississippi	1.73×10^{11}	5.84×10^{10}	2.42×10^{10}	5.73×10^{10}	5.00×10^{7}	2.10×10^{8}
California	1.59×10^{11}	6.88×10^{10}	2.25×10^{11}	1.42×10^{11}	1.68×10^{7}	1.19×10^{8}
Texas	2.38×10^{11}	1.48×10^{11}	2.91×10^{11}	2.52×10^{11}	1.76×10^{7}	2.67×10^{6}
Louisiana	2.74×10^{11}	9.03×10^{10}	2.32×10^{11}	2.96×10^{11}	5.40×10^{8}	2.80×10^{8}
All Regions	1.37×10^{11}	4.39×10^{10}	1.44×10^{11}	1.10×10^{11}	2.60×10^{8}	1.42×10^{8}

Table 2. Mean CFU/g according to pH.						
pH	Total Bacteria	Gram Neg.	Gram Pos.	*Staphylococcus*	*E.coli*	Coliforms
6.0	$1.48x10^{10}$	$2.85x10^{9}$	-	$4.38x10^{9}$	$6.22x10^{7}$	$1.43x10^{8}$
7.0	$6.40x10^{10}$	$1.04x10^{10}$	$1.86x10^{11}$	$6.50x10^{10}$	$8.87x10^{7}$	$1.52x10^{8}$
8.0	$1.43x10^{11}$	$3.86x10^{10}$	$1.35x10^{11}$	$1.21x10^{11}$	$4.03x10^{8}$	$1.82x10^{8}$
9.0	$1.96x10^{11}$	$8.09x10^{10}$	$1.56x10^{11}$	$1.36x10^{11}$	$6.95x10^{7}$	$7.07x10^{7}$

STUDIES USING RESTRICTION ENDONUCLEASES ON AVIAN ADENOVIRUSES ISOLATED FROM CHICKENS AND PIGEONS IN TAIWAN

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SUMMARY

Six adenoviruses were isolated from chickens showing respiratory signs and/or diarrhea. Another adenovirus was isolated from pigeons with diarrhea. The viruses were confirmed to be group 1 avian adenovirus (FAV) by the indirect immunoflourescent and agar-gel immunodiffusion tests. The DNA of these FAV was extracted from infected chicken kidney cell cultures and the restriction endonuclease assay was performed. The results indicated that four chicken isolates and one pigeon isolate belonged to group E and one chicken isolate belonged to group A based on the grouping proposed by Zask and Kisary (6). Another chicken isolate did not fit into an existing group. A set of primers was designed from the published nucleic acid sequence of CELO virus. Polymerase chain reactions using this set of primer could detect all seven isolates.

INTRODUCTION

Avian adenoviruses can be divided into 3 groups on the basis of their antigenicity. Group 1 virus was named fowl adenovirus (FAV). FAV could be recovered from clinically healthy or sick poultry such as turkeys, chickens, ducks, geese and pigeons. The clinical picture could include respiratory signs, diarrhea, inclusion body hepatitis, and also a decline in egg production (1,3,5). FAV had been grouped by the cross neutralization test and restriction endonuclease produced patterns of nucleic acid. By the cross neutralization test, FAV has been divided into 12 serotypes (FAV1-FAV12). On the basis of restriction endonuclease analysis using two enzymes, FAV has been placed into 5 genotypes. Each genotype appears to correspond only to certain serotypes (6). At present, although no apparent association has been found between pathogenicity and serotype, all pathogenic FAV isolates belong to E genotype (4). In this paper we described the isolation and characterization of FAV from chickens and pigeons in Taiwan and a PCR for rapid diagnosis of FAV.

MATERIALS AND METHODS

Virus isolates. Seven avian adenoviruses were isolated from Taiwan field cases. These isolates had been plaque-purified three times and were studied at a low number of passages. The history of the viruses is given in Table 1. All these viruses were isolated in chicken kidney cell culture or SPF chicken embryos via the yolk sac route. The isolated viruses revealed typical adenovirus morphology under electron microscope. The viruses were confirmed to be group 1 avian adenovirus by the indirect immunoflourescent and agar-gel immunodiffusion tests. None of these viruses agglutinated chicken RBC.

