PROCEEDINGS OF THE FIFTY-SECOND WESTERN POULTRY DISEASE CONFERENCE

March 8-11, 2003. Sacramento, California

WPDC SPECIAL RECOGNITION AWARD FOR ROLAND C. HARTMAN

Roland Hartman, or Rollie as many of his friends knew him, is no stranger to the egg industry of California, the U.S., and to the world. His contributions to the advancement of this industry for more than 72 active years of his life are extensive and more than we could even hope to itemize. As book author, trade journal editor, and writer he has been instrumental in spreading the news about the latest innovations and research in this dynamic industry for over seven decades.

Rollie was born in 1906 in Milwaukee, Wisconsin and grew up raising chickens in his backyard purchased from his local Woolworth's store. In 1926, Rollie wrote his first poultry article - on cooperative egg marketing - which appeared in the January 1926 issue of the Poultry Tribune. He went on to the University of Wisconsin where he received his B.S.A. degree in 1929 with a major in Agricultural Journalism and a minor in Poultry Husbandry.

His entire career since his graduation has been devoted to poultry journalism - a wonderful career based upon his formal and informal education. He began as an associate editor for Poultry Tribune and Hatchery Tribune in 1929 to 1932. He then became editor of Everybody's Poultry Magazine (1932-1945) published in Hanover, Pennsylvania. This was a very popular monthly publication with thousands of subscribers ($.25 per year).

Not to be bored with only one job at a time, in 1939, he founded the Poultry Digest of which he was the editor in 1939 to 1942, 1946 to 1947, and finally in 1970 to 1979. He remained a contributing editor after his formal retirement all the way to 1999.

Californians got to know Rollie in his term as editor for the Pacific Poultryman from 1947 to 1969. This was the period of immense growth in the California Egg Industry and one that owed a lot to Rollie for his keen sense of news for new viable technology. He was personally recognized by practically everyone in the California industry because he was an "on-site" person. He traveled to hundreds of individual farms for interviews and kept an immense file of notes on every interview. He was widely known for his keen ability to present information in both print and in public meetings where he was often invited to speak.

In 1980 to 1993, Rollie served as the executive secretary for Inland Empire Poultrymen Inc, a Southern California egg producer organization representing about 45% of California's layer population.

Rollie gained international recognition as the author of several best selling books on raising poultry. These include: *Hatchery Management* and *Keeping Chickens in Cages*. This latter book sold some 16, 000 copies (in English) world wide (and another 8,000 copies in Spanish) and was widely recognized as the definitive book on the subject.

He's a member of the Poultry Science Association, the World's Poultry Science Association, the American Poultry Historical Society and the Council for Agricultural Science and Technology.

In 1976, Rollie received the prestigious "Man of the Year" award from the Pacific Egg and Poultry Association. This was followed in 1995 when he was elected to the American Poultry Historical Society's Poultry Hall of Fame. In 2000, Mr. Hartman was presented with Pacific Egg and Poultry Association's first "Lifetime Achievement" award.

SPECIAL ACKNOWLEDGMENTS

The Western Poultry Disease Conference (WPDC) is honored to acknowledge the many contributions to the Conference. The financial contributions provide support for outstanding participants and to help pay for some of the costs of the Conference. Almost 40 organizations, companies and individuals have given substantial financial support. Many companies and organizations, including some that also contribute financially, send speakers at no expense to the Conference. We thank all these people, and acknowledge their support and contribution.

We are extremely pleased to acknowledge two contributors at the Benefactor level. They are the American Association of Avian Pathologists and Merial Select, Inc. Once again, our distinguished Patrons, Donors, Sustaining Members, and Friends of the Conference are listed on the following pages. We greatly appreciate their generosity and say thanks to them and their representatives.

Dr. Dave Willoughby would like to express his thanks to the many people at the California Department of Food and Agriculture who supported him with this year's conference. In addition, he is indebted to Dr. Richard Chin and the other members of the WPDC Executive Committee for their efforts in planning the program and managing all the elements necessary for a successful conference. Many of the conference presenters made difficult schedule changes and travel arrangements to attend the conference. He is grateful for their efforts and the excellent quality of their presentations, as they are the people who ensure the high quality of the WPDC. Dr. Willoughby would also like to thank all the moderators and others who work behind the scenes to make events run smoothly. Finally, he would like to express his sincere gratitude to all the attendees, whom without their continued support, the WPDC could not sustain the high standards accomplished for 52 years.

Many have provided special services that contribute to the continued success of this conference. The WPDC would like to thank Helen Moriyama, Rebecca Gonzales and Courtney Hufnagle, of the Fresno branch of the California Animal Health and Food Safety Laboratory System, for their secretarial support. For this year's meeting, the WPDC has contracted Conference & Event Services, of the University of California, Davis, for providing registration and budgetary support for the conference, in particular Ms. Teresa Brown.

We thank Dr. David Frame for editing and producing another outstanding Proceedings of this meeting. Dr. Frame is indebted to Ms. Sherry Nielson, Senior Secretary of The Utah State University Turkey Research Center, for her diligent proofreading and technical help in formatting the Proceedings for publication. We express our gratitude to all authors who submitted manuscripts – especially those who followed the instructions and submitted their papers on time! We again acknowledge and thank Ominpress (Madison, WI) for the handling and printing of this year's Proceedings, and to Microsearch Corporation (Saugus, MA) for their creation of the CD-ROM for this year's meeting. Once again, we acknowledge Bruce Patrick (Graphic Communications, Brigham Young University) for the cover design, and to Dr. Rocio Crespo for designing the CD cover and label.

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The **Proceedings** of the 52nd Western Poultry Disease Conference are not refereed, but are presented as a service and a source of information to those attending the conference and to others who wish to gain some insight as to the information presented. Copies of the Proceedings are available in either hardcopy or electronic (CD) formats. Currently, we are only able to accept payments by cheque.

Copies of these Proceedings are available from:

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3rd WPDC – 1954	C. M. Hamilton	Kermit Schaaf		
4th WPDC – 1955	E. M. Dickinson	W. H. Armstrong		
5th WPDC – 1956	D. E. Stover	E. E. Jones		
6th WPDC – 1957	D. V. Zander	H. E. Adler		
7th WPDC – 1958	H. E. Adler	E. E. Jones		
8th WPDC – 1959	R. D. Conrad	L. G. Raggi		
9th WPDC – 1960	L. G. Raggi	A. S. Rosenwald		
10th WPDC – 1961	A. S. Rosenwald	D. V. Zander		
11th WPDC – 1962	D. V. Zander	R. V. Lewis		
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16th WPDC – 1967	D. S. Clark	Roscoe Balch		
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18th WPDC – 1969	R. McCapes	Dean C. Young		
19th WPDC – 1970	D. C. Young	W. J. Mathey		
4th Poultry Health Sym. (PHS)		**1st combined WPDC & PHS, 1st listing of distinguished members**		
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5th PHS				
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44th WPDC – 1995	H. A. Medina	David D. Frame	W. M. Dungan (posthumous)	A. J. DaMassa Gabriel Galvan Walter F. Hughes W. D. Woodward R. Yamamoto
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53rd WPDC – 2004	D. H. Willoughby	Joan Jeffrey		

MINUTES OF THE 51ST WPDC ANNUAL BUSINESS MEETING

Secretary-Treasurer Chin called the meeting to order on Tuesday, 2 May 2002, at 3:50 PM, at the Marriott CasaMagna Resort, Puerto Vallarta, Mexico. President Takeshita could not make the meeting due to a family emergency. There were approximately 15 people in attendance.

APPROVAL OF 50TH WPDC BUSINESS MEETING MINUTES

The minutes from the 50th WPDC business meeting were reviewed and a motion was carried to approve them as printed in the Proceedings of the 27th ANECA and 51st WPDC.

ANNOUNCEMENTS

Secretary-Treasurer Chin acknowledged all the contributors; in particular, those contributing at the Benefactor level, which included the American Association of Avian Pathologists, Embrex, Inc., Intervet and Merial Select, Inc. He also thanked all the contributors for their generous donations. Secretary-Treasurer Chin acknowledged the efforts of the current WPDC officers and ANECA officers who jointly organized the meeting.

REPORT OF THE SECRETARY-TREASURER

Dr. R. Chin presented the Secretary-Treasurer report. There were 300 registrants for the 50th WPDC held at University of California, Davis, March 24-26, 2001. Contributions for the 50th WPDC were $48,890, with a total income of $94,630. There were expenses of $89,734 for the meeting, resulting in a net gain of $4,896. The current balance in the WPDC account is $60,601.47. Due to the increased expenses of the joint meeting in Mexico, Dr. Chin estimates a net loss of about $12,500 for the 2002 meeting. A short discuss ensued on the increased expenses for the current meeting.

REPORT OF THE PROCEEDINGS EDITOR

Dr. D. Frame presented the Proceedings Editor report. Due to the joint meeting, the production of the Proceedings, both a hard copy and electronic versions, were quite complicated. Difficulty occurred in getting all the Mexican papers along with the Spanish translations of the summaries for the English papers. Nonetheless, only a few papers were printed without the English or Spanish summary. There were a total of 194 papers. As in the past, all papers were submitted by E-mail. Total expenses for preparing, printing and shipping the Proceedings were $19,568. There were 700 Proceeding books printed, each with 382 pages, at a cost of $10.40/copy for printing. In addition, there were 1200 CD-ROM's produced at a cost of $6.45/CD for production. Dr. Frame noted that the CD's were easier and less expensive to produce. There are still some problems with authors not following the directions, in particular, problems with formatting, length and number of tables.

OLD BUSINESS

There was no old business.

NEW BUSINESS

Secretary-Treasurer Chin reported that the WPDC Executive Committee nominated Dr. Joan Jeffrey for Program Chair-elect of the 53rd WPDC in 2004. This was seconded. A motion was made and seconded to close nominations. Dr. Jeffrey was elected unanimously as program chair-elect. Secretary-Treasurer Chin nominated the following officers for 2002-2003:

Program Chair: Dr. David Willoughby
President: Dr. Barbara Daft
Local Arrangement Coordinator: Dr. Carol Cardona and Dr. A.S. Rosenwald
Contributions Chair: Dr. Ken Takeshita
Proceedings Editor: Dr. David Frame
Secretary-Treasurer: Dr. Rich Chin
Program Chair-elect: Dr. Joan Jeffrey

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

It was announced that the 52nd WPDC, in Sacramento, on March 9-11, 2003.

The location of the 53rd WPDC was discussed. Dr. Chin suggested the meeting be in Sacramento, CA, at the Holiday Inn. Dr. Cardona commented that Dr. Rosenwald had requested that the meeting be held at the University of California, Davis. It was moved, seconded and approved to have the 53rd WPDC, in 2004, in Sacramento, CA, at the Capitol Plaza Holiday Inn.

Once again, there was a discussion on when the WPDC Proceedings should be produced as CD's and/or hard copies. At the previous meeting, it was approved that a CD version of the proceedings be produced every 5 years, in conjunction with the joint meeting with ANECA. The CD would contain the 4 previous years and that year's proceedings. However, at this year's meeting, there was a high demand for the Proceedings to be in electronic format. It was suggested that the book would contain only abstracts and the CD could contain the entire manuscript. However, this would require more work for the proceedings editor. After further discussion, it was approved to make the proceedings available in both print and electronic versions. (Note: A survey conducted after the meeting found that 2/3 of the respondents preferred to have the electronic version.)

Secretary-Treasurer Chin commented that the financial accounting and registration of this year's meeting was very difficult with Veterinary Medicine Public Programs (VMPP). There were numerous personnel changes and it was difficult to keep track of all the accounting. Dr. Chin will investigate whether or not to stay with VMPP or change to another registration company for next year's meeting.

Secretary-Treasurer Chin passed the presidency to Dr. Barbara Daft who thanked those involved in the organization of the meeting, in particular Drs. Ernesto Soto and Victor Mireles, and adjourned the meeting at 4:30 PM.

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LOW PATHOGENIC H6N2 AVIAN INFLUENZA IN CALIFORNIA

David M. Castellan

SUMMARY

During the second week of February, 2000 chickens were submitted to the California Animal Health and Food Safety Laboratory (CAHFS) from two separate veterinarians on behalf of a backyard chicken owner in Ventura County and a commercial egg producer from San Bernardino County. Low Pathogenic H6 N2 Avian Influenza Virus (AIV) was isolated from chickens at both premises, representing the first recorded cases of Low Pathogenic H6 N2 AIV associated with chickens from California. Subsequent laboratory analysis indicated both viruses were closely related with no epidemiological link, indicating that migratory waterfowl or water birds may have been the original source of the virus. The primary pathological effects involved the respiratory and digestive systems.

A positive case was defined as a premises with positive virus isolation of H6N2 AIV or one with positive serology for AIV by AGID. Eight of fifteen egg layer premises tested were positive for H6N2 AIV out of a total of 15 ranches. Epidemiological findings include a mild increase in mortality from 0-2% above expected values over a two week period with consideration for strain and stage of production and a decrease in egg production ranging from 0-30% over a two week period. Risk factors identified included movement of layers, equipment and people, proximity to live bird market or positive premises or the owner observed waterfowl or water birds on the premises during the preceding 90 days. The role of concurrent disease in accounting for mortality, morbidity and decrease egg production requires further study.

Veterinarians and egg producers began implementing more rigid biosecurity measures as a result of the incursion of Low Pathogenic H6N2 AIV. More notably, a vaccine pilot project was initiated on an egg production premises using a killed H6N2 vaccine with the support of the U.S.D.A. and the California Department of Food and Agriculture. It is foundational, that successful prevention and control of H6N2 AIV using vaccine depends on a coordinated flock health plan that stresses biosecurity.

During 2001, H6N2 AIV was detected on 6 occasions including the first isolate from Northern California. A second H6N2 AIV subtype was identified at several of these locations. Two additional egg layer premises were also enlisted in the vaccine pilot project during 2001.

In February of 2002, a new series of related outbreaks of H6 N2 AIV occurred in San Diego County. A pathological tropism for the reproductive system resulted in more severe egg production drops of up to 60% over a 5-day interval on some affected premises. By mid-March, the H6N2 AIV was disseminated to Northern California layer and meat bird premises (Figures 1 & 2). The case definition was expanded to include premises with a positive Directigen® test. Molecular interpretations indicated a common source for all H6N2 AIV isolates, however some heterogeneity became evident by 2001 and by 2000 with the identification of at least two and possible three subtypes related to genetic re-assortment. Thirty-seven commercial layer premises and 55 commercial meat bird flocks representing approximately 31 premises are considered incident cases in California thus far in 2002. Both externally and internally derived sources have been responsible for virus introduction onto premises previously negative for H6N2 AIV.

Both H6N2 and H6N8 killed AIV vaccines have been used in conjunction with heightened biosecurity, to reduce the prevalence of H6N2 AIV and associated production losses. Previously positive and high-risk premises are currently enlisted in the *H6N2 AIV Vaccine Pilot Project*. Over eight million doses have been administered to date, using an initial dual series protocol. Field challenge is being assessed through the judicious use of sentinel birds in vaccinated flocks. Data is being collected that will assess vaccine efficacy and biosecurity. Variables to be assessed include details of the vaccination process, characteristics of vaccinated and sentinel birds as well as laboratory results.

Ongoing cooperative efforts among all shareholders are being directed in the areas of communication, research, education and outreach using a science-based approach to support decision-making. Research is aimed at both broad as well as focused studies including case-control methods, field research during and following clinical outbreaks as well as experimental studies. Industry leaders are developing sound, market-based strategies to further prevent and control H6N2 AIV, including aggressive biosecurity measures and vaccination.

Figure 1. 2002 California H6N2 incident commercial layer premises (n = 37).

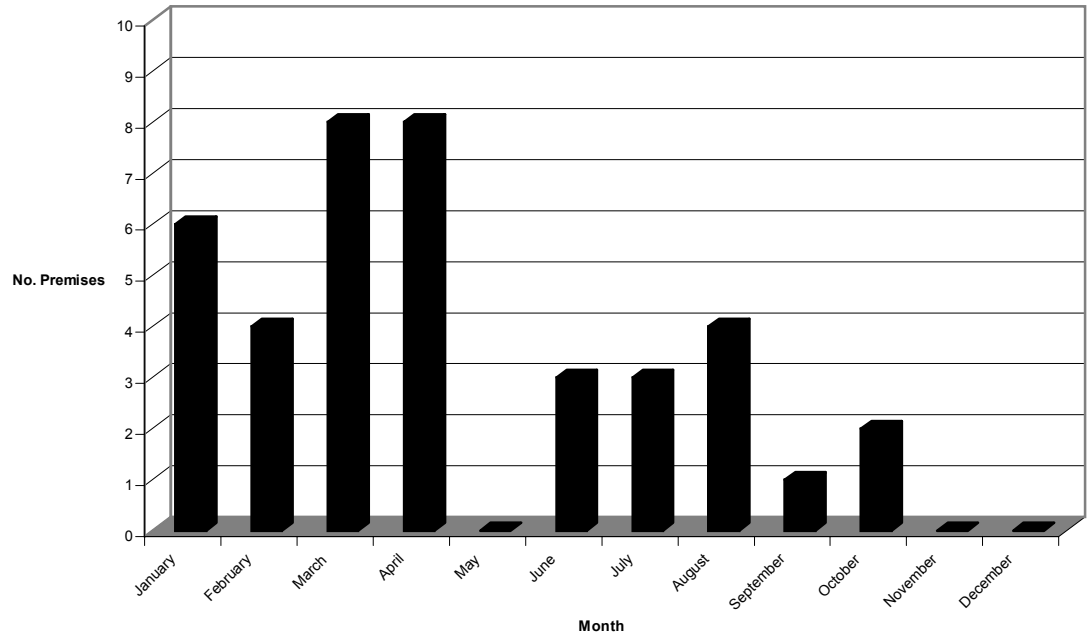
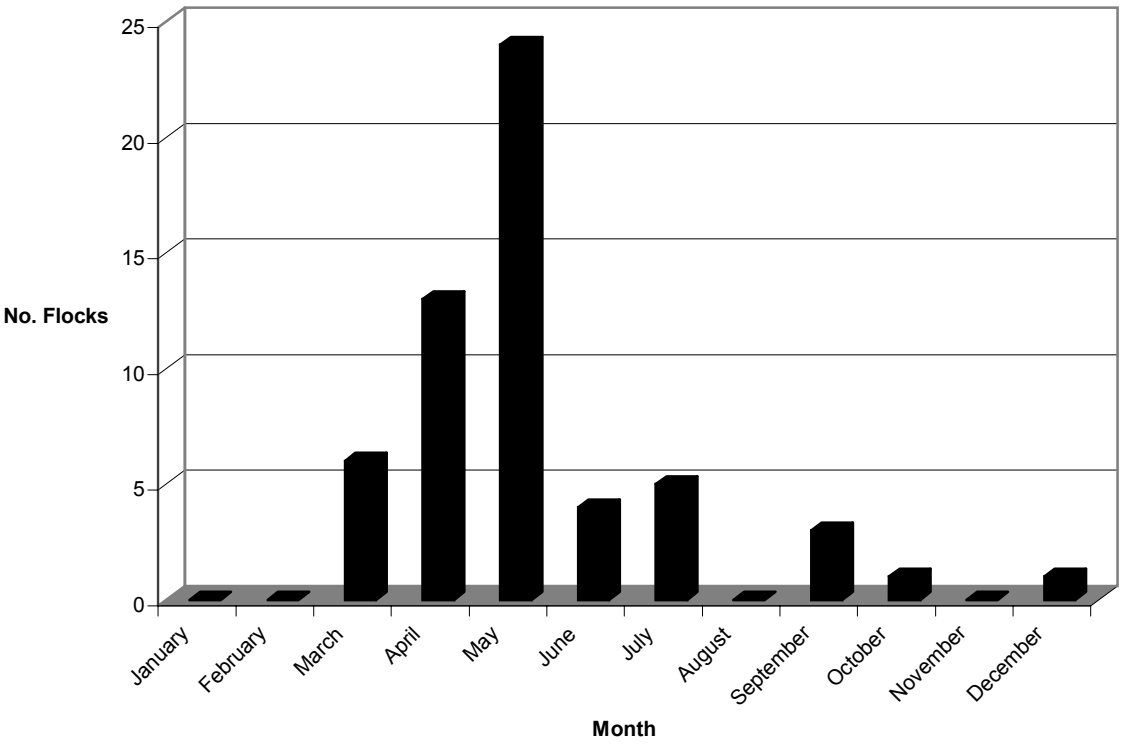


Figure 2. 2002 California H6N2 incident meat bird flocks (n = 55) on 31 premises.



CONTROL OF LOW PATHOGENIC AVIAN INFLUENZA IN VIRGINIA

M. A. Smeltzer

Regional Epidemiologist, USDA, APHIS, VS, Raleigh, NC

The commercial poultry industry of the Shenandoah Valley of Virginia is a highly concentrated industry of over 1000 poultry farms in a 3-4 county area. The industry in this area consists of both broiler and turkey production and their associated breeder flocks. In March of 2002, the initial case of avian influenza was identified in a turkey breeder flock. Laboratory serotyping identified this as a low pathogenic H7N2.

Highly Pathogenic Avian Influenza (HPAI) is a list A OIE reportable disease and subject to Federal action and response. In the past, all cases of HPAI have emerged from a LPAI serotype H5 or H7 that have circulated in a commercial industry for a period of time. This occurred in Pennsylvania in 1983, Mexico in 1995, and Italy in 2000.

Low Pathogenic Avian Influenza (LPAI) is under state authority for control programs. With increasing numbers of cases and limited resources the Commonwealth of Virginia requested assistance from USDA in dealing with this outbreak of LPAI H7N2.

In response to this request, a task force was formed to assist Virginia in this control effort. Programs were directed to prevent the spread to other poultry production areas, identify cases, and to eradicate H7N2 LPAI to preempt the emergence of HPAI. Authority for this program and depopulation orders were from the Commonwealth of Virginia.

In pursuit of these goals, the task force developed case criteria, surveillance methods, and epidemiologic investigations as well as assisting in depopulation and disposal protocols. By the end of June 2002, over 197 flocks had been identified, including meat turkeys, breeder turkeys, broilers, broiler breeders and table egg layers. A total of 4.7 million birds were affected by this outbreak.

Table 1. Types of poultry operations depopulated in the Shenandoah Valley.

	Breeders	Grow Out	Table Egg	Total
Chickens	29	13	2	44
Turkeys	26	127		153
Total	55	140	2	197

Laboratory tests available initially were the Agar Gel Immunodiffusion (AGID), a screening test for influenza A antibody and the Directigen® test, a rapid screening test for influenza A antigen. Confirmation by virus isolation was submitted to NVSL in Ames, Iowa. Prior to the outbreak the laboratory ran approximately 800 tests per week. It became apparent that the volume of testing needed would rapidly overwhelm the laboratory system. The task force assisted in personnel, equipment, and testing materials. Personnel and equipment were brought in to conduct RT-PCR testing at the regional laboratory in Harrisonburg for rapid confirmatory testing in the local area. It is essential that in large outbreaks confirmatory tests are readily and rapidly available for decision makers.

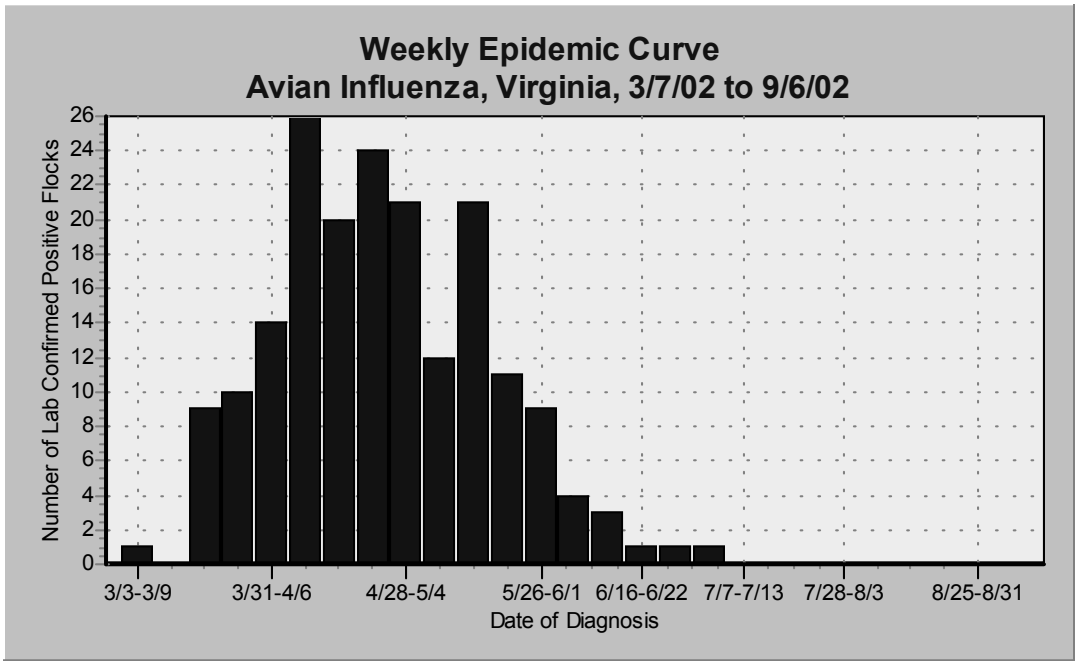
Initial cases were identified primarily from testing of birds with clinical symptoms and the associated area testing. Clinical symptoms were primarily respiratory signs such as snicking and sneezing. Drops in water and feed consumption were also noted. Breeder birds in addition to clinical symptoms showed egg production drops. High mortality was not associated with this LPAI in most cases. Turkeys showed the most significant respiratory lesions and were most often affected shortly after 10 weeks of age. Very few symptoms were noted in broilers.

Barrel (dead bird) surveillance became a main surveillance method at the end of April. On a specified day, each farm was tested weekly by this method. The grower would place ten birds of daily mortality from each house in plastic bags and place these in a barrel at the end of the farm drive way or entrance. Surveillance teams collected tracheal swabs from these birds for Directigen, PCR and virus isolation.

Pre-slaughter surveillance was also begun, requiring negative results on serology and tracheal swabs within 72 hours prior to movement off the farm to processing.

Molecular fingerprinting of this isolate confirms this as identical to the virus found in the Northeast the past 7 years. No direct ties however, were identified to definitively trace this to the Live Bird Markets of the

Northeast. Testing done on backyard flocks and local migratory waterfowl were negative for this serotype. Epidemiology studies conducted during this outbreak showed that growers using off farm mortality disposal were six times more likely to be positive. Rendering locations and drop off sites for daily mortality should be considered high risk areas. Much task force effort and education, as well as company programs, were directed to eliminating this point of spread. Use of only family members for farm labor showed indications of protective value. Airborne transmission was not believed to be involved. Movement of persons, vehicles and equipment appear to be the primary method of spread.



After the decision was made that positive flocks would be destroyed, carcass disposal became a primary issue. On farm burial was not permitted by environmental agencies. There was a three-week delay in local approval for landfill use to bury large numbers of birds. In-house composting was attempted on several flocks. With the numbers of birds, the size of birds and extended down time needed, in-house composting did not become a viable option in this outbreak. Incineration of the depopulated birds was also done for a period of time. Air curtain incinerators were brought to an isolated area to facilitate carcass disposal. These proved to be very expensive to operate, unable to keep up with the volume and resulting environmental (air and water) quality issues relating to smoke, ash and runoff became prohibitive. Landfill became the method of choice after arrangements were made with a county landfill, out of the area, able to handle the numbers of birds to be disposed of.

In conclusion, no evidence has been found to indicate this was introduced from local backyard flocks or migratory waterfowl. Off-farm mortality disposal methods such as rendering drop off sites need to have special consideration in regards to proper cleaning and disinfection of vehicles, persons, and containers prior to returning to farms. On-farm methods of dead bird disposal such as incineration and composting should be promoted and encouraged. Environmental agencies will play a major role in disposal decisions especially when emergencies are not officially declared. It is apparent that uniform national policies are needed to deal with LPAI. The availability or lack of indemnity will be essential as to how these programs develop and their ultimate success. This will be true whether dealing with large commercial companies or the smaller poultry system operations such as the LBM marketing system. State decisions in handling local animal disease issues such as controlled marketing, depopulation, and vaccination will be heavily influenced by national export policies.

THE ECONOMICS OF AVIAN INFLUENZA CONTROL

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ABSTRACT

When large numbers of flocks get low pathogenic avian influenza (AI), different types of control programs have different costs associated with them. Costs of several large outbreaks are shown and compared. The difference in AI outbreak costs per flock from the least to the most expensive is over 100-fold. A new model for controlling AI, incorporating the best features of different control measures, is proposed. This model would draw from poultry industry and government expertise to quickly, cooperatively and cost-effectively stop AI outbreaks. The advantages of the proposed program are that it requires no unethical destruction of healthy birds, requires no costly disposal, is cost effective and puts more of the costs of control on the producers with infected birds.

INTRODUCTION

It is well accepted that "stamping out" of highly pathogenic avian influenza (HPAI) is a suitable method for control and eradication of this deadly disease. Protection of a national industry and protection of an export market are the major reasons for destruction and disposal of HPAI infected poultry. The costs associated with destruction and disposal of infected flocks can be great. Often there are other costs of even greater magnitude including lost markets, down time, etc. In 1999-2000 an outbreak of H7N1 HPAI in Italy resulted in $112 million in compensation for destroyed birds, but it was estimated that indirect costs exceeded $400 million for a total cost of over $512 million.

Low pathogenic avian influenza (LPAI) control methods have been more variable. Avian influenza (AI) has been introduced into the U.S. poultry industry over 100 times in the last 25 years and usually few flocks are involved in each outbreak. In Minnesota, where most U.S. introductions of AI have been recorded, a program of monitoring, biosecurity and controlled marketing has been employed. In other areas the index flocks have been destroyed. The costs of the two approaches are not large when small numbers of flocks are involved. In Minnesota the costs of the disease may be $2 per bird (or more) in a market turkey flock with additional cost for delaying placement of the next flock. In states where destruction is practiced the cost is going to be the bird value, or in the case of birds in egg production it will be bird value plus the impact of lost production. Thus, costs of destroying a flock will range from $1 or $2 per bird for broilers to more than $100 per bird for turkey breeders. In the past 15 months, destruction of flocks or eggs due to LPAI was implemented over 10 times in the U.S. (Maine, Michigan, Connecticut, Pennsylvania, New York, North Carolina, Virginia, California, Texas, Ohio).

However, sometimes, large numbers of flocks are infected. There have been several notable outbreaks of LPAI that affected large numbers of poultry flocks: in 1978, 1988, 1991 and 1995 AI affected over 100 flocks of turkeys in Minnesota; in 1995 220 flocks of turkeys were affected in Utah; in 2000 to 2001 88 farms were affected in Italy; in 2000 to 2002 several flocks in California experienced AI; and in 2002 AI was found in Colorado and Virginia turkeys. The costs associated with those outbreaks are shown in Table 1. The difference in costs can be attributed to the species and type of birds infected, their age, flock size, as well as control methods employed, but most of the outbreaks involve turkeys.

CONTROL

With expanding worldwide interest in LPAI, it seems likely that there is room for a new approach that would replace the old paradigm of destroy and dispose. A new paradigm could incorporate the desirable attributes of all successful programs: it should be

effective; it should be rapid; it should be cost effective; and it should reward desired behavior.

Effectiveness. Actually all the LPAI control methods depend on biosecurity and have been shown to be effective in some situations and to be less than 100% effective in others. If applied correctly, all are demonstrably effective.

Speed. The Virginia 2002 LPAI outbreak, where destruction was employed, was controlled in 4 months. In the Utah 1995 LPAI outbreak, where vaccination & controlled marketing were employed, no new infected flocks were detected 6 weeks after vaccination was initiated. (Vaccine-induced antibody was present until the last vaccinated flock was marketed 10 weeks later.)

Cost effectiveness. According to the information in Table 1 the range in costs for LPAI and its control is from $4,000 per flock in the Italy 2001 outbreak where vaccination and controlled marketing were employed to $760,000 per flock in the Virginia 2002 outbreak where destruction was the primary control method.

Reward good behavior. One of the problems of some disease control programs is that the financial rewards go to those who fail to maintain healthy flocks. In stark contrast producers with healthy birds often find themselves caught in quarantine zones unable to move flocks, eggs or hatchlings. Because their birds are healthy they are ineligible for indemnity payments even though they suffer economic loss. This paradox sometimes causes extreme behavior by a producer who, in desperation, infects his birds to save his business. All LPAI control programs should be structured to reward producers for noninfected flocks.

A NEW PARADIGM

Economics of LPAI control require us to examine the attributes of all control methods to determine how they could be combined to develop an effective and economical control program. Rather than having a loose ad hoc program managed by industry veterinarians or a tight program managed by APHIS veterinarians a hybrid cooperative organization should be formed. Once LPAI is detected aggressive, well-thought-out measures should be initiated by a group of industry and government veterinarians.

Biosecurity. First, all off farm movement of dead birds and manure should be halted area wide, and all off farm movement of live birds or eggs should be controlled, as should movement of people and equipment.

Processing. A program of processing all virus negative (antibody positive or negative) meat birds of marketable age in the area should begin.

Scheduling. Placement schedules should be interrupted. No placement of chicks or poults should be allowed and downtime should be extended for LPAI infected premises.

Vaccination. The group should assess whether long-lived birds need to be vaccinated. Layer (and breeder) replacements should be vaccinated twice before being moved to the layer facility. Meat birds should be vaccinated if deemed to be at risk (if they are moving from brooder farm to infected grower farm for example). Vaccinated flocks are under quarantine. It is imperative, however that vaccine is available for emergency use. Thus vaccine banks should be established.

Area repopulation. After no new infected flocks are detected for four weeks the outbreak may be nearly over, controlled repopulation may begin. When all flocks are virus negative the outbreak is over but antibody positive flocks remain under quarantine.

Cost. The costs of this program would be borne by the affected individuals and companies with government providing diagnostic and logistical support. Companies and individuals with infected birds would experience more of the costs than their noninfected counterparts. These costs would include the costs of mortality, medication, condemnation, lost production, rescheduling, and vaccination. People with noninfected flocks might experience the costs of rescheduling and vaccination. The greatest cost is the forced rescheduling which would be greater for infected than noninfected farms. For a 10,000 bird turkey house to remain empty for an extra week it would cost about $1600. So if it was vacant for an extra 6 weeks, that would be only $9,600 per flock a far cry from the $760,000 per flock associated with the outbreak in Virginia.

CONCLUSION

Different types of low pathogenic avian influenza control programs have different costs associated with them. By incorporating the best features of different control programs it should be possible to develop a new integrated avian influenza control program that has a high probability of success at a reasonable cost. The advantages of the proposed program are that it requires no unethical destruction of healthy birds, requires no costly disposal, is cost effective and puts more of the costs of control on the producers with infected birds.

Table 1. Costs associated with large LPAI outbreaks.

Outbreak	Year	Serotype	Flocks	Cost*	Control**
Minnesota	1978	H6N1	141	$ 13.9 M	CM
Minnesota	1988	H2,H9N2	258	$ 5.1 M	CM
Minnesota	1991	Multiple	110	$ 1.3 M	CM
Minnesota	1995	H9N2	178	$ 7.4 M	CM
Utah	1995	H7N3	220	$ 2.6 M	Vac & CM
Italy	2000	H7N1	88	$ 10.3 M	Des & CM
			586	$ 2.6 M	Vac & CM
California	2000	H6N2	NA	NA	Vac & CM
Virginia	2002	H7N2	197	$149.0 M	Des & CM
Colorado	2002	H8N4	NA	NA	Vac & CM

*2002 dollars

** Biosecurity is assumed in all outbreaks, CM=controlled marketing, Vac=vaccination, Des=destruction NA=not available, costs have not been calculated yet.

VACCINES AND THEIR USE IN CONTROL OR ERADICATION OF AVIAN INFLUENZA

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SUMMARY

Avian influenza (AI) in domestic poultry is a national and international issue that negatively impacts animal health and trade in poultry and poultry products. Control programs for AI in poultry varies from tolerance of endemic low pathogenicity forms of AI to the extreme of implementing total depopulation programs for eradication of high pathogenicity AI. Vaccines have been used in control and eradication programs for AI based on needs of individual countries. Vaccine-induced protection is based upon antibodies produced against the surface glycoproteins, principally the hemagglutinin, but also the neuraminidase. This protection is specific only for individual subtypes of hemagglutinin (H1-15) and neuraminidase (N1-9) proteins. Avian influenza vaccines protect chickens and turkeys from clinical signs and death, and reduce respiratory and intestinal replication of a challenge virus containing homologous hemagglutinin protein. AI vaccines will not consistently prevent total replication of challenge virus; i.e. "sterilizing immunity." Vaccination should only be viewed as a single tool in an overall comprehensive control strategy that utilizes the practice of strict biosecurity, proper disinfection, limited human access to farms, adequate surveillance and quarantine measures, and methods of low risk elimination and disposal of infected birds.

INTRODUCTION

Maintaining poultry free from high pathogenicity (HP) avian influenza (AI), a list A disease for poultry as defined by the Office Internationale des Epizooties (OIE), is essential to the continuing trade in poultry and poultry products between nations (1). In addition, low pathogenicity (LP) AI has negatively impacted trade between individual countries, especially when involving H5 and H7 subtypes of LPAI. However, LPAI is not a list A or B disease of OIE, but H5 and H7 LPAI is being considered for addition to OIE code of Animal Health as a disease for eradication. In most trading countries, eradication by stamping-out is the preferred method for dealing with OIE List A diseases; i.e. HPAI. However, for many poultry diseases, including those on List A, judicious use of vaccines has been part of prevention, control, and/or eradication programs as determined by individual countries. In this paper, the primary focus will be on AI vaccines currently licensed for use and novel vaccines with a potential for use in the future.

BACKGROUND

Avian influenza is caused by type A orthomyxoviruses(10). Such viruses contain a single stranded, negative sense ribonucleic acid (RNA) genome divided into eight gene segments that code for

10 different proteins (10,15). Avian influenza viruses are pleomorphic with helical capsid symmetry and contain hemagglutinin and neuraminidase surface glycoprotein projections. Each AI virus strain will contain one of 15 different hemagglutinin subtypes (H1-15) as determined by the hemagglutinin inhibition (HI) test and one of 9 different neuraminidase subtypes (N1-9) as determined by the neuraminidase inhibition (NI) test. Furthermore, reactions to two internal proteins, the nucleoprotein and matrix protein, in agar gel immunodiffusion (AGID) test determine the type or species of the influenza virus. All AI viruses are Type A influenza viruses.

Avian influenza viruses are grouped into two broad pathotypes: 1) low virulent viruses, i.e. LPAI, and 2) high virulence viruses, i.e. HPAI (15). The LPAI viruses cause various clinical problems ranging from clinically inapparent infections to drops in egg production and mild respiratory disease. Severity of respiratory disease can be increased when accompanied by infections with other viral or bacterial pathogens. By contrast, HPAI viruses produce severe, highly fatal, systemic diseases affecting multiple internal organ systems. HPAI viruses are not endemic in commercial poultry and usually are associated with epizootics. The presence of HPAI in domestic poultry in a country is recognized as a legitimate trade barrier by OIE member nations. However, LPAI also may prevent trade between individual nations.

AVIAN INFLUENZA VACCINES AND VACCINATION

Various vaccine technologies have been shown experimentally to be effective for immunization of chickens and turkeys against AI virus. These technologies include traditional inactivated oil-based whole AI virus, vectored virus, subunit protein and DNA vaccines. By comparison, vaccine use in the field has been limited to inactivated oil-emulsion whole AI virus of various hemagglutinin subtypes and a recombinant fowlpox with AI H5 gene insert. Vaccination has been used as a strategy to minimize losses and reduce the incidence of disease, or vaccination has been combined with other strategies for the goal of total eradication, e.g. LPAI H7N1 in Italy 2000-2002. In most commercial poultry operations, vaccination against AI is not routinely practiced. However, it has been used in regions or within certain poultry industry segments with a high risk of exposure to LPAI; e.g. turkey breeders raised in geographic areas where HIN1 influenza is endemic in pigs.

Two surface glycoproteins of the virus (hemagglutinin and neuraminidase) stimulate the production of virus neutralizing antibodies that are responsible for vaccine-induced protection. Vaccines that induce immunity against the hemagglutinin provide the best protection against AI virus challenge, but protection is specific only against the homologous hemagglutinin subtype; i.e. a subtype H5 vaccine can protect only against other H5 AI viruses (9). In addition, vaccines against the neuraminidase surface glycoprotein also provide protection against AI virus challenge by homologous neuraminidase subtypes of AI viruses (5). By comparison, vaccines that target the conserved internal proteins of the virus such as the nucleoprotein or matrix protein do not provide protection against clinical signs and death (17).

Inactivated oil-emulsion whole AI virus vaccines have been used in the U.S. during the past 24 years, mainly against sporadic LPAI viruses in two situations: 1) high risk of transmission from wild waterfowl to domestic poultry, primarily in Minnesota turkeys raised on range, and 2) high risk of transmission of H1N1 swine AI viruses to turkey breeders. During 2001, 2.8 million doses of H1 influenza vaccine was used to protect turkey breeders from H1N1 and H1N2 swine influenza in the states of North Carolina, Ohio, Michigan, Illinois, Minnesota and Missouri, and 677,000 doses of inactivated H6N2 vaccine was used in layers in California (11). By contrast, inactivated AI vaccines use in HPAI outbreaks is of recent application. Specifically, vaccines were used in Mexico and Pakistan in the recent outbreaks of H5N2 and H7N3 HPAI, respectively. A recombinant fowl poxvirus vaccine with an H5 AI virus gene insert has been used in Mexico since 1998, and some has recently been used in El Salvador and Guatemala to control H5N2 LPAI in chickens.

Experimental studies have been used to assess the utility of vaccines in control of AI. Most studies have demonstrated the ability of vaccines to provide uniform protection of chickens and turkeys against clinical signs and death following challenge with H5 and H7 HPAI viruses (2,7,8,18,19). Extensive work has been done with H5 vaccines to demonstrate the ability to lowering infection rates and reducing quantity of challenge virus shed from respiratory and gastrointestinal tracts of vaccinated chickens (12). This translates into reduced contact transmission of the AI virus (12). Many of the vaccines are effective if given by single injection and provide protection for greater than 20 weeks (12). Protection has been demonstrated against both low and high doses of challenge virus. Furthermore, subtype H5 AI vaccine has been shown to provide protection against other H5 strains with 89.4% or greater hemagglutinin deduced amino acid sequence similarity and isolated over 38 years (14).

Currently, inactivated whole AI virus vaccines and a fowl pox-vectored vaccine with AI H5 hemagglutinin gene insert are used commercially in various countries of the world. These vaccines have

some disadvantages associated with the labor requirements for parenteral administration. However, an experimental recombinant Newcastle disease virus vaccine with an AI hemagglutinin gene insert shows some promise as a low cost, mass administered aerosol vaccine (16).

AVIAN INFLUENZA VACCINE LIMITATIONS AND DISADVANTAGES

Experimental studies with AI vaccines in specific-pathogen-free chickens have shown good-to-excellent protection. However, vaccine efficacy will always be less than demonstrated in the laboratory because of multiple field variables including improper vaccination technique, infections by immuno-suppressive viruses such as infectious bursal disease virus or hemorrhagic enteritis virus, and improper storage and handling of vaccines.

A critical issue for the use of vaccines in the field is the need to differentiate vaccinated birds from birds infected with the field virus. Differentiation is necessary for outbreak surveillance and continuation of trade. The AGID and commercial ELISA tests detect type A specific antibodies directed against the nucleoprotein and matrix protein, and are routinely used for serological monitoring of flocks. However, birds vaccinated with inactivated AI vaccines cannot be differentiated from birds challenged by field viruses using the AGID or ELISA tests. Most commonly, non-vaccinated sentinel birds are placed within vaccinated flocks to determine if field virus challenge has occurred. However, with some vaccines such as the recombinant fowlpox with AI gene inserts, current serologic monitoring can directly differentiate vaccinated from field challenged birds. Such vaccinated birds lack antibodies on AGID or ELISA tests but can have antibodies to the specific hemagglutinin gene as detected by hemagglutinin inhibition test (12). In other situations, alternative tests can be used such as differentiation based on detection of antibodies against neuraminidase subtypes if the vaccine and field challenge virus contain different neuraminidase subtypes (3). This latter approach was used in Italy during 2000-2002 H7N1 LPAI outbreak.

NOVEL VACCINES

Although inactivated oil-emulsion whole AI virus and fowlpox recombinant vaccines have been shown experimentally and in the field to be efficacious, other vaccine technologies have the potential for use in the future. Oil-emulsified vaccines containing only the hemagglutinin protein as obtained from a baculovirus-vector-insect-cell-culture system have shown to protect chickens from AI in experimental studies (4,13). Similar protein production systems utilizing cultures of yeast or bacteria with gene inserts have similar potential depending on the final purity of the hemagglutinin product and the ease of preventing inclusion of deleterious proteins from the bacterial or yeast vector.

Experimental studies have been conducted with DNA vaccines using hemagglutinin genes but the protection has been less consistent than with antigen-based vaccines. The current limitations for AI DNA vaccine use include: 1) use of high doses of the immunizing nucleic acids to induce protection; 2) the need to select proper expression vectors and adjuvants and to optimize their doses to improve protection and to lower the optimal dose of nucleic acids needed; 3) need for multiple immunizations to get adequate protection; and 4) best protection requires use of expensive gold particles and gene-gun technology. However, with improvements DNA vaccines may have future use in the field under current production systems. The greatest potential use in the future would be for vaccination by injection of 1-day-old birds or *in ovo* in the hatchery to reduce field vaccination costs.

An alternative to current costly parenteral vaccination methods is to vaccinate by using a mass administration method such as aerosol spray or drinking water administration. For example, use of the B1 vaccine strain of a lentogenic ND virus (paramyxovirus type 1 virus as a vector for AI hemagglutinin gene) has shown some protection against both velogenic Newcastle disease and AI challenge virus homologous to the AI hemagglutinin inserted in the recombinant vaccine (6,16).

REFERENCES

1. Alexander, D. J. Highly pathogenic avian influenza (fowl plague). In: OIE Manual of standards for diagnostic tests and vaccines. List A and B diseases of mammals, birds and bees, 3 ed. Office International des Epizooties, Paris. pp. 155-160. 1996.

2. Brugh, M., C. W.Beard, and H. D.Stone. Immunization of chickens and turkeys against avian influenza with monovalent and polyvalent oil emulsion vaccines. Am.J.Vet.Res. 40:165-169. 1979.

3. Capua, I., S. Marangon, P. Dalla, and U.Santucci. Vaccination for avian influenza in Italy. Vet.Rec. 147:7512000.

4. Crawford, J., B. Wilkinson, A. Vosnesensky, G. Smith, M. Garcia, H. Stone, and M. L. Perdue. Baculovirus-derived hemagglutinin vaccines protect against lethal influenza infections by avian H5 and H7 subtypes. Vaccine 17:2265-2274. 1999.

5. McNulty, M. S., G. M. Allan, and B. M. Adair. Efficacy of avian influenza neuraminidase-

specific vaccines in chickens. Avian Pathol. 15:107-115. 1986.

6. Nakaya, T., J. Cros, M. S. Park, Y. Nakaya, H. Y. Zheng, A. Sagrera, E. Villar, A. Garcia-Sastre, and P.Palese. Recombinant Newcastle disease virus as a vaccine vector. J.Virol. 75:11868-11873. 2001.

7. Stone, H. D. Efficacy of avian influenza oil-emulsion vaccines in chickens of various ages. Avian Dis. 31:483-490. 1987.

8. Stone, H. D. Optimization of hydrophile-lipophile balance for improved efficacy of Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis. 32:68-73. 1988.

9. Suarez, D. L. and S. Schultz-Cherry. The effect of eukaryotic expression vectors and adjuvants on DNA vaccines in chickens using an avian influenza model. Avian Dis. 44:861-868. 2000.

10. Swayne, D. E. Understanding the ecology and epidemiology of avian influenza viruses: implications for zoonotic potential. In: Emerging diseases of animals, C. C. Brown and C. A. Bolin, eds. ASM Press, Washington, D.C. pp. 101-130. 2000.

11. Swayne, D. E. Avian influenza vaccine use during 2001. In: Proceedings of the 104th annual meeting of the U.S. Animal Health Association, USAHA, Richmond, Virginia. pp. 469-471. 2001.

12. Swayne, D. E., J. R. Beck, and T. R. Mickle. Efficacy of recombinant fowl pox vaccine in protecting chickens against highly pathogenic Mexican-origin H5N2 avian influenza virus. Avian Dis. 41:910-922. 1997.

13. Swayne, D. E., J. R. Beck, M. L. Perdue, and C. W. Beard. Efficacy of vaccines in chickens against highly pathogenic Hong Kong H5N1 avian influenza. Avian Dis. 45:355-365. 2001.

14. Swayne, D. E., M. Garcia, J. R. Beck, N. Kinney, and D. L. Suarez. Protection against diverse highly pathogenic avian influenza viruses in chickens immunized with a recombinant fowl pox vaccine containing an H5 avian influenza hemagglutinin gene insert. Vaccine 18:1088-1095. 2000.

