SAES-422 Multistate Research Activity Termination Report (2004-2008)

Accomplishments

Objective 1. Identify reservoirs of infectious respiratory disease agents in wild birds and poultry.

The first comprehensive biological characterization of H5N1 high pathogenicity avian influenza (HPAI) virus from wild birds was completed on viruses that came from Mongolia. H5N1 HPAI virus has caused outbreaks of disease in poultry and wild birds of 50 countries in Asia, Europe and Africa. With field assistance of Wildlife Conservation Society, Food and Agricultural Organization and Government of Mongolia, H5N1 HPAI viruses were isolated from a dead Whooper Swan in Mongolia. In experimental studies, this virus expressed high lethality in chickens and young domestic ducks, and was easily passed between ducks by causal contact. The virus grew in many internal tissues including the brain and heart. Because the outbreak occurred where no poultry exist, this indicates the H5N1 HPAI virus spread into Mongolia by migrating wild birds, but is a virus that can infect poultry and cause severe disease. (SEPRL) (2006)

Avian influenza viruses of many different antigenic subtypes (H1-H16) are found commonly in wild birds, but only the H5 and H7 subtypes are known to have the potential for being highly pathogenic in poultry. The H5N1 subtype is of particular importance because of the widespread outbreaks of highly pathogenic avian influenza in Europe, Asia, and Africa, and extensive surveillance of wild birds was conducted in the Americas to evaluate the chance of these highly pathogenic viruses entering the U.S. through wild birds. Several low pathogenic H5N1 viruses were isolated in wild birds in collaboration with APHIS and USGS, and these viruses were sequenced and shown to be of North American lineage that are separate from the highly pathogenic H5N1 viruses found in Europe, Asia, and Africa. The biologic and sequence characterization of these viruses continue to provide evidence that highly pathogenic H5N1 viruses have not traveled to the Americas in wild birds, and clearly documents that low pathogenic H5N1 viruses are normally found at a low prevalence level in the Americas. This study also included experimental animal studies in collaboration with The Ohio State University that showed that these viruses did not replicate well in poultry and pose only a small threat of introduction to our poultry populations. (SEPRL) (2008)

Only a few avian influenza viruses (AIV) have been transmitted from wild bird reservoirs to poultry resulting in outbreaks, and predicting which AIV will cause an outbreak has not been possible. An infectivity model was developed which predicts transmissibility based on the intranasal bird mean infectious dose (BID50) test for chickens, turkeys, domestic ducks and geese, and Japanese quail. The quantity of avian influenza virus (AIV) needed to produce an

infection in poultry is dependent upon both the bird species and virus strain, and most AIV that caused outbreaks had BID50 of 3log10 or less of virus, while low infectivity was observed for viruses with 4.7log10 or greater BID50. Chickens were not easily infected with most wild bird AIV, but domestic ducks and geese, Japanese quail and turkeys were easily infected and they could serve as key bridging species for waterfowl-origin AIV crossing into domestic poultry. Furthermore, these data suggest mixing of poultry species during rearing and using outdoor production systems is a major risk factor for transmission of AIVs from wild birds to domestic poultry. (SEPRL) (2008)

A variety of wild bird species have been sporadically infected with and have died from infections with H5N1 high pathogenicity avian influenza viruses (HPAIV), but which species might be involved as urban and rural reservoirs and transmission hosts are unknown. In cooperative studies with University of Georgia, Southeastern Cooperative Wildlife Disease Study and Southeast Poultry Research Laboratory, infectivity and pathogenicity of three H5N1 HPAI viruses were determined for a variety of wild bird species. Swans, geese, gulls and house sparrows were highly susceptible to H5N1 HPAI virus and are good sentinels for detecting H5N1 HPAIV within an area since mortality in infected birds is high, while dabbling ducks (Genus Anas) were resistant to clinical disease and excreted low levels of virus. Pigeons were resistant to H5N1 HPAIV, and swans and geese were asymptomatic shedders for up to 5 days with a potential for short to intermediate transmission. These studies indicate that only some wild birds species could be involved with transmission of H5N1 HPAI viruses while others would be good sentinels for detection in wild bird populations. (SEPRL) (2008)