Restriction endonuclease analysis of virus DNA. For DNA extraction, the method of Shinagawa *et al.* (5) was used. The purified DNA of each isolate was split with the restriction enzymes *Hin*dIII and *Bam*HI. The digestion was carried out as recommended by the manufacturer (Promega) except that five times the amount of restriction enzymes was used to insure the complete digestion of the DNA. Digested DNA was submarine electrophoresed in 0.8% gel in TAE buffer. Electrophoresis was conducted at 1.5V/cm for 18 hours. Then the gel was stained with 0.5μg/ml ethidium bromide and photographs were taken under UV light.

Primers. Based on the published sequences of avian adenovirus CELO strain, a set of the primer was designed. The primers were 20 base pair for each and were located at the penton gene. The sequence of the primer PU1 was 5'-GGAACAGCATTAGATACCGG-3', and of PL1 was 5'-TCAGCAAATCCAGATACCGC-3'. PU1 is 15285-15304 nucleotide sequence of CELO and PL1 is the complementary sequence to nucleotides 15787-15806.

Table 1. The group 1 avian adenovirus isolated in Taiwan

Virus	Date of isolation	Location (County)	Avian type	Age (days)	Clinical signs	Adenovirus gene group	Mixed virus infection
TS-1	July 1997	Pintung	Broiler	7	Diarrhea, nephritis	E	IBV, ELPs, Reovirus
TS-2	Nov. 1997	Taichung	Broiler	28	Diarrhea, respiratory signs, nephritis	?	IBV, ELPs, Reovirus
TS-3	Jan. 1998	Taoyung	Broiler breeder	36	Diarrhea, respiratory signs	E	ILTV
TS-4	Aug. 1997	Taichung	Broiler	26	Respiratory signs, hepatitis	E	Reovirus
TS-5	July 1997	Taichung	Broiler	35	Diarrhea, nephritis, respiratory signs, bursitis	E	IBDV, ELPs
TS-6	July 1997	Miaoli	Broiler breeder	147	Respiratory signs	A	none
TS-7	June 1998	Tainan	Pigeon	30	Diarrhea	E	none

IBV= infectious bronchitis; ELPs= enterovirus like particles; ILTV= infectious laryngotracheitis virus; IBDV= infectious bursal disease virus.

Polymerase chain reaction. 0.5μl FAV DNA, 1μl each of 25μM primer PL1 and PU1 , 2μl of 10mM dNTPs, 1μl of 2U/μl Tag DNA, 10μl of 10X buffer solution(ProZyme), and 84.5μl of D2W were mixed and put on a thermal cycler. The reaction conditions for the PCR for amplifying the FAV DNA were denaturing: 95°C, 40sec; annealing: 50°C, 70sec; elongation: 72°C, 70sec. After a total of 30 cycles of reaction, final elongation was conducted at 72°C for 7 min, and the products were kept at 4°C.

Assay of the PCR product. 10μl of PCR product and 100bp DNA ladder was each mixed with 2μl of Blue/Orange 6X loading dye (Promega) and were electrophoresed in 2% agarose in TAE buffer solution. The assay condition was 100 volts for 30 min. The gel was stained with 0.5μg/ml ethidium bromide and photographs were taken under UV. The DNA of the PCR product was purified by a commercial kit (Omega, Ezna cycle-pure kit) following producer's instruction. The purified DNA was sequenced by automatic DNA sequencer (ABI PRISM 377, Applied Biosystems, USA).

RESULTS

Restriction endonuclease analysis. The results indicated that five of these adenoviruses (four chicken isolates and one pigeon isolate) belonged to E group and one isolate belonged to A group based on the grouping proposed by Zask and Kisary (6). Another adenovirus isolated from a chicken could not be fitted into an existing group.

Polymerase chain reaction. All the 7 Taiwan group 1 avian adenovirus isolates could produce a predicted 522bp PCR product. The PCR products could be digested by *Pst*I and *Sma* I and specific fragments obtained. The PCR products were sequenced and compared by BLAST programs. This revealed a 96% (473/490) homology with the penton gene of CELO virus.