15. Swayne, D. E. and D. L. Suarez. Highly pathogenic avian influenza. Rev Sci Tech Off Int Epiz 19:463-482. 2000.

16. Swayne, D. E., D. L. Suarez, S. Schultz-Cherry, T. M. Tumpey, D. J. King, T. Nakaya, P. Palese, and A. Garcia-Sastra. Recombinant Para-myxovirus type 1-avian influenza-H7 virus as a vaccine for protection of chickens against influenza and Newcastle disease. Avian Dis. 47:in press. 2003.

17. Webster, R. G., Y. Kawaoka, J. Taylor, R. Weinberg, and E. Paoletti. Efficacy of nucleoprotein and haemagglutinin antigens expressed in fowlpox virus as vaccine for influenza in chickens. Vaccine 9:303-308. 1991.

18. Wood, J. M., Y. Kawaoka, L. A. Newberry, E. Bordwell, and R. G. Webster. Standardization of inactivated H5N2 influenza vaccine and efficacy against lethal A/chicken/Pennsylvania/1370/83 infection. Avian Dis. 29:867-872. 1985.

19. Xie, Z. X. and H. D. Stone. Immune response to oil-emulsion vaccines with single or mixed antigens of Newcastle disease, avian influenza, and infectious bronchitis. Avian Dis. 34:154-162. 1990.

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NOVEL PERSPECTIVES FOR THE CONTROL OF AVIAN INFLUENZA

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INTRODUCTION

Recent epidemics of highly contagious animal diseases included in the list A of the OIE such as foot-and-mouth disease, classical swine fever and avian influenza (AI) have led to the implementation of stamping out policies resulting in the depopulation of millions of animals. The implementation of a control strategy based on culling of animals that are infected, suspected of being infected or suspected of being contaminated which is based only on the application of sanitary restrictions on farms, may not be sufficient to avoid the spread of infection, particularly in areas that have high animal densities thus resulting in mass depopulation.

With reference to AI, the EU directive that imposes the enforcement of a stamping out policy (6) was adopted in 1992 but was drafted in the 1980's. The poultry industry has undergone substantial changes in the last twenty years, mainly resulting in shorter production cycles and in greater animal densities per territorial unit. Due to these organisational changes, infectious diseases are significantly more difficult to control in reason of the greater number of susceptible animals reared per given unit of time and to the impossibility of respecting basic biosecurity measures.

The slaughter and destruction of great numbers of animals, also is also questionable from an ethical point of view, particularly when human health implications are negligible. For this reason, mass depopulation has raised serious concerns for the general public and has recently led to very high costs and economical losses for the Community budget, the Member States, the stakeholders and ultimately for the consumers.

In the EU, the use of currently licensed vaccines in such emergencies has been limited by the impossibility of differentiating vaccinated/infected from vaccinated/non-infected animals. The major concern was that through trade or movement of apparently uninfected animals or products, the disease could spread further or might be exported to other countries.

The following paper takes into account the possible strategies for the control of avian influenza infections, bearing in mind the new proposed definition of AI, the possibility of enforcing an emergency vaccination programme with the currently available products and on the opportunity of using other products, which are currently not licensed.

DEFINITION OF AVIAN INFLUENZA

Avian influenza viruses all belong to the *Influenzavirus A* genus of the *Orthomyxoviridae* family and are negative stranded, segmented RNA viruses. The influenza A viruses, can be divided into 15 subtypes on the basis of the haemagglutinin (H) antigens. In addition to the H antigen, influenza viruses possess one of nine neuraminidase (N) antigens. Virtually all H and N combinations have been isolated from birds, thus indicating the extreme antigenic variability, which is a hallmark of these viruses. Changes in the H and N composition of a virus may be brought about by genetic reassortment in host cells. In fact one of the consequences of genomic segmentation is that if co-infection by different viruses occurs in the same cell, progeny viruses may originate from the reassortment of parental genes originating from different viruses. Thus, since the influenza A virus genome consists of 8 segments, from two parental viruses 256 different combinations of progeny viruses may theoretically arise.

Current EU legislation (6) defines avian influenza as "an infection of poultry caused by any influenza A virus which has an intravenous pathogenicity index in six-week-old chickens greater than 1.2 or any infection with influenza A viruses of H5 or H7 subtype for which nucleotide sequencing has demonstrated the presence of multiple basic amino acids at the cleavage site of the haemagglutinin"…, however it has been proved that highly pathogenic avian influenza (HPAI) viruses emerge from low pathogenicity (LPAI) progenitors of the H5 and H7 subtypes. It therefore seems logical that not only HPAI viruses must be controlled, but also the LPAI progenitors (1), therefore with reference to the present paper, the term avian influenza applies to all avian influenza viruses of the H5 and H7 subtype, regardless of their virulence.

RATIONALE BEHIND THE USE OF VACCINES

In the event of an outbreak of avian influenza in an area with a high population density in which the application of rigorous biosecurity measures is incompatible with the modern rearing systems, vaccination should be considered as an option for controlling the spread of infection. The expected results of the implementation of a vaccination policy on the dynamics of infection are primarily those of reducing the susceptibility to infection (i.e. a higher dose of virus is necessary for establishing productive infection) and reducing the amount of virus shed into the environment. The association between a higher infective dose necessary to establish infection and less virus in the environment should be a valuable support to the eradication of infection.

Clearly, the efficacy of an emergency vaccination program is inversely correlated to the time span between the diagnosis in the index case and the implementation of mass vaccination. For this reason, it is imperative that if emergency vaccination is considered as a possible option in a given country, vaccine banks must be available.

CURRENTLY AVAILABLE VACCINES

CONVENTIONAL VACCINES

Inactivated homologous vaccines. These vaccines were originally prepared as "autogenous" vaccines, i.e, vaccines that contain the same avian influenza strain as the one causing the problems in the field. They contain an oil emulsion as adjuvant.

The efficacy of these vaccines in preventing clinical disease and in reducing the amount of virus shed in the environment has been proven through field evidence and experimental trials. The disadvantage of

this system is the impossibility of differentiating vaccinated from field exposed birds unless unvaccinated sentinels are kept in the shed. However, the management (identification, bleeding and swabbing) of sentinel birds during a vaccination campaign may be time-consuming and rather complicated, since they may be identified only with difficulty, and furthermore they may be substituted with seronegative birds in the attempt to escape restrictions imposed by public health officials.

Inactivated heterologous vaccines. These vaccines are manufactured in a similar way to the previous ones, although they differ in the fact that the virus strain used in the vaccine is of the same H type as the field virus but has a heterologous neuraminidase. In case of field exposure, clinical protection and reduction of viral shedding are ensured by the immune reaction induced by the homologous H group. The advantages of this system are that it allows the preparation of vaccine banks beforehand, and that it may be used in the framework of a "DIVA" (Differentiating Infected from Vaccinated Animals) strategy. The rationale behind the use of heterologous vaccination as a "negative marker vaccine" is that vaccinated/uninfected animals will not have antibodies against the neuraminidase induced by the field virus can therefore be used as a marker of natural infection. The disadvantage of this system is that it is a "tailored system" designed to support the eradication of the serotype that is causing the field infection.

For both homologous and heterologous vaccines, the degree of clinical protection and the reduction of shedding are positively influenced by the antigen mass in the vaccine, and are not strictly correlated to the degree of homology between the hemagglutinin gene of the strain included in the vaccine the hemagglutinin gene of the challenge virus. This fact, as mentioned above, enables the establishment of vaccine banks since the master seed does not contain the virus, which is present in the field and may contain an isolate (preferably of the same lineage) available before the epidemic.

RECOMBINANT VACCINES

A recombinant fowlpox virus expressing the H5 antigen has been licensed and is currently being used in Mexico (13). Experimental data has also been obtained for fowlpox virus recombinants expressing the H7 antigen (2). Other vectors have also been used to successfully deliver the H5 antigen such as the use of constructs using infectious laryngotracheitis virus (ILTV) (9).

However, the only field experience with a recombinant virus to control AI has been carried out in Mexico, (12) where it has been used in the vaccination campaign against a LPAI H5N2 virus.

No such product has been licensed in the EU to date.

TRADE IMPLICATIONS

Until recent times, vaccination against avian influenza viruses of the H5 and H7 subtypes, was not considered or practiced in developed countries since it implied export bans on live poultry and on poultry products. It should however be mentioned that in case of an infection with an H5 or H7 regardless of the virulence of the isolate, export bans have also been imposed.

While on one hand, the severe clinical signs caused by HPAI ensure a prompt diagnosis and therefore facilitate the implementation of a stamping-out policy, the inconspicuous nature of disease caused by viruses of low pathogenicity make this infection difficult to diagnose and only detectable with the implementation of appropriate surveillance programs. In fact, in several recent outbreaks, infection with a virus of low pathogenicity has only been detected when infection was already widespread, and often out of control.

In absence of vaccination, trade bans imposed on a given area last until freedom from infection can be demonstrated in the affected population. Similarly, in case of the enforcement of a vaccination policy, which does not enable the application of a "DIVA" strategy (either for the type of vaccine used, or because the monitoring system in place does not guarantee that infection is no longer circulating) also results in trade bans. On the contrary, if it is possible to demonstrate that the infection in not circulating in the vaccinated population trade bans may be lifted (5).

This possibility sheds light onto the control strategies, which may be applied for OIE list A diseases. In fact if it is possible to safeguard international trade by implementing a control strategy, which enables the differentiation between vaccinated /infected and vaccinated/non infected animals there should be no reason for allowing infection to spread and subsequently enforce a mass stamping out policy.

OPTIONS FOR CONTROL

Although it is extremely difficult to establish fixed rules for the control of infectious diseases in animal populations, due to an unpredictable number of variables, some basic scenarios may be hypothesized, and on the basis of the considerations made above some guidelines may be drawn and are reported in the table below:

Table 1. Guidelines for the application of emergency vaccination for avian influenza infections.

H5/H7 VIRUS PATHOGENICITY	INDEX CASE FLOCK	EVIDENCE OF SPREAD TO INDUSTRIAL CIRCUIT	POPULATION DENSITY IN AREA	POLICY
HPAI/LPAI	Backyard	No	High/Low	Stamping-out
HPAI/LPAI	Backyard	Yes	Low	Stamping-out
			High	Vaccination
HPAI/LPAI	Industrial	No	High/Low	Stamping-out
HPAI/LPAI	Industrial	Yes	Low	Stamping-out
			High	Vaccination

Fundamentally however, there are several crucial steps, which must be planned for if avian influenza represents a risk. Firstly the index case must be promptly identified. Generally speaking this does not represent a problem if the virus is of high pathogenicity, but it can be a serious concern if the virus if of low pathogenicity. For this reason countries or areas at risk of infection should implement specific surveillance systems to detect infection with AI as soon as it appears.

Secondly, a timely assessment of whether there has been spread to the industrial poultry population of that area must be performed. This is a crucial evaluation, which must be made available for decision makers.

Finally, if vaccination is the proposed strategy, vaccine banks should be available for immediate use and a contingency plan must be enforced. In addition a territorial strategy must be implemented to perform the adequate controls and assess whether the virus is circulating or not in the vaccinated population.

APPLICATIONS IN THE FIELD

Inactivated homologous vaccines. In recent times they have been used in the attempt to control avian influenza infections in Pakistan and in Mexico (12), but in the specific conditions they have not have been successful in eradicating the infection. In contrast, in one instance, in Utah (7), the use of this vaccination strategy has been successful. The reason for the discrepancy of the results probably lies in the efficacy of the direct control measures, which must be implemented to support a vaccination campaign.

Inactivated heterologous vaccines. This vaccination strategy has been used successfully over the years in Minnesota (8), however in these instances vaccination was never implemented to control infections caused by viruses of the H5 or H7 subtypes. In addition the heterologous neuraminidase was not used as a marker of infection.

Conversely, in Italy during 2000-2002 this strategy was used to supplement control measures for the eradication of the H7N1LPAI virus. In order to control the re-emergence of LPAI virus and to develop a novel control strategy, a coordinated set of measures, including strict biosecurity, a serologic monitoring programme and a "DIVA" (Differentiating Infected from Vaccinated Animals) strategy were enforced (4).

The "DIVA" strategy was based on the use of an inactivated oil emulsion heterologous vaccine containing the same haemagglutinin (H) subtype as the field virus, but a different neuraminidase (N), in this case an H7N3 strain. The possibility of using the diverse N group, to differentiate between vaccinated and naturally infected birds, was achieved through the development of an "*ad hoc*" serological test based on the detection of specific anti- N1 antibodies (3).

The control of the field situation was ensured through an intensive sero-surveillance programme aiming at the detection of the LPAI virus, through the regular testing of sentinel birds in vaccinated flocks and through the application of the anti-N1 antibody detection test. Serological monitoring was also enforced in unvaccinated flocks, located both inside and outside the vaccination area. In addition, the efficacy of the vaccination schemes was evaluated in the field through regular testing of selected flocks.

After the first year of vaccination, the epidemiological data collected, indicating that the H7N1 virus was not circulating any longer, was considered to be sufficient by the EU Commission to lift the marketing restrictions on fresh meat obtained from vaccinated poultry (5).

Recombinant vaccines. The only field experience with this vaccine has been carried out in Mexico, where it has been used in the vaccination campaign against the H5N2 virus. Avian influenza has not been eradicated in Mexico, probably because an eradication program based on a territorial strategy and including monitoring, vaccination and controlled marketing of infected birds was not established.

Recombinant live vectored vaccines also enable the differentiation between infected and vaccinated birds, since they do not induce the production of antibodies against the nucleoprotein antigen, which is common to all AI viruses. Therefore, only field infected birds will exhibit antibodies to the AGP or ELISA test directed towards the detection of group A (nucleoprotein) antibodies.

Since these vaccines have encountered some difficulties in licensing, their use is restricted to countries in which they are legally available. In addition, it must be mentioned that these vaccines will not replicate, and induce protective immunity, in birds that have had field exposure to the vector (ie fowlpox or infectious laryngotracheitis viruses) (9,10). Since serological positivity to these viruses is widespread (due to field exposure and vaccination) in the poultry population, and can be in some instances unpredictable, the use of these vectored vaccines in case of an emergency restricts their use to a population which is seronegative to the vector virus.

DISCUSSION

From the data presented above it appears that emergency vaccination should be considered when there is evidence of the introduction of a highly transmissible virus in a densely populated poultry area, or whenever the epidemiological situation indicates that there could be massive and rapid spread of infection. In addition, emergency vaccination should be considered where applicable, when economically (e.g. pedigree flocks) or rare (endangered) birds are at risk of infection.

Taking into account the advantages and disadvantages of the products and diagnostic tools which are currently available, if no recombinant products are licensed in that country, it would seem logical to use heterologous vaccination rather than homologous vaccination in case of an emergency. The main reason for this would be that it would enable the differentiation of vaccinated from naturally exposed birds, through the development/application of an appropriate test. At present only the anti-neuraminidase based test is available and has been validated. In our opinion however, this test represents a starting point on which future developments of the "DIVA" strategy can be based. The development of novel candidate vaccines and of additional tests which enable the detection of field infection in vaccinated populations should be a priority for pharmaceutical industries and for research institutions since for all the reasons listed above vaccination is already an option for the control of avian influenza.

If, on the other hand the country has access to licensed recombinant products, the use of these vaccines can be envisaged, bearing in mind that the immune status of the population against the vector could impede the replication of the vector virus and therefore the establishment of immunity.

In conclusion, recent events including devastating epidemics in densely populated poultry areas, public health concern on animal welfare issues and the introduction of novel technology into vaccinology and into the development of diagnostic companion tests have opened breaches into the control strategies for OIE List A diseases which were unthinkable of only a few years ago. Countries, areas and enterprises at risk of infection should imperatively enforce surveillance programs and have a contingency plan in case of an emergency, which may include vaccination. If the latter is considered as an option, among other issues the contingency plan must foresee the establishment of licensed vaccine banks, which enable the "DIVA" strategy thus safeguarding animal health, animal welfare and international trade.

REFERENCES

1. Anonymous. (2000). The Definition of Avian Influenza and the Use of Vaccination against Avian Influenza. EC Scientific Committee on Animal Health and Animal Welfare, Document Sanco/B3/AH/R17/2000, 1-38.
2. Boyle, D. B., P. Selleck, and H.G. Heine. (2000). Vaccinating chickens against avian influenza with fowlpox recombinants expressing the H7 haemagglutinin. Aust. Vet. J. 78: 44– 48. 2000.
3. Capua, I., C. Terregino, G. Cattoli, F. Mutinelli and J.F. Rodriguez. Development of a DIVA-Differentiating infected from vaccinated animals- strategy using a vaccine containing a heterologous neruraminidase for the control of avian influenza. Avian Path. 32 47-55. 2003.
4. CEC Commission Decision 2000/721/EC of 7 November 2000 on introducing vaccination to supplement the measures to control avian influenza in Italy and on specific movement control measures. Official Journal of the European Commission, L291, 33-36.
5. CEC Commission Decision 2001/847/CE of 30 November 2001 amending for the third time Decision 2000/721/EC to modify the Italian avian influenza vaccination programme and current trade

restrictions for fresh meat originating from vaccinated turkeys. Official Journal of the European Commission, L315, 61- 63.

6. CEC. (1992). Council Directive 92/40/EEC of 19 May 1992 introducing Community measures for the control of avian influenza. Official Journal of the European Commission, L167: 1-15.

7. Frame, D.D., B.J. Mc Cluskey, R.E. Buckner and F.D. Halls. Results of an H7N3 avian influenza vaccination program in commercial meat turkeys. Proceedings 45th Western Poultry Disease Conference, Cancun, Mexico, 1996.

8. Halvorson D.A. The control of mildly pathogenic avian influenza: a role for inactivated vaccine. Avian Path. 31: 5-12 2002.

9. Luschow D., O. Werner, T.C. Mettenleiter and W. Fuchs. Protection of chickens from lethal avian influenza A virus infection by live-virus vaccination with infectious laryngotracheitis virus recombinants expressing the hemagglutinin (H5) gene. Vaccine 19, 4249 – 4259. 2001.

10. Swayne, D.E., J.R. Beck and N. Kinney. Failure of a recombinant fowl poxvirus vaccine containing an avian influenza hemagglutinin gene to provide consistent protection against influenza in chickens preimmunized with a fowl pox vaccine. Avian Dis. 44: 132-137. 2000.

11. Swayne, D. E., J.R. Beck, M. Garcia and H.D. Stone. Influence of virus strain and antigen mass on the efficacy of H5 avian influenza inactivated vaccines. Avian Path. 28: 245-255. 1999.

12. Swayne, D.E. and D.L. Suarez. Highly pathogenic avian influenza. Rev. Sci. Tech. Off. Int. Epiz. 20: 463-482. 2000.

13. Villareal-Chavez, C. and E. Rivera Cruz. An update on avian influenza in Mexico. In: Proceedings of the 5th International Symposium on Avian Influenza. Georgia Center for Continuing Education, The University of Georgia, Athens Georgia USA, 14-17 April 2002. In press.

PROTECTIVE EFFICACY OF AN INACTIVATED AVIAN INFLUENZA VACCINE AGAINST CHALLENGE WITH A 2002 H7N2 AVIAN INFLUENZA ISOLATE

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SUMMARY

The outbreak of H7N2 low pathogenicity avian influenza (LPAI) in Virginia this past year raised questions about the potential of available vaccines to provide protection. The current study was undertaken to determine if an existing commercial H7N2 avian influenza vaccine could provide protection against a recent LPAI (H7N2) isolate. Low pathogenic A/Chicken/Pennslyvania/21342/97 was used as the vaccine strain and emulsified in a proprietary oil-based vaccine (Lohmann Animal Health, Waterville ME). Groups of 10 one-day-old and three-week-old turkeys (British United Turkeys of America, Lewisburg, WV) were immunized subcutaneously with either the inactivated vaccine or normal allantoic fluid emulsified in the same adjuvant. In vaccine trials, neither sham-vaccinated nor H7N2-vaccinated turkeys developed clinical signs or death following challenge with A/Turkey/Virginia/158512/02 (H7N2) LPAI virus. However, high titers of challenge virus could be detected from swabs collected from the oropharynx on days one-seven after challenge in the sham-vaccinated group. Low or undetectable viral titers were recovered from cloacal samples from the sham-vaccinated birds. The inactivated vaccine groups (1x and 2x vaccinated) had a significant reduction in titers of challenge virus shed from the oropharynx when compared to sham-vaccinated groups for days one-seven after challenge. These studies suggest that a currently available commercial H7N2 avian influenza vaccine can provide protection against a recent H7N2 avian influenza isolate.

THE INFLUENCE OF STRAIN AND THE EFFECT OF VACCINATION ON THE CLINICAL SIGNS OF LOW PATHOGENIC H6N2 AVIAN INFLUENZA VIRUS IN COMMERCIAL LAYERS

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SUMMARY

Epidemiologic study of 34 non-vaccinated flocks established that host strain impacted the severity of production losses and of mortality increases. Strain C experienced the most severe changes in production and mortality, while strains A and B seemed more resistant to clinical signs. Six additional flocks were vaccinated as pullets. These flocks had significantly less of a productions drop and almost no change in mortality in comparison with the 34 unvaccinated flocks. Vaccination also appeared to slow the spread of the virus across an infected farm.

Clinical signs associated with this H6N2 infection included decreased egg production and increased mortality predominately. Increased respiratory sounds and decreased feed consumption were noted on an inconsistent basis. The producers we questioned confirmed the previously published reports on the disease syndrome associated with this virus (1-3).

REFERENCES

1. Kinde, H., D.H. Read, B.M. Daft, J. Moore, F. Uzal, J. Mukai, and P.R. Woolcock. The occurrence of avian influenza A subtype H6N2 in commercial layer flocks in Southern California. Fifth International symposium on Avian Influenza, Athens, GA. 2002.

2. Webby, R. J., P.R. Woolcock, S.L. Krauss, D.B. Walker, P.S. Chin, K.F. Shortridge, and R.G. Webster. Multiple genotypes of non-pathogenic H6N2 Influenza viruses isolated from chickens in California. Proc. of the Fifth International Symposium on Avian Influenza, 5:17, 2002.

3. Woolcock, P.R., D.L. Suarez, and D. Kunev. Low pathogenicity Avian Influenza Virus (H6N2) in chickens in California, 2000-2001. Proc. of the Fifth International Symposium on Avian Influenza, 5:15, 2002.

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SEROLOGIC RESPONSE OF CHICKENS TO AVIAN INFLUENZA VACCINATION

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The USDA is considering the use of vaccines as a tool in the control of H5 and H7 low pathogenic avian influenza (AI) (1). Vaccines have long been used to prevent and reduce the occurrence and severity of disease. A secondary outcome of vaccination is the decreased shedding of the infectious agent and spread of the disease. Vaccination is also a major tool in the eradication and control of a disease including AI. Vaccination would augment quarantine and biosecurity efforts of producers.

Poultry producers usually practice all feasible combination of vaccines, routes of administration, and timing to "best" manipulate the avian immune response. The current "experimental" H6N2 AI vaccine being used in California is no exception. Although it is licensed in a specific fashion, what happens in the field is debatable. The present study was undertaken to define what is the chicken's serologic response to the H6N2 avian influenza vaccine.

Two commercial layer operations were selected based on their willingness to participate and use of

licensed available H6N2 AI product. Blood samples were collected prior to initial vaccination and approximately at 3, 5, 7 10, 14, 18, 21 and 28 days post-vaccination. A second vaccination was performed at about 28 days after the primary vaccination. Blood samples were again collected at 0, 4, 7, 14, 28 and 56 days past this second vaccination. Serum samples were tested by the agar gel immunodiffusion precipitin (AGIP) test and the Idexx ELISA test.

Preliminary results (Fig. 1) of one commercial flock demonstrated that greater than 50% positive AGID tests did not occur until approximately three weeks after the initial vaccination. Ninety percent of the birds were not positive until shortly after the second vaccination at 28 days post initial vaccination. The ELISA test closely matched the AGID test in number of positive samples.

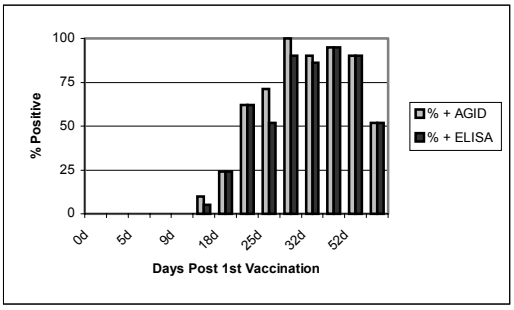

Figure 1. Percent of blood samples positive to Avian Influenza by AGID and ELISA.

The ELISA S/P ratio (Fig. 2) started to increase prior to reaching the positive limit (>0.5). This increase occurred about nine days after the initial vaccine was given and about 10 days before a sample was classified as positive. The S/P ratio and the number of positive samples decreased significantly seven weeks after the initial vaccination or four weeks past the second vaccination.

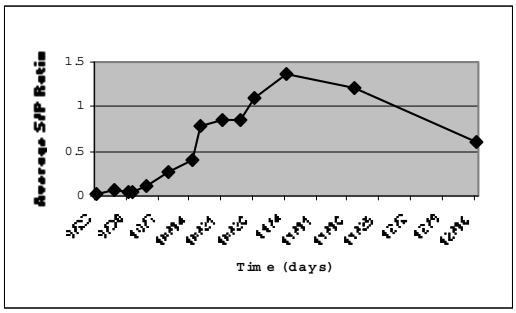

Figure 2. ELISA S/P ratio of blood samples plotted by time after vaccination.

Modifications in vaccination procedure with this experimental AI vaccine cannot be recommended based on the limited data of this study. Decreasing the dose and/or decreasing the number of vaccinations would markedly decrease the already short serologic response. It must be remembered that this serologic response is not a measure of the protective response. Protection may start earlier and last longer than the serology indicates. Studies are needed to determine the protective ability and time frame of this vaccine and correlated with the serologic response.

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REFERENCES

1. T.J. Myers, Michael David, Andrew Rhorer, and John Clifford. USDA Options for Regulatory Changes to Enhance the Prevention and Control of Avian Influenza. http://ag.ansc.purdue.edu/ispa/news/Reports/USDAoptionsAI.html.

GENETIC AND VIROLOGIC CHARACTERISTICS OF RECENT H5 SUBTYPE AVIAN INFLUENZA VIRUSES IN NORTH AMERICA

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SUMMARY

H5 subtype avian influenza viruses (AIVs) are one of most frequently isolated subtypes in the U.S. and have been related to two highly pathogenic AI outbreaks in North America. AI is endemic in wild birds in U.S., and the virus is routinely transmitted from this reservoir to poultry. However, isolation of H5 subtype AIV has been much less common in chickens and turkeys in the recent five years. In 2002, two different outbreaks of H5 occurred in chickens and turkeys. The first outbreak was in Texas and was identified as a H5N3 subtype AIV. The second outbreak was with a H5N2 virus isolated from a turkey farm in California. Based on phylogenetic analysis of the HA, NA, M and NS gene, the Texas and California isolates seem to be a separate introductions from wild waterfowl. Compared to other recent H5 isolates, the Texas H5N3 isolate had many unique changes in the HA gene including the HA cleavage site sequence of REKR/G (other recent isolates have the RETR/G which is a typical avirulent motif). This HA cleavage site sequence for H5 viruses may be minimum basic amino acid required for virulence, but the Texas isolate had glycosylation site at the amino acid position 12 (Asn 12), which may prevent the virus becoming highly pathogenic. Further, this isolate had the 24 amino acids deletion at the stalk region of NA gene, a characteristic of other chicken adapted influenza virus, and may indicate that this virus had actually been circulating in poultry for an extended period of time before it was isolated. Previous serologic evidence of positive H5N3 reinforces this conclusion. In experimental studies, the Texas isolate replicated better than other H5 isolates in chickens, which supports the idea that it is better adapted to chickens.

INTRODUCTION

Aquatic birds are believed to be a natural host and reservoir for influenza virus. All 15 hemagglutinin and 9 neuraminidase subtypes have been isolated from these species of wild waterfowl and shorebirds. Poultry are not considered to be a normal host for the virus, although transmission from wild birds to poultry occurs routinely. Although most HA subtypes have been found in poultry, particular emphasis is placed on the H5 or H7 hemagglutinin subtypes of AIV isolates because these are the only subtypes clearly shown to cause highly pathogenic avian influenza in poultry (1).

Several outbreaks of highly pathogenic avian influenza (HPAI) have occurred in North America involving the H5 subtype virus. In 1966, the prototype H5 virus, A/Turkey/Ontario/7732/66 (H5N9), caused a limited outbreak. In 1983, HPAI occurred in commercial poultry in Pennsylvania with devastating effects on the poultry industry. Retrospective genetic analysis of H5N2 isolates from live bird market (LBM) provided evidence that the LBMs was the likely source of infection for commercial poultry operation (2). With increased surveillance, sporadic isolation of H5N2 viruses continued from chickens inside and outside the live bird markets since 1986. After 1990, the number of H5 isolation decreased compared to the isolation in the 1980s. These coincided with the increase of the H7 subtype AIV isolation. Though, several H5N2 viruses isolated from chickens in live bird markets in 1993 were shown to have the potential to become virulent, most isolates after 1990 had characteristics analogous to those of avirulent H5 viruses. Isolation of H5 subtype AIV in chickens and turkeys is very rare since 1990 and no outbreak involving clinical signs of disease in domestic chickens and turkeys has been reported (3).

In 2002, two different outbreaks of H5 occurred in chickens and turkeys. The first outbreak was in Texas and the causative virus was identified as a H5N3 subtype AIV. The second outbreak was with a H5N2 virus isolated from a turkey farm in California. In this study, we determined the likely origin of these isolates and compared them with other recent non-chicken and non-turkey origin H5 subtype viruses from North America. Further, the replication competency and potential pathogenicity of recent H5 viruses in chickens were compared.

MATERIAL AND METHODS

Virus. Virus isolates for this study were obtained from the National Veterinary Services Laboratories in Ames Iowa, Texas Medical Diagnostic Laboratory in Gonzales Texas, and the California Animal Health and Food Safety Laboratory System - Fresno Branch in Fresno California. Viruses were received in allantoic fluid after passage in embryonated chicken eggs (ECE). Isolates were passaged one or two additional times at the Southeast Poultry Research Laboratory to make working stocks of the virus.

Pathogenicity in chickens. Eight 6-to-8 week-old chickens derived from an SPF flock were inoculated intravenously with 0.2 ml of a 1:10 dilution of bacteria-free AAF containing AIV. The chickens were observed daily for illness or death for 10 days post-inoculation (DPI).

14-day-old ECE passage system. Two H5N2 (A/Pheasant/NJ/1355/98 and A/Avian/NY/31588-3/00) and a H5N3 (A/CK/TX/167280-4/02) isolates of first passage in 10-day ECE were used as the parent viruses. The viruses were passed through a 14-day chicken embryo laboratory system that favors the emergence of HP derivatives.

RNA extraction and sequencing. RNA from the isolates used in this study was extracted with the RNeasy kit (Qiagen, Valencia, CA) from infected allantoic fluid from ECE. The entire coding region of hemagglutinin, neuraminidase, matrix, and non-structural genes were amplified by standard RT-PCR with the Qiagen one-step kit (Qiagen) and primers directed to the 12 or 13 conserved bases at the ends of each influenza viral RNA segment. PCR products were purified with the Qiagen gel extraction kit (Qiagen). Sequencing was performed with the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit (Perkin-Elmer**,** Foster City, CA) run on a 373A automated sequencer (Perkin-Elmer).

Nucleotide and amino acid sequence phylogenetic analysis. The nucleotide sequences were compared initially with Megalign program (DNASTAR, Madison, WI) using the Clustal W alignment algorithm. Pairwise sequence alignments were also performed in the Megalign program to determine nucleotide and amino acid sequence similarity. Phylogenetic comparisons of the aligned sequence for each gene segment were generated using the maximum parsimony method with 100 bootstrap replicates in a heuristic search using the PAUP 4.0b4 software (Sinauer Associates, Inc, Sunderland, MA).

Experimental chicken infection. Three Groups of three-week-old SPF White Rock chickens (n=10 birds per group) were inoculated intranasally with one of the three isolates. Tracheal and cloacal swabs were collected at three and seven DPI. Swabs were suspended in brain-heart-infusion (BHI) broth with antibiotics and the suspensions were injected into 10-day-old ECE for virus re-isolation and titration.

RESULTS

Isolation of viruses. In the past five years, several H5N2 viruses have been isolated from the LBMs. These viruses were from domestic ducks, game birds, and environmental samples. Other isolations have been made from quail, pheasant, and ducks as part of the surveillance programs required to sell birds to the LBMs. Isolation of H5N2 AIV from turkeys was made from frozen lung samples from breeder birds in California. Flocks had already been determined to be seropositive for AIV. The Texas H5N3 outbreak occurred at a farm owned by a semi-retired egg producer who kept a few chickens (white leghorns) and leased some of the houses to two LBM operators. The LBM operators used the houses to hold spent brown layers and spent broiler breeders. In April of 2002, clinical signs such as wheezing and swollen heads were observed in white leghorns and 16 out of 111 chickens were dead. Several H5N3 viruses were isolated from those chickens and also from brown layers that did not show clinical signs of disease. In 1999, there was serological evidence of H5N3 infection in a commercial turkey farm and in two premises with ducks destined for the LBM.

Pathogenicity of virus. Some isolates had been previously pathotyped as low pathogenic AIV (3). The chickens inoculated intravenously with representative H5 isolates remained clinically healthy during the 10-day observation period. The potential pathogenicity of two H5N2 and a H5N3 isolates were further assessed using 14-day-old ECE passage system. No HP derivatives were obtained from those viruses.

Sequence and phylogenetic analysis. The coding sequence for the HA1, NA, NS, and M gene segments from 24 H5 isolates were determined and compared with other sequences available in the database. The HA1 phylogenetic tree demonstrated a close relationship between all recent non-turkey and non-chicken origin H5 isolates, and these isolates were clearly different from the Pensylvania/83 lineage. Within these recent isolates, two distinct clusters were observed. One cluster of viruses contained only isolates obtained from 2001 and 2002. Similar tree topology was also observed in NA, MA, and NS phylogenetic tree with different NS subtype.

The California turkey isolate clustered with one of the lineage based on HA, M, and NS sequence. However, this isolate had unique N2 sequence and did not cluster with any other recent isolates.

The Texas H5N3 isolate clustered most closely with recent isolates (A/Enviroment/NY/5626-1/98)

obtained from non-LBM premises in 1998 (93.2% nucleotide sequence homology). Compared to other recent H5 isolates, the Texas isolate had many unique changes in the HA gene including the HA cleavage site sequence of REKR/G (most of the other recent isolates have RETR/G motif). The isolate had a glycosylation site at amino acid position 12 (Asn 12). We also analysed the N3 gene from the Texas H5N3 isolate and from other wild bird isolates, and they did not appear to be related. Further, the NA gene of Texas isolate had the 24 amino acids deletion at the stalk region that is not found in other wild bird isolates.

Replication and pathogenicity in experimentally infected chickens. SPF three-week-old chickens were infected intranasally with allantoic fluid ($10^{5.0}EID_{50}$) containing one of three viruses: A/Chicken/TX/167280-4/02, A/Turkey/CA/D0208651/02, and A/Duck/ME/151895-7A/02. The birds infected with turkey and duck isolates showed no clinical signs during observation period. However, birds infected with Texas isolate demonstrated depression at three dpi and two birds died at 6 days after infection. Much higher titer of virus was isolated from trachea at three and seven DPI in birds infected with Texas isolates compared to birds infected with the turkey and duck isolates. No virus was isolated in cloacal swab samples in birds infected with Texas and turkey isolates. Very low titer of virus was recovered from cloacal swab in birds infected with duck virus.

DISCUSSION

A study of AIV subtypes in LBMs and non-LBM premises shows the persistence of H5 subtype despite the efforts to control the infections. It is of concern because certain lineages of this subtype have caused HPAI outbreaks in U.S. Based on sequencing and animal experiment result, all the recent H5N2 viruses from the LBMs and non-LBMs were consistent with that of low pathogenic AIV. On the phylogenetic analysis, some isolates tend to cluster together, but it is not clear whether those isolates represent endemic infection in poultry or separate introduction from wild birds. It is more likely a separate introduction because the clustering coincided with the year of virus isolation and there was no genetic evidence of adaptation in poultry.

Unlike the H5N2 viruses, the Texas H5N3 virus had multiple basic amino acids at the cleavage site (R-E-K-R). This HA cleavage site sequence has the potential to be virulent motif if the carbohydrate residue near the cleavage site at amino acid position 12 (Asn 12) was removed. Thus, a single point mutation could potentially yield a HP virus by removing the glycosylation site near cleavage site. Phylogenetic analysis suggests that the Texas isolate originated from wild waterfowl like other H5 isolates in North America, but it has been circulating in poultry for an extended period of time before it was first isolated. The stalk deletion in the NA gene and previous observation of positive H5N3 serology reinforce this conclusion. Though this isolate was low pathogenic by standard pathotype test, this isolate replicated efficiently in chickens and produced clinical signs and mortality. Although the majority of recent H5 isolates are low pathogenic and represent typical avirulent waterfowl-like cleavage site sequence, the outbreak in Texas and the relationship between this poultry farm and LBM underscores the importance of ongoing surveillance and control efforts of the H5 subtype AIV in North America.

REFERENCES

1. Easterday, B. C., V. S. Hinshaw, and D. A. Halvorson. Influenza. In: Disease of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C.W. Beard, W. M. Reid, and H. W. Yoder, eds. Iowa State University Press, Ames, IA. pp. 583-606. 1997.

2. Suarez, D.L. and D.A. Senne. Sequence analysis of related low-pathogenic and highly pathogenic H5N2 avian influenza isolates from United States live bird markets and poultry farms from 1983 to 1989. Avian Dis. 44:356-364. 2000.

3. Panigrahy, B., D.A. Senne, and J.C. Pedersen. Avian influenza virus subtypes inside and outside the live bird markets, 1993-2000: a spatial and temporal relationship. Avian Dis. 46:298-307. 2002.

EVALUATION OF MOLECULAR MARKERS FOR PATHOGENICITY IN RECENT H7N2 AVIAN INFLUENZA ISOLATES FROM THE NORTHEASTERN UNITED STATES

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SUMMARY

Since 1994 low pathogenic H7N2 subtype avian influenza virus (AIV) has been routinely isolated from urban live bird markets (LBM's) in the northeastern U.S. and occasionally from commercial poultry in the region. The persistence of AIV in the LBM system for the past eight years has also allowed the virus to evolve and become adapted to replication in gallinaceous poultry. Historically, one outcome of the persistence of low pathogenic H5 or H7 AIV in poultry has been the emergence of the highly pathogenic form of the virus, in some cases within months to a few years after initial detection. Pathogenicity of AIV for chickens and turkeys has been highly correlated with the amino acid sequence at the hemagglutinin cleavage site, where a sequence with five or more basic amino acids for the H7 subtype is considered to fulfill the molecular criteria for highly pathogenic AIV. Between 1994 and 2002, five cleavage site sequences have been observed from 50 AIV isolates from several outbreaks involving this lineage. The earliest isolates, from 1994, which persisted until 1999, had two basic amino acids. In 1995, and from 1999 to 2002, sequences with three basic amino acids were observed. Finally in 2002 viruses with four basic amino acids at the cleavage site were isolated. The trend over the past eight years has been toward more basic amino acids at the cleavage site, and it is likely that a fifth basic amino acid would result in the emergence of a highly pathogenic virus.

INTRODUCTION

H7N2 avian influenza virus (AIV) has been routinely isolated from live bird markets (LBM's) in New York and New Jersey since 1994 (6,7,10). The virus has been transmitted to commercial poultry operations in Pennsylvania (1997-1998, 2001-2002) (12), Virginia (2002) and North Carolina (2002) resulting in outbreaks ranging from involvement of only a few premises to an outbreak involving nearly 200 farms in Virginia in 2002. Although the virus has remained low pathogenic, it has had a substantial economic impact.

Attempts at eradicating AIV's from the LBM's have been made; however, success has been limited. Probably because control efforts have not been as rigorous as those for commercial poultry and there are no uniform surveillance and control programs among the states in which the LBM's are located.

During the eight years that this virus lineage has circulated, the virus has become adapted to growth in gallinaceous poultry, which are not natural host species for AIV, and has established itself as a relatively stable genetic lineage. Although, host specific changes may occur in multiple genes, there may also be changes that affect pathogenicity. Specifically, the hemagglutinin (HA) protein cleavage site has been well established as a marker for AIV pathogenicity in chickens and turkeys (reviewed in 1). This study traces the changes which occur in the HA cleavage site in the recent North American H7N2 AIV's and evaluates them in regard to what is currently understood about the cleavage site sequence and pathogenic potential.

MATERIALS AND METHODS

Virus isolates. Influenza isolates were obtained from the repositories at the National Veterinary Services Laboratories or Southeast Poultry Research Laboratory. All isolations were made from tracheal or cloacal swabs from birds, primarily chickens or turkeys in live-bird markets or on poultry farms, or from environmental swabs of the premises of live-bird markets and poultry farms. AIV isolations were made in 9-11 day-old embryonating specific pathogen free chicken eggs as previously described (9). A total of 50 AIV isolates were included in this study.

Sequencing. RNA was isolated from the allantoic fluid (after no more than two passages in chicken embryos) with Trizol LS reagent (Life Technologies, Rockville, MD) in accordance with the manufacturers instructions. The entire coding regions of the HA gene segment was amplified with the Qiagen one-step RT-PCR kit (Qiagen Inc., Valencia, CA) using primers which included at least the conserved 12 or 13 nucleotides at the ends of the gene segment as previously described (11). The RT-PCR products were electrophoresed on 1.2% agarose gels and extracted with the Qiagen gel extraction kit (Qiagen) and were subsequently cloned into the pAMP1 vector (Gibco). Sequencing was performed with the ABI PRISM

BigDye terminator sequencing kit (Perkin-Elmer, Foster City, CA). Sequencing reactions were run on the PE 3700 automated sequencer (Perkin-Elmer).

Sequence analysis. Sequence analysis was carried out with the Lasergene software (DNAStar, Madison, WI). The ClustalV algorithm was used for multiple sequence alignments.

RESULTS

Hemagglutinin cleavage site sequence. Between 1994 and 2002 five HA cleavage site sequences were observed in H7 subtype AIV isolates from LBM's in NY, NJ and MA and from commercial poultry in PA, VA and NC. The three earliest H7 subtype isolates from 1994 and three H7N3 viruses from 1999, had the sequence: N-P-K-T-R /G, with two basic amino acids ("K" or "R") at the cleavage site. One isolate from 1995 and 1998 each, had a sequence with three basic amino acids: K-P-K-T-R /G. The predominant sequence from 1995 to 1999 (16 isolates) had only two basic amino acids, however the –2 amino acid changed from threonine to proline (N-P-K-P-R /G) (originally reported by Suarez, et al. (10). In 1998 an isolate with the sequence K-P-K-P-R /G was observed, this sequence, with three basic amino acids, became the most common sequence (23 isolates total) from 1999 until the present (sampling through mid-2002). The first sequence with four basic amino acids: K-P-K-K-R /G, was seen in early 2002 and was present in three isolates included in this study.

The codon for the proline at the –4 position is CCA, therefore to become an arginine, only a single nucleotide change is necessary (CCA to CGA), but a minimum of two changes are required for a substitution to a lysine (CCA to AAA or AGA).

DISCUSSION

Due to poor biosecurity, a constant influx of susceptible hosts, and the routine intermingling of many avian species including waterfowl and gallinaceous poultry, the urban LBM's provide a unique environment for the ecology and evolution of AIV. A single H7N2 AIV lineage has persisted in gallinaceous poultry in the LBM system for at least eight years. In many previous outbreaks of H5 or H7 subtype AIV in poultry, a low pathogenic avian influenza (LPAI) virus has evolved into highly pathogenic avian influenza (HPAI) (2,3,4,5).

The most important and best characterized molecular indicator of AIV pathogenicity in chickens and turkeys is the presence of multiple basic amino acids at the HA cleavage site. The presence of multiple basic amino acids (lysine and arginine) at the HA cleavage site allows the HA to be cleaved in a wide range of tissue types. Because cleavage of this protein is necessary for infection, this leads to systemic infection and subsequently manifests as HPAI. In contrast, LPAI infection remains localized in the respiratory and intestinal tracts (reviewed in 1).

Since 1994, the number of basic amino acids in the HA cleavage site of the H7 AIV's from this lineage has increased from two to four, indicating a trend toward more basic amino acids. Importantly, it is currently not clear whether the gene with four basic amino acids has a competitive advantage over the three basic amino acid motif, which has been predominant since 1999, and would therefore replace it as the most common sequence.

Based on what is currently known about the molecular basis of AIV pathogenicity, five basic amino acids at the cleavage site would be considered the minimum number to produce a highly pathogenic H7 subtype virus (8). One may conclude that if these viruses continue to circulate long enough, a cleavage site that is consistent with the highly pathogenic motif will eventually arise, although it is not possible to predict when that could occur. The current trend in the HA cleavage site also reinforces the need to eradicate AIV from the live-bird markets.

REFERENCES

1. Alexander, D.J. A review of avian influenza in different bird species. Vet. Microbiol. 74:3-13. 2000.
2. Donatelli, I., L. Campitelli, L. Di Trani, S. Puzelli, L. Selli, A. Fioretti, D.J. Alexander, M. Tollis, S. Krauss, and R.G. Webster. Characterization of H5N2 influenza viruses from Italian poultry. J. Gen. Virol. 82:623-30. 2001.
3. Garcia, M., J.M. Crawford, J.W. Latimer, E. Rivera-Cruz, and M.L. Perdue. Heterogeneity in the haemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. J. Gen. Virol. 77:1493-504. 1996.
4. Horimoto, T., E. Rivera, J. Pearson, D. Senne, S. Krauss, Y. Kawaoka, and R.G. Webster. Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. Virology 213:223-30. 1995.
5. Kawaoka, Y. and R.G. Webster. Evolution of the A/Chicken/Pennsylvania/83 (H5N2) influenza virus. Virology 146:130-7. 1985.
6. Pomeroy, B.S.E. Avian Influenza. in Proceedings of the 98th Annual Meeting of the U.S. Animal Health Association. Richmond, VA. pp. 512-523. 1994.
7. Senne, D. NVSL Report on Avian Influenza. in Proceedings of the 101st Annual Meeting of the U.S.

Animal Health Association. Richmond, VA. pp. 497-498. 1997.

8. Senne, D.A., B. Panigrahy, Y. Kawaoka, J.E. Pearson, J. Suss, M. Lipkind, H. Kida, and R.G. Webster. Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. Avian Dis. 40:425-37. 1996.

9. Spackman, E., D.A. Senne, T.J. Myers, L.L. Bulaga, L.P. Garber, M.L. Perdue, K. Lohman, L.T. Daum, and D.L. Suarez. Development of a real-time reverse transcriptase PCR assay for type A influenza virus and the avian H5 and H7 hemagglutinin subtypes. J. Clin. Microbiol. 40:3256-60. 2002.

10. Suarez, D.L., M. Garcia, J. Latimer, D. Senne, and M. Perdue. Phylogenetic analysis of H7 avian influenza viruses isolated from the live bird markets of the Northeast United States. J. Virol. 73:3567-73. 1999.

11. Suarez, D.L. and M.L. Perdue. Multiple alignment comparison of the non-structural genes of influenza A viruses. Virus Res. 54:59-69. 1998.

12. Ziegler, A.F., Davdison, S., Acland, H. and R.J.Eckroade. Characterization of H7N2 (Non-pathogenic) Avian Influenza Virus Infections in Commercial Layer, in Pennsylvania, 1997-98. Avian Dis. 43:142-149. 1999.

THE H7N3 AVIAN INFLUENZA OUTBREAK IN NORTHERN ITALY IN 2002

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INTRODUCTION

Between 1999 and 2001 North-eastern Italy was affected by four subsequent waves of avian influenza caused by a type A influenza virus of the H7N1. The first epidemic wave was caused by a virus of low pathogenicity, which subsequently mutated to a highly pathogenic (HPAI) virus of the same subtype (4). The HPAI epidemic occurred in the Veneto and Lombardia regions, which raise approximately 65% of Italy's industrially reared poultry. In addition, some areas affected by the epidemic (particularly south of Verona province), are densely populated poultry areas (DPPA), which count (in some municipalities of Verona province) over 70 000 birds raised per square kilometer. The HPAI epidemic caused directly or indirectly the death or culling of over 13 million birds that inevitably determined the disruption of the marketing system and great economic losses to the poultry industry and to the social community. Following eradication, the formerly HPAI infected areas were restocked. Four months after the stamping out of the last outbreak, LPAI re-emerged twice, thus determining the poultry industry to request and obtain, through the Italian veterinary authorities, vaccination against avian influenza of the H7 subtype.