Monitoring was conducted for avian influenza viruses in poultry and wild birds from samples from the U.S. and around the world. Avian influenza viruses are present in various wild birds and poultry throughout the world. Southeast Poultry Research Laboratory worked with several laboratories to monitor and study avian influenza viruses. No viruses were identified by molecular tests or were isolated from wild birds in Tunisia and Canada Geese in the USA. Some avian influenza (AI) viruses and Newcastle disease viruses (NDV) were obtained from samples from Iraq, Yemen and Nigeria. The Iraqi viruses included both virulent and non-virulent NDV, but antibodies to low pathogenic AI H9N2 viruses were detected. The Nigerian viruses were H5N1 high pathogenicity AI viruses and virulent Newcastle disease viruses. A virulent Newcastle disease virus was isolated from the Yemeni samples. These studies emphasize the need to continue to monitor poultry and wild birds worldwide for AI virus and NDV. (SEPRL) (2006)

Avian influenza virus (AIV) surveillance in poultry (commercial and backyard) and wild birds is ongoing at University of Delaware's Lasher Laboratory and Allen Laboratory, respectively. No AIV activity using USDA NAHLN approved agent detection (real time RT-PCR and antigen capture on oropharyngeal swabs) or antibody detection assays was observed in over 15,000 active (pre-slaughter) or passive (clinical disease cases) surveillance samples in commercial broilers. One backyard duck flock was found to be positive by real time RT PCR. Wild bird surveillance was initiated at the Allen Lab in Newark in October 2006 in cooperation with the Delaware

Department of Natural Resources & Environmental Control. Testing has yielded many real time RT-PCR positive cloacal samples. Only one H5 sample was identified but was determined by NVSL to be a non-Nl neuraminidase subtype. Virus isolation attempts on these samples are now being performed. Our ongoing collaboration with Dr. Richard Slemons (Ohio State University) and his research group yielded several AIV isolates from waterfowl and shorebirds from the Delmarva Peninsula region. The isolates will be characterized in poultry in laboratory trials. (Delaware) (2006)

Low path avian influenza virus isolates representing H5, H7, H6, and H3 subtypes recovered from wild waterfowl and shorebirds on the Delmarva peninsula were not pathogenic for two-week-old meat type turkeys and broiler chickens based on clinical signs and microscopic lesions. However, the viruses were recovered from the trachea or cloaca. (Delaware) (2007)

Cloacal swabs were collected from hunter killed or trap-nested wild ducks in the South Eastern USA. Three tested positive by Directigen antigen capture (AC) enzyme linked immunosorbent (ELISA). Five real time RT-PCR matrix-positive samples were negative for Newcastle Disease Virus (NDV) by the H inhibition test (HI) test. Five samples were submitted to National Veterinary Service Laboratory (NVSL) for subtyping. All were H1N1. The % identity of the H1 genes from the 5 isolates ranged from 85 to 98. Knowledge gained from these findings will be used for developing a more effective intervention program for AIVs. (Alabama) (2007)

Cloacal swab samples were obtained from various species of hunter-killed waterfowl from the southeastern (SE) United States (USA). Samples were processed by embryonated egg inoculation, hemagglutination, AC-ELISA, and real-time RT-PCR tests. Out of 704 swab samples tested, twelve were positive for AIV. Sequence analysis of the hemagglutinin gene of the isolates revealed that they are closely related (98%) to recent isolates (2003-2004) from Delaware and Canada, but only 90% related to an H10N7 isolated 30 years ago. Four isolates had 94-97% similarity to published H1N1 isolates including one from swine. Isolate Sp1 was highly pathogenic in embryos, produced a high hemagglutination titer and was positive for both AIV and Newcastle disease virus (NDV). Two AIV isolates also contained type 4 paramyxo viruses. Since none of the isolates were H5 or H7, it is unlikely that an AIV isolate, from wild ducks in the SE USA, could cause disease in commercial poultry. (Alabama) (2008)

