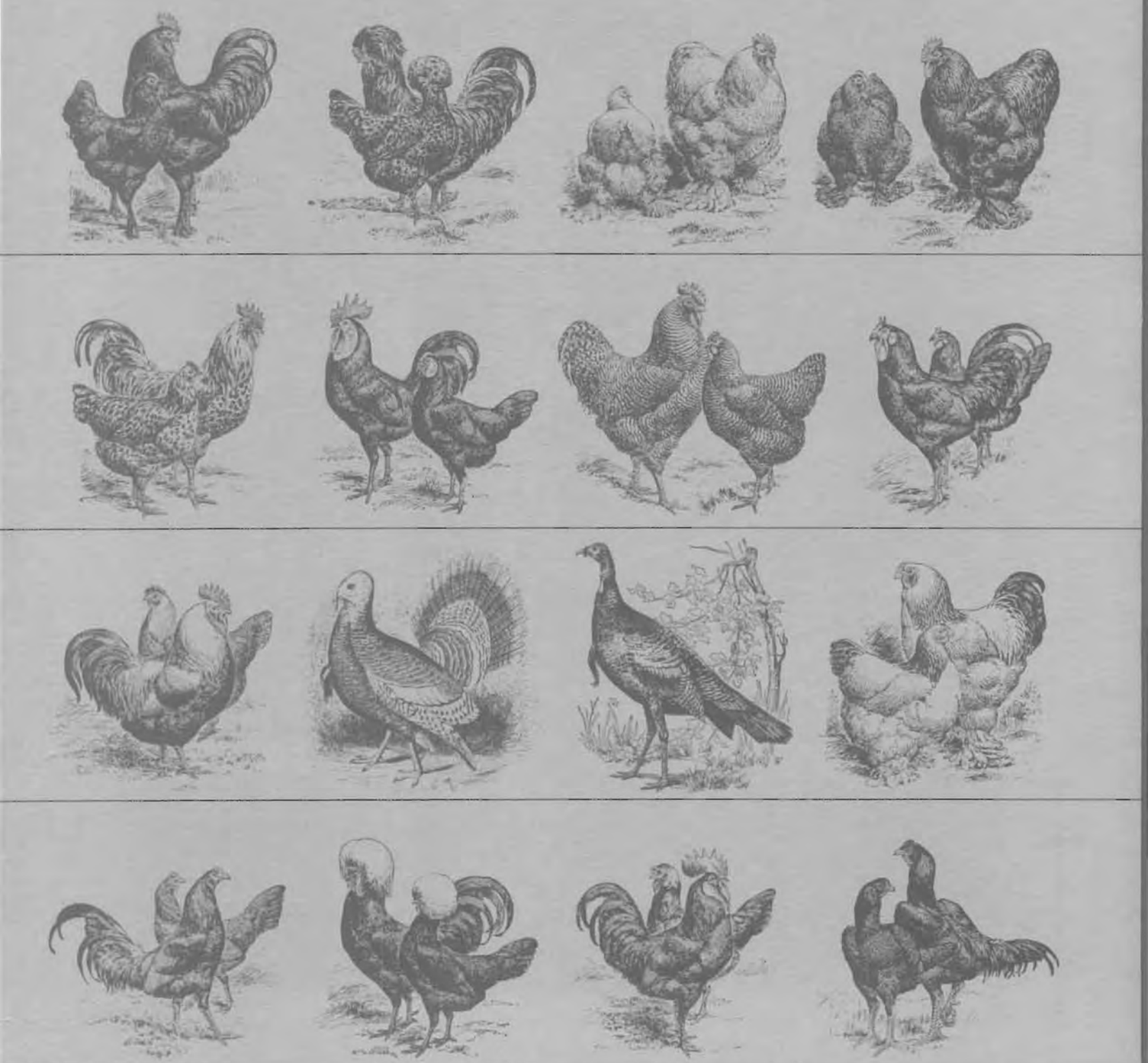
PROCEEDINGS OF THE FORTY-SECOND WESTERN POULTRY DISEASE CONFERENCE

February 28, March 1–2, 1993 · Sacramento, California



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WESTERN POULTRY DISEASE CONFERENCE

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42ND WESTERN POULTRY DISEASE CONFERENCE OFFICERS 1993

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The Proceedings of the 42nd Western Poultry Disease Conference 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. As has been stated in the past, these Proceedings are not considered or regarded as an "official publication" but simply as a source of information.

Copies of Proceedings are available from: A.S. Rosenwald
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SPECIAL ACKNOWLEDGMENT

The Western Poultry Disease Conference (WPDC) is pleased to acknowledge substantial contributions to its Speaker's Fund. These contributions provide support for outstanding scientists whose participation might otherwise not be possible. Over thirty-five organizations and companies 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.

We especially thank the American Association of Avian Pathologists for its generous program enrichment grant that helps bring speakers from other regions. WPDC also sends "thanks" to the Asociacion Nacional de Especialistas en Ciencias Avicolas de Mexico (ANECA) for jointly defraying expenses of several of our speakers. WPDC is most grateful to Pfizer Animal Health for sponsoring and hosting the **"Pfizer Classic"** as a welcoming reception at the Towe Ford Museum.

Our distinguished patrons, donors, and sustaining members are listed on the following pages. We greatly appreciate their generosity.

Many have provided special services that contribute to the continued success of this conference. We express special thanks to Bryan Mayeda and those who have helped him with the local arrangements at Sacramento; members of the translation crew (B. Lucio, M. Salem and V. Mireles), who make a major contribution in providing the excellent English/Spanish translation and other bilingual assistance; the program chair, Singh Dhillon; and all others who contribute to the program and host committees.

We express our gratitude to all authors who submitted manuscripts. We give thanks and acknowledgment to Brigham Young University, Department of Microbiology, who handled the publication of the proceedings. We especially thank Angela Shaeffer for typing and formatting the manuscripts and Jennifer Harrison for coordinating the computer desktop publishing of the proceedings. We also thank Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design and interior layout, and Brigham Young University Print Services for the excellent and timely printing of the Proceedings.

WPDC SPECIAL RECOGNITION AWARD

At this year's conference, the WPDC is pleased to present its Special Recognition Award to:

Royal A. Bagley

for his many contributions and for his many years of support and service to the poultry industry and to this conference.

DEDICATION OF THE 42ND WESTERN POULTRY DISEASE CONFERENCE TO THE MEMORY OF WALTER WHITE SADLER, DVM, MPH

1913-1992

Walt was born in Biloxi, Mississippi. He received his baccalaureate degree and DVM from Texas A and M University, and his MPH from the University of California. He was on the staff and faculty of the School of Veterinary Medicine, Davis, from 1950 until his retirement in 1983. He was Chair of the Department of Epidemology and Preventive Medicine from 1970 to 1977 and directed the Master of Preventive Medicine program in which he played a major designer role, from 1977 to 1983.

He was recognized internationally and travelled world-wide to help advance programs in poultry disease control and in public health. He was a rare and unusual teacher, especially one-on-one; he was a person of incisive intellect as an investigator and a real "people" person with a dry and wonderful sense of humor. But it is as the developer of the WPDC's financial program that he is specially remembered.

He was a founding member of the WPDC. In 1990, the organization recognized him for his many contributions to the poultry industries and the conference. Unfortunately he was unable to be present on that special occasion.

This dedication is a special recognition for his many contributions and as the developer of the WPDC's financial program. In 1960, a collection of $2.00 per participant was initiated: Dr. Sadler was designated as custodian of the funds. He willingly accepted the responsibility, but not the title, saying it implied janitorial status! Walt personally set up a bank account of his own for the funds and when contributions from industry grew, he established the University of California extramural fund or account which is still operating. W.W. died at his home on October 3, 1992. We all owe him a real debt for his enthusiasm, originality and foresight and for his constant vigilance on behalf of the poultry industry, general and specific public health, student well-being, the veterinary and ancillary professions--and especially for this unincorporated organization--the Western Poultry Disease Conference, which he helped establish. For all this and many other reasons, we proudly dedicate the 42nd Western Poultry Disease Conference (WPDC) to Walter White Sadler.

DEDICATION OF THE 1992 WPDC TO MARCUS M. JENSEN

For his many contributions to the Western Poultry Disease Conferences. Before 1986, the WPDC proceedings were assembled and mailed two to six months after the conference. In 1986, Drs. McMartin and Lucio combined to have the proceedings of the joint ANECA-WPDC available at the meeting in Puerto Vallarta. Since then, Dr. Marcus Jensen has been working tirelessly with a stellar crew at Brigham Young University to publish and transport beautifully designed and printed proceedings for the five conferences (1987, 1988, 1989, 1990 & 1992) held in the United States. Dedication of the 1992 conference is a small "thank you" to Marcus Jensen, which we could not "sneak" into the 1992 WPDC Proceedings. It is a tribute to Marcus' dedication to do a great job of getting complete useful proceedings for timely distribution for all of us and to his many other helpful innovations. Thank you, Marcus!

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MINUTES OF THE 1992 41ST WESTERN POULTRY DISEASE CONFERENCE BUSINESS MEETING

President Richard P. Chin called the meeting to order at 11:30 a.m. on March 2, 1992 at the Capitol Plaza Holiday Inn, Sacramento, California. The minutes of the 40th WPDC business meeting as printed in the 41st Proceedings were approved.

President Chin acknowledged with thanks the contributions of AAAP, ANECA, Patrons, Donors, Sustaining Members, and Friends of the Conference to the WPDC Speaker Funds as printed in the program. The Monday evening reception, "The Pfizer Express," sponsored by Pfizer Animal Health was also acknowledged.

Dr. A.S. Rosenwald, Treasurer, reported that the WPDC had a balance of $2000 after the 1991 meeting. Since then, a balance of $13,500 had been accumulated. A balance of approximately $5,000 is expected at the completion of the 1992 conference. Eleven speakers were invited to the 1992 conference.

A discussion on fees for students attending the meeting concluded with general agreement that students should be encouraged to attend and participate. A fee of $35, for banquet and Proceedings, or $10 for Proceedings only, was approved.

Dr. Chin made the following announcements:

1) The 1992 WPDC Achievement Awards would be presented to Dr. Henry E. Adler (posthumously), Dr. Raymond A. Bankowski, and Dr. Charles E. Whiteman.
2) That the 1992 WPDC would be dedicated to Dr. Marcus Jensen, Editor of the Proceeding.
3) The winner of the "Best Poster" would be selected by a committee (it was later determined to be Dr. R. Droual for his poster titled, "Severe Mortality in Broiler Chickens Associated with *Mycoplasma synoviae* and *Pasteurella gallinarum*").

In a discussion of a Local Arrangement Coordinator, it was decided that a Local Arrangement Coordinator would be desirable (later Dr. Bryan Mayeda accepted this position which was announced at the banquet).

Dr. Marcus Jensen, Editor of the Proceedings, reported that the 1992 Proceedings consisted of 95 pages of manuscripts and cost $5700 for 883 copies. He indicated that the 1992 proceedings was somewhat shorter than previous ones perhaps because of our emphasis to shorten some of the lengthy papers that we have had in the past. He will write instructions for the next Proceedings that will hopefully result in submissions that will not be too long or too short and still be informative.

Dr. Rosenwald announced that the 42nd WPDC will be held on Feb. 28, March 1 and 2, 1993 at the Capitol Plaza Holiday Inn, Sacramento, CA.

Dr. Yamamoto announced that an evaluation sheet will be handed out at the conference in order to obtain better feedback from the membership on the quality and direction of the conference.

The 1992 WPDC conference is expecting 280 registered participants from 23 states and 10 countries.

Dr. Hugo A. Medina was nominated for Program Chairperson-elect, and was elected unanimously. The officers for 1993 are constituted as follows:

Program Chairperson:	Dr. A. Singh Dhillon
President:	Dr. Rocky Terry
Secretary:	Dr. Richard Yamamoto
Treasurer:	Dr. Arnold S. Rosenwald
Local Arrangements, Chairperson:	Dr. Bryan Mayeda
Program Chairperson-elect:	Dr. Hugo A. Medina
Editor, Proceedings:	Dr. Marcus Jensen

President Chin passed the gavel over to the incoming President, Dr. Terry, and the meeting was adjourned at 12:15 p.m. (Outgoing Pres. Chin was presented with the President's plaque at the banquet).

Respectfully submitted,

Richard Yamamoto
Secretary, WPDC

TABLE OF CONTENTS

Note: Both the oral and poster presentations of the 42nd WPDC are listed below in alphabetical arrangement by presenting author. Authors and titles of all papers scheduled for presentation at the conference are listed.

"Snap Shots" from 1992 Awards Banquet

Special Recognition Awards

"Rosy" presenting to Ray Bankowski

"Rosy" presenting meeting Dedication Award to Marc Jensen

Dick presenting to Mrs. Henry Adler

Rocky Terry and Rich Chin: incoming & outgoing Presidents

Art presenting to Charles Whiteman

Best Poster Award presented to Bob Droual by Rocky

PROCEEDINGS OF THE FORTY-SECOND WESTERN POULTRY DISEASE CONFERENCE

POULT ENTERITIS: DIAGNOSTIC OBSERVATIONS OF SELECTED CASES

B. R. Charlton[A], B. C. Barr[B], J. Moore[B],
B. W. Nordhausen[B], and P. Woolcock[C]

UC Davis, California Veterinary Diagnostic Laboratory System,
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P.O. Box 1522, Turlock, CA 95381

Poult enteritis is a significant problem to the turkey industry. Diarrhea, vent pasting, decreased weight gains, and mortality have all been associated with poult enteritis. The term "poult enteritis" is used to describe an enteritis of uncertain etiology in turkeys about 2 to 4 weeks of age. Although bacterial and protozoal agents can cause enteritis, viral agents are thought to be primarily involved. Numerous viral agents have been detected in the digestive system of young turkeys. The pathogenic potentials of a several viral agents have been documented, but frequently the exact role of the virus in the disease is uncertain. The disease is further complicated by the detection of multiple agents.

The diagnosis of poult enteritis involves obtaining a complete history and performing a complete necropsy. The presence of bacterial and parasitic agents are demonstrated by culture, mucosal scrapings, and/or histopathology. The presence of viral agents can usually be demonstrated by direct electron microscopy (DEM), viral isolation, and transmission electron microscopy (TEM). Although these procedures can demonstrate the presence of various agents, the role of each agent in causing enteritis cannot be established. The following series of selected poult enteritis cases compared histopathology and transmissions electron microscopy of the intestinal mucosa with a non-poult enteritis case . The presence of viral particles on DEM examination of intestinal contents was used as the selection criteria of poult enteritis cases.

All cases were from commercial turkey operations and involved birds at 2 weeks of age. Clinical histories described birds as being noisy, off feed, droopy, and having a slight increase in mortality. Gross necropsy observation included litter accumulation in ventriculus, thin-walled ceca filled with foamy fluid contents. *Salmonella* species were isolated from several of the birds and most of the cases. One case had both coccidia schizonts and cryptosporidia oocysts, while a second case had an occasional coccidia oocyst. DEM resulted in visualizing 3 different viruses (rotavirus, reovirus, and entero-like virus) in the 4 poult enteritis cases. Histopathology and TEM photomicrographs of the intestinal mucosa will be presented.

NECROTIC ENTERITIS ASSOCIATED WITH COCCIDIOSIS IN TURKEYS

R. Droual, H.L. Shivaprasad, and R.P. Chin

California Veterinary Diagnostic Laboratory System - Fresno Branch,
University of California, Davis
2789 S. Orange Avenue, Fresno, California 93725

Necrotic enteritis is described mainly in broiler chickens where it is often associated with intestinal coccidiosis. There are very few reports of necrotic enteritis in turkeys in the literature. The present report describes an outbreak of necrotic enteritis in a flock of turkey breeder candidates which was associated with severe coccidiosis.

A flock of turkey breeder candidates was moved from the brooder house to the growout house at 5 weeks and 4 days of age. The litter in the growout house had been used for 2 weeks, immediately prior to the placement, by an older flock at one-half the normal stocking density. Within 1 week of being placed on the used litter, the mortality in the younger flock rose sharply. Poults from this flock were submitted to the California Veterinary Diagnostic Laboratory System - Fresno Branch for evaluation.

Necropsy revealed a necrotic enteritis involving mainly the lower intestinal tract. Scrapings of intestinal mucosa showed high numbers of coccidial oocysts. Histopathology confirmed a severe coccidiosis and necrotic enteritis associated with large rod-shaped bacteria. *Clostridium perfringens* and *Salmonella arizonae* were isolated from the intestines.

Amprolium was present in the feed in the brooder house but not in the growout house when the flock was transferred. One day after coccidiosis was diagnosed at our laboratory, the flock was treated with amprolium in the water for 5 days, after which amprolium was added to the feed. Two weeks after treatment began, mortality returned to normal. However, when breeders were selected at 17 weeks 3 days, the selected birds were found to be about 450 grams (1 pound) lighter than expected for birds of the same age and strain.

A more complete report is being submitted for publication in Avian Diseases.

CATARRHAL ENTERITIS IN TURKEYS ASSOCIATED WITH AN UNUSUAL FLAGELLATED PROTOZOAN

G. Cooper[A] and H. L. Shivaprasad[B]

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[A]Turlock Branch, 1550 N. Soderquist Rd., Turlock CA 95381
[B]Fresno Branch, 2789 S. Orange Ave., Fresno, California 93725

During the summer of 1992, outbreaks of catarrhal enteritis, associated with an unusual flagellated protozoan parasite, were seen in 6 California turkey flocks belonging to 3 separate companies. Flocks were uneven in size and had increased mortality rates and excessive numbers of culls. Affected birds were hunched up, ruffled, and depressed. Most had a foamy, yellowish diarrhea.

Gross necropsy of the birds revealed moderate dehydration. The small intestines were distended with fluid and ingesta, and large numbers of actively motile, flagellated protozoans were seen on microscopic examination of wet mount preparations taken from the jejunum and duodenum. The parasites were much less common in the ilium, and were not observed in the cecum.

On scanning electron micrographs, the organisms appeared to be attached to the surface of the intestinal epithelium by a ventral sucker-like apparatus, much like *Giardia*. The parasite was not definitively identified, but it closely resembles *Cochlosoma anatis*, a rarely reported flagellated protozoan found in the intestinal tract of ducks and turkeys[2].

In most of the cases reported in the literature, *C. anatis* was associated with other intestinal pathogens, and believed to be of questionable pathogenicity[2,3]. However, in Scotland, in 1945, a severe outbreak of catarrhal enteritis, which resulted in high mortality in turkeys, was directly attributed to *C. anatis*[1]. In the current outbreaks of catarrhal enteritis in California, a variety of intestinal pathogens were identified, including *Salmonella*, *Campylobacter jejuni*, picornavirus, and coroavirus-like particles, as well as the intestinal flagellates. The only organisms found consistently in all cases, however, were the intestinal flagellates.

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MICROBIAL ECOLOGY OF THE TURKEY JEJUNUM

T.Y. Morishita[1], K.M. Lam[2], and R.H. McCapes[2]

[1]Department of Veterinary Pathology, and
[2]Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine,
University of California, Davis California 95616

The succession of microbial flora from the turkey poult jejunum was explored on several commercial turkey ranches. A pattern of microbial flora was common to several ranches. The early microbial flora in the poult jejunum appeared to be variable from Day 0 to about 3 weeks of age. *E. coli* appeared to establish early in the poult's life, i.e. establishment at Day 1 on the ranch. Gram-positive organisms were also seen within the first few days of life and included such organisms as *Staphylococcus* species and *Enterococcus* species. Some Gram-negative enterics were also seen during the first 2 weeks of life and included *Klebsiella* species, *Proteus* species, and *Salmonella* species. After 3 weeks of age, a predominant flora consisting of *E. coli*, *Lactobacillus* species, and *Campylobacter jejuni* was noted in turkeys on ranches participating in the study.

ENTERITIS AND SO-CALLED RUNTING STUNTING SYNDROME IN GEORGIA BROILER CHICKS

Mark A. Goodwin,[A] James F. Davis,[AB] M. Stewart McNulty,[C]
John Brown,[D] and E. Craig Player[A]

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So-called runting stunting syndrome (RSS) in poultry around the world is characterized primarily by a failure of chicks to grow rapidly and feather normally[1,5,7]. However, these two clinical signs simply indicate the obvious; that chicks are failing to thrive is due in part to: 1) a definitive diagnosis can not be made without adequate diagnostic testing, 2) clinical signs are not synonymous with a diagnosis, and 3) a long list of metabolic, nutritional, toxic, and infectious agents will cause chicks not to thrive; just what constitutes RSS remains a diagnostic dilemma. We have recommended that the use of the imprecise acronym "RSS" be discontinued[3].

Several infectious agents have been associated with failures to thrive. However, clinicians and researchers agree that illness is exacerbated by nutritional, husbandry, and hygienic factors[1,5]. Recently the potential for roles played by many viruses have been emphasized[6]. Experimental infection of chicks with a combination of viruses and bacteria most closely mimics spontaneous illness[5].

A recent retrospective study in Georgia established that coccidiosis is the leading cause of microscopic diagnoses of enteritis among broiler chicks[3]. Viral and bacterial enteritides also were common.

Chicks that were microscopically diagnosed as having enteritis were infected with several viruses. In all but two cases, only small round viruses consistent with enterovirus were seen in small intestinal lesions. Enteroviruses FP3, 700, 612, and avian nephritis virus were isolat-

sional togavirus-like virus (TVLV) particles were seen[4]. The light-microscopic lesions in TVLV-infected chick intestines were different from intestinal lesions in enterovirus- or reovirus-infected chicks.

Epornithological data indicate that diagnoses of viral enteritis are: 1) first made when chicks are young (Fig. 1), are more common among certain broiler-producing companies (Fig. 2), and 3) may have a seasonal pattern (Fig. 3). Viral enteritis will often result in substantial financial losses for the poultry producer. Among Georgia broiler-producing companies in the present study, body weights predictably fell short of target at market age (range of lost weight were 0.15 to 0.63 pounds).

We do not speculate that a cause-and-effect relationship necessarily exists between intralesional enterovirus, reovirus, or togavirus and illness in chicks in the present study. Because many etiologies are strongly associated with failure of chicks to thrive, it is reasonable for us to describe future cases of enteritis as objectively as possible. Cases with an intralesional agent(s) should be described according to the presence of those agents (e.g., reovirus-associated enteritis; [RAE]). For example: RAE must be differentiated from cases of enteritis with intralesional togavirus-like particles, enterovirus-like particles, combinations of all three, or cases with none of the above.

Although finding intralesional virus in small intestinal segments from chicks that are failing to thrive and that have microscopic evidence of enteritis constitutes a reasonable criterion for making a diagnosis of viral enteritis; chicks without detectable virus infections and without bacterial or protozoal enteritis may also be small and abnormally feathered. Thus, even with the use of tools such as the transmission election microscope, conclusive evidence concerning the etiology of gastroenteritis usually remains elusive simply because the failure to find an agent is not synonymous with the absence of that agent. Better diagnostic tools are needed. In cases where an intralesional etiologic agent is not detected, a diagnosis of idiopathic enteritis should be made.

Information in this abstract will be published in *Avian Diseases*[2,3].

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Figure 1. Diagnoses of viral enteritis are common among young chickens.

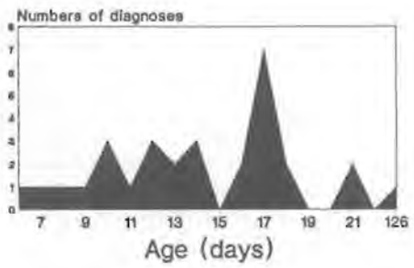


Figure 2. The incidence of viral enteritis varies among companies.

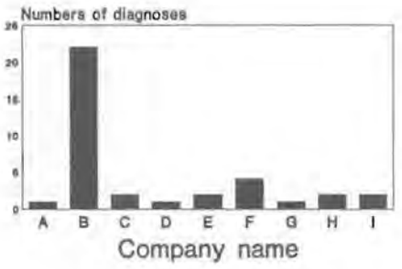
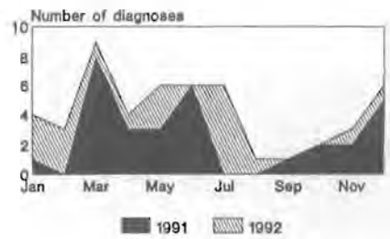


Figure 3. Temporal distribution diagnoses of viral enteritis for 1991-92.



TWO OUTBREAKS OF NEPHROTROPIC STRAINS OF INFECTIOUS BRONCHITIS ASSOCIATED WITH INFECTIOUS BURSAL DISEASE IN 6-WEEK-OLD PULLETS IN TWO DIFFERENT FARMS IN SOUTHERN CALIFORNIA

H. Kinde[A], B.M. Daft[A], Gregg Cutler[B], James T. Case[C]

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INTRODUCTION

Surveillance of infectious bronchitis virus (IBV) is a difficult task and perhaps very expensive to undertake on a routine diagnostic setup. However, it is very important in recognizing and understanding new and emerging serotypes. Determining the epidemiology and economic significance of the serotype of field isolates of IBV is important in the implementation of an immunization program.

Nephrotrophic strains of IBV have occurred very rarely in California, being reported only once previously[1]. This report describes lesions in the commercial flock of pullets infected with 2 nephropathogenic strains, a Connecticut strain, and a strain not identical to any of the 3 major strains of IBV (Massachusetts, Arkansas, or Connecticut).

CASE REPORT

A nephropathogenic Connecticut strain (flock A) and an undetermined strain (flock B) of infectious bronchitis virus were isolated from kidneys of 6-week-old commercial pullets from 2 different farms in southern California. Both farms were owned by the same person, however, flock B had a better management than flock A. The flocks were comprised of 2 strains of White Leghorn pullets housed in separate but adjacent buildings. Only one strain was affected on each premise. There was a total of 42,000 birds kept in 4 different houses in flock A. About 1 week prior to submission, this flock had a spike in mortality due to acute infectious bursal disease. At submission, there was clinical depression and a second spike in mortality averaging to 30 per day with a total death loss of 1213 birds. Ten birds, 6 live and 4 dead were necropsied. In flock B, there were 200,000 birds which were kept about 10,000 birds per house. Three houses were affected. A slight increase in mortality was noted, about 8 to 10 a day per house with a total of 336 birds at the time of submission. Eleven birds, 6 live and 5 dead were submitted for necropsy.

Dehydration was commonly seen in all 21 birds necropsied. Gross renal lesions consisted of swollen, pale kidneys with dilated tubules and ureters distended with urates. Three birds in flock B had watery discharges from the sinuses and showed mucoid exudates in tracheas. Some bursas showed marked atrophy. Microscopically renal changes were associated in the medullary zone and primarily consisted of an interstitial mononuclear cell infiltration, urate deposition, edema and cystic renal tubules often containing epithelial debris and heterophils. Three birds in flock B had lesions in tracheas comprised of mild thickening of the mucosa, desquamation of ciliated epithelia and squamous metaplasia. The bursas showed varying degrees of lesions. Some showed lymphoid necrosis, others had lymphoid depletion with mild atrophy of lymphoid follicles. Bacteriological cultures were negative from multiple tissues from birds in flock B. Bacteriology revealed large numbers of *Escherichia coli* from tissues of one bird in flock A. No culture was performed from tissues of the remaining birds in flock A. A suspension of kidney pools inoculated in 10-day-old chicken embryos were positive for coronavirus after second passage. Inoculated embryos showed stunting, curling, and clubbing of feathers. The coronavirus was confirmed by electron microscopy (flock B) and by use of monoclonal antibody based immunoperoxidase assay applied to the infected chorioallantoic membranes (flock A & B). The immunoperoxidase staining was performed using a group specific anti-IBV monoclonal antibody, provided by Dr. Syed Naqi from Cornell University, as the primary antibody. Formalin-fixed tissues were prepared as usual and incubated with the primary antibody which had specificity for the matrix proteins of IBV. Commercial ABC (avidin-biotin-complex) kits were used to visualize the primary antibody. Normal mouse serum was run in parallel as a negative control. Serotyping was done by Dr. Naqi by using serotype specific monoclonal

antibody based immunoperoxidase staining strains of IBV. The isolates were determined to be Connecticut strain (flock A) and a strain not identical to any of the 3 major strains (Connecticut, Massachusetts, and Arkansas) (flock B). Nephropathogenecity was determined using the isolate from flock B. A birnavirus was also isolated from tissue pools of birds in flock B. Nephropathogenecity study was not yet done using the Connecticut IBV strain.

DISCUSSION

Nephropathogenic strains of IBV have been identified throughout the world[1,2,3,4,5,6]. The reason for new emergence of IBV strains is uncertain. Investigators speculate that the virus may have acquired new tissue or organ tropism as a result of natural mutation or vaccination pressure[6].

The outbreaks limited to 1 strain of pullets on both farms under similar management may suggest perhaps, there exists a genetic susceptibility or predisposition. The bursal lesions are not unusual finding in recently vaccinated chicks, and thus, a birnavirus isolated from tissue pools (flock B) probably reflected a vaccine strain of IBV.

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

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IDENTIFICATION OF FIELD ISOLATES OF INFECTIOUS BRONCHITIS VIRUS IN CALIFORNIA

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Infectious bronchitis (IB), is a common disease of chickens. The virus of IB (IBV) is highly contagious and affects breeders and layers in addition to young chickens. Respiratory disease, nephrosis, egg production drops and shell quality problems have all been associated with IBV infections. Protecting commercial chickens against disease from exposure to environmental IBV by vaccination is a challenge exacerbated by the variety of "variant" serotypes that may be present.

An isolate of IBV from broilers in California was determined to be unique by antigenic characterization via virus neutralization (VN) tests. This California variant (CaV), was used with other reference strains of IBV (Mass., Ark., Conn.) to produce type specific antisera in specific-pathogen-free (SPF) leghorns. These antisera were then used for VN testing of isolates of IBV from commercial broilers. Sentinel SPF chickens and field broilers exhibiting clinical signs of IBV were used to obtain samples from tracheal swabs, cecal tonsils, and in 1 case kidneys, for the IBV isolations.

Eleven IBV isolates were collected over a 4 month period. Evaluation by VN with the type-specific antisera of the isolates revealed 4 isolates of IBV found in commercial vaccines, 6 CaV isolates, and an isolate that was not neutralized. No mixed infections were identified.

The isolation of the *strains of IBV* associated with vaccines is possibly due to persistence from vaccination. The origin of the CaV strain remains unclear. It could be found in birds on first run litter. In view of these results, further research on variant IBV isolates is necessary as are challenge studies to determine optimal vaccination schemes to maximize protection.

INFECTIOUS LARYNGOTRACHEITIS IN BROILERS

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Infectious laryngotracheitis (ILT) was diagnosed as the cause of a recent outbreak of respiratory disease in broilers in California. ILT has not been diagnosed in broilers in the California Veterinary Diagnostic Laboratory System for the last ten years[1]. This report presents the diagnostic findings of this outbreak.

The first case of the outbreak was a diagnostic challenge. Mucoid to hemorrhagic exudate was observed in the tracheas. Microscopic examination confirmed a severe acute tracheitis but intranuclear inclusion bodies diagnostic for ILT were not found. Birds were requested for serologic monitoring. A rising titer to ILT was demonstrated after a week by the Kirkegaard-Perry ELISA and considered indicative of ILT infection since the birds were not vaccinated for ILT. ILT virus was isolated subsequently, but not before other cases were received. The second case consisted of ten 45-day-old broilers with a clinical history of flock mortality doubling over a period of 2 days, birds gasping and blood in the tracheas. On presentation, the 3 live birds were gasping, coughing, and had moist rales. The combs and wattles had a cyanotic appearance. The tracheas were slightly hyperemic and contained copious mucus. No blood was found in the tracheas. Microscopic lesions consistent with ILT were found in the conjunctiva, trachea, and lung. Tracheal lesions were most consistent at the level of the tracheal bifurcation. ILT virus was isolated from the trachea. In the cases that followed, a few dead birds had gross lesions characteristic of ILT consisting of severe diffuse hyperemia and a fibrinous pseudomembrane in the larynx and trachea; however, the most consistent gross findings were conjunctivitis and mucoid tracheitis. Similar findings were reported in some broiler flocks during an ILT outbreak in Pennsylvania where the principal gross findings were mucus in the trachea and conjunctivitis[2].

The classical form of ILT is characterized by respiratory depression, gasping, and the expectoration of blood[3]. Severe laryngotracheitis, often with bloody exudate in the trachea is a typical finding[4]. Conjunctivitis and sinusitis may be the only gross findings in the less pathogenic form of the disease[4]. During this outbreak of ILT, most of the broilers submitted to our laboratory did not show the classical form of the disease. Gasping, coughing, moist rales, and slightly hyperemic tracheas containing copious mucus can be caused by respiratory diseases other than ILT, i.e., Newcastle disease, infectious bronchitis, and avian influenza. As this outbreak demonstrates, ILT cannot be reliably diagnosed based on clinical and gross lesions alone[3]. Laboratory confirmation should be obtained prior to the implementation of control procedures[3]. Poultry veterinarians, field service personnel and producers should be aware of this non-classical presentation of ILT in broilers.

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DIAGNOSTIC CHARACTERIZATION OF THREE UNUSUAL CONDITIONS IN POULTRY

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This diagnostic report will present information on three conditions observed over the past year at the Turlock Branch of the CVDLS—rhomboidal crystalline intranuclear inclusion bodies in pigeon hepatocytes, *Enterococcus durans* septicemia in newly hatched chickens, and wry neck deformity associated with *Mycoplasma meleagridis* (MM) in backyard turkeys.

Rhomboidal Intranuclear Inclusions: In one young adult pigeon (of 10 mixed-age birds with respiratory disease), the liver was slightly swollen and mottled. Histologically the hepatocytes were generally swollen with foamy cytoplasm and virtually all had finely granular pale eosinophilic intranuclear inclusion bodies. The nucleolus was usually prominent and central but, in some cells, was displaced eccentrically by the inclusion body. In several cells there were brightly eosinophilic and refractile elongate crystalline inclusions within the nucleus. These crystalline inclusions resembled closely those reported in dogs and other mammals but to our knowledge have not been reported in avian species. Ultrastructurally, the general inclusions appeared to consist of fine filaments in a rather loose array and no viral particles were observed. The crystalline inclusions consisted of very dense material with distinct pyramidal or rhomboidal shapes.

***Enterococcus durans* Septicemia**: In two submissions of chicks (one layer flock and one broiler flock) with high mortality in the first week after placement, a variety of neurologic signs, swollen livers and spleens, and occasional large retained yolks with watery blood-tinged contents were observed. Cultures of livers, yolk sacs, and brains yielded *Enterococcus durans*. In sections of brain there were focal areas of malacia in the brain stem and, more rarely, in the adjacent cerebellum, but there was little evidence of bacterial infection (no heterophil response) except for scattered fibrin thrombi in malacic lesions. Attempts to reproduce this condition in day-old chicks using one of the *Enterococcus* isolates clearly established a septicemic infection, but failed to produce the observed neurologic disorders and brain lesions.

Wry neck in Turkey Poults with MM Infection: In the years before MM control in primary breeders, it was fairly common to observe neck deformities in a variable percentage of turkey poults during late brooding or early grow-out. The last case I recall seeing in commercial turkeys was in the late 1960's. The present case consisted of two 5-week-old Bronze turkeys from a mixed backyard poultry flock (ducks, geese, chickens, and turkeys) with classical wry-neck. The necks could not be straightened, indicating skeletal deformity rather than a neurologic disorder. These poults had plaques of exudate in the thoracic and abdominal air sacs and *Mycoplasma meleagridis* was cultured. Histologic evidence was found for inflammation of cervical air sac diverticula with involvement of ossification centers and articular surfaces.

UNUSUAL BACTERIAL GRANULOMAS IN MULTIPLE VISCERAL ORGANS OF YOUNG CHICKENS

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INTRODUCTION

In recent years, an increased number of unusual bacterial granulomas have been observed in the spleens, livers, and less frequently other visceral organs of young chickens[2]. These granulomas have been observed at inspection in USDA inspected plants throughout the western, southwestern, easter, southeastern, and midwestern parts of the United States. The granulomas result in condemnation of effected viscera, and the lesions must be distinguished from malignant tumors caused by the avian leukosis complex. Histological lesions and bacteriological findings indicated that the lesions may be caused by *Eubacterium* species similar to those described in the turkey[1,3,4].