In compliance the guidelines indicated by the European Commission (1) the vaccination strategy proposed and applied was that of using an inactivated oil emulsion vaccine containing a strain with a homologous haemagglutinin (H) group and a heterologous neuraminidase (N) group (1). The reason for this was the possibility of using it as a natural "marker" vaccine, or more correctly a DIVA [Differentiating Infected from Vaccinated Animals] vaccine. The vaccination campaign lasted for 14 months and was associated to a coordinated territorial strategy aimed at establishing whether the field virus was still circulating, and ultimately resulted in the eradication of infection (3).

During the month of August 2002, serological positivity at the abattoir to an H7 virus were detected in three meat turkey flocks originating from Brescia province. This was a result of a surveillance program which was implemented in Italy following the epidemics which had occurred in the previous years. Intensive surveillance in the whole area did not allow the identification of additional outbreaks.

In October 2002, hemagglutination inhibition (HI) tests on serum samples from meat turkeys in the Brescia province were again found to be positive for antibodies to the H7 subtype of avian influenza. The following paper describes the clinical, pathological and epidemiological data obtained from the epidemic and reports the results of a preliminary phylogenetic analysis.

CASE REPORT

Following the implementation of a sero-surveillance program, serologically positive birds were detected in 12 further meat-turkey flocks in the

Mantova, Vicenza and Verona provinces. Virus isolation yielded an H7N3 subtype influenza A virus of low pathogenicity (LPAI). To 20th December 2002, 135 outbreaks have been notified of which 120 in meat turkeys, two in turkey breeders, two in broilers, two in layers, four in broiler breeders, three in guinea fowl, one in a live-bird market dealer, and one in a backyard flock.

Clinical and gross findings. In meat turkeys initially, clinical signs were completely inapparent, to the extent that evidence of infection was only detected at the abattoir as a result of a surveillance program. Subsequently instead, probably as a result of adaptation of the virus to the domestic host, general symptoms such as reluctance to feed, tendency to gather and ruffled feathers were accompanied by respiratory signs such as sinusitis, sneezing, rales and in some cases gasping. This clinical picture could be seen in adult birds as well as in poults in which clinical disease was particularly evident in birds aged 25-40 days. Morbidity was 100%, while mortality rates were generally low in the order of 3-7% depending on the age and general condition of the group. In a limited number of cases mortality rates reached 30%.

In turkey breeders a sharp drop in egg production (from 60% to 10%) and general signs such as reluctance to move, depression and drop in feed consumption could be seen. This clinical picture was not associated to any increased mortality.

Non-specific clinical signs were observed in the guinea fowl, broilers, broiler breeders, and in the layers.

On post mortem, in turkeys, the constant gross finding was acute pancreatitis. In meat turkeys this finding was associated with congestion of the lung and trachea and to airsacculitis. In breeders it was accompanied by egg yolk peritonitis. No post mortem alteration was present in any of the chickens affected.

Virological investigations. The H7N3 isolates obtained from the dead or humanely sacrificed birds were isolated in embryonated fowl's SPF eggs, and caused embryo mortality three to four days post inoculation. The virulence assays performed as indicated by Directive 92/40/EC, indicated that the isolate was of low pathogenicity. The intravenous pathogenicity index in 6 week-old SPF chickens was 0.0 and the deduced sequence of the cleavage site of the hemagglutinin molecule was of PEIPKGR*GLF and thus did not contain multiple basic amino acids, which are considered a marker for virulence.

Phylogenetic analysis performed on the hemagglutinin (H) gene indicated that this isolate is part of the Eurasian lineage of H7 viruses. The virus was related, but not identical, to the H7N1 virus that caused the 1999-2001 avian influenza epidemic in Italy. The virus was also unrelated to the H7N3 strain contained in an inactivated vaccine (A/ck/Pakistan/95) used in a "DIVA" (Differentiating Infected from Vaccinated Animals) vaccination strategy, which was used to control the re-emergence of a LPAI H7N1 virus following the 1999-2001 Italian epidemic (3). Sequence data obtained from early isolates indicate the presence of a neuraminidase stalk deletion and the absence of additional glycosilation sites at the globular head of the hemagglutinin molecule, which are considered a result of acquired adaptation to the domestic host (2, 7).

DISCUSSION

The findings reported above indicate that isolate A/ty/Italy/2002/H7N3 appears to be virus of novel introduction into the domestic poultry population of Northern Italy. The preliminary phylogenetic analysis clearly indicates that the H7 gene of the isolate is related to Italian 1999-2001 H7N1, but is not identical to it, and therefore a donation of the gene from the H7N1 virus can be ruled out. Similarly it is clear that the virus is unrelated to the A/Ck/Pakistan /95/H7N3 used as a vaccine strain used in the framework of a "DIVA" vaccination strategy.

The clinical and pathological lesions are similar, although less severe than those observed during the Italian 1999-2001 LPAI H7N1 epidemic (5). The milder clinical and pathological traits of the H7N3 infection could be related to the nature of the strain or to the lower degree of adaptation these isolates have to the domestic host.

During the past five years North–eastern Italy has experienced one outbreak of highly pathogenic avian influenza (HPAI) caused by a virus of the H5N2 subtype, three epidemic waves of LPAI of the H7N1 subtype, one epidemic of HPAI caused by mutation of the H7N1 LPAI virus, and in 2002 the recent outbreak of H7N3 described above. The resultant major economic losses following the recent avian influenza epidemics place the future of the industry at risk. The introduction of novel subtypes and the recurrent nature of infectious waves over the last five years suggest that treating the appearance of these influenza viruses as a 'veterinary emergencies' may no longer be appropriate from a clinical and economical viewpoint.

We believe it would be advisable to establish permanent surveillance programs for AI infections, and in defined risk areas, to implement long term control programs based on biosecurity, surveillance, and possibly on the application of a "DIVA" vaccination strategy. The adoption of such a vaccination strategy, and the subsequent preservation of a certifiable health status for poultry meat exports, should lead to a more stable economic environment for one of Italy's major fresh-meat export industries. Furthermore, decreased

susceptibility to field infection following vaccination, plus the rapid detection of any field challenge, should reduce the total number of infected animals and therefore minimize the number of birds stamped out during any outbreak -- an extremely desirable outcome in terms of bird welfare and public acceptance of intensive agriculture disease control strategies.

REFERENCES

1. Anonymous. The Definition of Avian Influenza and the Use of Vaccination against Avian Influenza. EC Scientific Committee on Animal Health and Animal Welfare, Document Sanco/B3/AH-/R17/2000, 1-38. 2000.

2. Banks, J., E.S. Speidel, E. Moore, L. Plowright, A. Piccirillo, I. Capua, P. Cordioli, A. Fioretti, and D.J. Alexander. Changes in the haemagglutinin and the neuraminidase genes prior to the emergence of highly pathogenic H7N1 avian influenza viruses in Italy. Arch. Virol. 146: 963-973. 2001.

3. Capua I., G. Cattoli, S. Marangon, L. Bortolotti, and G. Ortali. Strategies for the control of avian influenza in Italy. Vet. Rec. 150: 223. 2002.

4. Capua I. and S. Marangon. The avian Influenza Epidemic in Italy (1999-2000): a review. Avian Path. 29: 289-294. 2000.

5. Capua I. and F. Mutinelli. A color atlas and text on Avian Influenza. Papi Editore, Bologna, Italy. 2001.

6. Matrosovich M, N. Zhou, Y. Kawaoka and R. Webster. The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. J Virol. 73: 1146-1155. 1999.

THE RAPID LABORATORY DIAGNOSIS OF AVIAN INFLUENZA VIRUS BY A COMBINATION OF DOT-ELISA AND VIRUS ISOLATION

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SUMMARY

A monoclonal antibodies (Mab) based dot enzyme-linked immunosorbent assay (Dot-ELISA) has been developed that specifically detected the avian influenza virus (AIV) directly from clinical and field specimens. The novel Dot-ELISA detects antigens of all AIV subtypes when using group-specific Mab to AIV nucleic proteins and specifically detects H7N2 subtype when using H7N2 subtype-specific Mab (4). The Dot-ELISA was specific for AIV as no cross-reactions were obtained with other avian viruses.

A combination of Dot-ELISA and virus isolation was successfully used for the rapid laboratory diagnosis of AIV within 24 hours during the 2001/02 outbreak in Pennsylvania. Clinical and field specimens including tracheal swabs, cloacal swabs, environmental swabs and watery manure samples were collected from AIV affected and suspicious flocks and processed with viral transfer medium. These specimens were screened for the presence of antigens of AIV by the Dot-ELISA first, and then were inoculated into in embryonating chicken eggs (ECE) for virus isolation following standard procedures (1, 2) with the modification of daily withdrawing of allantoic fluid (AF) samples. After 20-24 hours and again 40-48 hours post inoculation, 0.2-0.5 ml of AF per egg was drawn (using a 1cc syringe with 25G1 needle through the inoculation hole using sterile technique) from the ECE that had been inoculated with a specimen which was positive for AIV by Dot-ELISA or from a clinically suspicious case of AIV infection. After AF samples were drawn, the ECE were resealed and placed beck to egg incubator for continuous incubation up to 72-to-96 hours. The early incubation AF samples were tested for AIV by the Dot-ELISA and hemagglutination (HA) test.

Among seven flocks affected with AIV during the 2001/02 outbreak in Pennsylvania (3), the Dot-ELISA detected AIV directly from clinical specimens before virus isolation from two of two broiler breeder flocks, and four of five broiler flocks. The presence of AIV was confirmed by virus isolation in ECE within 24 hours by means of the modified procedure.

Findings in this study indicated that the H7N2 virus present in a clinical specimen grew rapidly in ECE and yielded sufficient HA titers for AIV identification within 24 hours post inoculation if the specimen was positive or suspicious for AIV by the Dot-ELISA. The combination of AIV screening test by Dot-ELISA and virus isolation in ECE with early

withdrawing AF provides a rapid and effective laboratory diagnosis of AIV during an outbreak.

The Mab-based Dot-ELISA is a rapid same day test and comparable to the commercial Directigen® test in the detection of AIV antigens from clinical and field specimens. Sensitivity and specificity of the Dot-ELISA and comparison to the commercial Directigen® test in the detection of AIV will be discussed.

(A full-length article describing the Mab-based Dot-ELISA for detection of avian influenza virus has been submitted for publication in *Avian Diseases.*)

REFERENCES

1. David, E.S., D.A. Senne and C.W. Beard. Avian Influenza. In: Isolation and Identification of Avian Pathogens. 4th ed. Am Assoc Avian Pathol, Kennett Square, PA, pp. 150-155. 1998.

2. Easterday, B. C., V. S. Hinshaw, and D. A. Halvorson. Influenza. In: Diseases of Poultry, 10th ed, by Calnek, B. W., H. J. Barnes, C. W. Beard, L.R. McDougald, and Y.M. Saif. Iowa State University Press, Ames, pp. 583-605. 1997.

3. Eva Wallner-Pendleton, P.A. Dunn, D.C. Kradel, D. Shaw, H. Lu, P. Miller, and D.J. Henzler, D.J. Low Path H7N2 Avian Influenza in Pennsylvania: The Disease in Broilers and Broiler Breeders. North Atlantic Poultry Health and Management Conference. Portsmouth, New Hampshire. March 20-21, 2002.

4. Wang X, A.E. Castro, M.D. Castro, H. Lu, D. Weinstock, N. Soyster, W. Scheuchenzuber, M. Perdue. Production and evaluation criteria of specific monoclonal antibodies to the hemagglutinin of the H7N2 subtype of avian influenza virus. J Vet Diagn Invest. 12(6):503-9. 2000.

HIGHLY PATHOGENIC AVIAN INFLUENZA IN CHILE

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Influenza A viruses are generally believed to occur worldwide in reservoir hosts such as waterfowl and shorebirds. On occasion, influenza A viruses spill over from the reservoir hosts into unnatural hosts such as commercial chickens and turkeys causing outbreaks of avian influenza. It is interesting to note that despite presence of reservoir species in South America, no isolations of influenza A virus have been reported from wild birds or commercial poultry until recently.

In 2002, the first reported case of avian influenza in South America occurred in commercial chickens and turkeys in Chile. The virus isolated in May 2002 was characterized as an H7N3 subtype of low pathogenicity. Within a few weeks the virus mutated to a highly pathogenic virus with unusual molecular characteristics. This paper will summarize the outbreak and present information on the characteristics of the viruses isolated from the outbreak.

Case history. In late April and early May 2002, a flock of broiler-breeders experienced a slight drop in egg production and salpingo-peritonitis. The farm had approximately 540,000 breeders of various ages in multiple houses. A hatchery was also present in the premises. Infectious bronchitis (IB) was suspected as the cause of the clinical syndrome. On May 9, 2002, samples were collected from the flock and submitted to a private laboratory for virus isolation studies. No IB virus was isolated; however, an unidentified hemagglutinating virus was recovered. The isolate was submitted to the Federal laboratory Servicio Agricola Ganadero (SAG), Santiago, Chile, the official laboratory in Santiago (SAG) where a tentative diagnosis of avian influenza was made. As subtyping reagents for avian influenza virus (AIV) were not available at SAG, the isolate was sent to the National Veterinary Services Laboratories (NVSL), Ames, Iowa USA (the Office International des Epizooties reference laboratory for avian influenza in North America), for virus subtyping and characterization. The virus was characterized as H7N3 AIV of low pathogenicity.

On May 23, 2002, SAG officials were notified by the broiler-breeder company that a precipitous drop in egg production with high mortality was observed in several of the broiler-breeder houses in the index farm. Toxicity or poisoning was suspected. The following day, the farm was visited by SAG personnel where a presumptive diagnosis of highly pathogenic avian influenza (HPAI) was made based on clinical signs and lesions. Subsequently, serums collected from birds in several houses were found to be positive for antibodies to influenza A virus by the agar gel immunodiffusion

(AGID) test. However, samples collected for virus isolation were negative for the AIV.

The first week in June 2002, because of the high mortality and confirmed presence of low pathogenic (LP) H7N3 AIV earlier in the farm, a decision was made to depopulate the premises. At the time of depopulation, approximately 110,000 of the 540,000 broiler-breeders had died of the disease. Additional samples for virus isolation were collected from young birds previously negative for antibodies to AIV. Five unidentified hemagglutinating viruses were isolated from the samples. The 5 isolates were sent to the NVSL, Ames, Iowa USA and to the Veterinary Laboratory Agency (VLA), Weybridge, UK. All 5 isolates were characterized as HPAI H7N3 viruses at both laboratories.

A second outbreak involved a turkey breeder operation located within 2½ miles from the positive broiler breeder farm. The farm had a total of approximately 26,000 turkeys in 4 dark-out houses and another 24,000 turkeys in 4 separate breeding houses. The premises also had a hatchery. The first evidence of clinical disease was reported on June 1, 2002. H7N3 AIV was also confirmed and the premises was depopulated. The virus recovered from the turkey breeders was very closely related to the HPAI broiler breeder isolates. No additional premises were affected.

Avian influenza virus and the molecular basis for pathogenicity. Avian influenza viruses are segmented, negative sense, single-strand RNA viruses of the family *Orthomyxoviridae*. AIVs are classified as type A influenza viruses based on the antigenic nature of the nucleoprotein and matrix proteins. They are further divided into subtypes based on the antigenic nature of the surface proteins: hemagglutinin (H) and neuraminidase (N). Fifteen H and 9 N subtypes are currently recognized. Each AIV has one H and one N antigen on its surface, apparently in any combination. Most combinations have been recovered from reservoir species, i.e., waterfowl and shore birds.

Virulence of AIV for chickens and turkeys varies from a mild localized infection with no or few clinical signs, referred to as LPAI, to a systemic disease with high mortality, referred to as HPAI. To date, all HPAI has been caused by subtypes H5 or H7, although the overwhelming majority of H5 and H7 viruses are LPAI. Since 1959, 18 reports of HPAI have been documented; 7 caused by H5 subtype and 11 by H7 subtype. Available evidence suggests that HPAI viruses emerged from the LPAI virus precursors by mutations (1,2,3).

In recent years, the molecular basis for pathogenicity of AIV has been studied extensively. It is well established that virulence of AIV is a polygenic trait. However, the H plays a dominant role in virulence. The H protein is produced as an inactive precursor molecule (HA0), which must be cleaved by host cell proteases to be functional (4). Therefore, the amino acid composition at the cleavage site and presence of the appropriate host cellular proteases determine the virulence of the virus and tissue tropism. Isolates that have a few basic (arginine and lysine) amino acids at the cleavage site are cleaved by trypsin-like proteases found primarily in the intestinal and respiratory tracts of the avian host; therefore, restricting virus replication to those localized sites. When replication is restricted, the host can initiate an immune response, limiting cellular damage caused by the infection, and often recover with little or no consequence. On the contrary, isolates that have multiple basic amino acids at the cleavage site, i.e., HPAI viruses, the H is cleaved by a class of substilisin-related proteases such as furin and PC6 that are ubiquitous in most eukaryotic cells. Therefore, infections with HPAI viruses are systemic, leading to severe cellular damage, illness and death (5).

Characteristics of the Chilean H7N3 virus. The first isolate from the broiler-breeders recovered in May 2002, was characterized as LPAI H7N3. The isolate had a cleavage site (c.s.) amino acid sequence of PEKPKTR/GLF and an intravenous pathogenicity index (IVPI) of 0.0. However, subsequent H7N3 isolates recovered in June 2002 were characterized as HPAI. The HPAI isolates had a 10 amino acid insertion (shown in bold) with two motifs at the c.s.: PEKPKT**CSPLSRCRKT**R/GLF and PEKPKT**CSPLSRCRET**R/GLF, and IVPIs in the range of 2.8 – 3.0. Phylogenetic analysis showed that the Chilean LPAI and HPAI viruses were closely related. The amino acid sequences of the HPAI H7N3 viruses are unusual because the motifs do not conform to the multiple basic amino acid motif of known HPAI viruses. The finding will have a significant impact on the future molecular definition used to identify HPAI viruses.

Recent studies on the Chilean LP and HP viruses showed that the 10 amino acid insert found at the cs of the hemagglutinin was likely due to a recombination involving the nucleoprotein gene. The 30 nucleotide insert showed 100% homology with a 30 nucleotide segment found in the nucleoprotein gene. The mechanism by which recombination could have occurred is not known. Recombination events involving influenza A viruses are rare.

Significance of the outbreak. The HPAI H7N3 outbreak in Chile represents the second documented case where a low pathogenic H7 virus has mutated to a highly pathogenic virus after circulating in poultry. The first such case occurred in Italy in 1999-2000 when a low pathogenic H7N1 AIV mutated to a highly pathogenic virus, affecting more than 12 million chickens and turkeys in over 400 premises.

Phylogenetic analysis of the Chile isolates would suggest that the HPAI viruses most likely evolved from the LPAI precursor virus or a very closely related H7N3 virus.

It should be noted that AIV has never been reported in commercial poultry in any country in South America prior to the diagnosis of H7N3 AI in Chile. However, Chile has been monitoring commercial flocks for presence of antibodies to AIV since 2000. Since 2000, more than 69,000 serums from 2200 flock had been tested. No positive flocks were identified prior to the outbreak of H7N3 AI in 2002.

Summary. In July 2002, HPAI H7N3 was diagnosed in Chile; this was first report of HPAI in South America. The outbreak was limited to two premises; a large broiler-breeder farm and a nearby turkey-breeder farm. Approximately 110,000 of the 540,000 broiler-breeders died of the disease before the premises was depopulated. The initial isolate from the broiler-breeders recovered in May 2002, was characterized as LPAI H7N3. The isolate had an amino acid sequence at the hemagglutinin cleavage site c.s. of PEKPKTR/GLF and an intravenous pathogenicity index (IVPI) of 0.0. However, subsequent H7N3 isolates recovered in June 2002, were characterized as HPAI. The HPAI isolates had a 10 amino acid insertion (shown in bold) with two motifs at the c.s.: PEKPKT**CSPLSRCRKT**R/GLF and PEKPKT**CSPLSRCRET**R/GLF, and IVPIs of 2.8 – 3.0. Phylogenetic analysis showed that the Chilean LPAI and HPAI viruses were closely related. The amino acid sequences of the HPAI H7N3 viruses are unusual because the motifs do not conform to the multiple basic amino acid motif characteristic of known HPAI viruses. A recombination event with the nucleoprotein gene is believed to be the source of the 30 nucleotide insert at the c.s. It is likely that this event will have a significant impact on the molecular definition of HPAI viruses.

REFERENCES

1. Garcia, M., J. M. Crawford, J. W. Latimer, E. Rivera-Cruz, and M. L. Perdue. Heterogeneity in the hemagglutinin gene and emergence of the highly pathogenic phenotype among recent H5N2 avian influenza viruses from Mexico. J. Gen. Virol. 77:1493-1504. 1996.

2. Perdue, M., J. Crawford, M. Garcia, J. Latimer, and D. Swayne. Occurrrence and possible mechanisms of cleavage site insertions in the avian influenza hemagglutinin gene. Proceedings of the fourth International Symposium on Avian Influenza, Athers, GA. U. S. Animal Health Association pp. 182-193. 1998.

3. Rohm, C., T. Horimoto, Y. Kawaoka, J. Süss, and R. G. Webster. Do hemagglutinin genes of highly pathogenic avian influenza viruses constitute unique phylogenetic lineages? Virology 209:664-670. 1995.

4. Rott, R. Molecular basis of infectivity and pathogenicity of myxoviruses. Archives of Virol. 59:285-298. 1979.

5. Rott, R. The pathogenic determinant of influenza virus. Vet. Microbiol. 33:303-310. 1992.

EXOTIC NEWCASTLE DISEASE VIRUS CHARACTERIZATION

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Newcastle disease (ND) is considered to be the most important viral disease of poultry worldwide. Most all commercial poultry are vaccinated to reduce the clinical effects of ND and control spread of the disease. Despite the widespread use of ND vaccines, the disease continues to cause problems in many parts of the world. Between 1997 and 2001, more than 100 countries reported presence of ND to the Office International des Epizooties (OIE). The reports included those from the United States (1998, 2002-03), Canada (1997), Mexico (2000-2001), and Central America (2000). The recent outbreak of exotic ND (END) in backyard game birds and commercial poultry in southern California has brought renewed focus on the importance of this disease and the economic consequences following introductions of END into the United States.

The diagnosis of ND is not straightforward because the clinical signs and lesions of ND are not pathognomonic and because of the need to differentiate END from other clinicopathologic forms. Therefore, laboratory confirmation of ND is a critical component of surveillance and control programs. This paper will focus on the current regulatory policy, molecular markers of virulence, and laboratory tests currently used to characterize field isolates of ND.

Regulatory Policy for ND. Control programs for ND in the Unites States have evolved as we learn more about the disease and gain a better understanding of the molecular basis for pathogenicity. In the 1970s and

1980s, regulatory programs were aimed at control of only the most virulent strains of the virus, i.e., viscerotropic velogenic NDV (VVNDV). This required inoculating 6- to 8-week-old chickens from a specific-pathogen-free flock by the cloacal route and examining the moribund or dead chickens for lesions compatible with VVND. In addition, the pathotype of the virus was determined by the mean death time (MDT), the time required for the minimum lethal dose of virus to kill chicken embryos. The recommendation to expand the definition to include all velogenic ND regardless of its tropism was made in 1983; however, the change was not published until 1997. The definition included the neurotropic velogenic strains of ND such as Texas G. B., a strain that is widely used in many laboratories as the standard challenge virus to evaluate the efficacy of ND vaccines. In 1999, the Office International des Epizooties (OIE) revised the definition of ND to include a molecular virulence marker as well as the intracerebral pathogenicity index (ICPI). Under the new definition, velogenic and mesogenic strains meet the requirements for international reporting if diagnosed in commercial poultry. However, the United States still defines END in the 2002 edition of the 9 CFR (Part 94.0), as "velogenic Newcastle disease."

Molecular basis for pathogenicity. Over the last few years, our understanding of the molecular basis for pathogenicity of ND virus has become clear. The ND virus has two functional spike-like proteins projecting from the envelope: the hemagglutinin /neuraminidase (HN) and the fusion (F) proteins. The HN functions as the attachment protein and the receptor destroying protein while the F protein initiates infection by facilitating the fusion of the virus and endosomal membrane, thus enabling the virus genes to enter the cytoplasm of the cell where replication takes place. The F protein is produced as a nonfunctional precursor glycoprotein, F0, which is cleaved by host cell proteases into functional subunits F1 and F2 that remain bound together by disulphide bonds. Post translational cleavage of the F protein is essential for the virus to be infectious (5).

Virulence of NDV is associated with its ability to replicate in a wide variety of cell types. The ability to replicate in a wide variety of cells depends on the cleavability of the F protein; the cleavability is determined by the amino acid sequence at the F protein cleavage site. Virulent strains of NDV have multiple basic amino acids (arginine and lysine) at the cleavage site and the F protein is cleaved by proteases in a wide variety of cell types (6,7). Therefore, host cell proteases and the amino acid sequence at the F cleavage site determines the spread of virus in the host. Proteases that recognize multiple basic amino acid sequences are ubiquitous in most all cells within the host, resulting in the production of infectious virus in all cells infected with virulent virus. Consequently, an infection with virulent NDV results in a systemic infection that can severely damage cells and causes systemic disease, system failure, and death. Low virulent viruses, on the other hand, possess a single arginine at the F cleavage site and are cleaved by trypsin-like proteases present in respiratory and intestinal tracts. Therefore, low virulent viruses such as lentogenic NDV produce a mild, localized infection of the respiratory and intestinal tracts. Low virulent viruses induce good immune response in the host and are commonly used in vaccines.

The reliability of the amino acid sequence in predicting virulence has been widely accepted and has recently been incorporated into the OIE definition of ND (see below).

OIE definition of ND. Historically, the terms velogenic, mesogenic, and lentogenic have been used to describe the clinicopathologic forms of ND; velogenic representing the most virulent form, mesogenic viruses are intermediate in virulence, and lentogenic viruses are the least virulent form. In 1999, the OIE adopted a new definition for ND. The new definition replaced the traditional velogenic, mesogenic, and lentogenic pathotype terms with the following (2):

> "Newcastle disease is defined as an infection of birds caused by a virus of avian paramyxovirus serotype 1 (APMV-1) that meets one of the following criteria for virulence: a) The virus has an intracerebral pathogenicity index (ICPI) in day-old chicks (*Gallus gallus*) of 0.7 or greater. Or b) Multiple basic amino acids have been demonstrated in the virus (either directly or by deduction) at the C-terminus of the F2 protein and phenylalanine at residue 117, which is the N-terminus of the F1 protein. The term "multiple basic amino acids" refers to a least three arginine or lysine residues between residues 113 to 116. Failure to demonstrate the characteristic pattern of amino acid residues as described above would require characterization of the isolated virus by an ICPI test.
>
> In this definition, amino acid residues are numbered from the N-terminus of the amino acid sequence deduced from the nucleotide sequence of the F0 gene; 113-116

corresponds to residues -4 to -1 from the cleavage site."

The OIE definition caused some confusion because it only refers to virulent viruses as NDV, while providing no recommendation for a term to designate strains that do not meet the criteria for international reporting. The term avian paramyxovirus type 1 (APMV-1) is becoming widely used to describe isolates that do not meet the OIE definition. It is recommended that the term NDV be used only for virulent strains of APMV-1.

Intracerebral pathogenicity index (ICPI). The ICPI is an *in vivo* test performed in day-old chicks. It is shown to be a reliable test to assess the virulence of ND. The disadvantages of the test are the need to have day-old chicks available at any given time and the need for biosecured cages with a supplemental heat source. The test is performed by injecting 0.05 ml of fresh infectious allantoic fluid diluted 10^{-1} (in phosphate buffered saline, w/o antibiotics) into the cranium (location is not critical) of 10 one-day-old chicks. The chicks are observed daily for 8 days for signs of disease and death. Normal (alert, moving without coordination), sick (birds exhibiting signs of paralysis or are prostrate, but excluding chicks that are dull), and dead chicks are respectively assigned numerical scores of 0, 1, or 2, and the index is expressed as the weighted mean over the total number of observations made (1).

Sequencing. Reference laboratories now routinely sequence the region of the F gene that encodes the cleavage site (c.s.). The sequence information allows a fairly accurate assessment of the virulence potential of the virus. Although sequencing is widely used and accepted, it has limitations. It has been difficult to find a single primer set that will recognize all strains of NDV. Therefore, determining the F sequence for some isolates has not been successful.

Procedures for sequencing NDV have been described (8). In summary, the RNA is extracted from the isolate, transcribed into cDNA and the cDNA amplified by PCR with forward and reverse primers that flank the F cleavage site. The PCR product is then electrophoretically separated in an agarose gel and stained with ethidium bromide to visualize the target and confirm that it is the correct size. The product is then purified to remove excess primers, standardized to the optimal concentration and sequenced with an automated sequencer utilizing fluorescent dyes. Sequence is analyzed with sequencing software and the amino acid sequence deduced from the nucleotide sequence. The entire procedure can be performed in 1-2 days; considerably faster than *in vivo* tests such as ICPI or cloacal inoculation of chickens, both of which can take 8-10 days.

Most virulent viruses have the requirement for a pair of basic amino acids at positions 112 and 113, and 115 and 116, plus phenylalanine at position 117, i.e., ^{112}R/K-R-Q-K/R-R/F^{117}. Pigeon paramyxovirus type 1 (PPMV-1) isolates show some divergence from the general sequence of virulent viruses but do have multiple basic amino acids and phenylalanine at position 117. However, low virulent viruses do not have multiple basic amino acids at the c.s. and have the general sequence ^{112}G/E-K/R-Q-G/E-R^{116} with L (leucine) at position 117 (3).

Monoclonal antibodies. Avian paramyxovirus type 1 comprises a single serotype, i.e., APMV-1 isolates are antigenically similar and cannot be distinguished with polyclonal antiserum. However, some isolates can be distinguished from each other with monoclonal antibodies (Mabs). Mabs have been developed for NDV that can recognize epitopes that vary as little as one amino acid and, therefore, can be useful in detecting slight antigenic differences among strains. This has been helpful in differentiating, for example, the commonly used vaccine strains (B1 and LaSota) from other strains of APMV-1. Mabs have been especially helpful in the differentiation and identification of pigeon paramyxovirus type 1 (PPMV-1) from other strains of APMV-1. It should be noted that Mabs should never be used as the primary method to determine the virulence of an NDV isolate.

A limited number of Mabs (15C4, 10D11, B79, and AVS) are available for research and diagnostic purposes from the National Veterinary Services Laboratories, Ames, IA. The reactivity of the antibodies have been published (4).

REFERENCES

1. Allen, W. H., J. E. Lancaster, and B. Toth. Tests for virus characteristics. In: Newcastle Disease Vaccines. Food and Agriculture Organization of the United Nations, Rome, Italy. pp74-79. 1978.

2. Anonymous. Newcastle disease. In: Manual of Standards for Diagnostic Tests and Vaccines, fourth ed. Office International des Epizooties, Paris, France. pp. 221-232. 2000.

3. Collins, M. S., J. B. Bashiruddin, and D. J. Alexander. Deduced amino acid sequences at the fusion protein cleavage site of Newcastle disease viruses showing variation in antigenicity and pathogenicity. Archives of Virol. 128: 363-370. 1993.

4. Lana, D. P., D. B. Snyder, D. J. King, and W. W. Marquardt. Characterization of a battery of monoclonal antibodies for differentiation of Newcastle disease virus and pigeon paramyxovirus-1 strains. Avian Dis. 32:273-281. 1988.

5. Nagai, Y., Klenk, H-D, and R. Rott. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology 72:494-508. 1976.

6. Rott, R. Molecular basis of infectivity and pathogenicity of myxoviruses. Archives of Virol. 59:285-298. 1979.

7. Rott, R., and H-D. Klenk. Mocecular basis of infectivity and pathogenicity of Newcastle disease virus. In: Newcastle Disease. D. J. Alexander, ed. Kluwer Academic Publishers, Boston, MA. pp. 98-112. 1988.

8. Seal, B. S., D. J. King, and J. D. Bennett. Characterization of Newcastle disease virus isolates by reverse transcription PCR coupled to direct nucleotide sequencing and development of sequence database for pathotype prediction and molecular epidemiological analysis. J. Clin. Microbiol. 33(10):2624-2630. 1995.

PATHOLOGY OF EXOTIC NEWCASTLE DISEASE IN BACKYARD CHICKENS DURING THE 2002-2003 CALIFORNIA OUTBREAK

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SUMMARY

On October 1, 2002, Exotic Newcastle Disease (END) was diagnosed in a backyard flock of game chickens located in Los Angeles County. Subsequently, thousands of birds in Los Angeles, San Bernardino, San Diego, and Riverside Counties have been sampled by the Federal/State END task force and submitted for necropsy at the San Bernardino branch of the California Animal Health and Food Safety (CAHFS) laboratory system. In addition to the gross examination, virus isolation on tissue pools (lung, trachea, cecal tonsil, and spleen) from up to five birds from each flock was performed. Gross findings of chickens whose tissue pools were positive for Exotic Newcastle Disease Virus (ENDV) were summarized. Histopathology was performed on birds belonging to accessions (up to five birds per accession) that met the following criteria: 1) at least one bird had gross lesions compatible with END and 2) the tissue pool was positive for ENDV. Lesions compatible with END were defined as one or more of the following: 1) cloacal congestion/hemorrhage at the mucocutaneous junction, 2) congested and edematous conjunctiva, and 3) diphtheritic lesions of any portion of the alimentary or respiratory tract. In sexually mature birds (n=153), the most common gross lesions were congested /hemorrhagic/diphtheritic proximal tracheas (49%), diphtheritic/ulcerative foci involving the pharyngeal mucosa (33.3%), splenic enlargement (28.1%), cloacal congestion/hemorrhage at the mucocutaneous junction (19.6%), diphtheritic/ulcerative foci involving the esophageal mucosa (19%), cecal tonsil hemorrhage /necrosis (18.3%), subcutaneous edema/congestion of the head (18.3%), ulcerative/hemorrhagic foci involving the proventricular mucosa (17%) and hemorrhagic /diphtheritic foci of the laryngeal mucosa (17%). Diphtheritic foci involving the oral mucosa, conjunctivitis, cervical edema, congested/hemorrhagic intestine or colon and undersized spleens were also noted. In sexually immature birds (n=20), the most common gross lesions were tracheal congestion /hemorrhage (40%), cloacal congestion/hemorrhage (35%), diphtheritic foci involving the esophageal mucosa (20%), splenic enlargement (20%) and ulcerative/hemorrhagic foci involving the pro-ventriculus (15%). Preliminary histologic evaluation shows that the major lesions seen in this outbreak include: 1) splenic lymphoid necrosis with fibrinoid deposits, erythrophagocytosis, and hemosiderosis; 2) fibrinonecrotic mucosal inflammation with submucosal lymphoid necrosis involving the nasal tissues, oropharynx, larynx, trachea, esophagus, intestine, proventriculus, cecal tonsils, and cloaca; 3) circulatory disturbances characterized by hyperemia, protein-aceous edema, and hemorrhage; and 4) vascular lesions consisting of fibrinoid necrosis of small blood vessel walls, fibrinoid thrombosis of small vessels, and hypertrophy and hyperplasia of endothelial and adventitial cells. The lesions observed in these birds are similar to those previously described (1, 2). One important difference is the relatively high prevalence of diphtheritic lesions within the oropharynx, esophagus, and trachea that is seen with this strain of ENDV.

REFERENCES

1. Alexander, D. J., Newcastle Disease and other avian Paramyxoviridae infections. In: Diseases of Poultry, Ed by Calnek B. W. et al., Iowa State University Press, Ames Iowa, pp541-569. 1997.

2. Brown, C., D. J. King, and B. S. Seal. Pathogenesis of Newcastle Disease in chickens experimentally infected with viruses of different virulence. Veterinary Pathology 36:125-132. 1999.

DETECTION OF EXOTIC NEWCASTLE DISEASE (END) VIRUS IN CALIFORNIA

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SUMMARY

At the end of September 2002 exotic Newcastle disease (END) virus was isolated from tissues taken from two male game chicken carcasses that had been submitted for necropsy to the San Bernardino Laboratory of the California Animal health and Food Safety Laboratory System (CAHFS). The birds had been brought from premises located in Lancaster, Los Angeles County. The Avian Paramyxovirus (APMV) type 1 isolated was confirmed as END virus by the National Veterinary Services Laboratory (NVSL), Ames, IA.

Since that initial case the number of END-suspect specimens submitted for virus isolation has increased at a phenomenal rate and continues to increase. The Avian Virology laboratory of CAHFS, centered in Fresno usually expects to receive 80-150 requests for virus isolation per month; but with the END outbreak this had increased in November to 580.

To accommodate the increased requests for virus isolation, which were more than the Fresno Virology laboratory could handle, an additional facility was set up in late October at the San Bernardino Laboratory to deal with just END cases. Requests for END virus testing have now outgrown both facilities and as of late December an additional laboratory in a trailer is being set up at the San Bernardino laboratory to perform more than 700 END virus isolation tests/week. This is in addition to up to 250 END virus isolation tests/week at the Fresno laboratory. In the interim, when the caseload was more than the two laboratories could accommodate some specimens were sent to NVSL for virus isolation testing.

A simplified protocol for testing END-suspect cases was introduced early on. This, (a modification of that used at NVSL) was based on a three-day virus isolation-screening test for END virus. Tissue pools taken at necropsy or cloacal swabs (up to five were pooled) were frozen at -80°C in 15 ml conical centrifuge tubes. In the laboratory these were thawed and centrifuged at low speed (1500 Xg) for 10 minutes. Two ml of supernatant was added to 1.3 ml antibiotic mix consisting of penicillin G 11,300 IU/ml, streptomycin sulfate 2,300 IU/ml, gentamicin sulfate 1,150 μg/ml, kanamycin sulfate 750 μg/ml, mycostatin 20 IU/ml (all concentrations are final after addition of 2 ml sample) and maintained at room temperature for at least one hour before inoculation into the chorioallantoic sac of 9- or 10-day-old SPF embryonating chicken eggs. Each specimen was inoculated into three eggs and these were incubated at 37°C for up to 72 hours. Eggs were candled daily and embryos dying were removed and transferred to 4°C prior to harvest of chorioallantoic fluid (CAF). After 72 hours all eggs were chilled at 4°C and the CAF harvested. The CAF was tested for hemagglutinating (HA) activity with chicken erythrocytes. If no HA activity was detected the sample was reported out as negative. If HA activity was detected, a hemagglutinating inhibition test was performed using reference antisera to APMV 1, 2 and 3.

All specimens positive for APMV 1 by HI were sent to NVSL for characterization.

To date more than 100 isolations of APMV 1 characterized as END virus have been made, these are predominantly from game chickens.

EXOTIC NEWCASTLE DISEASE: CORRELATION BETWEEN GROSS LESIONS AND VIRUS ISOLATION

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The correlation between specific gross lesions and the results of virus isolation (VI) in backyard chickens submitted for diagnosis of Exotic Newcastle Disease (END) during the current outbreak in Southern California was analyzed, in order to determine the predictive value of gross changes for the diagnosis of END.

When VI positive cases (n= 65) were analyzed, diphtheritic tracheitis was found in 73% of the cases, diphtheritic oro-pharyngo-esophagitis in 58%, splenomegaly in 41%, proventricular hemorrhage in 32%, cecal tonsil hemorrhage in 29%, cloacal hemorrhage in 20%, and conjunctival hemorrhage in 11%. No gross lesions were observed in 9% of the cases.

When VI negative cases (n= 220) were analyzed, diphtheritic tracheitis was found in 27% of the cases, diphtheritic oro-pharyngo-esophagitis in 13%, splenomegaly in 18%, proventricular hemorrhage in 4%, cecal tonsil hemorrhage in 15%, cloacal hemorrhage in 10%, and conjunctival hemorrhage in 5%. No gross lesions were observed in 45% of the cases.

The cases were characterized on gross examination as compatible or non-compatible for END. END virus was isolated from 48% of the compatible cases analyzed (n=191), while no virus was isolated from 52% of these cases. Only 7% of the non-compatible cases (n=215) were positive for END VI, while 93% of them were negative.

These preliminary results, albeit from a limited number of cases, suggest that diphtheritic thracheitis and oro-pharyngo-esophagitis are the most reliable gross findings to establish a gross diagnosis of END. Gross pathology had an efficacy of approximately 50% in the diagnosis of END cases.

VACCINATION EFFORTS WITH VIROSOMES PRODUCED FROM NEWCASTLE DISEASE VIRUS

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SUMMARY

Outbreaks of highly virulent Newcastle disease virus (NDV) occur with regular frequency and are a major concern to the poultry industry internationally. Economic losses caused by low virulent (lentogenic) strains continue to result from decreased egg production in layers and airsacculitis in broilers. In an effort to protect chickens against Newcastle disease (ND), a non-replicating virosome-vaccine was produced by solubilization of Newcastle disease virus (NDV) with Triton X-100 followed by detergent removal with SM2 Bio-Beads. Biochemical analysis indicated that the NDV virosomes had similar characteristics as the parent virus and contained both the fusion (F) and hemagglutinin-neuraminidase (HN) proteins. To target the respiratory tract, SPF chickens were immunized intranasally and intratracheally with the NDV virosome vaccine. This was compared to a standard NDV (LaSota) live-virus vaccine for commercial poultry. Seroconversion (> 4-fold increase in hemagglutination inhibition (HI) antibody titers) was achieved in all vaccinated birds utilizing the virosome vaccine. Upon lethal challenge with a velogenic NDV strain (Texas GB), all birds receiving either vaccination method were protected against death. Antibody levels against NDV, as determined by ELISA and HI titer, were comparable when utilizing either vaccine and increased following virus challenge. These results demonstrate the potential of virosomes as an effective tool for ND vaccination.

IN OVO VACCINATION AGAINST NEWCASTLE DISEASE: FIELD SAFETY EVALUATION

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SUMMARY

An *in ovo* vaccine against Newcastle Disease has been developed using a novel technology based on antigen-antibody complex. Field trials were conducted to evaluate the safety of the vaccine in commercial broilers under field conditions. The vaccine was administered *in ovo* and was compared to conventional ND vaccination programs, which consisted of hatchery and field ND vaccination. Safety parameters evaluated included hatchability, post-hatch mortality, body weights, and field condemnation. Serological measurements were also carried out and evaluated. The data showed that there were no significant differences between the two groups in all of the above parameters. Both groups responded serologically to ND and IBV vaccinations at about the same serological level. These results suggest that the vaccine was safe to be administered *in ovo* to commercial broilers under these field conditions.

INTRODUCTION

Newcastle disease is a highly contagious respiratory disease of chickens as well as other avian species. The disease continues to be a major problem with a substantial economic impact on the poultry industry throughout the world. Good vaccination as well as sanitary veterinary measures are required to control the disease. Current ND vaccination practices are based on multiple vaccinations against the disease either through spray or drinking water during the life of the bird. The increasing problems associated with the control of the disease worldwide have emphasized the need for a better vaccine that would be accurately applied and have a significant degree of effectiveness.

In recent years and due to the availability of an automated mass egg injection system (the Invoject® System, Embrex Inc.,), the poultry industry has adopted the use of the *in ovo* route of administration to administer vaccines and biologicals to broilers at the time of transfer around day 18 of embryonic development (5). Currently, vaccines that can be used *in ovo* include Marek's Disease (MD) and Infectious Bursal Disease Virus (IBDV) vaccines (3, 5, 10). There have been several attempts at developing *in ovo* vaccines against Newcastle Disease Virus (NDV) (1-2, 6), Infectious Bronchitis Virus (IBV) (8-9), bacteria, and coccidiosis (reviewed in 5).

We have previously reported the successful development of an ND vaccine that can be delivered *in ovo* and was shown to be both safe and effective against a standard Velogenic Neurotropic NDV challenge (11). Herein, we report on the safety testing of this ND vaccine in broilers under field conditions. The objective of this work was to evaluate the safety of this ND vaccine in commercial broilers under field conditions.

MATERIALS AND METHODS

Vaccine preparation. The NDV vaccine was prepared as described previously (10-11). Briefly, the appropriate amount of Newcastle Disease Antiserum (NDA) was mixed with the appropriate amount of NDV strain La Sota Type B1 (NDV - Antibody complex vaccine), a virus stabilizer added, the vaccine dispensed into glass vials at 2000 doses/vial, then lyophilized. Virus titer (7) and anti ND activity in NDA (4) were determined prior to mixing. The vaccine was re-hydrated using commercially available MD diluent and injected at one dose on day 18 of embryonic development, using the Inovoject® System.

Vaccine evaluation criteria. Safety was evaluated based on hatchability, post-hatch mortality, field condemnation, and body weight at processing. Additionally, serological measurements were determined against multiple viruses.

Trial design. The trial was conducted at three geographically different locations in the eastern part of the United States. Each location included two groups: NDV-Antibody complex and Control groups. Each group included two houses of 20,000-25,000 birds/house for a total of 40,000-50,000 bird /group/location (location = farm). The only difference between the two groups was that the NDV-Antibody complex group received the vaccine *in ovo* while the Control group did not. The Control group received the routine post-hatch ND vaccination at each location. All *in ovo* or post-hatch vaccinations/medications, other than ND, were the same for both groups within each location. On injection day at transfer, eggs from

multiple breeder flocks/farm were randomized between the two groups. The NDV-Antibody complex vaccine was mixed with other *in ovo* vaccines, such as MD vaccines (in the same diluent bag) prior to administration. Hatchability, post-hatch mortality, field condemnation, and body weight were all collected and compared. Additionally, serology, using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (IDEXX, Inc.), was also determined against multiple viruses on serum samples collected from the three different farms at various intervals. Hemagglutination-Inhibition (HI) tests (HA=8) were conducted on serum samples collected from the three farms to determine NDV HI titers.

Statistical analysis. Hatchability data were analyzed using two way analysis of variance with treatment as fixed and flocks as randomized blocks. Descriptive statistics were used wherever appropriate. Titer values are presented as Geometric Mean Titer (GMT). HI titers are presented as Log_2 values.

RESULTS AND DISCUSSION

There was no significant differences in hatchability data within each location or combined. Overall hatchability for the three locations combined was 84.38 and 84.04% for the Control and NDV-Antibody complex groups, respectively. Post-hatch mortality, field condemnation, and body weights from the NDV-Antibody complex group were all the same as those of the Control group.

Serological measurements showed that levels of anti NDV antibody titers from the NDV-Antibody complex group were the same as that from the Control group. One noticeable difference in the response from the NDV- Antibody complex group was the apparent trends in antibody production, which showed a tendency towards continual increase in titer even when measured at about seven weeks of age. Values of HI titers (Table 1) showed similar patterns to those measured by ELISA. However, at earlier age, there were measurable levels by HI that were not detected by ELISA reflecting the isotypes of the antibodies being measured by each procedure. Both groups responded well and equally to the IBV vaccination. This is very important since ND and IB vaccines may not show satisfactory response with inappropriate ratio between the two of them (12 & 13). Additionally and although there was no vaccination against Infectious Bursal Disease Virus (IBDV), both group (all locations) showed about the same levels of antibodies against IBDV, suggesting field exposure to IBDV and similar immune response from both groups to the possible field exposure.

In conclusion, the new NDV- Antibody complex vaccine was shown to be safe when administered *in ovo* via the Inovoject® System to commercial broilers under these field conditions. The vaccine also induced active immunity against the virus measured in terms of antibody production. The vaccine did not interfere with the ability of the birds to respond to other hatchery or field vaccinations. This is the first time that an *in ovo* NDV vaccine is shown to be safe and efficacious in commercial broilers maintained under field conditions.

REFERENCES

1. Ahmad, J and J. M. Sharma. Evaluation of a modified live virus vaccine administrated *in ovo* to protect chickens against Newcastle disease. Am. J. Vet. Res. 53:1999-2004, 1992.

2. Ahmad, J., and J. M. Sharma. Protection against hemorrhagic enteritis and Newcastle disease in turkeys by embryo vaccination with monovalent and bivalent vaccines. Avian Dis. 37:485-491, 1993.

3. Haddad, E. E., C. E. Whitfill, A. P. Avakian, C. A. Ricks, P. D. Andrews, J. A. Thoma, and P. S. Wakenell. Efficacy of a novel Infectious Bursal disease virus immune complex vaccine in broiler chickens. Avian Dis. 41:882-889,1997.

4. Haddad, E. E., C. E. Whitfill, C. A. Ricks, T. Fredericksen, D. Rowe, L. Owen, A. Baldridge, L. Murray, and J. A. Thoma. Adaptation of the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay for the determination of virus-neutralizing antibodies using the virus-neutralization assay. Avian Dis. 38:755-761. 1994.

5. Ricks, C. A., A. Avakian, T. Bryan, R. Gidersleeve, E. Haddad, R. Ilich, S. King, L. Murray, P. Phelps, R. Poston, C. Whitfill, and C. Williams. *In ovo* vaccination technology. Adv. Vet. Med. 41:495-515, 1999.

6. Stone, H. D. In ovo vaccination of chicken embryos with experimental Newcastle disease and avian influenza oil-emulsion vaccines. Avian Dis. 41:856-863, 1997.

7. Villegas, P. Titration of biological suspensions. In: A laboratory manual for the isolation and identification of avian pathogens, 4th ed. D. E. Swayne, J. R. Glisson, M. W. Jackwood, J. E. Pearson, and W. M. Reed, eds.University of Pennsylvania, PA . pp 248-253. 1998.