Surveillance of 200 samples from non-migrating and migrating wild water fowl for AIVs from Alabama, Georgia and Florida were conducted in 2006 using both net caught ducks and hunter killed ducks. Samples were from non-migrating wood ducks, and migrating hooded mergansers, blue-winged teal, gadwall, and ring-necked ducks. Twenty samples were from net caught adults ducks, whereas the rest were hunter killed ducks. Six of the samples produced HA positive results. However, only one sample was positive for AIV using real time RT-PCR and antigen capture ELISA. This sample was sent to the NVSL in Ames, Iowa and found to be a low pathogenic H10N7. The H10 gene was isolated and amplified and it is being sequenced. (Alabama) Four viruses, three from turkeys and one from swine, were tested for their antigenic

relatedness using Hemagglutinin Inhibition (HI) test and Virus Neutralization (VN) test in cell culture. The viruses are: TK/IL/04 (H3N2); TK/OH/03 (H3N2); TK/NC/03 (H3N2) and SW/NC/03 (H3N2). The formula of Archetti and Horsfall was employed to express the antigenic relatedness of the different isolates. Results showed that turkey isolates are highly related (71-100 % similar), however the swine isolate was distantly related from the others (< 30% similar to the turkey isolates). The genetic analysis revealed a high degree of similarity between the turkey virus isolates which were less similar to the swine isolate. All eight genes were more than 99% similar between the three different turkey isolates, however, genes from swine isolate were 94-96% similar to the turkey isolates genes. (Ohio) (2006)

The potential presence of avian influenza virus (AIV) within the digestive tract and trachea of European Starlings from Ohio was investigated. Three hundred twenty eight digestive samples and 97 trachea samples were collected. Real-Time RT-PCR and standard RT-PCR confirmed eighteen AIV positive digestive tract samples and three AIV positive trachea samples. All virus isolation attempts were unsuccessful. Six Non-structural (NS) genes from AIV positive digestive tract samples were amplified, sequenced, and phylogenetically analyzed. Phylogenetic analysis revealed that the majority (five of six) of the NS genes isolated from Starlings belonged to NS subtype A. Those NS genes were most similar to the NS gene from a wild bird isolate from Ohio, A/Mallard/Ohio/667/2002 (H4N6). (Ohio) (2008)

Over 5000 poultry samples were tested by real-time RT-PCR (RRT-PCR) and over 3000 blood samples by AGID were tested and found to be negative for avian influenza from live bird markets and backyard flocks in the New England States. (Connecticut) (2005-2006)

Throughout New England, clinical samples from commercial poultry, back yard poultry, and live bird markets were tested at the Connecticut Veterinary Medical Diagnostic Laboratory by serological and real time PCR specific for H5 and H7. All were negative for H5 and 7 using these tests. (Connecticut) (2007)

60 wild bird samples were collected from Alabama and tested by virus isolation for avian influenza. All the samples were negative. (Alabama) (2005)

H3N2 viruses have been isolated from outbreaks in several states from turkey breeder flocks experiencing drops in egg production. The antigenic relatedness of four isolates, the IL isolate, OH isolate, an NC recent turkey isolate, and the swine vaccine used in Illinois, were compared and a high antigenic relatedness was observed between the turkey isolates, but only a 10% relatedness of the swine vaccine to the turkey isolates. The variability of circulating H3N2 viruses may affect how vaccination is used to control this problem. (Ohio) (2005)

Recently, the first H1N1 triple reassortant virus from swine was isolated and is currently being compared to recent H1N1 swine and human isolates. Viruses circulating in swine are a possible threat to commercial turkeys and it is important to recognize and characterize these isolates. (Ohio) (2007)

