



Cornell University
College of Veterinary Medicine

77th Northeastern Conference on Avian Diseases

June 15-17, 2005

**Poultry Diagnostic and Extension Services
Animal Health Diagnostic Center
Ithaca, New York 14853**

**New York State Department
of Agriculture and Markets**



Proceedings
77th Northeastern Conference on
Avian Diseases

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Ithaca, New York 14853

June 15th, 2005

Dear 77th NECAD Participant:

The organizing committee of the 77th Northeastern Conference on Avian Diseases welcomes you to the College of Veterinary Medicine, Cornell University and Ithaca, NY. We hope that you will enjoy the scientific presentations, the social events, and your stay in Ithaca. We acknowledge the support of Cornell University, New York State Department of Agriculture and Markets, the College of Veterinary Medicine, and the Animal Health Diagnostic Center. Generous financial support was received from our

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We also extend our thanks to Ms. Renee Cornelius and Nancy Bennett for their administrative help.

Special thanks to Ms. Karen LoParco for her help with organization, registration and general assistance to participants of the 77th NECAD.

If you need assistance during the course of the meeting, please feel free let us know. We will do our best to make your stay in Ithaca a pleasant one.

Yours truly,

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College of Veterinary Medicine

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***Student eligible for NECAD Award.**

THE ROLE OF VACCINATION ON THE CONTROL OF INFECTIOUS BURSAL DISEASE VIRUSES.

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Summary. Currently, vaccination is the only practical method for controlling Infectious bursal disease virus (IBDV) infections. The correct antigenic types of IBDV need to be selected for any successful vaccine program. To insure proper protection from maternal or active immunity, vaccine strains should be selected so their antigenicity closely matches the antigenic characteristics of wild-type viruses present in the bird's environment. Because of the diversity of viruses in the United States, identification of wild-type viruses and their antigenic characteristics is critical to setting up a vaccine program that will succeed.

The disease caused by infectious bursal disease virus (IBDV) varies from sub-clinical to one that is characterized by high morbidity and mortality. Immune suppression is a characteristic of all pathogenic forms of infectious bursal disease (IBD). Control of the immune suppression is critical because an immune compromised host is more susceptible to secondary infections by pathogens as well as opportunistic microorganisms. Furthermore, vaccinations for other diseases will be less effective.

The virion of IBDV has a single protein shell or capsid in an icosahedron geometry. It covers two segments of double-stranded RNA, is very stable and resistant to most chemicals and environmental stimuli. Consequently, the virus is difficult to eradicate and once introduced to a physical location it becomes endemic in the chicken population. Thus, vaccination is the most practical form of control.

Vaccination has been a successful mechanism for the control of IBDV infections and the subsequent immune suppression caused by the virus. This is partly because, IBDV infection elicits a strong immune response characterized primarily by the production of antibodies. Ironically, the virus subsequently suppresses this humoral immune response. Because IBDV infects immature B cells, infections early in the bird's life are more devastating to the immune system. Vaccination of breeder hens so maternal antibodies are transferred to the chick has been a very effective method for controlling IBDV infections early in the life of chickens. Unfortunately, these maternal antibodies wane and by 14 to 21 days of age, the chicks are susceptible to the virus. Usually at that age, the immune suppression is less severe during the infection and convalescent birds often recover most of their humoral immune functions. However, the transient immune suppression that occurs during the infection can lead to secondary infections and poor flock performance. To address this problem vaccination with live-attenuated IBDV at 10 – 14 days of age is often recommended. This vaccination stimulates an active immune response in the birds as the passive (maternal) immunity is waning. *In ovo* vaccination and day-of-age vaccination have been used to stimulate an active immune response in chicks as maternal immunity wanes. The efficacy of these vaccines has been debated because they are given at a time when maternal antibodies should be high. Immune complex vaccines, those that contain IBDV and specific antibody were approved for *in ovo* use in 1995. Although effective, their mechanism of action is still being investigated (2).

When everything is working properly, maternal antibodies keep endemic wild-type IBDV strains in the bird's environment in check until active immunity from the live-attenuated vaccines can take over and protect them for the remainder of their grow-out period. Problems arise when the maternal immunity or active immunity fail to thwart IBDV infections. Several problems can cause the failure of IBDV vaccines to protect chickens. They include improper or poorly timed vaccine delivery, immune suppression by other infectious agents, and the lack of apposite immunity due to newly emerging viruses that are antigenically divergent from vaccine strains. The role of antigenic drift which can lead to antigenically divergent strains of IBDV and vaccination failure has been the focus of our research.

Antigenic Drift. During the normal replication cycle of IBDV, mistakes or mutations occur in the viral genome. Many of these mutations are not tolerated and the viruses with them do not survive. Some however give the virus an advantage by enhancing its ability to replicate in the chicken. Antigenic drift is characterized by a change in the amino acid structure of a neutralizing epitope on the surface of the virus. Mutations in these areas often give the virus a replication advantage. In 1991, Heine and co-workers (5) reported that a change at amino acid 222 in the VP2 protein of IBDV was important in the antigenic drift from classic viruses to variant viruses. Studies have since shown that vaccines prepared with classic viruses do not adequately protect chickens from variant virus infections (6, 17). A single mutation event did not lead to the creation of variant viruses. Several mutations were needed to cause sufficient antigenic drift for variant viruses to replicate in the face of classic virus induced immunity. Furthermore, these viruses continue to mutate and drift antigenically. Studying this antigenic drift as it occurs in nature is difficult.

In the late 1990's we reported on the use of molecular techniques to study the genetic mutations that were occurring in IBDV strains isolated from commercial poultry (7, 9, 10, 11). Since genetic mutations have the potential to cause changes in the amino acid structure of the virus, these techniques were useful in identifying viruses that could be drifting antigenically. Restriction fragment length polymorphisms (RFLP) were detected in RT-PCR products from the VP2 gene of IBDV (10, 11). This gene was selected because sequence studies indicated a higher frequency of mutation was occurring in a specific region of the VP2 protein (1, 4, 5, 7, 14). The hypervariable region of VP2 as it was denoted became the focus of several diagnostic assays that attempted to identify antigenically divergent viruses (13, 15, 16). In 1998 and 1999, we reported on six molecular groups that correlated with antigenic differences in vaccine strains of IBDV (10, 11). Epidemiologic studies conducted in our laboratory from 2000 to 2005 indicated that IBDV continues to mutate and that the genetic diversity of viruses causing disease in commercial chickens was much greater than was originally thought (12).

Development of a successful vaccination program. Our epidemiologic studies demonstrated that the genetic diversity of IBDV was large. This suggests the potential for greater antigenic diversity than just classic and variant viruses. Recent studies in our laboratory focused on the extent of this antigenic diversity among IBDV strains and how it may be distributed geographically in the United States. Real-time RT-PCR, nucleotide sequence analysis and phylogenetic analysis indicated that variant IBDV strains fell into 4 distinct groups and that two of these groups were widely distributed across the U.S (12). Our data further indicated that commercially available variant vaccines were only associated with one of these widely distributed groups.

A key element in the development of successful vaccination programs for IBDV appears to be the ability to match vaccine antigenicity with the antigenic type of virus on the premises. In vaccination/challenge studies, most of the wild-type IBDV strains we tested were unable to replicate in broilers that had maternal immunity induced by commercially available classic and variant viruses (8). However, there were some viruses in one widely distributed phylogenetic group that were able to break through maternal antibodies induced by commercially available variant and classic vaccines. Control of these viruses is problematic since no commercial vaccines exist that match their antigenic profile. In these cases, autogenous vaccines have the greatest potential for controlling IBDV infection and the resulting immune suppression.

The use of autogenous vaccines has helped control IBD in local regions where outbreaks were not controlled by commercially available vaccines. Selecting the field virus to use in an autogenous vaccine is an important first step. Viruses from outbreaks of disease can be initially screened using real-time RT-PCR and hybridization probes. The real-time RT-PCR assay used in our laboratory can be used to identify nucleotide sequences that encode neutralizing epitopes on the IBDV VP2 protein (13). Using this assay to detect viruses that are different from commercial vaccines is the first step to identifying an autogenous vaccine candidate. Ultimately, nucleotide sequence analysis is recommended to confirm the existence of amino acid changes that could lead to antigenic diversity.

It should be stressed that true antigenic diversity can only be determined by vaccination/challenge studies. However, new information on the crystal structure of the IBDV VP2 protein suggests that we may be able to use molecular data to more accurately predict antigenic changes in IBDV strains (3). The three-dimensional structure of VP2 demonstrates that the hypervariable region is folded into a short column that protrudes above the molecule. The folding of this region is critical to our understanding of antigenicity because key amino acids in the hydrophilic peaks A and B are physically located adjacent to each other at the top of the column. Specific

amino acids in the minor epitopes of VP2 are also located at the top of this column. It is now possible to determine if an amino acid mutation will be buried in the molecule or exposed on the surface of the VP2 protein. Mutations on the surface are more likely to change antigenicity and contribute to antigenic drift.

Vaccination for IBD has been an effective control mechanism for this immunosuppressive disease. Regardless of the type of vaccine used, a successful vaccination program must be tailored to the antigenic type of IBDV present in the bird's environment.

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CHICKEN INFECTIOUS ANEMIA VIRUS INFECTION: IT IS A SERIOUS PROBLEM

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INTRODUCTION

Chicken infectious anemia virus (CIAV) has a world-wide distribution and most commercial flocks are likely to become infected. It is the only member of the genus Gyrovirus of the *Circoviridae*. The virus has a single-stranded, negative-sense DNA genome of approximately 2300 bases producing a single polycistronic message, which is translated into 3 proteins: VP1, VP2, and VP3. Because it does not code for the enzymes needed for DNA replication, CIAV depends on dividing cells for its own replication. Although it has been well established that CIAV infection can cause immunosuppression in addition to clinical disease, the importance of infection for the poultry industry has not always been clear. In this paper, I will briefly review new information on the regulation of virus transcription, the pathogenesis of infection, CIAV-induced immunosuppression, and prevention. Additional information on these topics can be found in a recent review by Miller and Schat (9).

VIRUS TRANSCRIPTION

Cardona et al. (3) showed that CIAV DNA can be detected in gonadal tissues of chickens, which are positive or negative for CIAV antibodies. These results suggest that CIAV might be present as a latent infection and that viral replication must be strictly controlled. The replication of CIAV is controlled by the promoter/enhancer (P/E) region which contains 4 direct repeats. These repeats resemble hormone response element (HRE) half sites. HRE's most often consist of two inverted half sites separated by approximately 15 nucleotides, but many exceptions to this general rule have been reported. Two direct repeats can function as a HRE especially when a SP1 site is located nearby the HRE half site. Miller et al. (7) used the CIAV P/E to examine expression of the enhanced green fluorescent protein (EGFP) gene in different cell lines including the LMH/2a, which is stably transfected with the estrogen receptor. The P/E construct used for these experiments included the 4 repeats and ended upstream of the transcription start point (TSP). This construct was able to express EGFP in DF-1, LMH, and LMH/2A cells. Expression of EGFP was enhanced in LMH/2A in the presence of estrogen but not in the other cell lines. Interestingly, expression was down regulated in all cell lines at the transcriptional level when a P/E construct was used that included sequences downstream of the TSP. The down regulation was caused by a hormonal repressor interacting with the HRE's and a second repressor interacting with the sequence at the TSP (Miller *et al.*, to be submitted). In conclusion, the regulation of CIAV transcription depends on the balance between activators such as estrogen and repressors. These findings are important for the understanding of the maintenance of latency and reactivation in SPF flocks.

PATHOGENESIS OF INFECTION

Until recently, most pathogenesis studies were done in chickens after intramuscular inoculation. Recently, two groups (12, 13) compared the effects of intramuscular and oral challenge. It was shown that relatively high doses of virus were needed to establish infection by the oral route, and that lesions developed later after oral infection than after intramuscular inoculation. These results are important to understand the pathogenesis in both SPF and commercial flocks. Breaks in commercial SPF flocks often occur after the onset of sexual maturity and are only detected because a few birds seroconvert, at that time the flock is considered positive (11). Unfortunately, there is no information available on the subsequent spread of infection within these flocks. We followed a SPF flock hatched from commercial SPF eggs. One of 90 chicks, maintained in colony cages, tested positive for antibodies and PCR at 4 weeks of age and was culled. Interestingly, the infection did not spread to cage mates. At 16 and 20 weeks of age 1/50 and 1/43 birds also tested positive for antibodies and one antibody negative hen was culled for a crooked toe and tested PCR positive (8). These results indicate that CIAV does not spread rapidly when birds are kept in cages and support the finding that a high threshold exist

for the establishment of infection. It is likely that the transcriptional control by two different repressors plays an important role when a low dose virus challenge occurs. In conditions when a high level of challenge virus is present (e.g., broilers!) these repressors may become overwhelmed and unable to control transcription.

The importance of maternal antibodies in preventing early infections has been shown by many groups (11), but few quantitative data supporting the effect of maternal antibodies on virus replication have been reported. We have shown by quantitative PCR (qPCR) and qRT-PCR assays (5) that chickens positive for maternal antibodies at 10 days of age were fully protected against viral replication after intramuscular challenge at 15 days of age. Interestingly, chickens were also protected at 30 days of age although the presence of maternal antibodies could not be demonstrated at 30 days of age using the Iddex ELISA kit (6).