8. Wakenell, P. S., and J. M. Sharma. Chicken embryonal vaccination with avian infectious bronchitis virus. Am. J. Vet. Res. 47:933-938, 1986.

9. Wakenell, P. S., J. M. Sharma, R. F. Slocombe. Embryo vaccination of chickens with infectious bronchitis virus: Histologic and ultrastructural lesion response and immunologic response to vaccination. Avian dis. 39:752-765, 1995

10. Whitfill, C. E., E. E. Haddad, C. A. Ricks, J. K. Skeeles, L. A. Newberry, J. N. Beasley, P. D.

Andrews, J. A. Thoma, and P. S. Wakenell. Determination of optimal formulation of a novel infectious bursal disease virus (IBDV) vaccine constructed by mixing bursal disease antisera with IBDV. Avian Dis. 39:687-699. 1995.

11. Eid E. Haddad, Craig E. Whitfill, Alan P. Avakian, Donald B. Link, and Patricia S. Wakenell. Safety and efficacy of a novel Newcastle disease vaccine administered in ovo to SPF and commercial broiler birds. Proceedings of the 51nd Western Poultry Disease Conference, Pp. 101-103. 2002.

12. Hanson, L. E., F. H. White, J. O. Alberts. Interference between Newcastle disease and Infectious Bronchitis viruses. Am. J. Vet. Res. Pp. 294-298. 1956.

13. Denise H. Thornton, and J. C. Muskett. Effect of Infectious bronchitis vaccination on the performance of live Newcastle disease vaccine. Vet. Rec. Pp. 467-468. 1975.

Table 1. Anti NDV hemagglutination inhibition (HI) serology data from participating locations.

Location	Group	Log_2 Mean HI Titer at age (days)						
		7	14	21	30	35	42	49
Location 1[1]	Control	2.60	4.30	6.20	5.95	4.50	4.75	4.60
	NDV-Antibody	2.30	1.70	1.50	3.45	3.95	8.15	8.55
Location 2[2]	Age (days)	13		25		46		
	Control	3.50		2.20		2.30		
	NDV-Antibody	4.80		5.30		4.70		
Location 3[3]	Age (days)	4	11	18	25	32	39	46
	Control	3.20	3.95	4.40	4.60	4.45	4.25	3.85
	NDV-Antibody	2.70	2.35	4.55	3.70	4.40	4.85	5.65

[1] Day of age serology was HI = 4.2 Log_2 (N = 99 from 9 breeder flocks)
[2] Day of age serology was HI = 5.2 Log_2 (N = 79 from 8 breeder flocks)
[3] Day of age serology was HI = 3.9 Log_2 (N = 50 from 5 breeder flocks)

ROLE OF WILD BIRDS IN THE TRANSMISSION OF AVIAN PNEUMOVIRUS

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Avian pneumovirus (APV) is a newly emergent member of the Paramyxoviridae family of viruses belonging to the genus *Metapneumovirus,* which causes a highly contagious acute respiratory tract infection in turkeys, and is characterized by coughing, sneezing, nasal discharge, tracheal rales, foamy conjunctivitis, and swollen sinuses. Uncomplicated cases have low mortality (2 to 5%) but infections accompanied by concurrent secondary infections can result in up to 25% mortality (5). In egg laying birds, a transient drop in egg production along with mild respiratory tract illness has been seen (5). APV was first detected in South Africa in 1978, and later it was detected in the United Kingdom, France, Spain, Germany, Italy, Netherlands, Chile, Israel, and Asia (1,5). In chickens, APV appears to cause a milder respiratory infection and has been associated with swollen head syndrome. The United States was free of APV infection until 1996, when an outbreak of upper respiratory system infection among turkeys in Colorado resulted in isolation of APV (6,8). The APV outbreak in Colorado was controlled by intense biosecurity measures and the disease has not been reported there since early 1997. APV was detected in 1997 in the north-central state of Minnesota where the disease caused by APV has continued its presence over the past five years. The APV infection in turkeys remains as a serious economic concern among the

turkey producers. In the year 2000, 36.5% flocks were serologically positive for APV (Dr. Dale Laur, Board of Animal Report, 2001 unpublished data). Recently a pneumovirus, named as human metapneumovirus (hMPV), has been isolated from young children with respiratory tract disease in the Netherlands (16). Studies (16,17) indicate that APV of turkey origin is closely related to hMPV. Both APV and hMPV have a high percentage of sequence similarity and similar genomic organization (17). Although the clinical signs in children due to hMPV infection were similar to that caused by human respiratory syncytial virus, phylogenetic analysis suggested that hMPV was more closely related to APV subtype C than to respiratory syncytial virus (7).

We have been conducting various studies on APV for the past five years. We experimentally reproduced the disease in turkeys (2,4) and demonstrated the histopathological and gross lesions produced by the APV isolate from Minnesota (4). We developed an RT-PCR assay (10), microindirect immunofluorescence test (3), and an immunohistochemical technique (4) for the detection of APV infection in turkeys. The APV from turkey origin was shown by our laboratory to be infective to broiler chickens (11), and its infectivity to ducks was demonstrated by us (12). Neonatal transmission of APV was also demonstrated (13). The vehicles or vectors of APV transmission remain unclear. Contaminated fomites can play a role in the transmission of APV. APV is shed from the respiratory tract in aerosol droplets. The presence of APV antigen was demonstrated in the reproductive tracts of breeder hens that were intranasally inoculated with a strain of APV by researchers in Europe. The authors assume that egg transmission of APV is a possibility in turkeys. It was suggested that migratory birds might be important in disease transmission. We have been investigating the role of wild birds in the transmission of APV in Minnesota. We have isolated APV from wild birds and have compared its sequence homology with APV from domestic turkeys. The results of this study and its significance will be presented.

REFERENCES

1. Alexander, D. J. 1997. Newcastle disease and other Paramyxoviridae infections, p. In *Diseases of Poultry*, 10th edition. (B.W. Barnes, H.J. Beard, C.W. McDougald, L. Saif, Jr, Eds). pp. 541-569, Iowa State University Press, Ames.

2. Jirjis, F. F., S. L. Noll, D. A. Halvorson, K. V. Nagaraja, E. L. Townsend, A.M. Sheikh, and D. P. Shaw. 2000**.** Avian pneumovirus infection in Minnesota turkeys: Experimental Reproduction of the disease. Avian Dis**.** 44:222-226.

3. Jirjis, F. F., S. L. Noll, D. A. Halvorson, K. V. Nagaraja, E. L. Townsend, S. M. Goyal, and D. P. Shaw. 2002a. Rapid detection of avian pneumovirus in tissue culture by microindirect immunofluorescence test. J Vet Diagn Invest. 14:172-175.

4. Jirjis, F. F., S. L. Noll, D. A. Halvorson, K. V. Nagaraja, and D. P. Shaw. 2002b. Pathogenesis of avian pneumovirus infection in turkeys. Vet Pathol. 39:300-310.

5. Jones, R. C. 1996**.** Avian pneumovirus infection: questions still unanswered. Avian Pathol. 25:639-648.

6. Kleven, S. H. 1997**.** Report of the Committee. Transmissible diseases of poultry and other avian species, p. 486-491. *In* Proceedings of the US Animal Health Association 101st Annual Meeting. 15-1720.

7. Njenga, M. K., H. M. Lwamba, K. V. Nagaraja, B. S. Seal, and D. A. Halvorson. 2002. Avian pneumovirus isolates from turkeys and wild birds in the United States have high homology with the newly described human metapneumovirus. In: Proceeding of the 53rd North Central Avian Disease Conference and Symposium on Impact of Poultry Health and Bio-terrorism on Food Safety. October 6-8, 2002, Minneapolis, Minnesota, USA., p 59.

8. Seal B. S. 1998**.** Matrix protein gene nucleotide and predicted amino acid sequence demonstrate that the first U.S. avian pneumovirus isolate is distinct from European strains. Virus Res. 58:45-52.

9. Shin, H. J., M. K. Njenga, B. McComb, D. A. Halvorson, and K. V. Nagaraja. 2000b. Avian pneumovirus (APV) RNA from wild and sentinel birds in the United States has genetic homology with RNA from APV isolates from domestic turkeys. J Clin Microbiol. 38:4282-4284**.**

10. Shin, H. J., G. Rajashekara, F. F. Jirjis, D. P. Shaw, S. M. Goyal, D. A. Halvorson, and K. V. Nagaraja. 2000a. Specific detection of avian pneumovirus (APV) US isolates by RT-PCR. Arch Virol. 145:1239-1246.

11. Shin, H. J., B. McComb, A. Back, D. P. Shaw, D. A. Halvorson, and K. V. Nagaraja. 2000c. Susceptibility of broiler chicks to infection by avain pneumovirus of turkey origin. Avian Dis. 44:797-802.

12. Shin, H. J., M. K. Njenga, D. A. Halvorson, D. P. Shaw, and K. V. Nagaraja. 2001. Susceptibility of ducks to avian pneumovirus of turkey origin. Am J Vet Res. 62:991-994.

13. Shin, H. J., F. F. Jirjis, M.C. Kumar, M. K. Njenga, D. P. Shaw, S. L. Noll, K. V. Nagaraja, and D. A. Halvorson. 2002a. Neonatal avian pneumovirus infection in commercial turkeys. Avian Dis. 46:234-244**.**

14. Shin, H. J., K. T. Cameron, J. A. Jacobs, E. A. Turpin, D. A. Halvorson, S. M. Goyal, K. V.

Nagaraja, M. C. Kumar, D. C. Lauer, B. S. Seal, and M. K. Njenga. 2002b. Molecular epidemiology of subgroup C avian pneumoviruses isolated in the United States and comparison with subgroup a and B viruses. J Clin Microbiol. 40:1687-1693.

15. Shin, H. J., K. V. Nagaraja, B. McComb, D. A. Halvorson, F. F. Jirjis, D.P. Shaw, B. S. Seal, and M. K. Njenga. 2002c. Isolation of avian pneumovirus from mallard ducks that is genetically similar to viruses isolated from neighboring commercial turkeys. Virus Res. 83:207-212.

16. van den Hoogen, B. G., J. C. de Jong, J. Groen, T. Kuiken, R. de Groot, R. A. Fouchier, and A. D. Osterhaus**.** 2001. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. Nat Med. 7:719-24.

17. van den Hoogen, B.G., T. M. Bestebroer, A. D. Osterhaus, and R. A. Fouchier. 2002. Analysis of the genomic sequence of a human metapneumovirus. Virology.295:119-132.

AVIAN PNEUMOVIRUS (APV) AND OTHER RESPIRATORY CHALLENGES IN TURKEYS IN THE MIDWEST: AN EXAMINATION OF ENVIRONMENTAL FACTORS THAT INDUCE A HIGHER INCIDENCE IN THE SPRING AND FALL

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ABSTRACT

Avian pneumovirus (APV) is known as rhinotracheitis in turkeys and swollen head syndrome in chickens. This disease has been identified since the 1980's in different parts of the world. In 1996 the virus was identified and isolated in the Midwest (Minnesota, Iowa, South and North Dakota) U.S.A.

An acute disease of the upper respiratory system, APV induces nasal and ocular discharge, rhinitis, and in extreme cases pneumonia. This disease has a high morbidity, where all ages of turkeys are susceptible, and the virus has a short incubation period (one to three days), with variable mortality, depending primarily on the influence of secondary bacteria infections.

The economic cost due to this disease has been estimated at about 15 million dollars a year (1999). At present, the APV problem persists, although the economic impacts are lower than they used to be. Historically the economic impacts have been primarily due to high mortality, cost of therapeutic procedures, reduction in weight, poor performance, presence of high air sac lesions and condemnation at time of processing.

Most of the research and applied technology associated with APV in Minnesota (Midwest) have been concentrated on the isolation, identification, virulence of the virus isolates, virus characterization, serology response, possible pathways of transmission, severity of the disease, and prevention. Prevention has been done by the use of killed and live virus "vaccination". Great emphasis has been placed on biosecurity practices and procedures, from the breeders all the way to processing plants. All these efforts have helped us to better understand some of the possible ways the disease is introduced and manifested in infected birds.

Several different endeavors have greatly contributed to a reduction in the effects of APV, especially in certain areas: improvements in biosecurity (field, livehaul, plants and other areas); a better understanding of the respiratory signs and symptoms; and improvements in husbandry. These efforts have all helped to reduce the number of positive flocks, as well as the severity level in infected ones. At the same time, prevention has been practiced by use of APV "Control Exposure" (C.E.), vaccination with either killed day-old APV sources or new APV licensed live virus vaccine, and reductions in the presence of other infections in the bird's environment.

Numerous potential carriers of the disease have been hypothesized and proven to be a factor in the presence of the disease for any given flock. Carriers include wild birds (geese, ducks, pigeons, seagulls, sparrows, etc.), farm equipment, livehaul crews and equipment, deadhaul equipment, and manure (particularly the transportation and spread over fields in close proximity to live turkey production units).

APV has been a year-round problem with its highest incidence during the spring and fall months. Features of the environment associated with these seasons have been investigated as a source of the APV

problem, including wild bird migrations, presence of wild birds close to the production units, manure spreading as fertilizer, crop planting and harvesting, and snow melt runoff, among others.

Historically Minnesota has been one of the largest turkey producers in the nation. During the last seven years there has been a significant reduction in the number of farms that grow birds on range. Production has been shifting significantly from range to confinement. Confinement has more advantages than disadvantages. At the same time, the producer growing birds in confinement needs to have the correct equipment for heating and ventilation capacity. It is important to maintain a stable environment with respect to temperature and moisture, regardless of the great variability in the weather outside. A friendly and comfortable confinement environment is needed to optimize bird growth and health.

During the last four years we have been working to minimize and/or prevent the exposure of APV to the turkey flocks. At the same time a great deal of work has been done to control and prevention some of the most common infectious diseases in the area like Newcastle Disease (NDV), Hemorrhagic Enteritis (HE), *Bordetella avium* (BA).

Minnesota is located in the upper Midwest, with the 45th parallel of latitude running through the middle of the state. In general, the weather is characterized by high variability and very distinct seasonality, as is the case for most mid-continental type climates. The state is large, composed of prairie, forest, and wetland ecosystems. Minnesota contains over 16,000 lakes, some of which have an influence on local climate and weather patterns.

We know that spring and fall represent transition periods between the extreme seasons of winter and summer. One of the characteristics of this transition period is the presence of a relatively large spread in daily temperatures between the high and low. This occurs as the combined result of changing sun angle and day length, changes in landscape vegetation, and distinct differences in air masses that migrate across the state during this time. We know too that large temperature ranges over short periods of time impose a physiological stress on birds. The bird's metabolic rate and respiratory functions are taxed in adjusting to large variations. This response to extreme temperature range forms the basis for a working hypothesis that such environmental stress may be a triggering mechanism associated with the incidence of APV in Minnesota flocks.

REFERENCES

1. Calnek, B.W. Diseases of Poultry. Iowa State University Press, Ames, Iowa, USA. 10th Edition 2000.

INDUCTION OF HUMORAL IMMUNE RESPONSES AGAINST IBDV IN BALB/C MICE USING DNA PLASMID ENCODING DIFFERENT LENGTH OF VP2 FRAGMENTS

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ABSTRACT

Direct DNA immunization using Balb/c mice was used to investigate the feasibility of using DNA vaccine encoding VP2 fragments to elicit protective immune responses against IBDV in chickens. The VP2 gene carries neutralizing epitopes against the virus but full length VP2 is not a suitable DNA vaccine candidate as it has been demonstrated to be an apoptotic inducer. We have generated 15 defined overlapping fragments with various lengths covering the 1.3kb VP2 using PCR and HK46 strain as template. They were then cloned into pRSET, an *E. coli* expression vector. Most of the fragments were highly expressed except that cover the middle region (~250bp) of the gene was found to be 5-10 times lower. Some fragments in the middle region showed no detectable expression. We also have constructed three overlapping fragments covering the whole VP2 and chosen as DNA vaccine candidates. The fragments contain the middle again showed expression levels much lower than the two fragments consisted of the 5' and the 3' end of the gene where high expressions were detected. These three fragments of the VP2 were then cloned into pCI-sp and pcDNA 3.1-sp, both eukaryotic expression vectors and tested in the mice system. Balb/c mice were inoculated intramuscularly with a mixture of the four plasmid constructs of the same vector at two weeks intervals for three time at 100ug/comstruct. Multiple booster doses (4x 30ug/construct) at 2 days intervals were followed. ELISA assays showed specific antibody was detected in serum samples after second booster and reaching a peak 1-week after the multiple boosters. Mice injected

with the pcDNA 3.1-sp vector group showed higher (~1.6x) specific antibody titer than mice injected with the pCI-sp vector group. Present study is the first report to illustrate and demonstrating using only VP2 and its fragment can induce specific antibody against IBDV. These results in total demonstrate the possibility of using DNA plasmids encoding VP2 gene fragments for DNA immunization.

(The full-length article will be published in *Vaccine*.)

INFECTIOUS BURSAL DISEASE VIRUS (IBDV) VARIANT STRAINS DELAWARE E AND MISSISSIPPI CHALLENGES IN SPF CHICKENS VACCINATED WITH AN IBDV-ANTIBODY COMPLEX VACCINE

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SUMMARY

Specific pathogen free (SPF) embryos were vaccinated *in ovo* with an IBDV-antibody (IBDV-Ab) complex vaccine at 18.5 days of incubation. Chickens were challenged at 21 days of age with either Delaware E (Del E) or Mississippi (Miss) variant IBDV. Birds were necropsied at 3 and 10 days after day 21 challenge. Non-vaccinated challenged chickens had bursal edema, significantly increased spleen/body weight ratios and decreased post-challenge weight gain while IBDV-Ab complex vaccinates were protected from these challenge virus affects. The average post-challenge body weight gain, spleen/body weight ratios, and incidence of bursal edema three days post challenge indicate that the IBDV-Ab complex vaccine protected SPF chickens from variant IBDV challenges.

INTRODUCTION

Infectious bursal disease virus (IBDV) is highly contagious and primarily infects the B cells of the bursa of Fabricius of young chickens. The accompanying disease, called infectious bursal disease or Gumboro, can be subclinical or acute and cause significant economic loss to the poultry industry annually (3). Economic losses are due not only to the clinical disease and mortality caused by IBDV, but also from the deleterious effect of immunosuppression of chickens at a young age. This immunosuppression places them at risk for secondary infections from a number of other poultry pathogens (3).

Serotype 1 IBDV is pathogenic to chickens (3). Serotype 1 variant IBDV have been described since the mid-1980s (11, 12). The antigenic variation between classic and variant serotype 1 IBDV (10) has been reported to cause vaccine failures when the antigenic structure of an IBDV deviates from the antigenic structure of the commonly used classic field vaccines. Variation in the viruses can be detected by monoclonal antibody, cross-virus neutralization, various molecular techniques, or a combination of these (4). Data suggest that some classic IBDV vaccines do not provide full protection against variant IBDV viruses (5, 6, 8, 9). The objective of this study was to determine the level of protection against variant IBDV challenges in birds stimulated by one administration of an IBDV-Ab complex vaccine containing the classic IBDV strain 2512.

MATERIALS AND METHODS

Experimental animals. Charles River SPAFAS SPF chickens were incubated, hatched, and housed at the Avian Research Center, Embrex, Inc, Durham, NC. Birds were housed in positive pressure isolator cages and provided feed and water *ad libitum*.

Viruses. The vaccine used is a commercially available IBDV-Ab complex vaccine containing the 2512 vaccine strain of IBDV (2, 7, 13). IBDV variant challenge viruses were provided by Dr. Joe Giambrone, Auburn University. Variants Del E (9) and Miss (5) were received and then propagated in Charles River SPAFAS CIAV-negative SPF chickens. Bursae were harvested 72 hours post-inoculation, homogenized, stored at -70ºC, and then titered by the CAM method (1).

Experimental design. Viable embryos were vaccinated with an IBDV-Ab complex vaccine, or left non-vaccinated. Vaccine was administered through the air cell end of the egg using a 2.5 mm 20 gauge needle at 18.5 days of incubation. On day 21 of age birds were challenged by eye-drop with $10^{3.5}$ EID_{50} of Del E or Miss challenge virus. Some birds in each group were left non-challenged. Three and ten days post challenge birds were euthanized, weighed, and necropsied. Gross bursal condition (normal, edema, atrophy) was recorded, as were body, bursa and spleen

weights. From these data, mean bursa/body weight ratio (B/BW), mean spleen/body weight ratio (S/BW), and mean body weight gain for the challenge period were calculated. Group means were compared by ANOVA and portioning of means by Student Neuman Keuls test (p=0.05).

RESULTS

Del E challenge. Three days after day 21 challenge, the mean B/BW ratio was significantly ($p<0.05$) lower in challenged and non-challenged IBDV-Ab complex vaccinates (Groups 3 and 4) as compared to non-vaccinated non-challenged (NVNC) controls of Group 1 (Table 1). There was no significant difference in mean S/BW ratio between Group 1 (NVNC) and Groups 3 and 4 (IBDV-Ab complex vaccinates). The non-vaccinated challenged chickens (Group 2) had a significantly ($p<0.05$) higher mean S/BW ratio than did Groups 1, 3, and 4. Group 2 also showed significantly ($p<0.05$) lower mean post-challenge weight gain than Groups 1 and 4. Six of the twelve (50%) non-vaccinated challenged birds from Group 2 had gross bursal edema, while none of the challenged IBDV-Ab complex vaccinates (Group 4) showed bursal edema.

Ten days post-challenge, mean B/BW ratio of Groups 2, 3, and 4 were significantly ($p<0.05$) lower than Group 1. Mean S/BW measures of the IBDV-Ab complex vaccinates (Groups 3 and 4) were not significantly different from each other and were either not significantly different or significantly lower ($p<0.05$) than that of the NVNC controls (Group 1). However, the mean S/BW ratio of the non-vaccinated challenged chickens (Group 2) were significantly ($p<0.05$) higher than those of Groups 1, 3, and 4. Group 2 also showed significantly ($p<0.05$) less mean post-challenge weight gain than Groups 1 and 4.

Miss challenge. Three days after day 21 Miss challenge, the mean B/BW ratio of IBDV-Ab complex vaccinates (Groups 3 and 4) were significantly ($p<0.05$) lower than NVNC birds (Group 1). There was no significant difference between the mean S/BW ratio in the NVNC birds (Group 1) and the IBDV-Ab complex vaccinates (Groups 3 and 4). However, mean S/BW ratio of Group 2 (NVNC) was significantly ($p<0.05$) higher than Groups 1, 3, and 4. Group 2 also showed significantly ($p<0.05$) lower mean post-challenge weight gain than Groups 1 and 4. Eleven of the eleven (100%) non-vaccinated challenged birds from Group 2 had gross bursal edema, while none of the challenged IBDV-Ab complex vaccinates (Group 4) showed bursal edema.

Ten days post-challenge, mean B/BW ratio of Groups 2, 3, and 4 were significantly ($p<0.05$) lower than Group 1. There was no significant difference between mean S/BW ratios in the Group 1 NVNC birds as compared to IBDV-Ab complex vaccinates (Groups 3 and 4). However, Group 2 had significantly ($p<0.05$) higher mean S/BW ratio than Groups 1, 3, and 4. Group 2 also showed significantly ($p<0.05$) less mean post-challenge weight gain than Groups 1 and 4.

DISCUSSION

Both challenge viruses caused increased mean S/BW ratios (splenomegaly), decreased post-challenge mean body weight gain, and bursal edema in the non-vaccinated control chickens. These data indicate that the non-vaccinated birds were highly susceptible to the challenges. Data from three days post-challenge indicate that lower B/BW ratios in Groups 3 and 4 were a result of the vaccine virus, because the lower B/BW ratios were present in both the challenged (Group 4) and un-challenged (Group 3) groups. With both challenge viruses, the IBDV-Ab complex vaccinates (Group 4) had normal S/BW ratios, normal post-challenge weight gain, and no acute bursal lesions when compared to the non-vaccinated challenged controls (Group 2) indicating that the vaccine fully protected SPF chickens from Del E and Miss variant challenges. It is hypothesized that the strength of the immune response to the classic 2512 strain in the IBDV-Ab complex vaccine provides protection to these variant IBDV.

REFERENCES

1. American Association of Avian Pathologists, *A Laboratory Manual for the Isolation and Identification of Avian Pathogens, Third Edition*. Pp. 186-191, Kennett Square, PA. 1989.

2. Avakian, A.P., Whitfill, Craig E., Haddad, E.E., van den Wijngaard, J-K, and Chettle, Nick J. *The Characteristics of Infectious Bursal Disease Virus-Antibody Complex Vaccines and their Application in Broilers with Maternal Immunity*. Proceedings of "COST-ACTION 839 "Immunosuppressive Viral Diseases in Poultry". Pulawy, Poland, 8-10 Nov., 2001. pp. 55-68.

3. Calnek, B.W. *Diseases of Poultry, Tenth Edition*. Pp. 721-738. Iowa State University Press, Ames Iowa. 1997.

4. Dormitorio, T.V., Giambrone, J.J., and Duck, L.W. Sequence comparison of the variable VP2 region of eight infectious bursal disease virus isolates. Avian Diseases 41:36-44, 1977.

5. Giambrone, J.J. Isolation of variant infectious bursal disease virus from commercial broiler chickens. Poult. Sci. 65:47. 1986.

6. Giambrone, J.J., Closser, J. Efficacy of live vaccines against serologic subtypes of infectious bursal disease virus. Avian Diseases 34:7-11, 1990.

7. Haddad, E.E, Whitfill, C.E., Avakian, A.P., Ricks, C.A., Andrews, P.D., Thoma, J.A., and Wakenell, P.S. *Efficacy of a Novel Infectious Bursal Disease Virus Immune Complex Vaccine in Broiler Chickens*. Avian Diseases, 41:882-889, 1997.

8. Jackwood, D.H., and Y.M. Saif. Antigenic diversity of infectious bursal disease viruses. Avian Dis. 31:766-770. 1987.

9. Rosenberger, J.K. S.S. Cloud, J. Gelb, E. Odor, and S. E. Dohms. Sentinel bird survey of Delmarva broiler flocks. Proc. 20th National Meeting on Poultry Health and Condemnation, Ocean City, Md. Pp. 94-101. 1985.

10. Rosenberger, J.K. S.S. Clound, and A. Metz. Use of infectious virus variant vaccines in broilers and broiler breeders. Proc. 36th WPDC, Davis, Ca. Pp. 105-106. 1987.

11. Rosenberger, J.K., and S.S. Cloud. 1986. Isolation and Characterization of variant infectious bursal disease viruses (abst.). J Am Vet Med Assoc. 189:357.

12. Saif, Y.M. 1984. Infectious bursal disease virus types. Proc. 19th Natl Meet Poult Health Condemn, Ocean City, MD. Pp. 105-107.

13. Whitfil, C.E., Haddad, E.E., Ricks, C.A., Skeeles, J.K., Newberry, L.A., Beasley, J.N., Andrews, P.D., Thoma, J.A., and Wakenell, P.S. *Determination of Optimum Formulation of a Novel Infectious Bursal Disease (IBDV) Vaccine Constructed by Mixing Bursal Disease Antibody with IBDV*. Avian Diseases 39:687-699, 1995.

Table 1. IBDV variant challenges in SPF chickens vaccinated with an IBDV-antibody complex vaccine

Challenge virus, day of sampling	Group	Vaccination	Chall.	Mean Bursa/Body Wt. (g)	Mean Spleen/Body Wt (g)	Mean Body Wt. Gain (g)	Number with Edema/ Number Examined
Del E challenge on day 21, necropsy on day 24	1	None	-	6.49^{A}	1.40^{B}	25.76^{B}	0/12
	2	None	+	4.68^{B}	2.53^{A}	17.31^{C}	6/12
	3	IBDV-Ab	-	2.03^{C}	1.48^{B}	DNI	0/10
	4	IBDV-Ab	+	1.92^{C}	1.49^{B}	34.47^{A}	0/12
Del E challenge on day 21, necropsy on day 31	1	None	-	6.77^{A}	1.90^{B}	113.28^{A}	
	2	None	+	1.49^{B}	2.72^{A}	90.98^{B}	
	3	IBDV-Ab	-	1.61^{B}	1.54^{BC}	DNI	
	4	IBDV-Ab	+	1.55^{B}	1.40^{C}	121.02^{A}	
Miss challenge on day 21, necropsy on day 24	1	None	-	6.49^{A}	1.40^{B}	25.76^{B}	0/12
	2	None	+	5.63^{A}	2.39^{A}	4.81^{C}	11/11*
	3	IBDV-Ab	-	2.03^{B}	1.48^{B}	DNI	0/10
	4	IBDV-Ab	+	2.03^{B}	1.42^{B}	34.19^{A}	0/12
Miss challenge on day 21, necropsy on day 31	1	None	-	6.77^{A}	1.90^{B}	113.28^{A}	
	2	None	+	1.41^{B}	2.37^{A}	78.69^{B}	
	3	IBDV-Ab	-	1.61^{B}	1.54^{B}	DNI	
	4	IBDV-Ab	+	1.64^{B}	1.74^{B}	114.48^{A}	

$^{A, B, C}$Means within columns with the same uppercase superscript are not significantly different using ANOVA and portioning of means by Student Neuman Keuls test (p = 0.05)
DNI = data not included due to insufficient sample size
*one bird died during the challenge period (day 21-24)

INFECTIOUS BURSAL DISEASE VIRUS PROTECTION IN COMMERCIAL BROILERS VACCINATED WITH MAREK'S DISEASE VACCINE +/- IBD VACCINE

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INTRODUCTION

Infectious bursal disease virus (IBDV) infections before two weeks of age can cause profound and long-lasting immune suppression in chickens. Although passive immunity is the most efficient way to prevent early field infections, some flocks still become challenged by two weeks of age. Recently, two studies have measured greater IBDV immunity in day of age Marek's vaccinated commercial broilers when IBD vaccine was also given. One study measured this effect using serum neutralization (3) and the other measured this effect using variant IBDV challenge (1). The objective of this study was to see if adding IBD vaccine to Marek's disease vaccine given *in ovo* would be both safe and enhance early IBDV protection in a commercial broiler flock.

MATERIALS AND METHODS

Day old commercial broilers were housed in Horsfall isolator units until the termination of the study at 21 days. Day of age broiler chicks came from a 39-week old breeder flock that had been vaccinated with two shots of bursal-derived vaccine. A sampling of chicks was bled for baseline maternal antibodies to IBDV using Idexx ELISA. All three vaccine groups received a full dose of conventional bivalent Marek's vaccine HVT/301B/1 from Fort Dodge. Two groups also received a dose of live IBDV vaccine—either Intervet's mild 89/03 or Fort Dodge's intermediate Bursine-2—mixed with the Marek's and given at 18 days of embryonation. Some birds were challenged at eight days of age with 3.0 EID_{50} of Jackwood Group-6 isolate 1174 (2), and evaluated at 15 days of age (n=15). Others were challenged at 11 days of age and evaluated at either 15 days (n=10) or 21 days of age (n=21). Non-challenged controls were evaluated at 8, 15 and 21 days (n=12 each time). Bursal protection was assessed by measuring bursa to body weight ratios (B:BW). Percent hatchability and 21-day livability were also recorded.

RESULTS

Hatchability (89% in Marek's-only vs. 88% with Bursine-2 and 92% with 89/03) and livability (94% vs. 92%) were not adversely affected by IBD vaccination. The day of age ELISA geometric mean titer was 3,448. In the vaccinated, non-challenged groups there were no significant B:BW differences between Marek's-alone and Marek's/IBD vaccinates at 8 and 21 days (Figure 1), but both IBD vaccine groups were lower at 15 days, suggesting both a vaccine "take" and subsequent bursal regeneration. Comparing the challenge groups at 15 days (Figure 2), both IBD vaccines significantly enhanced protection from an 8-day IBDV challenge compared to the Marek's-alone group (64% Bursine-2 and 68% 89/03 vs. 12% Marek's-only protection 7dpch.). However, only the Marek's plus Bursine-2 group had significantly enhanced protection from the 11-day IBDV challenge (100% vs. 30% and 40% protection 4dpch., respectively). The 21day evaluation of the 11-day challenge, however, showed no differences between vaccine groups, as all were unprotected.

DISCUSSION

Recent studies have demonstrated that day of age IBD vaccination can significantly enhance early Gumboro protection in commercial boilers vaccinated for Marek's and having either low or high starting maternal immunity to IBDV (1, 3). The current study demonstrates a similar finding with the concurrent *in ovo* vaccination for Marek's disease and Gumboro vaccine. Both the mild 89/03 and the intermediate Bursine-2 vaccines were safe—measured by % hatchability and livability—when given *in ovo* to broilers with marginally low levels of maternal IBDV immunity. In addition, both IBD vaccines gave significantly higher levels of protection against an 8-day variant IBDV challenge, compared to the Marek's-only group. The only difference between the two IBD vaccine groups was seen during the 15-day evaluation of the 11-day challenge. While the Bursine-2 group showed full protection 4 days post challenge, the 89/03 group had significant bursal atrophy similar to the

Marek's-only challenge group. Perhaps the active immune response to the less attenuated Bursine-2 vaccine was greater at the time when the maternal immunity was waning. However, the significant bursal atrophy in all groups at 10 days post challenge also suggests that the difference in immunity seen at 15 days likely reflected only a longer delay in the onset of the infection and pathogenesis of the challenge virus.

REFERENCES

1. Cookson, K.C., J.J. Giambrone and J. Rodenberg. Effect of day of age infectious bursal disease vaccination on early IBD protection in commercial broilers. 43rd Southern Conference on Avian Diseases. Page 52, #230. 2002.

2. Jackwood, D.J., S.E. Sommer and H.V. Knoblich. Amino acid comparison of infectious bursal disease viruses placed in the same or different molecular groups by RT/PCR-RFLP. Avian Dis. 45:330-339. 2001.

3. Knoblich, H.V., S.E. Sommer and D.J. Jackwood. Antibody titers to infectious bursal disease virus in broiler chicks after vaccination at one day of age with infectious bursal disease virus and Marek's disease virus. Avian Dis. 44:874-884. 2000.

Figure 1. Bursa:body weight of the vaccinated controls.

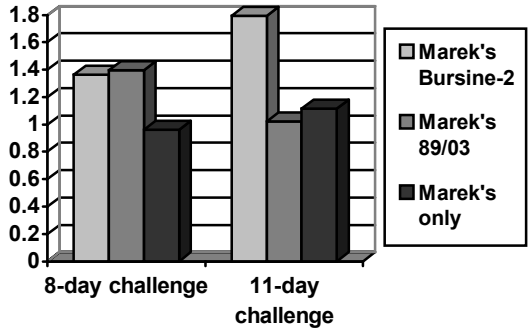
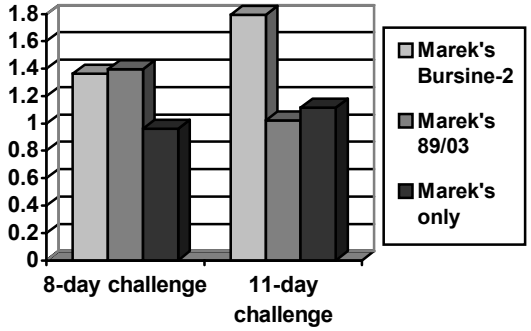


Figure 2. 15-day bursa: body weight of challenged groups.



CHICKEN ANEMIA VIRUS: SUBCLINICAL INFECTION IN BROILERS

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ABSTRACT

Results of a survey on the frequency and on the economical effects of horizontal transmission of chicken anemia virus infection in commercial broilers are reported. Serological tests at slaughter on 25 broiler flocks, using an ELISA kit, showed positive reaction in 20 (80%) flocks, of which five partially, and negative reaction in five (20%) flocks.

No significant differences in major production parameters were found between CAV positive and negative flocks in field conditions.

INTRODUCTION

Chicken anemia virus (CAV) infection occurs worldwide in chicken. The pathogen is a small single stranded DNA, non-enveloped virus, recently classified as *Gyrovirus*, of the *Circoviridae* family.

CAV causes a disease in very young chickens, characterized by serious anemia, lymphoid depletion (particularly thymus atrophy), diffused hemorrhages and increased mortality (5 to 30% and more). The disease occurs naturally in the progeny, when the breeder flocks become infected during laying period, even if without apparent effect on egg production,

fertility and hatchability. CAV is transmitted vertically through four to six weeks to the offspring, which develops disease in the first two to three weeks of age. More recently, it has been demonstrated that CAV can persist in the reproductive tissues for longer than previously thought and can be transmitted to offspring from chronically infected hens (2). The growth of recovered birds is stunted and often aggravated by dermatitis (blue wing) and other bacterial infections due to immunodepression (1, 4, 5). Morbidity and mortality are considerably enhanced if chicks are dually infected with CAV and Marek's disease virus (MDV) or infectious bursal disease virus (IBDV) or reovirus, due to more serious immunodepressive effect (1, 5, 10).

Circumstantial evidence indicates that commercial broiler chickens may become infected also by horizontal transmission from three to four weeks of age after disappearance of maternal antibodies. That often could result in slightly lower performance due to subclinical effect of CAV infection (8, 9), even if such condition could not be confirmed by others (4, 6, 7). In some serological investigations, antibodies to CAV were detected in sera collected at the processing plant from different percentage of broiler flocks, sometimes over 50% (4, 7, 8, 9).

In view of the serious damages of the disease because of infection via vertical transmission, of the high prevalence of CAV antibody found in clinically normal broiler flocks at the slaughter in some countries, and of the controversial economic effect of subclinical infection, we attempted to investigate frequency and effect of horizontal transmission of disease in Italy.

MATERIALS AND METHODS

Experimental design: Males broiler flocks, from parents of the same breed, regularly vaccinated against CAV during pullet period in an integrated big farm, were weekly inspected throughout the production period. The birds were regularly vaccinated against NDV at one and 15 days with NDV 6/10, against IBV at one day with attenuated H120 and against IBDV at 17 and 24 days with intermediate vaccine. The feed given was always from the same feed plant. Samples of blood were collected from 10 birds of each flock, randomly selected at the processing plant. From some flocks the blood samples were collected periodically from the first day of life in order to control the time of maternal antibody disappearance and the eventual time of active antibody prevalence. Flock performances were monitored for the most important productive parameters at slaughter, i.e., weight and feed conversion and for mortality.

Serology: Serum samples were examined for the presence of antibody to CAV by an enzyme linked immunosorbent assay (ELISA), using a kit of Idexx, Laboratories Inc., West Brook ME. The test was performed according to the guidelines of manufacturer.

RESULTS

Twenty-five broiler flocks, containing a total of about one million birds, were surveyed from June to November 2002. No specific clinical disease, including blue wing or dermatitis, was detected during the routine weekly inspection of the flocks.

Out of 25 flocks, examine at slaughter time, 20 (80%) resulted serologically positive to CAV, five of them only partially, and five (20%) resulted negative.

No statistically significant differences were found in correlation analysis between the three categories of broiler flocks (total or partial presence or absence of CAV antibody) and the major production parameters and mortality. The results are reported in table 1 and 2.

DISCUSSION

The prevalence of CAV infection in broiler flocks in the present survey, compared with serological results from surveys reported in other countries (7, 9), resulted considerably higher (80% against a maximum of 50%). However, the matter needs further more extensive investigations in the area.

The results here reported show that subclinical CAV infection seems to have no substantially significant effect on commercial broiler performance and profitability. The present findings confirm those of other two surveys (6, 7), but are in some disagreement with a third (9), the only so far reported by literature.

The time during the production period when subclinical infection with CAV occurs might be of considerable importance. Infection very soon after disappearance of maternal antibody, i.e., during the third to fourth week of age, might have more affect. A comparison under experimental conditions, with or without infection, of broilers at different ages, as from the third week, excluding many other infective or non-infective factors, might allow a more effective judgement, also with regard to the immunodepressive effect of CAV. But it is demonstrated that chicks with maternal antibody exposed to a very virulent strain of CAV intramuscularly or by contact in the first days of life showed normal hematocrit values until three weeks, seroconverted, and kept healthy for 63 days (11).

Also, the possible use of a very attenuated live vaccine within the third week of age has been debated with discordant opinions in various meetings on immunodepressive effects of viral origin.

In conclusion, further investigations on the matter are necessary, since few and conflicting data are available and there are many factors such as management, housing, breed, feed, etc., that may influence the performances of broilers.

REFERENCES

1. Bülow V.V., R.Rudolph and B. Fuchs. Erhohte Pathogenicitat des Erregers der aviaren infektiosen Anaemie by Huhnerkuken (CAA) bei simultaner Infektion mit dem virus der Marekschen Krankheit (MDV) Bursitisvirus (IBDV) oder Reticuloendotheliosis Virus (REV). J. Vet. Med. B 33: 93-116. 1986.
2. Cardona C., B. O. Wendelien and K A. Schat. Chicken infectious anemia virus in reproductive tract cells in infected hens and in their embryos. In: Proc. 49th West Poult. Dis. Conf., Sacramento, march 5-7. p 31. 2000.
3. Chettle N. J., R. Eddy, P. Y. Wyeth and S. A. Lister. An outbreak of disease due to chicken anemia agent in broiler chickens in England. Vet. Rec. 124: 211-215. 1989.
4. De Hert P., G. van den Bosch, R. Ducatelle, E. Uyttebroek and C. Schrier. Epidemiology and significance of chicken infectious anemia virus infections in broiler and broiler parents under nonvaccinated european circumstances. Avian. Dis. 45: 706-708. 2001
5. Engstrom B. E., O. Fossum and M. Luthman. Blue wing disease of chickens: experimental infection with a Swedish isolates of chicken anemia agent and avian reovirus. Avian Pathol. 17: 33-50. 1988.
6. Goodwin M. A., M. A. Smelzer, J. Brown, B. L. Murray and S. Mc Carter. Effect of so-called chicken anemia agent maternal antibody on chicks serologic conversion to viruses in the field. Avian Dis. 37: 542-545. 1993.
7. Jørgensen P. H., L. Ohe, O. L. Nielsen and M. Bisgaard. Influence of subclinical virus infections and other features on broiler flock performance. Br. Poult. Sci. 36: 455-463. 1995.
8. Mc Ilroy S. G., M. S. Mc Nulty, D. W. Bruce, J. A. Smith, E. A. Goodall and M. J. Alcon. Economic effects of clinical chicken anemia agent infection on profitable broiler production. Avian Dis. 36: 566-574. 1992.
9. Mc Nulty M. S., S. G. Mc Ilroy, D. W. Bruce and D. Todd. Economic effect of subclinical chicken anemia agent infection in broiler chicken. Avian Dis. 35: 263-268. 1991.
10. Yuasa N. T., T. Yamiguchi, T. Noguchi and I. Yoshida. Effect of infectious bursal disease virus infection on incidence of anemia by chicken anemia agent. Avian Dis. 24: 202-209. 1980.
11. 11Zanella A., P. Dallara, A. Lavazza, R. Marchi, A. Moreno Martin, T. Rampin and G. Tosi. Interaction between Marek's disease and chicken infectious anaemia viruses. 6th Int. Symp. on Marek's Disease, Montreal August 20-23, pp. 11-19 2000.

Table 1. Performance parameters in commercial broilers at 58 days of age correlated with serological condition to CAV.

POSITIVE				PARTLY POSITIVE				NEGATIVE			
No flocks	Weight g.	F.C. ratio	Mort. %	No flocks	Weight g.	F.C. ratio	Mort. %	No flocks	Weight g.	F.C. ratio	Mort. %
7 summer	3286	1.984	5.8	-	-	-	-	-	-	-	-
8 autumn	3521	2.011	4.7	5	3520	1.980	5.1	5	3526	1.990	5.5

Table 2. Performance parameters in two flocks of commercial broilers showing positive serological reaction at different age.

Flock	Age at slaughter (days)	Age at positivity (days)	Weight g.	F.C. rate	Mortality %
1	58	53	3390	2.119	6.5
2	58	38	3385	2.103	5.9

USE OF A CHICKEN ANEMIA VIRUS ANTIBODY TEST FOR MONITORING PROTECTIVE ANTIBODY TITERS

Valerie Leathers, Kathy Velek, Beth Myrick, J. Taxter, and R. Muñoz

SUMMARY

The 1:100 sample dilution shifts the linear ranges of the IDEXX CAV Antibody ELISA such that the relevant antibody titer levels can be assessed. Specifically, $\log_2$ VN ranges of 8-10 approximately correlate to the upper and lower ranges of the assay. A proposed titer calculation and titer ranges have been derived to help in the interpretation of the data.

The data presented in this paper supports the use of the IDEXX CAV Antibody ELISA at two different dilutions depending on the status of the flock. The 1:10 dilution should be used for optimal sensitivity such as monitoring a SPF flock for exposure to CAV. The 1:10 dilution can also be used for a rough estimation of correlation to VN titers. The 1:100 sample dilutions can be used for monitoring significant antibody titers to assess the neutralizing antibody status of the flock.

INTRODUCTION

The IDEXX CAV Antibody ELISA was originally validated to provide a positive or negative test result. It has become increasingly important for breeder companies to monitor neutralizing antibody titers to prevent vertical transmission of the virus and to protect progeny against chicken infectious anemia during the first few weeks. Antibody titers of $\log_2$ VN ≥ 8 have been reported to prevent breeder shedding of the virus; maternal antibody titers of $\log_2$ VN >9 are needed to prevent horizontal infection (1).

The IDEXX CAV Antibody ELISA uses an anti-CAV monoclonal antibody in a blocking format. The assay has excellent correlation to virus neutralization titers, however because of the blocking format, the dynamic range of the assay is limited. The following data supports the use of the IDEXX CAV Antibody ELISA for monitoring antibody titers. By increasing the sample dilution factor, the linear portion of the assay is shifted to allow for greater discrimination of the relevant neutralizing antibody titers.

MATERIALS AND METHODS

IDEXX CAV Antibody ELISA test kits were obtained from lots available in inventory. The test kits were tested according to standard assay protocol with the exception of the sample dilution step. Samples were diluted either at the normal 1:10 dilution or at a modified 1:100 dilution. All sample ODs were normalized to the negative control (S/N= sample OD650/negative control OD650).

RESULTS AND DISCUSSION

A panel of 40 samples with known VN titers was obtained and tested on the IDEXX CAV Antibody ELISA according to the standard assay protocol. Two different sample dilutions were used 1:10 and 1:100. The 1:100 dilutions were chosen because of an earlier sample dilution experiment covering the range of 1:20 - 1:320. The 1:100 dilution factor provided the best separation for moderate titer VN samples (data not shown). The 1:10 sample dilution has the optimal sensitivity however as seen in the top panel, the range of the assay does not clearly distinguish between samples with moderate to strong antibody titers. The linear range of the 1:100 sample dilutions roughly correlates to the $\log_2$ VN range of 8-10.

In order to obtain a titer calculation for the 1:100 sample dilutions, a series of positive samples (n=100) were serially diluted to obtain end point dilution values. Figure 2 is a graph of the S/N value for each sample tested at the 1:100 dilutions vs. the end point dilution for the same sample. The S/N value was then graphed against the log of the end dilution.

The correlation range for the IDEXX CAV Antibody ELISA is shown in Table 1 for the 1:10 and 1:100 sample dilutions. This experimental titer calculation can only be used with samples tested at the 1:100 dilutions (Table 2).

The data presented in this paper supports the use of the IDEXX CAV Antibody ELISA at two different dilutions depending on the status of the flock. The 1:10 dilution should be used for optimal sensitivity such as monitoring a SPF flock for exposure to CAV. The 1:10 dilution can also be used for a rough estimation of correlation to VN titers. The 1:100 sample dilutions can be used for monitoring significant antibody titers to assess the neutralizing antibody status of the flock.

REFERENCES

1. Malo, A. and Weingarten, M. Determination of the minimum protective neutralizing antibody titer to CAV in adult chickens. Intervet VSD Newsletter 11: 1-5. 1995.

Table 1. Correlation ranges for the IDEXX CAV antibody ELISA.

1:10 Sample Dilution			
VN Antibody Titer (log2)	Status	ELISA S/N	Result
< 4	Negative (No titer)	>0.6	Negative
5-7	Positive (Low Titers)	0.59-0.20	Positive
>8	Positive (Protective Titers)	<0.2	Positive
1:100 Sample Dilution			
VN Antibody Titer (log2)	Status	ELISA S/N	ELISA Titer
< 7	Negative/Low Titer Positive	>0.8	<1000
8-10	Positive (Moderate Protective Titers)	0.80-0.20	1000-8660
>10	Positive (High Protective Titers)	<0.2	>8660

Table 2. IDEXX CAV Ab ELISA titers/titer groups: 1:100 sample dilution.