DISCUSSION

Adenoviruses are widely distributed in poultry flocks. Group I adenoviruses are isolated from chickens, geese, ducks and turkeys. The exact role of most of these viruses as etiological agents of specific diseases in chickens remains uncertain. It has been shown that hypervirulent FAV belong to a subgroup of restriction endonuclease group E (2). In this study five out of seven avian adenovirus isolated in Taiwan in chickens and pigeons were placed in E group. Although most of them were isolated from clinical cases with a mixed virus infections, at least some of them are considered to be pathogenic. In a preliminary study, one of the E group isolates (TS-7 strain; a pigeon isolate) was showed to be hypervirulent to day-old SPF chicks and caused 50% mortality. Another E group isolate (TS-4 strain) induced inclusion body hepatitis and 20% mortality in day-old SPF chicks.

The designed primers could diagnose all the 7 Taiwan isolates, which belonged to different gene groups and which were isolated from different host species. The results indicate that these sequence are very conserved in group I adenovirus.

REFERENCES

1. Dhillon, A. S., R. W. Winterfield, H. L. Thacker, and D. S. Feldman. Lesions induced in the respiratory tract of chickens by serologically different adenoviruses. Avian Dis. 26:478-486. 1981.

2. Erny, K. M., D. A. Barr, and K. J. Fahey. Molecular characterization of high virulent fowl adenoviruses associated with outbreaks of inclusion body hepatitis. Avian Pathol. 20:597-606. 1991.

3. McFerran, J. B., and B. M. Adair. Avian adenoviruses – a review. Avian Pathol. 6:189-217. 1977.

4. Pallisier, J. A., K. M. Erny, and K. J. Fahey. Serological relationships with the group E fowl adenoviruses. Intervirology. 36:84-90. 1993.

5. Shinagawa, M., A. Matsuda, T. Ishiyama, H. Goto, and G. Sato. A rapid and simple method preparation of adenovirus DNA from infected cells. Microbiol. Immunol. 27:817-822. 1983.

6. Zask, L., and J. Kisary. Grouping of fowl adenoviruses based upon the restriction patterns of DNA generated by *Bam*HI and *Hind*III. Intervirology. 22:110-114. 1984.

VACCINATION OF TURKEYS AND CHICKENS AGAINST ORNITHOBACTERIUM RHINOTRACHEALE INFECTION

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Vaccination of young broiler chickens with inactivated vaccines was found to be effective, but the results were influenced negatively by the presence of maternal antibodies. Live vaccination was found to be feasible but so far no avirulent *Ornithobacterium rhinotracheale* strains have been found. Vaccination of broiler breeder chickens resulted in significant protection of their progeny up to 4 weeks of age against an aerosol challenge with *O. rhinotracheale.* Laboratory trials, in which intravenous challenges were performed, also showed protection against arthritis and liver degeneration. Maternal antibodies could be measured in egg-yolks and in the progeny up to 30 days of age of vaccinated breeders. The antibody level in the breeders was found to be constant up to at least 50 weeks of age.

EVALUATION OF A HIGHLY SENSITIVE AND SPECIFIC ELISA FOR DETECTION OF ANTIBODIES TO AVIAN INFLUENZA VIRUS

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An ELISA test kit has been developed for the detection of antibody to avian influenza virus (AI) in chicken serum. This assay has been designed in the microtiter plate format, in which purified AI antigen has been coated onto the solid phase. It is a rapid test method, which has been shown to be highly sensitive and specific, and has demonstrated high correlation with other serological methods.

For the evaluation of sensitivity, samples generated by Dr. David Swayne's laboratory at the Southeast Poultry Research Laboratory (SEPRL) in Athens, GA, were made available to IDEXX for evaluation under a Cooperative Research and Development Agreement with the USDA/ARS. Detection of seroconversion was assessed through the testing of temporal bleeds from birds experimentally infected with AI. Five specific pathogen free (SPF) birds were infected with AI subtype H5N2 and five with subtype H7N2 at SEPRL. Serum samples were collected from each bird over time and tested by agar gel precipitation (AGP), hemagglutination-inhibition (HI) and ELISA. ELISA testing for each of the samples indicated positive results for all five of the H7N2 infected birds by day 7, and four of the five H5N2 infected birds by day 7. By day 10 all five of the H5N2 infected birds tested positive on the ELISA. This response was similar to the results obtained by AGP testing and more sensitive than HI for the same samples.