IMMUNOSUPPRESSION

It was mentioned before that CIAV replicates in dividing cells and especially the hematocytoblasts in the bone marrow, thymocytes, and splenic T lymphocytes are target cells for CIAV (reviewed in 1, 9). The consequence of virus replication is the production of VP3 or apoptin causing apoptosis of precursor cells of innate and acquired immune responses. Virus-induced apoptosis of hematocytoblasts affects the development of heterophils and macrophages, both important components of innate immune responses. The effects on acquired immune responses are especially important when cytotoxic T lymphocytes (CTL) are needed to protect chickens against other infections. Replication of CIAV prevented the induction of CTL against reticuloendotheliosis virus (REV) 7 days after dual infection with CIAV and REV. The absence of REV-specific CTL was likely the cause of apoptosis and is not caused by decreased interferon (IFN)- γ or IL-2 production; because these cytokines were not affected by CIAV infection at 7 and 14 days post infection (dpi) as measured by RT-PCR (6). Others have reported that these cytokines can be down regulated but these effects are in general seen after 14 dpi. It is likely that replication of CIAV also effects precursors of dendritic cells (DC), which would impact the induction of immune responses. DC's are the key players in the induction of immune responses through the activation of Toll-like receptors by pathogen-associated molecular patterns (PAMS). Activated DC's produce IL-12 which will start the cascade needed for the development of cell-mediated immune responses. It is possible that CIAV VP2 interferes with activation pathways of immune responses, because VP2 has a dual phosphatase activity and can catalyze the removal of phosphate from both phosphotyrosine and phosphoserine/phosphothreonine substrates (10). Thus two of the three viral proteins may be involved in immunosuppression.

The importance of immunosuppression caused by CIAV replication is often difficult to determine because CIAV infections are subclinical when chickens are infected after maternal antibodies wane. However, it is most likely an important factor in condemnations of broilers with septicemia, which is the major cause of condemnations in the USA. In addition, the potential effects on CTL may cause vaccine breaks for many diseases [e.g., Marek's disease (4)] and may aggravate diseases where cell-mediated immune responses are important for recovery. In addition to immunosuppression, CIAV infection can also impact feed conversion and other economic parameters, although some publications show a lack of impact (reviewed in 11).

PREVENTION

Current vaccination strategies are directed toward breeder vaccination so that the offspring will be protected against clinical disease. Vaccination may not be needed if the birds have been naturally exposed. Barbour et al. (2) found no differences in replication of CIAV in broilers derived from naturally exposed and vaccinated breeders which had comparable antibody levels. It is unknown if the breeder vaccines can cause subclinical immunosuppression when administered to antibody negative breeders. Passive protection of broilers through maternal antibodies will leave broilers susceptible to infection, and thus immunosuppression, once maternal antibodies do not longer protect against virus replication. Unfortunately, the currently available breeder vaccines are not authorized for use in young birds, because these vaccines can cause lesions after inoculation in maternal antibody free one-day-old chickens. In my opinion, the development of appropriate vaccines for broilers will be important to protect chickens against the subclinical consequences of infection.

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BIOLOGICAL CHARACTERIZATION OF RECENT CHICKEN ANEMIA VIRUS ISOLATES

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Summary

The diseased commercial flocks in which chicken anemia virus (CAV) infections were identified (1), and the latest outbreaks in young broiler flocks (2) directed us to characterize biologically the recent chicken anemia virus isolates. Twelve CAV isolates were prepared from various flock types and genetic lines. These isolates were used to develop an *in vivo* system for biological CAV characterization. Two of the 12 isolates and the CIA-I prototype were also inoculated in day old chicks and surveyed over a period of 35 days. In each isolator half of the chicks were inoculated and the other half was infected by the contact with the injected chicks.

The chicken infectious anemia virus (CIAV) gains lately an extended appreciation. While formerly CIAV was recognized as an unimportant pathogen, increasing evidences indicate now that the virus is acting as a sophisticated parasite reviewed by Miller and Schat (3). Infections are manifested with either clinical or sub-clinical signs. In young chicks, the infection may display different signs and various degrees of severity. These signs include stunting, runting, increased mortality, anemia, bone marrow cell depletion, subcutaneous hemorrhage and atrophy of secondary lymphatic organs. These effects are caused because of the multi-potent efficacy of CIAV to infect stem cells of both the hematopoietic and lymphocytes cell lineages in the bone marrow and thymus.

The impact of chicken infectious anemia virus (CIAV) infection on commercial chicken flocks in Israel was examined by analyzing flocks with or without typical CIAV signs, signs of other diseases or apparently healthy flocks (1). In 23 flocks (broilers and layers) of ages up to 8 weeks typical signs of CIAV infection (stunting, gangrenous dermatitis, and secondary bacterial infections) were recorded. We also surveyed 63 flocks affected by other diseases such as tumors, respiratory diseases, or coccidiosis, and 20 flocks with no apparent clinical signs. The latter two groups were negative by CIAV-PCR, suggesting that typical CIAV clinical signs are associated with one step PCR-CIAV amplification. However, still a small amount of CIAV might be present in these flocks, acting to induce sub-clinical effects of CIAV infection. These data suggest a link between the presence of virus sequences and typical CIAV signs, and strengthen the concept that CIAV-infection has a negative economic impact for the chicken industry.

We also reported on an outbreak of chicken infectious anemia virus-related clinical signs in young broiler flocks derived from three broiler breeder flocks (2). The epidemiology and presence of CIAV sequences by PCR in the progeny, broiler flocks were determined, as well as the CIAV-antibody levels in the respective broiler breeder flocks. The three parent flocks were of a similar age, their serological response to CIAV was low and inconsistent, whereas their progeny broiler flocks showed the typical clinical signs of CIAV-infection and were CIAV-PCR positive. The survey also included three control unaffected broiler flocks, that originated from breeder flocks of similar ages and genetic origin, but were reared on other breeding farms, and differed in the maternal immunity that was transmitted to their progeny. Indeed their progeny flocks were negative for both clinical signs and CIAV-PCR. The present data exemplify the necessity of an adequate immunity of the breeder flock in order to avoid the acute disease in the progeny flocks.

The diseased commercial flocks in which CIAV- infections were identified (1) and the latest outbreaks in young broiler flocks (2) directed us to characterize biologically the recent chicken anemia virus isolates.

Twelve CAV isolates were prepared from various flock types and genetic lines. These isolates were used to develop an *in vivo* system for biological CAV characterization. We explored the inoculation of 7 ED embryonated eggs and analyzed the CAV replication and pathogenicity. At 18 ED the embryos were assayed for both pathologic and virus presence, by PCR. The system was found useful to compare the CAV replication and lesions of the 12 isolates.

To characterize the pathogenicity of the recent CIAV isolates and to establish criteria of in vivo disease reproduction, two of the 12 isolates and the CIA-I prototype were inoculated in day old chicks. The chicks were kept in isolators and were surveyed over a period of 35 days. In each isolator half of the chicks were inoculated and the other half was infected by the contact with the injected chicks. At 7, 14, 21, 28 and 35 days post inoculation 4 chicks of each sub-group were sampled. We surveyed the presence of CIAV sequences in various organs, the kinetics of antibody production, the growth rate of the chicks, the atrophy of lymphatic tissues and the CIAV lesions and anemia. For the first time we explored the involvement of feathers in the horizontal CIAV spread, and found them as a possible means of CIAV reservoir.

As the experiment was completed only in these days, we will publish the detailed results in the near future. However, several observations were evident:

- a) The CIAV-inoculated chicks develop typical signs, such as hemorrhages on the muscles and thymus, paleness and gangrenous dermatitis. These signs commenced at 21 and 28 dpi in the injected and in-contact chicks, respectively.
- b) On the histopathological examination of the thymus a typical change was observed; the border between the cortex and medulla disappeared, and hystiocyte hyperplasia emerged.
- c) The hematocrit decreased at 14-21 dpi in the injected chicks and at 21-28 dpi in the in-contact infected chicks.
- d) The spleen, thymus and bursa weight indexes were measured and the change kinetics were determined in both groups.
- e) The kinetics of CIAV-PCR was analyzed, showing the presence of positive, injected chicks already after 7 dpi, and one week later in the in-contact infected birds. DNA from four organs was purified and amplified, however to determine the chick PCR positivity, we considered one or more of the organs of a bird.
- f) Antibodies appeared in both sub-groups with a 7 days lag compared to the CIAV amplification. The specificity of the PCR compared to antibody detection was 98% and the specificity – 76%.
- g) When dissecting the results of CIAV-PCR of the various organs, the feathers were 89% specific and 83% sensitive compared to the other three organs.
- h) Histology was followed by that point only on the 35 dpi samples and was found 87.5% specific and 77% sensitive compared to the PCR on the same specimen.

In conclusion, we documented the infectivity in commercial flocks, obtained isolates and characterized their effect on two in vivo systems, embryonated eggs and day old-chicks. For the first time for CIAV, we also determined the kinetics of CIAV sequences in injected and in-contact chicks, and implicated the feathers in the horizontal spread.

Acknowledgement

This study was supported by Grant US-3535-04R from the USA-Israel Agricultural Research and Development Fund (BARD).

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DETECTION AND SEQUENCE ANALYSIS OF A CIRCOVIRUS IN COMMERCIAL PEKIN DUCKS IN NEW YORK.

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The family *Circoviridae* includes viruses that produce diverse infections in chickens, geese, pigeons, and pet birds. Circovirus infection is commonly associated with damage of lymphoreticular tissues. A new circovirus infection was reported in ducks in Germany in 2003. This new circovirus was detected in mulard ducks suffering from developmental and feathering disorders. The virus was also detected in Muscovy ducks in China. The presence of a similar duck circovirus has been detected by polymerase chain reaction (PCR) in samples of liver and lymphoid organs obtained from culled Pekin ducks on a duck farm in New York. A segment of the replication protein (Rep) of this virus exhibited nucleotide sequence similarities of 97% and 91% when compared to the viruses detected in Germany and China respectively. The clinical and pathological significance of this infection is still unknown. However, most of the birds studied showed bursal or thymic atrophy. The immunosuppressive properties of the circoviruses have been characterized in chickens and pet birds. Further research is being undertaken to determine the possible effect of this virus on the immune system of ducks.

NOVEL INSIGHTS INTO MAREK'S DISEASE VIRUS PATHOGENESIS.

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Marek's disease virus (MDV) is a member of the *Alphaherpesvirinae* and causes T cell lymphomas in chickens that lead to death of susceptible animals within 3 to 8 weeks post infection. Vaccination using modified live vaccines, however, is able to reliably control MDV infection. MDV – like many related viruses such as herpes simplex virus (HSV) or varicella zoster virus (VZV) – induce a downregulation of MHC class I molecules that is observed in the early stages of lytic infection, but absent in tumor cells that are latently infected and harbor integrated virus genomes. MDV does not encode an ICP47 homologous protein, which blocks TAP and as such causes downregulation of MHC class I in cells infected with HSV. The screening of mutant MDV that were reconstituted from a library of mutagenized bacterial artificial chromosome (BAC) clones revealed, however, that downregulation of MHC class I was absent when the protein kinase (pUS3) encoded in the unique-short region of the genome is absent. Since direct downregulation by expression of pUS3 alone was not observed, we concluded that interaction of pUS3 with another viral protein is required for its function in MHC class I downregulation. Another MDV protein, the secreted glycoprotein C (gC), was also implicated in MDV-mediated immunoevasion. Deletion of the open reading frame resulted in viruses that exhibited enhanced growth properties *in vitro*, while early growth *in vivo* was unaffected. Moreover, recombinant MDV gC was shown to possess complement-binding activity. Consistent with an immunomodulatory function of gC, virus mutants lacking gC exhibited reduced virulence in chickens. Additional animal experiments are conducted to evaluate the *in vivo* effects of a presence or absence of immunomodulatory molecules in Marek's disease.

ATTENUATION OF SEROTYPE 1 MAREK'S DISEASE VIRUS (MDV) BY DELETION OF OPEN READING FRAME RLORF4, BUT NOT RLORF5A

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The recent identification and cloning of RLORF4 and the finding that four of six attenuated strains of Marek's disease virus (MDV) contained deletions within RLORF4 suggested that it is involved in the attenuation process of MDV. To assess the role of RLORF4 in MDV pathogenesis, its coding sequence was deleted in the pRB-1B bacterial artificial chromosome clone. Additionally, RLORF5a was deleted separately to examine its importance for oncogenesis. MDV reconstituted from pRB-1B Δ RLORF5a (rRB-1B Δ RLORF5a) produced similar plaque sizes when compared to parental pRB-1B virus (rRB-1B). In contrast, virus reconstituted from pRB-1B Δ RLORF4 (rRB-1B Δ RLORF4) produced significantly larger plaques. Replication of the latter virus in cultured cells was increased when compared to rRB-1B or rRB-1B Δ RLORF5a using quantitative (q)PCR assays. *In vivo*, both deletion mutants and rRB-1B replicated at comparable levels at 4, 7, and 10 days post-inoculation (pi), as determined by virus isolation and qPCR assays. At 14 days pi, virus isolations from chickens infected with rRB-1B Δ RLORF4 were comparable to highly attenuated RB-1B and significantly lower than that from rRB-1B-infected birds. rRB-1B Δ RLORF5a was shown to produce tumors that were similar in number and kinetics to rRB-1B in P2a chickens. In stark contrast, none of the chickens inoculated with rRB-1B Δ RLORF4 died up to 13 weeks pi; however, two chickens had tumors at termination of the experiment. The data indicate that RLORF4 is involved in attenuation of MDV, although the function of RLORF4 is still unknown.

EFFICACY OF REDUCED DOSES OF HVT VACCINE ADMINISTERED *IN OVO* AGAINST CHALLENGE WITH MAREK'S DISEASE VIRUS IN SPECIFIC-PATHOGEN-FREE CHICKENS

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It is a common practice in hatcheries to use reduced doses of *in ovo*-administered herpesvirus of turkeys (HVT) vaccine to protect against Marek's disease (MD). However, there is little information to support this management practice. As such, the effectiveness of recommended, half and quarter doses of a commercially available HVT vaccine when administered *in ovo* to SPF chickens was evaluated against challenge with 250 plaque forming unit (PFU) of RB1B strain of MDV on day 5 post hatch. On 7, 14 and 21 days post-inoculation (dpi), a subset of birds from each vaccinated and control groups were euthanized and assessed for gross and histological lesions. Also, samples were collected for PCR analysis to detect and quantify MDV load. There was no significant difference in spleen and bursal weights as a % of bodyweight among most groups, except significantly lower bursal weights were observed on 21 dpi in unvaccinated challenged birds or those that received quarter dose of the HVT vaccine ($p < 0.05$). As suggested by clinical outcome and pathological data acquired on 7, 14 and 21 dpi, none of the HVT vaccine dose groups were protected against challenge with RB1B strain of MDV. However, there was a delay in development of lesions in the vaccinated groups when compared to the non-vaccinated challenged birds. On day 7 post-inoculation, the *gB* gene of HVT could be amplified from feather tip DNA of 3/5, 2/6 and 0/6 of birds belonging to full, half and quarter dose groups, respectively. However, on the same day, the *meq* gene of the challenge virus was not detectable in any of feather tip DNA samples. On 14 and 21 dpi, all the challenged birds irrespective of their groups showed amplification of MDV *meq* gene virus in feather tip, whereas the HVT *gB* gene was no longer detectable. When *meq* gene-positive samples were analyzed further by quantitative PCR using SYBR green chemistry, birds in the HVT full dose group had the lowest MDV load, while the same group showed the highest MDV load among other challenged groups on 21 dpi. However, this difference was not significant due to the large variation of the MDV load in the feather tip DNA.