GROSS FINDINGS

The spleen was the most commonly affected organ, and the liver was the second most frequently involved. A small number of cases had granulomas in the kidney, and sporadic cases had lesions in the lungs, pancreas, bursa, heart, and intestinal tract or mesentery. The affected spleens were enlarged (up to 3cm x 5cm) many times normal and often diffusely (less frequently multifocally) involved. The spleens were often symmetrical, but may have nodular or irregular surfaces. Spleens were often described as mostly solid to slightly mottled, white and hard; gritty or granular foci were only rarely observed grossly. The lesions were often grossly similar in appearance to lymphoid neoplasia. The livers varied from completely effaced with a dense, white infiltrate and 2 to 3 times normal size to multifocal to coalescing dense white foci. In the other tissues, the lesions were smaller than those of the spleen or liver and most often focal. Often the chickens were described as being in good flesh.

HISTOLOGICAL FINDINGS

Sections of the lesions were collected in 10% buffered formalin solution. Routine paraffin embedded tissues were sectioned at 6μm and examined by hematoxylin and eosin (H&E), Gomori methenamine silver (GMS), and Lillie-Twort (Gram stain). Typically, almost all of the spleen was replaced with multifocal to mostly coalescing granulomas surrounding large numbers of long filamentous to beaded bacteria. The bacteria stained intensely basophilic with H&E, were Gram-positive, and were GMS-positive. The central portion of the granulomas was composed of an eosinophilic coagulum of necrotic debris and contained large masses of entangled filamentous bacterial colonies. Some coccoid and rod-shaped bacterial fragments of variable Gram staining were present; these fragments were free or within the cytoplasm of multinucleated giant cells. There were numerous multinucleated giant cells and epithelioid macrophages present. There were fewer lymphocytes, mostly arranged in small nodular foci at the margins of the necrosis. A few cords of fibrous connective tissue encircling the granulomas and scattered foci of heterophils (often adjacent to necrosis) were present. In other tissues, similar, often focal, granulomas were observed.

BACTERIOLOGICAL FINDINGS

Spleens from 14 young chickens with grossly abnormal spleens were collected; one half of the spleen was placed in 10% buffered formalin and the other half frozen. Of the 14 spleens submitted, 10 had histological lesions of bacterial granulomas, and from these 10 cases, 6 isolates had phenotypically and morphologically similar bacteria, compatible with *Eubacterium* species. For culturing the spleens, a portion of spleen was sterilized on the external surface and the tissue was macerated using a stomacher. For aerobic cultures, samples were placed on 5% sheep blood agar, MacConkey agar, and chocolate agar and incubated in 7.5% CO_2 at 37 C for 10 days. For anaerobic cultures, samples were placed on pre-reduced *Brucella* agar and phenylethyl alcohol agar and incubated under anaerobic conditions at 37 C for 10 days. The isolated bacteria were all obligate anaerobes, Gram-positive rods in chains, did not form spores (confirmed with ethanol and heart sensitivity tests) and produced major amounts of lactic and butyric acids and minor amounts of acetic and succinic acids by gas-liquid chromatography. In addition, these organisms hydrolyzed aesculin, reduced nitrate and fermented glucose, lactose,

maltose, sucrose, fructose, and raffinose. Aesculin, starch, mannitol, and cellobiose were not fermented. Indole was not produced. The growth in liquid media was flocculent with granular sediment and without turbidity. Small numbers of varying aerobes were also cultured in 2 of the 6 spleens containing *Eubacterium*. Of the other 4 spleens containing granulomas, 1 yielded an aerobic Gram-positive rod in chains, 1 yielded an anaerobic Gram-positive rod (different from the other 6 isolates), and the other 2 cases yielded different species of *Pseudomonas*.

DISCUSSION

Microbiologically, the phenotype and morphology of the bacterial isolates identified in 6 of the 10 splenic granulomas most closely resemble members of the genus *Eubacterium*, and more specifically, *Eubacterium tortuosum*[5,6]. Additional tests are being conducted to confirm and type-strain the isolates. *Eubacterium tortuosum* (formerly *Catenabacterium*) has been described as a cause of granulomas in the livers of turkeys[1,3,4]. In turkeys, it has been speculated that the bacteria enter the portal circulation via an enteric lesion. The enteric lesions may have been the result of another bacterium such as *Streptococcus faecalis*. The histopathological lesions of *Eubacterium tortuosum* in the turkey differed from our findings in young chickens. The liver is the predominant site of the granulomas in the turkey, while the spleen appears more commonly affected in the chicken. In the turkey the lesions often appear as pin-point to 3mm diameter multifocal granulomas, whereas in the chickens, the granulomas are often coalescent and efface normal parenchyma. The bacteria within the chicken granulomas stain quite distinctively with H&E stain, while those observed in the turkey generally do not stain with H&E stain[2].

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RECURRENT OUTBREAKS OF A CUTANEOUS FORM OF PASTEURELLA MULTOCIDA INFECTION IN TURKEYS

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Within a 5 year period, the same turkey growout range area experienced annual outbreaks of cholera with a cutaneous *Pasteurella multocida* infection as the predominant lesion. Although, multiple flocks were rotated each year during late summer (August/September). All affected flocks were toms between 17 and 22 weeks of age.

Flock infection was characterized by early low insidious losses, then gradually increasing mortality as the disease progressed. Lesions early in the course of the outbreak were restricted to an ulcerative dermatitis and folliculitis slightly ventral and lateral to the tail. Often the skin lesions were bilateral. In many cases, only by close individual bird inspection, could the cutaneous lesions below the tail be noticed. Enlarged and discolored livers, but generally few respiratory abnormalities, were found at necropsy initially; but, as the course of the outbreak progressed, concurrent caseous pneumonia became more prevalent. Synovitis was also occasionally encountered. Histologic findings of skin lesions consisted of epidermal necrosis with multiple areas of heterophil

infiltration, and hemorrhage, necrosis, and cellulitis of the subcutis. The inflammatory cells extended along fascial planes and inter-myofibril spaces. Occasional discernible bacterial colonies were observed.

Although a variety of *P. multocida* serotypes were encountered over the 5 year period, serotypes 1 and 14 were the predominant isolates.

Only rarely were internal lesions present at processing; however, the prevalence of cutaneous tail ulcerations ranged from 40% to 90% of carcasses. Trimming was usually minimal, but occasionally the cutaneous infection would spread to include areas of the lower breast as well as the tail region.

Because of the age and stage of development of these turkeys, it is hypothesized that the infection, once introduced, was disseminated laterally within the flock through bird-to-bird transmission. It is probably that during self-preening, fighting, or picking at flockmates with blood-engorged developing tail feathers, the *P. multocida* was inoculated into feather follicles causing initial localized infection that later became systemic.

The reason for the temporal distribution of outbreaks is unclear. A variety of wild birds, squirrels, deer, and other wildlife frequented the range area throughout the year. It is likely that a reservoir exists somewhere in this wildlife population which seasonally reintroduces the *P. multocida*.

STAPHYLOCOCCUS HYICUS INFECTION IN GROWING TURKEYS

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Staphylococcus hyicus was diagnosed in a flock of 16-week-old breeder replacement hens. The disease was characterized by a gradual increase of lameness that continued over a period of 3 to 4 weeks. Approximately 2 to 3% of the flock was affected. Lesions consisted of slight synovitis of the hock, stifle joints, and osteomyelitis of the proximal tibia. *S. hyicus* was isolated from the osteomyelitis lesions and bone marrow of the clinically affected birds. Treatment consisted of penicillin in water which was effective in reducing the severity of the disease.

The disease was reproduced in 8-week-old male turkeys inoculated with *S. hyicus* organisms via I.V. route. Clinical signs of lameness, reluctance to move, and diarrhea developed within 1 week post-inoculation. Turkeys inoculated with *S. hyicus* via I.V. demonstrated synovitis at the hock joint, and osteomyelitis lesions at the tibial epiphysis and at the T-6 vertebrae. *S. hyicus* organisms were recovered from all lesions. *S. hyicus* was not recovered from the spleen or liver of most of the affected turkeys exhibiting pathological lesions described above. Turkeys inoculated with *S. hyicus* into the foot pad did not exhibit any clinical or pathological symptoms. *Staphylococcus xylosus, Staphylococcus lentus,* and other staphylococcal species were recovered from the liver of turkeys inoculated with *S. hyicus*. Inoculation of *S. xylosus* did not produce clinical or pathological symptoms. *Staphylococcus aureus* was not recovered out of the internal organs or joint lesions of the experimental birds.

Note: The above abstract will appear as a full length article in the journal Avian Diseases.

PASTEURELLA ANATIPERTIFER-LIKE BACTERIA ASSOCIATED WITH RESPIRATORY DISEASE IN PIGEONS

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Two isolates of a short, Gram-negative, non-fermenting, rod-shaped bacteria were isolated from pigeons in two unrelated cases of respiratory disease. These bacteria were associated with a mycoplasma in the lung of a pigeon and with coliform bacteria in the trachea of a pigeon in another case. They appeared to be involved in the production of lesions consisting of mucopurulent bronchopneumonia and tracheitis. Clinical signs varied from reduced racing performance in an adult flock to gasping and death among young pigeons from the other flock.

The Gram-negative, non-fermenting bacteria were positive for oxidase, but negative in other biochemical tests including urease, citrate, indole, and nitrate. Although not characterized fully, the bacteria appeared to resemble *Pasteurella anatipestifer* (PA). To determine whether they were PA, the isolates were studied and further characterized at the Cornell University Duck Research Laboratory.

At that laboratory, neither isolate could be distinguished from PA based on biochemical reactions. However, morphologically both bacteria differed from PA, and their colonies on solid media were opaque rather than transparent as with PA. Additionally, the pigeon isolates failed to be agglutinated by antisera to 15 serotypes of PA, and they had different patterns of antibiotic sensitivity than PA.

Thus, neither isolate from pigeons proved to be PA. Nevertheless, both pigeon isolates sufficiently resemble PA that they could be difficult to distinguish from PA, especially by laboratory workers who do not encounter PA often and are not very familiar with its characteristics. The classification and identity of the two pigeon isolates remains uncertain.

CALCIUM DEFICIENCY ASSOCIATED WITH POOR ABILITY TO FLY IN DOMESTIC FLYING MALLARDS

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Two hunting preserves in central Minnesota had problems with their domestic flying mallards being able to fly for at least the last 3 years. The operations were located within 16 km of each other. The ducklings were reared in an enclosed brooding building until 5 weeks of age. At that time they were moved to an outside fenced area with open-fronted shelters and grown there until 5 months of age. They were then moved to a large pond area and trained to return to catch pens located 0.4 km away from the ponds. The ducks were supposed to learn to fly to the pond and then return to the catch pens in the evening for feeding.

The ducks raised on these farms were slow to learn to fly and very unenthusiastic about doing it. Soon after release onto the pond area, many became weak and lethargic. They were even reluctant to enter the water. Some of the birds lost their equilibrium and would rock back and forth or fall forward when in a standing position. They also lost their wariness of the approach of humans. Later, groups of ducks could often be found

standing on the roads and could not be forced to fly. Mortality did not rise. Ten percent were severely affected, 80% affected to a moderate degree, and 10% were normal. Excessive amounts of whitish droppings were observed in the catch pens.

Live and dead ducks, 5 to 7 months of age, were submitted for laboratory examination from both farms in August and September of 1991. The live birds seemed normal in attitude but clumsy in their attempts to fly. It was found in necropsy that they were in good flesh and many had feed in the digestive tracts. The sternums contained large amounts of cartilage and were extremely flexible. The ribs were very soft and flexible. Bones of the limbs were firm and strong. No antibody to Newcastle disease or avian influenza viruses was detected in serum. Lead levels in livers were in the normal background range. No parasites were found nor were pathogenic bacteria isolated.

It was recommended that crushed oyster shell be provided *ad libitum* as a top dress to the feed and in special dispensers. One farm vigorously followed this recommendation and their ducks lost all deleterious signs and became agile strong flyers. The second farm did not report back that year.

In August of 1992, the second farm contacted the avian health laboratory again and reported that the previous year's ducks performed poorly in spite of the oyster shell supplementation. New ducks were submitted for evaluation and no findings different from 1991 were present. It was discovered in further questioning that this farm had not been very successful in getting their ducks to consume the crushed oyster shell. They were encouraged to make more aggressive attempts to supplement with crushed oyster shell by top dressing the feed, feeding area, and shore of the pond. After this approach, they reported marked improvement in the ducks.

It is likely that hypocalcemia was induced in the ducks when they were released into the pond area and consumed large amounts of wild feedstuffs. Many of the plant materials and insects available in the ponds probably contained low levels of calcium and relatively high levels of phosphorus. In the process of excreting the excess amounts of phosphorus absorbed from the diet, calcium was removed from the bone in order to provide the correct calcium/phosphorus in the urine. This calcium loss affected the ribs and sternums most severely because of their thinness. Tension from muscle pulling on the soft ribs and sternums was probably painful and discouraged the ducks from using the flight muscles.

HIGH MORTALITY IN YOUNG OSTRICHES

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SUMMARY

High mortality was present in a flock of 94 young ostriches hatched in 1992. The majority of the 10 birds necropsied had clostridium colitis and typhlitis. Treatment with broad spectrum antibiotics and vitamins in water failed to eliminate death losses.

INTRODUCTION

Ostrich farming began in the mid 18th century in South Africa and Algeria. Ostrich farms were established in California in 1886, and soon spread to Texas, Arkansas, Arizona, Florida, and Pennsylvania. The industry in the United States came to a sudden crash in 1914. It is commonly thought that the invention of the automobile made Ostrich plume hats impractical. More significantly, however, is the fact that ostrich feathers were over supplied at a time in Europe. World War I may have caused a major impact to the flourishment of the ostrich industry.

Ostrich leather is most valuable, and has reached markets such as shoes, clothing, handbags, wallets, belts, and small leather goods. Ostrich feathers possess a variety of color, size, shape, and quality. They are also sold as dyed plumes in the shape of numerous products including hats.

Ostriches have lean red meat similar in flavor and texture to beef with minimal marbling. The fat and cholesterol levels are comparable to poultry meat. Feed conversion efficiency and lean meat yield are similar to those of cattle.

CASE REPORTS

History: An ostrich farmer hatched 94 babies in Spring, Summer, and Fall of 1992. He was successful in raising 11 birds. The majority of chicks died at 1 to 6 weeks of age, and the remainder died from 7 to 16 weeks of age. The birds were reported to be unthrifty with anorexia with occasional diarrhea for a few days prior to death.

Brooding Conditions: The brooder house was a 3.66 X 7.32 m room with concrete floor that was heated by circulating hot water embedded in the concrete. The room was also heated by space heaters hanging from the ceiling. The brooder was attached to an outdoor 3.66 X 7.32 m fenced patio.

Necropsy Examinations: Ostrich 1 - September 22, 1992. Gross pathologic alterations in a 2-week-old bird were of pale yellowish liver. Most remarkable alterations were of diphtheritic necrotizing typhlitis involving both ceca.

Ostrich 2. The liver was pale yellow with some ecchymotic hemorrhages. The gizzard and proventriculus were full of feed. The proximal and mid intestines were dilated, thin walled, and contained foul smelling contents. A *Clostridium* species was isolated from the intestines of both ostriches. A *Klebsiella* species was isolated in low to moderate numbers from the liver, lungs, and intestine. Results of *Salmonella* isolation were negative.

Ostriches 3, 4, 5, 6, 7, and 8. Six frozen ostrich babies, 1 to 4 weeks old, that died during the first and second week of September were necropsied. Two of these 6 birds had necrotizing lesions of colitis and typhlitis. Thin-walled intestines with presence of fluid contents were present in the lumen of intestine of the remaining 4 birds. No other remarkable lesions were present. Alterations of typhlitis and colitis were present on microscopic examination.

Ostrich 9 - September 24, 1992. A young female ostrich chick, 1,706 grams in weight, was necropsied. The bird died 2 days post withdrawal of bacitracin from the drinking water. Gross pathologic alterations were of severe congestion of duodenum mucosa. The mid intestines were congested with adhesions of fibrinous tags to the mucosal surface. A *Clostridium* species was isolated from the mid intestines and from the cecum. Focal necrotizing enteritis was present on microscopic examination.

Ostrich 10 - September 29, 1992. A 12-day-old ostrich was necropsied with a history of anorexia and recumbency. The bird was emaciated and shank bones were rubbery. Gross pathologic alterations were of typhlitis and colitis. A long segment of colon was involved. A *Clostridium* species was isolated from the colon and cecum. Aerobic cultures from the liver, lungs and kidneys were negative for bacterial pathogens.

Treatment: The treatment using water soluble broad spectrum antibiotics, along with supplementation with vitamins, controlled illness for a few days. Withdrawal of antibiotics predisposed individual birds to go off feed, followed by death in a few days. Feeding of sick birds by gavage was cumbersome, and in the majority of cases the birds failed to recover.

EFFECT OF AGE AT EXPOSURE TO HYPOBARIC HYPOXIA AND DIETARY CHANGES ON MORTALITY DUE TO ASCITES

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INTRODUCTION

Hall and Machicao[6] first reported on a condition characterized by mortality with the accumulation of fluid in the abdomen in a commercial flock raised at high altitude. While originally confined to countries such as Bolivia, Peru, Colombia, Mexico, and South Africa, where poultry traditionally are raised at high altitude, this syndrome known as ascites or broiler pulmonary hypertension syndrome (BPHS) has now been reported in virtually all countries employing intensive poultry production practices using modern broiler strains. Key changes that identify pulmonary hypertension as the trigger for ascites include dilation of the right ventricle, increased right to total

ventricular weight ratios, and thickening of the pulmonary arteries. While all species show some degree of pulmonary artery hypertension upon exposure to hypoxia, only chickens, cattle, and humans actually decompensate and suffer adverse effects. In humans this syndrome is called Monge's disease. The disease is characterized by the appearance of intolerance to the lower oxygen (O_2) tensions experienced at altitude in a previously acclimatized person. The pathogenesis of this condition is not fully understood but may be primarily due to a decreased sensitivity of the respiratory center to carbon dioxide resulting in a hypoventilation and arterial desaturation[7].

Brisket disease has been recognized in cattle raised in the mountains of Colorado since 1889. Clinical signs and pathologic changes are very similar to those of chickens suffering from BPHS. The pathogenesis of this condition has been shown to be an early vasoconstriction (which can be alleviated by the administration of oxygen), followed by a medial muscular hypertrophy which increases the vasoconstrictive ability of the pulmonary vasculature and decreases the compliance[3,4,5].

Efforts have been made to identify the chemical mediators of the pulmonary vasoconstriction induced by hypoxia. One mediator which has been identified as having an effect is the local hydrogen ion concentration[1,2,10]. Acidosis, whether induced by hypercapnia or infusion of mineral acids, generally causes a pulmonary vasoconstriction. Alkalosis, on the other hand, causes a vasodilation, although this response is more variable.

Little information is available concerning the effect of the age of exposure to hypoxia on the development of pulmonary hypertension in birds. Classical management practices in countries where birds are reared at high altitude make this information important as broilers are generally hatched at lower altitudes and then transported to grow out houses at higher altitude on the day of hatch.

This work was conducted as a series of experiments designed to investigate the effect of age of exposure and dietary acidification or alkalinization on mortality due to ascites syndrome in birds reared at simulated high altitude.

MATERIALS AND METHODS

Experiment 1: One hundred Hubbard X Hubbard male chickens were obtained from a local hatchery on the day of hatch. Groups AA (altitude-altitude) and AN (altitude-ambient) were housed in a hypobaric chamber at an atmospheric pressure of 493 mm Hg (3500 meters altitude) on the day of hatch. Groups NA (ambient-altitude) and NN (ambient-ambient) were housed at ambient atmospheric pressure (366 meters altitude). Group AN was removed from the chamber on day 10 of the experiment and housed at ambient atmospheric pressure, and group NA was placed in the chamber and housed at a simulated altitude of 3500 meters.

Experiment 2: One hundred Hubbard X Hubbard male chickens were obtained from the same source on the day of hatch. Groups AAl (altitude-alkalinized) and AC (altitude-basal) were housed in a hypobaric chamber at an atmospheric pressure of approximately 526 mm Hg (3000 meters altitude). Groups NAl (ambient-alkalinized) and NC (ambient-basal) were housed at ambient atmospheric pressure (366 meters altitude). Groups AC and NC were fed *ad libitum* a basal broiler ration containing 24.7% protein (Table 1). Groups AAl and NAl were fed *ad libitum* the same basal broiler ration containing 1% sodium bicarbonate (alkalinized diet). Water was provided *ad libitum*.

Two additional replicate trials were conducted in exactly the same fashion. One replicate again utilized the alkalinized diet, and the second replicate used a diet acidified by the addition of 1% ammonium chloride (acidified diet) instead of the sodium bicarbonate.

All birds were weighed; and the weights were recorded on days 0, 7, 14, 21, 28, 35 and 42. Standard limb lead electrocardiograms (leads I, II, and III) were obtained on days 0, 14, 28, and 42; and with the exception of day 0, blood was obtained by venipuncture for PCV determination. At the termination of each experiment (day 51 - experiment 1; day 42 - experiment 2) any remaining birds were euthanized by asphyxiation with carbon dioxide. All birds dying during the course of the trial and those euthanized at the conclusion of the trial were necropsied; and heart, lung, liver, spleen and kidney weights were obtained.

The data were analyzed by the general linear models and least square means procedures for the Statistical Analysis Systems[11]. Data are reported as the least square means and standard error of the least square means. Mortality data were analyzed utilizing the Z test for two binomial proportions[9].

RESULTS

Experiment 1: There was no difference in mortality for birds exposed to simulated high altitude on the day of hatch (group AA) when compared to birds exposed at 10 days of age (group NA). Mortality was 0, 3, 7, 3, 3, and 3 birds per week for group AA and 0, 4, 9, 4, 3, and 1 bird per week for group NA. Mortality from ascites was dramatically reduced upon removal from the hypoxic environment with mortalities of 1, 4, 0, 0, 0, and 1 bird per week being recorded for group AN. Only 1 bird died from ascites (week 3) in group NN.

Experiment 2: Overall mortality due to BPHS was significantly decreased in birds fed the alkalinized diet (12/50 = 24%) when compared to controls fed the basal

ration (21/50 = 42%). Birds fed an acidified diet had a numerically increased mortality (13/25 = 52%) which approached statistical significance ($p=0.10$) when compared to control birds fed the basal ration (8/25 = 32%). These differences occurred even though there was no significant diet related effect on growth rate or body weight in birds raised in the chamber.

DISCUSSION

It appears that under the conditions of these studies, dietary alkalinization may offer a natural pharmacologic method for reducing mortality due to ascites syndrome. Sodium bicarbonate is both a common feed additive, and it occurs naturally in the blood; therefore the use of this compound would cause no concern pertaining to residues in processed poultry. Additionally, sodium bicarbonate is relatively inexpensive and its use would not add substantially to grow out costs. Naturally, the addition of sodium bicarbonate would require the reduction of other forms of added sodium during diet formulation because excess sodium intake is known to increase ascites mortality.

The mechanism of action of dietary alkalinization is not known. Reduction of body weight and reduction of packed cell volume do not appear to play a role in the reduction of mortality. If dietary alkalinization produces an alkalemia and this alkalemia in turn causes a pulmonary vasodilation[2], this would effectively increase the cross-sectional area of the pulmonary vessels resulting in a decreased resistance to flow and reduction of the pulmonary artery pressure. However, the alkalinized diet did not significantly reduce EKG amplitude, heart mass or the ratio of the right ventricular to total ventricular weight as would have been expected if pulmonary vascular dilation reduced the work required by the right ventricle to pump blood through the lungs. Other factors must also be considered, however, including possible effects of dietary alkalinization on cardiac output and respiratory drive.

At least up until 10 days of age, the age of exposure to hypobaric hypoxia does not appear to make a difference in subsequent mortality due to BPHS. It was hoped that brooding chicks at low altitude could result in a reduction of the mortality due to BPHS in countries that traditionally hatch chicks at low altitude and then grow them out a higher altitude. It appears from this study that management changes of this nature would have no effect.

Further work is needed to more fully establish the effectiveness and practicality of dietary alkalinization under actual field conditions. If dietary alkalinization is effective under field conditions, it could provide a safe and inexpensive tool in the management control of this condition.

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Table 1. Composition of basal broiler ration.

Ingredients	%	Calculated analysis	%
Yellow Corn	51.840	Protein	24.689
Soybean Meal DHL	41.500	Methionine	0.602
Limestone	2.085	Cystine	0.381
Microingredients[1]	1.500	Calcium	1.134
Dicalcium Phosphate	1.000	Total Phosphorus	0.718
Corn Oil	1.000	Available Phosphorus	0.451
Monobasic Sodium Phosphate (monohydrate)	0.475	Sodium	0.190
Sodium Chloride	0.247	Chloride	0.239
Choline Chloride	0.150	Potassium	1.045
D-L Methionine	0.200	ME (kcal/kg)	2886.21

[1]Supplied per kg of diet: vitamin A, 5010 IU; cholecalciferol, 1500 IU; vitamin E, 66 IU; menadione, 0.00152 g; riboflavin, 0.011 g; calcium pantothenate, 0.02g; niacin, 0.05g; vitamin B12, 20 ug; biotin, 0.0002 g; ethoxyquin, 0.0125%; thiamin, 0.011 g; folic acid, 0.004g; pyridoxine.HCL, 0.0045 g; inositol, 0.25 g; $FeSO_4.7H2O$, 0.33 g; $MnSO_4.H2O$, 0.33 g; KI, 0.0026 g; $CuSO_4.5H2O$, 0.0167 g; $ZnCO_3$, 0.115 g; $Na_2MoO_4.2H2O$, 0.0083 g; $NaSeO_3.5H2O$, 0.0033 g; $Cr(SO_4)_2.1H2O$, 0.02 g; sucrose diluent.

MYCOTOXICOSIS IN POULTRY: DIAGNOSIS, PREVENTION, AND CONTROL

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In the majority of the cases observed, mycotoxicosis behaved as a secondary pathogen instead of a primary agent. Thus, it is very uncommon to see a problem caused by mycotoxins alone. For this reason, it is difficult for the clinical avian pathologist to identify and to recognize the effects of mycotoxicosis in a flock.

We are in the habit of looking for the "classical pathological diseases" that are described in the textbooks. We often think first of some kind of immunodepressed diseases such as Gumboro, Marek, infectious anemia, or reoviruses, instead of thinking that maybe mycotoxicosis is the main problem. We have seen in many small and large broiler operations where bacterial, viral, and parasitic diseases (infectious bronchitis, pasteurellosis, mycoplasmosis, coccidiosis, Gumboro disease, Marek disease) almost disappear when the problem of mycotoxicosis is controlled. For many years, the pathologists have been reporting to us microscopic lesion related to mycotoxicosis; however, the techniques generally used to detect levels in feed and grains give levels of zero or less than 20-50 ppb. This situation has confused us, and led us to think the problem might be pesticides, or has caused us to work on programs of immunization against Gumboro disease or to look for "viral variants."

In November 1991, in a broiler operation of ½-million broilers per week, one flock of about 120,000 broilers had a mortality up to 8 weeks; however, in the last week, the mortality was 3%. We found only lesions similar to "ascites syndrome," but the age and the elevation below 1,400 m did not correspond with this problem. However, a microscopic study showed severe lesions for mycotoxicosis. From this experience we started to work on the determination of mycotoxins in livers, kidneys, feed, and grain in conjunction with the histopathological studies. The pathologist Juan Jose Enriquez had made some modification in the method of detecting each mycotoxin more specifically. For instance, closing the "lactona ring" with chlorine acid obtained better sensitivity.

The determination of mycotoxins in grain or feed is uncertain because there is a great variation of the levels in each batch and the type of sampling. Therefore it is difficult to determine when and how much mycotoxin the

birds are being exposed to. It would be useful to know where the mycotoxins come from in order to control and prevent the problem. The data in Table 1 may help establish some kind of correlation.

For accurate diagnosis, we strongly recommended that all of the following steps be covered: a) clinical signs and lesions, b) microscopic lesions, and c) quantity determination of mycotoxins in visceral organs.

The clinical signs more frequently encountered in the mycotoxins problems are: a) increasing mortality, more common by 5 to 6 weeks of age, b) most dead birds have lesions called "Circulatory-Toxic Hepatic-Kidney-Heart-Pneumonic Syndrome," c) difficulty in the determination of a specific pathologen or disease, and d) unexplained presence of either pasteurellosis, infectious coryza, infectious bronchitis, more severe reaction after vaccination with Gumboro or Newcastle disease or an increased incidence of inclusion body hepatitis because of some failure in the control group of the coccidiosis program.

It is recommended to reduce the time of feeding by 6 hours per day for 3 days in spite of the broiler age. If the respiratory signs or the mortality are reduced significantly, or if there is a better response to antibiotic therapy in the following 2-3 days, we may suspect a mycotoxicosis problem. On the other hand, if there is no response, we might suspect another immunodepressed problem.

In the last half of 1989, many large broiler and layer operations in Mexico began to import grains. From this time on, many pathological problems arose; inclusion body hepatitis became very pathogenic, and many respiratory diseases were associated with *Pasteurella multocida* serotype 5A and 4D, to mention some of the more significant problems. This situation led us to seek alternative means to prevent and control mycotoxicosis.

In October 1992, we conducted a study to determine the effects of 2 commercial mycotoxin adsorbents (Table 2). Each treatment was repeated 3 times with 650 broilers each. At 59 to 69 days (end of experiment) we took liver samples from each bird to determine the quantity of mycotoxins and the presence of microscopic lesions (Table 3). Serologic responses at 9 weeks of age were determined against Newcastle, bronchitis, and inclusion body hepatitis. The birds were vaccinated at 5 days of age with a triple killed emulsion vaccine.

The microscopic lesions found in liver were: 1) tixolex; mild congestion with a few distention of the sinusoidal spaces; mild fat metamorphosis, 2) mildbond; fat metamorphosis, severe, general, and chronic active. Two showed many foci of coagulative necrosis, mild periportal fibrosis, and some hyperplasia of bile tubes, 3) without adsorbent; heavy congestion of the sinusoidal spaces; disassociation of the hepatic cords, periportal fibrosis (chronic and active) and heavy steatosis; hyperplasia of the bile ducts, many spots of petechial and equimotic hemorrhages.

In order to control the mycotoxins effect in the birds, we obtained good results by restricting the time of feeding to 8 hours per day from 14 days of age up to the time of market. This practice was used to control the presentation of ascites syndrome (AS) in Mexico since the beginning of the 1970s. The presentation of the AS is directly related to the source of feed and its incidence increased at altitudes above 1,800 m and at lower temperatures.

Using this practice, respiratory outbreaks were few during an 8 month period in a large operation that had 52 farms; the mortality remained in the standard range (between 4-7% in 8- to 9-week-old birds).

Other experiments were conducted to observe this effect more precisely using 600 birds per pen with 10 repetitions. The results are shown in Table 4. Two different feeding programs were conducted. Group A with 8 hours of feeding from 14 days to market time (8 weeks), and Group B feeding ad libitum.

From one batch of sorghum, we conducted a study to determine the grade of adsorption for 6 commercial products. After exactly 144 hours, we made the determinations for each of them. The dose was the same for each one (0.05%).

In one flock of 104,000 broilers, the total mortality at 7 weeks of age was 3.69% with the 8 hours of feeding daily. But at 7 weeks of age, the feed was given ad libitum. The mortality started to increase and there was a respiratory outbreak. After the flock returned to the previous feeding program, the mortality decreased and the respiratory disease almost disappeared.

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Table 1. CIPP Average levels of mycotoxins found in 107 cases between June and October 1992.

SAMPLE	AFLATOXINS*ppm Alfa	Beta[1]	Beta[2]	Gama	TOTAL	OCRA-TOXIN ppb**	T-2 ppb***
FEED	2.89	1.07	1.31	1.56	6.83	21.62	2.2200
SORGHUM	4.30	2.40	2.95	2.21	11.86	16.06	2.1800
SOYBEAN MEAL	2.38	1.86	1.25	1.58	7.07	3.73	0.0670
CORN GLUTTEN	2.80	0.58	0.57	0.58	4.5	18.55	0.0003
LIVER	5.68	1.94	2.47	2.20	12.29	23.69	0.9110
KIDNEY	8.80	9.90	9.30	9.80	37.80	22.00	-------

* Thin Layer Chromatographic
** Fiber glass layer spectophotometry
*** Atomic absorbtion spectophotometry

ppm (mg/kg)
ppb (ug/kg)

Table 2. Economic and productive effect of two mycotoxin adsorbent in broilers.

ADSOR-BENT	DOSE kg TONS/FEED ADSORB.	TOTAL MORT. %	LIVE WEIGHT kg	DAILY GAIN grams	FEED CONV	PROD. INDEX	COST* kg
TX	0.7	6.87	2.544	36.87	2.234	153.8	$0.20
MB	2.5	7.22	2.563	37.14	2.215	155.6	$0.17
N/A	0.0	8.37	2.424	35.13	2.405	134.6	(0.20)

* Included fixed feed cost only ($2.878), adsorbent cost, and sale cost ($0.69).

Table 3. Levels of mycotoxins found in liver between 59 to 69 days of age with 8 hours of time feeding ad libitim per day from 14 days of age to market.

ADSORBENT	TOTAL AFLATOXIN PPM	OCRATOXIN PPB	T-2 PPB	RUBRATOXIN PPB
NO ADSORBENT	4.003	20.3	0.001	0.00101
TIXOLEX	0.329	11.6	0.009	0.00007
MILDBOND	0.215	15.0	0.018	0.00028

Table 4. Mycotoxicosis mortality. The effect of two different feeding programs.

WEEKS OF AGE	AD LIBITUM Mortality per week %	8 HOURS OF FEEDING DAILY Mortality per week %
4	2.98	0.55
5	1.68	0.31
6	4.31	0.89
7	3.71	1.05

NECROPSY SUMMARIES OF FLOCK HEALTH MONITORING IN BROILER COMPANIES IN 1992

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This presentation showed the results of field necropsy sessions conducted during 48 monitoring sessions in 20 broiler companies during 1992 and compared those results by regions of the United States.

There were 3 distinct geographic regions represented: 1) the Southeastern U.S. (Alabama, Mississippi, and Georgia/Florida), 2) California, and 3) the Northwestern U.S. (Washington and Oregon). These were compared with the composite of all reports and shown as the U.S. average and each region.

The reports used were those from necropsy sessions conducted as a part of technical service activities in the companies represented. Confidentiality was respected during reporting, and each company was given its comparison to national and regional averages at each session

as well as compared to its previous reports. Several types of reports will be shown:

1. Single Company with Quarterly Reports.
2. Multiple-complex Company.
3. Two Complexes in Different States.
4. 1992 Averages vs Regional Averages.
5. 1991 Averages vs 1992 Averages.

The reports show gross lesions that are reported according to an agreed set of norms, and are grouped under the categories: coccidiosis, enteric, molds/toxins, respiratory, skeletal, bursas, retained yolks, parasites, and I.P. (subcutaneous cellulitis).

The method of collecting and reporting this data was presented at the 1992 Western Poultry Disease Conference as a poster presentation, and is currently being used by several technical service veterinarians representing companies which provide service to broiler companies. There are many modifications of this type report in use today in broiler production, and standardization of the reporting format is the goal of the author.

Credit for introducing the author to this type of report is given to Dr. Birch McMurray, Seaboard Farms and Dr. Don Waldrip, Gold Kist. Inc.

THE ADVANTAGE OF USING LIGHT CONTROL IN GROWING BROILERS

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Field studies showed reduced condemnation and improved feed conversion in broilers grown with controlled lighting. Light intensity was reduced to 30% of the bulbs' total brightness starting at 1 week of age. There were no times of total darkness. Twenty-four hours of light were used for the last week before processing.

The flock got 3 daytime feedings and 2 nighttime feedings. When the feeders started, the 30% reduced level of light was gradually brought up to full light over a 20 minute period. When the feeders stopped, the lights were dimmed over a 20 minute period to the 30% level The feeding time was increased as the birds grew.

Mortalities late in the grow period were reduced, especially from sudden death syndrome. Birds developing misshapen legs were also less. Feed conversion was improved.