Further data demonstrating correlation of ELISA results to AGP and HI testing were obtained by testing an experimental SEPRL sample set. There was an overall correlation between the ELISA and AGP of 98.2% for 564 samples tested. The correlation between ELISA and HI testing was 98.6% based on testing of 72 postinoculation samples. Control samples collected prior to inoculation (n=110) tested negative on both HI and ELISA, representing 100% correlation for these samples. Additional sensitivity data were obtained through testing of serially diluted sera collected from avian influenza virus exposed birds, representing each of fourteen hemagglutinin (H) and neuraminidase (N) subtypes (sera obtained from National Veterinary Services Laboratories, Ames Iowa).

For evaluation of specificity, a total of 2184 chicken sera representing multiple sites in the United States and Europe were assayed on the AI ELISA. Samples included sera from broilers, primary breeders and broiler-breeders of various ages. The specificity of this field negative population was 99.6%.

The IDEXX Avian Influenza Antibody test kit described above provides good correlation to other serological methods in the detection of seropositive birds, while maintaining excellent specificity. The test will provide commercial poultry companies with a rapid, standardized method to screen populations for exposure to avian influenza virus.

THE NUCLEOPROTEIN OF NEWCASTLE DISEASE VIRUS : THE AVIAN IMMUNE RESPONSE TO rNP OF NDV IS NOT DIFFERENT FROM THE RESPONSE TO rNP OF AVIAN INFLUENZA VIRUS

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The nucleoprotein (NP) of influenza virus has been shown to induce a protective immune response (1). To investigate whether the NP of Newcastle disease virus (NDV) would induce equal broad immunity, the NP of a highly virulent NDV pathotype was cloned and sequenced. Comparison of the sequence with five other paramyxoviridae showed that the NDV-NP had a 29.5% similarity. The T-cell epitope (peptide 361-370) (2) of Sendai virus appears to be well conserved in the NDV-NP. The NDV-NP gene expressed in recombinant vaccinia (rVac) was electrophoretically and immunologically identical to the wild type NDV-NP. In order to study the immunoprotective capability we prepared 2 sets of recombinant vaccinia for immunization. The first rVac contained the NDV-HN gene (VHN) (3); the second contained the NDV-NP gene (VNP) (2). Groups of 18 G-B1 and G-B2 MHC congenic chickens were inoculated with 1.2 x 10^4 PFU of VNP at two-week intervals. Groups of 10 G-B1 and G-B2 chickens were similarly inoculated with VHN (1.2 x 10^4 PFU). In addition, equal numbers of birds were inoculated with wild type Vaccinia (WTV) and groups of 6-9 birds were vaccinated with attenuated NDV La Sota vaccine. Three weeks following the booster, the birds were lethally challenged with 0.1 ml 10^2 ELD_{50} of NDV (strain Texas GB). None of the WTV inoculated birds developed a substantial antibody titer and the survival rate was 0% upon lethal challenge. The birds inoculated with VHN showed low anti-HN titers as measured by haemagglutination inhibition (HI). This group had low ELISA titers. However, 80% of G-B1 birds survived while 70% of the G-B2 birds survived the lethal challenge. The birds inoculated with VNP developed no measurable anti-HN antibodies but had very high anti-NP titers as measured by ELISA. As in previous experiments, the anti-NP titer of the G-B1 birds was much higher than that for the G-B2 birds at the primary response (16,802 $\pm$ 484 vs. 7,722 $\pm$ 1,085). However, upon lethal challenge there was a 0% survival rate despite very high anti-NP ELISA titers (20,036 $\pm$ 215 and 19,608 $\pm$ 368 for G-B1 and G-B2, respectively). Thus, unlike in the murine influenza model, NDV-NP antibodies do not give any protection after induction with VNP. Our findings are similar to those reported for avian influenza (4).

REFERENCES

1. Andrew, M. E ,and B. F.Couper. Efficacy of influenza haemagglutinin and nucleoprotein as protective antigens against influenza virus infection in mice. Scand. J. Immunol., 28:81-85. 1988.