The full-length article will be published elsewhere.

A PRELIMINARY REPORT ON THE IMMUNE CHANGES OBSERVED FOLLOWING INOCULATION WITH AN *EIMERIA* PROTEIN ISOLATE IN BROILER CHICKENS

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Abstract: *Eimeria* infections in commercial flocks are costly to the chicken meat industry from expenses associated with disease prevention, treatment, or performance loss. Unlike some poultry infections, *Eimeria* doesn't typically result in mortality but rather reduces body weight gain and feed conversion efficiency, which negatively impacts producer profit. Currently, commercially available live oocyst vaccines have been associated with negative impacts on performance in small bird markets based on the short growout period and lack of time for compensatory gain following establishment of immunity. Thus a protein-based vaccine could have application to the industry. In this study, we evaluated the efficacy of using an *Eimeria* protein isolate by studying immune endpoints. Commercial broilers on the day of hatch were randomly segregated into 3 groups; vehicle-treated controls, commercially vaccinated, and protein antigen treated. Birds were treated on day 1 of age and boosted on day 21. On days 10, 14, 28, and 31 of age, 5 birds from each group were euthanized and evaluated for immune profile changes. Samples were collected for measurement of organ weight, cellularity, splenic leukocyte flow cytometry analysis, and mitogenic proliferation. In this prefatory study, no significant findings were observed. An increasing trend in organ size and tissue density in spleens of protein treated birds however, was noted. Studies are currently underway to further elucidate potential changes in immune status through an expanded protocol.

The full length article will be published in a refereed journal.

DEVELOPMENT OF IBV-S GENE SPECIFIC RECOMBINANT DNA VACCINE AND ITS APPLICATION *IN OVO* USING INTERFERON TYPE 1 AS AN ADJUVANT

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Abstract: Infectious Bronchitis Virus (IBV) is the etiologic agent of infectious bronchitis, an acute, highly contagious upper respiratory tract and urogenital disease of chickens. IBV replicates primarily in the respiratory tract, but also in the epithelial cells of the gut, kidney, and oviduct. The virus is distributed worldwide and is of considerable economic importance to the poultry industry, causing poor weight gain and feed-efficiency, and decline in egg quality and production. In addition, IBV is often a component of mixed infections that produce air-sacculitis and may result in condemnations of broilers. This disease is controlled by extensive vaccination programs using killed and attenuated vaccines.

In our laboratory, we have developed several different recombinant DNA vaccines expressing the IBV-S gene. We have used those vaccines *In Ovo*. They were expressed in the lymphoid organs of vaccinated embryos, but there was no full protection detected against an IBV challenge in four week old chickens. To improve the efficacy of our vaccine, we have used interferon type 1 as an adjuvant. This cytokine dramatically improved the resistance against the challenge in four week old chickens.

EFFICACY OF COMBINED INFECTIOUS BRONCHITIS/NEWCASTLE DISEASE VACCINES

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Viral interference is a phenomenon occurring in a simultaneous infection in which one virus can inhibit the infectious process of the other. This can be achieved through a variety of mechanisms such as rate of viral growth, interferon susceptibility, or direct biochemical interactions between the two strains. Multivalent vaccines combining Newcastle Disease Virus (NDV) and Infectious Bronchitis Virus (IBV) are commonly used in poultry production to alleviate costs and save time. However, there is evidence that IBV can actually hinder the growth of NDV (1,2,3,4), which creates concern about the ability of these vaccines to provide effective NDV protection. Live, multivalent vaccines containing one or more strains of both IBV and NDV are commonly used for vaccination in the poultry industry to save time and money. This project serves to examine the effects of viral interference on the ability of multivalent vaccines to provide effective immunity in chickens. In this trial, two combination vaccines were examined. Both vaccines used lentogenic NDV strain VG, with IBV Arkansas-type vaccine strain in one vaccine and IBV vaccine strains Massachusetts and Connecticut in the other. The efficacy of these vaccines was compared with monovalent vaccines of NDV-VG, IBV-Ark, and IBV-Mass using two-week-old broiler chicks. Three weeks post-vaccination, birds vaccinated with VG were challenged with velogenic, neurotropic NDV strain Texas GB. NDV protection was assessed by observing neurotropic signs over a two-week period following Texas GB challenge. Birds vaccinated with Ark were challenged with IBV-Ark DPI and birds vaccinated with Mass or Mass/Conn were challenged with Mass41. Viral interference was evaluated by monitoring post-vaccination viral growth via real-time PCR.

IBV growth appeared to be similar in all vaccine groups, and no apparent differences in IBV-antibody titers were found between the treatments. Protection from IBV challenges will be evaluated by inoculating specific pathogen free (SPF) eggs with tracheal swabs taken five days post-challenge and observing any embryonic lesions. Real-time PCR confirmed reduced viral growth for VG in birds vaccinated with a combined vaccine versus those vaccinated with VG alone. Birds receiving combined vaccines also showed lower NDV-antibody titers in the hemagglutination inhibition (HI) test than those vaccinated with only VG. However, following Texas GB challenge, no notable differences in the susceptibility of broilers vaccinated with the monovalent versus combination vaccines were observed.

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AVIAN INFECTIOUS BRONCHITIS VIRUS POSSESSES A REVERSIBLE SPIKE GLYCOPROTEIN THAT FUSES IN A LOW pH-DEPENDENT MANNER.

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Coronaviruses have been identified as the causative agent of respiratory disease in humans and animals, including recently reported severe acute respiratory syndrome (SARS). Although the replication and the assembly of this family of infectious agents have been well studied, virus entry into host cells has not been clearly defined. Although coronavirus fusion is generally considered to occur at neutral pH, but supporting studies rely on cell-cell fusion property that may not recapitulate the virus-cell fusion event occurred during viral entry. In addition, few reports have indicated that coronaviruses (i.e. TGEV, MHV-3, and SARS-CoV) enter in a low-pH dependent fashion. As a consequence, we investigated the entry of avian infectious bronchitis virus (IBV), a group 3 coronavirus. Our initial electron microscopic experiments identified viral particles in the endosomal compartments. We also found that IBV infection was sensitive to endosome acidification inhibitors such as, a lysosomotropic weak base (ammonium chloride), an ionophore (monensin), and a vacuolar proton-ATPase inhibitor (bafilomycin A1). Furthermore, Octadecylrhodamine labeled fluorescence dequenching assay (R18 FdQ) demonstrated that coronavirus-cell membrane fusion occurred in a low pH-dependent manner. The fusion activity was maximal at pH5.0 with a half maximum at pH5.5. However, in contrast to a typical class I low pH fusion protein like hemagglutinin of influenza A virus, coronaviruses were not inactivated by exposure to a pH5.0 buffer, suggesting that the trigger for coronavirus fusion is reversible. We conclude that IBV fuses in acidic endosomes using a reversible trigger instead of following a pH-neutral fusion at the cell surface.

CHARACTERIZATION OF INFECTIOUS BRONCHITIS ISOLATES DISCOVERED DURING THE 2004 AVIAN INFLUENZA OUTBREAK OF THE DELMARVA PENINSULA

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An outbreak of low path H7N2 avian influenza virus (AIV) was discovered on a small live bird market supply farm in Delaware in February of 2004. An extensive testing program was implemented throughout the Delmarva Peninsula in order to identify AIV positive broiler flocks and to prevent the spread of the virus to other farms. Testing focused on flocks that had high mortality, defined as 3 birds/1000/day or higher as well as flocks just prior to movement for slaughter. Respiratory disease was associated with the high mortality flocks. Although hundreds of high mortality flocks were tested, only the index and two additional cases of avian influenza were confirmed. Obviously there were other causes for the high mortality found throughout the Delmarva Peninsula. The large number of samples gathered during this outbreak presented an opportunity to see if other pathogens were involved. Virus isolation attempts of samples from high mortality flocks were performed in specific-pathogen-free (SPF) chicken embryos. Many of the embryos showed signs of stunting and curling, which are suggestive of infectious bronchitis virus (IBV) infection.

A total of 254 isolates from embryos with stunting or curling were tested for IBV by reverse transcriptase-polymerase chain reaction (RT-PCR) and 204 were positive. Initially, there was concern that a new variant strain of IBV had developed that was not controlled by current vaccines. However, sequencing of a portion of the S1 subunit of the spike gene revealed that all of the isolates had a high amino acid similarity (92.6% to 100%) to common vaccine strains; Arkansas, Connecticut, and Massachusetts.

CORRELATES OF IMMUNE PROTECTION IN GT5 VACCINATED CHICKENS AGAINST *MYCOPLASMA GALLISEPTICUM* INFECTION

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Mycoplasma gallisepticum infection in chickens is characterized by inflammatory responses in the trachea, air sacs, conjunctiva and other tissues. A live-attenuated GT5 vaccine has been shown to protect chickens against challenge with virulent *Mycoplasma gallisepticum* strain R_{low}. This study focuses on evaluating GT5 vaccine-mediated correlates of immune protection by examining the cellular infiltrates and numbers of mycoplasma-specific antibody secreting cells found within the trachea following R_{low} challenge. GT5 vaccinated chickens developed minimal tracheal lesions with few infiltrates of B cells, CD4+ and CD8+ cells when compared to sham vaccinated chickens, post-challenge. These lesions were characterized by few and scattered, discrete, subepithelial lymphocytic infiltrates reminiscent of mammalian secondary lymphoid follicles. These lymphofollicular structures consisted of distinct B cell central zones containing few IgG+ cells and peripheral CD4+/CD8+ T cell zones. The lymphofollicular aggregates were rarely observed in sham vaccinated chickens prior to day 12 post-challenge, but were incorporated within an increasingly more cellular inflammatory response that often progressed to severe inflammation. This was due to expansion of interfollicular zones by large numbers of infiltrating CD4+ and CD8+ T cells and a sizeable population of dispersed IgG- and IgA-secreting plasma cells. This response in sham vaccinated chickens appeared to be aberrant, in that mycoplasma persisted in the face of this vigorous immune response. GT5 vaccinated chickens also had higher *M. gallisepticum*-specific serum and mucosal IgG concentrations and significantly higher numbers of *M. gallisepticum*-specific IgG-and IgA-secreting plasma/B cells within the tracheas. These responses were observed as early as day 1 and 4 post-challenge. In addition, *M. gallisepticum* was not found to colonize the tracheas of GT5 vaccinated chickens. These results indicate the importance of antibody-mediated early clearance of the mycoplasma that is observed in GT5 vaccinated chickens.

PHYLOGENETIC ANALYSIS OF FOWL ADENOVIRUSES ISOLATED FROM CHICKENS WITH INCLUSION BODY HEPATITIS IN CANADA

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Fowl adenoviruses (FAdV) are ubiquitous in domesticated fowl and have a worldwide distribution. FAdVs have often been isolated from asymptomatic chickens, but several FAdV serotypes/strains have been associated with outbreaks inclusion body hepatitis in broilers (IBH). Typically, the disease affects 2-6 weeks old broilers. Outbreaks of IBH, causing various degrees of morbidity and mortality, have been occurring yearly in Southern Ontario. In the past two years similar problems have been often reported in western Canadian provinces (Manitoba, Saskatchewan, Alberta and British Columbia). We have characterized a total of one-hundred and thirty-seven fowl adenoviruses (FAdVs) isolated during outbreaks of IBH in Ontario and western Canadian provinces. The comparison was done by sequencing and phylogenetic analysis of the L1 loop of hexon protein amino acid (aa) sequences (1). Fifty-seven field isolates appeared to be highly related to FAdV-8a strains TR-59 (100%) and T-8 (98.9-99.5%), while 60 viruses showed highest percentages of identity to FAdV-11, strain 380 (96.2%). Twenty Canadian field isolates were 94.7-95.2% identical to FAdV-7, strain x11a.

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

AVIAN HEPATITIS E VIRUS IN AN OUTBREAK OF HEPATITIS SPLENOMEGALY SYNDROME AND FATTY LIVER HEMORRHAGE SYNDROME IN TWO FLAX-FED LAYER FLOCKS IN ONTARIO

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A commercial layer flock experienced high mortality averaging to 0.34% per week over a 20 week period, with a peak mortality of 0.9% in a single week. The initial diagnosis was hepatitis/splenomegaly syndrome (HSS). In the middle of the outbreak, a second flock on the same farm, 10 weeks younger also experienced high mortality and had similar clinical signs and lesions as in the first flock. Both flocks had been fed a regular layer ration and at 28 weeks of age the diet had been shifted to a ration fortified with 11% flax for the production of “designer” omega-3 enriched eggs. Reduced feed consumption, poor body weight gain and poor peak production were noticed prior to the appearance of lesions. 245 dead hens collected weekly from the 2 flocks were examined during the outbreak. Most birds had lesions confined to the liver and spleen. 43% were diagnosed with hepatitis splenomegaly syndrome (HSS) with lesions ranging from acute periportal lymphoplasmacytic cholangiohepatitis to chronic severe hepatocyte necrosis, hemorrhage, vasculitis and amyloidosis accompanied by acute to severe granulomatous splenitis and amyloidosis. 11% were diagnosed with fatty liver hemorrhage syndrome (FLHS), and 24% of the birds had combined lesions of HSS and FLHS. The diagnoses on the remaining birds examined were a mixture of other problems consistent with normal mortality in layer flocks. There were no bacterial or viral isolates from samples of the liver/bile and spleen, but 11 out of 21 bile samples of birds with HSS & HSS/FLHS lesions were positive to RT-PCR for Avian Hepatitis E Virus (AHEV). This is the first reporting of avian hepatitis virus in layer flocks in Canada. A retrospective epidemiological investigation of 24 previous flocks demonstrated a mortality pattern in flax fed flocks that was consistent with the flocks in this report but different from flocks fed regular layer rations.