A HISTOMORPHOMETRIC STUDY OF THE SKELETON OF BROILER CHICKENS KEPT UNDER DIFFERENT MANAGEMENT REGIMES

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The commonest skeletal deformity in broiler chickens is valgus or varus deformation of the intertarsal joint (VVD) which has previously been called twisted leg. VVD has been attributed to irregular growth plate development by Thorp[4] and to delayed cortical bone differentiation by Dammrich and Rodenhoff[2]. The incidence of VVD can be decreased by growing broiler chickens on a short photoperiod at an early age followed by increasing photoperiods up to processing[1]. The incidence can be increased by growing broiler chickens in battery cages[3]. The present study was designed to use histomorphometrics to compare bone development under different management regimes in an attempt to determine which pathogenesis may be most important in the development of

VVD. Chickens were selected for study from two experimental trials. In the first trial the incidence of skeletal deformities was 2.27% in birds on continual light and 0.95% in birds on a reduced photoperiod. In the second trial the incidence of skeletal deformities was 7% in batteries and 2% in floor pens.

Birds selected for study were injected intravenously with 15 mg of oxytetracycline (Liquamycin 100, Rogar/STB, Montreal, Quebec) at 15 and 20 days of age and weighed and necropsied at 21 days of age in the first trial. Birds in the second trial were injected in a similar manner at 15 and 21 days of age and weighed and necropsied at 22 days of age. The right tibiotarsus was collected from each bird and its length measured. A 3 mm transverse undecalcified section from the middle of each tibiotarsus was embedded in methyl methacrylate and sections cut for examination by fluorescent microscopy. A sagittal decalcified section was cut from the center of each proximal tibiotarsus, and routine histologic sections prepared. The circumference, cortical bone area and distance between the 2 oxytetracycline labels was measured from the transverse sections. The thickness of the zone of proliferation, the distance from the top of the zone of proliferation to the nearest invading metaphyseal vessel, and the number of metaphyseal vessels reaching to within 2.5 mm of the top of the zone of proliferation and within the central 6.2 mm of the metaphysis were measured from the sagittal sections.

The only significant difference found in the parameters measured in the first trial was a reduced circumference and cortical bone area in birds on the reduced photoperiod. This occurred in spite of no significant decrease in bone length or body weight with interrupted light. No significant differences were found in any of the parameters measured in the second trial. The results suggest that the interrupted light may affect cortical bone growth rather than endochondral bone growth. The thinner bones on the reduced photoperiod may reflect a difference in modelling though no difference in periosteal growth could be measured. It should be noted that there was considerable variation in most of the parameters measured. This variation and a relatively small number of birds studied may have masked some differences between treatments.

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COMPOSTING AS A METHOD FOR THE DISPOSAL OF POULTRY CARCASSES

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SUMMARY

As environmental concerns for air and water quality have emerged, the need for alternative methods for managing poultry farm mortality (carcasses) has also emerged. One alternative, composting, has gained acceptance. For the composting of poultry farm mortality, a prescribed amount of carcasses, litter, a bulking material (e.g. straw, peanut hulls, litter cake), and water provide the necessary mixture for converting these waste products into a humus-like material that is useful as a soil amendment. Over the last 3 years, field and controlled research has been on-going to characterize the microbiology of composting. Data from both field and specific pathogen challenge studies demonstrate that pathogens associated with carcasses are effectively destroyed by the composting process. Thus, composting, when properly managed, is a biosecure and relatively inexpensive means for handling poultry carcasses.

Every poultry production facility is faced with the reality of farm mortality. Disposing of these carcasses

has emerged as a significant environmental issue that stands as a potential obstacle to industry expansion[5].

Burial pits and incineration are still commonly used for the disposal of poultry carcasses; however, the potential for possible ground water and air quality deterioration may compromise the acceptability of these methods. Due to these emerging environmental concerns, alternative methods of disposal must be made available to the poultry producer. The organic farming practice of composting in which microorganisms transform organic wastes into a useful end product has emerged as one alternative that provides an environmentally and biologically safe method of converting daily mortality losses into humus-like material useful as a soil amendment[6,8,9]. For the composting of poultry farm mortality, a prescribed amount of carcasses, litter, straw, and water provide the necessary mixture[7,8,9]. In Alabama, composting has met with great success. At present, there are approximately 300 composters successfully operating in the State.

For composting to be a truly viable method for the disposal of poultry farm mortalities, it is paramount that the composting process results in inactivation of pathogenic (avian and human) microorganisms prior to land application. It has been documented that bacterial pathogens (e.g. *Listeria monocytogenes*) can be transmitted from farm animals to humans via land application of contaminated compost and manure used as fertilizer[11]. Therefore, in evaluating composting or any other method of carcass disposal, avoidance of both human and avian disease transmission must be a major consideration.

To address these microbiological questions, we have been involved over the last 3 years in field and controlled studies. Research has been directed toward characterizing the microbiology of mortality composting as well as determining the survival of specific pathogenic microorganisms. A summary of these studies[1,2,3,4] and the findings will be reported here.

COMPOSTER EVALUATION

Field Study #1

A 2-stage mortality composter (three primary and one secondary bins) operated on a broiler farm with a flock size of 86,000 in Clay County, Alabama, was investigated to determine changes in microbial populations, temperature and moisture occurring over a typical composting cycle. Composting was accomplished by sequentially layering carcasses (daily mortality), litter (manure and pine shavings), wheat straw, and water at a ratio (weight:weight) of 1:2:0.1:0.25 into primary bins. Each primary bin was filled to a final depth of approximately 1.5 m (4-6 layers) and held for 8-10 days post-filling, then transferred to the secondary bin for an additional 17-21 days. Compost temperatures were recorded daily. At 5-day intervals, 6 and 4 samples were obtained from each primary and secondary bin, respectively, and analyzed for moisture content and populations of total aerobic (APC) and coliform bacteria.

Field Study #2

A total of 36 composters primarily located in northern Alabama were sampled for the presence of viable *Salmonella*, *Listeria monocytogenes*, and *Campylobacter jejuni* as well as for populations of enteric, coliform and APC bacteria. Compost samples were obtained from the secondary bins of each unit, and were materials that had completed the secondary heating. Samples were obtained from a depth of *ca.* .5-.75 m using a soil test auger, then transported to Auburn University for analysis. The microbiological analyses for the three bacteria were conducted using standard USDA or FDA detection-isolation protocols.

Specific Pathogen Challenge Studies

A 2-stage composting unit was constructed according to published specifications at Auburn University, Department of Poultry Science Research Farm[1]. The unit consists of three primary bins (1.52 x 1.52 m), three secondary bins (1.52 x 2.44 m) and a litter storage area (2.44 x 3.96 m).

To generate a more complete microbiological profile, populations of fungi and coliform, aerobic, anaerobic, thermotolerant, and thermophilic bacteria were enumerated from samples obtained during typical 2-stage composting in the research composting unit under conditions similar to those used in the aforementioned field study.

During studies conducted to investigate biosecurity aspects of mortality composting, survival-inactivation of the pathogens *Salmonella enteritidis*, *S. typhimurium*, *S. senftenberg*, *Pasteurella multocida*, *L. monocytogenes*, *Escherichia coli* 0157:H7 and *Aspergillus fumigatus* or *A. flavus* was determined during the two-stage composting process with and without added bulking materials (carbon sources; i.e. wheat straw, peanut hulls). Each test culture was either inoculated directly onto carcasses or into tubes of brain heart infusion with 0.5% agar, and placed into the composter unit at 4 different, prescribed locations at the initiation of the composting process. For direct carcass inoculation studies, compost samples were periodically obtained and analyzed for the presence of the test culture, whereas in test tube studies sample tubes were obtained and analyzed for viability of test cultures using direct plating or enrichment techniques after completion of the primary and secondary composting cycle.

RESULTS OF MICROBIOLOGICAL SAFETY

Field Studies

Moisture content of the compost ranged from 25% to 45%. After the last layer was added to primary bins,

temperatures rapidly exceeded 50 C and generally stabilized until the materials were transferred to the secondary bin where another heating cycle occurred, generating temperatures in excess of 60 C. Temperatures then remained relatively constant over the remaining test period. Maximal temperatures (>60 C) were obtained during the secondary composting cycle.

APC populations remained high, ranging from 10^4 to $>10^7$ colony forming units (CFU) per gram of compost throughout the 30-day sampling period. Initially high populations of coliform bacteria (*ca.* 10^4 CFU/g) were reduced to nondetectable levels ($<10^1$ CFU/g); however, coliform counts remained high during the primary cycle and inactivation did not occur until materials were transferred to the secondary bin[3].

None of the samples from the 36 composters yielded viable *Salmonella, L. monocytogenes* or *C. jejuni*. Again, all samples were from compost that had received the recommended two-stage process. These data indicate that under the various field conditions in which these composters were operated, the target bacteria were effectively inactivated. Furthermore, data suggest that these composters were managed properly to ensure a biosecure process[2].

Challenge Studies

In studies to determine changes in populations of different classes of microorganisms during composting, it was found that only the population of coliform bacteria substantially changed (from $>10^6$ to $<10^1$ CFU/g). This indicates that coliform population may be a useful biological indicator for determining mortality composting efficacy[1,4].

In subsequent studies, specific pathogens were inoculated or placed into the materials used for preparation of carcass compost; that is, the compost process was "challenged" with various pathogens. In one study, carcasses were inoculated with *S. typhimurium* (10^6 CFU/carcass) and placed into the composting unit with varying amounts (0, 10, 20%) of wheat straw as a bulking material. Over the compost cycle, temperatures were determined and compost samples were obtained periodically and analyzed for *S. typhimurium*. With 20% wheat straw, which is twice the recommended level, temperatures reached 65 C; with 10% straw, 52 C; and with 0% straw, 50 C. Under these conditions, *S. typhimurium* were effectively inactivated. Although *S. typhimurium* were inactivated in compost with no added bulking material, this compost was of very poor quality as evidenced by a strong putrid odor.

In similar studies, tubes containing the aforementioned cultures of pathogenic bacteria (10^9 CFU) and fungi (10^7 spores) were placed at four locations into the compost bins during the daily layering procedures: 1.2 m deep (layer 2) at center, 1.2 m deep at front slats, 0.6 m deep (layer 4) at center, and 0.6 m deep at front slats. At the completion of primary composting, culture tubes were retrieved and analyzed for viability. A duplicate set of tubes of each test organism was initially placed into the primary bins; therefore, the second set of tubes was removed with the compost and placed into the secondary bins at the same relative positions as in the primary bins. This second set of tubes was removed and analyzed for viability at the completion of the secondary cycle. The results were similar for compost prepared either with wheat straw or peanut hulls as bulking agents/carbon sources. None of the bacterial pathogens were recovered following primary (or secondary) composting with 10 and 20% wheat straw or peanut hulls, while bacteria survived primary composting when no wheat straw was used. However, no viable test cultures were recovered under any test conditions following the secondary cycle. This indicated that composting effectively inactivated tested pathogenic microbes[2].

Furthermore, presumptive data from similar challenge experiments[10] in which carcasses were purposely infected with virulent Newcastle disease virus (NDV) or virulent infectious bursal disease virus (IBDV), indicated that both NDV and IBDV were inactivated during typical carcass composting.

CONCLUSION

Data collected during the last 3 years show that properly managed composting is a biosecure and relatively inexpensive means for managing poultry farm mortality in a manner that has less environmental impact than the traditional methods of burial and incineration. A well managed composter will generate temperatures (a minimum requirement of 55 C for 3 days) capable of destroying many avian and human pathogens that may be associated with carcasses. Data further indicate that aeration, via turning or transferring the compost is an effective means for enabling proper heat generation. Evaluation of composters on poultry farms in Alabama further support that composters are effectively providing the necessary conditions for pathogen inactivation and pose little risk of disease transmission.

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FERMENTATION OF POULTRY CARCASSES PRIOR TO RENDERING

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SUMMARY

Every poultry grower is faced with the reality of carcass disposal. Nationwide, this represents a tremendous amount of organic matter that requires environmentally and biologically safe disposal or utilization. Carcass disposal has been identified as a major problem facing the poultry industry. If poultry carcasses resulting from death by natural occurrences at such high levels of production are not disposed of by environmentally acceptable methods, future industry expansion will be limited or regulatory constraints will be imposed. Rendering carcasses into a valued protein by-product meal is an alternative. Removing poultry carcasses from the farm is environmentally acceptable and a valuable feed ingredient results. However, the spread of pathogenic microorganisms during routine pickup and transportation to a rendering facility presents a substantial threat. Lactic acid fermentation of poultry carcasses prior to transportation stabilizes carcass deterioration and minimizes pathogen threat. Unlike routine pickup of "fresh" carcasses, fermentation allows for long-term on-farm storage of poultry carcasses, reduced transportation costs and results in a usable feed ingredient.

Every turkey and broiler production facility is faced with the reality of carcass disposal. For a flock of 30,000 turkeys averaging 0.5% mortality weekly (9% total mortality), approximately 16,272 kg (17.9 tons) of carcasses require disposal during an 18 week growing period. For a flock of 50,000 broilers grown to 49 days of age averaging 0.1% daily mortality (4.9% total mortality), approximately 2,182 kg (2.4 tons) of carcasses require disposal[4].

CURRENT DISPOSAL METHODS

Burial pits are most commonly used for disposing of poultry carcasses. The decline in ground water quality where pits are located is a concern. Residue remaining in pits after years of use is recognized as another concern. Incineration is a biologically safe method of disposal; however, it tends to be slow, expensive, and generates the greatest number of nuisance complaints even when highly efficient incinerators are used.

Due to increasing burial or incineration costs and newly imposed local, state, and federal water and air quality standards, alternative methods of disposal are of interest to the poultry producer. As the poultry industry

expands, so also will the amount of waste generated on the farm. Therefore, the poultry industry must aggressively pursue efforts to protect the environment and maintain a good public image.

RENDERING POULTRY CARCASSES

Rendering can be used as a method for the conversion of poultry carcasses into a valued, biologically safe protein by-product meal. However, the spread of pathogenic microorganisms during routine pick up and transportation of poultry carcasses to a rendering facility is viewed as a potential threat. Removing poultry carcasses from the farm is most acceptable for the environment, and a valuable feed ingredient results.

One of the major concerns with centrally located carcass disposal sites is disease transmission. Sound biosecurity of disposal sites is essential to prevent disease transmission[5]. Central carcass disposal sites have been placed on trial in Minnesota and North Carolina[13,14]. Transportation costs have made this method expensive, approximately $.15/454 g (1 lb) in comparison to other alternatives such as burial or composting which cost less than $.01/454 g (1 lb) and $.03/454 g (1 lb), respectively[9,14].

Freezing carcasses for short-term storage prior to transportation to a rendering facility is effective. However, this method has also proven to be expensive. Large-capacity units are usually required because 90 kg or more of carcasses at near body temperature (41 C) may be encountered daily. Electrical costs for the operation of a high-capacity refrigeration equipment have been estimated to be approximately $.05 to .10/454 g for carcasses stored and picked up at weekly intervals[9,14].

FERMENTATION

Fermentation, a controlled natural process has been successfully used as a preservation method for foods and feeds for millennia, and has become well documented as a scientifically sound method for the preservation of organic materials[1]. Lactic acid fermentation of poultry carcasses prior to transportation inhibits carcass deterioration and minimizes pathogen threat[6,7,8,12].

Initial studies conducted by Dobbins[8] described methods for preserving poultry carcasses by lactic acid fermentation. Successful fermentation is enabled by the combination of prescribed amounts of farm carcasses with a fermentable carbohydrate source such as sugar, whey, molasses or ground corn[6,7,8,12,13]. In order for effective fermentation to occur, carcasses must be ground in 2.5 cm or less particles. Particle reduction is required for tissue acidification. Grinding aids the dispersion and mixing of intestinal anaerobic lactic acid-forming bacteria. Bacteria that produce lactic acid ferment the carbohydrate source, resulting in the production of volatile fatty acids and a subsequent decline in pH to below 4.5, which preserves the nutrients in the carcasses.

Pathogenic microorganisms associated with the carcasses are effectively inactivated or inhibited during the fermentation process via the decrease in pH[6,8,12,16]. Presumably, fermented material can be stored and will remain in a stable state for several months[6,8,12]. Therefore, fermentation could be initiated and continue on-farm until carcass amounts are sufficient to warrant the cost of transportation for rendering.

ON-FARM FERMENTATION

Laboratory Studies

Initial investigations have been conducted in small-scale vessels to evaluate the appropriate combination of ground poultry carcasses with fermentation carbohydrate and/or other additives required to assure rapid fermentation and biosecure stabilization that would result in long-term storage on the farm[6,7].

Results from laboratory studies indicate:

- Addition of at least 6% glucose or whey permeate or 8% whey to ground carcasses promoted fermentation as evidenced by a decline in pH from 5.6 to a range of 4.2 to 4.5 within 7 days.
- Ground corn at a level of 15% or greater was necessary to support adequate fermentation as indicated by a decline in pH to less than 4.5.
- Addition of an acidulant, bacterial culture, a protease enzyme, or antifungal agents failed to improve the fermentative process.
- Populations of indigenous coliform bacteria and added *Salmonella typhimurium* were reduced from moderately high (ca. 10^6 colony forming units (CFU)/g) levels to undetectable levels (<10 CFU/g).

Scale-up

Two experiments were conducted to address the scaling-up of an endogenous fermentation system of carcass stabilization from laboratory to on-farm use[11]. In both experiments, approximately 10 kg of ground carcasses were mixed with an appropriate carbohydrate and placed in a closed container with subsequent additions occurring on four consecutive days, resultant batch size of 50 kg.

Results from scale-up studies indicate:

- Batches fermented with sucrose (10%) or whey (10%) and subsequently stored at either 2 C or 25 C exhibited declines in pH from 5.8 to 4.1 (25 C) and from 5.8 to 4.8 (2 C).
- Batches fermented with whey (10%), whey permeate (10%) or ground corn (20%), stored at 25 C for 12 weeks, exhibited pH decreases from 5.8 to 4.6, 4.5 and 5.1 within 7 days. Lowest pH levels

for whey (4.0), whey permeate (4.3) and corn (4.8) occurred at 18 days and remained relatively constant.

- Initial coliform levels were greater than 10^6 CFU/g and declined to undetectable levels (<10 CFU/g) by the 18th day of fermentation.

On-Farm System

Two on-farm fermentation facilities have been installed on contract farms with capacities of 86,000 and 68,000 broilers to demonstrate the feasibility of on-farm endogenous fermentation of poultry carcasses[2,3]. A grinding unit was specifically designed and fabricated which allows for the simultaneous addition of a carbohydrate source during the grinding of carcasses[15]. Daily, broiler mortality is ground and ground corn was added at the 20% level. The mixture (mortality and carbohydrate) was directly fed into a 1,135 liters (300 gallon) capacity enclosed tank (PCO Tank #10951, Raven Industries, Sioux Falls, SD).

Weekly pH measurements were obtained from the fermentation tank(s) at 3 locations approximately 30 cm below the surface. Typically, the pH values of the fermented product decline below 5.0 within a 10-day period. All resulting ferment obtained from both farms was transported for rendering at the end of a typical grow-out cycle (45-49 days later). Over 13,636 kg of fermented carcasses have been processed into a usable feed ingredient[10].

Results from these studies indicated that fermentation can be adapted for the stabilized, pathogen-free storage of broiler carcasses during a typical 7-week growout. Unlike routine pickup of "fresh" mortalities, fermentation and subsequent storage of dead poultry reduces transportation costs by 90% and eliminates the potential for transmission of pathogenic microorganisms through poultry via rendered products.

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A NOVEL STRAIN OF INFECTIOUS BRONCHITIS VIRUS AFFECTING CHICKENS IN GREAT BRITAIN

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SUMMARY

Investigations of outbreaks of disease in broiler breeders and broiler flocks throughout Great Britain resulted in the isolation and identification of a variant strain of infectious bronchitis virus (IBV).
Studies with commercial vaccines showed that they did not confer protection against the novel strains of IBV.

INTRODUCTION

Infectious bronchitis virus (IBV) occurs worldwide in the domestic fowl[1]. Many types of IBV have been described which may differ either antigenically, in virulence or in tissue tropism from the original Massachusetts strain and from each other. There is considerable evidence that the virus periodically mutates or undergoes genetic recombination[2]. This variation between IBV serotypes makes it difficult to achieve complete protection by immunization.

Unusual outbreaks of disease in broiler breeders, broilers and later in commercial layers were reported in Great Britain in early 1991 and found to be associated with a novel strain of IBV, designated 793/B[3]. This paper deals with the clinical and virological aspects of the isolate.

MATERIALS AND METHODS

Clinical Signs. In broiler breeders the clinical signs most frequently reported were sudden drops in egg production, up to 50% in some cases with pale-shelled eggs, accompanied by respiratory symptoms, trembling, cyanosis and increased mortality. At postmortem examination the most prominent feature was oedema and myopathy of the pectoral muscles, tracheitis and congestion of the ovaries. In broilers both tracheitis and pneumonia were observed.

Virus Isolation and Identification. Suspensions of trachea and caecal tonsils were inoculated into the allantoic cavity of 9 to 11-day-old commercial SPF chicken embryos. All embryo lethal agents were identified following electron microscopy (EM) examination of concentrated samples.

Haemagglutinating (HA) antigens were also prepared[4] and used in HA hemaggulination-inhibiting/(HI) tests with reference strains of IBV.

Serology. Serum samples from flocks of IB vaccinated broiler breeders in which outbreaks of disease had occurred, were tested by IB/HI test using antigens prepared from M41 and the prototype novel strain 793/B.

Protection Studies. Three-week-old SPF chickens were vaccinated with live HI20 vaccine and challenged by aerosol exposure to isolate 793/B four weeks later. After 4 days the birds together with age-matched unvaccinated controls were sampled and virus isolation carried out on tracheas and kidneys. A similar group of chickens received HI20 vaccine at 3 weeks followed by inactivated monovalent oil emulsion vaccine at 7 weeks and challenged 6 weeks later.

RESULTS

Virus Isolation and Identification. Over 20 embryo infectious agents were isolated from tracheas and caecal tonsils which were subsequently identified by EM as IBV. Treatment of the concentrates with enzyme produced HA titres between 2^8 to 2^{10} . Monospecific antiserum to one of the isolates, 793/B, was used in two-way neutralization and HI tests with 12 reference strains of IBV. No significant neutralization or inhibition was recorded with any of the reference sera, although the homologous titres of 793/B were $2^{8.5}$ and 2^8 respectively. A further 14 'new' isolates were similarly tested with monospecific antisera to M41 and the Dutch isolates D274 and D1466 and in none of the tests was an HI titre of $>2^4$ recorded.

Serology. Low levels of M41 antibody were detected in the field sera up to 18 weeks of age, with insignificant levels to 793/B . Following vaccination with inactivated vaccine, a significant rise in M41 antibodies occurred; with a mean HI titre of $>2^8$. Antibody titres to 793/B were insignificant up to 22 weeks of age. At 30 weeks, approximately 5 weeks after the onset of clinical disease there was a significant rise in both M41 and 793/B anti-

bodies. Samples taken at 40 and 50 weeks showed a decline in M41 antibody levels but those to 793/B had been maintained.

Protection Studies. Following challenge clinical signs were observed in both vaccinated and controls 3 to 5 days post-challenge. Challenge virus was isolated from 21 of 30 tracheas and kidneys sampled from the vaccinated birds compared with 19 of 20 samples from the unvaccinated controls, 4 days after challenge. Following a further challenge, 4 weeks after the birds were revaccinated with inactivated IB vaccine, no significant respiratory signs were observed. However, challenge virus was reisolated from 14/15 tracheas and 9/15 kidneys of the vaccinated and from all the control samples, 4 days after challenge.

DISCUSSION

From the results of this study it is concluded that recent egg production and respiratory disease problems in broiler breeders, broilers and more recently commercial laying chickens in Great Britain, are associated with a strain of IBV which appears to be significantly different from strains of IBV from which current European IB vaccines are designed.

The mortality occurring during the acute phase of the disease and the myopathy affecting the pectoral muscles are unusual features to record at the onset of IB infection.

Using antigen prepared from the prototype novel strain 793/B, serological monitoring of numerous flocks of broiler-breeders and layers in Great Britain and other European countries have shown that the virus is widespread.

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COMPARISON OF cDNA AND OLIGONUCLEOTIDE PROBES FOR DETECTING NDV

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We have compared cDNA and oligonucleotide probes to detect Newcastle disease virus (NDV) viral RNA in the slot blot hybridization method. We have synthesized a sequence of oligonucleotides from the published data[1] at the Biotechnology Center, University of Connecticut, Storrs. The cDNA probe specific for NDV was prepared and characterized in our laboratory.

The cDNA and oligonucleotide probes were used simultaneously on viral RNAs from NDV isolates. Both probes hybridized most of the RNAs from NDV isolates and did not cross hybridized to other avian viral RNA and DNAs. When these probes were used at various concentrations of NDV-RNAs in slot blot hybridization, the cDNA probe hybridized NDV-RNA at lower concentration than the oligonucleotide probe. The application of both probes in the slot hybridization method tends to suggest their usefulness in detecting NDV. But the sensitivity of the cDNA probe was higher than the oligonucleotide probe.

The disadvantage of oligonucleotide probe (short probe) was that each oligonucleotide could be labeled with only a single reported molecule. Thus, oligonucleotides

are often cited as being 10 to 100 folds less sensitive than long probe. In this study, it was apparent that the cDNA probe (long probe) was a better choice for the detection of low number of sequestered NDV organisms in the tissues rather than the synthetic oligonucleotide probe.

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POULTRY VACCINES: AN OVERVIEW OF PRODUCTION, REGULATION AND QUALITY CONTROL

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DEVELOPMENT OF VACCINES

Companies producing vaccines in the United States must now have their facilities licensed by the USDA. Previously, small companies that produced vaccines only for intrastate use were not required to have a licensed facility. Recently, these regulations were changed in order to provide more uniformity in biologic products. When the facility is licensed, a USDA reviewer will be assigned to that company and will be responsible for decisions made concerning license approval.

Vaccines produced at companies located in the state of California are either federally licensed or are licensed by the state. Regulations pertaining to California state licensed products must not be in conflict with federal regulations, and a product cannot have a dual "federal/state" license. California is the only state that maintains its own licensing program.

For most vaccines currently produced, all companies must abide by the same procedures for obtaining a license for that vaccine. These procedures are detailed in a book called the "Code of Federal Regulations #9" (9 CFR). Some vaccines do not have licensing procedures outlined in the 9 CFR (new products, etc.). In these cases, a company must submit to the USDA a proposal detailing the steps that they wish to take in order to obtain a license for this vaccine. The USDA will then either approve (with or without comments) or disapprove the proposal.

If no vaccines are available for a particular disease and there exists a critical need, then a company has the option of pursuing a conditional license for that vaccine. Essentially this means that the company only has to prove vaccine safety before releasing the vaccine to the consumers. Eventually, however, the company will be required to submit the appropriate efficacy data to the USDA in order to acquire a full license for the vaccine.

Autogenous vaccines have regulations which apply to all autogenous products as a whole. In general, the autogenous product must be in answer to a specific need, must only be used for the location from where the organism originated (or in special cases, adjacent farms) and has a limited span of time in which it can be used. The main purposes of autogenous products are to address emergency needs, needs of specific locations or needs of minor species where no suitable substitute currently exists.

Companies initially will obtain an isolate (virus or bacterium), or will purchase a technology which includes an isolate. These isolates are generally acquired from a university or another research establishment. Occasionally, the isolate is "obtained" by culturing the organism from a competitor's live vaccine.

After the organism is in the company's possession, the culture is screened for contaminants and a plan is detailed for development of the product. This plan will anticipate whether the isolate needs to be further processed, i.e., attenuated, decontaminated, concentrated, etc. before a master seed stock is produced. Also, the most appropriate route(s) of application will be determined.

VACCINE PRODUCTION AND QUALITY CONTROL

The master seed stock is the parent seed from which all lots of vaccine are ultimately produced. Companies usually try to store as large a quantity of this as possible because loss of the master seed could mean loss of the vaccine.

From the master seed, progeny seeds or passes will be produced. The first passage beyond the master seed is usually designated the production seed. Frequently the USDA will limit the number of passes to 5 passes beyond the master seed. (The vaccine virus or bacterium can be no greater than passage # 5.) When the actual vaccine serial is produced, one of the progeny passes will be used as the inoculum rather than the master seed. In this way, the master seed can be preserved for long periods of time--usually many years.

Master seeds and all progeny seeds are subjected to numerous in vitro tests which include titer, sterility, purity (viral contamination), PPLO, COFAL, salmonellae, formaldehyde residue (inactivated vaccines), and moisture (lyophilized vaccines). The master seed is also tested for the presence of the organism that it is supposed to contain. After in house testing, the company will submit vials of the master and production seed(s) to the USDA for similar testing in order to verify the company's results.

In addition to proving that the master seed and all progeny are pure, companies must prove that the vaccine will be safe and efficacious. In general, safety tests are conducted using the master seed and all vaccine serials that are produced. Efficacy is established by determining the minimum dose required to protect a set percentage of birds as specified in the 9 CFR (usually 90%). This dose is established using the farthest passage beyond the master seed that would ever be used as vaccine. This is to hopefully prevent producing a vaccine that is significantly different from the one that was used to establish the minimum protective dose (MPD). An MPD must be established for each route of application that will be included on the vaccine label. Once this dose is determined and a "buffer" amount added, all vaccine produced must have at least this amount of organism per dose in order to be sold. The buffer amount is added to compensate for any loss of potency that might occur during storage of the vaccine up to the expiration date. (The buffer amount for most live vaccines is 1.2 log above the MPD.) Since this minimum dose required has already been determined, most live vaccine lots produced will not have to have a bird efficacy test conducted on each lot. The company only has to prove that each vaccine lot has achieved the required titer. Killed vaccines are an exception as the vaccine titer determined before inactivation may not be representative of the efficacy of the product. A vaccine can, however, have a much higher amount of organism per dose when the vaccine is sold--it just cannot be lower.

Since most of the above work has been done on a small scale in the company's research facility, parameters must be established for large scale production of the vaccine (scale up). Sometimes scale up can take a considerable amount of time and effort. Organisms frequently grow differently when produced in large quantities rather than small.

Once the safety and efficacy of the vaccine have been proven, the company is allowed to produce 3 test lots or serials of vaccine. These are used for testing the vaccine "in the field" (at actual commercial companies) and are designated as pre-licensing serials. When used for this field testing prior to licensure, these serials will have an experimental label. All of these serials (and all subsequent licensed serials) will be subjected to in vitro testing as outlined for the master and production seeds, safety testing and, in appropriate cases, efficacy testing. If the field test results are good, then the appropriate data and an accompanying production outline will be submitted to the USDA for (hopefully!) license approval.

The production outline (or recipe) for producing the vaccine is often submitted to the USDA shortly after the decision is made to pursue a license and while the organism is still in the research testing phase. Pertinent information is then added as often as necessary until the outline is complete. This helps expedite the time it takes for the USDA reviewer to examine the license application.

Some general comments to consider about the development of vaccines: 1) For chicken vaccines, laboratory tests are conducted using Specific Pathogen Free (SPF) birds. These birds are particularly "wimpy" and safety can be difficult to prove. However, if the vaccine is made safe enough for use in these birds, it may lose much of the efficacy needed in order to be a protective vaccine for commercial birds. 2) Since vaccine companies sometimes produce their own vaccines by using a competitor's vaccine as a source, there may not be a great amount of difference between competitors' vaccines. Check to see if the strain(s) used in the vaccines are the same or are different from company to company. With killed vaccines, the emulsifier or adjuvant used can account for substantial differences in quality despite uniformity in strain origin. 3) In general, vaccines produced and/or licensed for use in the United States are of a uniform and good quality at the time they leave the company's warehouse.

THE USE OF BIOTECHNOLOGY IN THE PRODUCTION OF POULTRY VACCINES

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INTRODUCTION

Biotechnology is being used to develop new vaccines for the control of avian diseases. Two different types of vaccines will be discussed. Subunit vaccines, which consist of only the immunogenic portion of a disease agent, and vectors (either bacterial or viral in nature) which are attenuated live agents that contain a foreign gene that codes for the immunogenic portion of another disease agent.

Techniques such as DNA cloning, hybridization, and the polymerase chain reaction (PCR) have increased our understanding of poultry disease agents at the molecular level. Understanding those techniques and their potential is necessary to comprehend the impact that biotechnology will have on the control of poultry diseases in the future. Thus, a brief overview, for some of those molecular techniques will also be discussed.

MOLECULAR TECHNIQUES OVERVIEW

DNA consists of purine (A & G) and pyrimidine (C & T) bases which contain the genetic code, and a sugar (deoxyribose) and phosphate backbone which makes up the structure of DNA. In double stranded DNA, A pairs with T and G pairs with C[10].

RNA contains bases like DNA, except that instead of T, it contains uracil (U). In addition, RNA has a ribose and phosphate backbone, whereas DNA utilizes a deoxyribose sugar. RNA is single stranded but can double back on itself to form stretches of double strands called hairpin loops. RNA is not as stable as DNA. It acts as a carrier of genetic information from the DNA to proteins. The flow of genetic information in a cell is as follows:

DNA ----> **mRNA** ----> **Proteins**
transcription translation

DNA ----> **DNA**
replication

Commercially available enzymes are used to manipulate and modify DNA in vitro. Those enzymes have many uses, some of which are to cut DNA and ligate it back together, to synthesize DNA or RNA, and to modify and determine the nucleic acid sequence of DNA. Some of the commonly used enzymes are listed below.

Restriction Enzymes. Recombinant DNA techniques were made possible by the discovery of restriction endonucleases (RE). Each RE is named after the bacterium from which it was isolated (i.e. *Eco*RI = *Escherichia coli*, *Hin*dIII = *Haemophilus influenzae*). Restriction endonucleases cut double stranded (ds) DNA at specific sequences called recognition sites. Digestion of DNA with REs generates either sticky or blunt ends which can be ligated back to each other, or to another piece of DNA.

Modifying Enzymes. Ligase brings two pieces of dsDNA with similar ends together. **DNA polymerase** synthesizes a complementary strand of DNA, using DNA as the template (Klenow fragment, T*aq* polymerase). **Reverse transcriptase** synthesizes a complementary strand of DNA using RNA as the template. **RNA dependent RNA polymerase** synthesizes **a** complementary strand of RNA using RNA as the template (found in many RNA viruses).

DNA Cloning. DNA cloning[4,8,9] is the insertion of a foreign gene into a bacterial plasmid (small, circular DNA that exists in the cell separate from the chromosomal DNA) or phage virus (viruses that infect bacteria). This technique is essential for creating subunit and vaccine vectors. Although there are many ways to clone genes, one way involves the following steps:

1. Purification of the gene of interest (usually mRNA).
2. Production of double stranded copy (c)DNA from the mRNA template[5] or PCR.
3. Restriction endonuclease digestion of the vector DNA (plasmid or phage).
4. Insertion (ligation) of the cDNA into the vector.
5. Transformation (uptake of DNA into a bacterial cell) of competent (bacteria capable of taking in DNA) *Escherichia coli* cells.
6. Identification of transformed bacteria containing cDNA inserts by insertional inactivation (color selection on artificial media).
7. Grow the transformed bacteria in culture medium and purify the recombinant DNA.

Polymerase Chain Reaction (PCR). The PCR amplifies very small quantities of DNA to detectable levels[3].

The discovery of a DNA polymerase (Taq polymerase) that is stable at very high temperatures, and the development of computerized temperature blocks have made the PCR possible. There are 3 steps in the PCR which are repeated 20 to 30 times. Those steps are: 1) Denaturation (95C), 2) Primer hybridization (30C-40C), and 3) Polymerization (72C).

Generally, the DNA to be amplified is flanked by a pair of synthetic primers. The target DNA is denatured by heating, and the primers are allowed to hybridize to the DNA by lowering the temperature of the sample. When the polymerization step is conducted, the sequence between the primers is copied, producing twice as much DNA as was originally present in the sample. By repeating the denaturation, hybridization, and polymerization steps many times, the target DNA is effectively amplified.

The utility of the PCR is still being discovered, but has been used for cloning experiments, DNA probe production, DNA sequencing, and many other genetic engineering techniques requiring large quantities of DNA. The PCR will play a key role in the synthesis of subunit vaccines or vaccine vectors for avian pathogens.