Titer Group	Titer Range	S/N Range	Interpretation
1	<1000	>0.8	Negative
2	1000-2460	0.8-0.55	Positive-Moderate (Protective)
3	2461-5050	0.54-0.35	Positive-Moderate (Protective)
4	5051-8660	0.34-0.2	Positive-Moderate (Protective)
5	>8660	<0.2	High Protective

ILTV DIAGNOSTICS REVISITED

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Infectious laryngotracheitis (ILT) is a highly contagious herpesvirus infection of chickens. The classical form of the disease is characterized by severe dyspnea, coughing, rales, and expectoration of bloody exudates. Mortality associated with ILT can be upwards of 50%. A milder form of the disease is characterized by mild tracheitis, swollen sinuses, and conjunctivitis, in the absence of mortality. ILT diagnosis is typically made by observation of clinical signs and histopathologic examination of tracheal lesions for the presence of type B intranuclear inclusion bodies. In addition, other methods utilized for diagnosing ILT include virus isolation in embryos via chorioallantoic membrane (CAM) or primary chick embryo cell culture, electron microscopy, direct and indirect fluorescent antibody detection, immunohistochemistry (IHC), and PCR. In this study, histopathology, virus isolation, immunohistochemistry, and nested PCR were evaluated for diagnosing ILT virus from clinical samples. The results indicate nested PCR and IHC gave the best correlation in diagnosing both the classical and mild forms of ILTV.

DEVELOPMENT OF MULTIPLEX PCR FOR AVIAN ENTERIC PATHOGENS

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SUMMARY

Avian reovirus (ARV), avian adenovirus (AAV), infectious bursal disease virus (IBDV) and chicken infectious anemia virus (CIAV) are all pathogenic viruses that have detrimental effects on the health of poultry. Birds infected with these viruses individually or as multiple infections have many clinical problems, such as immunosuppression, weight loss and enteritis, which result in reduced marketability and economic losses. Diagnostic detection of these pathogens plays a crucial role in the management of these diseases. The objective of this research was to develop a reliable, reproducible, cost effective, multiplex PCR (mPCR) which will allow for the identification of these four viruses simultaneously in one reaction. A multiplex PCR is developed and being optimized to simultaneously detect four avian pathogens. Four sets of primers specific for ARV, AAV, IBDV and CIAV were used in the test. The multiplex PCR DNA products consisted of 532 bp for ARV, 421 bp of AAV, 365 bp of IBDV and 676 bp of CIAV and were visualized by gel electrophoresis. The mPCR assay developed and evaluated in this study was found to be specific assay for ARV, AAV, IBDV, and CIAV with no amplification of nucleic acids from other avian pathogens. Further studies on mPCR assay are being conducted to test its sensitivity and specificity on clinical samples.

HYDROPERICARDIUM-HEPATITIS SYNDROME (ANGARA DISEASE) IN BROILERS IN THE NETHERLANDS

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ABSTRACT

In May 2002 we diagnosed two outbreaks of Hydropericardium-Hepatitis Syndrome (HHS or Angara Disease) in broiler flocks in the southern part of the Netherlands, this is the first report of HHS in Western Europe.

On a broiler farm with 95,000 broilers, mortality increased in one house at the age of 11 days (D11). Post mortem findings revealed severe hydropericarditis with straw-colored fluid in the pericardial sac. In some of the examined birds pale and swollen livers were seen, some livers with white foci. On histopathology inclusion bodies were seen in the liver, a fowl adenovirus serotype 4 (FAV4) was isolated from the liver of affected birds. On the 21st day of life total flock mortality was 30 %, and it was decided to cull the whole flock.

In spite of removing the HHS-infected flock, HHS problems started on day 23 in a second house on the same farm resulting in a total mortality on D35 of 30 % and on D31 a slight increase in mortality due to hydropericardium problems occurred in a third house on the same farm.

On another farm (distant from the first outbreak) a second HHS-outbreak occurred in offspring from the same broiler breeder flock. This case was also confirmed to be HHS, based on post mortem, histopathology and the isolation of a fowl adenovirus serotype 4 from the liver. In this outbreak mortality started at 20 days of age, total flock mortality was 20 %.

The two broiler farms originated from the same broiler breeder flock. In this flock FAV4 virus neutralizing antibodies were found, despite extensive sampling no virus could be isolated from the breeder flock.

Until now, the origin of this very pathogenic FAV4 could not be found; after the two outbreaks in broilers no other outbreaks have occurred. In case the breeder flock would have been the source of this vertically transmitted FAV, one would expect many more outbreaks.

As the FAV4 prevalence in breeder flocks is not known, a small scale serological survey was performed on 40 broiler breeder farms. From these 40 farms 20 were situated in a southern poultry-dense area around the FAV4-positive breeder farm. Another 20 broiler breeder farms were sampled in other regions in the Netherlands. The results of the survey showed that 50 % of the farms had virus-neutralizing antibodies towards FAV4; in the southern poultry region the number of seropositive farms was higher than in the other regions.

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

MAREK'S DISEASE IN TURKEYS: HISTORY AND CURRENT STATUS

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HISTORY

Marek's disease (MD) is the most common lymphoproliferative and neuropathic disease of domestic chickens and less commonly in turkeys and quails. The causative agent is Marek`s disease virus (MDV), a group of highly cell- associated oncogenic avian herpesviruses. Although several reports on naturally occurring lymphomas (MD-like conditions) in turkeys were published in the past (1, 2, 8, 13, 16, 17, 22, 24, 25, 26, 27) less attention has been paid to the disease in this species.

It has been shown that experimental inoculation of pathogenic MDV can cause a lymphomatus diseases in turkey (20, 25). Elmubarak et al. (10) found that experimentally inoculated turkey poults with pathogenic MDV become persistently viremic, although the levels of detectable circulating MDV were generally lower in turkeys than in similarly inoculated chickens. Gross lesions were most prevalent in liver and spleen, peripheral nerves were involved infrequently and the tumour formation was similar in chickens and in turkeys. As chickens, infection of turkeys with a virulent MDV also resulted in immunosuppression. Witter and Solomon (27) incidentally isolated a virulent MDV-like strain from a turkey (TK 809). The virus seemed partially adapted to turkeys, growing better than chicken MD viral strain in turkey cells *in vivo* and *vitro* (25). When comparing the pathogenicity of this turkey isolate (TK809) with virulent MDV strains they found that, it is oncogenic for both chickens and turkeys. However, the lesions incidence was greater overall in turkey inoculated with strain TK809 than those inoculated with other MD viral strains. Although the clinical manifestations of MD are similar in chickens and turkeys, there appears to be a fundamental difference in the mechanism of the disease induction by MDV in these two species. In chicken, MDV transforms T cells, whereas in the turkey, the B cell is likely to be the target cell for transformation by MDV (10,18). In contrast to this result, Powell et al. (21) found that cell lines established from MDV-induced turkey tumours to be T- lymphocytes. In addition, Nazerian and Sharma (19) investigated the susceptibility of different turkey lines to MDV and reported that small white Beltsville type seems to be resistant, while the commercial type (Nicholas) to be moderately susceptible to develop MD lesions.

CURRENT STATUS

Although commercial turkeys were appeared to be susceptible to infection with MDV, less attention has been paid to the disease in turkeys. Recently, reports on natural MDV outbreaks associated with tumors in commercial turkey flocks were described in several countries. The recent history has started in 1990 in France. In 1990 broiler farms located in the southwest of France, an increased incidence of MD accompanied with high condemnation rate at slaughter was observed. Shortly after that, a high incidences of tumor cases were also detected in turkey farms located in the same area (3). At the beginning of outbreaks black turkey line appeared to be more susceptible than others. However, the situation was changed within a short period of time, and later also commercial BUT-type turkeys in other parts of France were affected (14). Clinical signs and mortality occurred between 12 and 20 weeks of age and reached rates of above 80 %. The clinical signs were unspecific and included: growth retardation, unwillingness to move, dehydration, and in some cases lameness/paralysis were observed. In a number of experiments the disease could be reproduced in turkeys and in SPF chickens by injection of blood from diseased turkeys. The agent was identified as a serotype 1 MDV with serotype-specific

monoclonal antibodies (3). Up 1995 till now several reports on natural MD outbreaks in meat turkey were described in Israel (4,7), Switzerland (12), Germany (11,23) and Ukraine (15). The clinical signs were mostly observed up to the ninth week of age. The mortality rate varies between 15 – 60%. At necropsy, tumors in the visceral organs – especially in the liver and spleen – are the most predominant finding. Other visceral organs and the peripheral nerves are infrequently involved. Histological examination showed a pleomorphic cell infiltration with mainly mononuclear cells in the affected organs.

Laboratory diagnosis based on isolation of the virus or detection of antibodies is mostly associated with some difficulties, since the levels of detectable circulating MDV-1 in experimentally infected turkeys were generally lower than in similarly inoculated chickens (10); and in some cases attempts to re-isolate the virus from experimentally infected turkeys were unsuccessfully (26). Also, differential diagnosis of the neoplastic conditions is problematic for several reasons: the lymphoproliferative disease virus (LPDV) cannot be propagated in cell cultures, while MDV and Herpes virus turkey (HVT) antigens are share common epitopes, therefore serological diagnosis is not valid. In addition, retroviruses are transmitted vertically, which mostly associated with immunotolerance, and antibodies are not produced. The ability to distinguish between the viral genomes of the avian oncogenic viruses allowed us to identify MDV as the major infecting virus in commercial flocks with tumors (4,5,6,7).

The question, why turkeys have apparently become more susceptible to natural infection with MDV-1 and develop clinical disease, is difficult to answer; and many hypotheses such as genetic changes in currently commercial turkey lines, increasing virulence of the MD strains, raising chickens and turkey flocks together which might contribute to inter-species transmission of MDV, and the existence of variant MDV strains with genuine genomic or antigenic changes that are oncogenic in turkeys were discussed.

CONTROL ATTEMPTS

There is strong evidence that MDV spreads horizontally from MDV-1 infected chickens to turkeys when kept in close proximity. To minimize the risk of spreading, more attention to biosecurity and separation between chicken and turkey farms must be taken into consideration. In the affected areas in France MD-vaccination of turkeys using Rispens strain vaccine was introduced in 1991, and has mainly solved the problem (14). Similar approach was applied also in Switzerland since 1995 with the same sucess (12). However, vaccination attempts under experimental conditions using commercial CVI988 vaccine were ineffective against MDV-1 challenge (4). Previous vaccination attempts using HVT against MD-induced in turkeys were ineffective (9). Also Nazerian and Sharma (19) demonstrated a negligible protection with HVT-vaccination against MDV-1 challenge.

In conclusion, several aspects on the pathogenesis of MD infection in turkeys remains unclear, and further investigations are necessary. In addition, further solid scientific date on the efficacy of the vaccine in turkeys are required.

REFERENCES

1. Andrewes, C.H. and R.E. Glover. A case of Neurolymphomatosis in a turkey.Vet. Rec. 51: 934-935. 1939.

2. Busch, R.H. and L.E.,Jr.Williams. Case-report-A Marek disease-like condition in Florida turkeys. Avian Dis. 14:550-554. 1970.

3. Coudert, F., A. Vuillaume, M. Wyers and F. X. Legros. Une nouvelle pathologie chez la dinde-la maladie de Marek. Proceeding 1eres jouenees de la reserche avicole d'Angers, pp. 164-166. 1995.

4. Davidson I., M. Malkinson and Y. Weisman. Marek's disease in turkeys. I. A seven-year survey of commercial flocks and experimental infection using two field isolates. Avian Dis. 46: 260-267. 2002a.

5. Davidson I., M. Malkinson and Y. Weisman. Marek's disease in turkeys. II. Characterization of the viral glycoprotein B gene and antigen of a turkey strain of Marek's disease virus. Avian Dis. 46: 268-279. 2002b.

6. Davidson, I., A. Borovskay, S. Perl and M. Malkinson. Use of the polymerase chain reaction for the diagnosis of natural infection of chickens and turkeys with Marek's disease virus and reticuloendotheliosis virus. Avian Path. 24:69-94. 1995.

7. Davidson, I., Y. Weisman, S. Perl and M. Malkinson. Differential diagnosis of avian tumors by PCR and a case report of MDV-1 in turkeys. Proceedings of the 5th International Symposium on Marek´s Disease, East Lansing, pp. 311 – 316. 1996

8. Downham, K.D. (1939). Lymphomatosis of turkeys. Vet. J. 95:89-90. 1939.

9. Elmubarak, A.K., J.M. Sharma, R. L. Witter and V.L. Sanger. Marek's disease in turkeys: Lack of protection by vaccination. American Journal of Vet. Res. 43: 740 – 742. 1982.

10. Elmubarak, A.K., J.M. Sharma, R.L. Witter, K. Nazarian and V.L. Sanger. Induction of lymphomas and tumour antigen by Marek's disease virus in turkeys. Avian Dis. 25: 911 – 926. 1981.

11. Hafez, H.M., D. Lüschow, F. Fehler and H. L Shivaprasad. Marek's disease in commercial turkeys: Case report. Proceedings of Workshop on Molecular Pathogenesis of Marek`s disease and avian immunology, Limassol, Cyprus, p. 43. 2002.

12. Hafez, H.M.. High mortality in meat turkey flocks in Switzerland caused by visceral tumours unpublished date 1995.

13. Harnden, E.E. and H.C. Smith. A Case of turkey lymphomatosis. Poultry Sci. 22:2454-2462. 1943.

14. Kross-Landsman, I.. Marek's disease in turkeys. Proceedings of the 1st International Symposium on Turkey Diseases Berlin. Ed. H.M. Hafez and A. Mazaheri. ISBN 3-930511-53-3, pp. 34-38. 1998.

15. Livoschenko, E.M.. Marek's disease in turkeys. 6th Intl. Symp. on Marek's disease, Montreal, Canada. 2000.

16. McKee, G.S. A.M. Lucas, E.M.Denington, and F.C. Love. Separation of leukotic and non-leukotic lesions in turkeys on the ispection line. Avian Dis. 7:19-30. 1963.

17. McKee, G.S. Visceral lymphomatosis of turkeys. Poultry Sci. 42:1289. 1963

18. Nazerian K, Elmubarak AK and JM Sharma. Establishment of B-lymphoblastoid cell lines from Marek's disease virus-induced tumors in turkeys. Intl. J of Cancer 29: 63-68. 1982.

19. Nazerian, K. and J. M. Sharma. Pathogenesis of Marek's disease in turkeys. Proc. Int. Symp. Marek's disease, 23.-26. Juli, Ithaca, NY, pp. 262 – 267. 1984

20. Paul, P. S., J.H. Sautter and B.S. Pomeroy. Susceptibility of turkeys to Georgia strain of Marek's disease virus of chicken origin. Amer. J. Vet. Res. 38, 1653 – 1656. 1977.

21. Powell, P.C., K. Howes, A.M. Lawn, B.M. Mustill, L.N. Payne, M. Rennie and M.A. Thompson. Marek's disease in turkeys: The induction of lesions and the establishment of lymphoid cell lines. Avian Path 13: 201-214. 1984.

22. Simpson, C. F., D. W. Anthony and F. Young. Visceral lymphomatosis in a flock of turkeys. J. Amer. Vet. Med. Ass. 130: 93 – 96. 1957.

23. Voelckel, K., E. Bertram, I. Gimeno, U. Neumann and E. F. Kaleta. Evidence for Marek's disease in turkey in Germany: detection of MDV-1 using the polymerase chain reaction. Acta Virologica 43:143-147. 1999.

24. Voûte, E. J. and A.E. Wagenaar-Schaafsma. Een op de ziekte van Marek lijkende afwijking bij mestkalkoenen in Nederland. The Netherland J. Vet. Sci. 99: 166 – 169. 1974.

25. Witter, R. L., J. J. Solomon and J. M. Sharma. Response of turkeys to infection with virulent Marek's disease viruses of turkey and chicken origins. Amer. J. Vet. Res. 35:1325 – 1332. 1974.

26. Witter, R. L., K. Nazerian, H. G. Purchase and G. H. Burgoyne. Isolation from turkeys of a cell-associated herpesvirus antigenically related to Marek's disease virus. Amer. J. Vet. Res. 31: 525 – 538. 1970.

27. Witter, R.L. and J.J. Solomon. Epidemiology of a herpesvirus of turkeys: Possible sources and spread of infection in turkey flocks. Infect and Immun. 4: 356-361. 1971.

INTESTINAL MUCOSAL PATHOLOGY, EPITHELIAL APOPTOSIS AND PROLIFERATION IN MALABSORPTION SYNDROME-AFFECTED BROILERS

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ABSTRACT

Malabsorption-syndrome (MAS) is a well-known problem in broilers causing reduced growth and lesions in the intestinal mucosa. A disturbance in epithelial turnover, that is apoptosis and proliferation, was hypothesized in the pathogenesis of the intestinal lesions. Chickens were experimentally infected with MAS by inoculation at one day of age with intestinal homogenate obtained from MAS affected birds. Chickens were sacrificed daily and intestine tissue sample was collected. The early inflammatory changes in the mucosa, epithelial apoptosis (TUNEL assay), and cell proliferation (PCNA immunohistochemistry) were investigated. Infected chickens had reduced body weight starting at one-day post-infection (PI) and ended below half the weight of control chickens in the second week. At two days PI, infiltration of the lamina propria with heterophils (PMN leukocytes), vacuolization of the villus epithelium and hyperplasia of the crypts of Lieberkühn were observed. At five days PI, the crypt wall epithelia were flattened and the

crypt lumen were dilated and often filled with apoptotic bodies of epithelium and debris of degenerating heterophils emanating in crypt abscesses. The TUNEL assay revealed extensive apoptosis that initially affected the epithelium on the villi at the luminal surface and subsequently the crypt epithelium. The excessive apoptosis in the crypts has led to cystic dilatation of the crypts, which was further aggravated by blockage of the crypt openings. The apoptosis at the crypt openings affecting proliferating (progenitor) cells might be associated with differentiation or maturation defects. There has been severe atrophy of villi at seven days PI owing to the vast epithelial apoptosis at the villi and crypts. On the other hand, the epithelia in the crypts and on the villus wall in the infected chickens were positive for the cell proliferation marker (PCNA) indicating an accelerated epithelial turnover. In the control chickens PCNA positive cells were limited in the crypts. The results indicate that impaired epithelium renewal processes, mainly increased apoptosis, could be critical in the pathogenesis of the MAS enteropathology. These changes in epithelial turnover seem triggered by the inflammation and growth factors and/or cytokine produced by the heterophils and the epithelial cells.

(The full-length manuscript of this work will be submitted to *Avian Pathology*.)

MYCOPLASMA GALLISEPTICUM OUTBREAK IN PRIMARY TURKEY BREEDERS

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Mycoplasma gallisepticum (MG) causes one of the most economically significant diseases of chickens and turkeys. The disease is characterized by respiratory rales, coughing, nasal discharge, and frequently in turkeys, sinusitis. Often the disease is complicated with secondary bacterial infections such as *Escherichia coli*. MG can be transmitted through the egg to the progeny, as such, obtaining poults from MG-free breeder flocks is extremely important. All the primary turkey breeders in the United States participate in a voluntary program called the National Poultry Improvement Plan in which birds are tested periodically to ensure that they are free of MG.

Three 18-week-old turkeys were submitted to the Fresno branch of CAHFS with a history of respiratory signs and a presumptive diagnosis of aspergillosis. All birds had severe thickening of the air sacs due to fibrinous and/or caseous exudate. Microscopically there was lymphoplasmacytic inflammation with lymphoid nodules in the trachea, air sac and bronchi. In addition air sacs also had fibrinosuppurative inflammation. *E. coli* was isolated from the air sacs. Serology revealed two birds were positive for MG. Prompt reporting and testing of subsequent submissions of blood samples and swabs from additional birds confirmed the presence of MG in the flock. Extensive trace back and testing of birds on one ranch revealed that MG was widespread. As a result, 16,000 birds of young pure line stock, 15,000 young breeders, and more than 200,000 eggs had to be destroyed. However, the pedigree stock was retained and treated with Baytril, vaccinated for MG, and the eggs were dipped in a solution containing antibiotics. Through this process MG was eliminated in the breeders and the progeny have been negative for MG for more than a year now.

The loss of breeders, pure line stock and eggs, the cost of medication, vaccination, labor, laboratory testing, feeding and cleaning and disinfection resulted in substantial economic loss. The last MG outbreak in this company occurred more than 30 years ago. The source of MG could not be exactly determined but is speculated that lax biosecurity played a significant role in introducing MG in to the flock. Biosecurity has been upgraded since this outbreak and no additional cases have been detected.

CASE REPORT OF MYCOPLASMA IN A TURKEY BREEDER FLOCK

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INTRODUCTION

Mycoplasma gallisepticum (MG) is a significant respiratory disease in turkeys, and remains a primary concern in commercial poultry operations. Clinical signs and pathologic changes vary widely, depending on the strain of mycoplasma, and range from nasal discharge, sinusitis, and cough to extensive airsacculitis (1). The financial consequences of an outbreak can be significantly extensive, due to condemnations at slaughter, poor feed conversion, lowered egg production, and increased costs of medication and labor. The following case report gives the chronological order of events in a difficult case of mycoplasmosis in a turkey breeder flock caused by a variant field strain of MG.

CASE REPORT

The affected breeder ranch is composed of five houses, four houses for hens and one house for toms. House A and B had 56 week-old hens, house C and D had 54 week-old hens. House E had both 56 week-old and 54 week-old toms. Biosecurity included fencing around the perimeter, a shower-in/ shower-out facility, a crematorium for daily mortality, a boot wash station at the entrance of each building, and an insemination crew that worked one house daily at this facility only.

In October 2002, a submission of four hens and 20 sera samples from house A showed signs of coughing, respiratory difficulty, and facial swelling, were submitted to the California Animal Health and Food Safety, Turlock Branch Laboratory. Gross pathology findings included respiratory difficulty, unilateral swelling of orbital sinus with cloudy viscous exudate, pericarditis, and airsacculitis. Aerobic bacterial culture attempts were negative. Serological tests were positive for *Mycoplasma synoviae* (MS) hemagglutination inhibition (HI) test up to titer group 1:80 and enzyme-linked immunosorbent assay (ELISA) test at titer group 1. Diagnostic mycoplasma cultures were performed.

Five days after first submission, three additional turkey hens and 20 sera from house A were submitted. Gross pathology findings included airsacculitis, joint effusion, sinusitis, and pneumonia. Aerobic bacterial and fungal cultures were negative. Unaffected houses B, C, D, and E each had 20 sera submitted. Serological tests for the affected hens (house A) were positive for MG in serum plate agglutination (SPA) test, HI test at 1:20, and ELISA titer groups 4 and 8. MS HI titers were also positive at titer 1:40 and MS ELISA titer group 1. The serological tests from the unaffected hens (house B, C, D, and E) were unremarkable. Diagnostic mycoplasma cultures were performed.

Twelve days after the first submission, six hens and 18 sera were submitted from house A. Gross pathology findings included bilateral sinusitis with mucopurulent to purulent exudate, airsacculitis, and pericarditis. Secondary infections of sinus occurred with *Pasteurella* species and *Escherichia coli*.

Serological tests were positive for MG in SPA test, HI test to 1:160, and ELISA test to titer group 7. MS serology was positive on HI test to 1:80 and ELISA test to titer group 13. *Mycoplasma meleagridis* HI titer was positive at 1:20. Mycoplasma cultures were performed.

DNA probes were utilized after the third submission. Probes were first tested on tracheal and sinus swabs from the second and third submission and from field specimens of house A. DNA probes were MG positive and MS negative. Diagnostic mycoplasma cultures after 14 days were completed on the first submission. The tracheal and sinus cultures were MG positive when identified by immunoperoxidase. The cultures on the other submissions were also confirmed as MG.

Seventeen days after first submission, sera and tracheal specimens were submitted from house B, C, and D and sentinel turkeys from house A. House B, located closest to affected hens, was DNA MG probe positive and MS ELISA to titer group 4. House C had an MS ELISA titer group 3 without confirming DNA probe evidence. The sentinel turkeys were MG positive on SPA and ELISA titer group 4, while MS HI titer was 1:20.

House C, D, and E were sampled day 27 and 33 after first submission. Day 27 serology was MS ELISA titer group 1 and 4 in house D and titer group 1 in house E. Day 33, all houses were DNA MG probe positive with MG ELISA titer group 3 in house E and MS ELISA titer group 2 in house C and titer group 9 in

house E. Incidentally, diagnostic mycoplasma cultures were negative.

CONCLUSIONS

The beginning of the infection had respiratory signs and pathology changes compatible with a mycoplasmosis. Clinical history, signs, and test results confounded the diagnosis in the early stages of the investigation. The serology was positive for MS HI and ELISA first, then had MG seroconversion on SPA, HI, and ELISA. This trend was repeated when the infection spread to the naïve houses. The immune response of the birds peaked at levels of MS HI at 1:80 and MS ELISA at titer group 13.

Throughout the investigation, the MS, HI, and ELISA tests were positive before the MG tests with evidence of MG via DNA probes and/or cultures. A possible explanation is the field strain of MG with a surface antigen that elicits a cross-reacting immune response on MS tests. Interestingly, MS SPA, MS DNA probe, and diagnostic cultures did not test MS positive throughout the investigation. The cross-reactive antigen is involved with the MS ELISA and HI only. An experimental investigation is being performed to reproduce the results from this MG field strain to rule out a dual infection.

REFERENCE

1. Ley, DH and HW Yoder, Jr. Mycoplasmosis. *Mycoplasma gallisepticum* infection. In: Diseases of Poultry, 10th ed. BW Calnek, HJ Barnes, CW Beard, LR McDougal, and YM Saif, eds. Iowa State University Press, Ames, IA. pp 194-207. 1997.

BACTERIOLOGICAL INVESTIGATION ON *SALMONELLA* IN MEAT TURKEY FLOCKS

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Salmonella infection of turkeys continues to be an important economic and public health problem. In spite of significant improvement in technology and hygienic practice at all stages of turkey production accompanied with advanced improvement of public sanitation salmonellosis remains a persistent threat to human and animal health.

The fact that the processing plants are not able to reduce the pathogenic bacteria in poultry products means that effort must be made to reduce the *Salmonella* contamination of the live birds before despatch to processing plant. Currently, there is no legislation or obligation for testing meat turkey flocks for *Salmonella* before slaughtering. However, the fact remains that processing plants are not able to effectively reduce the incidents of pathogenic bacteria in poultry products. This fact has forced several companies in Europe to change the slaughter and processing system toward the logistic slaughtering of the flocks with a known status based on the history of individual farm and the results of a bacteriological examination before slaughtering in order to improve the quality and to reduce the cross contamination during the processing. The present study was carried out to investigate the occurrence of *Salmonella* infection in meat turkey prior to slaughter toward logistic slaughtering.

MATERIAL AND METHODS

Bacteriological examinations for detection of *Salmonella*. In year 2001 totally 1460 and in year 2002 totally 1350 commercial turkey flocks were monitored. Two socks samples were collected from each monitored flock 2-3 weeks prior to slaughter and examined bacteriologically. The samples were pre-enriched in Buffered Peptone Water (1:10) and incubated at 37°C for 24 hours. 0.1 ml of this pre-enriched broth was transferred to 9.9 ml Rappaport Vassiliadis (RV) enrichment broth (1:100) and incubated at 41.5°C for 48 hours. The RV broth was streaked on Brilliant Green Phenol Red Agar (BGA) and Rambach plates then incubated at 37°C for 24 hours. *Salmonella* suspected colonies were identified serologically using slide agglutination tests.

RESULTS AND CONCLUSION

In 2001 *Salmonella* of different serovars could be isolated from199 flocks out of tested 1460 flocks (13.7 %). Examination of the 1350 flocks monitored in year 2002 revealed positive results in 238 flocks (17.6%). Different serotypes of *Salmonella* were detected in both years (Table 1). The most frequently isolated serovars were belonging to serogroup B.

The most predominant serovar in year 2001 was S. Heidelberg and in year 2002 S. Saint-paul (90

flocks). *Salmonella typhimurium* as well as *Salmonella enteritidis* were isolated from 7 and 22 flocks respectively in year 2001. Two of isolated *S. enteritidis* were of phage type 4 while 5 isolated were of phage type 19. Fifteen out of 22 isolated *S. typhimurium* were belonging to DT104L, while 5 and 2 were DT120 and DT008 types respectively. In year 2002 *S. enteritidis* of phage type 4 could be detected in one flock, while *S. typhimurium* of DT104L type was found in 11 flocks.

In the present investigation *Salmonella* serovars of significant public health concern could be detected in monitored turkey flocks. All positive flocks were slaughtered and processed at the end of the day followed by thoroughly cleaning and disinfection of the slaughterhouse and equipments. Meat from positive flocks was not used for preparation of specific products. In addition, hygienic measures were intensified on positive farms. The obtained results still reinforce the fact that it is essential and important to continue and/or to start efforts on reducing foodborne infections.

Table 1. Results of isolated *Salmonella* serogroups**.**

	2001		2002	
	No. of positive	%	No. of positive	%
	N= 1460		n= 1350	
Serogroup B	167	84.0	214	89.9
Serogroup C	10	5.0	11	4.6
Serogroup D	7	3.5	1	0.4
Serogroup E	8	4.0	4	1.7
Others	7	3.5	8	3.4
Total	199		238	

n= Number of tested flocks

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EVALUATION OF POULVAC-ST IN COMMERCIAL BROILERS

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INTRODUCTION

A live *Salmonella typhimurium* vaccine is marketed under the name Poulvac ST by Fort Dodge Animal Health. Gene modification of the aro-A and serC genes was used to produce the vaccine organism from the parental strain. Loss of function of the aro-A gene blocks the biosynthetic pathway of the organism and results in the requirement of specific metabolites or aromatic amines not found in birds or mammals. The requirement for these specific metabolites results in retardation of *in vivo* growth of the vaccine organism, yet allows retention of broad cross protective properties to wild *Salmonella* serotypes.

The *Salmonella* reduction properties of this vaccine have been well documented during extensive field usage in commercial layer pullets and broiler breeders in the United States. The broiler industry has been slower to accept live *Salmonella* vaccines as an intervention method to reduce carcass *Salmonella* numbers at the processing plants. Coloe, et al. reported in the 1994 WPDC Proceedings on field trials in commercial meat turkeys and broilers in Australia. Flocks were placed in infected sheds and exposed to a field strain of *S. typhimurium* under natural conditions. Comparison of Day-1 vaccinated birds to non-vaccinated birds showed a significant reduction in lesions and mortality in the Poulvac ST vaccinated birds. Vaccination also led to a significant increase in bird weights at processing in both the turkeys and broilers. This increased productivity has also been observed in commercial broilers vaccinated with

Poulvac ST in the United States. Thus the following study was conducted to evaluate both the protective and performance properties of this vaccine in commercial broilers under a highly controlled pen study.

MATERIALS AND METHODS

A seven week floor pen study with 1,920 Ross x Ross broiler chickens was designed to compare the performance of broilers vaccinated with Poulvac ST live *Salmonella* vaccine to broilers not *Salmonella* vaccinated, and on a BMD/Stafac feed shuttle program in a controlled highly replicated floor pen trial. There were four different treatment groups replicated eight times for a total of 32 pens with 60 birds per pen at a density of 0.7 sq. ft. per bird. Birds were fed corn-soy based diets. The four groups were as follows: Non-vaccinated without growth promotants (NVU), Poulvac ST vaccinates without growth promotants (PVU), non-vaccinated with growth promotants (NVG), and Poulvac ST vaccinates with growth promotants (PVG).

All broilers were vaccinated for Mareks at Day 18 of incubation along with gentamicin antibiotic. Poulvac ST was applied at the hatchery by coarse spray at the dose rate of seven ml per 100 birds. Newcastle and Bronchitis were applied at the hatchery at Day 1 by coarse spray in accordance with normal commercial procedures. A booster dose of vaccine including Poulvac ST, Newcastle, and Bronchitis were given to all pens at Day 17 by coarse spray.

All birds were weighed by pen on Days 0 and 21, and by individual weights at Day 49. All mortality was added back into the pen weights for final analysis of feed conversion. Feed consumption was determined at Day 21 and Day 49. At Day 49, four birds per pen were taken and the pre-slaughter weight and hot, post evisceration weight were used to determine hot carcass yield. On Day 51 the breasts were removed (bone-out) from each bird and post-chill breast and carcass yield was determined.

Paper from the floors of all the chick delivery boxes were collected at delivery to the trial site, identified by treatment, and sent to a microbiology lab for evaluation. On day 49 all pens were sampled by drag swabs. At Day 50, two birds per pen (one male and one female) were processed at trial site and carcass rinses were conducted according to USDA specifications. The remaining birds were cooped and trucked at Day 50 to a commercial processing plant. The trial birds were processed as the first flock on the morning shift. Pre-chill carcass rinses were conducted on sixteen birds from the vaccinated group and sixteen from the non-vaccinated group. Ten post-chill carcass rinses were also conducted from each group. In addition, 25 cecal samples, each sample consisting of 5-paired ceca, were collected from both vaccinates (PV) and non-vaccinates (NC). All samples were sent to a microbiology lab for *Salmonella* evaluation.

CONCLUSIONS

This study was designed to compare the performance of broilers vaccinated with the *Salmonella* vaccine Poulvac ST to that of broilers not vaccinated against *Salmonella*. Each group was further divided into a BMD/Stafac feed shuttle program or a non-medicated feed program. Mortality and flock homogeneity (by Coeficient of Variation) were not significantly ($p>0.05$) affected by the use of *Salmonella* vaccine. Final average weights were heavier for the groups receiving Poulvac ST in each paired comparison as well as in the pooled comparison. Final average live weights were significantly heavier for vaccinated broilers on the growth promotant shuttle program. Final average weights were also heavier in the vaccinates when data were pooled. Week three feed conversions were not affected by the use of Poulvac ST. Final feed conversions of the Poulvac Vaccinated Unmedicated (PVU) birds were significantly lower than those of the Non Vaccinated Unmedicated (NVU) group. Pooled final feed conversions were significantly lower for the Poulvac Vaccinated (PV) treated birds than the Negative Controls (NC). Pooled carcass yield of the PV group was significantly higher (1.28%) than that of the NC group and there were no differences found between the pooled breast yield values.

Microbiology results of the paper from the floors of the chick delivery boxes were 50% positive for salmonella which serogrouped as B or C. Pen drag swabs at Day 50 were positive for salmonella in all four treatment groups. The PVU group had approximately 75% fewer Day 55 *Salmonella* positive carcasses than the other three treatment groups.

A cost analysis of the feed cost per pound of meat and return over feed was conducted. Input cost of vaccines and feed medication was included in the overall cost analysis. Revenue per bird of the PVU group was significantly greater than that of NVU group. Revenue per bird of the PVG and NVG groups was not different. Pooled Revenue/Bird of the pooled PV group was significantly greater than that of the pooled NC groups.

SUMMARY

These data suggest that Poulvac ST vaccine administered at the hatchery followed by a booster at 14 days provides substantial protection to broilers allowing performance to be minimally affected by

Salmonella organisms. Laboratory analysis for *Salmonella* findings were not consistent for the two Poulvac ST groups as the vaccinated group not fed growth promotants had significantly fewer *Salmonella* findings than did the vaccinated group fed growth promotants.

REFERENCES

1. Alderton, M.R., Fahey, K.J., and Coloe, P.J. Humoral responses and salmonellosis protection in chickens given a vitamin-dependent *Salmonella typhimurium* mutant. Avian Diseases 35:435-442. 1991.

2. Barbezange, C., Humbert, F., Rose, V., Lelande, F., and Salvat, G. Some Safety Aspects of *Salmonella* Vaccines for Poultry: Distribution and Persistence of Three *Salmonella typhimurium* Live Vaccines. Avian Diseases 44:968-976, 2000.

3. Coloe, P.J., Garraty, N.L., Christopher, W., Alderton, M.R., and Smith, S.C. Vaccination of poultry with *Salmonella typhimurium* STM-1. Laboratory and field evaluations. Proc. 43rd West. Po. Dis. Conf. 37-39. 1994

4. Methner, U., Berndt, A, and Steinbach, Gunther. Combination of Competitive Exclusion and Immunization with an Attenuated Live *Salmonella* Vaccine Strain in Chickens. Avian Diseases 45:631-638, 2001.

THE THREAT OF BIOTERRORISM TO THE POULTRY INDUSTRY: THE VETERINARY RESPONSE

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Recent events have made clear the United States is a potential target for terrorist activities designed to kill Americans, destroy critical infrastructures and destabilize the government (6). Serious concerns have been raised as to whether agriculture could also be a potential target. Questions have also been raised by some industry and governmental officials whether the federal government is capable of adequately responding to such emergencies and whether commercial agricultural production and processing companies could withstand the financial impact should a major agroterrorism attack occur (1).

Agriculture is one example of what can be called a "critical infrastructure," i.e., a system with out which our society could not function. Agriculture's contribution to the overall economy, exceeding a trillion dollars annually, accounts for approximately one sixth of the gross domestic product. Serving as the nation's largest employer it includes one out of every eight Americans, who are employed directly or indirectly in the many and varied facets of food production, processing, transportation, distribution and sales (5). Being a complex system of many interlocking subsystems, animal agriculture is in particular, vulnerable to disruption at many points, including maintenance of genetic stocks; replacement bird populations and distribution; live broiler, turkey and egg production; feed ingredient storage and transportation; feed production and distribution; animal transportation and processing; further processing; finished product storage, transportation and sales. Acting essentially as a pipeline, interference at any point, could eventually lead to the partial or total disruption of the end supply for the consumer. Within agriculture, the more integrated the subsystem, the more vulnerable to disruption; therefore poultry, being the most integrated of the animal production systems could also be considered the most vulnerable to terrorist attack (3).

Although, projections as to the potential economic effects that could result from a terrorist attack on the poultry industry are difficult to determine, (due to variances in the model used or differences in the depth, breadth and exact nature of the attack), some generalities can be stated. In Alabama, a simple scenario was developed to determine the potential economic impact on poultry processing personnel, resulting from a simulated attack projected to affect both regions of Alabama containing poultry (i.e. North and South) and subsequently causing all transportation and processing activities within and between the regions to cease. The scenario projected immediate unemployment affecting between 30,000-35,000 employees, thereby leading to a projected direct cost to the state of $30-35 million per month in unemployment benefits, with an overall projected economic impact (economic multiplier of 4) exceeding $120-140 million per month (3).

Presently, over 24 million people are employed in agriculture or allied industries in the United States. Although, all of these people are not employed in poultry, if for example 10 % of the total (2.4 million

people) were assumed to be employed in the poultry and production industries and affected by loss of employment, the total projected cost (using an average of $1000/month for unemployment benefits) would be $2.4 billion per month, with a total economic impact (using an economic multiplier of 4) calculated to exceed $9.6 billion per month. If such a scenario were to occur in the present economic climate, the major poultry production states could not be expected to have the additional financial resources necessary to respond adequately, leaving most of financial burden to the federal government (3). Additional catastrophic economic losses would also be expected to occur to company assets as well, but were not used in these calculations.

All emergencies, regardless of their eventual outcome, start as local problems. Should an emergency occur in the poultry industry, (for example large-scale mortality due to avian influenza), the first layer of recognition and response would be the local farmer and/or company serviceman. From this level of responsibility, contact would be made with higher-level live production management, who could then respond either directly or indirectly through the auspices of contracted private or company veterinarian(s). After assessment, a response would begin, likely including the removal of dead and moribund animals by the farmer and the administration of situation specific and appropriate medication(s) and/or vaccinations.

A sampling of moribund animals and/or dead animals would most probably be taken to the designated agency within the state responsible for veterinary diagnostics. Once definitive diagnosis had been made, state and federal law could if appropriate mandate a greater response. If the disease outbreak were suspected to be a "foreign animal disease," the state veterinary laboratory system and political leadership responsible for agriculture in the state would refer the case to the state or regionally assigned United States Department of Agriculture – Animal and Plant Health Inspection Service (USDA-APHIS) Veterinary Medical Officer. Upon referral, USDA-APHIS would assess the situation and if deemed appropriate, could activate the Emergency Programs system, which would respond with federally sponsored equipment and massive numbers of additional personnel. Other federal agencies having secondary roles in the response would most likely include the Commerce Department, Environmental Protection Agency (EPA), Federal Emergency Management Administration (FEMA) and the United States Department of Agriculture – Food Safety Inspection Service (USDA-FSIS). When suspected, or once proven that a terrorist attack actually occurred, the lead agency from an evidentiary standpoint would become the Federal Bureau of Investigation, thus making more complex the response. At each level, veterinary professionals would be involved in the decision making process as well as the containment and eradication response.

In theory, the system should and has in the past worked efficiently. Given a naturally occurring reportable disease such as avian influenza, the federal response has and should be expected to be massive. Past incidents of naturally occurring disease are however in themselves inadequate models by which future plans can be designed to project the required resources necessary to respond to an actual terrorist attack. Given, the vagaries of state and federal politics, infrastructure and personnel deficiencies, the system at present should be assumed to be inadequate to address this new type of scenario. Depending on the scope and nature of the attack, some states will be able to respond rapidly and decisively, while others will not. In all scenarios multiple, geographically dispersed targets should be considered the norm. As has been witnessed in both recent and more distant examples of natural outbreaks in the commercial poultry industry, delays should also be considered inevitable. One particularly disturbing scenario projects multifaceted attacks that cross commodities, geographical boundaries and/or the possibility of using animals as a vector for transmitting human disease. Such scenarios are not addressed in any existing emergency plans at either the state or federal level. Should such a scenario prove true, adequate federal resources at present would most likely not be available for simultaneous responses to multiple point attacks (2).

Given this rather pessimistic view, the question that remains is, what can be done? One possible solution, although controversial has suggested that states must be made more autonomous, so that they have a greater share of the trained veterinary, support personnel, equipment and infrastructure on site, to deal with that portion of the attack that might occur within their borders. If each state is equipped to independently deal with its own catastrophic situation, supplemented by resources that can only be provided by the federal government, the argument states the country as a whole becomes more empowered to diminish the delay and contain the economic impact.

One effort presently being designed to enhance the security and readiness of the nation's animal agricultural production and processing industries, is a proposed program entitled, The Poultry and Meat Production and Processing Information Sharing and Analysis Center (PMPP-ISAC). In direct collaboration with the National Infrastructure Protection Center (NIPC), the proposed PMPP-ISAC system is being designed to provide a mechanism by which vital security-related information can be moved more effectively between the multi-agency NIPC, (now part of the Office of Homeland Defense) and the many

diverse operations comprising the poultry and meat production and processing industries, using faculty from collaborating Land Grant Universities and Veterinary Colleges as research leaders, analysts and third party experts (4).

The PMPP-ISAC has been envisioned as a partnership of major poultry and meat production and processing companies, agricultural related banking and insurance companies, State and Federal Agencies and cooperating Land Grant University and Veterinary College entities. The PMPP-ISAC allows the nation's leading experts in poultry and meat production and processing located to share and assess threat intelligence information provided by its industry membership and NIPC and to then assist NIPC in preparing warnings of threats against the poultry and meat production and processing infrastructure (4).

The PMPP-ISAC is also part of a larger proposed plan entitled CANARI, or "Consolidated American Network for Agricultural Resource Intelligence," which is envisioned as a network of cooperating agencies at the Federal and State level, operating in a manner that exploits the strengths of the varied stakeholders, while providing a platform for better directed and coordinated resources and management tools at the state, local or commodity level. Representing a much- needed proactive approach for integrating surveillance, detection, identification and information dissemination the system is designed to better use state, local, commodity and private veterinary resources in prevention program development, as well as the rapid response capabilities of these "on-site" experts, should an agroterrorism event occur (3).

Recent change in the USDA certified laboratories and regional laboratory system also promises to be a good first step in providing a system better able to respond to local or regional emergencies. The expansion of resources and responsibilities should however, continue to include more of the state veterinary diagnostic laboratories, as yet not part of the enhanced system. Private resources must be included in the expansion of these capabilities, even including commodity based laboratories, which could play a positive role in the event of an attack.

Federal resources will be stretched too thin to adequately field a response to all of the areas of concern, should multiple states be attack simultaneously. Some states or even regions would likely be forced to depend at least initially upon their own resources, should a coordinated attack occur. The number of veterinarians and laboratory professionals, trained in foreign animal diseases, whether state, federal, company employed or private must be expanded dramatically and rapidly. Much can be done within the existing veterinary college curriculums to further this goal. Those already in practice must be given the means and opportunity, by which they to can be trained further. Foreign animal disease training should become a required topic for all continuing education requirements and licensing, even among small animal practitioners. An alternative to the presently required on-site training at USDA's Plum Island facilities must also be found to expedite the process.

In the case of a multi-front agroterrorism attack, trained veterinarians and laboratory professionals "on the ground" must be given the authority to act independently and make decisions that under other, less serious circumstances might have been solely reserved for the Federal Veterinary Medical Officers. Some State Veterinarian systems, using California as an example, are well prepared and experienced in acting semi-autonomously, using federal veterinarians in a consultative capacity and federal resources to supplement state sources. Other states are at best ill prepared for assuming such a role. Changes in the authority of any state veterinary diagnostic system, regardless of its degree of preparation is not and will not be possible, without dramatic changes in the federal law, which will also not be accomplished without pain and controversy. Many changes, as yet undefined will occur as a result of USDA-APHIS (Animal and Plant Health Inspection Services) resources and personnel being transferred to the Office of Homeland Defense. Regardless of the political outcome, the threat of agroterrorism attacks will not diminish and in fact will increase until which time the terrorists are either brought to justice or justice is brought to them. The probability of the end of terrorist activities in the foreseeable future is of now very dim. In the meantime, veterinarians, laboratory professionals, law enforcement and Homeland Security professionals must train and be trained, prepare and be prepared, so as to better ensure the continued availability of a safe, economical and readily available food supply.

REFERENCES

1. National Association of State Departments of Agriculture Research Foundation. The Animal Health Safeguarding Review Results and Recommendations. October, 2001.

2. National Research Council. Countering Agricultural Bioterrorism- Prepublication Edition. National Research Council of the National Academies, Washington, D.C. 2002.

3. Norton, R.A. The Consolidated American Network for Agricultural Resource Intelligence (CANARI) Addressing the Food Security Issues of the 21st Century – A Proposed Comprehensive Plan.

Report to the National Infrastructure Protection Center, U.S. Department of Justice, Washington, D.C. 2002.

4. Norton, R.A. The Poultry and Meat Production and Processing Information Sharing and Analysis Center (PMPP-ISAC) – A Proposed Industry, Federal and State Government and Land Grant University Cooperative Effort. Report to the National Infrastructure Protection Center, U.S. Department of Justice, Washington, D.C. 2002.

5. Parker, H.S. Agricultural Bioterrorism: A Federal Strategy to Meet the Threat. McNair Paper No. 65. Institute for National Strategic Studies, National Defense University, Washington, D.C. 2002.

6. Tenet, G.J. Worldwide threat-Converging dangers in a post 9/11 world. Unclassified testimony of Director of Central Intelligence George J. Tent before the Senate Armed Services Committee, 19 March, 2002.

ANTIBIOTIC ISSUES' DEFINITIONS: FOR THE RECORD

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SUMMARY

The staff veterinarian or manager for a commercial poultry company is likely to be involved in discussing topics related to antibiotics. In order to discuss the issue of antibiotic usage in an accurate and professional manner, the individual must be educated in the proper terminology to use.

BACKGROUND

Differentiation between therapeutic uses via the water administration is "blurred" with the feeding of growth promoters at "low levels" or sub-therapeutic levels. Fact is that sub-therapeutic usage has beneficial impact on sub-clinical disease, providing for safer food for humans and healthier animals.

Ionophore/Arsenicals and other chemical compounds used to control coccidiosis in poultry and improve production in livestock constitute nearly half of total antibiotic volume used in animals. The 2000 AHI Annual Survey found that 83% of animal antibiotics are used as therapeutics (disease treatment and prevention), not growth promotion. Thirty-five percent of the antibacterials (antimicrobials) are unique to animal production and are not used in human medicine (ionophores and arsenicals).

Antibiotics have been used safely for more than 40 years to maintain animal health and were first approved by FDA as feed additives in 1951. Antibiotics are vitally important to veterinarians and poultry producers who rely on these medicines to protect poultry flocks from disease. When used for health maintenance, antibiotics decrease the amount of feed needed, increase the rate of weight gain, and improve feed efficiency (i.e., improve performance). Humans also benefit from the safe, effective use of animal antibiotics in many ways. Antibiotics keep our food supply safe by preserving the health and safety of food animals and animal products such as meat, eggs and milk. When antibiotics are used to raise healthy food animals, there is less potential for contamination by intestinal breakage during processing of the meat. The intestines are stronger and in better condition in healthy animals.

DEFINITIONS

Antibiotic: The AVMA definition is "a chemical substance [class of drug] produced [fermented, synthesized] by a [living] microorganism, which has the capacity, in dilute solutions, to inhibit the growth of or to kill other microorganisms." [Note in brackets was added by author for clarification] "A substance can be classified as an antibiotic agent although it is without effect in vivo (within the body) or is too toxic to permit its use in the body" (Kirk-Othmer, p. 106).

Some consider ionophore anticoccidials to be excluded from the antibiotic classification, even though microorganisms produce them in fermentation, but others consider the ionophores as narrow spectrum (gram positive effect) antimicrobials. Ionophores are not medically important in human medicine, as they are actually toxic, and generally are not absorbed systemically.

Antibacterial: Includes a broader category of compounds (such as, an antibiotic or disinfectant). Whereas a living organism produces an antibiotic, an antibacterial can be any agent (such as, a fermentation or chemical product). Antibacterial activity is limited to bacteria.