This paper has been submitted for publication elsewhere and only an abstract is provided.

CASE REPORT: MYCOPLASMA IOWAE INFECTION IN COMMERCIAL TURKEYS

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A series of turkey cases were submitted to the Animal Diagnostic Laboratory at the Pennsylvania State University by a commercial turkey company over a three month period during 2004 and 2005. Most cases involved poults from 3 days to 3 weeks of age. The submitting complaints included increased “leg problems” beginning in the very early brooding period, and uneven growth and increased culls throughout the grow-out. Various treatments had been ineffective. The histories suggested that flocks composed of progeny from certain breeder flocks were more likely to be affected than others. Findings in some of the earlier cases were non-specific and inconsistent. As more cases were submitted, bilateral leg lesions were noted as common to several groups. Increased amounts of clear to variably turbid synovial fluid were more common in younger poults, and thickened epiphyses and metaphyses at the intertarsal joints and shortened long bones of the legs (lesions suggestive of chondrodystrophy) were evident in older poults. Other gross observations included stunted growth, dehydration, anorexia, and splenomegaly. Microscopic lesions included synovitis, perichondritis and chondritis. Impression smears of synovial fluid stained with modified Wright-Giemsa and Gram stains were negative for bacteria, and positive for mixed inflammatory cell infiltrates. Routine aerobic bacterial cultures and mycoplasmal cultures of affected joints consistently yielded no growth. Virus isolation attempts on synovium and tendon samples were negative for reovirus. Polymerase chain reaction (PCR) assays were performed on joint swabs, and a few samples were positive for *Mycoplasma sp.*, but negative for *Mycoplasma gallisepticum* (MG) and *Mycoplasma synoviae* (MS). Swabs and/ or PCR reaction products from these and additional cases were sent to the University of Georgia Poultry Diagnostic and Research Center for further work-up. *Mycoplasma iowae* (MI) was detected in one or more samples by PCR tests and/or culture in several of the cases. Pooled swabs from joints with gross lesions were the most likely samples to yield positive results. Serologic tests of affected flocks later in the grow-out were negative for antibodies to MG, MS and *Mycoplasma meleagridis* (MM). No nutritional deficiencies or imbalances were found. Paired house trials conducted on farms receiving poults from implicated breeder flocks comparing multi-vitamin supplementation versus enrofloxacin therapy showed no response to the former intervention, and positive response to the latter. We concluded that vertically transmitted MI infection was a major underlying problem in flocks represented in this series of cases.

CASE SERIES OF PROVENTRICULITIS, SPLENITIS / MARBLE SPLEEN SYNDROME IN BROILERS

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After the eradication of Low Pathogenic (LP) avian influenza (AI) H7N2 from Delmarva broilers in April, 2004, there was a renewed interest and agreement to submit clinical case to the Lasher Diagnostic Laboratory, U of DE. Besides multiple cases of respiratory disease and Gangrenous Dermatitis (GD), there were 11 cases of Inclusion Body Hepatitis (IBH) during the period of April 14 to Sept 30, 2004. At the same time a syndrome characterized by runting / stunting, pallor, depression and mortality was observed.

At necropsy the birds affected showed growth retardation, moderate, firm proventricular enlargement and marble or hypoplastic spleens, many with hypoplasia of the bursa of Fabricius and or thymus. The birds affected, in a few cases, also showed lesions consistent with IBH.

Of the 11 cases of IBH received, 5 of them also showed proventriculitis and 7 of them showed splenitis.

On the other hand, there were 33 cases observed where proventriculitis and splenitis were present but no IBH lesions were present. Most of the spleens showed moderate enlargement with an unusual "marbling" or hypoplasia and pale discoloration.

Histological evaluation showed a "viral origin proventriculitis" and severe loss of "T" lymphocyte areas as well as severe plasma blast infiltration.

There is no viral etiology determined in the proventriculitis/marble syndrome. However there was an isolation of a Hemorrhagic Enteritis Virus (HEV) in one of the cases.

METHODS FOLLOWED FOR COMPOSTING BROILER CHICKENS AND THEIR MANURE DURING AN OUTBREAK OF HIGHLY PATHOGENIC AVIAN INFLUENZA IN BRITISH COLUMBIA

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At the request of an Emergency Operations Center established to respond to an outbreak of highly pathogenic avian influenza virus in British Columbia, more than 200,000 broiler chickens on 4 farms were composted by the same method during a 2 week period in April, 2004. Composting operations commenced on the first farm 1 to 3 days after 65,000 market age broilers (39 days old) had been killed with CO₂. Barns were two story buildings and a garden tractor with a blade was used on the top floor to push birds and their litter through a hole in the floor. The birds and litter on the cement bottom floor were wetted with water and were pushed into windrows that were about 5 feet high and 12 feet wide at the base. The windrows were then covered with wood shavings. These windrows were left undisturbed for 4 to 6 days and were then moved with front-end loaders to an outside structure, prepared close to the barns. The structure consisted of two parallel rows of cement highway dividers (27 inches high) that were 250 feet long and 15 feet apart. A 6 inch layer of wood shavings was placed on the ground between the rows of dividers. Heavy black plastic was placed over the wood shaving and the surplus plastic was draped over the top of the dividers so as to contain all liquids within the structure. The tractors transporting the first stage compost from barns traveled between the rows of dividers and deposited their loads on the plastic lined floor. To avoid having tractors travel over the plastic, it was rolled out as required to lengthen the pile. Loads of compost and loads of wood shavings were added alternatively to the pile. In addition, 6 tons of pelleted broiler feed was added. To provide passive aeration, lengths of flexible perforated drainage pipe were laid over the compost mix, about 1.5 feet above the plastic floor. Ends of the pipes extended beyond the cement dividers. The completed pile was about 5 feet high and 250 feet long. The top of the pile was covered with vapor barrier fabric and this was covered with a one foot layer of wood shavings. The entire pile was then covered with black plastic. The ends of the aeration pipe extended through the plastic at the sides of the piles and air holes were also made at the top of the pile. The average temperature of piles in the barns just prior to their removal was about 40 C and after 1 to 9 days in the outside pile, it ranged from 39 to 45 C. Results were similar on all 4 farms. Based on available literature, the avian influenza virus should have been killed before the piles were removed from the barns. Odors from the compost piles were minimal. After about 40 days in the outdoor structure, the carcasses had degraded to the extent that the compost was considered suitable for disposal on land. The composting procedures provided biosecurity and proved to be efficient for disposal of poultry carcasses and other organic wastes in the face of an outbreak of highly pathogenic avian influenza virus.

DISEASES OF WATERFOWL AND THEIR CONTROL

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Important viral diseases of waterfowl are duck virus hepatitis (DVH), duck virus enteritis, avian influenza and parvovirus infection of Muscovy ducks and geese. Duck hepatitis virus type I (picornavirus) causes a highly contagious and fatal disease in young ducklings less than 3 weeks of age. The disease causes as high as 95% mortality. All ducks except Muscovies are susceptible. Duck virus hepatitis caused by type 2 (astrovirus) has only been reported from ducks in England, while type 3, also a picornavirus, has been isolated from ducks on some duck farms in the United States. Since DVH type 1 occurs primarily in young ducklings, vaccination of breeder ducks provides adequate protection in ducklings through maternal immunity. Duck virus enteritis also called duck plague is a highly contagious disease affecting anseriformes (ducks, geese and swans). It is caused by a herpesvirus and occurs worldwide except Australia. Muscovy ducks are highly susceptible. The disease is characterized by eruptive lesions in the gastrointestinal tract, tissue hemorrhages and atrophy of lymphoid organs. The disease is prevented by vaccination with a live attenuated virus vaccine.

Parvoviral infection of geese and Muscovy ducks is a serious disease of young goslings and Muscovy ducklings characterized by muscular weakness, serofibrinous pericarditis, perihepatitis and accumulation of large amounts of ascitic fluid in the abdominal cavity. Recently, some differences have been observed between goose and Muscovy duck isolates. The disease is transmitted by direct or indirect contact. The virus can also be transmitted through the egg. Vaccination of breeding flocks as well as goslings or ducklings is used for prevention. Avian influenza viruses have been frequently isolated from domestic and wild waterfowl but have not been a major cause of disease until recently in Asia; serious outbreaks with high mortality have been reported in ducks and geese due to antigenic type H5N1. Other viruses such as reovirus, adenovirus, circovirus, reticuloendothelial virus, rotavirus, poxvirus and Newcastle disease virus have been reported from waterfowl but their significance is not well known.

Bacterial diseases of economic importance are *Riemerella anatipestifer* infection, colibacillosis, salmonellosis and avian cholera. *R. anatipestifer* causes a septicemic disease in ducks and geese. It is characterized by incoordination, tremors of head, neck and legs, ataxia and death. Mortality can be as high as 75%. Gross lesions include fibrinous pericarditis, perihepatitis, airsacculitis, meningitis and salpingitis. At least 20 different serotypes have been reported; heterologous serotypes do not cross-protect. Treatment with antibiotics and sulfa drugs is effective if started early in an outbreak. Inactivated and live vaccines are used for prevention and control. *Escherichia coli* causes colisepticemia and omphalitis in ducks and geese. Different serotypes have been isolated from ducks; the predominant serotype is O:78. Salmonellosis has been reported in birds under 2 weeks of age. *Salmonella typhimurium* and *S. enteritidis* are the common serotypes involved. Fowl cholera caused by *Pasteurella multocida* can produce high mortality in ducks and geese. Other bacterial infections are staphylococcosis, streptococcosis, erysipelas and chlamydiosis.

Aspergillosis, coccidiosis and necrotic enteritis are not uncommon in anseriformes. Noninfectious diseases of ducks include ascitis, amyloidosis and aflatoxicosis.

DIFFICULTIES OF DECONTAMINATING SALMONELLA ENTERITIDIS CONTAMINATED POULTRY FACILITIES

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The Food and Drug Administration is proposing to enact a "Rule on the Prevention of *Salmonella enteritidis* (SE) in Shell Eggs during Production". This rule would contain required procedures for sanitation of layer houses contaminated with SE. It is a standard veterinary recommendation to clean and disinfect poultry layer houses between flocks. In an ideal situation a proper cleaning and disinfection should achieve a 10^4 or >99% reduction of the number of salmonella bacteria (1) in a hatchery. Most evaluations of cleaning and disinfection (C & D) procedures of commercial layer houses are qualitative tests that are based on the number of samples that test positive or negative for SE, irrespective whether there are 10^5 or 10 viable SE bacteria present per sample. Numerous reports have been published that report that C & D procedures did not consistently result in complete decontamination (2, 3, 4, 5) and incomplete cleaning is one of the reasons for the failure. Not all disinfectants are equally effective against SE (6). Other factors that undermine the effectiveness of C & D include presence of fluff, rodents, beetles (2, 3, 4, 5, 7, 8). While some researchers found persistent salmonella contamination on surfaces of buildings and equipment (4), others stated that no salmonella were detected on visibly clean and disinfected surfaces (7). Steam cleaning, 60°C, 100 per cent relative humidity and 30 PPM formaldehyde were effective in eliminating SE (9). The results of a survey conducted in several regions of the US also indicated a lower risk associated of SE contamination if poultry houses were cleaned and disinfected between flocks (10).

In practice C & D procedures are not routinely followed in all egg layer operations or do not meet the intended goal. The complex structure of the equipment and sheer size of large modern poultry layer facilities, cost of C&D and the associated down time, damage done to the equipment, labor safety and environmental regulations or lack of expertise in the application impede on the effectiveness of C & D. It has also been suggested that improper cleaning prior to disinfection can result in bacterial bloom and higher bacterial count post disinfection. In a previously reported study (11) in which we recorded the number of positive samples before and after each C & D step we found that with the exception of dry cleaning each washing and disinfection step resulted in a reduction of the number of positive samples provided a 2 week drying period was allowed after each step. Only the formaldehyde fumigation procedure, followed by a two-week drying period resulted in an apparent elimination of SE.

We report here the results of another decontamination study of an experimental poultry facility that had housed SE infected birds.

Materials and Methods

Two poultry isolation facilities that had each housed 40 laying hens infected with a SE page type 4 culture had to be terminally decontaminated at the end of an USDA approved challenge experiment. A mean count of 10^4 colony forming SE bacteria was present in one gram of cecal content in the birds in both isolators at depopulation. Both units were dry-cleaned within a week of depopulation. One unit (A) was washed down and thoroughly cleaned with a detergent, and disinfected with 3000 PPM sodium hypochlorite solution. Unit B was left dry-cleaned. Six weeks after depopulation both units were fumigated with formaldehyde. Unit B was washed and cleaned with detergent and disinfected again 10 weeks after depopulation and because of inconsistent test results both units were repeatedly disinfected with 6000 PPM sodium hypochlorite between 15 and 41 weeks following depopulation.

Results and Discussion

Wet cleaning resulted in visually such clean surfaces, which practically are not attainable in commercial poultry houses. The number of positive SE samples was not reduced following primary disinfection with 3000 PPM sodium hypochlorite. Formaldehyde fumigation appeared initially to reduce the number of positive SE samples in the wet and dry-cleaned units but subsequent tests revealed again an increase in positive tests in both units. Subsequent repeated disinfection with 6000 PPM sodium hypochlorite resulted in apparent complete

disinfection in Unit A (initially wet cleaned) at 41 weeks. The initially only dry cleaned unit B was still SE contaminated 44 weeks after depopulation.