2. Ward, M. D., F. Fuller, Y. Mehrotra, and E. V. De Buysscher. Nucleotide sequence and vaccinia expression of the nucleoprotein of a highly virulent strain of Newcastle disease virus (to be submitted to Avian Diseases).

3. Schaper, U., F. Fuller, M. Ward, Y. Mehrotra, and E. V. De Buysscher. Nucleotide sequences of the envelope protein genes of a highly virulent strain of Newcastle disease virus. Virol. 165:291-295. 1988.

4. Webster, R. G., Y. Kawaoka, J. Taylor, R. Weinberg, and P. Paoletti. Efficacy of nucleoprotein and haemagglutinin antigens expressed in fowlpox virus as vaccine for influenza in chickens. Vaccine; 9:303-308. 1991.

(A full-length paper is being submitted to *Avian Diseases*.)

ESCHERICHIA COLI INFECTION CHARACTERIZED BY SEPTICEMIA AND FIBRINOUS POLYSEROSITIS IN EGG-TYPE HENS AT THE START OF LAYING

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ABSTRACT

Some very severe outbreaks of *Escherichia coli* infection in hens, at the start of laying, on the same farm are reported. The outbreaks were characterized by no clinical signs, but high and sudden mortality, from 5 to 10%, with lesions of septicemia and fibrinous polyserositis. A gram-negative bacterium was always isolated in pure culture from tissues, with biochemical characteristics related to *E. coli,* but slightly different from the classical strains: lactose negative and non-motile. The isolates when serotyped belonged to somatic group O111. The isolates were sensitive to enrofloxacin, amoxicillin, colimycin, and partially to flumequine, which were used for therapy. The stress of starting to lay seems to be the most probable precipitating cause of the disease.

INTRODUCTION

The association of *Escherichia coli* with certain pathological conditions of poultry dates from the end of the last century. Many reports on the subject have been published (2, 6, 7, 11, 14, 17, 20, 21). *E. coli* affects poultry of all ages but young birds are more sensitive. The infection is considered to be one of the leading causes of economic loss for the poultry industry. Many different manifestations of *E. coli* infection have been described: septicemia, enteritis, granulomas, omphalitis, sinusitis, airsacculitis, arthritis, synovitis, peritonitis, pericarditis, cellulitis, and swollen head. Frequently *E. coli* infection takes place as a consequence of physical and chemical agents such as ammonia, moisture and dust, stress or other microbiological agents such as viruses and mycoplasmas (10, 15, 18, 22). Sometimes *E. coli* is the primary cause of disease particularly in young (4) but also in adult birds (6). Many serotypes of *E. coli* have been isolated everywhere in the world (5, 8, 13, 19, 21). Currently 173 O, 74 K, 53 H and 17 F antigens are recognized (3). The most frequent serotypes in poultry are O1, O2, O8, O35, and O78. Pathological conditions due to *E. coli* have been reproduced experimentally by different routes of inoculation, with mortality varying from 30 to 100% (9, 16, 21). Extensive research has been done on the susceptibility of *E. coli in vitro* and *in vivo* to chemotherapeutics and antibiotics and on the appearance of resistant strains (1, 5, 12, 19). The purpose of this paper is to report on three outbreaks of *E.coli* infection occurring in succession in three flocks of laying hens at the start of lay on the same farm. Signs, gross pathology, microbiology and therapeutic measures taken to control the disease are described.

MATERIALS AND METHODS

Birds. Three flocks, each with about 75,000 layers, 19 to 21-weeks-old, kept in cages in contiguous houses on the same farm, but housed at three different times and from separate growing houses; the first flock had been reared on the floor, the other two in cages until 16 weeks of age.