Antimicrobial: Includes a broader category of compounds. The AVMA definition is "an agent that kills bacteria [microbe] or suppresses their multiplication or growth. This includes antibiotics and synthetic agents. This excludes ionophores and

arsenicals." [Added by author] Although an antimicrobial is actually not limited to bacteria, it can inhibit or destroy all "microbes" (including protozoa, coccidia, fungi, mildew, etc.). For example, by definition, high concentrations of salt can be an antimicrobial. Some other terms might include "preservative, disinfectant, antiseptic, antifoulant, slimicide, and mildewcide" (Kirk-Othmer, p. 639).

Basically, it is noted that roxarsone (3-Nitro®; 3-nitro-4-hydroxyphenylarsonic acid) and nitarsone (Histostat®, 4-nitrophenylarsonic acid) are not antibiotics by definition, but instead are considered arsenicals with antimicrobial activity. Arsenicals have primary activity against protozoa, coccidia, and spirochetes (*Brachyspira* in swine with roxarsone and *Histomonas meleagridis* with nitarsone, for example). Both roxarsone and nitarsone are not approved or used in human medicine. There are no antibiotic resistance concerns for humans because of the use of these arsenical products in poultry and swine.

Antibiotic Resistance: A natural property of bacteria that confers the capacity to inactivate or exclude antibiotics or a mechanism that blocks the inhibitory or killing effects of antibiotics. Resistance may be either acquired or intrinsic.

Acquired resistance occurs from a mutation that occurred under the selective pressure of having the antibiotic in the environment. The bacteria can also acquire the mutated resistant gene from other bacteria, plasmids, or viruses.

Intrinsic resistance is a natural process by which a bacterium is less sensitive to an antimicrobial. An example of natural resistance is inability to interfere with the cell wall structure or a metabolic pathway.

Resistant defines a bacterium that does not respond to an antibiotic. Less susceptible refers to when an antibiotic is no longer effective at the prescribed dosage or tested level (which may not always be the same). Susceptible describes the sensitivity a bacterium has to an antibiotic.

Disease treatment: Any specific procedure used for the cure or the amelioration of a disease (Taber's 18th and Stedman's 27th ed., Medical Dictionaries).

Disease control: Ongoing operations (programs, procedures) aimed at reducing a disease (Taber's 18th and Stedman's 27th ed., Medical Dictionaries). This might be limited to an antibiotic label claim or expanded to include biosecurity or disinfection plans.

Disease prevention: Hindering the occurrence of disease in a susceptible population (Taber's 18th and Stedman's 27th ed., Medical Dictionaries).

Judicious: "Having or showing sound judgment" (Webster's New World, 1995). The AVMA's position statement on judicious use is: "When the decision is reached to use antimicrobials for therapy, veterinarians should strive to optimize therapeutic efficacy and minimize resistance to antimicrobials to protect public and animal health." The AVMA position is consistent with the Veterinarian's Oath stating "protection of animal health, the relief of animal suffering, the conservation of animal resources, the promotion of public health". The AVMA website has excellent Judicious Use Guidelines for poultry.

Health maintenance: Shifting the population balance of the microflora in the gastrointestinal tract. Improving nutrient utilization, results in healthy growth. Feed efficiency and average daily gain are indicators of response.

Prudent: Exercising sound judgment in practical matters or managing carefully (Webster's New World, 1995).

Therapeutic: Treatment and control of specific bacterial disease(s). Typically the antibiotic is dosed at relatively higher levels. Some definitions limit the use to 14-days or less administration.

Sub-therapeutic: Prevention of bacterial disease. Typically the antibiotic is dosed at relatively lower levels (less than 200 grams per ton of feed) for greater than 14-days. Also called "growth promotion" level.

Non-therapeutic: Similar to sub-therapeutic. The term was coined by the Union of Concerned Scientists in a non-peer reviewed self-published economics article ("Hoggin It", C. Benbrook, 2001). The UCS term "non-therapeutic" includes all products with growth promotion claims plus disease control claims. It implies that any use other than for treatment of an ill animal is unnecessary and constitutes misuse. "Non-therapeutic" is an activist-coined term that has no regulatory or scientific accuracy outside the activist domain.(http://www.asmusa.org/pasrc/browncom.htm).

Growth promotion: Increase rate of weight gain and improve feed efficiency. Dr. Mireles (2002) presents that the mechanism by which growth promoters improve performance probably involves the inflammatory response. "As such, they may not promote growth but rather allow potential growth to occur. Growth promoter supplementation appears to exert a protective mechanism during the inflammatory response, which probably leads to increased survival and improved overall well-being." Refer to health maintenance.

Sub-MIC: The action of an antibiotic when administered at subinhibitory (sub-MIC) levels in an animal. In vivo sub-MIC levels of antibiotics may affect bacteria by (1) modification of the bacterial structure, (2) decreasing adhesion to epithelial surfaces, (3) affecting the production of virulence factors, and (4) utilizing nutrients necessary for growth. Some MIC (minimum inhibitory concentration) determinations are based on breakpoints specifically derived for veterinary medicine, while others utilize human (tetracyclines,

beta-lactams) breakpoints (Roche, 1998). Sub-MIC is not equivalent to sub-therapeutic.

RESOURCES

Alpharma Inc. Animal Health sponsors "For the Record". This is a series of newsletters presented by Alpharma to create a better understanding of the issues surrounding the use of antibiotics in food-producing animals. As the nation's premier source of medicated feed additives, ALPHARMA has committed to supporting your freedom to use those compounds, through continued research and development, promotion of prudent use, funding of producer education, and fighting junk science with facts. www.alpharma.com/ahd.

Alpharma Animal Health is a global leader in the development, registration, manufacturing and marketing of pharmaceutical products and technologies for food producing animals. Through prudent use, these various products maintain good health and robust growth, enhance reproductive efficiency and treat disease in cattle, swine and poultry. They are integral, health-management tools, necessary to meet the world's growing demand for a safe, abundant and affordable food supply.

American Council on Science and Health. Founded in 1978 to advocate the use of sound science in policy making, ACSH's board of 350 doctors, scientists and advisors balances with scientific fact the inflammatory media coverage of smoking, AIDS, alcohol, nutrition, the environment, drug safety, biotechnology, food safety, and other topics. www.acsh.org

The Animal Health Institute (AHI) is the U.S. trade association that represents manufacturers of animal health care products -- the pharmaceuticals, vaccines and feed additives used to produce a safe supply of meat, milk, poultry and eggs, and the veterinary medicines that help pets live longer, healthier lives. The Coalition for Animal Health (CAH) is an alliance representing livestock and poultry producers, veterinarians, pet owners, and animal drug and feed makers. Formed in the early 1990s to fight for changes to FDA's inefficient approval process for needed animal drugs, the group now serves as a unified voice in opposing irrational changes in animal-health regulation. CAH members include AHI, National Turkey Federation (NTF), plus numerous others. www.ahi.org

American Veterinary Medical Association website has several Judicious Use Guidelines (http://www.avma.org/scienact/jtua/poultry/poultry00.asp). The AVMA represents over 67,000 veterinarians working in all aspects of veterinary medicine. Also visit http://www.avma.org/scienact/jtua/default.asp and http://www.fda/gov/cvm/fsi/JudUse.htm.

Bayer Animal Health. The www.healthypoultry.com web site was launched to help concerned consumers understand how Baytril® (enrofloxacin) 3.23% Concentrate Antimicrobial Solution is used in poultry and to provide information to the veterinary community. A special section of the site provides technical information for veterinarians and poultry producers and includes selected submissions to the FDA Docket relating to the upcoming hearing.

Center for Consumer Freedom. A coalition made up primarily of restaurant and tavern owners, CCF began in response to attacks on consumer choices in the areas of smoking, drinking and eating. Their efforts, including magazine advertising and web sites, aim to "shine the light on these groups [so], like fungus, they'll dry up and blow away," according to the group's Director of Communication. www.consumerfreedom.com

Kirk-Othmer Concise Encyclopedia of Chemical Technology. Martin Grayson, editor. 1985, John Wiley and Sons.

THE ANTIBIOTIC RESISTANCE DEBATE: AN INDUSTRY PERSPECTIVE ON THE USE OF VIRGINIAMYCIN (STAFAC®) IN POULTRY

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INTRODUCTION

Virginiamycin (Stafac®) is a composite streptogramin feed additive antibiotic that has been used safely and effectively in the United States to prevent and control disease and improve performance in poultry for more than 20 years. In 1998 the use of Virginiamycin was questioned following the launch by Aventis of Synercid®, another composite streptogramin antibiotic (quinupristin/dalfopristin), designed to treat

Vancomycin Resistant Enterococal infections (VREs) in humans.

SYNERCID® AND VIRGINIAMYCIN

A number of presentations by a group of scientists from the Centers for Disease Control and Prevention (C.D.C.) have been made suggesting that Virginiamycin use in poultry and livestock has created a huge reservoir of enterococci resistant to both Virginiamycin and Synercid®, and that this can compromise the effectiveness of Synercid® (J. McClellan, et al., 2001; T. Chiller, et al., 2002; S. Rossiter, et al., 2000; F. Angulo, et al., 2000a; T. Karchmer, et al., 2000; N. Marano, et al., 1999; K. Gay, et al., 2002; F. Angulo, et al., 2000b). A published review of these presentations, however, has uncovered several flaws and biases leading the author to conclude that "the wrong conclusions from bad science can lead to even more serious consequences" (R.A. Norton, 2000b). Others have questioned the connection between the use of feed additive antibiotics in poultry and livestock and the increased problems with antibiotic resistance in human medicine, and have asked that future policy decisions be made on sound science instead of theoretical assumptions (J. Acar, et.al., 2000; I. Phillips, 1999, D. Sahm, 2000, D. Price 2000; R.A. Norton, 2000a).

The theory proposed by the group of scientists at the C.D.C. is that the high incidence of spreptogramin resistant enterococci (SRE) often found in raw poultry and pork meat was caused by the use of Virginiamycin, and that these SRE can be passed to the human population through the food chain creating a large reservoir of SRE. However, when one looks at the results of a very extensive survey on streptogramin resistant *Enterococcus faecium* (SREF) which included more than 1000 clinical isolates from North America (U.S. and Canada) conducted at the time Synercid® was launched in the United States (R.N. Jones, et al., 1998), a different picture emerges. Of the 2 most common enterococal species isolated from human clinical specimens, *E. faecalis* and *E. faecium*, only *E. faecium* is sensitive to Virginiamycin and Synercid®, *E. faecalis* is naturally resistant to both. The results of this survey showed that after 30 years of Virginiamycin use in food animals only 0.2% of the isolates tested were resistant to Synercid®. These results clearly indicate that the transmission of enterococci via the food chain is more theoretical than real. These results are further confirmed by C.D.C.'s own research (Rossiter, et al., 2000) that showed a carriage rate of only 1% SREF isolated from stool samples of human volunteers, after 30 years of Virginiamycin use in poultry and livestock. In contrast, the carriage rate of SREF isolated from raw poultry meat was 61%. It has never been disputed that the use of Virginiamycin in animals will lead to various levels of resistance in the *E. faecium* population of those animals. What has been disputed is the assumption that the SREF from those animals will colonize humans and therefore become a reservoir of resistant genes for the normal bacterial flora of human beings as suggested by the scientists from the C.D.C. (J. McClellan et al., 2001; T. Chiller, *et.al*., 2002; S. Rossiter, et al., 2000; F. Angulo, et al., 2000a; T. Karchmer, et al., 2000; K. Gay, et al., 2002; F. Angulo, et al., 2000b). First of all, poultry and pork meat is not consumed raw; therefore, the cooking process destroys any *E. faecium* originally present. Secondly, scientific research has clearly demonstrated that *E. faecium* from humans do not colonize chickens (J. A. Johnson, 1999; R.J. Willems, et al., 2000; S. Qaiyumi, et al., 2000) and *E. faecium* from animal origin do not colonize humans, even when massive doses are ingested (M. Bloom, 2000 and T.L. Sorensen, et al., 2001).

Another argument frequently made is that the continued use of Virginiamycin in chickens and other food animals may compromise the effectiveness of Synercid® (Rossiter, et al., 2000; F. Angulo, et al, 2000a; T. Karchmer, et al., 2000; F. Angulo, et al., 2000b; J. McClellan, et al., 2001). However, a recently published drug sensitivity survey on *E. faecium* isolated from humans (McDonald, et al., 2001), showed that in spite of the widespread use of Synercid® in U.S. hospitals Synercid®-resistance in *E. faecium* remains low (0.8%).

Further proof of the host-specificity and uniqueness of the *E. faecium* associated with human nosocomial infections has recently been provided (Williems, et al., 2001). These researchers have identified a specific *E. faecium* subpopulation containing a variant of the esp gene responsible for causing epidemics in human hospitals across three continents. The variant gene was absent in all isolates from healthy individuals and 98 isolates from animals.

ZYVOX® AND SYNERCID®

Zyvox® (Linezolid), a new class of antibiotic has been developed and launched by Pharmacia for the treatment of VRE infections in the U.S. (Anon., 1999). The main advantages of Zyvox® over Synercid® are that unlike Synercid®, Zyvox® has good antibacterial activity against both *E. faecium* and *E. faecalis*, which greatly facilitates treatment initiation for critically ill hospital patients. Since Synercid® is only active against *E. faecium*, speciation of pathogenic enterococal isolates is required, a process that further complicates and delays the onset of treatment. Another advantage is that unlike Synercid®, Zyvox® can be administered orally instead of intravenously allowing

patients to be discharged sooner from the hospital with the consequent savings. Finally, unlike Synercid®, Zyvox® exhibits fewer side effects and none of the arthralgia and myalgia often experienced by patients treated with Synercid® (D.M. Livermore, 2000). It is due to all of these advantages that Zyvox® is quickly replacing Synercid® as the drug of choice for the treatment of VRE infections in U.S. hospitals.

THE RISK ASSESSMENT

A science-based risk assessment of Virginiamycin in regards to the creation of SREF has recently been completed and presented to the scientific community (L.A. Cox, Jr., and D.A. Popken, 2002; L.A. Cox, Jr., and K.W. Bafundo, 2002). Even when worst-case scenario conditions were used, this risk assessment proves scientifically and conclusively that the risk to human health from Virginiamycin use in food animals is negligible.

THE FOCUS ON FOOD ANIMALS

A distressing aspect of the entire antibiotic resistance debate is related to its strict focus on antibiotic use in food animals. Clearly, of the 2 distinct animal populations, food animals and companion animals, and as pointed out by others (R.A. Norton, 2000a; D.A. Barber, 2001), companion animals are a much more likely source of antibiotic resistance transmission to humans than food animals. First of all, recent polls have shown that a large percentage of the U.S. human population considers their pets as members of the family, and a significant percentage has admitted to letting their pets sleep in bed with them. Many pets and their owners live in intimate contact and frequently there is tongue to face and mouth to mouth contact. Where is the chance for a significant germ exchange greater, between a baby or a child and his/her puppy or kitten? Or between that baby or child and a raw piece of chicken? I have witnessed many dogs lick their owners' faces and lips, including babies and children, but I am yet to witness a baby or a child lick a raw piece of chicken. One should remember that before chicken is consumed it is cooked, and during this process the bacteria that might have been on it are destroyed. Dogs and other companion animals get treated with the same classes of antibiotics used in human medicine with little to no supervision by any regulatory agency, in much the same way as those prescribed by physicians. So it is difficult to understand why scientists are most concerned with antibiotic use in food animals and not companion animals. Does it make any sense to ban the use of antibiotics in food animals over antibiotic-resistance concerns? As expressed by other experts, "we have so many problems in hospitals that it is hard to imagine that veterinary uses are significantly contributing to microbial resistance in humans" (D. Sahm, 2000), and "if all animal uses of antibiotics were terminated today, there is no evidence that human health would measurably benefit, while animal health would certainly suffer, and possibly human health as a consequence" (I. Phillips, 1999). So if scientists are truly concerned about reducing antibiotic resistance, they should be talking more about reducing unnecessary antibiotic use in medical facilities and hospitals, instead of food animals. And, if animals were to be considered at all as potential sources of antibiotic-resistance, antibiotic use in companion animals instead of food animals would be the first and most logical place to start. So, one has to wonder if there are other factors, such as the fear of taking on the American Medical Association or the American Veterinary Medical Association, or on the known emotional bond between people and their pets, that are causing that the debate primarily be centered around antibiotic use in food animals.

UNANSWERED QUESTIONS

Other questions that common sense dictates one must ask, are the following:

Why is resistance to Vancomycin in human hospitals such a problem if Vancomycin or an antibiotic in the same class has never been used in food animals in the United States?

How is banning the prophylactic use of antibiotics in food animals going to reduce antibiotic resistance problems in the human population when every year several hundreds of thousand people come to the United States from countries where antibiotics are sold indiscriminately over the counter? Most of these people are carrying a natural flora of bacteria with multiple-resistance to the antibiotics used in human medicine. Unfortunately and due to their socio-economic status, these new residents of the United States most often end up working as food handlers at restaurants or food processing plants where they present the greatest risk for transmission via the food chain.

If contact with live germs from food animals is such a danger, why do food animal veterinarians, food animal producers, slaughterhouse and food animal processing plant workers willingly expose themselves on a daily basis to such a danger? Why do life insurance companies not recognize them as a higher risk?

Is it not better to feed small doses of antibiotics that leave no meat residues to food animals to prevent disease rather than wait until they become ill and then have to use much larger doses that can leave meat

residues? Remember, the old saying, "An ounce of prevention is worth a pound of cure". That is very true in the case of preventing diseases in food animals as we have learned from the recent experience in Europe. For example, in Denmark, following the ban of several antibiotics fed in small doses to prevent diseases in food animals, there has been a 30% increase in the use of antibiotics for therapeutic purposes (DANMAP, 2001; S. Muirhead, 2001).

If antibiotic use in food animals is at the root of the antibiotic-resistance problem in human medicine, why is it that only a small number of the many types of bacteria carrying multiple resistant to antibiotics can be acquired by eating foods of animal origin?

BIBLIOGRAPHY

Acar, J., M. Casewell, J. Freeman, C. Friis and H. Goosens. 2000. Avoparcin and virginiamycin as animal growth promoters: a plea for science in decision-making. Clinical Microbiology and Infectious Disease 8: 477-482.

Angulo, F., N. Marano, C. Mackison, C. Gregg, R. Sokolow, E. Debess, L. Gilbert, J. Benson, B. Hill, C. McDonald, 2000a. Isolation of quinupristin /dalfopristin-resistant *Enterococcus faecium* from human stool specimens and retail chicken products in the U.S.-associated with use of virginiamycin in poultry? 1st International Conference on Enterococci, Banff, Canada, February.

Angulo, F., N. Marano, S. Johnson, C. Mackinson, L. Gilbert, M. Park, E. Debess, B. Taylor, J. Madden, B. Hill, K. Joyce, F. Tenover, L. Archibald, and the EIP enterococci study, 2000b. Monitoring for the seeds of antimicrobial resistance in the food supply. 2nd International Conference on Emerging infectious Diseases. Atlanta, GA, July.

Anon., 1999. Linezolid in phase III. Deutsche Apotheker Zeitung, 139: 4128-4129.

Barber, D.A. 2001. Commentary: New per-spectives on transmission of foodborne pathogens and antimicrobial resistance. JAVMA, Vol. 218, 10: 1559-1561.

Blom, M. 2000. Ingestion of vancomycin resistant *Enterococcus faecium* strains of food animal origin by healthy human volunteers: a randomized double blind study. Abstract 162, ICAAC, Toronto, Canada.

Chiller, T., McClellan, S. Rossiter, J. Stevenson, K. Gay, K. Joyce, K. Weeks, F. Angulo and the EIP Enterococci Working Group, 2002. Antimicrobial resistance of enterococci isolated from outpatient stools in the United States, 1998-2001. Infectious Diseases Society of America, Chicago, IL.

Cox, Jr., L.A. and D.A. Popken, 2002. Human Health Risk Assessment for Virginiamycin Use in Chickens. 2002 Conference on Antimicrobial Resistance, Science-Prevention-Control, National Foundation for Infectious Diseases, June 27-29, Hyatt Regency Hotel, Bethesda, MD., p. 43.

Cox, Jr., L.A. and K.W. Bafundo, 2002. Human Health Risks from Use of Virginiamycin in Chickens, 37th National Meeting on Poultry Health & Processing, October 9-11, Clarion Resort, Ocean City, MD, 109-116.

DANMAP 2001. Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, foods and humans in Denmark.

Gay, K., K. Joyce, J. Stevenson, F. Angulo, T. Barrett, and the NARMS Working Group, 2002. Quinupristin/dalfopristin-Resistant *Enterococcus Faecium* Isolated from Human Stools, Retail Chicken, and Retail Pork: EIP Enterococci Project. International Conference on Emerging Infectious Diseases, Atlanta, GA, March.

Johnson, J.A. 1999. Comparison of quinupristin /dalfopristin resistant *Enterococcus faecium* from clinical and poultry sources. Antibiotic Use in Animals: Food Safety and Risk Assessment. Conference Proceedings, May 1999.

Johnson, J.A., J. Hayes and D. Wagner. 2000. Comparison of streptogramin resistant *Enterococcus faecium* from poultry and humans. Conference Proceedings of the American Association of Avian Pathologists, presented at the 137th Meeting of the American Veterinary Medical Association, Salt Lake City, Utah.

Jones, R., C.H. Ballow, D.J. Biedenback, J.A. Deinhart, and J.J. Schentag. 1998. Antimicrobial activity of quinupristin-dalfopristin (RP 59500, Synercid®) tested against over 28,000 recent clinical isolates from 200 medical centers in the United States and Canada. Diagn. Microbiol. Infect. Dis. 30: 437-451.

Karchmer, T., C. Mackinson, M. Sullivan, S. Johnson, L. Gilbert, M. Park, E. Debess, R. Sokolow, F. Tenover, K. Joyce, F. Angulo. Presence of quinupristin/dalfopristin-resistance *Enterococcus faecium* in stool specimens from healthy people and in retail chicken products in the U.S. 4th Decennial International Conference on Nosocomial and Healthcare-Associated Infections. Atlanta, GA, February.

Livermore, D.M., 2000. Quinupristin/dalfopristin and linezolid: where, when, which and whether to use? J. of Antimicrobial Chemotherapy, 46: 347-350.

McClellan, J., K. Joyce, S. Rossiter, T. Barrett, F. Angulo, and the NARMS Enterococci working group, 2001. High-Level Gentamycin Resistant Enterococci and Quinupristin/Dalfopristin Resistant *E. faecium* from Ground Pork Purchased from Grocery Stores. The 41st Interscience Conference on Antimicrobial

Agents and Chemotherapy annual meeting, Chicago, IL.

McDonald, L.C., S. Rossiter, C. Mackinson, Y.Y. Wang, S. Johnson, M. Sullivan, R. Sokolow, E. Debess, L Gilbert, J. Benson, B. Hill and F. J. Angulo. 2001. Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. N. Engl. J. Med. 345 (16): 1155-1160.

Muirhead, S., 2001. Therapeutic antibiotic use rises in Denmark. Feedstuffs: December 31: 3.

Norton, R.A., 2000a. Opinion: Antimicrobial resistance debate far from complete. Feedstuffs: October 2, 8.

Norton, R.A., 2000b. Commentary on Science and Public Policy: Antimicrobial Resistance: A Looming Crisis?, WATT Poultry USA, November, 34-39.

Phillips, I., 1999. Assessing the evidence that antibiotic growth promoters influence human infections. J. of Hospital Infection: 43: 173-178.

Price, D., 2000. Opinion: Real antibiotics issue can not be overlooked. Feedstuffs: September 4, 8.

Qaiyumi, S., A.C. McIntosh, C.K. MacKinson, V. Bohrer and J.A. Johnson. 2000. Effect of virginiamycin on *Enterococcus* colonization in a chicken model. Abstract 151, ICAAC, Toronto, Canada.

Rossiter, S., K. Joyce, B. Hill, N. Marano. Isolation of quinupristin/dalfopristin-resistant *Enterococcus faecium* from human stool specimens and chickens purchased from grocery stores in the United States-use of virginiamycin may compromise new human antibiotic. 137th American Veterinary Medical Association Annual Meeting, Salt Lake City, UT.

Sahm, D., 2000. Antibiotic resistance getting 'more attention than it deserves,' expert says. Food Chemical News, July 17: 18-19.

Sorensen, T.L., M. Blom, D. Monnet, N. Frimodt-Moller, R. L. Poulsen and F. Espersen. 2001. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. N. Engl. J. Med. 345 (16): 1161-1166.

Willems, R.J.L., J. Top, N. van den Braak, A. van Belkum, H. Endtz, D. Mevius, E. Stobberingh, A. van den Bogaard and H. van Embden. 2000. Host specificity of vancomycin-resistant *Enterococcus faecium*. J. Infect. Dis. 182: 816-823.

Willems, R.J.L., W. Homan, J. Top, M. van Santen-Verheuvel, D. Tribe, X. Manzioros, C. Gaillard, C., Vandenbroucke-Grauls, E. Mascini, E. van Kregten, J. van Embden and M. Bonten. 2001. Variant esp gene as a marker of a distinct genetic lineage of vancomycin-resistant *Enterococcus faecium* spreading in hospitals. The Lancet 357: 853-855.

CAMPYLOBACTER JEJUNI ORAL GAVAGE IN YOUNG BROILER CHICKEN

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One hundred and seventy day old broiler chicks were obtained from a local hatchery. Blood samples were collected from 10 chicks and the chicks were necropsied, composite samples of intestines cultured for the presence of *Campylobacter jejuni*. The serum samples were tested for IBDV, MG, MS and Pullorum typhoid antibodies.

The remaining 160 chicks were subdivided into ten groups of 16 chicks in each group. The chicks were raised on wire in isolation chambers. The feed and water was provided at libitum. Three groups of 16 chicks were inoculated with approximately 0.5 ml of $1x10^8$ CFU of *C. jejuni* of chicken origin by crop gavage at 13 days of age. Similarly one group of chickens was inoculated with the *C. jejuni* of human origin. Another four groups of chicks were inoculated similarly and also were vaccinated with a commercial infectious bursal disease vaccine. One group was vaccinated with only infectious bursal disease vaccine and another group was kept as uninoculated control group.

At 8, 15 and 22 days post inoculation, three chicks were collected at random from each treatment group, euthanized, necropsied, intestine tissues cultured for *C. jejuni* enumeration, and the tissues of intestine collected for histopathology. All chicks were weighed at 7, 14, 21, 28, and 35 days of age, and statistical analysis was performed. The study was terminated at day 35.

Clinical signs of diarrhea were not present in any of the inoculated groups. Reduced body weights were not observed at 21, 28 or 35 days of age. A mortality of 6.25 percent was present in three out of eight inoculated groups with lesions of septicemia.

Results of culturing of the distal intestine and ceca composite samples were three to four logs higher as compared to duodenum and mid intestine composite samples. Tests performed by PCR on DNA extraction

of the distal intestine and ceca composite samples were quite similar to the culture method results. The *Campylobacter*-uninoculated birds were negative for these bacteria as determined by PCR analysis of DNA extractions and culturing methods.

COMPARISON OF PATHOGENIC OR ENVIRONMENTAL ESCHERICHIA COLI FROM BROILER CHICKENS AND BROILER HOUSE LITTER

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INTRODUCTION

The objective of this study was to characterize the *Escherichia coli (E. coli)* causing airsacculitis or cellulitis in broiler flocks and to ascertain the occurrence of these pathogenic types in the broiler flock environment. Lesion and litter isolates were matched by house and flock of origin and compared for similarity in serotype, and by pulsed-field gel electrophoresis (PFGE).

MATERIALS AND METHODS

E. coli isolates were collected from the broiler house litter, and lesions of either cellulitis or colibacillosis in six flocks. Litter isolates were obtained by culture from grab samples or from drag swabs. The isolates were cultured on MacConkey and blood agar, and identification was confirmed using standard biochemical tests. Serotyping was performed by the *E. coli* Reference Center (ECRC) in State College, PA. Genotyping of isolates from two flocks was performed by PFGE using a rapid method (Gautom, 1997). Plugs were digested with the restriction enzyme*SfiI* and electrophoresis was carried out using a BioRad Chef-Dr III system with run times of 30 h at 6 volts and 120 degree included angle and switch times of 2 to 30 seconds.

RESULTS

The variation among serotype for lesion isolates was less that for litter in all six flocks. There were two to six serotypes identified from lesions within a flock, while up to 13 serotypes were identified from litter isolates. A large percentage of isolates were not typed, i.e. did not react against the panel of antisera used by the ECRC. Up to 84% of lesion isolates and 45% of litter isolates were not typable (NT) using standard reference antisera. The occurrence of matching serotypes among lesion and litter isolates was extremely low, excluding NT isolates. Three of six flocks had no matching serotypes, two ranches had one match and one ranch had three matches. Common pathogenic serotypes identified included O1, O2, O5 and O78. Serotype O119 was identified on 50% of farms. Statistical analysis of the serotype data showed a strong dependence of serotype on isolate source, meaning that there was a high probability that a particular serotype would be associated with lesions or with litter, but not with both. This finding was also supported by comparing the macrorestriction profiles generated by pulsed field gel electrophoresis of lesion and litter isolates in two flocks. Sixteen unique macrorestriction profiles (genotypes) were found among lesion isolates and 11 among litter isolates. Most isolates that were NT by serologic methods were typed using PFGE.

CONCLUSIONS

Serotyping revealed significant differences between litter and lesion isolates of *E. coli*, suggesting that the majority of *E. coli* in the broiler house environment are not involved in causing airsacculitis or cellulitis. PFGE proved to be a useful means of differentiating *E. coli* isolates that were not typable by serologic methods. Defining the composition of *E. coli* populations in commercial poultry production may enhance our understanding of the epidemiology of *E. coli* related diseases.

REFERENCES

1. Gautom, R. K. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Micro. 35:2977-80. 1997.

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THE MULTIPLE FACES OF CLOSTRIDIAL INFECTIONS IN OSTRICHES

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Clostridial infections in ostriches are probably one of the most common problems observed in ostriches raised under commercial farming conditions. Different strains of clostridium including *C. perfringens*, *C. colinum*, *C. septicum*, *C. sordellii* have been reported to be involved in enterotoxemic problems in ostriches (4). Clostridial infections in ostriches may be related to different syndromes ranging from hyperacute mortality in young ostriches to toxemic paresis in adult ostrich breeders. *Clostridium perfringens* (A and D) and probably other types of clostridia seem to be part of the natural intestinal flora of healthy adult birds, but under certain conditions these clostridia may become pathogenic and cause severe disease and mortality in ostriches.

Young ostrich chicks are prone to suffer from clostridial enteritis. The lack of a normal flora in the intestines, the management and grazing conditions and the stress caused by other factors such as overcrowding and low rearing temperatures, are probably the main factors involved in the dynamics of the development of clostridial diseases in young ostrich chicks.

Hyperacute enterotoxemia caused by *C. sordellii* (7), *C. perfringens*, *C. difficile* (3) and *C. colinum* (6) have been observed in young ostrich chicks as early as 9 -10 days of age causing mortality rates of 5% to 95%. Clostridial infections in very young chicks up to 2-3 weeks are characterized by a sharp increase in mortality, watery diarrhea, and non-specific enteritis with desquamation of the epithelium and severe dehydration. Typical lesions of necrotic enteritis at this age are rare, and in most of the cases the disease is diagnosed by making direct smears of the intestinal content, staining and examination under a light microscope.

One of the most outstanding findings in suspected clostridial enteritis is the fact that examination of stained smears from intestinal content reveals only gram positive clostridium like bacteria without the presence of gram negative flora.

Isolation and identification of the type of clostridium involved may be carried out by accepted methods of bacteriology and specific immuno-fluorescence.

Depending on the management procedures carried out at the farms, the movement of young ostrich chicks from a clean rearing environment to lucerne grazing fields may increase in many cases the appearance of clostridial enteritis. These cases are more common when the grazing field has been used previously for grazing and the contamination with clostridial spores may be relatively high, inducing disease in the ostrich chicks. In one case in Israel, *C. sordellii* was the only clostridia found causing hemorrhagic enteritis in ostriches after being moved from the rearing houses to a lucerne field used previously to keep growing ostriches. Large numbers of *C. sordellii* spores were found on samples taken from the lucerne pasture (2).

Control of clostridial diseases during the rearing period up to 2 months of age can be achieved by the use of zinc bacitracin in the feed (6).

Clostridial hemorrhagic enteritis is a hyper-acute form of clostridiasis usually observed in ostriches 1-6 months of age. The outcome of the disease is very rapid with apparently healthy ostriches dying suddenly without any signs of disease or diarrhea. Clostridial hemorrhagic enteritis seems to be more common during the early winter and spring and is probably related to changes in the weather conditions and the tendency of ostriches to peck or eat muddy ground. During post mortem examination the small intestine is found filled with fresh blood, in most of the ostriches dying during the acute phase this may be the only macroscopic change. Dying ostriches during the latter phases of the outbreak may show the typical towel like appearance of the epithelium and in some cases focal necrosis of the liver. The rapid outcome of the disease and the sharp increase in the mortality (up to 10% in one day) makes it imperative to start treatment as soon as possible. Ampicillin or oxytetracycline in the

drinking water have been very successful in reducing mortality and spreading of the disease.

Typical necrotic enteritis is more often observed in ostriches after 6 months of age. The outbreaks are in many cases seasonally related (early winter) and may affect adult breeders causing usually sporadic but stubborn mortality among the ostriches within a pen. The disease in adult ostriches is less acute and affected ostriches show severe signs of disease. They move very slowly and seem to be in pain and shock. Sick birds die between 24 and 48 hours from the first clinical signs. Post mortem examination of affected birds reveals large areas of necrosis along the small intestine. Most of the affected ostriches will show a swollen dark and enlarged liver with areas of focal necrosis. Attempts to treat affected ostriches showing clinical signs have only very limited success. Treatment of the flock in the drinking water may stop mortality in the affected pen within 48 hrs; in some cases the disease may reappear in the pen within a few weeks from the first outbreak.

Partial and total paralysis of the legs, wings, and neck may be in some cases related to clostridial toxemia. *C. botulinum* (1) and *C. chauvoei* (5) have been reported to be probably involved in cases of neurological impairment in ostriches. Intoxication is due to ingestion of contaminated meat from carcasses of dead animals. These kind of clostridial problems are sporadic and of low economic importance, but differential diagnosis in such suspected cases must include encephalitis caused by encephalitis due to arboviruses and Newcastle disease.

REFERENCES

1. Allwright, D. M., M. Wilson and W.J.J. Van Rensburg. Botulism in ostriches (Struthio camelus). Avian. Pathol. 23(1):183-186.1994.

2. Ashash, E. Personal comunication.2002.

3. Frazier, K.S., A.J. Herron., M.E. Gaskin., and N.H. Altman. Diagnosis of enteritis and enterotoxemia due to Clostridium difficile in captive ostriches (Struthio camelus). J. Vet. Diagnostic Investigation. 5: 623-625. 1993.

4. Huchzermeyer. F. W. Bacterial infections In: Diseases of ostriches and other ratites. Agricultural Research Council. Onderstepoort Veterinary Institute. pp.161-162. 1998.

5. Lublin, A., S. Mechani., H.I. Horowitz and Y. Weisman. A paralytic-like disease of the ostrich (Struthio camelus massaicus) associated with Clostridium chauvoei infection.
Vet. Rec. 132:273-275. 1993.

6. Perelman, B. Medical problems in ostriches raised under intensive conditions. In: Proc. Post Graduate Education in Veterinary Medicine. Utrecht Holland. pp. 1-26.1993.

7. Poonacha, K. B. and J.M Donahue. Acute clostridial hepatitis in an ostrich. J. Vet. Diagnostic Investigation.9: 208-210. 1997.

AN ANALYSIS OF THE PERFORMANCE OF COMMERCIAL BROILERS IN THE USA FROM 1997 TO 2001

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ABSTRACT

It is well known that significant improvements have been made in the performance of commercial broilers in recent years but actual data is often anecdotal and not widely available. In the USA a database has been developed that provides accurate, frequent information from individual poultry units on many aspects of poultry production (AGRI Stats Inc., Fort Wayne, IN). This database has been used to analyze changes in calorie conversion (CC) a measure of feed efficiency, number of days to produce a 2.27 kg broiler (DAYS), final bird weight (FBW), and % mortality (MORT) from 1997 to 2001. There was a linear decrease in CC and DAYS but no change in % MORT. By contrast there was a linear increase in FBW during this period. There were no differences in CC or DAYS from January to June but these traits showed a significant increase in July. This was followed by a steep decline during August, September, and October. FBW was similar from January to June but showed a significant decrease in July. No consistent variation in monthly MORT was noted. It is concluded that improvements in the productivity of broilers have been achieved during the period from 1997 to 2001, but that more attention should be given to the problem of broiler management during the summer months.

(The full-length article will be published in *Poultry Science*.)

COCCIDIOSIS IMMUNITY IN COMMERCIALLY GROWN BROILER CHICKENS

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INTRODUCTION

Coccidia cause damage to the epithelial and sub-epithelial tissues of the intestines, which may lead to an inability of the affected birds to absorb nutrients. This inability may subsequently lead to lower growth rate, impaired feed efficiency, and even mortality. Coccidia are ubiquitous and pose significant threat to the health of the chickens. The coccidia are very prolific organisms and the wall of the oocyst provides a good protective covering to aid in the survival of organism in the environment. However, these hardy organisms are susceptible to desiccation and heat for extended periods.

For years, coccidiosis control has been primarily by the use of anticoccidial compounds, although there are other options that fit into many coccidiosis management strategies. The basic function of the anticoccidial product is to reduce infection pressure and thus allow the animals opportunity to optimize performance.

The control of coccidiosis in broiler production is twofold. The first function is to keep infection pressure low; the main objective is to prevent devastating losses by avoiding or minimizing the buildup of infection pressure. The advantages of low or tolerable infection pressure levels in the host are: (1) minimal destruction of host epithelial tissue, (2) less drug selection pressure for resistance, and (3) the development of natural host immunity.

The second function of controlling coccidiosis is to manage drug resistance. Coccidia are extremely adaptable and because of this characteristic resistance becomes an issue with these organisms. This is more apparent with products that are highly efficacious.

There is a notion that the anticoccidial drugs being fed for the control of coccidiosis do allow for the chickens to develop immunity to coccidia.

SUMMARY

Two trials were conducted to measure the level of immunity in commercially grown broiler chickens. The anticoccidial drugs used in the first and second trials were Clinacox/Bio-Cox and Maxiban/Coban, respectively. The birds were older than 35 days of age and were selected randomly from 20 broiler farms. In the first test, birds were from 11 farms and in the second test birds were from nine farms. Six healthy birds were selected from each farm. The coccidia used were recently isolated from commercial broiler farms in the area. The predominant coccidia species were *E. maxima* and *E. tenella.* Birds were brought in from commercial broiler farms, tagged, weighed, inoculated and placed in a floor pen. Birds used for positive controls were grown in coccidia free environment prior to the inoculation. Between 144 and 156 hr post-inoculation (pi) all birds were weighed, euthanized and intestines evaluated for gross lesions, using a 0 to 4 scale (0, negative and 4, severe). Scrapings were taken from the upper, mid-gut and cecal areas to be evaluated microscopically for the level of parasitism, using a 0 to 4 scale (0, negative and 4 severe).

Immunity to *E. maxima* and *E. tenella*, as measured by the levels of parasitism found in the birds between 144 and 156 hours post inoculation was highly variable. The level of immunity in the birds that were from the 11 farms in the first group was very erratic. The anticoccidial program used was Clinacox/BioCox, The levels of protection to *E. maxima* were as follows: four farms had birds that had protection that was 60% when compared to the positive controls. Three farms had birds that had protection that was 30% better than the controls and four farms had birds that had no protection. Only birds from two farms exhibited fairly good control; the protection level was greater than 70%. Protection levels to *E. tenella* were as follows: one farm had birds that had protection that was above 60% better than the controls. Birds from 10 farms had no protection against the *E. tenella* challenge when compared to the positive controls.

In the second evaluation, the birds were on the Maxiban/Coban control program. The level of protection in the birds to *E. maxima* was variable but less erratic than the birds in the first evaluation. The level of protection ranged to 73 to 100%. The level of protection to *E. tenella* was also variable, eight of the farms had birds that had immunity that ranged from 80% to 94%. One farm had birds that had no protective immunity to the challenge.

The low levels of immunity and the high variations in the levels of immunity among the groups of chickens might be reflective of the anticoccidial program used. The program(s) used might influence

the prevalence of the coccidia species present in the litter of the chicken houses. This factor will influence the species of coccidia that the host will be exposed and therefore will influence the type and level of immunity to be achieved by the resident host population.

NICARBAZIN IN SHUTTLE PROGRAMS USING ENVIRONMENTAL HOUSING

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Nicarbazin (NI) has been used for many years as a starter anticoccidial during cool seasons to complement ionophore coccidiostat programs. The increased use of environmental housing (tunnel ventilated and cool-cell equipped) makes the use of nicarbazin a possibility during any season.

A commercial farm study was conducted in the summer of 2002 in Arkansas. 18,000 straight-run broiler chickens (Ross X Hubbard) were placed in each of 4 environmentally configured houses and programs were selected to compare the effects of nicarbazin (NI) in two feeding programs using either salinomycin (SAL) or semduramicin (SEM) in starter and grower feed. Nicarbazin was fed at two levels in starter feed; 90g/t (0.8#/ton) and 113 g/t (1.0#/ton) using semduramicin as the grower anticoccidial. Each house represented a treatment. Birds were marketed at 54 days of age. Data collection included body weight, feed conversion, and mortality. Environmental measurements of temperature and water consumption were recorded daily for each house. Bird density [~0.90 ft2/bird], temperature, lighting, feeder and water space were the same for all experimental groups.

Chicks for this trial were split between the four houses so that all breeder flocks were divided evenly between each house. All birds removed from all houses starting on day 0 were examined grossly to determine probable cause of death and recorded on the house mortality record.

Standard diet formulations were those used by the broiler company. All treatment diet mixing was conducted at a company research facility feed mill. Mixed feed was stored in bulk storage bins and labeled with treatment identity and further identified with color code. Starter feed (1.1 pounds per bird) was milled and delivered at the same time. Grower feed (3.5 pounds per bird) was milled at least twice. Withdrawal feeds were divided into two feeds.

DATA COLLECTED

- Body weights: by house at 54 days. A sub-sample weight of 55 male and 55 female birds was collected to ensure uniformity and consistency of the house weights compared to the farm weight.
- Feed amounts added and removed from each house days 0 through study end.
- Mortality, daily, by house.
- Daily observations of facility and birds, daily facility temperature and water consumption.
- Coccidiosis lesion scores.

RESULTS

- There were no gross or microscopic lesions of coccidiosis.
- Temperature and water consumption values were uniform among houses.
- There were no differences in mortality among houses.
- There was an advantage in body weight and feed conversion using a 90 gram NI starter program.
- The results for the 113 gram NI starter program were similar to the farm averages.
- The nicarbazin treatments were pooled and compared to the farm averages. These results are shown in Table 1

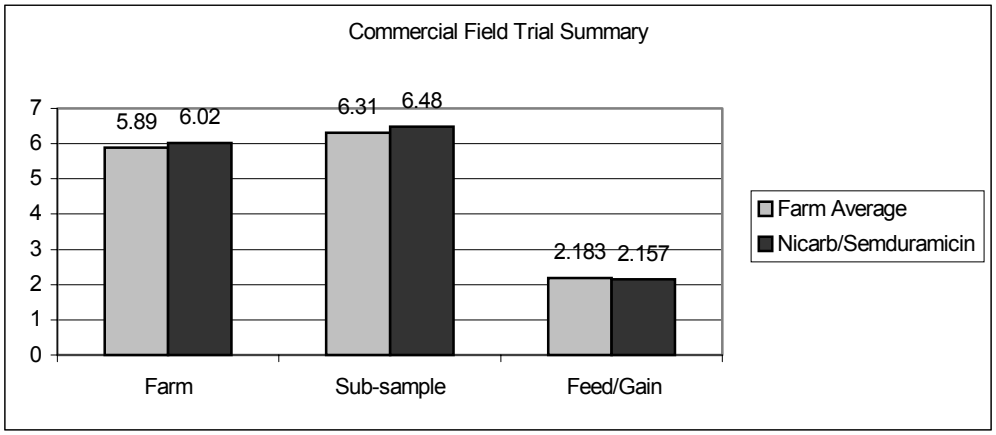
CONCLUSION

This commercial study demonstrates that nicarbazin can be successfully fed during warm weather in environmental housing.

Table 1.

Treatment	Body Wt (lbs)	Sub-sample Wt. (lbs)	Feed/Gain	Mortality
Farm Average	5.89	6.31	2.183	5.46%
semduramicin	6.02	Nicarbazin/ 6.48	2.157	5.09%

Figure 1.



THE USE OF A VIABILITY ASSAY TO FORMULATE A HIGH DEFINITION COCCIDIOSIS VACCINE

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SUMMARY

Determination of coccidial oocyst viability has traditionally been done using *in vivo* methods such as lesion score or oocyst shedding following the oral delivery of a dose determined by microscopic enumeration. The microscope is readily used to determine number of sporulated vs non-sporulated oocysts per ml, but the determination of the percent of live oocysts is not possible using a microscope. This introduces a source of uncontrolled variability when multiple suspensions of sporulated oocysts are used to formulate a live coccidiosis vaccine. An *in vitro* assay has been developed to measure the viability of sporocysts of *E. tenella*, *E. maxima* and *E. acervulina* (1). The assay is based on the use of a non-vital stain, ethidium bromide. The assay requires the excystation of sporocysts and depends on the permeability of sporocysts from *Eimeria* or any other sporocyst-forming protozoan to this non-vital dye. Preliminary experiments confirmed that vaccine from a suspension of *E. tenella* oocysts assessed non-vital by ethidium bromide staining were also not capable of generating resistance to a coccidiosis challenge. As the proportion of oocysts from the non-vital suspension increased in the vaccinating dose, there was a concomitant decrease in the resistance of the bird to oral challenge as indicated by lesion scores. When the immunizing inoculum contained 1000 viable oocysts or less, lesion scores were not different than non-vaccinated controls. This study confirmed that the *in vitro* viability assay correlates to the ability to confer resistance to a coccidiosis challenge. A second experiment was done to test the use of the viability assay to formulate vaccines from suspensions of oocysts that ranged from 24 to 52 weeks old and 16 to 82% viability. Results indicated that suspensions of *E. acervulina* varying widely in viability and age were all capable of generating resistance to an oral challenge providing the vaccine was formulated on a viable oocyst basis.

INTRODUCTION

Coccidiosis is caused by *Eimeria* species, which are protozoa of the Apicomplexia parasite family. Avian coccidiosis is an enteric disease that causes loss of production and mortality in the poultry industry. The primary means of control (~90%) is through the use of various feed additive medications; however, some live oocyst vaccines are also commercially available. While these vaccines have not been broadly accepted by the poultry industry for use in rapidly growing broilers, they are widely used for broiler breeders where rapid growth is not a requirement for commercial success of the vaccine. There has been renewed interest in the use of live oocyst vaccines for commercial broilers due to recent regulatory activities in the EU to ban certain antibiotic growth promoters and increasing consumer demand worldwide for less use of drugs in animal production practice.

Several species of *Eimeria* exist but the most problematic for rapidly growing broilers are *Eimeria tenella, Eimeria acervulina* and *Eimeria maxima*. *Eimeria* have two predominant stages in their life cycle. The exogenous phase occurs as a result of gametogony or sexual phase of the parasite life cycle. Diploid oocysts which are produced as a result of gametogony are deposited in the intestinal tract and collect in the fecal material in the litter of grower houses. The oocysts undergo sporulation in the litter to produce 4 sporocysts each containing 2 sporozoites. The endogenous part of the life cycle begins when the sporulated oocysts are ingested by the bird. Infection of the intestinal cell lining is key to pathogenesis of the parasite; however, it is also key to building resistance or immunity to *Eimeria*. Fowls, once recovered from a coccidia infection have been shown to be resistant to subsequent infections. This immunity or resistance appears to be long lived (at least for the life span of the bird). Vaccination or pre-exposure with controlled amounts (low dose) of sporulated oocysts into poultry has shown to be an effective method for inducing resistance to avian coccidiosis. Although many attempts have been made to generate vaccines against avian coccidiosis, only live vaccines given through the oral route have proven to be commercially useful. In order to generate a multi species coccidiosis vaccine that is both safe and effective, one must determine percent of viability of each individual species in order to formulate the vaccine with as little variation and excess as possible.