British researchers reported that SE persisted in broiler and layer breeder houses for a year after depopulation, primarily in fluff and up to 26 weeks in contaminated poultry feed (8). Studies in Pennsylvania compared the effectiveness of dry and wet cleaning prior to disinfection of SE contaminated houses and found no significant differences. A similar study in Maine produced inconclusive results because of the low isolation rate of SE from wet-cleaned and disinfected or dry cleaned previously SE contaminated houses. All flocks in this study had been vaccinated with SE bacterin and no SE was isolated from the eggs from any of the flocks in that study (Opitz, unpublished).

Our study in a small experimental poultry facility confirmed that complete decontamination of large commercial layer houses would be an unrealistic goal. Rodents play an important role as reservoir and amplifier of SE contamination in poultry houses (12) and are considered the most important risk factor for the next flock in the house (2). Other risk factors as well can undo the results of the best C & D (5, 7). There is apparent consensus that removal of manure, feed, dust, fluff and other organic, potentially SE contaminated matter that can be picked up by birds either by ingestion or inhalation needs to be removed from poultry houses. Thorough dry-cleaning of some facilities may suffice to reduce the risk of SE infection hens or eggs provided other sanitary measures, especially rodent control, are given the same attention. Additionally vaccinations against SE are a prudent measure to reduce the risk of egg infection.

From a veterinary point of view, a proper wet cleaning and disinfection procedure is still the preferred method of sanitizing a poultry house because it is directed against a broad spectrum of other potential disease risks. However, it will be difficult to determine the effectiveness and enforce a specific federally mandated sanitation procedure because of the multitude of variables involved. Local authorities should determine the most effective sanitation method. A good dry-cleaning may be better in some situations than wet cleaning and disinfection. On the other hand, the effectiveness of pest control, and specifically rodent control, and vaccinations against SE can be determined. These methods need to be part of any program aimed at preventing contamination of table eggs during production on every premise with history of SE contamination.

Acknowledgement

We would like to thank Brenda Kennedy-Wade for her assistance with the cleaning and disinfection of the poultry isolation units and Emily Giles for her help in the laboratory testing the environmental samples.

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ALTERNATIVE CONTROL FOR AVIAN INFLUENZA

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Abstract

A new model for controlling avian influenza, 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 poultry disease outbreaks. The advantages of the proposed program are that it utilizes the industry infrastructure, utilizes regulatory power, requires no unethical destruction of healthy birds, requires no costly disposal, is cost effective and puts more of the control costs on the producers with infected birds.

Introduction

Stamping out (designation of infected zones, imposition of quarantines, slaughter and destruction of infected and susceptible animals and intensive monitoring) is a well-recognized but unproven strategy for emergency livestock diseases.

The word “quarantine” dates from 1423. The city of Venice, Italy, confined sailors aboard ships at anchor for 40 days to safeguard the city from plague. The word derives from “quaresma” for 40

According to Dr Erhard Kaleta, “...clubbing of farmed animals originated during severe outbreaks of a disease which is now termed rinderpest in the year 1711 in Italy. It was the pope Clement 11th who – after deliberations with some of his cardinals and medical advisors – decided to eradicate rinderpest in all cattle by clubbing and deep burying. Very soon responsible authorities in other European countries and later also in the United States of America followed this divine advice (2).”

In the late 19th century funds were provided to the Bureau of Animal Industry, U.S.D.A., to pay indemnity for the quarantine and slaughter of cattle affected with bovine pleuropneumonia and the disease was successfully eradicated. In the first three decades of the 20th century, using this familiar approach foot and mouth disease and fowl plague were eradicated six times and twice respectively (4).

Even though the public finds it distasteful, there is support in the veterinary community for stamping out when applied to emergency (O.I.E. List A) diseases. This support is strong but not universal. Recently (Oct. 1, 01 to Sept 30, 02) eggs or poultry were destroyed as a result of low pathogenic avian influenza (LPAI) infections in 10 states. Support for this approach may exist for small outbreaks, but what happens if hundreds of flocks are involved? It is often said that stamping out is the most cost effective strategy; however, recent stamping out programs involving poultry disease all eclipsed the \$100 million mark in their total costs (Virginia, California, Italy, Netherlands and British Columbia). Further, destruction efforts have sometimes contributed to the spread of AI.

It is questionable whether the modern poultry industry can tolerate this expensive, unproven, draconian and dramatic method of disease control much longer. The question of whether an alternative strategy would have been more effective has not been asked. In the absence of research trials to document the advantage of this archaic approach, regulatory officials should examine and document instances where emergency diseases were satisfactorily brought under control with a different approach. Low pathogenic avian influenza outbreaks have been effectively controlled by vaccination and controlled marketing as well as by stamping out, but for substantially less money. It was recently pointed out that stamping out programs for low path AI may cost 10 to 100 times more than controlled marketing (1).

Certainly most veterinarians are in agreement that eliminating susceptible animals will contribute to eradication of a pathogen, but elimination of susceptible animals is not equivalent to destruction. Times have changed since the pope embarked on a rinderpest eradication campaign:

- Microbiology was discovered,
- Our arsenal of disease control tools has expanded,
- Agriculture has evolved from a scattered poultry population disseminated throughout rural areas to dense populations on industrial farms in various rural areas,
- The consuming public has become increasingly suspicious of dramatic televised scenes of destruction and disposal of animals and
- Questions are being raised about the ethics of killing and disposing of healthy animals.

A proposal

Because industry-driven controlled marketing programs as well as government-driven stamping out programs have been successful, a thoughtful examination of stamping out programs leads to the idea that their success is related, not to the destruction of infected, susceptible and convalescent poultry, but to the enforced downtime, designation of infected zones, imposition of quarantines, and intensive monitoring. There is nothing special about killing and burying or burning poultry because disease outbreaks have been stopped by alternative means. Thus we can infer that it is the government's authority to quarantine, order cleaning and disinfecting, monitor and permit repopulation that accounts for its success in controlling disease. These strengths in government programs match up well with the major weakness of industry programs.

The modern poultry industry is driven by the companies' needs for meat and eggs. The weakness of industry-driven disease control is that this need for a continuous supply of meat and eggs may cause companies to act in ways that do not contribute to disease control and may actually contribute to disease spread.

A new hybrid disease control program is proposed that encompasses the best that industry and government programs have to offer. Industry and regulatory veterinarians, in a cooperative arrangement, could initiate well-thought out measures when a disease outbreak occurs. For example:

- **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. The group can immediately do an epidemiological assessment.
- **Processing and scheduling.** The cooperative group should initiate a program of processing all healthy meat birds of marketable age in the area. Placement schedules should be interrupted. No placement of chicks or poults should be allowed and downtime should be extended for infected premises.
- **Vaccination.** Just as the Italians gave us quarantine and slaughter, now it is the Italians that have conclusively demonstrated the effectiveness of controlled vaccination for avian influenza (4).The cooperative 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 may be held under quarantine. It is imperative, however, that vaccine is available for emergency use.
- **Area repopulation.** After no new infected flocks are detected for an agreed upon period of time, 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 non-infected counterparts. These costs would include the costs of mortality, medication, condemnation, lost production, and vaccination. People with non-infected flocks might experience the costs of rescheduling and vaccination. The cost of

rescheduling is far less than the cost of destruction, and governments could redirect their financial support to aid farmers while their barns are empty.

Conclusion

It is no longer necessary to consider exposed, diseased or convalescent poultry as “evil.” In the scientific age, we now recognize that disease control programs with totally different approaches can have the same outcome. Combining the best features of existing programs has the potential to improve the existing disease control strategies and to reduce the objections that have been raised about them.

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END – THE CALIFORNIA EXPERIENCE SEPTEMBER 25, 2002 TO SEPTEMBER 16, 2003

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An outbreak of Exotic Newcastle Disease (END) began in Southern California in the urban Los Angeles area on September 25, 2002. The infected chickens were game fowl used for cock fighting. While cock fighting is not legal in California, ownership of game fowl for display and breeding is allowed. Throughout the period of this outbreak both game fowl flocks and small hobby flocks were referred to as “backyard flocks”.

California had a previous experience with END in 1971 to 1973. At that time over 116,000 square kilometers were under quarantine. Almost 12 million chickens were destroyed. The US government’s cost of eradication was over \$56 million and took over 3700 persons and three years. It took the poultry industry many more years to fully recover.

In the current experience, the index case was brought to the California Animal Health and Food Safety Laboratory in San Bernardino at the recommendation of a local veterinarian. About 10 of 200 birds had died. Clinical signs included mucoid discharge from the mouth, difficulty breathing and swollen sinuses. Necropsy was performed and additional lesions were observed including gastrointestinal and pharyngeal hemorrhages. END was immediately suspected. A confirmed diagnosis was made and reported on October 1, 2002. The California Department of Food and Agriculture began an investigation and eradication efforts began immediately. It is important to note that a non poultry veterinarian recognized this disease as out of the ordinary and sent it to the laboratory for identification.

Dealing with the owners of the game fowl flocks proved to be very difficult. Ethnic differences were not well understood by the officials sent to deal with the disease. Many of the owners did not speak English. Defining the population at risk was not easy. Many birds were moved by owners in the course of routine activities as well as to escape possible depopulation after the eradication effort began. A state quarantine was put in place over parts of the Southern California counties on November 13, 2002 and a federal quarantine on November 21.

The commercial poultry industry, predominantly involved in commercial egg production, began self monitoring by reporting general bird health, egg production and mortality on a daily basis. Eventually a program of END monitoring was established. For those production companies with private veterinary supervision, swab kits were sent weekly and returned to the Laboratory by overnight mail. A system of dead bird pick up by state and federal authorities was established for those commercial producers without private veterinary supervision. Initially, monitoring for virus was by virus isolation but soon a PCR test was developed, validated and became the standard test.

An industry advisory committee was established. It met weekly with state and government officials and private poultry veterinarians. These weekly meetings were very valuable in distributing information as well as gaining mutual respect and trust for those in industry and regulatory positions.

Infections were confined to backyard premises until the first commercial outbreak on December 21, 2002. Twenty two commercial premises out of over 100 were depopulated in the outbreak. This totaled over 3.2 million birds out of a total population of nearly 12 million in Southern California.

In all, over 18,000 total premises were quarantined. More than 2400 backyard and 22 commercial flocks were depopulated. The peak number of people involved in the eradication efforts from the United States Department of Agriculture and the California Department of Food and Agriculture was over 1700. The expenditure on eradication was nearly \$200 million. The last commercial flock affected was found on March 26, 2003. The last backyard affected flock was found on May 31, 2003. State and federal quarantines were released September 16, 2003.

An epidemiological investigation was conducted. The initial commercial flock infected was believed to be from employee association with game fowl flocks. Thirty percent of affected commercial flocks had workers who admitted to an association with game fowl. Forty one percent of affected flocks had egg processing and marketing links. Dirty egg flats and shipping racks may have been involved. The industry quickly responded with egg processing plant biosecurity measures that included the use of one way disposable egg flats and not returning pallets to the laying facilities.

Forty seven percent of affected commercial flocks were in close proximity to affected backyard flocks with active exotic Newcastle cases. Forty one percent of affected flocks were associated with infection in other company owned facilities. Stringent biosecurity measures were instituted. They included washing and disinfecting all vehicles and equipment entering facilities and providing company owned clothing and shoes for employees. Change rooms or areas were provided for storing clean clothes and for cleanly changing.

A continuing END surveillance program was initiated. This program is voluntary and consists of several options. Flock owners may report on overall health, egg production and mortality, they can do serological monitoring for Newcastle disease or they can do swabbing of dead birds for PCR testing. It was planned that all will do some swabbing of dead several times during the surveillance period which was expected to last at least one year. The program continues on a voluntary basis. Newcastle as well as Avian Influenza testing is done at the California Animal Health and Food Safety Laboratory at no charge to the owner

Education programs for backyard flock owners have been started. Issues of biosecurity and bird health are covered. Backyard bird owner organizations are participating in these educational projects. Improved cultural and ethnic understanding will help relations with regulatory officials. It is believed that the initial introduction of END in this outbreak was from game fowl brought illegally from Mexico.

IBV VACCINATION. CAN WE KEEP UP WITH VARIANTS?

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Infectious bronchitis (IB) is among the most common and challenging of all poultry diseases to control. IB is highly contagious and results significant economic losses in commercial broiler, layer and breeder chickens. The causative coronavirus, infectious bronchitis virus (IBV), frequently causes respiratory disease in young chickens and egg production losses in hens. In addition, some strains of the virus exhibit a renal (kidney) tropism and produce up to 30% mortality in affected flocks. Nephropathogenic IB caused a serious outbreak in the state of Pennsylvania from 1997-2000 (16).

IBV, a member of the coronavirus family, is the causative agent of IB. The virus targets the trachea and other respiratory tissues. Damage to defense mechanisms of the respiratory system which are responsible for trapping and clearing inhaled bacteria often predisposes chickens to secondary bacterial infections. *E. coli* and other bacteria may colonize the respiratory tract, produce toxic products causing airsacculitis, perihepatitis, and pericarditis. Chickens inoculated with IBV and *E. coli* were found to have more severe and persistent respiratory lesions than those inoculated with IBV alone (10). Other factors such as ammonia, temperature fluctuations, and social stress (pecking) contribute to the susceptibility of layer type chickens to bacterial infections (14). In addition, immunosuppressive infections with IBDV in combination with IBV reduced macrophage activity vs. *E. coli* (10) and increased IBV persistence (13,15)

IBV is perhaps best known for its existence as numerous antigenic types or serotypes. Although antigenic variation of the virus has been recognized for years, it is only since the mid-late 1990s that the scientific community has had the capability to truly appreciate the magnitude of the genetic diversity of the virus.

IBV is uniquely suited to undergo mutation during its replication cycle. Replication of the virus' RNA genome is error-prone resulting in mutations. The major target for mutation is the gene encoding the spike (S) envelope protein that the virus uses to attach to the host cell. Mutations in S result in antigenic changes and the emergence of variant strains as well as subtypes of recognized serotypes. The S protein gene is able to tolerate numerous mutations without compromising the virus' ability to replicate and cause disease.