Bacteriology. Liver, spleen and brain from necropsied birds were cultured on MacConkey agar, blood-agar (Difco) and Gassner VMC agar (Oxoid) media. Liver and ovaries were cultured in enrichment tetrathionate broth (Difco) overnight at 42°C, plated onto XLT4 agar plates (Difco), and incubated at 42°C to check for *Salmonella* species. Metabolic profiles for each isolate were compiled using a miniaturized microorganism differentiation system (Enterotube, Beckton Dickinson or API System, Bio Mérieux), designed for the identification of *Enterobacteriaceae*. An 8-12 hours broth culture was prepared for each isolate and swabbed onto the surface of a 150x15 mm Müller-Hinton agar plate (Difco). Paper discs, impregnated with the antibiotic to be tested, were laid on the medium. The plates were incubated 24 hours at 37°C and inhibition zones were measured. Cultures of *E coli*, isolated from two episodes, were serotyped by Biovac BP61, Beaucouze Cedex, France.

RESULTS

Birds. The birds apparently showed no signs of disease. Egg production was not significantly different from the standard, except in the first group affected. The mortality was high, from 5 to 10% (standard 1.5%) for a period of about 14 weeks in the different flocks, with three phases related to antibiotic treatments. Splenomegaly, fibrinous pericarditis, perihepatitis, oophoritis and lung congestion were evident in nearly all birds. The birds were in good body condition.

Bacteriology. Cultures obtained at different times from liver, spleen and brain of many birds resulted in the isolation

in pure culture of a gram-negative bacterium, non-motile, lactose negative, however with the other characteristics of *E. coli*. All samples were negative for *Salmonella*. The biochemical characteristics of numerous isolates in the three outbreaks were always the same and those of *E. coli*, but differing from the classical strains in being non-motile and lactose negative. These isolates of *E. coli* were serotyped as belonging to somatic group O 111. The organism was sensitive to enrofloxacin, amoxicillin, apramycin, colymicin, gentamycin and ampicillin. It was also sensitive to flumequine initially. It was resistant to lincomycin, kanamycin, tetracycline, chloramphenicol and nalidixic acid.

Medication. A single treatment for 7-10 days of enrofloxacin, amoxicillin, colimycin or flumequine used at recommended doses did not control the disease; at least three treatment cycles of 7-10 days were necessary over about 14 weeks for control.

DISCUSSION

The disease first appeared in the flock which had been reared on the floor at a different farm. The other two flocks were affected 3 weeks and 5 months later. All flocks were affected at 19-20 weeks of age. Two older flocks on the same farm in contiguous houses did not show any signs of disease. Layers from the same brooder houses as the second and third affected flocks but housed on a separate farm of the same company were not affected. This indicates that the strain of *E. coli* in question was most likely introduced by the group first affected. The course of the disease in every group had three phases related to treatment. After treatment mortality returned to normal, except in the last affected flock when it persisted over normal but without further treatments. Enrofloxacin, amoxicillin and colimycin controlled the disease but at least 3 cycles of treatment were necessary.

The serological characterization of the *E coli* isolates showed that they belonged to serotype O 111. The isolation of such a serotype has apparently been reported only once (5). Lesions resembling those seen in the outbreaks have been reproduced by intramuscular and oro-nasal inoculation of the isolate into 21-week-old layers (Zanella *et al.*, unpublished observation).

As long as the intestinal mucosal barrier is intact, the normal microflora of birds is likely to inhibit the translocation of pathogenic *E. coli* from intestine to the blood-stream and internal organs. When the barriers are damaged, perhaps by stress due to the start of egg production, pathogenic bacteria can be isolated from the blood. Consequently, the birds develop septicaemia. If the stressed chickens do not develop the acute form of the disease with death, or recover, their humoral immune system could respond by antibody production (15). In these outbreaks no detectable specific antibodies were found in the blood-stream of a sample of cohabiting birds by the rapid agglutination test, using a suspension of isolated *E coli* as antigen. To explain these outbreaks it is possible that it may be an individual susceptibility related to MHC haplotype of the birds or the stress of laying. In both possibilities, *E. coli* entering the submucosa could induce the production of IgA antibodies responsible for local immunity in resistant birds without inducing a systemic reaction. This could explain the differences between the birds dying and those surviving without symptoms.

In conclusion, although the pathogenesis of *E. coli* infection is poorly understood, there is a general agreement that stress and a following bacteremia are essential factors for the development of clinical disease.

REFERENCES

1. Aleson Sanz R. M., and M. I .M. Aleson Sanz. Sensibilitad de Escherichia coli a los antibioticos: evolucion 1984-1995. Proc. 45th West. Poult. Dis. Conf. Cancun, Mexico. pp. 379-381. 1996.