Nucleic Acid Sequencing. Nucleic acid sequencing is a method whereby the order of the nucleotides (A, C, G, and T) in a gene are determined. Two types of DNA sequencing exist, but only one is practical and routinely used. **Chemical sequencing** (Maxam and Gilbert) involves the use of hazardous chemicals to cleave the DNA after each base[9]. The procedures are difficult, expensive, and potentially hazardous since one of the chemicals used is hydrazine (rocket fuel).

Sanger dideoxynucleotide chain termination sequencing is the standard sequencing method used in most laboratories[8,9]. Dideoxynucleotides (ddATP, ddCTP, ddGTP, ddTTP) are similar to deoxynucleotides except that when they are incorporated into a growing DNA sequence, the addition of other bases is impossible. When one of the dideoxynucleotides is used in a chain elongation reaction, it causes premature chain termination at that particular base. Since this is a random event, different lengths of DNA are produced. When 4 separate reactions (each with one of the 4 dideoxynucleotides) are run and the different lengths of DNA synthesized are separated using polyacrylamide gel electrophoresis, the sequence can be determined.

SUBUNIT VACCINES

Subunit vaccines are vaccines that contain a part or "subunit" of the disease agent. That subunit is usually an immunogenic protein expressed (synthesized) in bacteria or cell culture, purified, and given directly to the bird or with an adjuvant. Since subunit vaccines are individual proteins, the risk of using live attenuated agents as a vaccine is eliminated. Another advantage of subunit vaccines is that a large amount of protein can be synthesized, whereas extraction of even a small amount of that immunogen from the disease agent may be difficult.

Problems encountered with subunit vaccines are proper glycosylation and protein folding which are necessary for immunogenicity of some proteins. In addition, the proteins of interest must be purified away form other cellular contaminants.

The most popular bacterium used for the expression of subunit vaccines is *E. coli*. Numerous plasmids designed for the insertion and expression of a foreign gene in *E. coli* as well as purification kits are commercially available.

Yeast (*Saccharomyces cerevisiae*) has also been used to express foreign proteins with the advantage that proteins are glycosylated in eukaryotic expression systems.

Baculovirus (*Autographa californica* nuclear polyhedrosis virus) which grows in continuous insect cell lines (Sf9, Sf21, MG1) is a common eukaryotic expression system[7]. High levels of protein expression are achieved by inserting a foreign gene into the baculovirus gene that codes for the polyhedrin protein (not necessary for replication). This system exploits the extremely efficient polyhedrin promoter which results in a high level of protein synthesis. When insect cells are infected with the recombinant virus, the foreign protein is synthesized.

Another common eukaryotic expression system is based on simian virus 40 (SV40) transformed cells. African green monkey kidney cells (COS cells) transformed by a defective mutant of SV40 will express high levels of a foreign protein when a plasmid containing an SV40 origin of replication and the gene for the foreign protein is inserted into the COS cells.

Synthetic Subunit Vaccines. Other subunit vaccines consist of peptides 10 to 40 amino acids long that can be synthesized in the laboratory[1]. Once an immunogenic epitope on a protein is identified and the sequence is determined, that portion of the protein can be manufactured synthetically. Since those peptides are usually not immunogenic, they are commonly coupled to a large carrier molecule before they are given to the bird. The major advantage of this type of vaccine is that they can be readily changed and quickly synthesized for use against new antigenic variants of the disease agent.

VECTORS

Bacterial and viral vectors are being developed for use in poultry. In general, genes from prokaryotes (bacteria and mycoplasma) are inserted into bacterial vectors, whereas eukaryotic genes (viruses and protozoans) are inserted into viral vectors.

Bacterial Vectors. Recombinant avirulent *Salmonella* species and *E. coli* that colonize and replicate in the host

and express one or more foreign proteins, are being developed for use as poultry vaccines[2,6]. Genetic modifications (deletions) that inactivate the genes for adenylate cyclase and cyclic AMP are commonly used to render the bacteria avirulent. Foreign genes are inserted into the avirulent bacterium (homologous recombination, transposon mutagenesis) at the site of the deletion. Those genes are designed such that the bacterium now expresses the foreign protein.

Virus Vectors. Virus vectors are created by inserting foreign genes into a nonessential region of the virus genome[1]. Once foreign genes are inserted into the virus, the virus is called a vector since it makes protein from the foreign gene and presents that protein to the immune system of the bird. Birds vaccinated with a virus vector containing a foreign gene respond immunologically to the virus vector as well as to the foreign protein.

Advantages of using a virus vector are that proper glycosylation and presentation of the protein is often achieved. In addition, more than one foreign gene can be cloned into the virus vector allowing for the construction of a multivalent vaccine. This is also true for bacterial vectors. A disadvantage of viral vaccine vectors is that they are expensive to produce.

Double stranded DNA viruses are being used as vectors because their genome is easy to manipulate. Some of the virus vectors being developed include, vaccinia virus, fowl poxvirus, herpes virus (HVT), and adenovirus.

CONCLUSIONS

Biotechnology must be used to develop more efficacious, less expensive, or safer vaccines; otherwise, those vaccines will be of little practical value. It will be difficult to obtain a better vaccine for some disease agents using biotechnology because extremely effective vaccines already exist for those diseases.

Selection of a subunit or vector-based vaccine will depend on the disease agent, the immune response of the bird to that agent, and the nature of the immunogenic protein to be expressed. Selecting the best system is often difficult and sometimes several systems must be tested before an efficacious vaccine is obtained.

All of this technology must rely on a thorough understanding of the disease agent and the bird's immune response to that agent. Each disease agent is different. For some agents, such as avian influenza virus or newcastle disease virus, the selection of an immunogenic protein to be used in a genetically engineered vaccine is relatively clear. Engineering vaccines for other disease agents like coccidia will be more difficult because we simply do not know enough about that parasite/host relationship at this time.

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HOW TO CALCULATE VACCINE EFFICACY

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Dichotomous data (dead/alive, yes/no) are usually analyzed using a chi-square test. This permits, for example, the statement that vaccination (yes/no) of chickens is significantly associated with death (yes/no) after challenge. What clinicians really want to know is "How efficacious is this vaccine?" Using simple arithmetic we show how to answer this question.

CONTROL OF VERY VIRULENT INFECTIOUS BURSAL DISEASE (GUMBORO DISEASE) IN THE NETHERIANDS WITH SO CALLED "HOT" VACCINES

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SUMMARY

Intermediate type vaccines could prevent infectious bursal (Gumboro) disease outbreaks caused by a very virulent serotype I virus only to a limited extent. They failed in situations of high infection pressure. Vaccination failures were due to the inability of the intermediate vaccines to break through maternal antibody in time, as compared with the virulent virus, and the deficient timing of vaccination.

These problems were overcome by the use of "hotter" vaccines and guidance of vaccinations by application of a mathematical formula to calculate the optimal vaccination age in broilers. This formula was developed on the basis of extensive serological testing in a field experiment involving nearly 29 million birds on 96 problem replacement layer and 714 broiler flocks vaccinated with either the LZ 228 E (Mycofarm) or the Bursa Plus (Duphar) vaccine. Only 4 minor disease outbreaks occurred. The "hot" vaccines had no adverse side effects. Technical results were the same as in undiseased flocks vaccinated with intermediate type vaccines.

INTRODUCTION

Immunity against infectious bursal disease virus (IBDV) is mainly antibody mediated and passive immunity is transferred with antibodies in the yolk to young chicks. Breeders either are vaccinated only with live vaccine or with live and inactivated (oil emulsion) vaccines (OEV). It was supposed that chickens coming from OEV vaccinated breeders would be hatched with antibody concentrations that were so high that vaccination with live vaccine would be necessary only after 4 weeks of age or not at all.

Most broilers in the Netherlands had not been vaccinated for a period of about 5 years since 1980 and there were no major problems. Most likely we benefited from the use of inactivated vaccines.

In 1986, we experienced serious losses in broilers and later in replacement layers as a result of a very virulent form of Gumboro disease; this was similar to previous outbreaks, first in Belgium and then later in Germany, England, and Middle Eastern countries. The causative virus appeared to be of the classical serotype 1 and not a serological variant. Obviously the passive immunity evoked by the OEV was not sufficient; birds experienced the disease around 4 weeks of age.

To cope with the disease, broilers were vaccinated at about 2 weeks of age with live vaccines, like in earlier days. Vaccines were used with an intermediate residual pathogenicity like D78, LZ 228TC, and Bursine 2. Undoubtedly these vaccinations limited the disease. However, repeated outbreaks occurred on many farms (broiler

and replacement layer), especially in concentrated growing areas.

This paper is a survey of the work done to cope with the disease by better timing of the vaccinations and the use of more virulent ("hotter") vaccines.

RESULTS OF EXPERIMENTAL WORK

The first important problem was to find the optimal age for vaccination in the course of the decreasing maternal antibody concentration. Experimental vaccination of broilers hatched with intermediate and high titres (as measured with the IDEXX ELISA), under controlled conditions, revealed that in most so-called "Emulsion birds" (coming from OEV vaccinated parents), vaccination from day 17 onwards gave a quick and good immunity. Immunity (antibody) developed slower and to lower titres when birds were vaccinated at an earlier age and with higher maternal antibody concentrations. Vaccines took best when the mean ELISA titres were $\leq$ 1:500 and preferably around 1:350. For the purpose of measuring titres, the ELISA test had the disadvantage of being able to reliably measure the relatively higher titres but less reliable to measure the lower titres. In the beginning we established the vaccination age subjectively, guided by our experience. Later we were able to develop a mathematical formula to estimate the optimal vaccination age more precisely.

In commercial flocks there appeared to be a great variation (spread) of titres in just hatched chicks and especially between hatches from different breeder flocks. Even titres of flocks vaccinated with OEV sometimes were surprisingly low. This meant that there was no general optimal vaccination age. This ignorance of the dynamics of the titre decline, contributed to vaccination failures.

We tried to overcome the disadvantages of this spread by multiple vaccinations. This perhaps helped, but not in situations of high infection pressure (poultry density).

We demonstrated experimentally that vaccination failures were mainly due to the ability of the field virus to break through a considerable higher concentration of maternal antibody than the (intermediate) vaccine viruses. This meant that in a situation of high infection pressure, it invaded chicken flocks many days before any vaccine virus could.

Most likely infection takes place as follows: once the field virus is present on a farm after a first outbreak, and especially when the farm is not disinfected completely (and this is difficult particularly on replacement layer farms), chicks hatched with low maternal antibody concentrations after some days start multiplying and excreting the virus. After some cycles the virus is built up to high concentrations in the litter and is then picked up by other chicks that have an antibody titre that can be overcome by the field virus but not by the vaccine virus. In such flocks a vaccination comes always too late.

It was concluded that in a situation of high infection pressure a more virulent vaccine virus (which means more residual pathogenicity) would be essential to overcome the problem.

However, we were aware that no vaccine could be found that could overcome a maternal antibody titre as high as the virulent virus could and at the same time would not be pathogenic.

EXPERIMENTAL VACCINATIONS IN THE FIELD

Licensed by the Veterinary Authorities, experimental vaccinations were performed during a year on 11 problem broiler farms (mortality of 10 to 30% in 6 or more successive flocks) situated in a poultry concentration area. The vaccine used was Bursa-Vac Gumboro (IBDV), produced by Sterwin Laboratories, Millsboro, DE, USA. This vaccine has a relatively high residual pathogenicity. While the disease regularly reoccurred on conventionally (intermediate vaccine) vaccinated farms in the area, it did not on the 11 experimental farms.

From Oct. 1990 to Dec. 1991, we were licensed for experimental vaccination of a greater number of (neighboring) problem farms with 2 other so called "hot" vaccines; the LZ 228E (Mycofarm Nederlands B.V., P.O. Box 8, 3730 AA de Bilt, The Netherlands) and the Bursa Plus (Solvay Duphar B.V., P.O. Box 2, 1380 AA Weesp, The Netherlands). These vaccines were slightly less "hot" than the Sterwin vaccine (caused no mortality in SPF birds).

Conditions were arranged for a precise monitoring of the success of the vaccination. In broilers this was done by an estimation of the best day of vaccination based on antibody measurement in just hatched chicks, followed by measurement at the day of vaccination and the day before slaughter. This could be done since great experience had been built up with broilers in the previous field experiment. In replacement layers, often a second titre measurement was done before calculating the vaccination day. Moreover these birds were vaccinated twice with a week interval.

A total number of 28,830,840 birds were vaccinated with either of the 2 vaccines on 96 replacement layer and 714 broiler problem farms (or neighboring problem farms). None of the replacement layer farms experienced the disease. In only 4 broiler farms Gumboro occurred. Three of the outbreaks occurred in a very small house of about 5,000 birds that had not been incorporated in the serological testing. The fourth was more serious, in one of 4 big houses, the other 3 remaining free. Losses in the affected house were 12.4%.

So these "hot" vaccines were able to cope excellently with the field virus. There were no differences between

the technical results of 26 "hot vaccinated" and 95 "conventionally (mostly D78) vaccinated" farms (same integration, same feed, same way of calculation etc.). Both categories had not suffered from the disease.

CALCULATION OF AGE OF VACCINATION

From the many serological data, the mathematical distribution of the maternally derived Gumboro titres could be calculated. Details will be published elsewhere. It appeared that square root (SQRT) transformed titres of young broilers (1- or 2-days-old) were distributed normally and that the average SQRT declined 2.82 per day.

From this comes the procedure and formula for calculation of the vaccination age if vaccination should be done at a mean ELISA titre of 1:500. Take blood samples of preferably 24 or 18 just hatched chicks by decapitation and perform an ELISA test. Take square roots of individual titres and calculate the MEAN SQRT. The best vaccination age follows from

$$(\text{MEAN SQRT} - 22.36)/2.82\ (+\ 1)$$

where 22.36 is the SQRT of 500, that is the titre at which we decided to vaccinate. If we want to vaccinate at a different titre, for example 1:350 we substitute 22.36 by the SQRT of 350; (+ 1) is added since it is usual to call just hatched chicks 1-day-old chicks.

If serum is taken from 2- or 3-day-old chicks 2 or 3 should be added. We recommend to take samples from chicks that are not older than 3 days. Otherwise one may run the risk of being too late for vaccination.

Titres can be so low that vaccination is required at, for example, day 4. In these cases we decided not to vaccinate before day 8 due to a possible immunosuppressive effect of the vaccine virus. It is well known that the immunosuppressive effect of IBDV decreases with increasing age of infection.

It appeared possible to use the SQRT of only the Mean Titre (SQRT MEAN), thus removing the necessity to take SQRT's of individual titres. Flocks with titres up to 1:1500, that have in most cases a coefficient of variation (CV) $> 50\%$, always had to be vaccinated at day 8 (see above), making calculation redundant.

In flocks with titres from 1:1500 and 1:4500 and a $25 < CV < 50\ \%$ there was a chance of about 7% that the calculation using the SQRT MEAN had a result different from that calculated using the MEAN SQRT and with higher and lower CV's this chance was $\leq 3\%$.

Also with titres $>$ 1:4500 and a CV $> 50\ \%$ (that are unusual with these higher titres) the chance on a different result was only 4%. However, if the CV was $< 50\%$ the chance on a different result was 11-21%.

In nearly all cases a different result meant that the age of optimal vaccination calculated with the simpler SQRT MEAN method was 1 or 2 days (maximally) later than that calculated with the SQRT MEAN method. In case of the above combination of high titre ($>$ 1:4500) and low CV ($<$ 50 %) 1 day could be subtracted from the result obtained with the SQRT MEAN calculation. Then there is a chance of 80 to 90 % that the vaccination is 1 day before time. It is questionable whether this is significant in these situations of high titres and hence "late" vaccinations.

In replacement layers, the average of SQRT transformed usually decreased more slowly. Titre decline showed a large and unpredictable variation even between the same brand of birds housed on different farms. Therefore a mathematical formula for these birds could not yet be developed. We therefore tested these birds at least twice within 10 to 14 day intervals. From the calculated trend it is possible to predict the optimal vaccination age rather accurately.

DISCUSSION

Two major factors explained the failure of vaccinations with intermediate vaccines against the very virulent IBDV field strain. One was the great variation (spread) of maternally derived antibody titres in just hatched chicks and between hatches from different breeder flocks, so that broiler flocks had to be vaccinated at various ages according to these titres. Therefore a vital part of the success of any vaccine depended on an exact as possible timing of the optimal age of vaccination on the basis of serological testing. We found the ELISA to be the only practicle test available to work with under field conditions where results have to be generated quickly. This test, with its limitations, helped a lot in discovering vaccination failures.

The mathematical formula developed from the results of thousands of sera examined during the field experiments now enables us to estimate the optimal vaccination age rather exactly.

The second factor was the inability of intermediate vaccines to break through maternal antibody levels as compared with the virulent virus in the field. This was overcome by the use of "hotter" vaccines. Their success may be explained by their property to break through slightly more residual maternal antibody than the intermediate vaccines. But it probably also depends to a great extent on their ability to multiply better in the host, resulting in more and longer excretion than with the intermediate vaccines.

The concept "hot" is of course relative. These vaccine strains, in our opinion, are only slightly more virulent than the intermediate vaccines like D 78, LZ 228TC and Bursine 2. Under field conditions, technical results of vaccinated flocks were the same as those of flocks vaccinated with intermediate vaccines. Here it should be

stated that we did not apply these vaccines in birds younger than 8 days. This means that these so called "hot" vaccines in a field situation have no adverse effect as compared with intermediate vaccines.

A RECOMBINANT HVT VACCINE EXPRESSING NEWCASTLE DISEASE VIRUS ANTIGENS PROTECTS CHICKS AGAINST A LETHAL NEWCASTLE DISEASE CHALLENGE

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Over the past decade, the increased interest in molecular biology and biotechnology has spawned new products for the treatment, prevention, and control of animal diseases. Marek's disease and Newcastle disease are two common viral diseases of chickens which have been controlled primarily by vaccination. Various vaccines are available for both Marek's disease virus (MDV) and Newcastle disease virus (NDV). Most (if not all) of the commercially available vaccines are prepared by conventional means from avirulent or low virulent viruses which are immunogenically related to MDV and/or NDV. This report evaluates a new genetically engineered vaccine for its efficacy and safety in protecting birds against Newcastle disease (ND) challenge. The new recombinant vaccine utilizes the turkey herpesvirus (HVT) as a vector for expressing the fusion (F) and hemagglutinin neuraminidase (HN) proteins of NDV.

Vaccines and vaccination. Three recombinant vaccine viruses were evaluated. All three utilized the HVT as the expression vector. Recombinant virus 048 contained the F NDV protein, 049 the HN NDV protein and 050 contained both F and HN. The viruses were administered to day-old birds by the subcutaneous (SC) or intraperitoneal (IP) route. The amount of vaccine administered (dose) was varied dependent upon the trial (see table 1).

A commercial ND vaccine was used for comparison purposes. A B1 strain of NDV was used and administered by the intraocular/intranasal (IO/IN) method. The dose which was administered in all trials was that recommended by the manufacturer. This vaccine was titered in embryos and the dose was calculated to be $5x10^5$ embryo lethal dose 50% (ELD_{50}).

Birds and housing. All birds used were obtained from specific pathogen free (SPF) eggs (Hyvac Co., Dallas Center, IA). Following hatch, birds were moved into either battery brooders contained in BL2 containment rooms or Horsfall-Bauer units. At time of challenge, birds in the battery brooders were moved into floor pens and the containment status of the rooms were changed to BL3. Birds placed in the Horsfall-Bauer units remained in the units for the duration of the trial. The birds were offered feed and water ad libitum.

Challenge virus. All birds were challenged with the Texas GB NDV. A large pool of challenge virus was obtained by inoculating chick embryos and harvesting the allantoic fluid. The challenge virus was frozen and stored at -70 C for future use. Prior to each challenge, the challenge virus was titered in chick embryos.

Antibody and virus titration. Serologic antibody titers to NDV were determined by the beta hemagglutination inhibition (HI) assay, using 10 hemagglutination units of antigen, according to procedures previously described[1]. Newcastle disease virus was titrated in 10-day-old SPF chick embryos using methods described previously[2,3].

Experimental design. *Following hatch*, chicks were randomly assigned to treatment groups and placed into battery brooder units. Extra birds (8 to 10) were euthanized and bled for serologic antibody determination. Birds were administered the vaccine virus (as described above) following the placement of all chicks. Control birds were not vaccinated. In trials 2, 3, and 4 unvacci-

nated sentinel birds were wing banded and placed with the group receiving the 050 vaccine the day following vaccination. The sentinel birds were commingled with the vaccinated birds and remained so for the duration of the trial. Blood samples were collected the day prior to challenge. Birds were challenged at 3 weeks of age. The challenge dose and route were dependent upon the trial design (see Table 1). Birds were observed daily for a period of 2 weeks at which time all surviving birds were euthanized and necropsied. All birds which died during the trial were necropsied on the day of death. Mortality and morbidity were noted. Moribund birds and/or birds which were nonambulatory were euthanized and considered as mortalities. Blood samples were collected at the end of the trial from surviving birds.

RESULTS & DISCUSSION

The results of the challenge studies are shown below in Table 1. The results of the HI assays revealed that all chicks were free of detectable maternal antibody. All control birds had HI titers less than 1:4 the day prior to challenge. Negative control birds had HI titers less than 1:4 at the termination of the trial. Birds vaccinated with the B1 commercial vaccine developed HI titers ranging from 1:8 to 1:1024 prior to challenge. The birds vaccinated with the recombinant vaccines developed low (or no) HI titers ranging from 1:2 to 1:32. The geometric mean titers for the B1 vaccine and the recombinant 050 vaccine are shown in Table 2.

Birds that were necropsied following challenge with the Texas GB NDV challenge virus displayed no gross lesions. There was no evidence that any of the vaccines caused unwanted side effects, such as granulomas from injection, tumors, etc. Similarly, there were no clinical signs of disease of any kind throughout the vaccination (prechallenge) period.

SUMMARY

The recombinant vaccines containing the NDV fusion (F) protein, alone or in combination with the hemagglutinin neuraminidase (HN) protein, were protective. This protection ranged from 80% to 100%. The recombinant vaccine that contained only the HN protein conveyed little protection (20%). The commercial B1 vaccine was 100% protective in all trials. Birds vaccinated with the commercial B1 vaccine developed moderate to high HI titers. The birds vaccinated with the recombinant vaccine developed low or no HI titers. There was no indication that any vaccine (recombinant or commercial) caused any deleterious or unwanted effects on the birds. The recombinant vaccine did not spread to unvaccinated sentinel birds placed in contact with vaccinated birds.

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Table 1. Efficacy of a recombinant Newcastle disease vaccine virus.

Trial 1

Vaccine Grp	No. Birds	Vaccine	Dose[A] log10	Adm Rt	Challenge Dose[A] / Adm Rt	Mort	% Mort	Morb	% Morb	% Protection
No Vaccine	10	-	-	-	-	0	0	0	0	-
No Vaccine	20	-	-	-	0.6 / IM	20	100%	0	0%	0%
Comm. Vacc.	18	B1	5.7	IO/IN	"	0	0%	0	0%	100%
Recom. F	19	048	4.6	SC	"	0	0%	0	0%	100%
Recom. HN	20	049	4.5	SC	"	14	70%	2	10%	20%
Recom. F+HN	20	050	4.2	SC	"	0	0%	0	0%	100%

Trial 2

Vaccine Grp	No. Birds	Vaccine	Dose^ Log10	Adm Rt	Challenge Dose^ / Adm Rt	Mort	% Mort	Morb	% Morb	% Protection
No Vaccine	10	-	-	-	-	0	0	0	0	-
No Vaccine	20	-	-	-	0.6 / IM	20	100%	0	0%	0%
Comm. Vacc.	19	B1	5.7	IO/IN	"	0	0%	0	0%	100%
Recom. F	20	048	3.8	SC	"	0	0%	0	0%	100%
Recom. F+HN	20	050	3.9	SC	"	0	0%	0	0%	100%
Recom. F+HN	20	050	3.3	SC	"	0	0%	0	0%	100%
Sent. Birds	10	-	-	-	"	9	90%	0	0%	10%

Trial 3

Vaccine Grp	No. Birds	Vaccine	Dose^ Log10	Adm Rt	Challenge Dose^ / Adm Rt	Mort	% Mort	Morb	% Morb	% Protection
No Vaccine	10	-	-	-	-	0	0	0	0	-
No Vaccine	20	-	-	-	4.0 / IM	20	100%	0	0%	0%
Comm. Vacc.	29	B1	5.7	IO/IN	"	0	0%	0	0%	100%
Recom. F+HN	30	050	2.9	SC	"	2	7%	0	0%	93%
Recom. F+HN	30	050	3.3	SC	"	6	20%	0	0%	80%
Recom. F+HN	30	050	3.8	SC	"	5	17%	0	0%	83%
Sent. Birds	10	-	-	-	"	10	100%	0	0%	0%

Trial 4

Vaccine Grp	No. Birds	Vaccine	Dose^ Log10	Adm Rt	Challenge Dose^ / Adm Rt	Mort	% Mort	Morb	% Morb	% Protection
No Vaccine	10	-	-	-	-	0	0	0	0	-
No Vaccine	25	-	-	-	4.0 / IM	25	100%	0	0%	0%
Comm. Vacc.	25	B1	5.7	IO/IN	"	0	0%	0	0%	100%
Recom. F+HN	25	050	2.9	SC	"	1	4%	1	4%	92%
Recom. F+HN	24	050	3.3	SC	"	0	0%	0	0%	100%
Recom. F+HN	22	050	3.8	SC	"	2	9%	1	5%	86%
Sent. Birds	8	-	-	-	"	8	100%	0	0%	0%

Trial 5

Vaccine Grp	No. Birds	Vaccine	Dose[A] log10	Adm Rt	Challenge Dose[A] / Adm Rt	Mort	% Mort	Morb	% Morb	% Protection
No Vaccine	10	-	-	-	-	0	0	0	0	-
No Vaccine	18	-	-	-	4.0 / IO-IN	18	100%	0	0%	0%
Comm. Vacc.	20	B1	-	IO/IN	"	0	0%	0	0%	100%
Recom. F	20	048	3.5	SC	"	0	0%	0	0%	100%
Recom. F+HN	19	050	3.5	SC	"	0	0%	0	0%	100%
Recom. F+HN	20	050	3.0	SC	"	0	0%	0	0%	100%
Recom. F+HN	19	050	3.5	IP	"	0	0%	0	0%	100%

[A] Dose of Commercial B1 and Texas GB Challenge NDV in ELD_{50}
Dose of Recombinant Vaccines in plaque forming units (PFUs)

TABLE 2. Geometric Mean Titers (GMTs) of Birds Vaccinated with a Commercial B1 ND Vaccine and a Recombinant ND Vaccine.

Trial No.	Dose of Recom. 050 (log10 PFUs)	GMTs (log2) Recom. 050	GMTs (log2) Commercial B1
1	4.2	1.8	4.7
2	3.9	2.2	4.9
	3.3	1.3	
3	2.9	3.9	7.3
	3.3	3.7	
	3.8	3.7	
4	2.9	1.9	7.7
	3.3	2.0	
	3.8	1.8	

A RECOMBINANT FOWLPOX VACCINE EXPRESSING NDV ANTIGENS PROTECTS AGAINST NEWCASTLE DISEASE AND FOWLPOX

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INTRODUCTION

Newcastle disease virus (NDV) is the causative agent of a severe and highly contagious disease of poultry. The economic loss caused by this disease warrants control by vaccination or by quarantine, with slaughter of flocks in a confirmed outbreak. NDV is unique in that different NDV isolates can be classified based upon a broad spectrum of virulence and pathogenicity, but show little antigenic variability. Due to the conserved antigenicity of NDV isolates, vaccination with mild or apathogenic NDV strains provides a broad range of cross-protection against virulent strains. The hemagglutinin-neuraminidase (HN) and fusion (F) proteins are glycoproteins found on the surface of the NDV particle and both are considered to be involved in the generation of immunity.

Fowlpox virus (FPV) is the prototype species of the *Avipoxvirus* genus of the family *Poxviridae*. This virus is a large (~300 kb) double stranded DNA virus. FPV has been used successfully for many years as a live attenuated vaccine. We have developed a fowlpox delivery system (viral vector) expressing the HN and F antigens of NDV. This vaccine, when administered to 1-day-old chicks, elicits protective immunity against both FPV and NDV. In this paper, we report the construction of this vaccine and present data from various animal trials, demonstrating its high degree of efficacy .

RESULTS

Construction of recombinant FPV vaccines. The HN and F genes from the B1 strain of NDV were isolated as cDNA clones, using oligo dT primed poly A selected mRNA. The genes were subsequently engineered to be under the control of synthetic pox virus promoters, and DNA cassettes containing the NDV HN, NDV F, and NDV HN+F genes were constructed. DNA cassettes containing the NDV genes were inserted into plasmids flanked by FPV DNA homology regions. The three plasmids containing the various NDV gene cassettes were transfected individually into cells previously infected with FPV (an attenuated strain) and the desired recombinant viruses were purified by plaque assays. The recombinant viruses containing the HN and the HN+F cassettes were screened by plaque hybridization, using NDV HN DNA as a probe. A bacterial marker gene (*E.coli lacZ*) was included in the F gene cassette, and the recombinant virus containing the F gene cassette was screened by a β-galactosidase enzymatic plaque assay. Recombinant viruses containing the NDV HN, F and HN+F cassettes were selected and plaque purified. The insertion of the NDV (HN and F) genes by homologous recombination was confirmed by Southern blot analysis with DNA probes specific for both the NDV genes and the FPV DNA used to mediate the homologous recombination event.

***In vitro* expression.** Expression of both NDV genes is under control of identical but separate synthetic promoter elements. This synthetic promoter functions as both an early and late promoter, directing expression throughout the reproductive cycle of the virus. Black plaque experiments were conducted examining HN and F expression from high titer stocks of FPV/NDV-HN, FPV/NDV-F, and FPV/NDV-HN+F. Infected CEF cell monolayers were fixed with methanol and then probed separately with 2 NDV specific monoclonal antibodies (MAbs). MAb 5-3F2 and MAb 3-1G5 specifically react with HN and F respectively. Infected cell monolayers of the parental strain of FPV were included as controls. Anti-HN MAb 5-3F2 was shown to react with FPV/NDV-HN and FPV/NDV-HN+F, but not with FPV/NDV-F. Anti-F Mab 3-1G5 was shown to react with FPV/NDV-F and FPV/NDV-HN+F, but not with FPV/NDV-HN. Neither MAb was found to react with cells infected with the parental strain of FPV.

Efficacy. Groups of 1-day-old SPF chicks (HyVac Inc.) were immunized with recombinant fowlpox viruses that expressed either the HN antigen, the F antigen , or both antigens. Non vaccinated controls were also included. Three weeks post-vaccination, the birds were challenged intramuscularly with either virulent NDV or virulent FPV (Table 1). The challenged chicks were observed daily for 14 days for clinical signs and death due

to NDV. Non-vaccinated control birds showed 100% mortality. FPV/NDV-HN+F vaccinated birds showed 100% protection against FPV challenge. Birds vaccinated with FPV/NDV-HN showed 95% protection compared with 85% seen with birds immunized with FPV/NDV-F. These results suggested that recombinants expressing HN and F alone provided only partial protection. When both NDV proteins were combined into the same virus FPV/NDV-HN+F, an enhancement of protection against lethal NDV challenge was obtained, resulting in a lower protective dose. The chicks that were challenged with FPV were scored for pox lesions. Non-vaccinated control birds showed no protection against FPV lesions. Birds vaccinated with FPV/NDV-HN+F were completely protected from FPV lesions.

Duration of immunity. The duration of immunity conferred by vaccination with FPV/NDV-HN+F was examined. A group of SPF chicks was immunized with FPV/NDV-HN+F at 1 day of age and then challenged 6 weeks post-vaccination with either NDV or FPV. Complete protection was observed against both NDV and FPV challenge in FPV/NDV-HN+F vaccinated birds, where as non-vaccinated controls were totally susceptible to both viruses. These results suggest that the duration of immunity afforded by vaccination with FPV-NDV-HN+F would span the life of a broiler bird (6 weeks).

Safety. The effect of vaccinating hens in lay with the recombinant FPV/NDV HN+F was evaluated by assessing egg production post-vaccination. One group of 50 hens was vaccinated and a second group of 50 hens, housed under conditions identical to the vaccinated group, served as non-vaccinated controls. Daily egg production was monitored for 4 weeks post-vaccination. No differences were observed in egg production between the 2 groups of hens, indicating this vaccine will not adversely affect egg production in laying hens.

Effect of maternal antibody. A study was conducted to determine whether FPV/NDV-HN+F could actively immunize chicks in the presence of maternal antibodies to both NDV and FPV. Chicks obtained from NDV and FPV immunized flocks were vaccinated with FPV/NDV-HN+F and 3 weeks after vaccination, they were challenged with either virulent NDV or virulent FPV. Clinical responses were compared with non-vaccinated chicks from the same flock and with non-vaccinated chicks from an antibody negative flock (Table 2). Chicks derived from antibody negative flocks showed 100% mortality. Protection against NDV challenge, in non-vaccinated chicks known to have maternally derived antibody against NDV, ranged from 30 to 60%. Protection levels increased, to a range of 75 to 85%, when the maternal antibody positive chicks were vaccinated with FPV/NDV-HN+F suggesting an active immunization. The increase in NDV protection from 30% to 75% (flock 1) and 55% to 85% (flock 2) clearly demonstrated the ability of FPV/NDV-HN+F to partially overcome maternal antibody to both NDV and FPV. A decrease in FPV protection (90%) was observed in flock 1, suggesting some inhibition of FPV replication.

CONCLUSIONS

Several FPV/NDV recombinant viruses were evaluated for their potential use as a poultry vaccine. Recombinant vaccine FPV/NDV-HN+F, expressing both the HN and F glycoproteins of NDV, was shown to be superior to recombinant vaccines expressing either the HN or F proteins alone. The duration of immunity (both NDV and FPV) conferred by immunization with FPV/NDV-HN+F was determined to be complete for at least 6 weeks after vaccination. Vaccination of laying hens with FPV/NDV-HN+F did not adversely affect egg production. Increased protection levels to virulent NDV were seen in chicks known to have maternally derived antibody against NDV, after vaccination with FPV/NDV-HN+F. This result suggests that FPV/NDV-HN+F can actively immunize and partially overcome maternally derived antibodies to both NDV and FPV.

Table 1. Immunity conferred by Fowlpox recombinant vaccines vectoring different genes from Newcastle disease virus

VIRUS	DOSE[b]	Challenge[a]	
		NDV	FPV
FPV/NDV-HN	8×10^5	95	NT[c]
FPV/NDV-F	2×10^4	85	NT
FPV/NDV-HN+F	2×10^3	100	100
Controls	none	0	0

[a] Percent protection following challenge 3 weeks post-vaccination
[b] PFU/0.1 ml dose
[c] Not tested

Table 2. Ability of recombinant vaccine FPV/NDV-HN+F to vaccinate chicks with maternal antibody.

Flock Vaccination History	Hen Antibody			Challenge[a]			
				NDV		FPV	
	NDV-HI	NDV ELISA	FPV-AGP	Vacc	Controls	Vacc	Controls
1 NDV + FPV	1:36	1:1738	Neg	75	30	90	0
2 NDV + FPV	1:64	1:2852	Neg	85	55	100	0
3 NDV only	1:92	1:4324	Neg	80	60	95	0
4 None	Neg	Neg	Neg	--	0	--	0

[a] Percent protection following challenge 3 weeks post-vaccination

COMMERCIAL BROILER STUDIES OF MAREK'S DISEASE VACCINATION IN OVO

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INTRODUCTION

In ovo vaccination techniques provide a means to elicit active immunity at the earliest opportunity in a bird's life. Consequently, *in ovo* technology could assist the poultry industry worldwide to significantly improve vaccination efficacy[1]. The present studies evaluated the administration of a Marek's disease vaccine *in ovo*, and the resulting benefit in commercial poultry production. Some of these data appear in Miles *et al*[2].