New mutant IBV strains are subject to immunological selection so that only the most antigenically novel variants persist in poultry populations. A new variant that is not antigenically novel, e.g. one similar to a vaccine strain used on the farm, will not persist in the flock because the vaccine-induced immunity will eliminate it from the population. Conversely, newly mutated variants that are antigenically distinct from vaccine strains will, in essence, have a far greater potential to escape vaccine-induced immunity, persist in flocks, and have the potential to cause disease.

The primary sources of new IBV variants are commercial layer flocks. IBV variants arise most frequently in multiple age layer operations. These farms provide all the necessary conditions favoring the emergence of new variants. Layer flocks of different ages frequently numbering in excess of a million birds, are housed in close proximity. Periodic introduction of new pullets, and the continual re-infection and recycling of IBV in the layers, results in a greater opportunity for infection and spread than occurs on farms using a single age, "all in-all out" management system. Novel variants build up in layer complexes over time since the premises are rarely, if ever, cleaned and disinfected. Importantly, vaccine induced immunological mechanisms provide a selective pressure for the most antigenically novel variants as new variants arise on a frequent basis.

Our laboratory has identified many new IBV variants isolated from commercial poultry farms. One of the more interesting variants is PA/1220/98, originally isolated from 20-week-old commercial layer pullets with respiratory noise. The birds had been vaccinated at 3, 5, and 12-weeks of age with Massachusetts (Mass) + Connecticut (Conn), Mass + Conn, and Holland, respectively. First recognized in Pennsylvania in 1998, isolates highly related to PA/1220/98 have been recovered in California, Iowa, and Ontario, Canada.

PA/1220/98 is genetically highly unique and unrelated to live vaccines Massachusetts, Connecticut, Arkansas, and DE/072/92. All of the isolates have come from layer or layer pullet flocks, many of which were located on large multiage farm complexes. In layers, egg production was decreased and accompanied by shell quality problems. The recognition of a unique widespread genotype unrelated to any other known IBV provides an opportunity to speculate how avian pathogens might be spread across long distances.

Another important and common source of pathogenic IBV is live attenuated vaccines that have undergone reversion to virulence under field conditions (12). This problem has been observed during the production of broiler chickens. Attenuated vaccines contain subpopulations that differ in their virulence (level of attenuation) and even slightly in their antigenic characteristics (subtype). Live IBV vaccines are considered to be unstable due to the presence of the different subpopulations and their ability to change (shift) depending on the host (embryonated egg vs. chicken) in which they are grown. For example, back-passage of embryonated egg-derived vaccine in the chicken results in shifting of the viral subpopulations in favor of a predominant virulent subpopulation over the attenuated subpopulation found in the original vaccine. Similarly, a slight shift or change in the antigenic characteristics of the vaccine may also occur through back-passage. Over time, and in the absence of farm clean out and disinfection, the back-passaged vaccine becomes established as a significant IBV field challenge causing disease losses. Indeed, laboratory studies have shown that vaccines may revert to partial or full virulence within only 3-6 back-passages in chickens (6). Practices associated with enhancing vaccine back-passage should be discontinued. These include over-diluting (cutting) IBV attenuated vaccines, using sprayers that apply wide-ranging and large particle sizes, and using a vaccine strain(s) only on an inconsistent or seasonal (winter) basis. Pathogenic IBV vaccine-derived field isolates are now thought to be the primary cause of many of the Arkansas vaccine problems in broilers on Delmarva in the middle 1990s (12). The back-passage of vaccine should be considered as a source of infection in situations where the incidence of isolation of a given serotype of IBV is high in regions where a vaccine serotype has been used with little or no apparent reduction in disease incidence.

DIAGNOSIS OF VARIANTS

The first step in controlling a suspect IB problem is confirming that the problem is in fact IB. Diagnosis is essential. Affected flocks generally present with a clinical history such as ongoing egg production and/or shell quality problems. The presence of respiratory disease signs may be inconsistent. However, signs alone are not sufficient for a diagnosis as other diseases as well as dietary and management issues may contribute to or cause production losses. Laboratory tests are needed to confirm an IB diagnosis. ELISA serology is inexpensive and specifically identifies IBV antibodies produced in response to infection. An ongoing ELISA serology program in flocks can provide a basis for analysis of samples to determine if IBV antibody levels are rising in response to infection. The challenge is to be able to differentiate between IBV vaccination responses, if in fact the flock is receiving live or killed vaccinations just prior to or during lay. In flocks given killed IB at 10-12 weeks, the age most layers are vaccinated as pullets, antibody titers fall to undetectable levels by 35-40 weeks of age. Moreover, live vaccines administered during production generally do not result in high ELISA IBV antibody responses. In contrast, ELISA IBV antibody responses of layers to virulent field strain infection are generally much greater than responses to live IBV vaccine strains, and in effect boost titers to higher levels.

In our experience, the use of hemagglutination-inhibition or virus-neutralization tests on sera from commercial layers to identify a causative IBV infection does not yield reliable results. Antibodies produced by layers in response to many IBV vaccinations and field exposures over their life become broadly cross-reactive and non-specific and are thus not indicative of the strains responsible for recent disease episodes (3).

Virus isolation and identification is critical for diagnostic purposes. Virus isolation may be accomplished through sentinel studies using IBV-vaccinated or susceptible chickens (5) or directly from the affected commercial layers. We prefer samples of trachea or lung rather than cecal tonsil specimens because the latter often are persistently infected with IBV vaccine strains as well as other viruses and complicate isolation. Identification of IBV is much faster and easier today with the application of reverse transcription polymerase chain reaction (RT-PCR) assays for the virus (7,8,9). Samples of isolated IBV may be shipped to laboratories for RT-PCR and results may be obtained as rapidly as several weeks. For testing in the USA, IBV specimens originating from other countries must be inactivated prior to shipment and accompanied by a government-issued import permit.

CONTROL OF IB THROUGH VACCINATION

As mentioned earlier, IB can be very difficult to control. Only a very limited number of vaccines are available given the tremendous number of recognized strains of the virus. However, effective control strategies can be developed to reduce the impact of the disease.

Layers. Effective IB control programs begin with well-conceived primary and follow-up booster vaccinations using live attenuated strains. Application of attenuated vaccines to the respiratory tract (eyedrop and aerosol) is recommended for primary immunization. Strains of lower virulence should be used for primary and the first booster vaccinations with more virulent strains for the subsequent boosters. Spray is preferred to drinking water for booster vaccination. Fine particle sprays should be reserved for the third and fourth boosters. A satisfactory immune response to killed IBV vaccination requires successful live IBV priming. In addition to application route, proper vaccine strain selection is vital. Layers will likely experience field challenges with a variety of IBV antigenic strains so a successful control program must provide the flock with maximum cross-protection against the diverse field strains. The best way to achieve a high degree of cross-protection is to immunize with different IBV strains. In the case of live vaccines, only approved IBV attenuated strains may be applied in a given country or state within the country. If possible, select vaccine strains that are different for each live IBV vaccination. For example, if approved, select a different serotype or strain for follow-up boosters than the one used for prior vaccinations. The use of the same IBV vaccine for all primary and booster vaccinations is not recommended because the immune response will not have sufficient antigen diversification to provide a high degree of cross-protection needed in the field.

Best results for controlling IB are achieved by using a vaccine strain that is identical or highly similar to the causative field strain(s). On multiage layer flock complexes, variants are frequent causes of production problems and these strains are often very different than any of the commercially available live or killed vaccine strains. Some vaccine manufacturers may produce killed autogenous IBV vaccines, that when given at 10-12 weeks of age, reduce the impact of variant IB losses, particularly in layers from onset of production to 35 weeks of age. Inactivated vaccines may provide protection of layers as they transition from the pullet farm to large multiage flock complexes. Another control strategy has been to initiate a live IBV booster program in hens during production. Vaccinations, using approved commercially available strains, are given, generally by spray, at regular intervals, every 8-10 weeks or as needed to maintain immunity in flocks. Flocks may benefit even though the strains used for vaccination are different than a variant strain because of generalized protection afforded by regular booster vaccination. Again, diversification of the vaccine strains is the key to achieving cross-protection. Potential production losses must be considered whenever using live attenuated vaccines so initiating a live booster program in an ongoing multiple age flock complex may be risky. Single age layer farms do not generally have the high incidence of variants as do multiple-age flock complexes and often do not require a live booster vaccination program in the layer house.

Broiler Breeders. Broiler breeders in the USA are raised on single-aged farms, so the strategies used to control IB are quite different than in commercial layers. For example, drinking water vaccination of broiler breeder pullets is more common than spray. Inactivated vaccines are not consistently given to pullets. When used however, inactivated vaccines are given at about 18 weeks of age, 6-8 weeks later than in commercial layers.

Broilers. Broiler chickens are routinely vaccinated in the USA. Only live vaccines are used, however, because inactivated vaccines are expensive and are not particularly effective for broilers. Live vaccines are commonly combined with Newcastle disease virus (NDV) vaccines such as B1 or LaSota, and are given via coarse spray in the hatchery and the field between the ages of 14 and 18 days. Live vaccines are available for four serotypes of IBV; Mass, Conn, Ark and Delaware 072. The vaccine serotypes are generally given as bivalent (e.g. Mass + Conn or Mass + Ark) combinations to broilers. Application of the vaccines should be done in a way to minimize back-passage and reversion to virulence as discussed above. Selection of appropriate vaccine serotypes is essential to achieve the best protection against endemic field strains of IBV. Some vaccine combinations (e.g. Mass + Ark) give better cross-protection against variants than others (e.g. Mass + Conn) (4).

Of course, vaccination programs are only effective when combined with other efforts such as farm biosecurity, ongoing clean-out and disinfection programs, and control of immunosuppression. More information on IB and IBV is available (1,2).

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CURRENT PRACTICES TO CONTROL INFECTIOUS LARYNGOTRACHEITIS IN THE U.S.

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Infectious laryngotracheitis (ILT) is an upper respiratory disease of chickens causing tracheitis associated with respiratory distress, variable degrees of morbidity, and varying levels of excess mortality. The causative agent is an alphaherpesvirus. Lesions found will vary in severity from a mild, mucoid tracheitis to caseous tracheitis to very severe, necro-hemorrhagic tracheitis. Diagnoses are obtained for the most part by gross examination. Confirmatory tests are histopathology (intranuclear inclusion bodies found in epithelial syncytia in both trachea and lung tissues), virus isolation (chorioallantoic membrane inoculation of nine to eleven-day-old embryonating eggs), direct fluorescent antibody, and PCR.

The commercial egg and meat-type breeder industry and the commercial broiler industry vary in the way ILT is controlled. The long-lived bird industry relies on both vaccination plus biosecurity while the broiler industry relies on biosecurity alone. The broiler industry does not utilize routine vaccination for commercial broilers as high-quality-water vaccination is required and the vaccine reaction is often sufficiently severe to be economically undesirable. Outbreaks in broiler growing areas are usually traced to exposure to materials from long-lived flocks (egg layers or breeders) that are carrying vaccine virus (4).

Biosecurity measures recommended and used to varying degrees to prevent introduction of ILT virus onto farms include 1) proper dead bird disposal procedures, 2) properly cleaned and disinfected equipment for transport of birds, 3) clean clothing, footwear, and headgear for personnel involved in bird transport, 4) footwear and hands sanitized prior to entry of each poultry house, 5) clean coveralls, boots, and headgear required for all visitors, 6) assurance that necessary visitors (flock supervisors, veterinarians, etc.) have not visited flocks likely carrying high levels of ILT virus prior to arrival at the farm, 7) preclusion of visitors such as feed truck drivers, gas delivery people, meter readers, etc. near or into entryways of houses, 8) locked entry doors at all times to prevent unwanted entry of visitors, 9) installation of perimeter fencing with locked gates at the farm entry, and 10) assurance that employees do not have exposure to other poultry. This list is a partial list and includes the major areas of concern.

Should outbreaks of ILT occur in a broiler producing area, the companies involved normally would use an industry driven respiratory disease reporting system in which the breaks are tracked and communicated to the industry. Using this information, decisions would be made whether or not to vaccinate. The most common method of vaccinating commercial broiler flocks is to use the water route using a high-tittered chick-embryo-origin (CEO) vaccine at 12 to 15 days of age (2). Flocks in close proximity to the outbreak that are under 4 weeks of age would be vaccinated at that time but flocks over 4 weeks of age would not. Increased biosecurity measures would be used for flocks over 4 weeks of age and early marketing is often practiced. Vaccination of broilers older than 28 days may result in increasingly severe reactions with poor weight gains and feed conversions. Vaccination of broilers in a high-risk area at 12 days of age would continue until reports of outbreaks have subsided.

During an outbreak situation in broilers, measures taken to avoid ILT from spreading from an infected broiler premise during movement to the slaughter plant include the following: 1) slaughter birds during the last shift at the end of the week to allow maximum downtime before resuming slaughter, 2) select transport routes that minimize possible contamination of flocks along routes to the plant, and 3) thoroughly clean and disinfect the equipment used for pickup and transport of birds after use.

Following bird removal, steps taken to minimize spread of ILT virus from materials in the house are 1) quarantine the farm to avoid moving any contaminated materials prior to decontamination, 2) heat the house to 100°F for 72 hours to inactivate the ILT virus, 3) remove litter and place in a covered pile for several weeks to

allow die-off of the virus, 4) wash house with water and detergent, 5) wet all surfaces with a disinfectant effective against ILT virus, and 6) allow the house to set idle for 3 weeks following disinfectant application before placement of the next flock.

Long-lived birds such as commercial layers, egg-type breeders, and meat-type breeders rely on vaccination for preventing ILT in most cases. In some areas of the country in egg-type chickens, breaks of vaccine-related viruses are seen either due to an increased virulence of the virus or poor quality administration of vaccines. There are some areas of the country in which long-lived birds are not vaccinated and reliance on biosecurity measures alone is used for prevention.