2. Barnes H. J., and F. Lozano. Colibacillosis in poultry. In: Pzifer Veterinary Practicum, Pfizer Animal Health, Lee's Summit, MO. p. 45. 1994.

3. Barnes H. J., and W. B. Gross. Colibacillosis. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, Iowa. pp.131-141. 1997.

4. Cheville N. F., and L. H. Arp. Comparative pathologic findings of Escherichia coli infection in birds. J. Am. Vet. Med. Assoc. 173:584-587. 1978.

5. Cloud S. S., J. K. Roserberger, P .A. Fries, R. A. Wilson, and E.M. Odor. In vitro and in vivo characterization of avian Escherichia coli. I. Serotypes, metabolic activity and antibiotic sensitivity. Avian Dis. 29:1084-1093. 1985.

6. Dhillon A. S., and D. K. Jack. Two outbreaks of colibacillosis in commercial caged layers. Avian Dis. 40:742-746. 1996.

7. Emery D. A., K. W. Nagaraya, D. P. Shaw, J. A. Newman, and D. G. White. Virulence factor of Escherichia coli associated with colisepticemia in chickens and turkeys. Avian Dis. 36:504-571. 1992.

8. Glanz P. J., S. Narotsky ,and G. Bubash. Escherichia coli serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis. 6:322-328. 1962.

9. Gross W. B. Pathological changes of an Escherichia coli infection in chickens and turkeys. Am. J. Vet. Res. 18:724. 1957.

10. Gross W. B. Factors affectings the development of respiratory disease complex in chickens. Avian Dis. 34:607-610. 1990.

11. Gross W. B., and P. B. Siegel. Coliform peritonitis of chickens. Avian Dis. 3:370-373. 1959.

12. Heller E. D., and H. W. Smith. The incidence of antibiotic resistances and other characteristics amongst Escherichia coli strains causing fatal infections in chickens: the utilization of these characteristics to study the epidemiology of the infections. Comb. J. Hyg. 71:771-781. 1973.

13. Hemsley R. V. , D. A. Barnum, and D. G. Ingram. Biochemical and serological studies of avian strains of Escherichia coli. Avian Dis. 11:90-97. 1967.

14. Hjärré A., and G. Wramby. Undersokningar over en med specifika granulom forlopande hons sjukdom orsakad av mukoida Kolibacterier Koli-granulom. Skand. Vet. Tidsskr. 35:449-507. 1945.

15. Leitner G. ,and E.D. Heller. Colonization of Escherichia coli in young turkeys and chickens. Avian. Dis. 36:211-220. 1992.

16. McGruder E. D. Evaluation of an experimental Escherichia coli airsacculitis model in broiler chicks. Proc. 45th West. Poult. Dis. Conf. Cancun, Mexico. pp. 128-130. 1996.

17. Morris M. P. Broiler cellulitis update. Broiler Industry March. pp. 36-39. 1994.

18. Oyetunde O. O. F., R. G. Thomson, and H. C. Carlson. Aerosol exposure of ammonia dust and Escherichia coli in broiler chickens. Can. Vet. J. 19:187-193. 1978.

19. Rosemberger J. K., and S. S. Chand. Characterization of Escherichia coli isolates from Delmarva broiler chickens. Proc. 16th Natl. Meet. Poult. Health and Condemn., Delmarva, Md. p.104. 1981.

20. Sojka W. J. Escherichia coli in domestic animals and poultry. Commonwealth Agricultural Bureau, Farnham Royal England. pp. 157-169. 1965.

21. Sojka W. J., and R. B. A. Carnagham. Escherichia coli in poultry. Res. Vet. Sci., 2:340-351. 1961.

22. Weinach O. M., G. H. Snoeyembos, C. F. Smyser, and A. S. Soerjadi-Liem. Influence of Mycoplasma gallisepticum, infectious, bronchitis and cyclophosphamide on chickens protected by native intestinal microflora against Salmonella typhimurium and Escherichia coli. Avian Dis. 48:416-425. 1984.

THE FORTY NINTH WESTERN POULTRY DISEASE CONFERENCE
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