In the past few years, H3N2 viruses have been isolated from commercial turkey breeder flocks. Search continues for unique influenza viruses in different species. In addition, TR H1N1 virus was isolated from pigs with respiratory symptom at an Ohio County Fair in late 2007. Importantly, twenty-six people that came in contact with the infected pigs developed respiratory disease. Isolated viruses from two individuals, a fair exhibitor and the exhibitor's father, were laboratory confirmed as H1N1 subtype by the Centers for Disease Control and Prevention. Genome sequencing showed almost 100% identity between human and pig isolates. Genetically similar virus was also isolated from pigs in Kansas and this new reassortant virus appears to move across the Midwest. In addition to these specific H1N1 strains, we continuously isolate H1 and H3 subtype viruses from pigs with severe respiratory symptoms in Ohio. (Ohio) (2008)

The survivability of poultry adapted and duck isolates in poultry litter and chicken feces was determined. Ongoing experiments are being conducted in vitro by mixing litter with different amounts of virus, and in vivo by monitoring the survivability of viruses shed in chicken feces. So far we have found that poultry adapted viruses (A/Ck/CA/431/00(H6N2)) persist longer in contact with litter and feces than wild bird virus (A/Mallard/MN/355779/00(H5N2)). (PDRC) (2008)

A comparison of the detection of antibodies against serotypes 1 and 2 IBDV by commercial ELISA kits indicated that currently available commercial ELISA kits detected antibodies elicited by the two serotypes of IBDV. Hence, the prevalence of serotype 2 antibodies in the flocks should be considered while determining antibody profiles of the flocks against serotype 1 viruses. (Ohio) (2005)

Infectious bursal disease virus (IBDV) exists in several different antigenic and pathogenic forms. The immune suppression caused by this virus in young chickens is not always associated with clinical signs of disease. The antigenic Variant viruses originally described in the United States, typically do not cause clinical signs of disease but can cause a marked immune suppression via the destruction of B lymphocytes. Using a reverse-transcriptase polymerase chain reaction (RT-PCR) assay we conducted a survey of asymptomatic chicken flocks in Europe for IBDV. Restriction fragment length polymorphisms in the VP2 gene of four viruses from Spain and four viruses from France indicated they may be different from the Classic and very virulent (vv) IBDV strains found throughout Europe. Nucleotide sequence and phylogenetic analysis of the hypervariable region of the VP2 gene indicated that all eight viruses were more similar to U.S. Variant viruses than Classic viruses. In two viruses, one from France and one from Spain, Threonine was observed at amino acid position 222 and Serine was found at position 254. These two substitution mutations are characteristic of the Delaware Variant viruses. In addition, all eight viruses had mutated amino acid position 318 from Glycine to Aspartic acid; another substitution mutation commonly found in U.S. Variant viruses. Although importation restrictions prevented us from directly testing the antigenicity of these viruses, their nucleotide and predicted amino acid sequences strongly suggest they may be antigenically unique compared to Classic and vvIBDV commonly found in Europe. (Ohio State U) (2006)

Domestic pigeons and other hobby birds can be infected with exotic Newcastle disease (END) virus, and this presents a risk of spread of the virus to poultry. The potential role of racing pigeons in that dissemination was examined by evaluating their susceptibility to infection and disease. Susceptible and Newcastle disease (ND) vaccinated pigeons were infected by eye drop and intranasally with an END virus isolate recovered during the 2002-03 END outbreak in the Southwestern U. S. Pigeons were readily infected and shed virus from both the respiratory and intestinal tract, but they were more resistant to disease with a virus dosage that would cause high mortality in chickens. Vaccination reduced the virus shed from infected pigeons and thereby reduced but did not eliminate the risk of transmitting virus to other birds. The results provide a basis for establishing regulations concerning the vaccination as well as the movement and flying of racing pigeons in a quarantine zone during an END outbreak. (SEPRL) (2006)