Three types of vaccines are available for use: 1) chick-embryo-origin, 2) tissue-culture-origin, and 3) recombinant pox-vectored vaccines.

Chick-embryo-origin (CEO) vaccine is best applied by the eyedrop method but also can be mass applied by spray or water routes. The use of CEO vaccine comes with some risk of reversion to virulence upon several passages through birds. Poor vaccination technique leads to recycling of the vaccine virus among the flock members and has resulted in numerous outbreaks of a relatively high pathogenic virus when passed to a susceptible flock.

Tissue-culture-origin (TCO) vaccine must be applied by the eyedrop method and spread following vaccination is nearly non-detectable. The use of TCO vaccine has not led to known outbreaks of ILT. The relative protection from the use of TCO vaccine compared to CEO vaccine has been shown to be similar in laboratory tests (1). TCO vaccine is not used widely in the egg industry due to its requirement for eyedrop application.

A recombinant pox-vectored ILT vaccine has recently come onto the market for long-lived birds and is applied by the wing-web method as pox normally is applied. The relative level and longevity of protection obtained from use of the recombinant has not yet been thoroughly investigated. Success in using the recombinant pox-vectored vaccine requires very high quality administration technique plus no exposure to pox prior to vaccination. Some reports of vaccine failure have been reported, but have been traced back to either poor administration of vaccine or pox exposure prior to vaccination.

The following are typical vaccination programs used for different levels of risk:

Low risk

- 1) No vaccination, only biosecurity
- 2) One application of TCO vaccine by eyedrop, 10 weeks of age
- 3) One application of pox-vectored recombinant, 8 to 10 weeks

Moderate risk

- 1) One application of CEO vaccine at 8 to 10 weeks by eyedrop
- 2) One application of pox-vectored recombinant at 8 to 10 weeks + CEO vaccine by spray or water at 15 weeks

High risk

- 1) Two applications of CEO vaccine at 6 to 8 weeks by eyedrop + at 13 to 14 weeks by eyedrop
- 2) Two applications of CEO vaccine at 6 to 8 weeks by eyedrop + at 13 to 15 weeks by spray or water
- 3) Two applications of CEO vaccine at 6 to 8 weeks by spray or water + at 13 to 15 weeks by spray or water
- 4) One application of recombinant pox-vectored vaccine at 6 to 8 weeks + CEO vaccine at 13 to 15 weeks by spray or water

Outbreaks of ILT in long-lived flocks have been successfully controlled by vaccination in the face of the outbreak as the spread of ILT is relatively slow. All birds in a biounit would need to receive vaccine or the CEO vaccine used would likely spread to other flocks and proceed to become more pathogenic. Two full doses of CEO vaccine are applied by the water route. One dose is given early in the day directly followed by the

second dose to assure full coverage of the flock members (3). The spray method has also had some success in the face of an outbreak, again applying 2 full doses of vaccine with an interval of 2 to 3 hours between applications.

Various schemes to eradicate ILT have been put forth in the past using various classifications of states based on the type of vaccine used. The effort would require import restrictions to prevent movement of birds that could possibly carry CEO ILT vaccine virus. In these plans, eventual freedom from ILT for a state would be achieved when no vaccine is used and no imports of possibly infected birds are allowed.

In summary, ILT breaks in the US are limited to spread of CEO vaccine strains to non-vaccinated broiler chickens or within long-lived, vaccinated flocks that are not effectively protected against exposure to CEO vaccine strain viruses.

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CURRENT USDA, APHIS, POLICY ON VACCINATION AS A TOOL IN THE ERADICATION OF AVIAN INFLUENZA (AI)

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USDA, APHIS, VS, Mission

The USDA, APHIS, VS, protects and improves the health, quality, and marketability of our nation's animals and animal products, and veterinary biologics by: preventing, controlling, and/or eliminating animal disease, and monitoring and promoting animal health and productivity, and licensing veterinary biological products intended for use in the treatment or diagnosis of diseases in animals.

Background

Eradication and biosecurity are the first line of defense against all AI viruses. Preventing the introduction of AI by eliminating all contact between commercial poultry and wild birds, swine farms, and live bird markets is a common and successful practice. However, occasionally AI is introduced into the commercial poultry population.

Under conditions of high poultry density or multiple poultry establishments in one area, eradication and biosecurity alone are not likely to be successful control strategies (4). A successful strategy requires reducing the susceptibility and density of the poultry population. The components of a control strategy can vary but generally include five categories: (1) biosecurity (including quarantine); (2) diagnostic and surveillance; (3) elimination of infected poultry; (4) decreasing host susceptibility to the pathogen (for example, through vaccination); and (5) education of personnel in the animal production chain and allied industries to better understand how diseases are transmitted so personnel with responsibility to prevent transmission or spread can be incorporated into action plans (2).

For many years, APHIS policy regarding the control of AI and the related production and distribution of AI vaccines remained unchanged. Then, in 1995, restriction on the production and use of H7 subtype vaccines was added to the restriction that existed since 1983 on the use of H5 subtype vaccines. At the same time, APHIS concluded that the restrictions imposed on AI vaccine production and use during the 1983 high pathogenicity avian influenza (HPAI) eradication campaign should be modified to allow H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI.

As we approach the eradication of specific animal diseases in the United States, it will be necessary to refine regulations, policies, and procedures pertaining to veterinary biological product availability for these diseases. In addition, eradication may require establishment of a vaccine bank.

Current APHIS policy

This presentation will review the current USDA, APHIS, policy on vaccination as a tool in the eradication of AI and the development of the AI vaccine bank.

The current APHIS policy, as described in VS Memorandum No. 565.12, allows "H5 and H7 vaccines to be used as a tool for combating any potential outbreak of HPAI in the United States (5)." AI vaccines may be prepared from any serotype, including H5 and H7, and may be recommended for use in chickens or turkeys

subject to the requirements and restrictions specified in VS Memorandum No. 800.85 (5). VS Memorandum No. 800.85 allows H5 and H7 vaccines to only be used under the supervision or control of USDA, APHIS, VS, as part of an official USDA animal disease control program. The USDA, APHIS, VS, Center for Veterinary Biologics (CVB), implements the provisions of the Virus-Serum-Toxin Act to ensure that veterinary biologics available for the diagnosis, prevention, and treatment of animal disease are pure, safe, potent, and effective.

APHIS supports the general concept of vaccination as a tool in the eradication of notifiable AI. However, vaccination should be available as part of a science-based influenza control strategy that includes: (1) enhanced biosecurity; (2) an eradication plan; (3) controlled vaccination for flocks deemed to be at risk; (4) suitable monitoring of all flocks at risk and of all vaccinated flocks; and (5) a repopulation plan (1, 2, 3). The management of AI must continue to be based on sound scientific principles. However, innovative strategies will be required to eliminate these persistent and adaptive viruses.

Research and development are urgently needed to obtain a better understanding of risk factors and implement more effective control measures. A need exists for significant sustained financial investment in national and regional infrastructures to ensure that most states work in a coordinated and harmonized manner to implement required surveillance and control measures. It is in the interest of USDA, APHIS, VS, and states to make these investments to protect animal health.

Acknowledgements

The authors thank Mr. Andy Rhorer, Dr. David Swayne, and Dr. Bruce Carter, and Dr. Martin Smeltzer for their excellent technical assistance.

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VIROLOGICAL STUDIES ON A CASE OF SARCOMAS IN WHITE LEGHORN CHICKENS

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SUMMARY. An outbreak of subcutaneous sarcomas in commercial white Leghorn egg layers was observed in the North Eastern United States. Subcutaneous tumors were confined to three flocks distributed in two locations and belonging to the same company. The tumors were first observed grossly at approximately 7 weeks of age and persisted throughout the economic life of the flocks. Most of the tumors observed during the growing period were present on the facial region or around the head, wings and legs. There was no gross evidence of bursal or visceral involvement. Microscopically, most tumors were undifferentiated sarcomas and myxomas. There was no gross or microscopic evidence of Marek's disease or lymphoid leukosis. Reticuloendotheliosis virus (REV) proviral DNA was not detected by PCR either in tumors or in cell cultures. Avian leukosis viruses (ALV) were isolated from tumors, plasma and serum. Upon virus neutralization, the viruses appeared at least partially related antigenically to ALV subgroups A and B, but not to subgroup J. Sequencing of the variable and hypervariable regions of gp85 in the envelope gene revealed that the viruses involved are closely related to avian myeloblastosis virus type 1 (AMV-1). The affected flocks seroconverted to avian leukosis virus. In vivo studies are under way to attempt to reproduce the sarcomas in commercial egg layers.

HISTOLOGIC ASPECTS OF NEWLY OBSERVED SARCOMAS IN WHITE LEGHORNS

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In the summer of 2004, three flocks of commercial white Leghorn egg laying chickens became affected by an outbreak of subcutaneous sarcomas. These subcutaneous lesions were first observed in birds as young as 7 weeks of age. These tumors ranged from semi-firm to very soft, and many had a gelatinous consistency when incised. The most common anatomic location for these neoplasms was on the head in a periocular location, typically associated with either the upper or lower palpebra. However, similar tumors were also seen in the subcutaneous tissues of the wings and legs. These tumors persisted for the life of the birds and most of the periocular masses obstructed vision of the affected side. Therefore, many were ulcerated and scabbed at the time of euthanasia.

None of the birds that were necropsied had visible evidence of either Marek's disease or lymphoid leukosis. All birds examined had mild to moderate splenomegaly, which was histologically confirmed to be due to increased numbers of germinal centers and ellipsoids. In all birds, the cloacal bursae were determined to be of normal size relative to the age of the birds and histologically, they were undergoing what was believed to be physiologic regression.

All tumors that were examined histologically were composed of a mesenchymal population of neoplastic cells that ranged from spindle-shaped to stellate to multinucleated to megalocytic cells with abundant cytoplasm. Whilst regions of some tumors were sparsely cellular with an abundance of extracellular matrix, other portions of tumors were more densely cellular and reduced amount of matrix. Mitotically active cells were rarely seen in the examined neoplasms and there was little nuclear or cytoplasmic pleomorphism in the tumor cells. Interstitial matrix varied from very loose, pale blue, myxomatous matrix to amorphous, eosinophilic, more fibrous matrix. Scattered through some of the sections were areas of necrosis admixed with heterophilic infiltration.

Morphologic diagnoses ranged from myxoma/myxosarcoma to fibroma/fibrosarcoma, depending on the cellular morphology and matrix produced by the neoplastic cells. Determination of malignancy was more difficult. Whilst these were believed to be viral induced tumors, that does not exclude the possibility that these are benign neoplasms. The absence of mitotic figures, lack of local invasion, and generally minimal cellular pleomorphism indicate that these tumors were likely benign neoplasms.

THE EFFECT OF FASTING AND NON-FASTING MOLTING PROGRAMS ON *SALMONELLA ENTERICA* SEROVAR ENTERITIDIS TRANSMISSION AND ISOLATION IN LAYING HENS

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Forced molting appears to increase the susceptibility of hens to *Salmonella enterica* serovar Enteritidis (SE) infection and shedding. Fasting as part of a molting program has been shown to exacerbate SE lesion formation; however, the use of non-fasting molting regimes employing reduced crude protein and metabolizable energy diets appears to decrease the severity of lesions. The reasons for this are not fully clear but it is believed that changes in gut microenvironment as well as general physiological stress and immunosuppression associated with prolonged fasting may play major roles. The purpose of this study was to determine whether 4 different molting programs currently employed by the industry differ in their effect on horizontal transmission, colonization, and shedding of SE.

In each of 2 trials, 64 pre-molt hens (32 Bovan, 32 Hyline) were randomly assigned to 16 Horsfall-Bauer isolators, 4 birds per unit, 8 birds per breed. On day 0, 1 bird from each isolator was randomly selected and orally inoculated with a field isolate of SE (1x10⁹, 1x10⁸ CFU / ml, trial 1, 2). These "shedders" were held for 24 hrs in separate isolators without feed and then returned to their original locations. Un-inoculated contact birds were started on 1 of 4 randomly assigned molting programs (A,B,C,D), 4 isolators per program. The molting programs were as follows: A - 4 days of fasting followed by 14% CP & 1260kCal ME/lb for 31days; B - 10 day of fasting followed by 11.75% CP & 995kCal ME/lb for 18 days and then 14% CP & 1260 kCal ME/lb for the remaining 7 days; C- 9.5%CP & 650 kCal ME/lb for 28days and then 14% CP & 1260 kCal ME/lb for the remaining 7 days; D - 9.5% CP & 650 kCal ME/lb for 14 days, 11.75% CP & 955 kCal ME/lb for 7 days and then 14%CP & 1260 kCal ME/lb for the remaining 14 days. Colorectal swabs and plasma were obtained from all birds on day 0 prior to inoculation and at 1,2,3,4,5, and 6 weeks post-inoculation (PI). Colorectal swabs were enriched for 24 hrs in TT-Hajna broth (37°C) then plated on XLD4 medium (37°C). Birds were considered positive based on the appearance of distinctive black colonies. Plasma was tested by ELISA for SE antibodies (S/N ≥ 0.75 = negative, .074-0.60 = suspect, ≤ 0.59 = positive).

Among inoculated "shedders" 85%, 100%, 50%, and 85.5% were culture positive within the first 2 weeks PI for programs A, B, C, and D respectively. Among all birds, significant effects on the rate of SE isolation were observed due to both trial and molting program. In trail, birds on programs A and B were more likely to culture positive compared to C and D. There appeared to be no breed differences. Disregarding the trial effect, an examination of cumulative % SE culture positives over time for trials 1 and 2 combined demonstrated that the non-fasting programs (C and D) resulted in fewer SE isolations. It also appeared that most horizontal transmission took place within the first 2 weeks and that birds on the fasting programs (A and B) were not only colonized at a higher rate but shed SE longer than those on non-fasting programs. There did not appear to be any significant difference in S/N ratio over time between trials, breeds or among molting programs. However, if S/N ratios were classified as negative, suspect or positive and the % of each plotted over time in a non-cumulative manner, a trend reflective of the culture data emerged. Interestingly, it appeared that not all birds culturing positive necessarily seroconverted.