MATERIALS AND METHODS

In three studies, a series of trials were conducted at two integrated commercial broiler companies. The INOVOJECT® system (Figure 1) was used to inject broiler hatching eggs in groups of 36 or 42 eggs at a time, while the eggs were held in incubation trays. The system punches a small hole in the blunt end of the egg and vaccine is injected below the chorioallantoic membrane. The system then transfers eggs into hatcher trays in groups of 144 or 168 at a time. The system is designed to vaccinate and transfer 20,000 eggs each hour. Injection needles and punches are cleaned and sanitized in place between each injection. During these trials, samples were collected to monitor sanitation of needles and vaccine lines. Samples of sterile saline and vaccine dispensed through the needles on the INOVOJECT® system were collected during and after each trial. These samples were cultured for microbiological growth. In Studies 1 and 2, live production performance of broilers vaccinated *in ovo* on Day 17 or 18 of incubation with HVT and SB-1 Marek's disease vaccines using the INOVOJECT® egg injection system was compared to broilers injected with the same vaccines on day of hatch by the conventional subcutaneous method. In Study 3, safety and efficacy of a bivalent HVT/SB-1 vaccine administered to embryos *in ovo* using the INOVOJECT® egg injection system was compared to broilers injected with the same vaccine on day of hatch by the conventional subcutaneous method. A subsample of 100 birds from each treatment group, in Study 3, was challenged with Marek's disease virus after hatching. Both *in ovo* and conventionally treated eggs and chicks were subjected to similar incubation and grow-out conditions in all studies.

RESULTS AND DISCUSSION

Table 1 shows results of production performance after the INOVOJECT® system was used. Differences in early mortality and average body weight varied between studies, being either numerically greater or less in birds vaccinated *in ovo*. However, in all three studies final livability was greater, and feed conversion, cost of production, and condemnations were numerically lower in the INOVOJECT® treated birds. In two trials (Study 3, bivalent Marek's vaccine administered) where a subsample of birds was challenged with Marek's disease at five days posthatch, protection was essentially equal (data not shown). Previous research by Sharma and Burmester[3] showed that *in ovo* vaccination with HVT vaccine at Day 18 of embryonation provided earlier protection than vaccination at hatch. Of the 1,709 microbiological samples collected from vaccine lines and needles, 6 were positive for bacterial growth and 3 were positive for mold growth.

These production and microbiological results indicate that the INOVOJECT® system is safe, efficacious, and cost effective for the administration of Marek's disease vaccine. Consequently, the INOVOJECT® can replace existing conventional Marek's vaccination in commercial hatcheries. Furthermore, by providing access to the avian embryo, the INOVOJECT® system will provide the basis for introducing a variety of novel biotechnology based products capable of improving bird health and performance posthatch. The next decade is likely to see the development of many such products, suitable for *in ovo* administration, including novel vaccines, immune modifiers, and performance regulators.

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Table 1. Live production performance of broilers vaccinated for Marek's disease with the INOVOJECT® (*In Ovo)* system versus conventionally (Conv.) vaccinated birds.

Production Variable	Study 1		Study 2		Study 3	
Number of Trials	28		14		2	
Paired vs. Non-Paired	Non-Paired		Paired		Paired	
Marek's Vaccine Used	HVT + SB-1		HVT + SB-1		BIVALENT HVT/SB-1	
Treatment	**Conv.**	***In Ovo***	**Conv.**	***In Ovo***	**Conv.**	***In Ovo***
Number of Eggs Evaluated	2,885,464	2,885,464	NA	NA	64,800	64,800
% Hatchability	83.91	83.75	NA	NA	86.29	85.73
Number of Chicks Placed *	9,739,225	582,500	257,575	260,575	42,600	42,600
% 1 Week Mortality	0.75	0.54	1.58	1.89	0.45	0.40
% Final Livability	96.68	96.70	96.38	96.65	96.97	97.56
Average Body Weight (lb)	4.65	4.59	4.58	4.57	4.76	4.96
Feed Conversion	2.00	1.98	2.08	2.05	2.08	2.05
% Total Condemns	0.73	0.63	0.53	0.52	1.32	0.68
% Marek's Condemns	0.04	0.01	0.01	0.01	0.00	0.00
Production Cost ($/lb)	.1607	.1597	.1909	.1883	.1641	.1605
Production Cost ($/kg)	.3543	.3521	.4208	.4151	.3618	.3538

* In Study 1 conventional production data includes chicks not included in hatchability trials.
NA = Data not available.

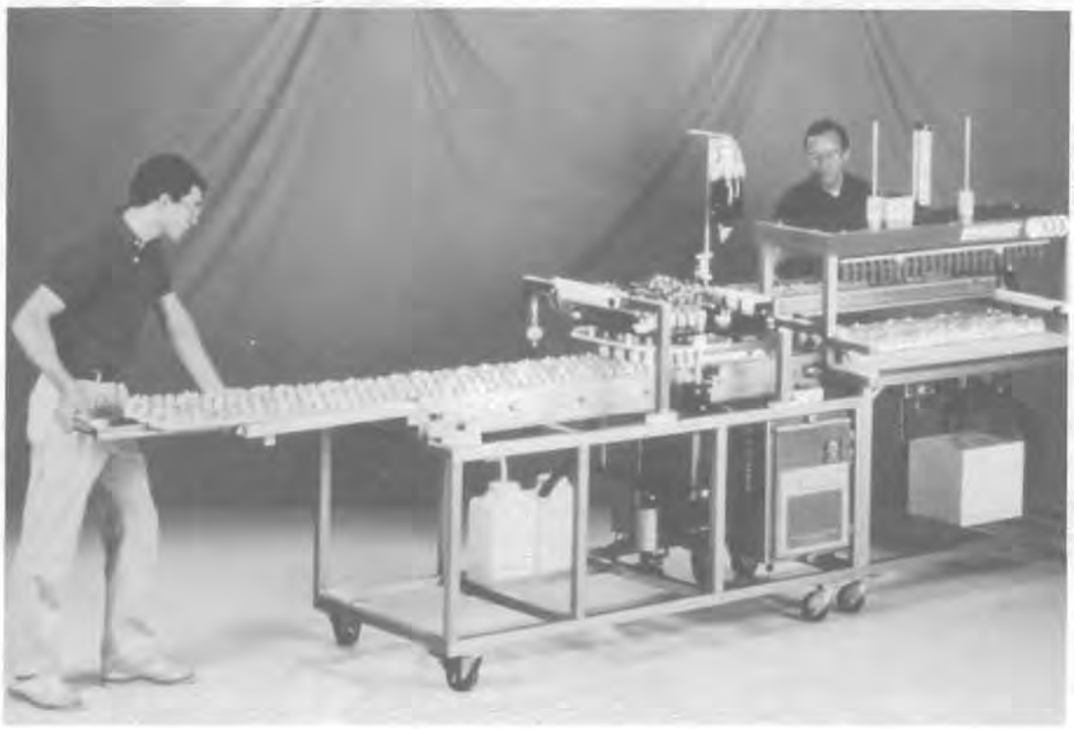

Figure 1. The INOVOJECT® system.

FIELD SAFETY AND IMMUNOGENICITY OF LIVE AVIAN ENCEPHALOMYELITIS (AE), FOWL POX AND COMBINED AE + FOWL POX VACCINES IN CHICKENS

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INTRODUCTION

Avian Encephalomyelitis (AE) is a viral infection of young chickens. The disease is characterized by ataxia which progresses to paralysis and tremors of the head and neck. Clinical outbreaks of AE are usually observed in birds between 1 and 3 weeks of age. Infected adults usually show no clinical signs except a transient drop in egg production. The causative agent of the disease is an enterovirus belonging to the *Picornaviridae* family. Although the disease is world wide in distribution, it can be controlled by an effective vaccination program in the breeders.

Fowl pox (FP) is a slow spreading viral disease of chickens, turkeys, and other birds characterized by cutaneous lesions on unfeathered skin on the head, neck, legs and feet, and/or by diphtheric lesions in the upper digestive and respiratory tract. The disease is caused by a large, DNA pox virus. In the mild cutaneous form of the disease, the mortality is usually low. A high mortality rate is seen in case of generalized infection or in diphtheric form of the disease. The disease in chickens and turkeys can be prevented by vaccination.

The objective of this study was to evaluate the efficacy and field safety of Tri Bio's freeze dried, live AE, Fowl Pox and combined AE + Fowl Pox vaccines in chickens

as part of the requirements for licensing the products by the USDA.

MATERIALS AND METHODS

Vaccines: The AE vaccine used in this study consisted of a freeze-dried preparation of live AE virus (Calnek strain) grown in specific pathogen free (SPF) chicken embryos.

The Fowl Pox vaccine consisted of a lyophilized preparation of live fowl pox virus grown in SPF chicken eggs.

The combined AE + Fowl Pox vaccine consisted of lyophilized preparations of live AE and fowl pox viruses grown in SPF chicken embryos and mixed together so as to present both the vaccines in one vial.

Prior to use, all the vaccines were reconstituted with sterile diluent following the manufacturer's directions for use circulars that comes along with the products.

Chickens: SPF chickens were used for studying the efficacy of the vaccines under laboratory conditions. For evaluation of the safety and efficacy of the vaccines under field conditions, commercial layer type chickens located at different geographical areas of the country were used.

Method of Vaccination and Challenge: The vaccines were administered to chickens following the manufacturer's directions for use circulars. For evaluation of the safety of the vaccines, mortality rates at three weeks before and after vaccination were compared. Vaccine efficacy was determined on the basis of actual challenge against virulent strains of
the vaccine viruses.

RESULTS

The results of efficacy and field safety studies on the AE vaccine are presented in Table 1. The vaccine when administered orally through drinking water provided 80% to 100% protection against challenge when tested under laboratory conditions. The vaccine was also tested to be efficacious when evaluated under field conditions. The field vaccinated birds when challenged intracerebrally against virulent strains of the AE virus, 100% of the vaccinates were protected. The vaccine was also found to be equally efficacious when administered via wing-web route.

Table 2 represents the safety and efficacy of Tri Bio's Fowl Pox vaccine in chickens administered either through wing-web or oral route. The vaccine proved to be highly efficacious when administered via wing-web route rather than administering it orally through drinking water. The protection against challenge ranged from 95% to 100% for the wing-web administered vaccine, compared to 20% to 80% for the same vaccine administered orally through drinking water. The vaccine also proved to be efficacious when tested under field conditions. More than 95% of the vaccinates showed "takes" and when challenged against virulent strains of the virus, 100% of the field vaccinated birds were protected.

The results of safety and efficacy study of the combined AE + Fowl Pox virus vaccines are shown in Table 3. The combined vaccine protected more than 90% of the vaccinates when challenged against either AE or fowl pox.

DISCUSSION

The present study did not reveal any significant difference in the efficacy of Tri Bio's AE vaccine administered either orally through drinking water or via wing-web route. The rate of protection against intracebral challenge ranged from 80% to 100% depending upon the titer of the vaccine virus in each dose of the vaccine. The vaccine also proved to be safe and effective when tested under field conditions.

The protective efficacy of the Fowl Pox vaccine administered via wing-web route ranged from 95% to 100%. The same vaccine, when administered orally through drinking water, did not perform as good as compared to the wing-web route of administration. We found about 80% protection against challenge when the vaccine was administered orally at a maximum rate of 1,000,000 ID_{50}/dose. In contrast to this, the wing-web administered vaccine provided 95% to 100% protection at a minimum rate of 50 to 100 ID_{50}/dose. These results corresponded with some of the earlier findings[1,2,3] and represented a higher rate of protection than that described by Sarma and Sharma[4]. Our findings indicate the feasibility of mass administration of fowl pox vaccine by drinking water provided that the virus concentration in the vaccine is sufficiently high.

Incorporation of both AE and fowl pox viruses in one preparation to make the combined vaccine did not effect the protective efficacy of either AE or fowl pox vaccine against challenge.

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Table 1. Safety and efficacy of Tri Bio's chicken embryo origin, live AE virus vaccine in chickens.

No. of birds vacc.	Route of admin.	Age at vacc. (wk.)	Titer per dose (Id_{50})	No. of birds chall.	No. of birds pos.	% protection
30	Oral	8	10	30	6	80.0
30	Oral	8	25	30	3	90.0
30	Oral	8	100	30	2	93.3
20	Oral	8	501	20	0	100.0
205,186*	Oral	10	>630	25	0	100.0
20	WW	8-10	32	20	1	95.0

* Birds vaccinated for field safety trial. Average mortality at 3 weeks before and 3 weeks after vaccination in the field trials was 0.2 and 0.3%. The percentage positive in the unvaccinated controls ranged from 90.0 to 100.0%.

Table 2. Safety and efficacy of Tri Bio's chicken embryo origin, live fowl pox virus vaccine in chickens.

No. of birds vacc.	Route of admin.	Age at vacc. (wk.)	Titer per dose (Id_{50})	No. of birds chall.	No. of birds pos.	% protection
30	WW	8	50	30	0	100.0
20	WW	9	100	20	1	95.0
30	WW	8	316	30	0	100.0
30	WW	8	794	30	0	100.0
140,409*	WW	8-10	>794	25	0	100.0
20	ORAL	10	10,000	20	16	20.0
20	ORAL	10	100,000	20	12	40.0
26	ORAL	8	1,000,000	26	7	73.0
20	ORAL	9	1,000,000	20	4	80.0

* Birds vaccinated for field safety trial. Average mortality at 3 weeks before and 3 weeks after vaccination in the field trial was 0.1%. The percentage positive in the unvaccinated controls ranged from 90.0 to 100.0%.

Table 3. Safety and efficacy of Tri Bio's chicken embryo origin, combined live AE + fowl pox virus vaccine in chickens.

No. of birds vacc.	Titer/Dose (ID_{50})		No. of birds Challenged		% protection	
	AE	FP	AE	FP	AE	FP
30	7943	126	-	30	-	100.0
30	50	1995	30	-	93.3	-
30	-	63	-	30	-	100.0
30	50	-	30	-	100.0	-
60	32	79	30	30	100.0	90.0
26,309*	>794	>2,500	25	25	100.0	100.0

* Birds vaccinated for field safety trial. Average mortality at 3 weeks before and 3 weeks after vaccination in the field trial was 0.1%. The age at vaccination ranged from 8-10 weeks. Vaccines were administered by wing-web route. The percentage positive in the unvaccinated controls ranged from 90 to 100%

VACCINATION TRIALS WITH A COMBINED OIL ADJUVANT NEWCASTLE AND FOWL CHOLERA VACCINE

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A combined inactivated oil emulsion vaccine for Newcastle disease (ND) and fowl cholera (FC) was tried in chickens. Serum samples collected after vaccination revealed that protective titers to ND and FC could be developed after vaccination. Challenge test results also indicated that protection levels of 100% against ND and 90% against fowl cholera could be achieved after booster vaccination with this new combined vaccine.

INTRODUCTION

Newcastle disease and fowl cholera have been recognized as the most important devastating pathogens of poultry in Egypt[10] and all over the world[1]. Prophylactic measures for protection of chickens against both diseases include the using either living attenuated[1,5] or inactivated vaccines[3,4,7]. Inactivated products have become increasingly popular because they contain no live organisms which may adversely affect growth, egg production, hatchability and fertility of the breeders, or be shed through the egg causing clinical disease in the chick[4]. Another reason is that they now contain better emulsions which are more syringable, induce less severe localization reaction, and contain more efficient adjuvants[3]. These improved emulsions allow slow release of antigen over a long period of time resulting in higher, longer lasting, and more uniform antibody titers in pullets than can be attained from continued use of live vaccines during lay[5]. The resulting more uniform levels of maternal protection make immunization of progeny more predictable.

In addition, more popular combinations of killed vaccines are now commercially available than ever before which contain multiple antigens. Two of these combined vaccines include Newcastle disease (ND) and infectious bronchitis (IB), and malabsorption syndrome (MA) and viral arthritis (VA) viral antigens[12]. Trivalent combined vaccines containing ND, IB, and *Hemophilus gallinarum* (HG), or ND, fowl pox (FP) and fowl typhoid (FT) have been tried successfully in chickens[8,9].

The aim of this investigation was to study the possibility of producing a combined inactivated vaccine for ND and FC which can be used to induce protective immunity against both diseases.

MATERIALS AND METHODS

A total of 200 white leghorn susceptible chickens were used in this study. They were obtained from a laboratory hatchery as 1-day-old chickens and raised in an isolated facility to 50 days of age. Serum samples collected from these chickens before experimentation were negative for *Pasteurella multocida*, and ND antibodies.

Chickens were then divided into 4 groups (50 each). The first, second, and third groups received ND, FC and combined (ND + FC) vaccines respectively, while the fourth group was kept as non-vaccinated controls.

Blood samples were collected from all groups at weekly intervals after the vaccinations.

Vaccines used in this study were locally prepared by Veterinary Serum and Vaccine Research Institute, Cairo, Egypt. The inactivated Newcastle disease vaccine was prepared from the F strain of NDV propagated in the allantoic cavity of embryonated chicken eggs. Then formalin was added to the harvested fluids for virus inactivation. A sample before inactivation was taken and titrated in 9-day-old embryonated chicken eggs for detection of EID_{50}/ml and the hemagglutination (HA) titer. The vaccination dose was 0.5 ml which contained $10^{8.6}$ EID_{50}.

FC vaccine was a trivalent formalin-inactivated, oil-adjuvant vaccine prepared from local isolates of *P. multocida*. The combined (ND + FC) vaccine was prepared by adding equal amounts of double the antigen content of each vaccine. Then the mixture was adjuvinated with oil.

The humoral immune response to ND was evaluated by measuring of geometric-mean-titer (GMT) of hemagglutination inhibition (HI) antibodies[1] . For immunity to FC, GMT of anti *P. multocida* antibodies were measured by the IHA[2].

Bioassays were conducted by challenging vaccinated birds either with velogenic viscerotropic NDV (10^5 ELD_{50}) as previously recommended[1] or with 10 LD_{50} of virulent *P. multocida* serotypes used in preparing the FC vaccine[2].

All the experimental birds were observed for the development of either signs of the disease and/or mortalities during the period of 14 days after challenge.

For statistical analysis, the analysis of variance was conducted[11].

RESULTS

Non significant differences ($P > 0.05$) in GMT of *P. multocida* antibodies were noted between the groups of chickens vaccinated solely with FC vaccine and the group that received the combined (ND + FC) vaccine (Table 1). Challenge test results also revealed that the protection afforded by FC vaccine was not significantly ($P > 0.05$) different from that afforded by the combined vaccine. The protection against virulent *P. multocida* challenge reached 92% with a mean survival time of 7 days and lesion score of 1 in chickens that succumbed to challenge infection. Meanwhile, protection was 90%, with a mean survival time of 6.8 days, and a lesion score of 1.2, in chickens vaccinated with the combined vaccine, but died due to infection (Table 2).

As illustrated in Table 3, protective ND antibody titers could be developed due to vaccination with either the inactivated ND vaccine alone or the combined vaccine. Non significant differences ($P > 0.05$) were noted between HI titers of both groups. On challenging the immunity of vaccinated candidates of both groups with VVND virus 3 weeks after primary vaccination, significant differences between the two groups were noted. The protection was 100% among the ND vaccinated group while it was 90% among the group vaccinated with the combined vaccine. Two weeks after boostering, 100% protection was observed in both groups.

DISCUSSION

In a previous study we have shown by experimental and field trials that simultaneous vaccination against ND and FC could be adopted successfully in chickens[6]. No mutual enhancement or competition was observed between the two simultaneously inoculated vaccines. Results of the present investigation demonstrated that the combined vaccine elicited the production of protective antibody titers against both diseases. Challenge test results also indicated that full protection against challenge infection could be achieved after vaccination with 2 doses of the combined vaccine.

These results coincide with the previous findings reported by others[8,9,12] who successfully immunized chickens by combined bacterial and viral vaccines. The combined inactivated vaccines have the advantage of providing protection against more than one disease at the same time, thus, reducing vaccination expenses and number of vaccinations per farm as well as saving time and labor

besides reducing the stress reactions. Due to these practical and financial benefits, the use of combined vaccines has proved to be very popular, especially when vaccination of broilers and primary vaccination of breeding stock are concerned.

Although the data of this study are encouraging , extensive field trials have to be conducted by using this new combined vaccine before a final conclusion could be reached. The duration of immunity and the keeping quality of this vaccine is now under investigation.

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Table 1. Average GMT of *P. multocida* antibodies at different intervals after vaccination with FC or the combined (FC + ND) vaccines.

Type of vaccine	Average GMT		
	2nd week	4th week	2nd week after boostering
FC vaccine	184	368	2560
combined FC + ND vaccine	171	394	2744
Control	0	0	0

Table 2. Challenge test results of chickens vaccinated with FC or combined FC + ND vaccines after inoculation with virulent *P. multocida* organism.

Type of vaccine	A alive/dead	% alive	B MST	C Lesion score
FC vaccine	18/20	90	7.0	1.0
Combined FC+ND vaccine	18/20	90	6.8	1.0
Control	0	0	0	0

A = No. alive 14 days post challenge/No. challenged
B = Mean survival time
C = Score of 0 to 4 for all dead chickens

Table 3. Anti ND serological responses of chickens vaccinated with inactivated ND or combined (FC + ND) vaccines.

Type of vaccine	Average Log_2 HI titers			
	1st week	2nd week	3rd week	3 weeks after boostering
Inactivated ND vaccine	3.2	4.5	6.4	7
combined FC + ND vaccine	3.0	4.2	5.6	6.5
Control	0	0	0	0

INCREASING THE YIELD OF EIMERIA TENELLA OOCYSTS IN CELL CULTURE[A]

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The purpose of this project was to interrupt the life cycle of *Eimeria tenella*, the parasite that causes cecal coccidiosis in chickens, by blocking the fertilization process with a panel of previously produced monoclonal antibodies (MAbs) directed against the gamont (microgametocyte and macrogametocyte) antigens. The screening of MAbs necessitated the establishment of parasite development and reproduction in a reliable and quantitative cell culture system. Several different parameters were investigated to maximize and stabilize oocyst production in vitro. The first step was to produce oocysts that were genetically adapted to in vitro culture conditions. A strain of *E. tenella* originally isolated from white leghorn chickens was grown in cell culture. The resultant oocysts were harvested and subsequently passaged through chickens. Oocysts were then collected from chicken manure and passaged back into cell culture. This process of alternating from cell culture to chickens and back to cell culture has been repeated several times. Cell culture consisted of infecting monolayers of primary chicken kidney cells grown in 24-well tissue culture plates in 89% RPMI medium, 10% fetal bovine serum, and 1% penicillin/streptomycin (pH 7.2) at 40.5 C in an atmosphere of 5% CO_2. We have now developed a strain of *E. tenella* that is consistently producing over 200% more oocysts in cell culture than the original strain. Refining this cell culture system will allow us to screen our panel of MAbs and determine which MAbs or combinations of MAbs are capable of inhibiting *E. tenella* fertilization. Ultimately, we hope to identify and isolate target antigens on parasitic gamonts which can be incorporated into an efficacious subunit vaccine. Such an in vitro system could also be used to screen other potential anticoccidial agents.

[A]The full length article will be submitted for publication in Avian Diseases.

PROTECTIVE EFFICACY OF AN ADJUVANTED NEWCASTLE DISEASE VIRUS VACCINE

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Commercial day-old turkey poults with maternal antibodies to Newcastle disease virus (NDV) were vaccinated with different preparations of adjuvanted killed NDV.

Immunostimulating complexes (ISCOMs) with whole virus, detergent treated virus, and subunit virus preparations were given subcutaneous (s/c) to day-old turkey poults. The subunit ISCOM preparations were also combined with an adjuvant and an immunomodulator separately or together in combination. The protective efficacy of each of these vaccine preparations was evaluated by challenge with a velogenic neurotrophic strain of NDV when the turkeys were 11 weeks of age.

Results from this study suggest the potential use of a subunit virus ISCOM vaccine given s/c together in combination with an adjuvant and immunomodulator in turkey poults at 1 day of age.

AN EVALUATION OF THE EFFECT OF LIVE IBD VACCINATION ON SUCCESSIVE FLOCKS THROUGH THE SAME HOUSE

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Vaccine programs in commercial poultry are compared based on side-by-side comparisons. Several flocks are often used for each treatment. The flocks are then grouped by treatment and compared. A program is selected based on the performance results.

Vaccines are made and tested for their ability to protect poultry from viral or bacterial challenge. These same vaccines are then tested and evaluated in commercial poultry for their ability to affect performance. We assume there is a positive relationship between protection and flock performance.

In concentrated food animal production environment, evaluation of a vaccine program in a "one time pass," using multiple flocks, is potentially short cited. Vaccines not only "protect" or "don't protect" the birds in a flock, they also influence the "resident" challenge virus.

Vaccines can influence the resident challenge virus positively or negatively. An example of a negative effect is a vaccine which becomes stronger than the existing field challenge through bird passage. An example of a positive effect is a vaccine that protects 100% of the birds in the flock from resident virus replication and it therefore eventually ceases to exist. Positive and negative effects occur in commercial poultry during every grow-out.

The evaluation of a virus and a vaccine's effect on the evolution of that virus is more of an issue for viruses that survive in the environment for long periods of time. In commercial poultry, infectious bursal disease (IBD) virus is widely recognized as the most economically significant virus within this criteria.

A vaccine for IBD was evaluated over a series of flocks through the same house. The evaluation documented pathology to the bursa of Fabricius and overall performance of each flock.

Bursae were taken at 7, 14, 21, 28, and 40 days of age and evaluated for lymphoid depletion. Performance was quantitated by using a commercial company's own calculations which yield the total cost per pound of bird.

Performance improved over the course of the year in a somewhat irregular but consistent fashion. The sixth flock was approximately 1¢ per 454 gm (1 pound) lower cost than the first flock.

Lymphoid depletion of the bursa was similar at 7, 14, and 28 days of age in the 6 flocks evaluated. The bursae evaluated at 21 and 40 days of age showed a trend towards less severe damage in the final flocks when compared to the initial flocks.

IBD vaccine should be evaluated not only for its ability to protect a bird from challenge, but also, and perhaps more importantly, for its effect on the evolution of the "resident" IBD challenge virus. Study of the evolution of the resident virus is difficult and requires continued study both in the laboratory and in the chicken house.

PROTECTIVE DOSE LEVELS OF NOVEL BDA-BLEN INFECTIOUS BURSAL DISEASE VACCINE IN SPF AND BROILER BIRDS

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INTRODUCTION

The infectious bursal disease (IBD) vaccine used in these studies was prepared by mixing IBD-BLEN™ and chicken bursal disease antiserum (BDA) together in the appropriate ratio to form the final lyophilized complex IBD vaccine product to be administered at day of age by subcutaneous injection. SPF birds were housed in isolation units and broiler birds were maintained in isolated self contained floor pens.

RESULTS

Hy-Vac SPF chicks and Cobb X Cobb broiler birds were vaccinated at day of age, using either IBD-BLEN™ (100 EID_{50}) without BDA or three doses of the complex vaccine: a) 100 EID_{50} IBD-BLEN™ + 24 units BDA, b) 20 EID_{50} IBD-BLEN™ + 4.8 units BDA, and c) 4 EID_{50} IBD-BLEN™ + 1 unit BDA. Parameters measured were visual observation of bursal infection, bursal to body weight ratios, ELISA values for IBDV serum antibody titer, and evaluation of IBD immunity against USDA IBD challenge strain given by eyedrop.

All SPF birds receiving either the IBD-BLEN™ (100 EID_{50}) vaccine or the three doses of complex vaccine (a-c, above), developed protective IBDV antibody titers when measured at Days 22 and 32 of age and were protected against a USDA IBD challenge strain given at Day 29 of age. At Day 8 of age, all bursas in the groups receiving the three doses of the complex vaccine were free from visual signs of infection, whereas all bursas showed visual signs of chronic bursal infection in the birds receiving the 100 EID_{50} of IBD-BLEN™ without BDA. BDA delayed bursal infection due to the vaccine virus when compared to the IBD-BLEN™ without BDA at Day 8 of age.

In the Cobb X Cobb broiler birds, the only vaccine preparation that did not infect bursas by Day 35 of age, stimulate protective IBDV antibody titers at Day 35 of age, or protect against USDA IBD challenge strain at Day 32 of age, was the dose listed in "c" above (4 EID_{50} IBD-BLEN™ + 1 unit BDA).

CONCLUSIONS

In summary, the BDA-IBD-BLEN complex IBD vaccine maintains protective efficacy in broiler birds against USDA IBD challenge strain at the two doses of 100 EID_{50} IBD-BLEN™ + 24 units BDA (release dose) and 20 EID_{50} IBD-BLEN™ + 4.8 units BDA (minimum protective dose). The high levels of maternally derived anti-IBDV antibodies in broilers prevents the 4 EID_{50} IBD-BLEN™ + 1 unit BDA dose from protecting against IBD challenge (unlike in the SPF type birds). BDA delays the vaccine virus from infecting bursas in SPF birds until after Day 8 of age.

PRELIMINARY EVALUATION OF ANTIGENS FOR USE IN A NEWLY DEVELOPED ELISA FOR DETECTION OF ANTIBODIES TO HEMOPHILUS PARAGALLINARUM IN CHICKENS

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Infectious coryza is a highly contagious, upper respiratory disease of chickens caused by *Hemophilus paragallinarum* (*Hpg*). In southern California, the disease typically causes severe temporary depression in egg production and is estimated to cost the industry $2 million annually. Control of the disease is difficult because current diagnostic tests are not suitable as management tools to assess or monitor the vaccinal or disease status of incoming or resident flocks.

It is for this reason that we recently reported preliminary results of a newly developed ELISA for detection of antibodies to *Hpg* in leghorn layer chickens[1]. The assay utilized a cell surface heat extract of a local strain of *Hpg* (EW/91) as the antigen source. It was able to detect increasing levels of antibody in SPF chickens inoculated with the EW/91 or Modesto strains of *Hpg*. It was also able to detect increasing levels of antibody in chickens exposed to the EW/91 strain in drinking water when housed under uncontrolled commercial conditions[2]. However, it had difficulty differentiating between control, infected, and vaccinated groups of chickens when housed under controlled conditions. Furthermore, the assay lacked acceptable day-to-day precision and positive to negative ratios to warrant further evaluation of its potential as a diagnostically useful test.

Over the past 2 months, we modified the assay by changing the antigen to formalinized whole-cells of *Hpg*. This modification showed promise in that it gave acceptable day-to-day precision (C.V. $< 10\%$) and positive to negative ratios (P/N $\simeq 12$) and it clearly differentiated between control and vaccinated groups of chickens. However, this differentiation may have been non-specific because the antigen was derived from the vaccine utilized to vaccinate the chickens. Furthermore, the modified assay did not detect antibodies in chickens experimentally infected with the EW/91 and Modesto strains of *Hpg*. Nevertheless, since formalinized cells of *Hpg* show promise and since there is a considerable need for a sensitive specific serologic test, we plan to further try various antigens in an attempt to optimize and validate an ELISA for Infectious coryza.

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MAREK'S DISEASE RESEARCH—PRESENT DIRECTIONS AND FUTURE OBJECTIVES

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Marek's disease (MD) has been studied intensively for 30 years, ever since the disease was first reproduced with regularity in genetically susceptible chickens[7]. The focus of studies has naturally changed over this period. The first task was to identify and characterize the etiologic agent, a highly cell-associated herpesvirus. This was soon followed by the development of highly effective vaccines by attenuation of oncogenic strains of MD virus (MDV) and by the isolation of low-virulence, or related but nononcogenic, avian herpesviruses. It was only after the largely successful control of this devastating disease that attention was directed to really understanding its epizootiology, pathogenesis and immunology, and the role of the herpesvirus as an oncogenic agent. In more recent years, the failure of vaccines to consistently control the disease to the extent desired has driven research toward defining the molecular basis to oncogenicity and immunity. Over these many years, MD research has served both as a model of oncogenic herpesvirology, and as a means to devise methods to control a disease of great economic significance.

This paper will examine the present directions of research on MD and speculate on what the future objectives might be. It will draw heavily from papers presented at the Fourth International Symposium on Marek's Disease held in Amsterdam, The Netherlands, in September, 1992, as part of the World's Poultry Congress. Papers on MD constituted nearly 15% of the Proceedings, attesting to the continued importance of research on the disease.

PATHOGENESIS

Studies in the field of pathogenesis are disappointingly scanty at the present time; although, a few areas of research have some exciting possibilities. Recent work has dealt with: 1) identification of the target cells for infection, latency or transformation with MDV, or turkey herpesvirus (HVT), 2) interaction between MDV and other infections, e.g., avian leukosis virus (ALV), chicken infectious anemia virus (CIAV), reticuloendotheliosis virus (REV), 3) the role of cytokines in latency, and 4) chromosomal aberrations in tumor cell lines.

Target cells. Although the B cell was considered to be the primary target for early MDV infection[47], a role for splenic ellipsoid-associated reticulum cells (EARC) was postulated based on studies with monoclonal antibodies[20]; however, more discriminating studies[21] confirmed that lymphocytes, not EARC are, indeed, the major cells infected in the spleen.

It was discovered, through the use of a local-lesion model[8], that a variety (at least 7) of T cell subsets can become infected and transform[44]. This pointed to the likelihood that the consistent finding of only CD4+ cells in MD tumor cell lines is due to specific timing of events during pathogenesis rather than a unique susceptibility of that particular target cell.

Kudo et al.[24] attempted to determine the importance of CD4+ and CD8+ cells in vaccinal immunity and lymphoma development by depletion methods. They concluded that CD4+ cells were required for lymphomas, and that vaccination with HVT decreased the number of these cells. Because there were very few chickens per experimental group, the conclusions must be interpreted with caution.

Holland et al.[17] used *in situ* hybridization and immunofluorescence to show that both CD4+ and CD8+ T cells become latently infected with HVT.

Interaction between MDV and other viruses. Studies on the capability of serotype-2 MDV to enhance lymphoma induction by ALV[3], apparently through enhanced transcription of retroviral genes[40], were extended when it was shown that a similar phenomenon occurs with B-cell tumors induced by another retrovirus, REV[2].

Studies continue on the ability of immunosuppressive viruses like CIAV to enhance MDV infection levels and tumor incidence[12].

Cytokines. Volpini et al.[53] have extended our knowledge about the ability of cytokine generated during immune responses to modulate the expression of MDV genome in latently infected splenocytes or tumor cell lines. It was found that more than one kind of cytokine from conditioned media (CM) can be involved in modulating latency in splenocytes, one of which has many of the characteristics of interferon. Also, splenocytes collected during the early latent period responded differently to 2 types of CM when compared to those harvested in later stages of latency.

Chromosomal aberrations. MSB-1, the first MD cell line described[1], was reported to have an increased

length short arm of one homolog of chromosome 1. Bloom[4] showed that it was due to a duplication (1p+) of a segment of that arm. Now, Moore and colleagues at Cornell University have extended the findings very significantly by showing that it is a consistent change; 14 of 15 MD cell lines demonstrated the change. ALV- or REV-transformed lines, and nontransformed cells did not show the duplication. It was learned that the duplication is due to amplified genomic DNA sequences. This exciting finding raises the possibility that the aberration may be essential to transformation of lymphocytes by MDV.

IMMUNOLOGY

From recent reviews[43,45,46] it can be concluded that the immunological basis for immunity after natural infection with MDV or after vaccination is still poorly understood. Although humoral immunity has a role, cell-mediated immunity (CMI) responses are involved to a large extent, and those have been very difficult to study with the cell-associated MDV. Nonetheless, some progress is being made.

Passively acquired maternal antibodies are partially protective, and at least some of these have virus-neutralizing activity, including those against the herpes simplex virus (HSV) gB homolog of MDV[11,33]. In addition to those previously described, Lee and Witter[25] recently detected a non virus-neutralizing antibody that protects against effects of early virus replication, but not against lymphoma development. Inserting MDV genes into baculoviruses to produce antigens, as with the gC[34] and the gB[35] homologs of HSV or pp38[10] offers a new approach for eliciting very specific antibodies to study their neutralizing activity and protective capacity.

It is still too early to speculate on which antigens are most important in CMI against MDV. However, a new approach to identifying antigens and determining the significance of cytotoxic T lymphocytes (CTL) by inserting MDV genes into REV-transformed lymphocytes was recently described by Pratt et al.[38] (see section on Molecular Biology). This technology should make CMI research much more productive.