Endemic Newcastle disease virus (NDV) is frequently recovered from wild bird species, but little is known about the distribution, genetic diversity, and the potential of these viruses to cause disease in poultry. A total of 249 NDV isolates from cloacal samples collected during 1986 to 2005 in the U.S. from apparently healthy waterfowl and shorebirds (WS) and 19 NDV live bird markets (LBMs) were used to characterize the distribution of genotypes and their potential for virulence. Phylogenetic analysis of the fusion protein identified a total of 9 novel genotypes among the class I viruses and new subgroups among genotypes I and II of the class II viruses, demonstrating a higher genetic diversity than previously recognized. All of the WS and LBM viruses were predicted to be lentogenic based upon either sequencing of the fusion cleavage site, intracerebral pathogenicity index, or mean death time in embryo assays. The USDA real-time RT-PCR (RRT-PCR) assay that targets the NDV matrix gene was able to identify nearly all of the class II NDV viruses tested, but failed to detect the class I LBM and WS viruses. The class 1 NDV viruses are commonly found in wild birds and have been detected in poultry in live bird markets in the U.S., but in part because of the difficulty in detecting these strains they pose an unknown risk of spread to our commercial poultry populations. (SEPRL) (2008)

A serological survey was conducted in the US poultry industry to determine the prevalence of antibodies to avian paramyxovirus 2, 3, 4, 6, 7, 8, and 9. (PDRC) (2008)

As a part of a West Nile virus surveillance program in the Houston Metropolitan Area and in Rhode Island, extracts from brain from 5608 dead birds representing 21 avian orders, were cultured in Vero cells. Sixteen Newcastle disease virus isolates were recovered from birds of the order Columbiformes. These viruses were identified as pigeon paramyxoviruses from genotype VIb by partial genomic sequencing and phylogenetic analysis. All viruses corresponded to virulent phenotypes upon analysis of the fusion protein cleavage site, and intracerebral pathogenicity tests suggested mesogenic pathotypes (0.98-1.35); however none were detected with the U.S. validated real time RT-PCR assay which targets the fusion gene. A new real time PCR test was developed to identify this group of viruses. (SEPRL) (2008)

To better address the role of wild birds in aMPV epidemiology, it is important to determine the presence and distribution of aMPV in wild bird populations outside endemic areas. Serum samples and oral swabs were collected from wild birds captured in Georgia, South Carolina, Arkansas and Ohio where aMPV infection in turkeys have not been reported previously. Antibodies to aMPV were identified in five of the fifteen species tested; American coots, American crows, Canada geese, cattle egrets, and rock pigeons. The presence of aMPVs in oral swabs collected from wild bird species with the highest percentage of aMPV seropositive serum samples was detected. Sequence analysis of the matrix, attachment gene and short hydrophobic genes revealed that these viruses belong to subtype C aMPVs. The detection of aMPV antibodies and the presence of the virus in wild birds outside endemic areas demonstrate that wild birds can serve as a reservoir of subtype C aMPVs and may provide a potential mechanism to spread aMPV to poultry in other regions of the United States. (SEPRL) (2008)

Routine virus isolation attempts from respiratory disease accessions from Delmarva commercial broiler chickens yielded four isolates of a variant of IBV based on S1 gene sequencing. This variant is similar to a 2004 cecal tonsil isolate (K0401737 ct) from commercial broilers in California, recovered by Dr. Peter Woolcock's laboratory and sequenced by Dr. Mark Jackwood. The potential role of this variant to cause disease in vaccinated or unvaccinated chickens under laboratory conditions has not been established. Other field isolates from broilers were Arkansas, Massachusetts or Connecticut S 1 genotypes. (Delaware) (2006)

Using real time RT-PCR, over 200 infectious bronchitis virus (IBV) isolates were characterized as either GA08 or GA07. Also identified were Arkansas vaccine type viruses. (PDRC) (2008)

Mycoplasmas from more than 25 cases in Georgia were isolated and characterized in 2008. Mycoplasma was isolated by inoculation in modified Frey's broth and agar and identified by immunofluorescence. Approximately 110 mycoplasmas were characterized by PCR and target genes were characterized (*mgc2* and IGSR for *Mycoplasma gallisepticum* and *vlhA* for *Mycoplasma synoviae)*. (PDRC) (2008)