Assessment of viability in coccidial oocysts has been studied extensively. Several papers describe the use of infectivity of sporozoites in cell culture as a method to assess efficacy of anticoccidial drugs (2). In addition, several dye-based viability assays have been described, but all assays depend on the use of sporozoites as the life form (3, 4). For example, fluorescein diacetate and propidium iodide have been used to determine viability. Although *in vitro* sporozoite infectivity and dye assays give useful information regarding the viability and infectivity of sporozoites, the dynamic process of sporozoite release can result in selection of the population, resulting in a poor correlation with the original oocyst population. In contrast, sporocyst release is a more passive process. Therefore, sporocyst staining was considered a better alternative in terms of being indicative of the viability of the oocyst population. Ethidium bromide was chosen as a dye to detect non-viable organisms. Experiments were designed and performed to demonstrate 1) Oocysts heated at 50C for 30 min were dead based on infectivity assays of purified sporozoites on baby hamster kidney cells. 2) Sporocysts can be purified from dead oocysts at the same rate of efficiency as from non-heated oocysts (control oocysts), and 3) Sporocysts can be stained with a non-vital stain to indicate mortality. The results of the assay can be quantified using conventional fluorescent microscopy or FACScan analysis. The selected staining procedure is linear, robust, and has low variability.

MATERIALS AND METHODS

Production of *Eimeria tenella* and *Eimeria acervulina*. *E. tenella* and *E. acervulina* oocysts used in this study were obtained from the master seed facility at Viridus Animal Health, LLC, Lincoln, NE. Oocysts were produced in three week old SPF leghorns (HyVac). Feces were homogenized and filtered. The oocysts were floated, decanted and sporulated. The suspension was sanitized, filtered, resuspended and stored at 4-7ºC.

Infectivity Assay. Single cell clones of Baby Hamster Kidney Cells were screened for susceptibility to infection by *E. tenella*. Sporozoites were then added to the wells ranging from approximately 300 to 10,000 sporozoites per well and the cells were incubated for 48-60 hrs. To assess infectivity, the number of schizonts was counted.

Purification of sporocysts from killed and viable oocysts. To demonstrate that sporocyst viability is indicative of oocyst viability, dye penetration of live and dead sporocysts was tested. Sporocysts were purified from a production lot of *E. tenella* oocysts (Control Sporocysts) and from *E. tenella* oocysts that had been heat killed (Dead Sporocysts). These sporocysts were then used to optimize viability staining conditions (Dibner et al, 2002). The assay was then used to assess sporocyst viability using Control sporocysts, Dead Sporocysts, and a 50/50 mixture of Control and Dead Sporocysts.

Immunogenicity assay. Ross X Arbor Acres broilers were immunized with sporulated *E. acervulina* oocysts by oral gavage at day of age. There were eight treatments consisting of positive and negative controls and six experimental vaccines. Treatments were as follows: 1) Non vaccinated, non challenged; 2) Non vaccinated, challenged; 3) oocysts 52 wk old, 16% viability; 4) oocysts 32 wk old, 53% viability; and 5) oocysts 24 wk old, 66% viability. Treatments 6-8 were all from the same lot of oocysts, but were stored under different conditions resulting in the following viabilities: 6) 79%, 7) 79%, and 8) 82%. There were 12 pen replicates per treatment, six males and six females, with eight birds per pen. Birds were challenged on day 22 using an oral gavage of viable sporulated *E. acervulina* oocysts. Lesion scores based on those described by Johnson and Reid (1970) were read on day four after challenge. Scorers were provided with birds at a lesion scoring station and were blind to the treatments.

Analysis. Performance data and log transformed lesion scores were subjected to analysis of variance using the General Linear Models procedure (SAS, 1996) and mean differences ($P<.05$) were determined using single degree of freedom contrasts.

RESULTS

Infectivity of heat-killed sporozoites. Infectivity of heated and non-heated oocysts was determined by preparing oocysts that were heated at 50 C for 30 min, while the Controls were placed on ice for 30 min. Sporozoites were purified from each of the two samples, and quantitated. Baby hamster kidney cells were inoculated with sporozoites ranging from approximately 300 to 10,000 sporozoites per well. No trophozoites or merozoites were observed when BHK12A cells were inoculated with as many as 10,000 heat-inactivated sporozoites per well. Sporozoites that were purified from heat treated oocysts were non-motile on cell culture, did not invade nor develop intracellularly. In the wells inoculated with Control sporozoites, the sporozoites were typically motile; trophozoites and merozoites were observed in all inoculated wells including the 300 sporozoites per well samples. This indicates that the oocysts were $\geq$ 95% dead by this procedure.

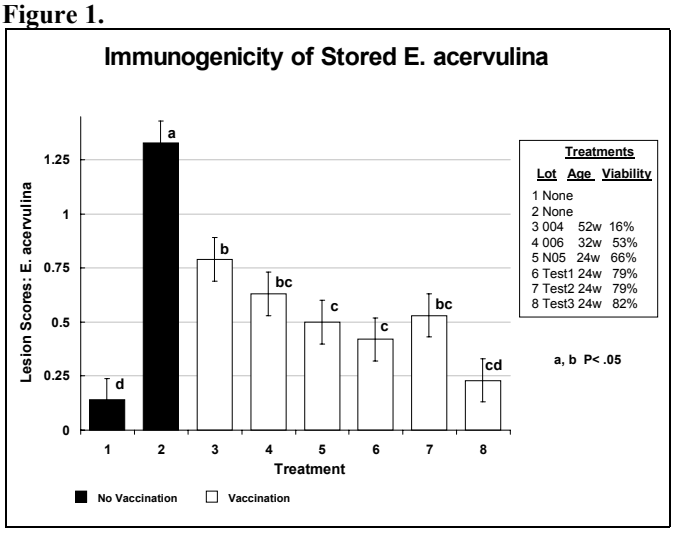
Recovery of sporocysts from heat-killed oocysts. To assess the accuracy of the ethidium bromide assay for non-viable sporocysts, control (untreated oocysts) and dead oocysts (heated at 50 C) were obtained from two different lots of oocysts. Sporocysts were purified from control oocysts, dead oocysts, and a 50/50 mixture of control and live oocysts. The percent recovery of sporocysts from each of these preparations was similar and did not appear to be influenced by the number of dead oocysts used for sporocyst purification. The resulting staining indicated that the Control Sporocysts were 91% viable (unstained), the Dead Sporocysts were 100% non-viable and the 50/50 mixture was 50% non-viable (50% stained). Varying proportions of control and heat-killed (50C) sporocysts indicated that the assay was linear ($R^2 = .998$).

Use of ethidium bromide assay to formulate vaccine from stored *E. acervulina* on a viable oocyst basis. Three *E. acervulina* suspensions 52, 32 and 24 weeks of age were tested for viability. In addition, three samples from a shelf-life experiment were also tested. The shelf-life experiment involved use of various suspension media and storage conditions to determine the effect on shelf life. Viability ranged from 16 (52 week old oocysts) to 82% (24 week old oocysts). Figure 1 shows the lesion score results from this experiment.

First, it is clear that the challenge was effective, resulting in a significant difference between non-vaccinated non- challenged controls (trt 1) and non-vaccinated challenged controls (trt 2). It is also clear that vaccines prepared from suspensions with a wide range of viabilities and ages were all efficacious in conferring resistance to the oral challenge. Although there is a general improvement in lesion scores as a function of viability and age, year old oocysts of only 16% viability were efficacious when formulated on a viable sporulated oocyst basis.

In summary, results demonstrate that the ethidium bromide assay detects non-viable sporocysts that reflect the original oocyst population and that formulation of the vaccine based on viable sporulated oocysts/bird will yield consistent efficacy across a wide range of age and viability.

Figure 1.



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REFERENCES

1. Dibner, J.J., M.L. Kitchell and M.A. Pfannenstiel. Viability assay for sporocyst-forming protozoa. United States Patent 6334340, 2002.

2. McDougald, L.R. and R.B. Galloway. *Eimeria tenella*: anticoccidial drug activity in cell cultures. Exp.
Parasit. 34:189-196, 1973.

3. Fuller, A.L., J. Golden, and L.R. McDougald. Flow cytometric analysis of the response of *Eimeria tenella* (coccidia) sporozoites to coccidiocidal effects of ionophores. J. Parasit. 81: 985-988, 1995.

4. Brown, S.M.A., V. McDonald, H. Denton and G. H. Coombs. The use of a new viability assay to determine the susceptibility of *Cryptosporidium* and *Eimeria* sporozoites to respiratory inhibitors and extremes of pH. FEMS Microbio. Letters 142:203-208, 1996.

5. SAS Users Guide: Statistics, Version 6.12Edition. SAS Inst., Inc. Cary, N.C. 1996.

COMPARATIVE EFFICACY OF A HIGH DEFINITION COCCIDIOSIS VACCINE AND IONOPHORES IN BROILERS ON USED LITTER

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This report describes 45-day performance and intestinal lesion evolution of broilers treated at day of age with a spray cabinet applied high definition coccidiosis vaccine (HDCV); trade name ADVENT™ Coccidiosis Control. The HDCV treatment was compared with contemporary controls fed either monensin (MON: 90 g/ton) or salinomycin (SAL: 60 g/ton). Straight run day old Ross X Hubbard broilers were placed 63/pen on litter previously used in a coccidiosis challenge experiment. Standard starter, grower and finisher diets were fed to all birds and all diets were medicated with 50 g/ton bacitracin methylenedisalicylate. Birds and feed were weighed at 20, 34 and 45 days of age (Table 1), and two birds per pen were sacrificed at 14, 21, 28 and 35 days of age to determine *Eimeria* lesions according to the method of Johnson & Reid, 1970 (Table 2). Performance data and log transformed lesion scores were subjected to analysis of variance using the General Linear Models procedure (SAS, 1996) and mean differences (P<.05) were determined using single degree of freedom contrasts.

Table 1. Growth performance results.

	Bodyweight (kg)				Feed/Gain			
DAYS	MON	SAL	HDCV	SEM	MON	SAL	HDCV	SEM
20	0.676a	0.678a	0.654b	0.006	1.311a	1.301a	1.328b	0.008
34	1.732	1.732	1.712	0.020	1.594a	1.610b	1.627b	0.008
45	2.496	2.494	2.461	0.048	1.860a	1.865ab	1.886b	0.007

Table 2. Lesion score results.

DAY	14			21			28			35		
	Ea	Em	Et	Ea	Em	Et	Ea	Em	Et	Ea	Em	Et
HDCV	0.3a	0.0	0.1	2.3a	0.1	0.0	0.0b	0.8	0.0	0.0	0.5	0.0
MON	0.0b	0.0	0.0	0.7b	0.0	0.0	0.0b	1.1	0.0	0.0	0.4	0.0
SAL	0.1b	0.0	0.1	0.4b	0.0	0.0	0.5a	0.8	0.0	0.0	0.3	0.0
SEM	0.0	0.0	0.1	0.3	0.1	0.0	0.2	0.2	0.0	0.0	0.1	0.0

Performance results indicated transiently lower body weights for the HDCV treatment at 20 days with no treatment differences at the other time points. Feed efficiency for MON and SAL was greater than HDTV at 20 and 34 days, and for MON at 45 days. Lesion scores indicated greater cycling of *E. acervulina* for HDCV at 14 and 21 days than MON or SAL, and the presence of some minor cycling for SAL at 28 days ($P<.05$). Lesions for *E. maxima* and *E. tenella* were present during the course of the experiment but did not differ due to treatment. All three treatments successfully prevented any outbreak of coccidiosis in this environment.

REFERENCES

1. Johnson, J. and W.M. Ried. Anticoccidial drugs: Lesion scoring techniques in battery and floor penexperiments with chickens. Exp. Parasit. 28:30-36, 1970.

2. SAS Users Guide: Statistics, Version 6.12 Edition. SAS Inst., Inc. Cary, N.C. 1996.

INCIDENCE OF BREAST BLISTERS IN TURKEYS AND THEIR EFFECT UPON MEAT QUALITY

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SUMMARY

"Enlarged Sternal Bursa"(ESB), otherwise called "breast blister," is a common problem in the commercial turkey industry. This study examined the effects of the presence of breast blisters on indices of meat quality such as the decline in post mortem pH, L* value, % thaw losses, and the % cook loss. At 14 weeks of age, 5.6 % of the birds had fully developed breast blisters. Fourteen birds with blisters and a similar number of birds without blisters (controls) were processed at 18 weeks of age. The breast meat from birds with ESB showed a more rapid decline in postmortem muscle pH (from 6.04 to 5.80) as compared to the controls (from 6.18 to 6.20) during the first 4 hours post mortem. Also the average % cook loss of the ESB breast meat samples (19.55 %) was more than that of birds with no blisters (1.09 %).

INTRODUCTION

Breast blisters are a chronic skin condition affecting the unfeathered skin over the keel of the breast of the turkey and occur most frequently in male birds. Breast blisters cost the turkey industry millions of dollars each year because the lesions occur on the most valuable part of the carcass. They must be trimmed at the processing plant thereby resulting in lost yield, downgrading, and slowing of the processing line. There are many factors that affect the

development of blisters including the strain, age, sex, body weight and feather cover of the birds, and floor type, litter quality, lighting program, stocking density (Kamyab et al.). In this study our main focus was to study the effect of breast blisters on the quality of the meat especially the pattern of pH decline post mortem, the L* value of the breast meat and also the % drip loss and % cook loss of breast meat samples when compared to the breast meat from birds without blisters.

MATERIALS AND METHODS

A flock of 250 day-old debeaked Nicholas tom turkeys were placed in 48 pens in groups of 15 birds per pen. Each pen was 4.18 m^2 excluding the feeders and waterers, thus providing 0.28 m^2 per bird. Rations were formulated according to a commercial feeding program and feed and water were provided *ad libitum.* Pine shavings were used for litter, which was not changed during the trial and a standard lighting regimen of 22 hours light and 2 hours dark was provided. Mortality was recorded daily. At 14 weeks of age, each bird was held upside down and the keel area examined by manually palpating, and visually inspecting, the unfeathered skin for the presence of breast blisters. Lesions were classified as (a) Normal - where the skin was tightly adhered to the keel area, (b) Loose skin - thickened or normal skin on the keel area that was not tightly adhered to the keel, (c) Button – a condition in which a thickened hard focal area was present on the skin, (d) Cord – a compartmentalization of the sternal bursa with fibrous tissue, and (e) Blister – a fluid filled bursa over the skin. Fourteen birds were identified with blisters, and with 14 normal birds (controls), were separated and processed according to standard procedures employed at the University of Arkansas Poultry Processing Plant. Breast muscle pH was recorded at 0.25 hr, 2 hr, 4 hr, and 24 hr post mortem using a standard CORNING pH Probe. The L* value (a measurement of color lightness) of the breast fillets was recorded using a standard MINOLTA CHROMAMETER Model MR-300. The probe was placed on the lateral aspect of the cut edge of the left fillet, three values were recorded at three different points, and an average value was calculated. After 24 hours the weight of the breast meat samples was recorded and the samples were sealed in freezer bags and freezed at -20^0 C overnight. The samples were then thawed to room temperature, and the weight recorded after thawing, to provide the thaw loss or the drip loss of the meat sample. The samples were then heated at an oven temperature of 75^0 C and the weight recorded after cooking. The difference in the thaw weight and cook weight gives the cook loss of the meat sample. This procedure was repeated for the blister and the normal (control) meat samples.

RESULTS

Among the 250 birds examined, 14 birds (5.6 %) had blisters, 55 birds had cords (22%), 58 birds had loose skin (23.2%), 16 birds had buttons (6.4%), and 92 birds showed no lesions (36.8%). The 14 birds with blisters and 14 normal birds (controls) were selected and processed and the breast samples were tested. The breast muscle pH of the controls showed a pH change of 6.18 to 6.20 whereas the blister group showed a significant ($p<0.05$) pH decline from 6.04 to 5.80 at 4 hours post mortem .The 2 hr and 24 hr post mortem L* values of the meat from the blister group was 43.0 and 48.4, which was not significantly different from the L* values of the control group (44.0 and 48.6 respectively). There was no significant difference between the % thaw loss/drip loss of the blister group (1.44 %) and the controls (1.35%). Interestingly, there was a wide difference in the % cook loss of the meat from the blister group (19.55%) and the control group (1.35%) indicating that the moisture retention capacity of the meat of the blister birds was poor.

DISCUSSION

Results of this study show that breast blister or enlarged sternal bursa is a major concern not only because of economic losses due to breast trimming, but also because of affects upon measures of meat quality such as a rapid decline in post mortem pH. This leads to a reduction in the keeping quality or the shelf life of meat and to protein denaturation thereby reducing nutritive value. Percentage cook loss is an important consideration for an industry that is looking for meat with a high moisture retention capability for further processed products. Since the meat from blister birds shows increased % cook losses, the use of meat from these birds for further processing is compromised.

REFERENCES

1. Barbut, S. (1993). Color measurements for evaluating the pale soft exudative (PSE) occurrence in turkey meat. *Food research International.* 26.

2. Tilley, B.J., Barnes, H.J., Scott, R., Rives, D.V., Brewer, C.E., and Gerig, T.M. (1996). Litter and commercial strain influence of focal ulcerative dermatitis ("breast buttons"). *Journal of applied Poultry Research* 5:39-50.

3. Kamyab, A. (2001). Enlarged sternal bursa and focal ulcerative dermatitis in male turkeys. *World's Poultry Science journal (Review)* 57:5-12.

LAYING HENS IN EUROPE: COPING WITH EU REGULATIONS

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SUMMARY

On July 19, 1999, the Council of Agriculture of the European Union (EU) published minimum standards for the protection of laying hens. This European Council (EC) Directive bans the use of battery cages for laying hens by the year 2012 (1).

The main points of the directive are: Starting January 1, 2012, conventional cages will be prohibited. Since January 1, 2003, conventional cages must have suitable claw shorteners and provide at least 550 cm^2 of space per hen. It is forbidden to install new or replacement conventional cages since that date.

The new standards (750 cm^2/hen plus access to a nest box and 15 cm perch space per hen) will apply to enriched cages (must provide a nest box, littered scratching area, and perch) from January 1, 2002. Since January 1, 2003, all new or replacement cages must be enriched ones.

New and replacement non-cage systems (new and replacement) must fulfill the new standards since January 1, 2002. These standards include: At least 1/3 of ground surface must be litter; at least 15 cm perch space per hen must be provided; there should be no more than 120 hens per communal nest (1 m^2 per nest); density should be no more than nine hens per m^2 of usable area.

There is a transition period for existing non-cage systems: they must fulfill the new standards from January 1, 2007 on.

Currently the proportion of hens within the EU in non-cage systems varies among countries. Unenriched cages are being phased out. Any new cages installed from 2003, and all cages from 2012, must be enriched (must provide a nest box, littered scratching area, and perch).

This directive lays down the minimum standards for member states of the EU. If single states feel the need to install more stringent regulations, the are free to do so. As an example, the German government banned the installation of cages from 2003 on, and keeping hens in standard cages will be banned starting 2007. Even the 'new' enriched cages will be banned from 2012 on, therefore the only remaining alternative will be in free-range or deep litter systems – or to give up laying hens.

In response to the new regulations, egg production seems to decrease within the EU. To meet current consumer demand, more eggs need to be imported from countries outside of the EU – not underlying these regulations.

This is not the first directive with a major impact onto the poultry industry. The EC Directive 98/58/EC (2) of July 20, 1998 focuses on the protection of animals kept for farming purposes. In Article 3, it is stated "Member States shall make provision to ensure that the owners or keepers take all reasonable steps to ensure the welfare of animals under their care and to ensure that those animals are not caused any unnecessary pain, suffering or injury." In the annex of this directive, there is a specific paragraph about 'mutilations' like beak trimming. Hadorn et al. (3) found improved feed conversion rates and a significantly decreased mortality in hens with shortened beaks, but, despite these results, there are many attempts, especially from welfare groups, to ban it.

Will all these regulations finally lead to an end of commercial layer operations in the EU, or is there light at the end of the tunnel? Article 10 of the directive says: Not later than 1 January 2005 the Commission shall submit to the Council a report, drawn up on the basis of an opinion from the Scientific Veterinary Committee, on the various systems of rearing laying hens, and in particular on those covered by this Directive, taking account both of pathological, zootechnical, physiological, and ethological aspects of the various systems and of their health and environmental impact. That report shall also be drawn up on the basis of a study of the socio-economic implications of the various systems and their effects on the Community's economic partners. This should finally lead to appropriate proposals taking into account the conclusions of the report and the outcome of the World Trade Organization negotiations. The Council shall act within 12 months.

REFERENCES

1. Council Directive 1999/74/EC of 19 July 1999 laying down minimum standards for the protection of laying hens Official Journal L 203, 03/08/1999 P. 0053 – 0057.

2. Council Directive 98/58/EC of 20 July 1998 concerning the protection of animals kept for farming purposes. Official Journal L 221, 08/08/1998 P. 0023 – 0027.

3. Hadorn R. et al.: Einfluss des Schnabeltouchierens auf braune Jung- und Legehennen. Agrarforschung 2000/7, P. 62-67.

CASE REPORT: POULTRY PRODUCTION NEAR TOMSK, RUSSIA (SIBERIA)

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In August 2001 I spent two weeks evaluating a fully integrated poultry complex near Tomsk, Russia (Siberia). It included broilers as well as layers and employed 800 or more. There was a further processing plant that made sausage, smoked chicken, and powdered eggs.

The primary disease problem in broilers was ascites and coccidiosis. All are raised in cages. A light control program was started with good results. Vaccine for coccidiosis control was suggested for broiler breeders raised on a satellite farm.

Layers showed satisfactory performance. Induced molting was a new concept and there was considerable interest in trying the program.

Slides will be used to describe the physical plant that had 42 poultry houses, a feed mill, egg grading facility, two hatcheries, and a steam generating plant.

HEMARTHROSIS IN TURKEYS

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A few birds in an 18-week-old flock of turkey breeder replacement hens were sitting much of the time on their hock joints. These birds were reluctant to move even if they were forced to. In each of these birds, there was swelling of one hock joint with red discoloration of the skin over the joint. The swelling and red discoloration was caused by the accumulation of large amount of unclotted blood in the joint cavity. Close examination of the affected joints and their associated structures revealed complete avulsion of the intertarsal lateral collateral ligament from its attachment to the lateral condyle of the tibiotarsus. There was marked hemorrhage at and around the insertion of the ligament on the lateral condyle of the tibiotarsus. The muscles and tendons above the affected joint were edematous.

Although avulsion of the ligaments of the intertarsal joints has been reported in turkeys and chickens, this is the first report of hemarthrosis in turkeys. The lateral and medial collateral ligaments run between the distal end of the tibiotarsus and the proximal end of tarsometatarsus; these ligaments, together with the joint capsule, keep the hock joint in position. It is most likely that the avulsion is caused by physical stress on the hock joint.

UNDERSTANDING SEROLOGY RESULTS WITH PROCESS BEHAVIOR (SHEWHART) CHARTS

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ABSTRACT

Serologic data from flock monitoring programs provide an opportunity to understand patterns of pathogen exposure. Such data are typically used to estimate the prevalence of a pathogen within a given population. In some instances, raw OD values, s/p values, or standardized OD values are also used to assess the degree of pathogen exposure within a given population. When these data are compared to historical

data, the comparison usually compares the current value(s) with the most recent historical value(s). Rarely, these two values are identical. More often, the values are different. The challenge for veterinarians is to determine how much these values differ from each other.

Process behavior (Shewhart) charts are tools that are used to determine the extent of the difference between/among data collected from a production process. The essence of process behavior charts is the ability to filter out normal variation, thus permitting the identification of special variation when differences between/among data are meaningfully different. Process behavior charts, by definition, filter out 99-100% of the normal variation within the data set from a production process. Thus, serologic data from production systems' pathogen monitoring programs, placed onto process behavior charts, permits system veterinarians to accurately interpret pathogen exposure.

Examples of process behavior charts applied to serologic data will be presented in the poster.

SEROLOGICAL SURVEY FOR NEWCASTLE DISEASE IN NON-COMMERCIAL LAYERS THAT BELONG TO A SOCIAL PROGRAM FROM ARGENTINA CALLED PRO-HUERTA

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SUMMARY

A commercial Newcastle Disease Virus (NDV) enzyme-linked immunosorbent assay (ELISA) was performed on 400 serum samples for NDV antibodies. The layers belong to a program called *Pro-Huerta*, in which chickens are given to families so that they can raise them in their backyard. Since Argentina has been declared free of velogenic NDV with vaccination since 1997 and these birds can be a potential source of the virus, the purpose of this study was not only to check for the presence of antibodies, but also to analyze and try possible ways of sanitary controls.

INTRODUCTION

Argentina, a country located in the tip of South America was declared free of velogenic Newcastle disease virus (NDV) with vaccination in 1997. Since there is a social program for food security called *Pro-Huerta* that belong to INTA (Instituto Nacional de Tecnología Agropecuaria) where chickens are given to poor families, the present study was done to evaluate the sanitary status to NDV and recommend ways of control. This program provides 10 chickens per family so that they can raise them in their backyard to self-produce food. However these birds can be a potential source of NDV.

MATERIALS AND METHODS

A commercial Newcastle Disease Virus (NDV) enzyme-linked immunosorbent assay (ELISA) was performed on 400 serum samples to check for NDV antibodies. Families with chickens that received the benefit of *Pro-Huerta* were visited and serum samples were obtained from those chickens.

RESULTS

Eight percent of the samples were positive for NDV although there was no evidence of disease.

DISCUSSION

Previous results obtained from hens that do not belong to this program were presented during the last AVMA meeting (Buscaglia et al., 2002). In that study very low-income families with chickens were visited and serum samples were obtained, and in-parallel samples were also collected from a commercial layer farm with a known vaccination schedule. The results obtained from the chickens that did not receive any

vaccine and the *Pro-Huerta* chickens are very similar. Although it is very difficult to implement a vaccination program in backyard chickens, a booklet was written by students that collaborated during the sampling to be given to those families, together with personalized instructions. However, since the *Pro-Huerta* chickens are not the only backyard chickens it would be interesting to consider the possibility to control periodically backyard chickens.

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REFERENCES

1. Buscaglia, Celina, Prío, María. Verónica, Prío Lofeudo, Graciela , Risso, Miguel. Atilio. Comparison of the Enzyme Linked Immunosorbent Assay for the Detection of Antibody to Newcastle Disease Between Commercial Flocks and Non-commercial Chickens Raised in the Backyard of Poor Families From Argentina. PP38, 139th AVMA annual convention notes, Nashville TN, July 2002.

SENSITIVITY OF FIELD ISOLATES OF EIMERIA TO SALINOMYCIN FOLLOWING USE OF DICLAZURIL AND A COCCIDIOSIS VACCINE IN BROILERS

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SUMMARY

Judged by ability to suppress oocyst production, salinomycin (SAL) in combination with roxarsone (ROX) and bacitracin methylene disalicylate (BMD) was only partially effective against isolates of Eimeria following four successive flocks given these drugs. Efficacy was slightly improved after four flocks in the absence of SAL and ROX. Efficacy was almost fully restored when birds were given diclazuril (DIC) and BMD in the starter and/or grower feeds for two flocks followed by vaccination with Coccivac-B® (CoB) for two flocks, or if vaccinated with CoB for four flocks.

Ionophores such as salinomycin (SAL) are widely used for the control of coccidiosis in broiler chickens but evidence has been obtained that they are not as effective as when first introduced (1). It has been proposed that vaccines containing drug-sensitive strains of Eimeria such as Coccivac-B® (CoB) could repopulate broiler houses with these strains and that, following vaccination, efficacy of ionophores would be improved (2). Although sensitive strains may replace existing strains any "resistant" parasites will not necessarily be eliminated and therefore it was also suggested that the synthetic drug diclazuril (DIC) be used for one or at most two flocks to eliminate, or substantially reduce, the numbers of "resistant" organisms prior to vaccine use. Synthetic drugs are mainly used in the starter feed but it is important to establish whether inclusion in the grower feed is necessary since any "resistant" parasites could survive in the litter for the brief period drugs are used in the starter ration. In this study the efficacy of SAL against isolates of Eimeria following four successive flocks given either DIC (in the starter and/or grower feeds) followed by vaccination with CoB, or the vaccine alone, is reported.

METHODS

Floor-pen experiment. Pens were seeded with oocysts of three species of *Eimeria* (*E. acervulina*, *E. maxima*, and *E. tenella*) that were partially resistant to SAL. Four successive flocks of broilers were then reared to seven weeks of age in the pens (50 birds/pen) with two weeks between flocks. Birds were given a starter feed from 0-21 days of age, a grower feed from 21-42 days of age, and a withdrawal feed from 42-49 days of age. There were six treatments each with eight randomly allocated pens. Treatments included: a) SAL (66 ppm) for four flocks; b) no SAL for four flocks; c) DIC (1 ppm) in the starter feed and SAL in the grower feed for two flocks followed by vaccination with CoB for two flocks; d) SAL in the starter feed and DIC in the grower feed for two flocks followed by CoB for two flocks; e) DIC in both starter and grower feeds for two flocks followed by CoB for two flocks; f) CoB for four flocks. Birds treated with SAL were also given roxarsone (ROX; 50 ppm). All birds received bacitracin methylene disalicylate (BMD; 55 ppm). Vaccination was carried out by spray at the hatchery using a Spraycox® machine.

Battery experiment. A battery cage experiment was conducted to investigate the efficacy of SAL (66 ppm) against isolates of *Eimeria* species obtained from the litter of the pens after the fourth flock. Four replicates of five birds in cages were given SAL + ROX + BMD (66, 50, and 55 ppm respectively) in the feed and inoculated with 1000 oocysts of the isolates. Oocyst production in the feces from 5 to 8 days later was measured and expressed as a % of that of birds that received BMD alone (controls).

RESULTS

Floor-pen experiment. For the first two flocks, the weight gain and feed conversion of birds given DIC was better than that of birds given SAL or CoB and oocyst production was almost completely suppressed. No lesions attributable to coccidiosis were observed in birds from any of the treatments.

Battery experiment. The oocyst production of isolates from pens where birds had received SAL for four flocks was 38% of controls indicating that the isolates were partially resistant to SAL. Oocyst production of isolates from pens that had not received SAL was 23% of controls indicating that the efficacy of SAL had improved in the absence of medication. Oocyst production of isolates from pens of birds that had received DIC in the starter, grower, or starter and grower for two flocks followed by CoB for two flocks was 3%, 3% and 1% of controls respectively, indicating that the efficacy of SAL had been fully restored. Oocyst production of isolates from pens of birds that had been vaccinated for four flocks was 5% of controls also indicating restoration of SAL efficacy. It is concluded that efficacy of SAL may be improved after vaccination of broilers with CoB or after use of DIC in broiler feeds followed by vaccination.

DISCUSSION

Results of this experiment show that the efficacy of SAL against isolates of Eimeria was improved following four successive flocks in which birds were given DIC followed by vaccination with CoB or when vaccinated alone. It is suggested that this improvement is due to the replacement of strains partially resistant to SAL with vaccine strains that are drug-sensitive. Interestingly, a slight improvement in efficacy was observed in the absence of any treatment suggesting that, in the absence of drug selection-pressure, restoration of sensitivity may gradually occur. The results suggest that alternation of ionophores with an effective synthetic drug and vaccine containing sensitive strains, or with such a vaccine alone, may ameliorate the problem of drug resistance, and that this approach could contribute to the long-term control of coccidiosis.

REFERENCES

1. Chapman, H. D., and A. B. Hacker. Sensitivity of field isolates of Eimeria from two broiler complexes to anticoccidial drugs in the chicken. Poult. Sci. 73:1404-1408. 1994.

2. Chapman, H. D., T. E. Cherry, H. D. Danforth, G. Richards, M. W. Shirley, and R. B. Williams. Sustainable coccidiosis control in poultry production: the role of live vaccines. Int. J. Parasitol. 32:617-629.2002.

SAFETY AND EFFICACY OF A HIGH DEFINITION COCCIDIOSIS VACCINE

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SUMMARY

This report describes experiments to determine the safety and efficacy of a new coccidiosis vaccine. ADVENT™ Coccidiosis Control contains a highly defined quantity of live, viable oocysts from the three most commercially relevant species of *Eimeria* - *E. acervulina* (strain VND-A10), *E. maxima* (strain VND-M27), and *E. tenella* (strain VND-T49). The strains have been selected to have the robust protection necessary for today's broilers and today's field challenges without including any unnecessary species. A unique methodology has been developed that inactivates extraneous agents including viruses highly resistant to inactivation, chick anemia virus and infectious bursal disease virus. The procedures

inactivate extraneous bacterial contaminants such as *Salmonella* spp. The coccidial strains in ADVENT have been maintained in the laboratory for 4–7 years and were selected for broad immunogenicity and undiminished oocyst yield. Screening for ionophore resistance confirmed sensitivity of the strains. An *in vitro* potency method – the VIACYST[SM] Assay, has been used to determine the viability of sporocysts of each of the three *Eimeria* strains (1). Studies confirm that the *in vitro* viability assay correlates to the ability to confer resistance to a coccidiosis challenge and permits accurate and consistent vaccine formulation (2). Oral immunization with ADVENT results in resistance to significant challenges of oocysts: at least 300,000 viable sporulated oocysts of *E. acervulina*, and 40,000 viable sporulated oocysts of *E. maxima* or *E. tenella*. Doses resulting in immunity have been evaluated both on the basis of lesion score reduction and post-challenge broiler performance. The manufacturing technology used for production of ADVENT results in coccidiosis control that is consistent from lot to lot and vial to vial. The resulting vaccine provides protection without excess immune stimulation and is a consistent and pure source of contemporary coccidial oocysts.

INTRODUCTION

Avian coccidiosis is an enteric disease that causes loss of production and mortality in the poultry industry. Coccidiosis is a chronic problem in modern poultry production. Increasing development of resistance to anticoccidials has led to the practice of rotation of coccidiosis control measures; most often multiple drugs or vaccines are each used for part of the year. This leads to complications in the management of the entire health program. There is room for improvement in coccidiosis control, as drug treatments are perceived to be unreliable and vaccines are perceived to be unpredictable. Furthermore, the high cost of drug development has deterred development of new types of medication and there is increasing pressure to eliminate preventive drug use in animal production. ADVENT was designed to meet the need for a more flexible and reliable coccidiosis control program. Safety has been maximized by improving oocyst purification and eliminating extraneous pathogens. In addition, the use of the VIACYST assay to formulate the vaccine on the basis of number of viable sporulated oocysts improves product consistency and minimizes the chance of under- or over-dosing the birds with live oocysts.

Infection of the intestinal cell lining is key to pathogenesis of coccidia; however, it is also key to building resistance or immunity to the parasite. Although many attempts have been made to generate vaccines against avian coccidiosis, only the live vaccines given through the oral route have proven to be effective in a commercial setting. Vaccination for coccidiosis requires the administration of a controlled dose of *Eimeria*. This results in a sub-clinical coccidial infection. The dose is controlled to be enough to cause immunity but too low to cause disease. Too few oocysts means incomplete protection while too many means unnecessary performance declines following vaccination. The optimal immunizing dose in ADVENT has been determined on each species individually and each serial is uniquely formulated based on the viability of the constituent bulk species. The development of any form of immunity requires immune cell proliferation and substantial protein synthesis, which results in some diversion of nutrients away from growth. This diversion has been minimized for ADVENT through development of technology that provides for consistent and accurate formulation.

MATERIALS AND METHODS

Production of *E. acervulina, E. maxima,* and *E. tenella*. Oocysts used in these studies were obtained from the Novus International Animal Research facility in O'Fallon, MO or the Viridus Animal Health facility, Lincoln, NE. Oocysts were produced in three-week-old SPF leghorns (HyVac) or Hubbard HiY broilers. Oocysts were separated by flotation, sporulated, sanitized, and stored under refrigeration.

Extraneous contaminants. Purity of Master Seed and Working Seed cultures and each lot of bulk oocysts to be blended into final vaccine is evaluated to meet USDA requirements. Ability of the sterilent and the processing methodology to inactivate viruses was evaluated using two viruses, chicken anemia virus (CAV) and infectious bursal disease virus (IBDV), selected for their stability, resistance to disinfectants and importance to the poultry industry. The testing procedure included addition of a pre-determined amount of CAV and IBDV virus prior to the sterilent step. The treated feces were then subjected to the sanitization procedure. The resulting oocyst suspension was then tested for presence of CAV and IBDV using indirect fluorescent antibody.

Safety of ADVENT. This study was conducted to confirm the safety of the minimum dose for each species, and to test the effect of an excess dose (approximately 10x) on bird mortality over a 14-day period after administration. Vaccines were administered either by spraying on birds (using a vaccine spray cabinet) or by spraying on feed (in the form of Oasis® Hatchling Supplement). There were eight birds per pen and five replicate pens of males and five of females for each vaccine treatment. Non-vaccinated controls had four pens of males and four pens of females rather than five. Birds were vaccinated

on day 0 and observed for 14 days. Flooring was provided throughout the study to ensure that birds were exposed to oocysts shed in the excreta. Livability, body weights and feed consumption were determined on days 0, 7 and 14.

Efficacy of ADVENT vaccine. Peterson Arbor Acres broilers were immunized by spray onto day old chicks (25 ml/100 birds) or onto feed (in the form of Oasis® hatchling supplement) with ADVENT vaccine diluted to contain four doses per ml. There were six treatments of six pen replicates of each sex. Birds were placed in battery cages. Flooring was provided for 21 days to facilitate parasite cycling. Treatments were as follows: 1) non-vaccinated, non-challenged; 2) non-vaccinated, challenged; 3) vaccinated (spray on feed), non-challenged; 4) vaccinated (spray on feed), challenged; 5) spray on birds, non–challenged; and 6) spray on birds, challenged. Birds were challenged on day 24 using an oral gavage of 300,000 viable sporulated *E. acervulina* oocysts, and 40,000 each of viable sporulated *E. maxima* and *E. tenella* oocysts. Lesion scores based on those described by Johnson and Reid (3) were read on two birds/pen for all three species on days four and six after challenge. Scorers were provided with birds at a lesion scoring station and were blind to the treatments.

Statistical analysis. Data were analyzed using the ANOVA procedure of SAS and mean differences ($P<.05$) were determined using single degree of freedom contrasts (4).

RESULTS

Extraneous contaminants. Ability of the sterilent used during oocyst processing to inactivate bacteria (including *Salmonella*) and fungi was confirmed in accordance with procedures described in the Code of Federal Regulations (9CFR), Title 9. Further, it was demonstrated that the sterilent and procedures used were sufficient in eliminating at least 4-5 $\log_{10}$ of IBDV and CAV.

Safety of ADVENT. This study confirmed the safety of the minimum dose for each species as well as a dose approximately 10 times that for each species. Livability in this study was very good. Of the 768 birds present when the study began, a total of eight birds died (about 1%) by the end of the study at day 14. Livability ranged from a low of 98.4% (non-vaccinated control treatment) to 100% (10x dose sprayed on birds and 10x dose sprayed on feed). Only one mortality, a non-vaccinated control bird, was found to have lesions. There was no significant effect of any vaccination treatment on body weight or feed conversion at day seven or day 14.

Efficacy of ADVENT. Table 1 shows the performance data and lesion scores for an efficacy trial using the ADVENT formulation. Birds were vaccinated as described and challenged on day 24. The post-challenge body weight of the non-vaccinated, non-challenged controls was significantly greater than any group except the birds sprayed directly with the vaccine. Post-challenge performance of all vaccinated birds was significantly better than the non-vaccinated, challenged controls for body weight and feed conversion. Birds that were vaccinated but not challenged had cumulative feed efficiency similar to the non-vaccinated controls.

Lesion score data confirm the effect of the challenge, with all non-vaccinated, challenged treatments giving scores significantly greater than any of the other treatments. Birds that were vaccinated had scores significantly smaller than the non-vaccinated birds. There were no differences in performance or lesion scores associated with the two routes of vaccine administration.

In summary, these results demonstrate that ADVENT is a safe and effective means of coccidiosis control in rapidly growing commercial broilers. The safety results from the ability to formulate the vaccine based on viable sporulated oocysts, which reduces variability in potency from serial to serial. Safety of a large dose of ADVENT was confirmed by the excellent livability and a lack of mortality in broilers. Efficacy data indicate that the vaccine produces a robust immunity that is reflected in both lesion score protection and improved bird performance.

Table 1. Performance and Lesion Scores for Birds Vaccinated with ADVENT and Given a Challenge by Oral Gavage

	Non-Vaccinated		Spray on Feed		Spray on Birds		
	Non-Challenged	Challenged	Non-Challenged	Challenged	Non-Challenged	Challenged	SEM
Post Challenge (Day 30)							
Body Weight (g)	1328a	1054d	1248b	1202bc	1307ab	1216c	27.0
Cumulative Feed to Gain	1.562c	1.748a	1.568c	1.632b	1.563c	1.621b	0.016
Lesion Score							
E. acervulina	0.17c	1.71a	0.21c	0.71b	0.08c	0.88b	0.16
E. maxima	0.06c	1.29a	0.15bc	0.42bc	0.02c	0.55b	0.16
E. tenella	0.07c	2.44a	0.22c	1.56b	0.07c	1.64b	0.13

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REFERENCES

1. Dibner, J.J., M.L. Kitchell and M.A. Pfannenstiel. Viability assay for sporocyst-forming protozoa. United States Patent 6334340, 2002.

2. Dibner, J.J. M.A. Pfannenstiel, M.L. Kitchell, and T.J. Miller. The use of a viability assay to formulate a high definition coccidiosis vaccine. Proc. 52nd West. Poult. Dis. Conf., Davis, California. (in press). 2003.

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

4. SAS Users Guide: Statistics, Version 6.12 Edition. SAS Inst., Inc. Cary, N.C. 1996.

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AIRSACCULITIS IN TURKEYS ASSOCIATED WITH CANDIDIASIS

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SUMMARY

Yeasts are ubiquitous in the environment and are usually considered opportunistic pathogens. Although there are hundreds of yeast species, only a few produce disease in animals. The most common yeast isolated from poultry is *Candida albicans*. Candidiasis in poultry is predominately associated with the digestive tract with lesions typically seen in the crop. Immunosuppression, extensive use of antibiotics, unsanitary watering conditions, and overcrowding have been contributing factors to candidiasis of the digestive tract (1). Here we report the finding of candidiasis associated with the respiratory system in turkeys.

This report reviews ten cases of airsacculitis in turkeys from which *Candida* spp. were isolated. These cases were submitted to the Fresno branch laboratory of the California Animal Health & Food Safety Laboratory System from six different ranches of two independent poultry companies. All turkeys were commercial breeders, ranging in ages between 2- and 52-wk-old. Both males and females were affected. Grossly, the air sacs were severely thickened and opaque with severe accumulation of white to yellow, creamy to caseous exudate. Histologically in the air sacs, there was severe fibrinoheterophilic and pyogranulomatous inflammation. In some sections, this inflammation was associated with plant material and pseudomycelia. In addition, many birds had pneumonia suggestive of a bacterial infection.

Various species of *Candida* were isolated from the air sacs, including *Candida albicans*, *C. dubliniensis*, *C. famata* and *C. krusei*. With the finding of plant material in the air sacs of turkeys from numerous cases, the *Candida* species are most likely opportunistic pathogens. In addition, *Escherichia coli*, *Ornithobacterium rhinotracheale*, *Pseudomonas aeruginosa*, *Proteus* spp. and *Staphylococcus* spp. were isolated from the air sacs and/or lungs.

The genus *Candida* belongs to the family Cryptococcaceae within the Deuteromycetes (fungi Imperfecti), and contains approximately 200 species. These yeasts can be found in the environment, on many plants, and as part of the normal flora within the digestive tract of mammals and birds and on the mucous membranes of humans. *Candida albicans* accounts for up to 70% of *Candida* species isolated from sites of infection in animals and has been reported as a causative agent of all types of candidiasis. *Candida dubliniensis* is phenotypically similar to *C. albicans* and has been recovered from the oral cavity of immunosuppressed human patients. *Candida famata* is a common environmental isolate and usually isolated from the skin. *Candida krusei* has been isolated from environmental samples, but is an emerging opportunistic pathogen that has been associated with some forms of infant diarrhea and patients with fungemia. It has also been reported to colonize the respiratory, digestive, and urinary tracts of patients with granulocytopenia.

REFERENCES

1. Mayeda, B. Candidiasis in turkeys and chickens in the Sacramento Valley of California. Avian Dis. 3:232-243. 1961.

EFFECT OF ONION INGESTION IN WHITE CHINESE GEESE

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Sudden increased in mortality was observed in two different flocks of mature breeder geese. The first case occurred in November 2001, where 6% mortality was observed in a flock of 1400 breeders of 11 different goose breeds. Most of the birds (95%) that died were White Chinese geese. The second case happened in August 2002, mortality as high as 8.4% was observed in a two-day period in a flock of White Chinese geese (634 birds). In both cases, birds had been fed a maintenance ration (110-160g/day/bird) plus free choice of green onions (*Allium ascalonicum*) for 7-10 days. Necropsy and histologic findings were similar in both cases. Grossly birds had pale epicardium with random petechiation, sanguinous fluid accumulated in the pericardial sac of a few birds, and liver and spleen were mildly swollen. Histologically, there was accumulation of hemosiderin in hepatocytes, Kupffer cells of the liver, macrophages and renal tubules. There was also moderate to severe hepatic necrosis, splenitis, and renal tubular nephrosis. No significant bacteria were isolated from liver or intestine. Livers contained heavy metals in expected concentrations for poultry, except above normal, but non-toxic, iron and copper concentrations. Increased concentration of iron probably was secondary to hemolysis and was compatible with the accumulation of hemosiderin observed histologically. In both cases, after onions were removed from the diet mortality in the flock went back to normal.

In areas where onions are grown is common practice to use culled onions as a source of feed for livestock. Since toxic effects of onions have been reported in cattle, cats, horse, dogs, and sheep(2), most farmers use onions in strictly limited quantities. However, there are no reports describing the effects of onion in avian species.

The toxic components in *Allium* species are sulfur-containing compounds, especially alk(en)yl-cysteine sulfoxides(1). These compounds interfere with the hexose monophosphate pathway and consequently lead to damage of red blood cell membrane, hemolysis and denaturation of hemoglobin.

In order to confirm the diagnosis of onion poisoning, a ration consisting of 75% (wet weight basis) green onions and 25% maintenance ration was fed *at libitum* to three mature White Chinese geese for 21 days. Blood for complete hematology and biochemistry studies was collected from the birds on days 0, 3, 6, 9, 12, 15, 18, and 21. During this period, the red blood cell count and hematocrit slowly decreased while polychromasia increased. On day 21 birds were euthanized and a full necropsy performed. No significant gross changes were observed. Histologically, livers contained moderate amounts of hemosiderin in the hepatocytes and Kupffer cells. In addition, livers contained toxic levels of copper and above normal, but non-toxic, of iron. No significant bacteria were isolated. This experimental study demonstrated that anemia and liver pathology could be caused with ingestion of onion; however, it is not clear what the relation is between the high levels of copper found in the livers and onion ingestion.

REFERENCES

1. Burrows, G.E., and R. J. Tyrl. Liliaceae. In:

Toxic Plants of North America. Iowa State University Press, Ames, Iowa. Pp. 752-805. 2001.

2. Valli, V.E. O. The hematopoietic system. In: Pathology of Domestic Animals, 4th ed. K.V. F. Jubb, P.C. Kennedy and N. Palmer, eds. Academic Press, Inc., San Diego. Pp. 84-238. 1993.

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HEMAGGLUTINATION-INHIBITION ANTIBODIES AND PROTECTION CONFERRED BY BI- OR TRIVALENT VACCINES OF HAEMOPHILUS PARAGALLINARUM AGAINST ISOLATES OF THE PREVALENT HEMAGGLUTININ SEROVARS IDENTIFIED IN MEXICO

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Haemophilus paragallinarum is the causative agent of infectious coryza, an acute upper respiratory disease of chickens (*Gallus gallus*). The greatest economic losses result from poor growth performance in growing birds and marked reduction (10-40%) in egg production in layers (1). Two related schemes have been used to serotype this bacterium. The Page scheme was originally developed with the use of a slide agglutination test to recognize the three serovars, A, B and C (2). Dr. Rimler showed that the three Page serovars represent distinct immunovars, since inactivated vaccines based in any one Page serovar provide no protection against the other two Page serovars (3). On the other hand, the Blackall serotyping scheme recognizes nine serovars, organized into three serogroups. Thus, the nine currently recognized hemagglutinin serovars are termed A-1, A-2, A-3, A-4, B-1, C-1, C-2, C-3, and C-4 (4). Based on the use of this scheme, we have identified serovars A-1, A-2, B-1, and C-2 in Mexico (5).

Commercial vaccines of *H. paragallinarum* included Page serovars A and C (bivalent) or A, B, and C (trivalent). The purpose of present study was to determine protection conferred by bi- or trivalent vaccines against prevalent serovars identified in Mexico. Reference strains 221 (A-1), 0222 (B-1), H-18 (C-1), and Modesto (C-2) were included in vaccines. Chickens were vaccinated in two occasions with a bivalent (A-1 and C-1) or trivalent (A-1, B-1, and C-2) combination. Three weeks after second vaccination, groups of chikens were challenged with isolates belonging to serovars A-1, A-2, B-1, and C-2. Obtained conferred protection in chickens by bivalent vaccine were 80%, 80%, 30% and 70% against challenge with isolates of serovars A-1, A-2, B-1, and C-2, respectively. Similarly, obtained conferred protection in chickens by trivalent vaccine were 80%, 80%, 70% and 80% against challenge with isolates of serovars A-1, A-2, B-1, and C-2. Hemagglutination-inhibition antibodies against serovar B-1 were not detected in bivalent vaccinated chickens. Obtained results indicate that the use of trivalent vaccines of *H. paragallinarum* could provide protection against prevalent serovars identified in Mexico.