Malkinson et al.[27] have taken the novel approach of comparing the HSV gB homologs in MDV and HVT based on conformational characteristics of the antigen as found in the membrane of infected cells. Their high degree of relatedness by this criterion provides an explanation for the cross reactivity between serotype 1 MDV and HVT. Some sharing of intertypic antigens was found with serotype 2 MDV, but there were unique conformational epitopes as well, perhaps explaining the synergistic effect seen with bivalent vaccines.

CONTROL

Genetic resistance continues to demand some attention, but probably not as much as it should, given the markedly superior response to MD vaccines of genetically resistant strains of chickens[7]. Gavora[15] noted that marker-assisted selection (e.g., selection based on MHC haplotype) may be complemented in the future by use of gene transfer techniques. Examples of the latter could include the introduction of genes like the HSV gD homolog to render cells resistant to infection (as has been shown with HSV), or the use transgenes coding for antisense mRNA.

Witter[56] reviewed progress in the prevention and control of MD during the past 4 years. He noted that whereas condemnations in some areas are unchanged or have been reduced, some countries have experienced increasing MD losses which represent a significant concern at the present time, perhaps due to one or more of the following: increased virulence of MDV isolates, decreased genetic resistance, immunosuppression by other agents, and poor vaccine handling and administration. Witter (personal communication) screened 8 isolates from vaccinated flocks with excessive losses and found a high preponderance of very virulent MDVs, but only one isolate of high enough virulence to possibly be considered a new pathotype; thus, he believed that there may be a gradual increase in overall virulence, but apparently no major shift.

Comparisons of various commercially available vaccines continue, especially combinations resulting in bi- or polyvalent vaccines, in attempts to improve the degree of protective synergism[26,42,54,56,57], and field studies on the practical application of *in ovo* vaccination are being conducted[29]. The exciting introduction of experimental recombinant viruses is discussed below (see Molecular Biology); these will no doubt command considerable attention in the next few years as potential next-generation vaccines.

Enhancement of vaccinal immunity through the use of an adjuvant, Acemannan, an acetylated mannose polymer, is being tested as one more weapon to improve the protective index of MD vaccines[36]. It is believed that the adjuvant stimulates the host immune response to live or inactivated vaccines.

MOLECULAR BIOLOGY

Without question, the most active area of MD research currently is the careful and detailed examination of the virus genome. It can be presumed that by identifying and characterizing the nature and significance of the viral genes, it will be possible to determine which are involved

in various aspects of the disease. Immunologically important genes are of great importance since they can be targeted for enhanced expression or for insertion in other vector systems. However, improved control could also be possible through careful manipulation of genes responsible for key steps in the pathogenesis of the disease or in the oncogenic response itself. In any case, our overall knowledge of oncogenic herpesvirology will be advanced by continued study of MDV as perhaps the best model available. One aim might be to sequence the entire genome. This massive project is presently being done in a focussed, piece-meal fashion by a variety of laboratories, but cooperation amongst different teams to accomplish the job more efficiently must be considered.

Velicer and Brunovskis[52] reviewed work leading to our present understanding of the genomic structure of MDV. They noted several key findings: 1) The genomic structures of all 3 related serotypes of MDV are similar to one another and to the alphaherpesviruses (prototype, HSV) rather than to the gammaherpesviruses (prototype Epstein-Barr virus). This is of importance in pointing to HSV as the virus in which genomic homologs might be found; indeed, several have already been identified. The development of restriction-enzyme maps was crucial to this finding as well as to general gene-location studies. 2) Key genes that have been identified and characterized to various degrees include: a) those which code for the MDV-A (now called gp57-65; a homolog of HSV gC) and MDV-B (gp100, gp60, gp49; a homolog of HSV gB) antigens, both of which are of immunological importance; b) a 1.8kb gene family associated with truncated RNAs in attenuated MDV and perhaps involved with maintenance of the transformed state in lymphoblastoid cell lines; c) a gene (called meq) with homology to the leucine-zipper class of nuclear oncogenes; and d) a gene which encodes a phosphoprotein (pp38) which is also expressed in lymphoid tumor cells. They speculated that possibly more than one gene contributes to MDV's oncogenic properties.

The MDV system offers some advantages over others in locating genes involved in oncogenicity or in immunity: 1) oncogenic viruses can be attenuated by cultivation *in vitro*, and the parent and derivative strains can be compared for genomic differences; 2) strains naturally different in oncogenic potential can be compared; 3) oncogenicity can be determined by *in vivo* analysis; 4) MD tumor cell lines can be used to search for genes that are being transcribed and thus potentially important to transformation; and 5) gene products that stimulate either antibodies or CMI can be determined, leading to identification of the respective genes. Some of the current work related to these points follows.

Oncogenicity-related genes. Fukuchi and colleagues[13] provided the detailed restriction-enzyme map which has formed the basis for much of the work in this area. Viral genes could contribute to an oncogenic response directly through the expression of a viral oncogene(s), or indirectly either by affecting cellular genes involved in regulation of cellular growth, or by affecting the host cell range, virus replication, or other features important in pathogenesis. Comparing viruses of different oncogenic potential has been very helpful in identifying areas of the MDV genome that might be involved. For instance, an expansion of a 132bp repeat in the *Bam*HI D and H regions has been associated with loss of oncogenicity[14,28,49]; oncogenic strains have 2 to 3 copies of this repeat whereas attenuated strains have more than 10 copies[19]. This expansion is thought to disrupt the production of a 1.8kb transcript which is truncated in the case of attenuated viruses[5]; thus the 1.8kb transcript may be tumorigenic. Inhibition of the 1.8kb gene family by antisense oligonucleotide caused proliferation of a tumor cell line to cease[23], further supporting the importance of the transcript in maintaining the tumorigenic state. Another change occurs in a 1.2 kb subfragment of the *Bam*HI L region, where there is a deletion of 200 base pairs[55].

Determining the regions transcribed in MD tumor cell lines which are non virus-producers could give clues regarding genes involved in transformation or the maintenance of the transformed state. This approach has revealed the presence of a gene, called *meq*, in the IR_L region, which has homology to the leucine-zipper class (*fos*/*jun*) of nuclear oncogenes[22]. Also, Ohashi and Schat[37] found 3 transcripts from the *Bam*HI A region and 1 from the *Bam*HI L region. Monoclonal antibodies were used to identify a phosphoprotein, pp38, produced by oncogenic MD viruses but apparently not by nononcogenic virus strains[18,48]. Expression of pp38 is variable and can be enhanced by increased expression of a homolog of the HSV ICP4, demonstrating the complexity of genomic activity[39]. Thus there are several candidate genes already uncovered; likely there will be more.

As prospective tumorigenic genes are identified, it will be important to clone and test them in defined systems, e.g., by transfection into potentially transformable target cells; or to determine if their insertion into nononcogenic virus strains will confer oncogenicity; or to find out if mutants in which the suspect gene has been deleted or "turned off" lose their oncogenic potential.

Immunity-related genes. Several antigens are either common to all 3 serotypes, e.g., p79[48], or related among the serotypes, e.g., gp 57-65 and gp 100, gp60, gp49[6]. The localization, cloning, and characterization of the respective genes now allows very specific testing of gene products for immunogenicity. The best example of this is the recent use of the HSV gB homolog gene from MDV in a recombinant fowlpox virus[33,58] and in HVT[41] for use as vaccines against MD. This approach is suitable for testing a variety of genes; in the same study in which gB

was found protective, a fowlpox recombinant expressing the gene for pp38 failed to induce antibodies or protect chickens against MDV challenge[33].

Testing the importance of potential immunity-related genes in CMI is much more difficult, but a breakthrough was recently reported by Pratt et al.[38]. They developed a system in which REV-transformed lymphoblastoid cell lines, susceptible to MHC-restricted lysis by cytotoxic T lymphocytes (CTL), were transfected with MDV genes which in turn rendered the cells susceptible to MDV-specific CTL. This approach now allows a systematic examination of candidate antigens significant in antiviral CMI[51].

MDV and HVT as vectors. The use of the chicken and turkey herpesviruses as vectors for other immunogens is of interest. First, it is necessary to determine appropriate insertion sites for new genes so as to not disrupt essential functions of replication, or inhibit the immunogenic properties of the virus against MD. A marker gene, such as the *Lac*Z gene of *E. coli*, has been used for this purpose[9,16,30,50]. Live recombinant MDV (CVI-988) and HVT vaccines expressing the Newcastle disease (ND) virus fusion factor gene protected against both MD and ND[31,32], and the gB gene from MDV was successfully inserted and expressed in HVT in efforts to enhance the protective effect of the vaccine against MD[41].

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STUDY TO ERADICATE AVIAN LEUKOSIS VIRUS FROM A CAGED WHITE LEGHORN POPULATION

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INTRODUCTION

In 1960, Rubin developed the first in vitro test for avian leukosis virus (ALV)[6] and his findings stimulated research that led to development of flocks of chickens that were free of this pathogen[4]. While the test was not suitable for commercial application, findings reported by Rubin have been fundamental to control lymphoid leukosis. He reported that congenital transmission of ALV was often erratic, in that only a low percentage of embryos

from some hens might be infected[7]. Also, those studies showed that chickens infected as embryos were immunologically tolerant and that infected males did not influence the incidence of congenital transmission.

The erratic nature of congenital transmission led to the notion that hens were intermittent shedders of virus. However, it is now recognized that ALV infected hens may consistently shed virus into albumen and yet some of their embryos escape infection. Of practical importance was the discovery that hens, which congenitally transmit infection, could usually be detected by testing for group specific (gs) antigen of ALV in egg albumen or other specimens[8]. This led to development of rapid test procedures for detecting ALV-infected birds.

A number of poultry breeding companies became interested in eradicating ALV following the discovery that White Leghorns test-positive for gs antigen in egg albumen were less productive than those which were test-negative[10]. This report updates results of our program to eradicate exogenous ALV from Single Comb White Leghorn breeding stock maintained at Agriculture Canada's Centre for Food and Animal Research (CFAR) in Ottawa.

MATERIALS AND METHODS

Tests for viral antigen and antibody. Enzyme-linked immunosorbant assay (ELISA) procedures for detecting gs antigen and methods followed in preparation of egg albumen and feather pulp for testing, have been reported[8]. Levels of gs antigen resulting from infection with exogenous ALV are usually higher than those associated with expression of endogenous viral (ev) genes[11]. ELISA kits purchased from IDEXX Inc., Portland, Maine, were used to detect antibody to ALV. An ELISA reader was used to record optical density (OD) readings and cut off points for interpretation of reactions are given below. Serum collected for antibody studies was always from chickens older than 14 weeks of age.

Chickens and management. All strains of chickens were developed and maintained at CFAR. Control strains were randombred whereas the selected strains were selected for high egg production and other traits[2]. For each generation there were 300 to 400 hens in each of 3 control strains and approximately 1100 in each of 6 selected strains. Strains are listed in Table 1 and are grouped according to their relationship. Strains 1, 3, and 5 were from the same genetic base; Strains 2, 4, and 6 were from a second base and Strains 8, 9, and 7 were from a third base.

Chicks were started in the upper deck of a 3 tier brooding and rearing (B&R) cage unit and there were approximately 75 per cage. To reduce the number of chicks in direct contact early in the brooding period, in 1987 the upper deck cages were divided with a solid metal partition. At 6 weeks the chicks in the upper deck of each unit were randomized among the 3 tiers of the unit.

The year of hatch is given to identify the generation of chickens and the 1983 hatch was the first generation tested for infection with ALV. B&R cages were randomized to a specific strain. Except for the 1987 and 1989 generations, dam families were randomized throughout B&R cages so that one family could be in 6 or more different cages. To limit spread of infection from an infected family, the 1987 and 1989 generation dam families of selected strains were divided between two (half) B&R cages. Control strains continued to be randomized throughout pens because there were usually only 1 to 3 chicks per family.

Test program for infection with ALV. The basic program was to test albumen of one egg per hen of each generation by ELISA for gs antigen. However, the 1985 generation was retained for a second year and was tested before (8.5 months) and after (21.5 months) molting. In all generations, breeders were selected from hens whose eggs had OD readings <0.4.

In an attempt to detect early infection, the 1987 hatch was tested for gs antigen in feather pulp. All chicks were tested at 0 to 12 days and those with OD readings greater than 1.8 were killed. Chicks were again tested at 22 to 31 days and the same cut off point was used to eliminate chicks.

Tests for antibody were also conducted on serum collected from a random sample of each strain of the 1987 hatch. Information from those studies was also applied in an effort to eliminate hens most likely to have been exposed to the virus. For that generation, none of the hens that had been reared in a given B&R cage were used as breeders: (a) if at 14 weeks of age, 30% or more had ELISA antibody OD readings greater than 0.25 or (b) if as adult hens, 10% or more had gs antigen OD readings in albumen greater than 1.0, and (c) in the case of Strains 3 and 5, only if 20% or more of the hens had serum antibody readings greater than 0.25. Testing of the 1992 generation for gs antigen in egg albumen is in progress. However, 89 to 183 chickens from each of Strains 3, 5, 7, and 8 have been tested for antibody and were considered positive if OD readings were above 0.25.

RESULTS AND DISCUSSION

Table 1 shows that when testing for gs antigen in albumen commenced in 1983, the percentage of test-positive hens ranged from 22-24 in 3 control strains, and from 2 to 13 in selected strains. As previously reported, the difference in incidence between control and selected strains was attributed to the fact that infected chickens tended to be less productive and were usually eliminated as breeders in the selected strains[10].

Eliminating hens of the 1983 generation as breeders that had gs antigen levels greater than 0.4, did not alter the incidence of infection in the 1984 generation. However, by applying the same test procedures in 1984, there was a substantial drop in the incidence of infection in the 1985 generation.

The additional tests and modified management practices applied in 1987 may have contributed to our achieving a low incidence of infection in all strains of that generation. Of particular interest was the drop from 15% to 0% in Strain 5. Feather pulp was tested for gs antigen because in previous studies it had proven useful for identifying infected chicks early in life[11]. However, in this study, interpretation of tests on feather pulp proved difficult because levels of endogenous gs antigen were higher than anticipated. The endogenous antigen was probably associated with the ev3 gene, which was subsequently found to be prevalent in the population[5]. In tests on feather pulp, 12% of 12,273 chicks had readings greater than 1.8 and these were eliminated.

As horizontal spread of infection would be expected to be greatest while chickens were in the B&R cages, breeders from the 1987 generation were from B&R cages where there had been the lowest incidence of infection. Despite these additional precautions, the incidence of infection in Strain 7, based on tests for gs antigen in egg albumen, unexpectedly jumped from 2% in 1987 to 17% in the 1989 generation. Results for Strain 7 were again high in the 1990 generation. Also in 1990 there was a slight increase in the percent positives in Strain 8 (Table 1). Although test results from the 1992 generation for viral antigen in albumen are not yet available, antibody was detected in approximately 30% of the chickens in both Strains 7 and 8. In contrast only 2% of Strains 3 and 5 were test-positive.

As previously reported, chickens whose eggs have non-detectable levels of gs antigen, can congenitally transmit infection[9]. While eliminating hens as breeders that shed high levels of the antigen would eliminate many of the infected hens, these results show that testing one egg per hen per generation was not adequate to eradicate ALV from the White Leghorn population under study. It is noteworthy that a similar program, carried out in parallel on a meat-type population at CFAR, was successful in eliminating exogenous ALV[9]. Results of that study suggested that horizontal spread of infection was much slower among meat-type birds than among White Leghorns. While the mechanism of resistance in the meat-type chickens is not understood, studies have shown that replication of leukosis-sarcoma viruses is less extensive in meat-type than in White Leghorn embryos and chickens[1,3].

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Table 1. Percentage of hens by generation that were test-positive for group specific antigen of avian leukosis virus in egg albumen[1].

	% Positive					
Strain[2]	1983	1984	1985	1987	1989	1990
1 S	7	9	2	4	1	0
3 S	7	9	3	1	1	0
5 C	22	24	15	0	1	0
2 S	2	1	1	1	1	0
4 S	2	1	1	0	1	0
6 S-C	2	1	1	1	1	0
8 S	13	9	2	1	1	4
9 S	13	9	3	2	1	0
7 C	23	18	5	2	17	17
10 C	24	25	8	3	3	1

[1]ELISA, optical density of 1.0 or more.
[2]S = selected strain, C = unselected control strain, S-C = selected before 1982

CHICKEN INFECTIOUS ANEMIA IN ARGENTINA

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Chicken infectious anemia (CIA) was first described in Japan by Yuasa et al. in 1979[11]. It is characterized by severe anemia and lymphoid depletion followed by thymus atrophy, bone marrow aplasia, low hematocrit values, and reduced body weight. Due to the lymphoid depletion that is the cause of the immunosuppression which can complicate the disease, field cases of CIA are frequently characterized by secondary bacterial infections (e.g., blue wing disease)[5], Marek's disease vaccination breaks[1], enhanced problems with bursal disease[10], and poor flock performance.

During the last 2 years, increasing numbers of broiler flocks in Argentina were reported to have problems suggestive of CIA and the complications described. Materials were obtained from some of these flocks for further study. This paper presents the isolation, identification and serological determination of chicken infectious anemia virus (CIAV) in Argentina.

MATERIALS AND METHODS

Chickens. Chickens were obtained from the specific-pathogen-free flock of the Department of Avian and Aquatic Animal Medicine (DAAAM), New York State College of Veterinary Medicine, Cornell University. Experimental chickens were housed in filtered-air, laminar flow isolators for the entire experimental period.

Virus strains. Cux-1 strain of CIAV[1] was obtained with permission from the USDA by the DAAAM from Mr. R. Wellenstein (SPAFAS, Inc., Norwich, Connecticut), after an unknown number of passages in MSB-1 cells. Cux-1 CIAV was used to infect MSB-1 cells for

the indirect fluorescent antibody (IFA) test as described by McNulty et al[9]. The same strain has recently been obtained with permission of the Argentinean Ministerio de Economía y Obras y Servicios Púbicos, Secretaría de Agricultura, Ganadería y Pesca (SENASA) from DAAAM. Two virus isolates were obtained at Universidad Nacional de La Plate (UNLP) from 2- to 3-week-old broiler flocks with clinical signs and/or lesions suggestive of CIA, anemia, poor flock performance, and gangrenous dermatitis (blue wing).

Livers obtained from these flocks were homogenized, disrupted by 2 freeze/thaw cycles and clarified by low-speed centrifugation. The supernatant fluid was extracted with chloroform and part of it was filtered through 50 nm filters. One-day-old SPF chicks were inoculated intramuscularly with 0.2 ml of the supernatant fluids into the leg and breast muscle. Uninoculated controls were kept separately or in the same isolator as the infected chicks (contact controls). Blood samples were collected at 12 and 14 days post infection (DPI) to determine hematocrit values and to collect serum. Chicks were examined for CIA lesions and livers were collected to prepare inocula for future virus passage *in vivo* and for inoculation of MSB-1 cells. Small portions of liver, spleen, and thymus were prepared for cryostat sections for the detection of viral antigens by immunofluorescence. Livers, spleens, thymuses, bursas of Fabricius, femurs, and kidneys were collected for histopathologic examination.

MSB-1 cells. MSB-1 cells were obtained by DAAAM at passage 96 from Dr. R.L. Witter (USDA Regional Poultry Research Laboratory, East Lansing, MI) and by UNLP at passage 148 from DAAAM. Cells were cultured in LM medium[2] with 10% fetal bovine serum at 41 C in 5% CO_2.

Monoclonal antibodies. Supernatant fluids from hybridoma 51.3 were used to detect the presence of CIAV-specific antigens. This hybridoma produces monoclonal antibodies (MAb) against the Cux-1 strain of CIAV. This MAb cross-reacts with at least 2 other strains of CIAV isolated in the USA[3].

FITC-conjugated antisera. Fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (RAM) and FITC-conjugated rabbit anti-chicken IgG were obtained from Zymed (San Francisco, CA). Both conjugates were used at dilutions that did not cause specific fluorescence with CAV-infected MSB-1 cells in the absence of CIAV-specific antibodies[8].

Fluorescent antibody assays. Indirect FA assays were used for the detection of a) CIAV in frozen sections of tissues obtained from experimentally infected and control chicks and b) antibodies in field sera and sera from experimentally-infected chicks. Cux-1-infected and control MSB-1 were used as positive and negative antigen preparations for the detection of antibodies as described by Lucio et al[7]. MAb 51.3 and RAM FITC were used to examine antigens for CIAV in frozen sections.

Histopathology. Tissues collected from field cases, inoculated, and control chickens were fixed in 10% buffered formalin, paraffin-embedded sectioned and stained with hematoxylin and eosin and examined by light microscopy.

Serology. Antibodies to CIAV were tested against Cux-1-infected and non-infected MSB-1 cells by IFA assays as described[7].

RESULTS

Virus isolation. Inoculation of 1-day-old chicks with chloroform-extracted materials from the 2 flocks resulted in the development of lesions associated with CIAV infection. Mild changes were observed with the inoculation of the 50 nm filtered material. Hematocrit values were often below 20% and occasionally as low as 7% to 12%. Macroscopic lesions consisted of marked thymus atrophy, yellow bone marrow, and anemia (pale muscles and visceral organs). The thymus and spleen tissues were characterized by an extensive depletion of lymphocytes. The major changes in the bone marrow consisted of atrophy and aplasia. Hematopoietic cells were replaced by adipose tissue. None of the uninoculated controls developed lesions and all had hematocrit values ranging from 30% to 37%.

Detection of CIAV antigens in tissues. Scattered positive cells were detected in frozen sections of tissues from infected birds after staining with the CIAV-specific MAb 51.3. Fluorescence was not observed in sections from infected chicks when PBS was used or in sections from uninoculated controls reacting with MAb 51.3 or PBS.

Serology. Sera collected from inoculated chicks from each chick isolation, in periods ranging from 12 to 18 DPI, were pooled and tested for antibodies to CIAV. Sera were negative for CIAV antibodies when tested at a 1:200 dilution by IFA test. Field sera samples belonging to the breeder flocks from where the broilers with clinical disease were obtained, were found positive in the IFA test.

DISCUSSION

This is the first report of the isolation and identification of CIAV in Argentina. The 2 isolates were obtained from different broiler flocks. These broiler flocks had problems suggestive of CIA, such as poor flock performance, poor weight gain, anemia, and mortality.

The identification of the virus strains as CIAV is based on: a) the lesions induced in inoculated chickens were compatible with those reported for CIA, b) these

lesions were also reproduced in chickens inoculated with materials treated with chloroform and passed through 50 nm filters, and c) the positive staining with MAb 51.3 of cells in the thymus and livers of experimentally inoculated birds demonstrated the presence of CIAV infection. The staining of these tissues was not caused by a reaction with the FITC conjugate alone[8].

The presence of antibodies in the breeder flocks that originated the broilers with anemia and in the flocks that presented Marek's Disease "vaccination failures" (C. Buscaglia, unpublished data), showed serological evidences of the disease. The possibility of aggravated field problems by other pathogens is not ruled out. For example, the combination of reovirus and CIAV causes the lesions of the "blue wing" disease[4,5,6].

The absence of CAV antibodies in sera from inoculated chicks was expected. Yuasa et al.[12] described that seroconversion in chicks inoculated at 1 day of age generally occurred only after 3 or more weeks post infection.

The results reported here present evidence of the presence of CIAV in Argentina. It will be important to determine the impact of its presence on the poultry industry and the distribution of CIA in the country.

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COMPARATIVE SUSCEPTIBILITY OF MSB-1 AND CU-147 LYMPHOBLASTOID CELL LINES TO CHICKEN INFECTIOUS ANEMIA VIRUS

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Diagnosis of chicken infectious anemia (CIA) is not easy. Clinical signs, gross and microscopic lesions, and observation of an immunosuppression syndrome (i.e., a pattern of bacterial, coccidial, or viral secondary infections) in a chicken flock are suggestive of CIA[2], but not enough for a definitive diagnosis[5]. Several techniques for detection of viral DNA (Soiné et al., in press), particles[4], or antigen[7] in chicken tissues have been described, and are without doubt very helpful in reaching a definitive diagnosis. Little or no progress on virus isolation has been made since the original report by Goryo et al. in 1985[6].

Isolation of the virus is desirable to confirm a diagnosis, and to study the characteristics of the etiological agent. Unfortunately, isolation of CIAV is not easy, requiring up to 10 passages in MSB-1 cells[2], a lymphoblastoid cell line derived from Marek's disease tumors[1]. In addition, some isolates do not readily replicate in MSB-1 cells (Lucio, unpublished data; Nordgreen and Odor, personal communication, 1992), and it is necessary to resort to multiplication of the virus in susceptible chickens.

MDCC-CU147 CU147[8] is a Marek's disease lymphoblastoid cell line derived from Marek's disease local tumors[3]. Whereas MSB-1 cells are of the CD4+ phenotype, CU147 cells were found to be CD8+.

Comparative susceptibility tests of the two cell lines showed that CU147 cells were markedly more susceptible than MSB-1 cells to Cux-1 ClAV infection, and that only CU147 cells were susceptible to CIA-1 virus. Implications will be discussed.

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TERMINATION OF SALMONELLA ENTERITIDIS SHEDDING AND CARRIAGE BY TREATMENT WITH ENROFLOXACIN FOLLOWED BY APPLICATION OF INTESTINAL MICROFLORA

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Considering the importance of the subject, we would like to take this opportunity to report on the successful termination of *Salmonella enteritidis* (SE) shedding and carriage in chickens achieved in laboratory as well as in field experiments. Extensive publications are being prepared.

In previous research the following had been established:

a) Treatment with different antibacterial drugs does not result in termination of salmonella carrier state (*S. infantis, S. typhimurium*, SE). After treatment with enrofloxacin (Baytril), which according to our research findings is the most effective drug, even in a double does during a 3 week period, *S. typhimurium* could be reisolated within 48 hours.

b) Preventive application of normal adult chicken intestinal microflora (intestinal homogenates of SPF birds in which selected flora was propagated in vivo, prepared and stored under strictly anaerobic conditions at the Poultry Health Institute) to newly hatched chicks induced a very significant colonization resistance against challenge with a virulent SE strain. However, used in infected birds (even after low infection doses of 10-100 colony forming units (CFU) per 1-7-week-old bird), the concept could only provoke a significant reduction in reisolation score, but never could end the carrier state nor the shedding of the agent. Administration of this microflora to older birds had no effect. On the other hand, in the new series of experiments, a combined treatment of Baytril followed twice by flora application resulted in total termination of SE carrier state, under laboratory as well as field conditions. The treatment comprised of an oral application of enrofloxacin (10 mg/kg body weight per day continuously through drinking water or administered in two doses) during 10 days followed by flora treatment at the second and the fifth day after finishing the enrofloxacin medications. Under laboratory conditions a single flora application at the second day after medications was not effective.

Under laboratory conditions, chicks housed on litter were orally inoculated on the 4th day of life with 10^5 CFU of a virulent and strongly colonizing S.e. strain (PT 4). During the 4 weeks preceding the treatment SE could be isolated from cloacal samples, caecal droppings, litter, caecum, liver, and spleen. Also the birds were serologically positive 10 days after infection (tested by ELISA monoclonal antibody sandwich method) by Dr. F.G. van Zijdeveld, Central Veterinary Institute, The Netherlands). Up to the end of the experiment (8 weeks after treatment) SE could be isolated from controls and from fecal and litter samples, but not from treated birds nor from environmental samples. At the end of the experiment, control and experimental birds were intramuscularly injected with methylcorticosteron and examined bacteriologically 4 days later. SE could be isolated from the controls but not from the treated birds, including those which were challenged orally with 4×10^4 CFU 4 weeks after treatment. In these experiments, the birds were housed in the same experimental rooms on on the same liter during the entire trial period.

In the field, 11 flocks with a total of 101,000 meat type parent birds were successfully treated with the above method. The birds were SE infected which was confirmed by culture and serologically. Three flocks received the total treatment at the reproduction farm, after they had been placed there while infected. Five flocks were medicated during 8 days at the rearing farm and after transfer to the reproduction farms 2 additional days of medication were applied, followed by the double flora treatment. Three flocks received the total treatment at the rearing farm.

These 11 flocks were housed at 11 different farms in 19 houses and were examined after treatment as follows: 1) at 4, 11, 18, 48 and 112 days after treatment 150 caecal droppings were examined per house, 2) at 18 days after treatment, 20 birds per house were examined 4 days after corticosterone injection (examination was performed on caecal content, liver, spleen, heart blood and ovary). So in total 14,250 fecal samples and 380 birds were examined bacteriologically, and 3) at 6, 10 and 16 weeks after treatment, 24 birds per house were examined serologically (total 1,368 birds). Four hatcheries incubating the eggs from the farms concerned were informed about the situation and fluff, egg shells, deads in shell and culled chicks were examined bacteriologically.

All the samples mentioned were completely salmonella negative after bacteriological examination and all sera became negative for SE 2 and 3 months after treatment.

After this period, all flocks were tested every 4 weeks bacteriologically (150 caecal choppings per house) and serologically (24 birds per house); the oldest during the next 5 months (in total 8 months) and the youngest during 2 months (in total 5 months).

From the 11 flocks included in this study, 9 remained negative during this period while 2 flocks which received the total treatment at the rearing farm became positive (bacteriologically) as well as serologically 4 months after treatment while housed in 2 different production farms.

It is our opinion that this concept offers the possibility to terminate salmonella flock infections (including SE) and to induce a solid colonization resistance in older birds. SE challenges in the mentioned field flocks will show how much longer this protective effect will last than the 4 weeks established in the mentioned challenge experiments to date.

We think that the above findings can be explained with the favorable properties of enrofloxacin as follows: 1) very high antimicrobial activity at low dose, 2) good resorption from the intestinal tract and effective tissue distribution, 3) accumulation in macrophages, 4) ipophilic properties enhances accumulation in a.o. yolk follicles, 5) high concentration of the active and very stable product in the feces, and 6) no effect on (obligate) anaerobes.
These result in a very effective treatment of the birds and the contaminated environment. The medication also creates a fresh seed-bed for the applied flora, which induces a very solid resistance against a possible reinfection from the environment or birds exterior (feathers).

LIPID-CONJUGATED ISCOM VACCINE FOR SALMONELLA

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The development of vaccines to prevent human and poultry diseases has been a major accomplishment in the field of immunology. Some diseases have been eliminated through the use of vaccines. However, while existing vaccines have certainly diminished the incidence, morbidity, and mortality of a large number of infectious diseases, salmonellosis is still very difficult to prevent and control through vaccines in domestic animals. Many investigators have sought to prevent salmonellosis through vaccination. Safe and efficacious vaccines capable of inducing long-lasting immunity are still unavailable. Current vaccines consist of inactivated whole-cell preparations. The protection given by these inactivated whole cell vaccines is of short duration, and adverse effects of lipopolysaccharide are always seen. Bacterins and attenuated live cultures for use as vaccines in the prevention of avian salmonellosis have never been applied widely in field conditions. Currently used oil-emulsion bacterins have serious drawbacks, such as production of granulomatous lesions at the injection site.

Various surface components of bacteria have been used as vaccines. Interest in the development of an effective vaccine has led researchers to explore the possibility of using outer-membrane proteins (OMPs) from gram-negative bacteria in a potential vaccine. OMPs of salmonellae have been shown to afford protection in animal models. Reports indicate that proteins from salmonellae have offered protection against experimental infection. Protein antigens have been shown to produce antibodies of higher affinity and induce cell mediated immunity that can result in long lasting protection against bacterial infection. However these proteins need to be immunopotentiated to be highly effective.

Adjuvants, such as aluminum hydroxide, aluminum phosphate, and water-in-oil emulsions localize the antigen at the site of injection. This localized antigen induces an inflammatory response and attracts immunocompetent cells. The humoral arm of the immune system is primed and activated. Phospholipid vesicles (liposomes) have been shown to enhance the antibody response to protein antigens. Macrophages can efficiently internalize and degrade liposomes and play a major role in the immune response towards a liposome associated antigen.

Increased immunogenicity of subunit vaccines has been achieved by incorporating them in immunostimulating complexes (ISCOMs). ISCOMs are stable molecular structures in which protein or polypeptide antigens are incorporated into a matrix composed of Quil-A, an

adjuvant glycoside. ISCOMs have proven to be an effective adjuvant system having many advantages over conventional adjuvants. ISCOM vaccines have determinants for both humoral and cellular immunity and hence afford better protective immunity. Considerable humoral, delayed-type hypersensitivity, and cytotoxic T-cell immune reactions have been shown to be produced against antigens when they were incorporated into the ISCOM structure. ISCOM vaccines have been used widely in a majority of viral infections and have been found to be highly effective in affording protection to animals.

In this study, outer-membrane proteins (OMPs) from *Salmonella heidelberg* were incorporated in lipid-conjugated ISCOMs and examined for their use in a vaccine for turkeys against homologous and heterologous *Salmonella* challenge. Two types of lipid-conjugated ISCOMs were examined: ISCOM-phospholipid and ISCOM-sphingolipid preparations. The turkeys were challenged with one of three *Salmonella* serotypes: *S. heidelberg*, *S. reading*, or *S. enteritidis*. The turkeys were monitored for clinical signs, shedding pattern post-challenge, and clearance of the challenge *Salmonella* from selected internal organs. The vaccine containing OMP with either of the lipid-conjugated ISCOM preparations produced significantly greater ($P < 0.01$) immune response than OMP alone. Cloacal swabs of birds given OMP+ISCOM-phospholipid and challenged with a homologous serotype were completely negative for *Salmonella*. A certain degree of cross-protection against heterologous *Salmonella* was observed in birds receiving either OMP-ISCOM vaccines. Isolation of *Salmonella* from internal organs was significantly lower ($P < 0.0001$) in vaccinated turkeys than in unvaccinated controls.

DETECTION OF SALMONELLA SPECIES USING POLYMERASE CHAIN REACTION

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Salmonella species have been reported to cause septicemia, enteric fever, and gastroenteritis. Standard procedures of isolation and identification of *Salmonella* spp. using growth media are slow and often these methods fail to detect the presence of bacteria which may in fact be present. More sensitive and rapid methods are therefore required. In this study, we have used molecular DNA technology to detect *Salmonella* spp. We have constructed a recombinant pUC8 plasmid carrying a *Hin*dIII digested fragment of *Salmonella enteritidis*. When the recombinant plasmid was used as a probe in a dot blot hybridization, our plasmid (C7 probe) detected all *Salmonella* spp. samples that we have tested but did not hybridize with other enteric bacteria. From the DNA sequence of the cloned fragment, we made two synthetic primers: NK1 and NK2. When used in polymerase chain reaction, NK1 and NK2 directed amplification of DNA of *Salmonella* spp. yielding an expected 2.1 kbp product, but did not amplify DNA of other enteric bacteria.

Further, in our study we have utilized C7 clone as a DNA probe in Southern blot analysis. The C7 probe enabled us to differentiate *Salmonella enteritidis* isolates from other salmonellae and enteric bacteria used in this study.

EFFICACY OF SARAFLOXACIN FOR THE CONTROL OF MORTALITY ASSOCIATED WITH E. COLI INFECTIONS IN BROILER CHICKENS AND TURKEYS

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Sarafloxacin, a fluoroquinolone, was evaluated for the control of mortality associated with *Escherichia coli* in broilers and turkeys.

In both species, dose titration studies were conducted in which a pathogenic serotype of *E. coli* (O1, O2, or O78) was injected into the breast muscle and treatment immediately commenced. Based on a reduction of mortality associated with *E. coli*, the doses of 20 ppm for broilers and 30 ppm for turkeys for 5 consecutive days in the drinking water were selected.

Four replicated-pen field trials, with a total of 4,200 male and female, 4- to 8-week-old turkeys, were conducted comparing 30 ppm sarafloxacin to a nonmedicated control on spontaneous outbreaks of colibacillosis. Fifteen-day mortality was 4.2% in the sarafloxacin-treated group compared to 10.0% in the nonmedicated controls. In broilers, 6 replicated-pen field trials with spontaneous outbreaks were conducted to evaluate the 20 ppm dose. These studies involved 18,000 straight run broilers, 1 - 5 weeks of age. Fifteen-day mortality was 2.0% in the sarafloxacin-treated group compared to 5.5% in the nonmedicated controls.