Several US ILTV strains and field isolates were genotyped by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) into nine different genotypes. All the commercial poultry isolates were identified within genotypes IV, V, and VI. Based on the PCR-RFLP Group IV isolates were characterized as genetically identical to the chicken embryo origin (CEO) vaccines, group V as genetically closely related to the CEO vaccines, and group VI as genetically different to the vaccine strains. The objective of this study was to determine the pathogenicity and growth characteristics of six ILTV commercial poultry isolates as compared to the CEO vaccine. Two isolates representative of PCR-RFLP groups IV, V, VI were selected. Differences in disease severity, viral tissue distribution in chickens, and plaque formation ability in cell culture were observed among viral genotypes IV, V, and VI, and between V-A and V-B isolates. Mild respiratory clinical signs were produced by IV-A, IV-B and the CEO vaccine, while VI-A and VI-B isolates produced severe respiratory signs, severe depression, and during the peak

of clinical signs both isolates were re-isolated from conjunctiva, sinus, trachea and thymus. Overall, differences in pathogenicity and growth characteristics were observed among genetically closely related US ILTV isolates, however, complete genomes will be necessary to identify molecular determinants linked to the pathogenic viral phenotypes. (PDRC) (2008)

Objective 2. Develop improved diagnostic capabilities including real time PCR as well as other rapid on-farm tests for economically important respiratory diseases.

Real time multiplex RT-PCR for avian influenza for subtypes H5, H7, and H9 and multiplex PCR or RT-PCR tests for Mycoplasmas and Infectious bronchitis infections were developed at the Guangxi Veterinary Research Institute Nanning, China. Plans are to continue to optimize the test and collaborate with the National Veterinary Services Laboratory (Ames, IA) and other experiment stations to test the new protocols. (Connecticut) (2006)

A microsphere-based 4-plex assay for the detection and subtyping of H5 and H7 subtype avian influenza (AI) virus was developed and evaluated. Four sets of probes were designed to detect Matrix (M), H7 and Eurasian (EA) and North American (NA) lineages of H5 genes, respectively. The assay successfully detected all 94 AI virus strains of 15 different HA subtypes tested, while differentiating 25 NA-H5, 11 EA-H5, and 11 H7 strains simultaneously. Detection limit determined with *in vitro* transcribed RNA was 1 pg for M, H5 and H7 probes. There was no difference in sensitivity between the monoplex and 4-plex assay. In addition, intra-plate % coefficient of variation (CV) was below 1, and inter-plate % CV remained below 5, proving the high precision and reproducibility of the assay. Furthermore, allantoic fluid and clinical swab samples that were tested without RNA extraction were equal to the pure RNA samples in sensitivity. (Ohio) (2008)

The use of AI control strategies using coordinated vaccination and monitoring with a serological high-throughput approach are being evaluated. Although vaccination has been applied successfully, rapid, specific, and sensitive serological assays, that allow differentiating infected from vaccinated animals (DIVA), has not been utilized together with AI vaccination, mainly because the lack of these assays. In this proposal, the validation of N1 and N2 ELISAs are emphasized as the assays required for the implementation of a DIVA control strategy, using a heterologous neuraminidase vaccination approach, for H5N1, H5N2, H9N2, H3N2 and H1N1 poultry infections. An N1-ELISA was developed and showed to be an effective and rapid assay to identify exposure to challenge virus during a DIVA vaccination strategy. Currently, N2 ELISAs are being developed. (PDRC) (2008)