These findings are consistent with those of other researchers and suggest that in terms of the incidence of SE subsequent to molt; non-fasting programs are superior to those that employ fasting. For the sake of context it is worth noting that a parallel study revealed the cost of egg production (¢ / egg) to be only slightly higher for full-fed programs (A=3.46, B=3.41, C=3.49, D=3.60).

77th NECAD Business Meeting

MINUTES OF THE 76TH NECAD BUSINESS MEETING

June 11, 2004
State College, PA

Prior to the start of the formal business meeting, the awards for Best Graduate Student Presentation were announced. Co-winners were:

Ms Sharonda Meade, paper entitled: The Effects of Social Stress on Immunological and Physiological Parameters in Poults.

Mr. Maung Myint, paper entitled: Prevalence and Risk Factors for *Salmonella* Contamination of Retail Poultry Meat in Maryland.

Dr. Patti Dunn called the meeting to order at 11:30am. The minutes of the 75th NECAD business meeting were unanimously approved without discussion (Henzler moved, Lucio seconded).

76th NECAD Budget Report

Dr. Dunn indicated that \$3,300 was received from Dr. Opitz following the 75th NECAD. There were 18 sponsors including AAAP for the 76th NECAD. Approximately \$3,000 will be transferred to Cornell for the 77th NECAD.

Committee Reports

Dr. Dohms provided a summary of the *Mycoplasma* Committee Report (p.44). A survey of states in the Eastern US indicated minimal *Mycoplasma* activity with only a few positive (MG) flocks being reported. There was no discussion. Dr. Dohms indicated that he would be willing to continue as chair. The report was unanimously approved (Bennington moved, Tablante seconded).

A verbal summary of the Avian Influenza Committee Report was not provided, but the report was unanimously approved as entered in the proceedings (Calnek moved, Lucio seconded).

There was no *Salmonella* Committee Report. The current chair, Dr. Miguel, was not present at the meeting. There was some discussion as to retaining the committee. It was moved that the work of the committee should continue and that a new chair be identified by the next meeting (Henzler moved, Dohms seconded, unanimously approved).

Old Business

The "Small Flock Resolution" tabled at last years meeting was discussed. Dr. Tablante indicated that Delaware had formulated guidelines and legislation requiring registration with the state of individuals raising live poultry (commercial and small flocks). Similar legislation was being proposed in Maryland. Dr. Kradel indicated that the USDA premise ID program was moving forward and that it may also include small flocks in 3-4 years. It was felt that the USDA program would likely address the issues raised in the "Small Flock Resolution". A committee was formed (Pendleton, Opitz, Pierson, and Dunn as chair; Mallinson and Clauer nominated in absentia) to pursue the matter further.

New Business

The 77th NECAD will be held at Cornell University with Dr. Lucio as committee chair. Dr. Lucio commended Dr. Dunn and the organizing committee of the 76th NECAD for having done an excellent job. Pending discussion with his colleagues, Dr. Bennington suggested that the 78th NECAD could be held at the University of Guelph.

It was proposed that North Carolina State University be invited to join NECAD. There was concern that this might infringe on SCAD. Dr. Pierson said that he would further investigate the matter. It was so moved (Calnek), seconded (Henzler), and unanimously approved.

Regarding graduate student participation, Dr. Dohms suggested that the evaluation forms for the graduate student competition be posted on the 77th NECAD website so that students could see the criteria on which they would be judged. Dr. Tablante indicated concern that the number of graduate students attending NECAD was down and encouraged faculty to promote the meeting among their students. Dr. Calnek supported this and emphasized that NECAD was an excellent venue for student presentations. Dr. Key suggested that funds might be solicited from sponsors to increase the monetary awards for competing students. Dr. Lucio suggested that funds transferred from the previous meeting might be used to support student travel. Dr. Kahn felt that the transferred funds provided a necessary buffer to cover potential cost over-runs for the meeting. Dr. Gelb indicated that travel expenses were an issue but that the decline in attendance may be more of a reflection of the number of students actually doing research in the field. Dr. Tablante felt that the meeting did not receive enough publicity and suggested that the AAAP, ACPV email lists be used to disseminate the meeting announcement as well as website for organizations like USDA and the Association for State Veterinarians. Ag Experiment Stations, Deans, Departments Heads, were also mentioned as a means of dissemination. Dr. Tablante suggested that solicitations for sponsorship begin immediately after the meeting because there was substantial competition with other meetings. Dr. Dunn indicated that some companies said that (December) 6 months before the meeting was too early for them to commit to sponsorship. There were no motions following this discussion.

The Organizing Committee for the 77th NECAD will include: Dr. Lucio, Chair; Dr. Dunn, Past Chair; Dr. Bennington, Chair Elect; Dr. Pierson, Secretary; and additional faculty and staff from Cornell University.

A motion to adjourn the meeting was made (Gelb), seconded (Fabricant), and unanimously approved. The meeting adjourned at 12:10pm.

AVIAN INFLUENZA SUBCOMMITTEE REPORT

Northeast Conference on Avian Diseases
June 17, 2005

Committee members: Mariano Salem (DE), Donald Hoenig (ME), Benjamin Lucio-Martinez (NY), Mazhar Kahn (CT), William Pierson (VA), Eric Gingrich (PA), Susan Trock (Chair, NY)

No highly pathogenic avian influenza was reported from any state in the Northeast during calendar year 2004.

Connecticut

Connecticut is continuing surveillance for avian influenza including testing of back yard flocks under the State program. The large egg production farm which was positive for a low pathogenic H₇N₂ avian influenza virus in 2003 has now tested negative and continues with a voluntary surveillance program. Vaccination for avian influenza at this facility has been discontinued with no evidence of recurrence. The quarantine has been listed for over six months.

Monitoring at the commercial flocks vaccinated with H7 subtype vaccine:

<u>Location</u>	<u>Virus isolation</u>	<u>Directogen</u>	<u>RRT-PCR</u>	<u>HI-sentinels</u>
Farm 1	0/33*	0/133	0/189	0/1180
Farm 2	0/59	0/166	0/220	0/680
Farm 3 (pullet farm)	0/12	0/89	0/134	0/430
Farm 4	0/32	0/146	0/142	no data

* = number positive/number tested

AI Surveillance Program Summary:

<u>State</u>	<u>AGID</u>	<u>Number of Tests</u>		
		<u>Directogen</u>	<u>Binax</u>	
Connecticut	1,274	1,316	278	
Rhode Island	190	nd	nd	
Massachusetts	4,511	nd	nd	
Total		6,395	1,316	278

All samples were negative for evidence of avian influenza.

Delaware/Maryland/Virginia (2 counties)

Beginning in February 2004, Delmarva suffered an outbreak of low pathogenic H₇N₂ avian influenza. Only three farms were found infected in the whole peninsula. The infected birds were humanely euthanized and composted in house. The first case was diagnosed in Feb 5th. This was a non-commercial farm growing "red and white" broilers for the live market in New York and New Jersey. The second case (Flock 2) was found on Feb 9th approximately one mile outside the five-mile quarantine area around the index flock. On March 5, 2004, a second commercial farm (Flock 3) was found infected in Maryland approximately 55 miles away from Flock 2 infected farm. This last flock was identified after testing hundreds of farms as part of enhanced surveillance. No flocks in Virginia were positive.

The Directogen Flu-A and RRT-PCR tests were used for the diagnostics and monitoring of tracheal swabs in cases submitted for surveillance and suspect flocks. The AGID test was also used on infected farms.

During the outbreak/surveillance period the following test were conducted:

<u>Laboratory</u>	<u>No. Farms</u>	<u>No. Houses</u>	<u>No. RRT-PCR Tests</u>
Lasher Lab	1,101	2,671	5,342
NVSL	1,276	3,193	6,386
Totals	2,377	5,864	11,728

An important part of the control efforts involved testing all flocks on the Delmarva prior to the birds moving to the processing plant. Surveillance for avian influenza has continued after the outbreak including clinical cases with mortality >3 birds /1000 that are three weeks of age or older. Also, starting in April, 2005 an active surveillance program has initiated. The surveillance program includes RRT-PCR testing for flocks prior to slaughter at the rate of one flock per processing plant per day.

Number of tests after April 14, 2004(end of AI outbreak) to May 23, 2005:

<u>Test</u>	<u>Number Clinical Cases (Respiratory signs)</u>	<u>Number Active surveillance</u>	<u>Number Positive</u>
Directogen	574	0	0
RRT-PCR	746	269	0

No positive samples have been found since March 5th, 2004.

Maine

Maine is now sampling 30 birds per flock for avian influenza surveillance purposes. There have been no positive findings in the state over the past year. Mr. Bill Morrison has been hired to oversee the avian influenza program as well as the Salmonella Enteritidis Risk Reduction Program for the state.

New York

Beginning January 2003 and continuing through the current time, New York State implemented a program of voluntary quarterly depopulation, cleaning and disinfection of all live bird markets.

Since January 2004 we tested 88 markets in the New York City area. Forty-nine (49) of these markets were negative for avian influenza on all testing. Of the 39 markets that tested positive for low pathogenic H₇N₂ avian influenza, 20 of them were positive only one time all year. Eleven (11) markets tested positive twice. Only eight (8) markets tested positive more than twice last year.

In the summer 2004, New York enacted a change in the regulation governing birds moving into the live bird marketing system. A new requirement was that those hauling birds directly to the end stage live bird markets must utilize an all-season working, mechanical crate washer and an all-season vehicle wash facility. This was initially accepted on an emergency basis and in early 2005 was accepted as a permanent change of regulation.

We have also tested wholesaler/poultry transporter facilities and delivery vehicles interdicted at markets. Since January 1, 2005, we have results from 33 of these samplings. Only one, on January 3, 2005, was positive for a low pathogenic H₇N₂ avian influenza virus.

During 2004, a total of 258 New York State-grown flocks tested for antibodies to avian influenza before movement to the live-bird markets and all were found negative.

Virginia

The Virginia Department of Agriculture performed 30,469 AGID tests between April 1, 2004 and April 1, 2005. Since pooled samples are run on each flock, this number roughly represents 7,619 flocks. All flocks moving to slaughter within the state are being tested prior to movement. For permitting purposes, testing of all flocks moving into the state for slaughter is also required (most of these flocks originate from North Carolina). No positive samples or flocks were found.

Pennsylvania (Dr. Eric Gingerich).

Pennsylvania has not had any breaks of either H5 or H7 avian influenza (AI) in the last year (May 2004 – May 2005). The Pennsylvania multi-faceted AI surveillance program in place detected only two H4N2 isolates from a waterfowl flock in follow-up diagnostic work after showing positive on antibody testing from Pennsylvania's active surveillance program.

Active surveillance is conducted in several ways. One way is through a voluntary sampling program for both egg and meat type flocks. Approximately 88% of the egg layer flocks are monitored by submission of 12 to 30 eggs each month for yolk antibody testing (Agar Gel Immunodiffusion Test). Most all turkey and broiler flocks are sampled at slaughter, 10 to 20 sera per flock.

The Pennsylvania Department of Agriculture conducts routine surveillance of the 10 largest poultry auction markets by obtaining samples from a subset of each of the lots brought in to an auction on one day. Tracheal or oropharyngeal swabs are obtained from gallinaceous birds while cloacal swabs are obtained from waterfowl. PDA also routinely monitors three live bird markets in Philadelphia taking both environmental and bird samples. No positive samples have been detected during the past year.

Another large aspect of active monitoring is for shipment of birds to the live bird market system. Samples are taken either 10 days prior to shipment or on a routine schedule if the farm is on the AI Monitored Program.

Passive surveillance, submission of diagnostic specimens to the a diagnostic laboratory from flocks that are showing signs of AI, is conducted daily from examination of necropsy cases not only by the 3 Pennsylvania Diagnostic Laboratory System laboratories but also by day-to-day examination of flocks for signs of AI by flock supervisors, flock owners, and allied industry technical services persons.

Approximately 210,000 tests for antibody and 3950 virus isolation tests for AI will be performed in fiscal year 2004/2005 (July 2004 to June 2005) by the Pennsylvania Animal Disease Laboratory System (PADLS) for the above mentioned programs.

Thanks go to Dr. Nan Hanshaw-Roberts and Dr. David Henzler of the Pennsylvania Department of Agriculture and Kim Sprout at the University of Pennsylvania for help in compiling this information.

AVIAN MYCOPLASMA SUBCOMMITTEE REPORT

Northeast Conference on Avian Diseases
June 17, 2005

After consulting with avian mycoplasma researchers in the eastern U.S., it appears that there is little *Mycoplasma gallisepticum* activity in commercial poultry operations in our region. However *M. gallisepticum* live vaccines are commonly found in egg layer operations. There were several *Mycoplasma synoviae* (MS) outbreaks. One integrator in Arkansas broke with MS in week-old breeder pullets that spread from the initial premise to other flocks. In August 2004, the University of Delaware Lasher Lab in Georgetown determined that they were infected with MS based on ELISA tests.

At the Newark campus, 150 swabs were received from Lasher Lab or from the company Veterinarian. Swabs were pooled (3/pool) cultured in Frey Broth and plated on PPLO agar. Colonies were very atypical in appearance. PCR results from 7 pools were MS positive using the Lauerman primer set.

Cultures were also inoculated into 10 day old SPF chickens using the intranasal and air sac routes of inoculation. However, MS ELISA testing was negative after inoculation. The MS plate agglutination was also negative. It appears that this isolate is highly attenuated.

The company Veterinarian treated infected flocks with tetracycline every other week which appeared to eliminate the MS infections in his operations.

The new *Mycoplasma gallisepticum* fowl pox vaccine produce by Bioimmune, Inc. appears to provide some good protection in field trials. Dr. Kleven reviewed the data and thought the results were promising. The advantage of the vectored vaccine is that no live *Mycoplasma gallisepticum* organisms enter the flocks.

Report submitted by J. E. Dohms

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The proceedings will also be posted at the following address after June 30, 2005

<http://diaglab.vet.cornell.edu/avian/necad.asp>

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