Additional studies were conducted at commercial production units. In broilers, 3 flocks (62,500 birds), approximately 4 weeks of age, experienced an outbreak of colibacillosis and were treated with sarafloxacin. Daily mortality dropped from 0.25% 24 hours prior to medication to 0.07% at the end of the 5 days of medication. Seven turkey flocks (100,000 birds), ranging from 3 - 10 weeks of age, experienced a colibacillosis outbreak and were treated with sarafloxacin. Daily mortality dropped from 0.29% 24 hours prior to medication to 0.06% at the end of the 5 days of medication.

RESURGENCE OF MYCOPLASMA GALLISEPTICUM WORLDWIDE

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Since the mid-1980's, there has been increasing serological evidence of more frequent incidence of *Mycoplasma gallisepticum* (MG) in poultry flocks around the world.

The early evidence came in the Middle East, and later in the UK in commercial egg layer flocks. Primary breeders had been clear for some considerable time, following intensive testing using the rapid plate method with slaughter of positive birds.

Layer flocks seroconverted often about 35 to 40 weeks of age. This has latterly become earlier in the production cycle, but has seldom been found in the rearing period.

Isolated broiler breeder flocks were identified as positive to MG and their offspring hatched and reared in isolation, possibly because of the size of the breeder flock. Offspring mortality was frequently higher than offspring of similar age breeder flocks. Seroconversion in commercial layer flocks often was not accompanied by detectable production changes.

These early cases were followed by suggestions of positive flocks in Italy, Spain, and Greece, but these were declared negative after diluting 1:5 or 1:10, so it was decided that misinterpretation of results existed in some cases.

Since that time, true positives have been found in Greece, Italy, Thailand, Malaysia, Hungary, UK and Nepal. These were positive to MG with no cross-reaction to *M. synoviae*, and which persisted after dilution and which latterly have been confirmed by positive results using ELISA technology.

In the UK, most of the cases have been in commercial layers and turkeys; broiler breeders and commercial layer breeders remaining negative. Elsewhere in the world, broiler breeders have been principally involved.

The percentage positive is often low to medium with something like 5% to 8% seropositive. However, cases as high as 75% in turkeys in the UK and 80% in broiler breeders in Nepal have been found.

In some cases it has been stated that offspring have been compromised in health, response to vaccination, and in growth rate, but in many cases, no detectable effect has been noticed.

Improved diagnostic techniques, more frequent monitoring, and better isolation techniques, coupled with accurate production effects are clearly becoming necessary, with action plans predetermined before the clinical situation becomes serious worldwide.

MYCOPLASMA GALLISEPTICUM INFECTION IN CHUKAR PARTRIDGES, PHEASANTS, AND PEACOCKS*

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Mycoplasma gallisepticum (MG) infection is commonly known as chronic respiratory disease of chickens and infectious sinusitis of turkeys. Clinical signs are usually slow to develop and the disease often takes a chronic course, with birds displaying poor physical condition and weight loss. In breeder flocks, there is a decrease in egg production. MG has also been isolated from natural infections in pheasants, chukar partridges, peafowl, bobwhite quail, and ducks[1].

MG was diagnosed in a backyard flock of 40-50 breeder chukars experiencing a marked drop in egg production with severe sinusitis, conjunctivitis, and eventually death. Nine 3-month-old offspring of these birds were submitted to the California Veterinary Diagnostic Laboratory System - Fresno branch with a 6-week history of watery eyes, swollen sinuses, and death due to emaciation and respiratory distress. Necropsy revealed moderate to severe conjunctivitis and caseous infraorbital sinusitis. Histopathological examination revealed moderate lymphoplasmacytic conjunctivitis and pleocellular sinusitis. Birds were seropositive for MG on the rapid plate and hemagglutination-inhibition tests, and MG was isolated from the sinuses.

The breeder chukar population consisted predominantly of adult birds of unknown origin collected at several field trials. Clinical signs first appeared 1 month into production (early May), and by the end of June all breeders had died or were culled. The pen was then disinfected and left vacant for 2 months. Approximately 50 offspring, while raised in an adjoining pen, became clinically affected at about 2.5 months of age. They were later moved to another pen to make room for newly purchased day-old chukars. These, too started showing clinical signs at about 2.5 months of age. All chukars were unresponsive to terramycin and only transiently responsive to Tylan (clinical signs returned in about 6 weeks in all groups).

Together with chukars, the house contained other species of game birds, including pheasants (125), peafowl (6), bobwhite quail (750), chicken (8), and mallard ducks (2). The house was divided into multiple pens separated by chicken wire, but all species had been in direct contact with the chukars. Of these species, only pheasants and peacocks displayed similar signs and were confirmed positive for MG serologically. However, unlike the chukars, the pheasants and peacocks responded favorably to Tylan.

*This case report will be submitted to Avian Diseases.

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FURTHER STUDIES ON LIVE MG VACCINE STRAIN 6/85

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INTRODUCTION

Mycoplasma gallisepticum (MG) infection continues to cause economic losses in the commercial layer industry in the U.S. Increased mortality, increased feed conversion ratios, decreases in egg production, and exacerbation of other respiratory agents are common sequelae of MG infection of layers.

DISCUSSION

Vaccination for MG remains an accepted method of decreasing losses from MG infection. Modified, live, and inactivated vaccines are utilized during the pullet rearing period to prepare the bird for the hen house challenge.

Inactivated bacterins have demonstrated their ability to decrease egg production losses in MG positive flocks. However, the bacterins are expensive and application by injection is costly as well as inaccurate at times.

Live vaccines have gained favor in recent years. The commercially licensed F-strain has shown to protect hens against airsacculitis and egg production losses from MG field strain. However, the F-strain does have some detrimental effects on the oviduct and is almost fully pathogenic to turkeys.

In January 1992, a new modified live strain of MG vaccine was licensed by the USDA. This 6/85 strain, as a vaccine, has shown excellent results protecting layers against clinical signs and egg drops from MG virulent field challenge. It is apathogenic for chickens and turkeys and is genetically stable[1,2].

Since the introduction of the MG 6/85 strain vaccine (Mycovac-Lr, Intervet Inc. Millsboro, Delaware) millions of doses have been applied to commercial layers throughout the U.S. Results have been positive in that protection is perceived to be equal to F-strain, but less damage to the oviduct and respiratory system has resulted in better livability, feed conversion, and egg shell quality in the Mycovac-L*r vaccinated chickens.

Mycovac-L. Since Mycovac-L*r does not cause chickens to seroconvert to the serum plate agglutination(SPA) test, the time of infection from the field challenge can be determined by a positive SPA test. The level of field challenge can then be monitored for the premise.Present commercial ELISA systems do not consistently detect antibodies to Mycovac-L*r. Since antibodies are not protective in MG infections, this is not an immunological issue but does pose a difficulty in serological detection of vaccination application efficacy. An effective way to monitor the effectiveness of Mycovac-L*r vaccination is to use the SPA test to detect the onset of field challenge and egg production data to compare performance of Mycovac-L*r vaccinated chickens to other programs.

Unlike F-strain, Mycovac-L*r does not perform well when administered by eye drop or coarse spray (Table 1). A fine spray (20 microns or less) is recommended as the preferred route of application. This allows quick colonization of the trachea which is important to the efficacy of the MG 6/85 strain.

There is concern in the turkey industry that live MG vaccine for use in chickens could accidentally infect a turkey flock causing economic losses. This has occurred with the F-strain[3]. A study was performed by an independent agency to determine the effects of Mycovac-L*r in turkeys. The results of this study demonstrate that Mg 6/85 vaccine is safe when administered to turkeys and that it does not cause positive MG titers as measured by SPA and HI. However, the MG 6/85 vaccine did not protect turkeys against airsacculities lesions after challenge with high doses of MG R strain.

SUMMARY

The MG 6/85 vaccine, Mycovac-L*r, is as effective as F-strain in preventing economic losses from MG field infection. However, its mild effect on the airsacs and oviduct allow an improvement in livability, feed conversion, and egg shell quality. The apathogenicity of Mycovac-L*r vaccine for turkeys decreases the concern of accidental contamination of commercial turkey flocks.

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Table 1. Airsac lesions[A] in chickens vaccinated with MG 6/85 vaccine by coarse and fine spray and challenged at 9 and 12 weeks post-vaccination with MG R strain.

	Average airsac lesion scores following challenge[B]:	
	9 weeks	12 weeks
Coarse	1.0	0.4
Fine	0.3	0.2

[A]0 = bright clearairsacs; 1= slight cloudiness; 2 = heavy cloudiness; 3 = heavy cloudiness or cheesy exudate or thickening; 4 = any combination of 3.
[B]Average lesion scores of 8 birds.

THE USE OF rDNA OLIGONUCLEOTIDE PROBES FOR DETECTION OF AVIAN MYCOPLASMAS

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INTRODUCTION

Hybridization with DNA probes has been proposed as a supplement to current diagnostic methods due to the high degree of specificity and ability to detect the etiological agent during early stages of infection. There is no dependence on the production of antibodies or influence of antigenic variation, and secondary infections with other bacteria including mycoplasmas do not affect the reaction. DNA probes based on restriction fragments and produced by random cloning have been reported for *Mycoplasma gallisepticum* (MG), *M. synoviae* (MS), and *M. iowae* (MI) by various groups. *In vitro* amplification by the polymerase chain reaction (PCR) has also been described for these important pathogenic mycoplasmas.

DNA probes complementary to ribosomal RNA (rRNA) and rRNA genes, so-called rDNA probes, have properties which make them particularly useful for the detection and identification of microorganisms. Ribosomal RNA is ubiquitous in all life forms and present in up to 10^4 copies per cell in rapidly dividing bacteria. Ribosomal RNA from about 50 *Mycoplasma* species has been at lest partially sequences and the sequences have been deposited in the GenBank® data base. There are regions in the 168 rRNA molecule of different evolutionary variability which makes it possible to exploit sequence infor-

mation for studies of bacterial taxonomy and evolution[6]. Sequence homology has shown that the mycoplasmas cluster in 5 phylogenetic groups, and the relationship between different species can be estimated[5].

MATERIALS AND METHODS

Design and construction of rDNA oligonucleotide probe: Oligonucleotide probes used in this study were selected to be complementary to the evolutionarily variable V8 region of 16S ribosomal RNA of the relevant *Mycoplasma* species, as described elsewhere[1]. The 16S rRNA sequence of MG, MS, and MI were compared with the relevant sequence of each other, and of other avian or phylogenetically related mycoplasmas by computer-assisted sequence alignment. The oligonucleotides were synthesized by the phosphoramidite method and end-labeled with ^{32}P using T4 polynucleotide kinase.

Filter hybridization: Hybridization was carried out with the rRNA of the target organisms, of which many copies are present, in contrast to single-copy genomic sequences. Samples from different mycoplasma broth cultures were applied to nitrocellulose membranes using a dot-blot filtration manifold, without prior purification of nucleic acid[2]. Ribosomal RNA was made accessible for hybridization by heating the filter in a boiling solution of SSC/SDS. The amount of mycoplasma rRNA accessible to the probes was checked by hybridization with the mycoplasma group specific H900 probe which reacts with conserved regions of the 23S rRNA molecule. This method is more relevant than direct estimates of the number of viable cells applied to the filter, especially in clinical samples, since the actual number of ribosomes in the mycoplasma cells, the binding efficiency of nucleic acids, and the efficiency of lysis in making the sites available for hybridization are taken into account. Hybridization conditions were described by Johansson et al[3].

RESULTS

The specificities of the probes was determined by filter hybridization with each of the other species as well as *M.meleagridis*, *M. gallinarum*, and *M. pirum* (a non-avian mycoplasma used for comparison). The detection level of the rDNA probe for the homologous mycoplasma was equivalent to about 3 X 10^4 mycoplasma cells applied to the filter, and there were no cross-reactions. The possibility of cross-reaction between MG and Ms was particularly tested, using many strains, in view of the similarity between these species with respect to biological properties and the possibility of mixed infections. Moreover, antigenic cross-reactions and shared gene sequences between these species are well-documented. The specificity of the rDNA oligonucleotide probes, as we found, was predicted since the phylogenetic distance between these species is fairly large, which finds expression in sequences of the variable regions of the 16S rRNA.

Particular emphasis was placed on studying the reactivity of the MG probe with a wide spectrum of MG strains in view of the intraspecies heterogeneity of this mycoplasma. MG strains tested included the type strain F631, widely used laboratory strains; F-vaccine; 3 variant strains from the USA; 8 field isolates from Sweden and 4 from Israel, all gave strong hybridization signals with the homologous rDNA probe. These data suggest that the VS region of the 16S rRNA gene is highly conserved within the MG species.

DISCUSSION

The selection of rDNA oligonucleotide probes for different organisms in the same region of the gene coding for the 16S ribosomal RNA provides a handy tool for specific identification of the product resulting from *in vitro* amplification of this gene by PCR. However, the oligonucleotide probes can also be used for direct and specific detection of rRNA of mycoplasmas in cultures or clinical samples. Direct hybridization may be the preferred method when the infecting organism is present in high enough titer to be detected without amplification, such as in the trachea during acute stages of infection[4]. In this case, increasing the number of birds sampled may be more effective in detecting infection than a very high degree of sensitivity in a limited number of samples. By using DNA probe hybridization, some of the pitfalls of *in vitro* amplification can be avoided, such as contamination by extraneous DNA and interference by components of the clinical sample (e.g. mucus). Cost-efficacy calculations should also be taken into account when evaluating the applicability of diagnostic tests under field conditions.

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COMMERCIAL DNA PROBE TEST KITS FOR MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SYNOVIAE: A FIELD REPORT

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Mycoplasma gallisepticum (MG) is an economically significant organisms and is the causative agent of one of the most challenging respiratory diseases in chicken and turkey flocks. The National Poultry Improvement Plan (NPIP) has published guidelines for the testing program aimed at eliminating MG from breeding flocks.

The most commonly used initial screening test for MG is the rapid serum plate agglutination test (SPA). The SPA, however, is prone to false positive reactions requiring subsequent confirming test protocols. Presumptive positive samples are usually tested using the ELISA or hemagglutination inhibition test (HI). The HI is more specific than the SPA, but traditionally shows wide variations due to lack of uniformity between antigen preparations and laboratory techniques. The ELISA is sensitive and specific, but can also be prone to non-specific reactor problems. Once a flock is presumed positive, traditional culture procedures are used to isolate the organism for confirmation. Culture can ultimately identify the organism, but has several disadvantages. First of all, the organism is relatively fastidious and slow growing. Therefore, NPIP requires a minimum wait of 21 days to confirm even a negative flock if SPA and HI or ELISA are positive. Secondly, organism growth may also be inhibited by damage during sample transport to the lab or by growth inhibitors in the media. Thirdly, overgrowth of competing organisms can cause additional time delays and expense due to the need to resample.

The global distribution of MG and *Mycoplasma synoviae* (MS) organisms and the difficulty in expeditiously confirming the presence of mycoplasma in a flock has prompted the development of two commercially available MG and MS DNA probe test kits. The USDA licensed kits are specific, sensitive, non-isotopic, and can be completed within 8 hours (2 to 3 hours hands-on time) for either a positive or negative result. In 1992, NPIP endorsed the PCR based DNA probe for MG to confirm the positive or negative status of the flock. Several case studies have been reported, including:

MG Field Study #1: The MG DNA probe incorporates two levels of specificity to MG. The test protocol is specific to the amplification of the DNA and the enzyme conjugated probe has been constructed with a second level of specificity to a DNA sequence unique to MG. Verified negative flocks were sampled and tested negative using the MG DNA probe. Other related *Mycoplasma* species have also been thoroughly tested, including:

Species	Results
Mycoplasma synoviae	Negative
Mycoplasma gallinaceum	Negative
Mycoplasma gallinarum	Negative
Mycoplasma meleagridis	Negative
Mycoplasma iowae	Negative
Mycoplasma pullorum	Negative

MG Study #2: Dr. Janet Bradbury from the University of Liverpool, Department of Veterinary Pathology, South Wirral, England tested cultures of MG strains and mycoplasma-like related strains for DNA probe confirmation. The cultures were from a variety of hosts and sampling sites (trachea, eye, cloaca, etc.). The MG DNA probe results correlated 100% with known positive and negative MG results.

MG Study #3: A commercial turkey flock exhibited clinical symptoms of swollen sinuses at day 65 while maintaining negative serological results for SPA and HI through day 75. The MG DNA probe confirmed 20/20 tracheal swabs positive for MG at day 75. Positive culture results were not obtained until day 90, partly due to overgrowth of earlier cultures. At day 90, only 2/10 sinus cultures and 0/10 tracheal cultures were positive for MG.

A new USDA licensed commercial DNA probe test kit for MS is now also available. The kit performance in the field is described below:

MS Study #1: The MS DNA probe incorporates two levels of specificity to MS. The test protocol is specific to the amplification of the DNA and the enzyme conjugated probe has been constructed with a second level of specificity to a DNA sequence unique to MS. Verified negative flocks were sampled and tested negative using the MS DNA probe. Other related *Mycoplasma* species have also been thoroughly tested, including:

Species	Results
Mycoplasma gallisepticum	Negative
Mycoplasma gallinaceum	Negative
Mycoplasma gallinarum	Negative
Mycoplasma meleagridis	Negative
Mycoplasma iowae	Negative
Mycoplasma pullorum	Negative

MS Study #2: A flock presented as somewhat symptomatic for MS. The SPA and HI test results were MS positive and culture was initiated. Samples were sent to the lab for MS DNA probe testing and all samples tested positive using the probe test kit.

MS Study #3: Tracheal swabs were collected from an experimentally infected field flock and tested in culture and with the MS DNA probe.

The results were as follows:

	# of Samples	Results
MS culture results:	1/30	MS Positive
	14/30	Overgrowth of non-MS organisms
	7/30	"Mycoplasma-like," FA negative
	8/30	MS Negative
MS DNA Probe:	30/30	MS Positive

SUMMARY

At the present time, the DNA probe promises to be a very cost effective replacement for culture with considerable time savings for both positive and negative samples when used as a confirmatory test for both MG and MS presumptive positive samples. Commercial DNA probes for both MG and MS can facilitate sample handling and transport, eliminate the inherent problems of culture overgrowth, save valuable time by producing same-day results, and provide clear, specific confirmatory answers.

COMPARISON OF POLYMERASE CHAIN REACTION AND ISOLATION PROCEDURES IN THE DIAGNOSIS OF MYCOPLASMA GALLISEPTICUM FROM CLINICAL SPECIMENS

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Thirty-five tissue samples, consisting of sinus or joint exudate from turkeys of clinical cases submitted to the California Veterinary Diagnostic Laboratory System - Fresno Branch, were collected between 5/89 and 9/92 and saved at -70 C. Mycoplasma cultures were performed on all samples at the time of submissions. *Mycoplasma gallisepticum* (MG) was isolated from 10 of the 35 cases by routine procedures and identified by indirect fluorescent antibody test[1]. Polymerase chain reaction (PCR) for detection of MG[2] was performed on all 35 frozen samples. All exudates that were positive for MG by culture were positive for MG by PCR and all samples that were negative for MG by culture were also negative by MG-PCR. Thus, MG-PCR appears to be a promising diagnostic tool on clinical specimens.

The advantages of the MG-PCR are: 1) high sensitivity and specificity, 2) rapidity of the test—total testing requires less than 8 hours, 3) ability to detect MG in long-term frozen samples—it is very difficult to recover MG from samples frozen for a long period of time, and

4) ability to detect small amounts of MG. Additionally, the cost is comparable to isolation procedures[2]. Furthermore, PCR can be used as a quick confirmation test on isolated colonies or tissues.

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DETECTION OF MYCOPLASMA SYNOVIAE BY POLYMERASE CHAIN REACTION*

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Recently, our laboratory has successfully developed a highly sensitive and specific procedure using the polymerase chain reaction (PCR) to detect *Mycoplasma gallisepticum*[1], *Mycoplasma meleagridis*[2], and *Mycoplasma iowae*[3]. Previous studies indicated that the PCR method was 1000 to 100,000 times more sensitive than dot blot hybridization[1,2,3]. The objective of the present study was to develop a species-specific polymerase chain reaction to detect *Mycoplasma synoviae* (MS).

A MS species-specific recombinant subclone, pMS156-20, of approximately 1.1 kbp was partially sequenced. Based on the sequence data, a pair of 25 base primers were synthesized to develop a MS polymerase chain reaction (MS-PCR). The primers amplified target DNA of approximately 1.1 kbp and had excellent specificity over a range of annealing temperature from 60 C to 68 C. The primers amplified 100 pg of 26 strains or isolates of MS, but not 3 strains of *M. gallisepticum* (S6, K810, A5969), 15 other avian mycoplasma species, nor pUC8 plasmid. The minimum amount of target DNA detected by MS-PCR was 10 fg, which was 100,000 times more sensitive than the dot blot methodology using MS recombinant DNA probes. The specificity of MS-PCR product detected by gel electrophoresis was confirmed by Southern blot hybridization using an internal probe derived from pMS156-20.

*This paper has been accepted for publication in Avian Pathology.

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MYCOPLASMA SYNOVIAE DIAGNOSIS USING THE POLYMERASE CHAIN REACTION TECHNIQUE

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A polymerase chain reaction (PCR) test for *Mycoplasma synoviae* (MS) was developed and evaluated as a diagnostic test. PCR primers were selected from the variable regions of the 16S rRNA sequence of MS. The MS PCR positively identified 55 individual isolates of MS in comparison to negative results for 44 individual isolates of 9 other species of avian mycoplasmas. Tracheal swabs from specific-pathogen-free chickens inoculated with MS tested positive, and *M. gallinarum* and noninoculated controls tested negative, respectively, for 3 weeks postinoculation (the duration of the study). During an MS epornitic involving broiler breeders, commercial layers, and backyard chickens, the PCR was compared to hemagglutination inhibition serology (positive = 1:80 titer) and to isolation and identification by culture. Analysis of field data showed a sensitivity of the PCR test (ability to identify MS infection) of 83% and a specificity (ability to correctly identify no infection) of 90%.

DIAGNOSIS OF MYCOPLASMA GALLISEPTICUM AND MYCOPLASMA SYNOVIAE USING DNA PROBE POLYMERASE CHAIN REACTION TEST KITS

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Mycoplasma gallisepticum (MG) and *Mycoplasma synoviae* (MS) are important respiratory tract and systemic pathogens of commercial poultry and are regarded as economically significant causes of disease impacting the efficiency of poultry production worldwide[3,7]. The most desirable strategy to reduce the adverse economic impact of MG and MS disease in commercial poultry is a surveillance, control, and eradication program as exemplified by the National Poultry Improvement Plan[1] (NPIP). A key to the success of this program is the ability to make a prompt, accurate, definitive diagnosis whenever MG or MS infections are suspected. The most commonly employed Gold Standard for the definitive diagnosis of MG and MS infections has been culture, isolation and identification of the organism. Recently, DNA probes and the polymerase chain reaction (PCR) have been developed for the detection of avian mycoplasmas[2,5]. This presentation describes our experience using commercially developed DNA test kits, along with serology and culture, to diagnose MG and MS in chickens and turkeys.

MATERIALS AND METHODS

Mycoplasma culture and identification. Frey's agar and broth media with 15% swine serum (FMS) were used for culture and isolation of mycoplasmas[4]. *Mycoplasma* spp. were identified by direct immunofluorescence[4,6] (IF) using fluorescein-conjugated rabbit antiserum provided by S. H. Kleven (Dept. of Avian Medicine, University of Georgia, Athens, Georgia).

POSTERS

CANDIDATE VACCINE STRAINS FOR THE PREVENTION OF COLIBACILLOSIS

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Escherichia coli infection in turkeys and chickens is observed in several forms, the most common being colibacillosis. This disease is characterized by septicemia, air sacculitis, pericarditis, and perihepatitis. Colibacillosis frequently develops as a secondary event subsequent to mycoplasma or viral infection or environmental stress.

The long term goal of our research is to develop a live attenuated vaccine that will be effective in the prevention of colibacillosis. Our initial work has concentrated on producing a vaccine that is effective for turkeys, but we believe that this vaccine will also be useful in other avian species.

To date, our efforts have concentrated on the investigation of the effect of deletions in the *carAB* operon and the *tolC* gene on virulence of the bacterium. Mutations in the *carAB* operon produce a requirement for arginine and uracil for growth while a mutation in the *tolC* gene renders the bacteria more sensitive to detergents and some antibiotics. We have produced these mutations in avian isolates of *E. coli* belonging to serogroups 01, 02, and 078.

To evaluate the relative virulence, day-old chicks were challenged by subcutaneous injection with the mutants and the wildtype isolates that they had been derived from. We observed a significant decrease in virulence of derivatives containing a mutation in the *carAB* operon. For example, when 100x the LD_{50} (for the wildtype) was given, no more than 50% of the chicks died in 7 days following challenge. In contrast, a mutation in the *tolC* gene resulted in no apparent loss of virulence when compared to the wildtype bacteria. The failure of the *tolC* mutation to decrease virulence was also observed when chicks were challenged by the intratracheal route. It appears that the *tolC* mutation is not attenuating and will therefore not be useful for vaccine strain production.

Research on *E. coli* infections in turkeys has been hampered by the lack of a good experimental model. We have developed a turkey model for colibacillosis that mimics the normal disease process. Seven-week-old turkey poults were exposed to an attenuated strain of haemorrhagic enteritis virus (HEV) followed 1 week later by *E. coli* given intratracheally. In the 7-day period following challenge with *E. coli*, we observed 75% mortality. No mortality was observed in the absence of attenuated HEV. In this model, not only were symptoms typical of colibacillosis observed, but synergy between the viral and bacterial infection also occurred.

Turkey poults were orally vaccinated at 4 weeks of age with either wildtype *E. coli* or the *carAB* mutant derived from it. Vaccination with either type of *E. coli* resulted in complete protection from infection with the homologous wildtype strain using the model described here.

FIELD APPLICATION AND COMPARISON OF LOCALLY PREPARED ALUMINUM HYDROXIDE AND COMMERCIAL INACTIVATED OILY ADJUVANT VACCINE AGAINST NEWCASTLE DISEASE IN BROILER

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INTRODUCTION

In Egypt, Newcastle disease (ND) is well established and widespread throughout the country, commonly occuring in an acute and subacute form. The control of ND is carried out by vaccination either with live or inactivated vaccines. The use of inactivated ND vaccine is considered an effective method of inducing immunity in chicks[1,3,9].

The use of aluminium hydroxide [AL(OH_3)] adsorbed vaccine has been reported[7]. The present studies were done to compare in the field the locally produced inactivated ND vaccine adsorbed in aluminium hydroxide gel with the commercial oily emulsion (OE) inactivated ND vaccine.

MATERIALS AND METHODS

In these studies, the following vaccines were used:

1. Locally produced inactivated ND virus in Al. hydroxide.
2. Commercial inactivated ND virus in OE.
3. Live attenuated commercially produced Hitchner B_1 and La Sota strains.
4. Local velogenic viscerotropic ND virus (VVNDV)[9].

Nine groups of Hubbard broiler 4-day-old chicks of 14,000 each were used. The chicks were reared on different farms of the General Poultry Organization. The first 2 groups had received the locally produced inactivated vaccine through subcutaneous (SC) injection with 0.3 ml for each chick (individual dose equal to $10^{8.6}$ EID_{50}) and simultaneously vaccinated with Hitchner B_1 via eye drop. The second group was boostered on the 28th day with Hitchner B_1 through drinking water.

The third and fourth groups received OE commercial vaccine instead of the local vaccine. The vaccination program and booster were exactly the same as group 1 and 2.

The 5th and 6th groups were vaccinated with locally produced inactivated vaccine by SC injection and simultaneously with HB_1 through dipping. The 6th group was boostered on the 28th day of age with HB_1 in drinking water.

The 7th and 8th groups received the same program of vaccination and booster but using the OE commercial vaccine instead of locally produced Al. hydroxide inactivated vaccine. The 9th group was used as vaccinated controls; the group had received the normal vaccination program for broilers specially used for poultry organization farms. This program include dipping at 4 days of age, followed by aerosol vaccination at 12 and 20 days of age. HB_1 was used in all the previous vaccinations. At 30 days of age, the chicks were vaccinated with La Sota NDV by spray.

RESULTS AND DISCUSSION

In this investigation, vaccination programs with different dead and live vaccines of NDV were evaluated by serological test, response to challenge with VVNDV and variations in the final body weight.

Effective vaccination of a flock having a varying passive immune status can be very difficult. Also administration of vaccine to chicks in the presence of significant maternal antibodies increase the rate of removal of antibodies and renders the chicks more responsive to a subsequent application of the vaccine[8]. These findings agreed with our results when using passively immunized chicks with antibody titers that ranged from $\log_2$ 4.2 to 5.3 at 1 day of age. These titers sharply decline 15 days post vaccination (PV) with inactivated and live attenuated vaccines. In all vaccinated groups, the minimum and maximum observed titers were $\log_2$ 1.3 to 2.6 in groups 7 and 4 respectively. Hemagglutination inhibition (HI) determination at the 30th and 42nd day PV and revaccination of groups 2, 4, 6, and 8 at the 28th day of age with HB_1 in drinking water, showed that there was a moderate increase in HI response at the 30th day, followed by significant increases in HI titers at the 42nd day; especial-

ly in the revaccinated groups which gave 5.8, 5.4, 5.7, and 5.3 $\log_2$ HI titer in groups 2, 4, 6, and 8 respectively.

Criteria for evaluation of challenge exposure responses include , morbidity , mortality and virus reisolation 6 days post challenge[10]. The minimal protection against VVNDV was observed 2 weeks post vaccination in all groups and ranged from 70% to 85% in groups 7 and 6 respectively. At 3 and 5 weeks PV, the protection percentage increased to acceptable levels of immunity in most of the challenged groups, with the exception of the 9th group which was used as the vaccinated controls. The protection percentages were 70%, 75%, and 80% at 2, 3, and 5 weeks PV. These results agree with an other study[4] that states, "if the time interval between the primary and secondary vaccination is less than 21 days , the antibodies produced by the first dose of vaccine is more likely to interfere with the multiplication of the second dose of virus."

The effect of the different vaccination programs on the final body weight was determined at 1, 14, and 28 days of age and just before slaughter. It revealed that the highest weights were obtained by birds of groups 1 and 2 which gave average weights of 1309 and 1313 gm at 45 days of age.

In summary the choice of vaccination program may be based on equating the degree of immunity necessary, costs involved and the exposure to local field virus both in terms of its virulence and prevalence. Also, vaccination with inactivated vaccines at an early age avoids the problem associated with the injection of inactivated vaccine which may cause residual tissue reactions, and consequently the carcasses might be downgraded and made unsuitable for human consumption.

The vaccination program of groups 1 and 2 which received local AL. hydroxide inactivated vaccine plus HB_1 by eye drop vaccination have the potential to induce higher HI titers and 100% protection due to the individual application of both vaccines either by SC or eye drop for inactivated and HB_1 respectively. This finding agreed with published findings[4]. Also, the higher gain of body weight was obtained by groups 1 and 2. So there is the distinct possibility of successfully using AL. hydroxide inactivated NDV vaccine for broiler vaccination program.

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SOME STUDIES ON THE VACCINAL STRAIN OF INFECTIOUS BURSAL DISEASE

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INTRODUCTION

Infectious bursal disease (IBD) is considered one of the most serious diseases in chickens. The disease causes severe economic losses, and birds that survive the infection may have reduced immune response to subsequent infections and attacks by other diseases[1,5,6].

The disease has been reported in Egypt[3], and control measures, including vaccination, should be done. This study points out the importance of preparing infectious bursal disease virus (IBDV) vaccines.

The aim of this project was to study the properties of IBDV in order to produce a local live attenuated or inactivated oil emulsion vaccine.

MATERIALS AND METHODS

The IBDV used in this study was American Bursavac M strain having a titer of 10^8 EID_{50}/ml.

Embryonated Chicken Eggs (ECE) 9 to 11 days old were obtained from General Poultry Company for virus propagation and titration.

For studying pathogenicity and different routes of inoculation, 60 ECE were divided into 2 equal groups. The first group was inoculated via allantoic cavity (AC) while the other via chorioallantoic membrane (CAM). The percentage of positive embryos[4,9,11,12] as well as the EID_{50}/ml were calculated after titration[2,15].

The effect of different virus dilutions from 10^1 to 10^4 was studied. Four equal groups (20 ECE) each were inoculated via AC. After 7 days incubation, the embryos were collected, homogenized[10] and titrated. For studying the best day of virus harvest, 60 ECE were inoculated with 10^3 dilutions of IBDV via AC. The eggs were collected at intervals of 24, 48, 72, 96, 120, 144, and 168 hours post inoculation, then chilled and titrated.

The keeping quality of the freeze-dried virus was examined at different temperatures. The lyophilized virus kept at room temperature was titrated weekly for 6 months, while the samples kept at refrigerator (+4 C) and deep freezer (-20 C) temperatures were titrated monthly for 12 months.

Heat stability of the virus fluid samples after 2 holding periods of 2 hours at 56 C was studied and the EID_{50} was determined.

RESULTS

Our results showed that the pathogenicity of IBDV to ECE was 90% and 75% when the virus was inoculated via AC and CAM respectively. Allantoic cavity was the best route of virus inoculation as it gave a titer of $10^{7.4}$/ml. EID_{50}, compared to $10^{6.7}$/ml EID_{50} for CAM inoculation.

The best virus dilution was 10^{-3}, as it gave a titer of $10^{7.75}$/ml when inoculated in ECE via AC. Three days post inoculation of ECE, high virus titers could be obtained; the EID_{50}/ml was $10^{7.5}$.

The results of keeping the quality of the freezedried live IBDV vaccine showed that the average loss of virus titer was 1.9 log. after 12 months storage in the refrigerator. After 6 months storage at room temperature, there was a 6.3 log. drop in the virus titer from the original virus, while there was no significant loss in the virus titer in samples preserved in the deep freezer (- 20 C).

IBDV is heat stable for 6 hours at 56 C which gave a titer of 10^8/EID_{50} and survived until 21 hours with a drop in titer to $10^{2.1}$/ml EID_{50}.

DISCUSSION

Some studies on the properties of IBD virus have been tried successfully by others[4,7,8,12]. In order to produce IBDV vaccine in the future the, our experimental and laboratory trials indicated satisfactory results.

It was found that the pathogenicity and best route of inoculation was the allantoic cavity, and this result disagreed with that obtained by Hitchner[8] who stated that the CAM was the route of choice.

The suitable time for virus harvest was 12 hours post inoculation. IBD virus was stable at 56 C for 6 hours. These results agreed with the previous finding observed by others[4,8,12].

A dilution of 10^3 was the best dilution for virus propagation. The keeping quality of vaccine stored at - 20 C for more than 12 months showed that there was no loss in virus titer, while those kept at room temperature for 6 months and + 4 C showed a reduction in virus titer equal to 6.3 and 1.9 log respectively.

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MORPHOMETRIC RELATIONS BURSA/SPLEEN IN INFECTIOUS BURSAL DISEASE (IBD)

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Macromorphometric observation can be used in evaluating the immune system[4], when IBD size and aspect of bursa is relevant. Bursal/body weight ratio has been used more for experimental evaluation; however, for field conditions, performing this technique requires the use of a precision scale. Since 1989 in South America, Botero and Uribe[1] observed, under field conditions, simultaneous presentation of bursal atrophy and splenomegaly in IBD-affected birds. Since then, people in Latin American areas have been using the relation Bursa/spleen sizes as an additional element in the diagnostic and evaluation of IBD. Bursal atrophy is well reported. The finding of splenomegaly has been mentioned in some reports[2], and denied in others[3]. Bursal atrophy and splenomegaly are not pathognomonic conditions. This present study evaluates how IBD affects the ratio bursa/spleen, under controlled challenge conditions, and its possible applications for diagnostic or evaluation purposes.

MATERIALS AND METHODS

Thirty SPF birds, divided in 3 groups of 10, caged in isolated conditions, were used for a challenge trial. The experimental design is showed in Table 1. At necropsy,

bursa and spleen diameters and weight, and body weight were recorded, tabulated, and used for statistical analysis. The average between width and length was used for spleen.

RESULTS

From Table 2, all columns proved the analysis of variance ($P<0.05$). All the used parameters differentiate between affected and nonaffected birds with significant differences ($P<0.05$). The spleen/body weight ratio have a highly significant difference ($P<0.01$), indicating the presentation of splenomegaly.