The hemagglutinin gene of an avian influenza virus [AIV; A/duck/NC/674964/07 (H5N2)] was cloned and expressed in a baculovirus system (H5-Bac). In parallel a recombinant hemagglutinin

of A/Vietnam/1203/04 (H5N1) was expressed in mammalian cells, purified and used for generation of H5 specific monoclonal antibodies (mAb). The purified H5-Bac was used to develop a competitive ELISA (cELISA) to detect H5 antibodies in a species-independent approach using one of the established H5-specific mAb as the competitor antibody. The cELISA performed with influenza antibody free sera or sera of animals infected with other than H5 encoding AIV showed no significant inhibition of H5-mAb binding, indicating a high test specificity. In contrast, sera of poultry (chickens, turkeys, ducks) experimentally infected with H5-encoding AIV were able to significantly inhibit the binding of the mAb in a species independent approach. Comparison of the results of the cELISA with results obtained by hemagglutination inhibition (HI) assay showed a gradient of the sensitivity (turkeys>ducks>chicken). The described results show that H5 specific antibodies in sera can be detected in a species-independent approach by using a recombinant protein. (PDRC) (2008)

Real-time RT-PCR detection and sequence analysis of the VP2 hyper variable region of Indian vvIBDV isolates indicated the value of real time RT-PCR in screening field samples for the presence of vvIBDV strains. The use of real-time RT-PCR for screening field strains is highly recommended. (Indiana) (2007)

A PCR-restriction fragment length polymorphism (RFLP) test determined that most of the ILTVs isolated from commercial poultry outbreaks in Alabama and Georgia were of vaccine origin. This is important because a diagnosis of live virulent ILTV could result in the loss of export market. This would cost millions of dollars in losses for commercial US poultry companies. (Georgia) (2007)

One of the attractive advantages of ILTV recombinant vaccines is that they can be used to implement a vaccination strategy where differentiation of infected from vaccinated animals (DIVA) is feasible. However, a DIVA vaccination strategy using the new ILT recombinant vaccines has not been studied because of the lack of specific reagents. Assays are being developed to evaluate the ILTV immune responses elicited by these recombinant vaccines. ILTV glycoproteins, expressed by the recombinant vaccines, have been cloned and expressed, and a third glycoprotein expressed during ILTV infection. Currently, testing is being conducted for the presence of antibodies against these glycoproteins in serum samples from ILTV infected birds, and as expected antibodies against the three glycoproteins were detected. Serum samples from recombinant vaccinated birds at different times post-vaccination has been collected at the moment for analysis. (PDRC) (2008)

The ILTV genome is approximately 155 kb in length (Thureen and Keeler, 2006). The unique short region of the ILTV genome is ~13.2 kb in length and is bracketed by inverted repeats ~11.2 kb in length. The inverted repeats allow the ILTV genome to exist in two isomeric forms. The unique short region contains 13 open reading frames, including six structural glycoprotein genes. These proteins are important in virus attachment and maturation and are also the primary antigenic determinants recognized by the avian immune system. Currently, a comparative

sequence analysis is being conducted of the unique short region of three ILTV field isolates isolated over a 20-year period in order to determine sequence variability and evolution and to identify targets for future molecular diagnostic development. Progress has been made on sequencing two of the strains (632 (1985) and 9030 (1995)), concentrating initially on the gI and gE gene region. For comparison purposes we are simultaneously sequencing the unique short region of the NVSL challenge virus, and representative CEO and TCO vaccine viruses. When complete, this project will generate ~78 kb of ILTV DNA sequence. (Delaware, Georgia) (2008)

The palatine cleft will be investigated as a potential sample site for the improved molecular detection of pathogenic avian mycoplasmas (Zavala, Ferguson). A comprehensive clinical study was conducted to evaluate the suitability of the palatine cleft as an alternative sampling site for molecular detection of avian pathogenic mycoplasmas (MG and MS). The palatine cleft is a suitable alternative sampling site for the detection of MG and MS. (PDRC) (2008)

Prompt detection of Newcastle disease virus (vNDV) is difficult due to the broad genetic variability of the viruses. A large number of loNDV viruses have been recently identified in samples recovered from wild birds and from poultry in live bird markets (LBMs); however, the M-gene assay failed to detect the majority of these viruses. In order to enhance our ability to detect endemic loNDV viral strains circulating in poultry and prevent future outbreaks, a multiplex real time RT-PCR assay has been developed that allows identification of a broad range of viruses. It is designed to work in conjunction with the existing M-gene assay. (SEPRL) (2008)