REFERENCES

1. Blackall, P. J., M. Matsumoto, and R. Yamamoto. Infectious coryza. In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 179-190. 1997.

2. Page, L. A. Haemophilus infections in chickens. 1. Characteristics of 12 Haemophilus isolates recovered from diseased chickens. Am. J. Vet. Res. 23:85-95. 1962.

3. Rimler, R. B., R. B. Davis, and R. K. Page. Infectious coryza: cross-protection studies, using seven strains of Haemophilus gallinarum. Am. J. Vet. Res. 38:1587-1589. 1977.

4. Soriano, V. E., G. Téllez, and R. P. Fernández. Proposal of the Blackall scheme for hemagglutinin serotyping of Haemophilus paragallinarum. Proc. International Pasteurellaceae Society Conference, Banff, Canada. p.30. 2002.

5. Soriano, V. E., P. J. Blackall, S. M. Dabo, G. Téllez, G. A. García-Delgado, and R. P. Fernández. Serotyping of Haemophilus paragallinarum isolates from Mexico by the Kume hemagglutinin scheme.

SALMONELLA SPP. IN BROILER BREEDER FLOCKS AND HATCHERIES IN NORTHEN ITALY: 2 YEARS OF BACTERIOLOGICAL MONITORING

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SUMMARY

Between 2001 and 2002 isolation of *Salmonella* spp. from 26 vaccinated broiler breeder flocks and two hatcheries in Northern part of Italy has significantly decreased compared with previous studies on unvaccinated flocks. In 2000, 23 of 799 samples (2.8%) were positive for *Salmonella* spp., while in 2001-2002 only eight of 1348 (0.6%) samples were positive. Biosecurity, Normal Avian Gut Flora (NAGF) application at the hatchery level, and double vaccination with rough-live and killed vaccines represent good means to control and eradicate *Salmonella* spp. infections in breeder flocks.

Two years of monitoring for *Salmonella* spp. have been performed in 26 broiler breeder flocks (5000 to 30000 birds) and in two hatcheries (one million eggs/weeks) between 2001-2002. Monthly controls have been executed on breeders; liver and intestine (pools); environmental swabs from the eggs-stocking rooms; swabs from the dressing rooms of the husbandry; pipped eggs and fluff; and environmental swabs from the whole building (offices, dressing rooms, eggs stocking rooms, incubators, setters, candling and vaccination rooms, vaccination machines, eggs trays and waste disposal rooms).

In 2002 NAGF at hatching and vaccination against *Salmonella gallinarum* (live vaccine) at seven weeks of age and *Salmonella enteritidis* (emulsified vaccine) at 18 weeks of age have been applied to every breeder.

In 2001, 23 of 799 (2.8%) samples were positive for *Salmonella* spp. In 2002 just eight of 1348 (0.6%) samples were positive: 4 isolates were serogroup C1 (environmental samples from the eggs stocking rooms) and 4 isolates were serogroup C2 (three fecal samples and one sample from the dressing room).

In 2001, 81 of 410 (19.8%) samples from the hatcheries were positive. In 2002, 33 of 724 samples (4.5%) from the same hatcheries were positive. Serogroups and sources of isolation were the following:

- 7 C1= fluff and environmental samples
- 3 C2= fluff and environmental samples
- 4 E= fluff and environmental samples
- 16 G= fluff and environmental samples and pipped eggs
- 3 F= only environmental samples

The reduction of Salmonella isolates observed during this monitoring could be referred to NAGF application at hatchery level and double vaccination against *Salmonella gallinarum* and *Salmonella enteritidis* as also confirmed by Mirandé and Leonard (2001) in the United States. A further improvement in controlling *Salmonella* infections can be obtained by implementation of biosecurity measures.

REFERENCES

1. Mirandé, A. and J. Leonard. Reduction of Salmonella in the United States poultry industry through vaccination. Proc. 51st West. Poult. Dis. Conf., Puerto Vallarta, Mexico pp 7-9. 2002.

REDUCING DOA'S AT PROCESSING PLANTS: TIME OF DEATH DETERMINATION

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Broilers that arrive dead at processing plants or "Dead-On-Arrival" (DOA) are a significant cost to poultry integrators. To better characterize where in the livehaul process DOA mortality occurs, an important step would be the development of criteria to determine the time of death of birds at receiving/hanging. The time of death criteria to be investigated in this study includes the development of rigor mortis in muscle groups, and rectal temperature correlated to ambient temperature.

Broilers that were scheduled for processing from 40-49 days of age were transported from the farm to environmentally controlled rooms at the University of Georgia. The birds were euthanized, weighed (g), and placed in dorsal recumbency on a necropsy table. Assessments of rigor mortis and rectal temperature were made at ambient temperatures of 21.1± 1^{O} C in Study 1 and 4.4±1^{O} C in Study 2. Rectal temperatures were performed at 30-minute intervals. Measurements of rigor mortis were made at 10 and 30-minute intervals, both were performed to determine if repeated manipulation of a muscle group had any detrimental effect on rigor mortis development. Evaluation of rigor mortis was done through extension of the thigh, extension and flexion of the hock joint, and extension of the beak muscles. Evaluations for the degree of stiffening were scored as none (1), slight (2), moderate (3), advanced (4), or rigid (5).

Table 1. Average rectal temperature measurements (°C) following death in study 1 and 2.

Time (min)	**0**	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**240**	Bird no.
Study 1(21.1^{O}C)	42.0	41.1	39.5	37.7	36.2	34.7	33.6	32.7	32.3	n=27
Study 2(4.4 ^{O} C)	41.6	40.6	38.2	36.0	33.5	31.3	28.8	25.9	23.8	n=12

Rigor mortis assessments made by extension of the thigh and extension and flexion of the hock joint showed slight development (score of 2 or greater) by 10 minutes post mortem (PM). Assessments made of by extension of the thigh in Study 1 and 2 (n=39) demonstrated full rigor (score of 5) by 60 minutes PM. Rigor mortis assessments made by extension and flexion of the hock joint in Study 1 and 2 (n=39) showed full development of rigor mortis by 70 minutes PM. Frequency of measurement and ambient temperature had little effect on rigor mortis development in the thigh and hock joint musculature. Rigor mortis assessments made by extension of the beak musculature, showed slight development of rigor mortis as early as 30 minutes PM. Of the birds measured at 10-minute intervals (n=17), 56% demonstrated full rigor at 120 minutes PM and 94% at 180 minutes PM. Of the birds measured at 30-minute intervals (n=10), 69% demonstrated full rigor at 120 minutes PM and 100% at 180 minutes PM. Birds in Study 2 (n=12) showed a delay in onset and full development of rigor mortis in the beak musculature. Average body weights for birds in Study 1 and 2 were 2215 g and 2668 g, respectively.

Rectal temperature assessments in Study 1 showed a gradual decrease in temperature following death until a plateau was reached after 180 minutes upon which slower body cooling ensued. Rectal temperature assessments at a lower ambient temperature (Study 2) showed a more rapid decline in body temperature and a plateau of cooling was not reached, as in Study 1. It was noted that the cooling rate of larger birds was slower than smaller birds. Further studies and analysis of birds in different weight ranges and ambient temperatures are in progress. Rigor mortis assessments showed some degree of variability in the onset and full development of rigor mortis at both ambient temperatures. The primary objective of this research was to create "body cooling curves" and rigor mortis findings at different ambient temperatures for use in field DOA analysis. The preliminary field applications of these findings have shown promise in determining a range for time of death in DOA mortality. Limitations of rectal temperature findings include the unknown temperature at the time of death as well as body cooling effects from the trailer micro-environment. Limitations of rigor mortis assessments are due to the breakdown of rigor mortis that can occur through manipulation and movement of carcasses in the livehaul process.

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

SQUAMOUS CELL CARCINOMA IN A CHICKEN'S CROP

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A 54-month old fighting chicken was presented for examination of a cervical swelling. The bird died during physical examination. Necropsy revealed a necrotizing ingluvial lesion. Microscopic examination confirmed an invasive squamous cell carcinoma. Polymerase chain reaction and ultrastructural examination failed to identify a viral component.

EVALUATION OF AVIAN ESCHERICHIA COLI'S ABILITY TO SURVIVE WITHIN MACROPHAGES

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SUMMARY

The organism *Escherichia coli* can be an avirulent commensal or highly virulent pathogen. In poultry, *E. coli* can cause a variety of ailments such as swollen head syndrome, cellulitis, or septicemia; these and other diseases are collectively referred to as colibacillosis (1). Colibacillosis continues to increase production costs in the poultry industry due to high mortality and downgrading of poultry carcasses at slaughter (5). This is aggravated by the fact that preventative measures against the disease are lacking (1). The treatment of affected flocks with antibiotics, the primary control measure of colibacillosis, is resulting in an increase of treatment failures due to increased antibiotic resistance in the bacterial populations of poultry (3). It is for these reasons our laboratory is performing research in efforts to identify virulence factors in avian *E. coli*.

A previous study by Wooley et al. indicated that pathogenic avian *E. coli* isolate V1 might have the ability to survive in macrophages and disseminate systemically using this ability (7). In this study, 12-day-old SPF chicken embryos were challenged with approximately 100 colony forming units of avian *E. coli* isolate V1, and 5-10 embryos were selected daily for culturing and histopathology. In the dying embryos, histopathological findings indicated that dead macrophages seen in cardiac tissue and spleens were surrounded by intact *E. coli* bacteria and frequently had multiple intact bacteria within the dying macrophage. Due to this observation, we embarked on a study to determine if V1 and 19 other well-characterized avian *E. coli* isolates have the ability to survive macrophage engulfment *in vitro*.

Using immortalized avian macrophage cell line HD11 survival of isolate V1 was not detected; however, problems with establishing the assay were apparent in that survival of the positive control isolate (ATCC isolate 14028, *Salmonella choleraesuis* subsp. *choleraesuis* serotype typhimurium) was also unable to be detected. Since obtaining the avian macrophage cell line MQ-NCSU (courtesy of Dr. M. Qureshi at North Carolina State University), we are developing a protocol to determine the ability of an avian *E. coli* isolate to escape killing by MQ-NCSU macrophages (2,4,6).

Although resident respiratory system macrophages are rare in avian species, these cells are elicited in response to colonization of the respiratory tract with *E. coli* or other bacteria. Studies involving primary avian cell cultures of heterophils and macrophages will also be performed with the avian *E. coli* isolates tested in this study.

REFERENCES

1. Barnes, H. J. Colibacillosis in Poultry, vol. 3. Pfizer Inc., Lee's Summit, Missouri. 1994.

2. Hamrick, T., E. Havell, J. Horton, and P. Orndorff. Host and bacterial factors involved in the innate ability of mouse macrophages to eliminate internalized unopsonized Escherichia coli. Infect. Immun. 68:125-132. 2000.

3. Hofacre, C. L., A. R. de Cotret, J. J. Maurer, A. Garritty, and S. G. Thayer. Presence of

fluoroquinolones-resistant coliforms in poultry litter. Avian Dis. 44:963-967. 2000.

4. Lissner, C., R. Swanson, and A. O'Brien. Genetic control of the innate resistance of mice to Salmonella typhimurium: expression of the lty gene in peritoneal and splenic macrophages isolated in vitro. J. Immunol. 131:3006-3013. 1983.

5. Morris, M. Poultry health issue. Poultry Times. July 3:11. 1989.

6. Qureshi, M., L. Miller, H. Lillehoj, and M. Ficken. Establishment and characterization of a chicken mononuclear cell line. Vet. Immun. and Immunopath. 26:237-250. 1990.

7. Wooley, R. E., P. Gibbs, T. Brown, and J. Maurer. Chicken embryo lethality assay for determining the virulence of avian Escherichia coli isolates. Avian Dis. 44:318-324. 2000.

COMPARATIVE EVALUATION OF DUPLEX PCR ASSAYS FOR *MYCOPLASMA GALLISEPTICUM* AND *MYCOPLASMA SYNOVIAE*

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Mycoplasma gallisepticum (MG) causes chronic respiratory disease of chickens and infectious sinusitis of turkeys. *Mycoplasma synoviae* (MS) causes subclinical to mild upper respiratory infection of chickens and may also cause systemic infectious synovitis of chickens and turkeys.

Rapid diagnosis of MG and MS is important in the control of these disease agents. Polymerase chain reaction (PCR) assays have been very helpful to the poultry industry in rapid detection and confirmation of these disease agents. Initially standard MG and MS PCR assays were run separately to obtain maximum sensitivity of the test. This paper is presented in response to resent requests by poultry producers for a standard PCR assay that combines the MG and MS as one test (duplex) and for evaluation of a new set of primers for MG and MS developed in Brazil.

MATERIAL AND METHODS

Stock cultures of MG and MS were processed for DNA extraction using the Gentra DNA Extraction Kit following the manufacturers protocol. Various combinations of 4 MG and 4 MS specific primers plus one general primer were evaluated by performing mono- and duplex PCR assays with serial 10-fold dilutions of the MG and MS DNA singly or combined. Three types of DNA polymerase enzymes (AmpliTaq Gold, Roche; Expanded High-fidelity PCR System, Roche; Taq DNA polymerase-recombinant, Invitrogen) were used to evaluate sensitivity with the various combinations of primers in the duplex PCR assays. A standard thermal cycler program was used (1 cycle at 94ºC for 2 min; 40 cycles at 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 30 sec; 1 cycle at 72ºC for 5 min) for all combinations except the duplex assay using Brazil primers which were run at 53ºC annealing temperature[1]. The amplicons were visualized after electrophoresis for 1.5 hr at 125V in an agarose/Synergel (1.5% agarose equivalent) with ethidium bromide and recorded using UV transillumination and photography.

Sequence of primers:

- MGLF 5'-GAG CTA ATC TGT AAA GTT GGT C-3'[1]
- MGLR 5'-GCT TCC TTG CGG TTA GCA AC-3'[1]
- MGBF 5'-GCG ATG ACG TGT AGT TAT GC-3'
- MGBR 5'-GGA GCG AAT ACT TCG GTG C-3'
- MSLF 5'-GAG AAG CAA AAT AGT GAT ATC A-3'[1]
- MSLR 5'-CAG TCG TCT CCG AAG TTA ACA A-3'[1]
- MSBF 5'-GCT TGA CGG TAC CAT GTC-3'
- MSBR 5'-GCC TTA GGC AGT CGT CTC-3'
- GMLR 5'-CGC TTG CAA CCT ATG TAT TAC C-3'[1]

RESULTS

Preliminary evaluations of the 3 DNA polymerase enzymes indicated that the recombinant Taq DNA polymerase (Invitrogen) gave the greatest sensitivity and this Taq DNA polymerase was used for the remainder of the experiments. Six combinations of the 4 MG primers and 4 MS primers and one general primer were evaluated as duplex PCR assays (1 - MGL

F&R + MSL F&R; 2 – MGB F & MGL R + MSB F & MSL R; 3 – MGB F&R + MSL F&R; 4 – MGL F&R + MSB F&R; 5 – MGB F & GML R + MSB F & GML R; 6 – MGB F&R + MSB F&R). Each set of the 2 MG and 2 MS PCR primers were run separately with the MG and MS DNA dilutions. Two combinations of primers (1 and 5) showing the highest and relatively equivalent sensitivity will be used for more extensive evaluation.

DISCUSSION

Disease caused by MG and MS continues to show up sporadically in private and commercial flocks resulting in economic loss, thus continuous monitoring of breeder flocks is imperative. A sensitive MG and MS duplex PCR assay would be less expensive that the procedures preformed separately. These preliminary results with the two combinations (1 and 5) of MG and MS primers as duplex PCR assays give sensitivity equivalent to the individual PCR assays.

REFERENCE

1. Lauerman, LH. Mycoplasma PCR assays. *In*. Nucleic Acid Amplification Assays for Diagnosis of Animal Diseases. Lloyd H. Lauerman, ed, Published by American Association of Veterinary Laboratory Diagnosticians, pp 41- 43 and 51, 1998.

PINE SHAVINGS AND SAND USED AS POULTRY LITTER AND THEIR RESPECTIVE BACTERIAL LEVELS

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SUMMARY

Bacterial loads associated with sand and pine shavings litter in a seven-week broiler trial was ascertained. The trial consisted 16 pens divided equally between litter types. Samples were collected weekly and plated on one of three media types. Water activity and moisture levels were determined. Overall, sand samples exhibited lower bacterial counts, water activity and moisture levels compared to pine shavings, indicating sand in these characteristics, may be a viable litter alternative to pine shavings.

INTRODUCTION

Availability of pine shavings has been decreasing due to alternative uses (1). As a result, sand has emerged as a possible alternative, due to its favorable particle size, porosity, and durability. Bilgili et al. (2,3) showed little or no difference in live performance, carcass quality, and deboning yields, when sand was used. Additionally aerobic bacterial counts were either lower or had no difference in sand. The present experiment was designed to compare total aerobic, anaerobic, enteric bacterial counts; water activity and moisture over a 7-week broiler grow out experiment.

MATERIAL AND METHODS

One-day-old broiler chicks were placed evenly into 16 pens (eight containing pine shavings, eight containing sand). Litter was collected weekly, for seven weeks, from three areas within each pen and pooled. Aerobic, anaerobic, and enteric bacteria were enumerated using Plate Count Agar (PCA), Reduced Blood Agar (RBA) and Maconkey Agar (MA). Each sample was diluted with sterile physiological saline (0.75% NaCl) to a final dilution of $1:10^4$ for MA and $1:10^6$ for PCA and RBA. These dilutions were spiral plated in triplicate and incubated under the appropriate conditions (37^o C) for 24 hours. Colonies were quantified using a digital plate reader and average bacterial count for each media/litter type obtained. The resulting average for each pen was than analyzed using a T test.

RESULTS AND DISCUSSION

Results were similar to those reported previously (4) with pine shavings associated with higher overall bacterial counts. PCA bacterial counts peaked on week four with both litter types (10^9 CFU/g). RBA counts peaked by week three with both litter types (10^8 CFU/g). MA levels fluctuated from week to week regardless of litter type. Bacterial load differences between litter types were not as dramatic as previously reported (4). Possibly because the litter from the

present experiment had previously had a flock reared on it and thus had higher initial bacterial load, while the previous experiment had unused litter.

Water activity was marginally higher in pine shavings, increasing in both litter types until week four (0.90 aw). Moisture level, a measure of total water in the sample, was significantly higher ($P<0.001$) in pine shavings compared to sand, with pine shaving typically having 10% more water than sand. The only exception to this was the initial measurement taken at week one.

From the data reported here and from other research (2,3,4) it could be concluded sand is a good alternative to pine shavings. Further research is needed to determine if there are differences in the types of bacteria present on each litter type, since differences in pathogenic bacteria could affect both bird and food safety.

REFERENCES

1. Malone, G.W. Litter types and management. In: Proc. Natl. Broiler Housing Seminar. pp. 14-22. 1992

2. Bilgili, S.F., G.I. Montenegro, J.B. Hess, and M.K. Eckman. Sand as litter for rearing broiler chickens. J. Appl. Poul. Res. 8:345-351. 1999.

3. Bilgili, S.F., G.I. Montenegro, J.B. Hess, and M.K. Eckman. Live performance, carcass quality, and deboning yields of broilers reared on sand as a litter source. J. Appl. Poul. Res. 9:352-361. 1999.

4. Macklin, K.S., R.A. Norton, J.B. Hess, S.F. Bilgili, M.K. Eckman, J.P. Blake, C. Wang, M. Noble and J.T. Krehling. Bacterial levels associated with sand and pine shavings when used as poultry litter. Poul. Sci. 81 (Suppl. 1): S102. 2002.

THE THREAT OF BIOTERRORISM TO THE POULTRY INDUSTRY: THE VETERINARY RESPONSE AS PART OF A COORDINATED SYSTEMS APPROACH TO DETECTION, CONTAINMENT, AND MITIGATION

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Animal agriculture is an example of a "critical infrastructure", or a system with out which society could not function. Being a complex system of many interlocking subsystems, animal agriculture is particularly subject to disruption. Acting essentially as a pipeline, interference at any single point could lead to the partial or total disruption for the consumer. The poultry industry, being the most integrated of the animal industries, is also the most vulnerable to terrorist attack. Strategies to address specific animal agriculture vulnerabilities have recently evolved into the use of the military concept of mitigating the potential for "asymmetric targeting". Asymmetric targets, the poultry industry being an example, are untraditional and unexpected assets, usually poorly defended and highly leveraged. Once disrupted, they tend not to be easily made functional again, nor damages easily contained, since they possess "force multipliers" which can cascade damages to other collateral elements. Understanding these characteristics allows governmental planners to develop better defensive strategies as well as identify additional resources needed for emergency response.

Using the poultry industry as a model, a comprehensive agroterrorism defense plan entitled the Consolidated American Network for Agricultural Resource Intelligence (CANARI) has been developed. The operational goals of the CANARI system were to provide a proactive system for the detection, identification, and containment of potential terrorist attacks on animal agriculture, as well as offering a mechanism by which the response interval between initial identification of an emergency and the eventual federal response is lessened. Using a "bottom-up" approach the system considers commodity, state, and even private personnel as essential, while providing mechanisms by which they can be equipped and trained to be the first line of defense for terrorist attacks. The system also dramatically increases the number of veterinary professionals trained and equipped to deal with foreign animal diseases, should these agents be used in an attack.

CANARI is designed to integrate the presently disparate elements by fostering a cooperative network of local, state, federal agencies, as well as commodity entities and interested non-governmental organizations, such as the American Veterinary Medical Association or other professional groups. Using a "market driven approach" the proposed system is designed to encourage commodity membership and cooperation through positive incentives rather than regulatory duress. In the plan, commodity "cooperators" are

encouraged to join through the positive influences of wholesale and retail end users or through financial incentives provided by insurance carriers and financial organizations. Encouragement is also garnered through internal corporate desires to better insure brand integrity or even create new premium niche "quality assured" consumer products. Using this approach, industry trade organizations can also be expected to encourage cooperation with CANARI as part of membership requirements.

Educational organizations, including Land Grant Institutions and Veterinary Colleges, are integral participants in the CANARI system, fostering a uniquely cooperative relationship that helps bridge the gap between governmental agencies and private enterprise. Besides providing for their traditional role of education, training and outreach, Land Grant Institutions and Veterinary Colleges offer the advantage of uniquely qualified expertise that might otherwise not be available to a comprehensive agroterrorism defense network like CANARI. Collectively, the various components of the CANARI system are designed to work synergistically to provide better assurance of the continued availability of a safe, economical, and readily available food supply.

OREGANO ESSENTIAL OIL REDUCES NECROTIC ENTERITIS IN BROILERS

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ABSTRACT

An experiment was designed to evaluate the performance of chicks feed Orego-Stim® in the presence of a necrotic enteritis (NE) challenge. Orego-Stim® is an all natural extract of a specially selected cultivar of *Origanum vulgare.* Orego-Stim® was compared with Bacitracin Methylene Disalicylate (BMD). A 22% protein corn-soybean meal diet served as the basal diet. A Necrotic Enteritis Model was utilized in order to insure the experimental chicks would have a NE challenge.

The NE Model consisted of incorporating 15% fishmeal into the feed, at the expense of 15% of the basal diet, during the 0 to 11 day starter period (fishmeal was not added to the feed during the 11 to 29 day growing period). All of the chicks (with the exception of the Negative Control) were gavaged with *Eimeria acervulina* and *Eimeria maxima* oocysts at 15 days of age followed by gavage with a *Clostridium perfringens* broth at 18 days of age. At 29 days of age the experiment was terminated and all chicks were scored for NE lesions.

Orego-Stim® was fed at levels of 330 and 660 g/MT of feed from 0 to 29 days of age. The chicks fed Orego-Stim® were compared with chicks that received no additive in the feed (Negative Control and Positive Control) and chicks that received 27.5 g/MT of BMD from 0 to 29 days of age.

At 11 days of age the weight and feed conversion of the chicks fed both levels of Orego-Stim®, and the chicks fed the BMD, were approximately equal. Both levels of Orego-Stim® had numerically better growth and feed conversion than the Negative Control and the Positive Control chicks.

From 11 to 29 days of age the chicks fed the 660 g/MT of Orego-Stim® had the best weight gain and feed conversion of all treatments. The chicks fed the BMD had numerically less weight gain and poorer feed conversion when compared with the chicks feed 660 g/MT Orego-Stim.

The Positive Control chicks (those chicks gavaged with oocysts and *C. perfringens* broth) had the poorest weight gain, feed conversion, and the highest NE score, which indicated there was an effective NE challenge. The lesion scoring at 29 days of age showed that Orego-Stim® was as effective as BMD in reducing the severity of NE. The lesion score for both products was significantly better than the lesion score observed for the Positive Control chicks.

INTRODUCTION

Sub-therapeutic levels of antibiotics have been added to animal feeds for nearly 50 years. So-called growth promoting antibiotics improve growth rate, feed efficiency and mortality under commercial conditions. With the increased usage of antibiotics to treat a number of microbial diseases in humans it has become apparent that antibiotic resistance is a serious problem. Regulatory agencies in Europe have effectively banned the use of sub-therapeutic levels of antibiotics in feeds.

Experience has shown that when growth promoting antibiotics are removed from the feed, intestinal problems, most notably necrotic enteritis, seriously impair production efficiency.

This research was conducted to evaluate the use of a naturally occurring product to control these enteric problems, specifically NE. Many studies have shown the essential oils from the oregano plant, *Origanum spp,* have antimicrobial activity. The product Orego-Stim® (Meriden Animal Health Ltd.) contains these essential oils at standardized concentrations from specially selected cultivars of *Origanum vulgar.*

MATERIALS AND METHODS

Day-old male chicks (Cobb x Cobb) were randomly assigned to starting batteries to provide three replicates of 25 birds for each of the five treatments for the 0 to 11 day of age starting period. At 11 days of age the chicks were re-assigned at random (within each treatment) to provide six replicates of 10 birds per replicate for the growing period (small and unthrifty birds were discarded). The basal diet used throughout the experiment was a 22% protein, corn-soybean meal diet fed in mash form. The basal diet did not contain an antibiotic, coccidiostats, or any other form of medication.

A Necrotic Enteritis Model was utilized in this experiment in order to insure that the chicks would have a NE challenge. The NE Model consisted of incorporating 15% fishmeal into the feed, at the expense of 15% of the basal diet, during the 0 to 11 day starter period (fishmeal was not added to the feed during the 11 to 29 day growing period). All of the chicks (with the exception of Treatment 1, the Negative Control) were orally gavaged with $1x10^5$ oocysts of a mixture of *Eimeria acervulina* and *Eimeria maxima* at 15 days of age. At 18 days all birds (with the exception of the Negative Control) were orally gavaged with a broth culture of *Clostridium perfringens*. The birds were administered a fresh broth culture once daily for up to three days. The number of days of administration was decided based on the overall appearance of the birds and the appearance of the intestine of extra infected birds.

The Orego-Stim® (at two levels, 330 and 660 grams/MT) and Bacitracin Methylene Disalicylate (BMD) (at 27.5 g/MT) were mixed into the appropriate basal diet from 0 to 29 days of age.

All birds were weighed by cage at 0, 11, and 29 days of age. Weight of feed consumed was determined for each cage during the starting period (0 to 11 days of age), and during the growing period, (11 to 29 days of age). Percent mortality was calculated, by cage, at the end of the starting period and at the end of the growing period. At the end of the experiment (29 days) all surviving birds were sacrificed and intestinal lesions were scored for NE (0=normal, 1=mild, 2=moderate, 3=severe).

RESULTS AND DISCUSSION

The results of the experiment are shown in Table 1. At the end of the starting period (11 days of age) the weight and feed conversion of the chicks fed both levels of Orego-Stim® and the chicks fed BMD were approximately equal. Both of the Orego-Stim® treatments and the BMD treatment were approximately 2-5% better in weight and 4-6 points better in adjusted feed conversion as compared to both the Negative Control and the Positive Control. These effects were statistically significant for adjusted feed conversion. There appeared to be no consistent affect of treatment on mortality.

During the growing period (11 to 29 days), the Positive Control chicks (those gavaged with oocysts and C. perfringens broth) had the poorest weight gain, adjusted feed conversion, and significantly higher NE score as compared with all of the other treatments. These results indicate there was an effective NE challenge.

The Orego-Stim®, at 330 and 660 g/MT, significantly reduced the NE lesion score, when compared with the Positive Control, and achieved a score that was equivalent to the lesion score shown by the chicks receiving 27.5 g/MT of BMD. The treatments had no statistically significant affect on gain, adjusted feed conversion, or mortality. The birds that received Orego-Stim® at 660 g/MT had the highest weight gain, even when compared to the Negative Control birds (uninfected birds). The birds that received the BMD in the feed had numerically less weight gain and a numerically poorer feed conversion when compared with the birds that received Orego-Sim® at the 660 g/MT level. In the case of the birds fed Orego-Stim® at 330 g/MT one of the replicates had a high NE lesion score (1.33), 20% mortality, a high feed conversion (2.090), and a low weight gain (694 g). When this replicate was removed from the average, the remaining 5 replicates performed better than the birds that received BMD, and nearly equivalent to the birds that reveived Orego-Stim® at 660 g/MT.

These results indicate that the Orego-Stim® gave effective protection against the necrotic enteritis challenge model. The level of protection achieved by the Orego-Stim® was similar to the protection provided by BMD at 27.5 g/MT.

Table 1. Performance results, 0 to 11 and 11 to 29 days of age.

			******O to 11 Days******			**********11 to 29 Days**********			
Treatment	Add. Level g/MT	C p & Oocysts[1]	Wt., g	Adj. Feed Conv.[2,3]	Mort.,%	Gain, g	Adj. Feed Conv.[3]	Mort.,%	NE Score[2]
1. Neg, Control	None	No	289	1.080[a]	4.00	803	1.642	1.67	0.21[c]
2. Pos. Control	None	Yes	289	1.073[a]	4.00	791	1.738	1.67	1.23[a]
3. Orego-Stim®	330	Yes	302	1.027[b]	6.67	792 (812)[4]	1.738 (1.670)[4]	3.33	0.77[b] (0.65)[4]
4. Orego-Stim®	660	Yes	296	1.023[b]	4.00	821	1.638	1.67	0.57[b]
5. BMD	27.5	Yes	303	1.030[b]	6.67	807	1.645	0.00	0.60[b]

[1]Chicks gavaged with oocysts at 15 days of age and *C. perfringens* at 18 days of age.
[2]Values in the same column that have different superscripts are significantly different, (P>0.05).
[3]Feed conversion was adjusted for mortality
[4]Average of 5 replicates.

OREGANO ESSENTIAL OIL REDUCES THE EXPRESSION OF COCCIDIOSIS IN BROILERS

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ABSTRACT

Orego-Stim® was fed to chicks that had been challenged with coccidiosis oocysts in order to evaluate the ability of this product to protect against a coccidiosis challenge. Orego-Stim® is an all natural extract of a specially selected cultivar of *Origanum vulgare.* Orego-Stim® was added to a corn-soybean meal mash basal diet at levels of 330 and 660 g/MT of feed from 11 to 19 days of age. The chicks fed Orego-Stim® were compared with chicks that received no additive in the feed (Negative Control and Positive Control) and chicks that received 55 g/MT of Salinomycin from 11 to 19 days of age.

All of the chicks (with the exception of the Negative Control) were gavaged with a mixed population of *Eimeria* spp. sporulated oocysts ($1x10^5$ oocysts per bird) at 15 days of age. At 19 days of age the experiment was terminated and all chicks were scored for intestinal coccidiosis lesions.

From 11 to 19 days of age the chicks fed the 660 g/MT of Orego-Stim® had the best weight gain and feed conversion of all treatments. The chicks that received the Salinomycin had numerically less weight gain and poorer feed conversion and a higher coccidiosis lesion score, when compared with the chicks fed 660 g/MT Orego-Stim®.

The chicks that received the 330 g/MT level of Orego-Stim® had a coccidiosis lesion score that was significantly better than the Positive Control chicks, and not significantly different from the chicks that received the 660 g/MT level of OregoStim® or Salinomycin. Growth performance on the 330 g/MT level of Orego-Stim® was also numerically better than the Positive Control chicks, and not much different from the Orego-Stim® 660 g/MT and the Salinomycin.

The Positive Control chicks (those chicks gavaged with oocysts) had the poorest weight gain, feed conversion, and the highest lesion score of all treatments. This indicated there was an effective coccidiosis challenge.

The lesion scoring, as well as growth performance, showed that Orego-Stim® was as effective as Salinomycin in reducing the severity of coccidiosis. The lesion score for both products was significantly better than the lesion score observed for the Positive Control chicks.

INTRODUCTION

So-called growth promoting antibiotics improve growth rate, feed efficiency and mortality under commercial conditions. With the increased usage of antibiotics to treat a number of microbial diseases in humans it has become apparent that antibiotic

resistance is a serious problem. Regulatory agencies in Europe have effectively banned the use of sub-therapeutic levels of antibiotics as well as the use of ionophore coccidiostats in feeds. There is also a growing consumer resistance to the consumption of poultry that has received feed containing chemicals and/or drugs.

This research was conducted to evaluate the use of a naturally occurring product to control coccidiosis. Many studies have shown the essential oils from the oregano plant, *Origanum spp,* have antimicrobial activity. The product Orego-Stim® (Meriden Animal Health Ltd.) contains these essential oils at standardized concentrations from specially selected cultivars of *Origanum vulgar*.

MATERIALS AND METHODS

Day-old male chicks (Cobb x Cobb) were randomly assigned to starting batteries and grown to 11 days of age. At 11 days of age the chicks were re-assigned at random to provide six replicates of 10 birds per replicate for the growing period (small and unthrifty birds were discarded). The basal diet used throughout the experiment was a 22% protein, corn-soybean meal diet, fed in mash form. The basal diet did not contain an antibiotic, coccidiostats, or any other form of medication. The Orego-Stim® (at two levels, 330 and 660 grams/MT) and Salinomycin (at 55 g/MT) were mixed into the basal diet from 11 to 19 days of age.

In order to provide a coccidiosis challenge all of the chicks (with the exception of Treatment 1, the Negative Control) were orally gavaged with $1x10^5$ oocysts of a mixed population of *Eimeria* spp. sporulated oocysts at 15 days of age.

The experimental growing period was from 11 to 19 days of age. Bird weight, weight of feed consumed, feed conversion, and mortality were all determined, by cage, at the end of the growing period. The feed conversion was adjusted for mortality. At the end of the experiment (19 days) all surviving birds were sacrificed and scored for coccidiosis intestinal lesions.

RESULTS AND DISCUSSION

The results of the experiment are shown in Table 1. Positive Control chicks (those gavaged with oocysts) had the poorest weight gain, adjusted feed conversion, and significantly higher intestinal coccidiosis score as compared with all of the other treatments. These results indicate there was an effective coccidiosis challenge.

The Orego-Stim®, at 330 and 660 g/MT, significantly reduced the coccidiosis lesion score, when compared with the Positive Control, and achieved a score that was equivalent to the lesion score shown by the chicks receiving 55 g/MT of Salinomycin. The treatments had no statistically significant affect on weight gain, adjusted feed conversion, or mortality. The birds that received Orego-Stim® at 660 g/MT had the highest weight gain, even when compared to the Negative Control birds (uninfected birds). The birds that received Salinomycin had numerically less weight gain and a numerically poorer feed conversion when compared with the birds that received Orego-Sim® at the 6^0 g/MT level.

Growth performance on the 330 g/MT level of Orego-Stim® was numerically better than the Positive Control chicks, and not much different from the Orego-Stim® 660 g/MT and the Salinomycin treatment. The chicks that received the 330 g/MT level of Orego-Stim® had a coccidiosis lesion score that was significantly better than the Positive Control chicks, and not significantly different from the chicks that received the 660 g/MT level of OregoStim® or Salinomycin.

These results indicate that the Orego-Stim® gave effective protection against the coccidiosis challenge. The level of protection achieved by the Orego-Stim® was similar to the protection provided by Salinomycin at 55 g/MTon.

Table 1. Performance results, 11 to 19 days.

			********** 11 to 29 Days **********			
Treatment	Additive Level g/MT	Oocysts[1]	Gain, g	Adj. Feed Conv.[2]	Mort.,%	Coccidiosis Lesion Score[3]
1. Neg, Control	None	Yes	433	1.512	0.00	4.33[a]
2. Pos. Control	None	No	457	1.473	1.67	0.00[c]
3. Orego-Stim®	330	Yes	443	1.487	0.00	3.35[b]
4. Orego-Stim®	660	Yes	458	1.465	1.67	3.08[b]
5. Salinomycin	55	Yes	444	1.502	1.67	3.25[b]

[1]Chicks gavaged with oocysts at 15 days of age.
[2]Feed conversion was adjusted for mortality.
[3]Values in the same column that have different superscripts are significantly different (P>0.05)

ORGANIC ACIDS EFFECTIVE IN PREVENTING NECROTIC ENTERITIS

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ABSTRACT

An experiment was conducted to evaluate the ability of chicks to resist a necrotic enteritis (NE) challenge when they received PI-Acidifier+ in the drinking water. PI-Acidifier+ is a liquid product containing a mixture of organic acids with a preponderance of propionic acid. PI-Acidifier+ was compared with Bacitracin Methylene Disalicylate (BMD). A 22% protein corn-soybean meal diet served as the basal diet. A Necrotic Enteritis Model was utilized in order to insure the experimental chicks would have a NE challenge.

The NE Model consisted of incorporating 15% fishmeal into the feed, at the expense of 15% of the basal diet during the 0 to 11 day starter period, (fishmeal was not added to the feed during the 11 to 29 day growing period). All of the chicks (with the exception of the Negative Control) were gavaged with *Eimeria acervulina* and *Eimeria maxima* oocysts at 15 days of age followed by gavage with a *Clostridium perfringens* broth at 18 days of age. At 29 days of age the experiment was terminated and all chicks were scored for NE lesions.

PI-Acidifier+ was incorporated into the drinking water at levels of 3.91 and 7.82 ml/l, from 0 to 29 days of age. The chicks that received PI-Acidifier+ were compared with chicks that received no additive in the feed or water (Negative Control and Positive Control) and chicks that received 27.5 g/MT of BMD in the feed from 0 to 29 days of age.

At 11 days of age there were no significant differences in chick weight or feed conversion. However, the chicks that received the high level of PI-Acidifier+ had an elevated mortality. This treatment also had the poorest weight, feed consumption, and feed conversion..

From 11 to 29 days of age the chicks that received the PI-Acidifier+ were not significantly different from the chicks that were fed BMD for both NE score and feed conversion. In comparison to the chicks fed BMD, the weight gain of the chicks receiving the low level of PI-Acidifier+ was not significantly different (though numerically lower), and the weight gain of the chicks receiving the higher level of PI-Acidifier+ was significantly lower. The Negative Control chicks had the best gain, feed conversion, mortality, and NE score of all treatments.

The Positive Control chicks (those chicks gavaged with oocysts and *C. perfringens* broth) had the highest NE score, which indicated there was an effective NE challenge. The lesion scoring at 29 days of age showed that PI-Acidifier+ was as effective as BMD in reducing the severity of NE. The lesion score for both products was significantly better than the lesion score observed for the Positive Control chicks.

INTRODUCTION

Sub-therapeutic levels of antibiotics have been added to animal feeds for nearly 50 years. So-called growth promoting antibiotics improve growth rate, feed efficiency, and mortality under commercial conditions. With the increased usage of antibiotics to treat a number of microbial diseases in humans it has become apparent that antibiotic resistance is a serious problem. Regulatory agencies in Europe have effectively banned the use of sub-therapeutic levels of antibiotics in feeds. Experience has shown that when growth promoting antibiotics are removed from the feed, intestinal problems, most notably necrotic enteritis, seriously impairs production efficiency.

This research was conducted to evaluate the use of an alternative, non-antibiotic, product to control these enteric problems, specifically NE. Many studies have shown that the short chain organic acids have antimicrobial activity. Propionic acid has both bacteriostatic and bactericidal activity. Propionic acid is also classified as Generally Recognized as Safe (GRAS). PI-Acidifier+ is based primarily on propionic acid with lesser amounts of other organic acids. All of the ingredients are classified as GRAS.

MATERIALS AND METHODS

Day-old male chicks (Cobb x Cobb) were randomly assigned to starting batteries to provide three replicates of 25 birds for each of the five treatments for the 0 to 11 day of age starting period. At 11 days of age the chicks were re-assigned at random (within each treatment) to provide six replicates of 10 birds per replicate for the growing period (small and unthrifty

birds were discarded). The basal diet used throughout the experiment was a 22% protein, corn-soybean meal diet fed in mash form. The basal diet did not contain an antibiotic, coccidiostats, or any other form of medication.

A Necrotic Enteritis Model was utilized in this experiment in order to insure that the chicks would have a NE challenge. The NE Model consisted of incorporating 15% fishmeal into the feed, at the expense of 15% of the basal diet, during the 0 to 11 day starter period (fishmeal was not added to the feed during the 11 to 29 day growing period). All of the chicks (with the exception of Treatment 1, the Negative Control) were orally gavaged with $1x10^5$ oocysts of a mixture of *Eimeria acervulina* and *Eimeria maxima* at 15 days of age. At 18 days all birds (with the exception of the Negative Control) were orally gavaged with a broth culture of *Clostridium perfringens*. The birds were administered a fresh broth culture once daily for up to three days. The number of days of administration was decided based on the overall appearance of the birds and the appearance of the intestine of extra, infected birds.

The PI-Acidifier+ was mixed fresh daily (from 0 to 29 days of age) into the drinking water for those chicks that were assigned to receive the PI-Acidifier+. Two levels of PI-Acidifier+ were tested, 3.91 ml/l and 7.82 ml/l. Bacitracin Methylene Disalicylate (BMD) (at 27.5 g/MT) was mixed into the appropriate basal diet from 0 to 29 days of age.

All birds were weighed, by cage, at 0, 11 and 29 days of age. Weight of feed consumed was determined for each cage during the starting period (0 to 11 days of age), and during the growing period, (11 to 29 days of age). Percent mortality was calculated, by cage, at the end of the starting period and at the end of the growing period. At the end of the experiment (29 days) all surviving birds were sacrificed and intestinal lesions were scored for NE (0=normal, 1=mild, 2=moderate, 3=severe).

RESULTS AND DISCUSSION

The results of the experiment are shown in Table 1. During the starting period (11 days of age) there were no significant differences in weight or feed conversion. There were significant differences in mortality. The chicks receiving PI-Acidifier+ at 7.82 ml/l had the highest mortality, as well as the poorest weight and adjusted feed conversion. A possible explanation is that the higher level of PI-Acidifier+ decreased water consumption, which could have resulted in the elevated mortality and decreased feed consumption and growth.

The Negative Control chicks (no oocysts or Clostridia and no additives) had the best performance during the growing period (11 to 29 days). The Positive Control chicks (with oocysts and Clostridia but no additives) had the highest NE score and the poorest adjusted feed conversion. These results show there was an effective enteritis challenge.

The chicks that received the highest level of PI-Acidfier+ during the growing period had a weight gain that was significantly lower than the Negative Control, Positive Control, and BMD treated chicks. The 7.82 ml/l level of PI-Acidifier+ may be too high for maximum performance. However, this higher level had a NE score that was significantly better than the Positive Control, and not different from the BMD treated chicks.

The chicks receiving the lower level of PI-Acidifier+ (3.91 ml/l) had weight gain, feed conversion, mortality, and NE score that was not significanty different from the BMD treated chicks. The NE score was significantly better than the Positive Control.

These results indicate that the PI-Acidifier+ gave an effective reduction in the incidence of necrotic enteritis. The level of protection achieved by the PI-Acidifier+ was similar to the protection provided by BMD at 27.5 g/MT.

Table 1. Performance results, 0 to 11 and 11 to 29 days of age.

		******O to 11 Days******			**********11 to 29 Days**********			
Treatment	C p & Oocysts[1]	Wt., g	Adj. Feed Conv.[2]	Mort.,%[3]	Gain, g[3]	Adj. Feed Conv.[2,3]	Mort.,%	NE Score[3]
1. Neg. Control	No	295	1.071	4.00[ab]	913[a]	1.571[b]	0.00	0.12[a]
2. Pos. Control	Yes	283	1.081	1.33[b]	832[b]	1.681[a]	3.33	1.05[c]
3. PI-Acidifier+ 3.91 ml/l	Yes	281	1.079	1.33[b]	799[bc]	1.648[a]	1.67	0.56[b]
4. PI-Acidifier+ 7.82 ml/l	Yes	268	1.095	12.00[a]	742[c]	1.627[ab]	0.00	0.42[b]
5. BMD 27.5 g/MT	Yes	293	1.093	2.67[ab]	848[ab]	1.624[ab]	1.67	0.34[b]

[1]Chicks gavaged with oocysts at 15 days of age and *C. perfringens* at 18 days of age.
[2]Feed conversion was adjusted for mortality
[3]Values in the same column that have different superscripts are significantly different, (P>0.05).
[4]Average of 5 replicates.

PELISTEGA EUROPAEA: A "NEW" BACTERIUM IN PIGEONS

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SUMMARY

Pelistega europaea is a bacterium associated with respiratory disease in pigeons. Its first characterization was published in 1998, and 16s rDNA sequence analysis of a representative strain indicated that this taxon belongs to the beta-subclass of the *Proteobacteria* with *Taylorella equigenitalis* as its closest neighbor (about 94.8% similarity). *Pelistega* is composed of several genetically distinct sub-groups. However, these genomovars cannot be differentiated by phenotypic tests and they were placed in a single species, *Pelistega europaea.*

The *Pelistega* genus and its single species, *Pelistega europaea,* consists of Gram-negative bacilli, capsulated, not sporulating, and non-motile. The size has certain variability but, generally, it is 0.2 to 0.4 μm in diameter and 1 to 2 μm in length (examination carried out on 16 to 24 hours old cultures and obtained on blood agar). They do not grow on MacConkey agar, and are oxidase positive. They are gelatine, OMPG, citrate and indole – negative; 60 – 90 % of the strains are esculin negative.

Since April 1999, our laboratory has isolated *Pelistega europaea* in several cases; usually connected with other pathogens such as trichomonads, *Aspergillus*, various bacteria, and Circovirus. In most of the cases, it was not the primary pathogen isolated from the case. Pigeons were submitted for either poor performance in homing pigeons or poor growth and increased mortality in squabs, although one of the cases was submitted for respiratory problems. The only finding in postmortem examination was small amounts of mucus in the trachea, but histopathology revealed a severe tracheitis and pneumonia.

Described as a primary pathogen of the respiratory tract, *P. europaea* was also isolated from the peritoneal cavity of a pigeon with chronic peritonitis, and in another case, when the underlying problem was an infection with PMV 1, from the liver.

REFERENCES

1. Andreasen, J.R. and T. Sandhu. *Pasteurella anatipestifer*-like bacteria associated with respiratory disease in pigeons. *Avian Dis*., 1993, 37, 908-911.

2. Vandamme, P., P. Segers., M. Ryll, J. Hommez, M. Vancanneyt, R. Coopman, R. De Baere, Y. Van de Peer, K. Kersters, R. De Wachter, and K.H. Hinz. *Pelistega europaea* gen. nov., sp. nov., a bacterium associated with respiratory disease in pigeons: taxonomic structure and phylogenetic allocation. *Int. J. Syst. Bacteriol*., 1998, 48, 431-440.

3. Euzéby, J.P. Dictionnaire de Bactériologie Vétérinaire: http://www.bacterio.cict.fr/bacdico/garde.html or http://www.bacdico.net.

PREVALENCE OF CAMPYLOBACTER AND SALMONELLA SPP. IN COMMERCIAL MEAT DUCKS

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Campylobacter and *Salmonella* spp. continue to be important food safety pathogens in commercial poultry operations. While prevalence has been determined in a number of studies of broiler chicken farms and processing plants, there is little information about prevalence in specialty poultry species. One earlier study reported 100% recovery from cloacal swabs on a commercial duck operation in California (1). We sampled three different duck flocks during the summer of 2001 for *Campylobacter* and *Salmonella*. Sampling of birds took place at six points along the continuum from farm to final product. Cloacal swabs were conducted at the farm and after transportation to the processing plant, while swabs of skin were done at four points in the processing plant. *Campylobacter* and *Salmonella* were cultured and identified using commercially available selective media and standard methods. Drag swabs of the environment or equipment involved at each sampling point were also tested. Of the three farms tested, one of three had a low prevalence on the farm and no further recovery post-transport or during processing. Two of three farms had a moderate to high prevalence on the farm, which increased post-transport, but then decreased throughout processing. Prevalence data gathered was used to determine potential critical control points for food safety at the farm and processing stations.

REFERENCES

1. Kasrazadeh, M. and C. Genigeorgis. Origin and Prevalence of Campylobacter jejuni in Ducks and Duck Meat at the Farm and Processing Plant Level. J. Food Prot. 50:321-326. 1987.

(The full-length article will be submitted for publication in *Poultry Science*.)