All affected birds at 7 days post-infection (P.I.) had bursas smaller than the spleen; all nonaffected birds had bursas bigger than the spleen (Figure 1). Using the bursa/spleen diameter ratio (B/SD) (Figure 2) $B/SD>1$ means that the bursa is bigger than the spleen; $B/SD<1$ means that bursa is smaller. B/SD differentiates affected vs. nonaffected birds with high levels of significance ($P<0.01$). From obtained data, it is possible to conclude:

- At 7 days P.I., there is evidence of splenomegaly.
- The combined effect of splenomegaly and bursal atrophy induces a big change in the B/SD.
- B/SD can be used in helping in the evaluation and diagnosis of IBD.
- Experimental data agree with field observation.

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Table 1.

Group & Group name ( )	Vaccine[a]	Challenge[b]	Necropsy
Non Vacc-Non Chall (NV-NCh)	-	-	42 days
Non Vacc.-Chall[c] (NV-Ch)	-	35 days	42 days
Vacc.-Chall (V-Ch)	21 days	35 days	42 days

a. IBD inactivated vaccine
b. Standard Challenge Virus
c. At days 3, 4, and 5 postchallenge, 1 bird was necropsied for verification of challenge efficacy.

Table 2.

Group	Bursa/body Wt. ratio	Spleen/body Wt. ratio	Bursa/spleen Wt. ratio	Bursa/spleen Diam. ratio
NV-NCh	$4.650^{a,A}$	$2.052^{a,A}$	$2.349^{a,A}$	$1.431^{a,A}$
NV-Ch	$2.797^{b,A}$	$3.011^{b,B}$	$0.946^{b,B}$	$0.730^{b,B}$
V-CH	$4.453^{a,A}$	$1.922^{a,A}$	$2.332^{a,A}$	$1.336^{a,A}$

Small letters in same column indicate significant difference ($P<0.05$), capital indicate high significant difference ($P<0.01$).

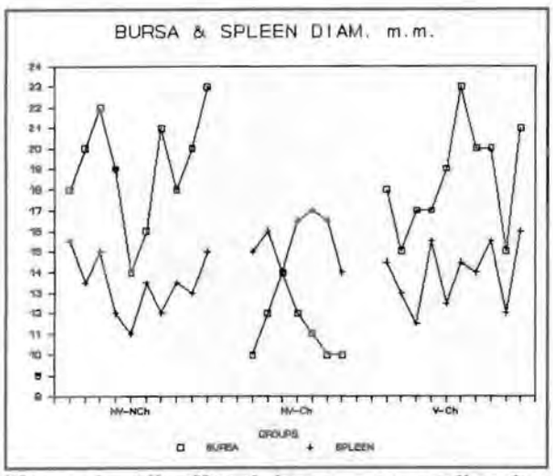

Figure 1. All affected bursas are smaller than spleen.

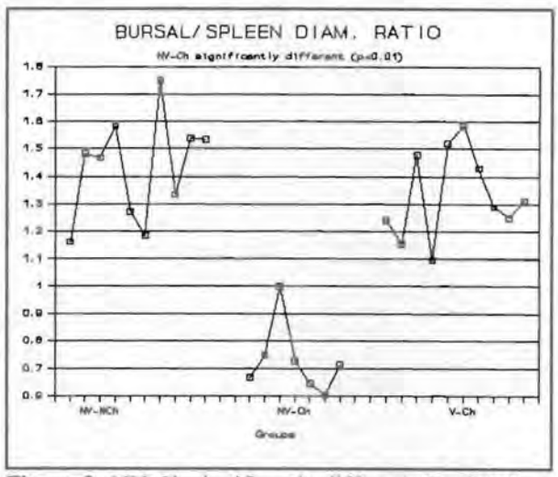

Figure 2. NV-Ch significantly different ($P < 0.01$).

ASCITES—CURRENT SITUATION

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An epidemiological survey of ascites and heart attacks was conducted at the beginning of 1992. Surveys were sent to all U.S. broiler production managers and health professionals. Responses represented over 75% of the industry.

An average ascites and heart attack mortality of 1.4% was reported with a range in the highest month of 1.9% and in the lowest month of 0.3%. Ascites mortality was highest in Arkansas and the West Coast (1.9%) and lowest in Delmarva and in Georgia and Alabama (both 1.2%).

Ascites was highest in January and February, with 73% and 68% of respondents listing these among the worst months respectively, and was reported to be lowest in June, July, August, and September by about half of the respondents.

Based on a 1 to 5 scale (5 highest), nutritional, genetic, environmental, management, and disease factors were related to ascites. Factors that averaged 3.0 or above were season, sex, average daily gain, breed, altitude, ventilation type, stocking density, respiratory disease, and chick problems.

Chick quality, chick source, down time, gas use, clean out between flocks, half-house brooding, brooder type, vaccination program, high early mortality, *E. coli*, leg problems, drinker type, diet, and feeding program ranked between 2.0 and 2.9. Factors with scores of 1.9 or below were incubator type, age of breeder flock, vaccination type, litter type, medication, and feed additive or anticoccidial. Comments given 3 or more times (of 84 responses) were:

Sex: higher in males, 47 (responses).

Breed: higher in Ross or Ross X, 27; higher in Cobb or Cobb X, 10; higher in Hubbard, 3.

Ventilation: higher with poor ventilation, 16; higher with natural ventilation/lower with power ventilation, 9; lower with positive pressure, 3; higher with negative pressure, 3.

Grower Rank: more ascites with higher rank, 18; less ascites with higher rank, 11; higher ascites with lower rank, 6.

Age of Breeder flock: higher from older flocks, 14 (specified as 40 to 60 weeks and older).

Vaccination: higher with spray vaccination, 8; higher with more virulent vaccines, 4; higher with greater reaction, 8.

Drinkers: higher with open drinkers, 11; lower with nipple drinkers, 7.

Diet: higher with high energy, 20; higher with high protein, 3.

Feeding Program: lower with lighting program, 5; lower with time feeding, 3; lower with restrictive feeding, 3.

Other: higher on hardwood litter, 5; higher with aspergillosis, 7; higher with pancake brooders, 8.

Ascites mortality was worst in smaller complexes (2.3% in companies under 400,000 birds weekly) and progressively lower in larger complexes (1.1% over 800,000 weekly). More ascites occurred as bird size increased. Birds under 1.8 kg (4.0 pounds) were reported to have mortality of 0.9% compared to 2.3% mortality for birds over 2.6 kg (5.7 pounds). No consistent relationship was found between ascites mortality and down time, frequency of clean out, stocking density or percent of flocks part-house brooded or raised sex separately.

Early growth restriction, achieved by light restriction, meal feeding or reduced energy and/or protein, was reported to reduce ascites on a large scale. Other important practices to control ascites included reduced stocking density, improved ventilation, control of respiratory disease and breed selection.

Because of the strong seasonal pattern of ascites, control measures are most important from mid-December through mid-March. Since many of the factors that contribute to ascites occur before 2 weeks of age, ascites control must be a part of an overall health, nutrition, and management program.

TOPOGRAPHIC ANATOMY OF THE CRANIAL BONES IN RELATION TO CNS DISEASES IN BIRDS

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The topographic organization of cranial bones in poultry plays a vital role in understanding the various components of the brain lodged in it. The avian brain differs to a great extent from the mammalian brain. Many avian diseases affect the central nervous system. Recently, Kuenzel and Blahser[5] identified GnRH neurons in the entire zone of brain in the chick. Volmerl and Firman[7] created lesions on the subfornical organ and organum vasculosum laminae terminalis to study the effect of water intake in broiler chicks by using stereotaxic equipment and also by placing electrodes. To undertake such experiments, topography of the cranial bones, their shape, and their structure is essential.

Hence, the present study was conducted to understand the shape of cranial bones and their topographic organization in relation to brain morphology.

MATERIALS AND METHODS

The heads of 30 adult and young (8 to 12 weeks of age) birds were procured from poultry farms and slaughter houses. Out of these, 10 adult heads were perfused with 10% formol-saline fixatives. Sagittal sections of the fixed skulls were macerated to obtain intact skulls and loose cranial bones.

RESULTS AND DISCUSSION

The bones of the cranium in poultry birds were separated at the time of and after hatching. The cranial bones formed a part of two large orbits, the hemispherical tympanic cavity on the sides, and a small cranium inside. Feduccia described that much of the brain case generally becomes fused in the adult and leaves little trace of the original structure[2]. Hofstad mentioned that avian encephalitis, Merek's disease, Newcastle disease, and also vitamin A and E deficiencies affect mid-brain and the cerebellum[3].

The parts of the brain that occupy the cranium were apparent in the sagittal section of the skull. The cerebral hemisphere and the olfactory lobes were lodged in the rostral cranial fossa, where they were formed, to a great extent, by the frontal bones. Each frontal bone was sickle shaped with wider caudal and narrower cranial ends (see Figure 1). The orbital surface was concave in both the directions and formed the medial wall of the orbit.

The medial margin articulated with the interorbital septum and about its middle formed an opening for the olfactory nerve.

The optic lobes and optic chiasma occupy the middle cranial fossa, which was formed by the petrous temporal bone (see Figure 1). The internal surface was divided into cranial and medial surfaces by a prominent petrosal crest (see Figure 1). The cranial division was smooth and deeply concave which lodged the optic lobes or rostral colliculus (see Figure 1). King[4] mentioned that the brain of fowl differs greatly from mammals. The cerebral cortex is poorly developed. The rostral colliculus has developed into optic lobes.

The cerebellum was lodged in the cranium, formed by parietals and occipital bones. The two parietals formed the caudal part of the cranium and were quadrilateral in shape (see Figure 1). The cranial surface was smooth and concave. Its caudal part was directed toward the caudal compartment of the cranium and was overlapped by the supraoccipital bone. The 2 divisions of the internal surface were separated by a median concave ridge. The medulla oblongata, which was lodged within the supraoccipital bone and connected with the spinal cord at foramen magnum, was chosen as a site for killing the bird in order to loosen the feathers for dry picking[1]. The occipital bone consisted of basioccipital, exoccipital, and supraoccipital (see Figure 1). The 2 halves of supraoccipital were joined on its median border, but Lucas and Stettenheim described it as a median supraoccipital bone[6]. Above the condyl was a large club-shaped foramen magnum by which the cranial cavity communicated with the vertebral canal. Ventrolateral to the condyl was present a hypoglossal foramen to transmit the hypoglossal nerve. Slightly below and lateral to the above foramen were 2 foramen: the medial, which transmitted the ninth and eleventh cranial nerves, and the lateral, which led to the jugular foramen and carotid canal. Subfornical organ and organum vasculosum laminae terminalis are the regions of the brain that border the ventricle. To create these lesions, topography of cranial bones is essential.

Thus, the topography of these cranial bones will provide a suitable site to approach the brain and its associated structures to conduct various research in terms of disease diagnosis and allied experiments.

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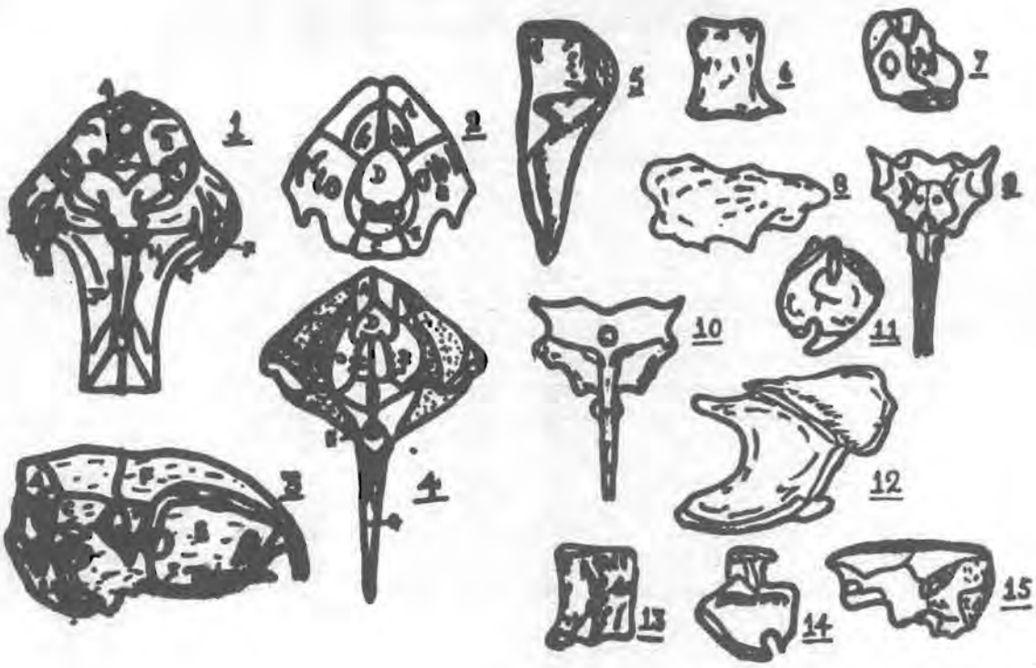

Figure 1. Legends: 1. Supraoccipital (A) Exoccipital (B) Basioccipital (C) squamous temporal (F,G) optic foramen (H) Olfactory foramen (J). 2. Supraoccipital (A) Exoccipital (B) Basioccipital (C) Foramen Magnum (D) Occipital condyle (E). 3. Supraoccipital (A) Exoccipital (B) Squamous temporal (C) Ethmoid (E) Frontal (F) Sphenoid (L). 4. Squamous occipital (A) Exoccipital (B) Basioccipital (C) pituitary fossa (E) petrous temporal (F) Body of sphenoid (G) petrosal crest (J). 5. Right frontal bone. 6. Right parietal. 7. Internal view petrous temporal bone. 8. Right squamous temporal. 9. Dorsal view of sphenoid. 10. Ventral view of sphenoid. 11. Right interorbital plate. 12. Ethmoid with horizontal and perpendicular plate. 13. Right parietal internal view. 14. Right post orbital plate. 15. Medial view of right squamous temporal.

IDENTIFICATION OF MAJOR IMMUNOGENIC PROTEINS OF AVIAN REOVIRUS USING MONOCLONAL AND POLYCLONAL ANTIBODIES[1]

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A simple and improved procedure of radioimmunoprecipitation (RIPA) for the identification of major immunogenic proteins of avian reovirus using murine monoclonal (MAbs) and chicken polyclonal antibodies is described. An indirect approach utilizing species-specific anti-IgM, IgG or antibodies followed by the precipitation of the immune complexes using Protein A, improved the sensitivity of the RIPA enabling the antigenic analysis of the major antigenic proteins of avian reovirus. The results indicated that this virus is more immunogenic in the

natural chicken host, as compared to BALB/c mice. A direct correlation between a strong neutralizing antibody response and an increased precipitation of the sigma (σ) proteins of the virus was observed. The results also demonstrated a strong association between the conformational viral epitopes of the 3 classes of proteins, i.e., large (λ), medium (μ), and small (σ).

The standard techniques of RIPA involve the utilization of bacterial proteins with natural nonspecific activity towards the Fc regions of immunoglobulins (Ig). The popular commercial reagents are *Staphylococcus aureus* protein A and *Streptococcus* species protein G. Both protein A and protein G have been reported to react with most mammalian Ig. However, it is known that both of these bacterial proteins possess faint reactivities or do not react at all with mouse IgM or chicken Ig. Also, these proteins fail to immunoprecipitate IgGs with low avidities towards the respective viral epitopes. To date, the only microorganisms which possess Fc receptors for Ig of chicken origin are those expressed on the surface of *Haemophilus somnus* and *Mycoplasma synoviae*. However, these reagents are not yet commercialized for laboratory use.

Due to these obvious reasons, direct addition of protein A to the antigen-antibody complexes do not prove useful in the RIPA when antibodies of either polyclonal chicken or murine monoclonal (IgM or IgG with low avidity) origins are used. However, inclusion of an additional incubation step, using anti-IgM, IgG, or chicken IgG reagent antibodies, followed by the precipitation of immune complexes by Protein A, enabled an appreciable immunoprecipitation of virus-specified polypeptides. Several interesting observations were made following the immunoprecipitation of avian reovirus lysates by this improved RIPA method. Distinctly clearer precipitation profiles, as indicated by intense bands representing σ proteins, especially that of σB[1], were noticed in the case of chicken antisera as compared to those obtained by mice antisera. This also correlated with higher viral neutralization titers obtained by the chicken antisera suggesting the putative roles of the σ proteins in the induction of neutralizing antibody titers in the host. On the other hand, the precipitation of λ, μ and the other σ polypeptides did not vary significantly between the antisera with higher and lower viral neutralization titers.

All MAbs reacted with the viral proteins of the 3 classes, λ, μ, and σ. A weak neutralizing MAb A4 was able to precipitate σC while none of the other nonneutralizaing IgM MAbs used in this study showed this reactivity. Although this prompted us to speculate that σC, the homologue of σ1 polypeptide of mammalian reovirus, may be the major antigen responsible for virus neutralization, the data obtained in this study are not substantial enough to prove it. It is hence essential to produce more potent neutralizing MAbs that can singly precipitate σC of avian reovirus. Whether the reactivities of most MAbs to multiple polypeptide bands are due to the close association between the reovirus polypeptides, as reported in the case of μ and s proteins of serotype 3 mammalian reovirus, is not clear at present. However, the specificity of the low avidity IgM MAbs A17, 626, 630, and 668 and neutralizing (IgG) MAb, A4, and finally the chicken antisera towards the reovirus was confirmed using the improved RIPA procedure.

[1]A full length article entitled "Radioimmunoprecipitation of avian reovirus polypeptides using virus-specific murine monoclonal (IgM and IgG) and chicken polyclonal antibodies" will be published in Journal of Virological Methods (1993).

POTENCY TESTING OF MAREK'S DISEASE VACCINES CONTAINING CEFTIOFUR SODIUM

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SUMMARY

This study was conducted to investigate the effect of ceftiofur sodium (NAXCEL®) on the protection given by Marek's Disease vaccines when included with the vaccine at a dose of 0.12 mg ceftiofur per day-old chicken. The vaccines used were HVT, SB-1, and HVT+SB-1 with a titer of 1000 pfu per 0.20 mL dose. Treatments included: negative control, positive control; HVT vaccine, HVT+ceftiofur; SB-1 vaccine, SB-1+ceftiofur; HVT+SB-1, and HVT+SB-1+ceftiofur. The presence of Marek's Disease lesions and mortality (calculated as percentages per treatment) were analyzed statistically. The results of this study indicate that the inclusion of 0.12 mg ceftiofur in a 1000 pfu dose of Marek's Disease vac-

cine (HVT, SB-1, or HVT+SB-1) per day-old chick had no negative effect on the protection given by the vaccine.

INTRODUCTION

Ceftiofur sodium (NAXCEL®), a broad-spectrum veterinary cephalosporin, has been found to be effective both in vitro and in vivo against *Escherichia coli* which is a primary infectious agent involved in terminal bacterial infections in young chickens. The approved use of ceftiofur in chicks is for subcutaneous injection in day-old birds for the control of colibacillosis. In the hatchery, under practical conditions of use, ceftiofur could be given concurrent with Marek's Disease vaccine. In vitro testing has been completed which indicates that ceftiofur has no apparent adverse effects on the vaccine potency. To confirm this observation, an in vivo study in the target species was conducted. The study was replicated over time (two replicates).

MATERIALS AND METHODS

Vaccine. Tri-Bio Laboratories' Marek's Disease vaccines were used as follows:
HVT (cell associated), titer 1000 pfu/dose; SB-1 (cell associated), titer 1000 pfu/dose;
HVT + SB-1 (cell associated), titer 500 pfu each serotype/dose.

Antibiotic. Ceftiofur sodium at a dosage level of 0.12 mg ceftiofur per chick.

Housing and Husbandry. Tri-Bio Laboratories test houses 3.05 x 3.85 m (10 x 12 ft) with individual heating and ventilation systems. Birds were placed on new wood shavings, fed a commercially prepared chicken feed (Purina, St. Louis, MO) and had water available *ad libitum*.

Chickens. One-day old, mixed sex specific pathogen and maternal antibody free chickens from Hy Vac Laboratory Eggs Co., Gowrie, IA.

Method of Vaccination and Challenge. The vaccine (with or without ceftiofur) was administered by subcutaneous injection on the neck region at the dose of 0.2 ml per chick. Virus challenge was done at five days post-vaccination using a virulent strain of Marek's Disease virus, Strain RB-1B (500 pfu/chick) in both replicates of the study.

Observations. All birds were observed twice daily for signs of depression. Dead birds were collected daily and necropsied to determine cause of death. Remaining birds were necropsied at the end of the 8 week observation period and examined for gross lesions of Marek's disease. The presence of a Marek's Disease lesion in any one organ specified was classified as a positive Marek's Disease case.

STATISTICAL ANALYSIS

The presence of Marek's Disease lesions and mortality (calculated as percentages per house) were the variables of interest. The percentages were transformed using the Freeman-Tukey double arcsin transformation to satisfy the assumptions of the analysis of variance.

RESULTS AND DISCUSSION

Mortality and lesions were less in all groups receiving ceftiofur with the vaccine than in groups receiving each respective vaccine without ceftiofur (Table 1). The group receiving the HVT+SB-1+ceftiofur had significantly fewer lesions ($P < 0.05$) than the group receiving the HVT+SB-1 vaccine; but none of the other comparisons were significant at $\alpha = 0.05$. The results of this study indicate that the inclusion of 0.12 mg ceftiofur in a 1000 pfu dose of Marek's Disease vaccine (HVT, SB-1, or HVT+SB-1) per day-old chick had no negative effect on the protection given by any of the vaccines.

Table 1. Mean Percentages and Least Squares Means of Freeman-Tukey Transformed Percentages of Presence of Marek's Lesions and Mortality

Treatment	Percent with Marek's Lesions		Percent Mortality	
	Percent	Transformed	Percent	Transformed
Negative Control	0.0	4.9	0.0	4.9
Positive Control	81.1	63.7	74.6	59.7
HVT	6.3	15.1	4.0	10.9
HVT/Ceft	5.0	14.1	0.0	4.5
SB-1	13.6	20.6	9.2	5.2
SB-1/Ceft	6.7	16.0	4.6	13.7
HVT/SB-1	12.9	21.6*	6.6	15.9
HVT/SB-1/Ceft	2.1	8.8	0.0	4.3

* Significant at $P < 0.05$

A NEW VIRAL DISEASE OF PIGEONS? PARTICLES RESEMBLING CIRCOVIRUS IN THE BURSA OF FABRICIUS

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Members of the newly formed family *Circoviridae* have been implicated in causing Psittacine Beak and Feather Disease (PBFD) in psittacines and infectious anemia in chickens (CAA). Porcine circovirus also belongs to this family. In this paper we will describe viral particles resembling circovirus in the bursa of Fabricius of 10 pigeons.

These pigeons range in age from 4 to 5 months and both male and female birds were involved. Histologically these birds had basophilic globules of various sizes, 5 to 25 μm in diameter distributed in the cytoplasm of the various bursal follicular cells. Some of the cells contained so many of these globules as well as large particles that they obscured the cell architecture. Electron microscopy of the bursa from some of the birds revealed large irregular electron dense gray areas in both the cytoplasm and nucleus. These electron dense areas contained viral particles which were icosahedral in morphology and

ranged in diameter from 15 to 18 nm. These particles were present either loosely arranged or arranged in paracrystalline array. Similar basophilic globular inclusions were seen in the mononuclear cells of spleen and cecal tonsils of a few pigeons. Similar inclusion bodies have been described in the bursa of Fabricius in chickens due to chicken anemia agent and in the bursa, thymus, and other organs of psittacines due to PBFD agent.

Most of the pigeons which had basophilic globular cells in the bursa also had concurrent infections such as salmonellosis, paramyxovirus-1 infection, pigeon herpes virus infection, hepatic and cerebral trichomoniasis, and adenovirus infection. The clinical histories of the birds submitted to the laboratory included diarrhea, neurological signs, loss of weight, listlessness, lethargy, etc. Most of the pigeons which had basophilic globular cells in the bursa also had various degrees of lymphoid depletion and cyst formation. It is, therefore, possible that this virus may cause immunosuppression in pigeons predisposing them to a variety of secondary bacterial, parasitic, fungal, and viral infections. The pathogenesis of this virus is not known.

Preliminary studies on the bursa of two pigeons were negative for PBFD antigen by using immunoperoxidase, DNA probe, and PCR techniques suggesting that this virus is not related to PBFD agent. Work is underway to see if this virus is related to the chicken anemia agent of chickens.

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SOME ASPECTS OF HYPERVITAMINOSIS A IN HENS

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INTRODUCTION

In chronically intoxicated animals, vitamin A toxicity signs include nervous disorders (weakness, hyperirritability, twitching, convulsions, paralysis), skeletal malformation (especially spontaneous fractures), liver dysfunction, and skin disorders. In general, non-ruminants appear to be able to tolerate vitamin A at levels of intake equivalent to about 10-fold their requirements[1]. For chickens, it was about 8-fold increase above their requirements[2]. As for hypervitaminosis A in adult hens, biochemical changes in their tissues were not studied yet.

MATERIALS AND METHODS

Rhode Island Red hens, 10-months-old, were used in the study and were kept in their individual cages. There were 10 hens in each experimental hen group. In the first and second experiments, 300,000 IU vitamin A/kg of diet were used during 90 days. Control diet was supplemented by 10,000 IU vitamin A/kg. In the third and fourth experiments, 250,000 IU vitamin A per hen a day were administered by gavage for 15 to 25 days.

Biochemical tissue characteristics were determined according to the methods described elsewhere[3].

RESULTS

The results of the first and second experiments indicated that a 30-fold increase of vitamin A in the hen's diet significantly decreased egg yolk carotenoids (from 14.41 to 4.11 mkg/g) and vitamin E (from 189.9 to 93.6 mkg/g) levels. At the end of experimental period, the vitamin E concentration in the hen's liver decreased as well (from 12.6 to 4.3 mkg/g).

Vitamin A was accumulated in the egg yolk (from 6.36 up to 49.6 mkg/g) and liver (from 1595.1 up to 15463.9 mkg/g).

Using a 30-fold increase of vitamin A in the hen's diet, it was impossible to find any hypervitaminosis A signs.

Vitamin A was more toxic in the third and fourth experiments. Using a 210-fold vitamin A dose, it was possible to observe clinical signs of hypervitaminosis A after 4 to 6 days of vitamin A feeding. It was observed that hens had either one or both eyes stuck closed, probably due to drying out of the crusty exudate when the eyes were closed during the period of inactivity. Forced opening of the eyelids several times a day made it possible for chicks to see and start eating. The eyelids and corners of the mouth were reddened.

Clinical signs of hypervitaminosis A in hens were similar to those described by others for broilers[2]: anorexia, conjunctivitis, and inflammatory lesions of the nares and mouth.

Egg production decreased very rapidly and after 2 weeks it terminated. The last egg was layed on day 13 of the experiment.

Egg yolk vitamin A concentration increased gradually from 4.7-5.4 mkg/g at the beginning of the experiment up to 9.7-12.9 mkg/g after 4 days of vitamin feeding, 35.6-37.9 mkg/g after 7 to 10 days of feeding, and 58.3-60.8 mkg/g at the end of the experiment.

Egg yolk carotenoids level decreased from 21.5-26.5 mkg/g at the beginning of the experiment to 11.8-12.4 mkg/g after 7 to 9 days of feeding, 6.6-6.8 mkg/g after 10 to 11 days of feeding, and 3.6-3.7 mkg/g at the end of the experiment.

Vitamin A accumulated in the liver. After 11 to 13 days of feeding, the liver vitamin A concentration was 5145.6-6750.0 mkg/g; after 15 days of the experiment, 10562.5 mkg/g and at the end of the experimental period, 12000 mkg/g.

In the other tissues, vitamin A concentration increased as well. At the end of the experiment, in the kidney it was about 632-836 mkg/g, in the spleen 30.8-60.3 mkg/g, in the heart 25.3-40.0 mkg/g, and in the brain, 6.0-13.3 mkg/g.

The hens fed 250,000 IU vitamin A per day had a significantly ($P < 0.001$) lower rate of liver mitochondria phosphorylation and ratio ADP/O. In the mitochondria isolated from the hen's livers of the experimental group, the succinate dehydrogenase and cytochrome oxidase activities decreased as well.

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GIARDIASIS IN A BUDGERIGAR

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INTRODUCTION

Giardiasis is caused by an intestinal protozoan parasite. It is the most common flagellate found in animals and birds[10]. A single species, *Giardia intestinalis*, is responsible for infecting a wide variety of animals including man[1]. Commercial poultry (chickens, turkeys, and game birds) are apparently resistant to clinical infection. The parasite does, however, cause serious health problems in budgerigar and cockatiel aviaries. Except in budgerigar fledglings, mortality is rare[7,9]. In California, approximately 50% of the budgerigars and cockatiels are believed to be infected with giardia[6]. Recovered birds may be carriers of the parasite and thus act as a source of infection to pen mates[7]. Transmission occurs directly via the fecal-oral route by ingestion of infective giardial cysts[5,6,7].

Although a single species causes disease in a number of different animals, zoonosis has not been clearly established[1].

The following report describes a case of giardiasis diagnosed by examination of the Gram stain of a swab of cloacal contents smeared on a glass microscope slide.

HISTORY AND CASE REPORT

A local veterinary practitioner, Dr. Kocher, examined a $\leq$ 6 month old budgerigar on September 22, 1992. The owner complained that the bird was sneezing, had a stained vent, and had perched abnormally since its purchase three months previously. Physical examination revealed the bird to be alert and active, but slightly fluffed. Mild tail bobbing was evident. The bird was slightly thin with a prominent keel. The feathers above

the cere were wet and matted. The droppings were of normal consistency. The abnormal perching was attributed to mild weakness.

Dr. Kocher submitted a glass microscope slide to the Animal Diagnostic Laboratory, Pennsylvania State University containing a smear of a cloacal swab from the budgerigar. A Gram stain was requested.

Examination of the stained slide revealed numerous Gram-negative, tennis racquet-shaped organisms. The organisms were identified as giardial trophozoites. A fresh dropping was requested. A direct smear of it was prepared in saline and examined*. Again giardial trophozoites were seen as well as an occasional cyst, thereby confirming the diagnosis of giardiasis.

The bird was treated with flagyl (metronidazole) 1.5 mg dose daily for 5 days, and 3 weeks later, the treatment was repeated. A follow-up microscopic examination of a dropping on November 12, 1992 failed to reveal any giardial trophozoites or cysts. On December 7, 1992, the owner reported that the bird was active and in good health. It was eating, had normal droppings and had stopped sneezing.

DISCUSSION AND SUMMARY

A diagnosis of giardiasis can be easily and quickly accomplished by microscopic examination of a Gram stain of fecal material (dropping, cloacal swab). The trophozoite stains pink and has a characteristic shape (piriform)[2,3]. The budgerigar responded favorably to treatment.

Other laboratory procedures for detecting giardia include the direct smear, flotation, and an enzyme-linked immunosorbent assay (ELISA)[5]. In the direct smear technique, a tiny amount of a fresh dropping is mixed with physiologic saline on a microscopic glass slide and examined for the rapid movement of the giardial trophozoite[1,7]. The motion looks like a leaf falling or a leaf tumbling in a stream[8]. (The dropping must not be refrigerated, frozen, or several hours old, or the motility of the trophozoites will not be observed[1]). Reducing the light intensity on the microscope aids in seeing the transparent trophozoite[1]. While adding a drop of 2% Lugol's iodine kills the trophozoite, the structural details of the trophozoite and cyst are made easier to see[1,9]. Nevertheless, giardial cysts are difficult to identify with this technique because of their lack of movement and small size[1]. If trophozoites are not identified in a direct smear, then further tests for cysts can be conducted.

A concentration procedure using a zinc sulfate flotation technique is the preferred method for detecting giardial cysts[1,4,5]. Other types of test solutions (sugar, salt) distort the cysts, making them unrecognizable[1,5]. Because cyst shedding occurs sporadically, several examinations for cysts should be made[1,6,7].

An ELISA (antigen detection) is available but does not offer any significant advantages over the current laboratory procedures used to detect giardia[1,4]. The ELISA is costly and technically difficult compared to other laboratory procedures used to identify giardial trophozoites and cysts[1].

A trichrome stain is recommended for studying the internal structures and detail of the giardial trophozoite and cyst[6,10].

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ISOLATION AND IDENTIFICATION OF AVIAN BRONCHITIS VIRUSES IN MEXICO—1992

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Between January and October 1992, 18 suggestive cases of infectious bronchitis (IB) were studied. Sixteen of them were from broiler farms and 2 from layers. The suggestive cases were from different parts of the country.

From the 18 cases, 10 were positive for coronavirus. Five of the isolates were shown by hemagglutination and hemagglutination inhibition tests to be vaccine strains of the Newcastle disease virus; they were neutralized by positive antisera against Newcastle disease virus and were negative for IB antisera.

Cross virus serum neutralization tests with Arkansas, Massachusetts, and Connecticut were made by the Alfa method (constant sera-diluted virus) in 9- to 11-day-old SPF chicken embryos. From these tests, 8 of the isolates were shown to be Massachusetts, 1 to be Arkansas, and one not determined.

ELISA ANTIBODY DETECTION AS A RELIABLE TOOL FOR THE DIAGNOSIS OF SALMONELLA ENTERITIDIS

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Salmonella enteritidis is an increasingly important pathogen of poultry. It has been isolated from broiler, breeder and commercial egg laying flocks. In addition to causing disease in poultry , *S. enteritidis* continues to be implicated in many cases of human food poisoning through the consumption of eggs, food containing eggs, or contaminated meat.

Monitoring flocks for *S. enteritidis* is typically accomplished via bacteriological or serological method. Disadvantages of bacteriological examination are that it is labor intensive and time consuming. Detection of the organism is further complicated because bacterial excretion can be intermittant and antibiotics in the sample can inhibit bacterial growth. Disadvantages of serological methods, such as agglutination tests, are a lack of sensitivity and a lack of specificity. For this reason, a highly specific and highly sensitive test which is suitable for high volume testing has been developed.

Sensitivity. The sensitivity of the test has been established by orally infecting known negative chickens at 2 days of age. Seroconversion of all birds was detected within 7 days of exposure to *S. enteritidis*.

Specificity. Specificity of the test is ensured by the choice of the antigen used. Unlike other serologial methods which mostly use lipopolysaccharides (LPS or somatic) as an antigen, this ELISA is based on flagellar antigen. The advantage over LPS is shown in Table 1.

As shown in Table 1, the somatic (or LPS) antigenic sites of *S. enteritidis* are present in salmonellae of both the B and the D group. The flagellar antigenic site (g,m) is only present in *S. enteritidis*.

In a trial with over 1100 breeder flocks where an ELISA with the same characteristics had been compared to culture, none of the flocks were missed and none of the flocks were falsely labelled as positive by the ELISA test. There were flocks which tested negative on culture and positive on ELISA , but further culturing of these flocks revealed the presence of *S. enteritidis*.

In summary, the ELISA should be considered for screening poultry flocks for the presence of *S. enteritidis*. When used in conjunction with culture as the confirmatory test, it should prove to be a reliable method to detect positive flocks in a large poultry population.

Table 1. The types of flagellar and somatic antigens of various salmonellae.

Serovar	Group	Antigens	
		Somatic	Flagellar
heidelberg	B	1, 4, 12	r
agona	B	1, 4, 12	f, g, s
derby	B	1, 4, 12	f, g
typhimurium	B	1, 4, 12	i
kingston	B	1, 4, 12, 27	g, s, t
gallinarum-pullorum	D	1, 9, 12	--
enteritidis	D	1, 9, 12	g, m
berta	D	1, 9, 12	f, g, t
panama	D	1, 9, 12	l, v

RODENT CONTROL IN POULTRY FACILITIES

Dick Rossow

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This slide set is intended to provide information in such a manner as to allow: 1) a high level of competency regarding the seriousness of rodent-related problems, and 2) an understanding of how to establish an effective rodent control program. It incorporates knowledge acquired through research activities conducted at the Purina Health Industries Research Unit at Gray Summit, Missouri as well as numerous field experiences. It also includes recommendations developed through extensive use of infrared videography which allowed careful observation of rodent activity in the most natural circumstances.