Previously it was not possible to genetically manipulate aMPV-C viruses for pathogenesis study and recombinant vaccine development. Project scientists at SEPRL have developed a reverse genetics system for rescue of recombinant aMPV-C viruses from cloned DNAs. A number of full-length cDNA clones containing the wild-type aMPV-V genome, gene deletions or chimeric genomes were constructed and recombinant infectious viruses were rescued from these clones. The successful development of the reverse genetic technology for aMPV-C provides a powerful tool for developing bivalent or multivalent recombinant vaccines and for identifying pathogenic determinants of the virus. (SEPRL) (2008)

Often diagnosis of avianpox virus infections is done by histopathology without virus isolation. In such cases, the information about the genetic make-up of the etiologic agent is not known. In order to genetically characterize such infections, in this study, two methods were used for isolation of DNA from formalin-fixed, paraffin-embedded tissue sections. In one case, the tissue sections were first de-paraffinized and then Quagen DNeasy Blood & tissue extraction kit was used for DNA isolation. In the other method, Wax free DNA extraction kit without de-paraffinization was employed. The isolated DNA was used for PCR amplification of specific gene(s). (Illinois) (2008)

Avian poxviruses produce cytoplasmic inclusion bodies. In order to determine the presence of A-type inclusion-body gene, four A-type inclusion body gene specific primers were used.

Amplification of fragments between 124 bp and 508 bp was accomplished by PCR. Amplified products were evaluated by their nucleotide sequence. (Illinois) (2008)

A majority of field strains of fowlpox virus reveal full-length reticuloendotheliosis (REV) integration in their genomes while vaccine strains contain long terminal repeats (LTR) of REV. DNA isolated from formalin fixed, paraffin-embedded fowlpox virus infected tissue sections was used to determine the presence of REV. Three primer sets that amplify between 118bp and 419bp of REV envelope gene were used in PCR amplification. While in the control sample amplification was observed with all primer sets, only 118bp and 227bp size products were obtained in the DNA from the infected tissue sections. (Illinois) (2008)

Objective 3. Investigate the pathogenesis and polymicrobial interactions of specific infectious agents associated with poultry respiratory diseases.

Triple reassortant (TR) H3N2 influenza viruses have been isolated from turkeys in the United States since 2003. These TR H3N2 virus infections have been associated with drastic declines in egg production in breeder turkeys although co-infection with multiple agents could have been responsible for exacerbating the clinical signs. It has been confirmed experimentally that TR H3N2 influenza virus alone can cause drastic reduction/complete cessation of egg production and pathology of the reproductive tract in 26-week-old breeder turkeys. High levels of virus replication and abundant distribution of avian-specific α2,3 sialic acid-galactose receptors in the oviduct of these turkeys has been confirmed. (Ohio) (2008)

The interspecies transmission of different H3N2 influenza viruses between turkeys and swine were examined in an aerosol transmission experimental model. Of the viruses tested viruses, TK/IL/04 (H3N2); TK/OH/03 (H3N2); TK/NC/03 (H3N2); SW/NC/03 (H3N2); A/TK/OH/88 (H1N1) and A/SW/OH/06 (H1N1), only TK/OH/03 efficiently transmitted from pigs to turkeys (replicated for two days or more in both species). All pigs seroconverted with mean HI titer of 1:360 and 50% of turkeys seroconverted with mean HI titer of 1:80. The TK/IL/04 and TK/NC/03 replicated well in pigs, but were detected in turkeys for only one day. Only pigs seroconverted. A/TK/OH/88 and A/SW/OH/06 replicated efficiently in pigs but were not detected at all in turkeys. Only pigs seroconverted. In the reverse experiment of transmission from experimentally infected turkeys to pigs four viruses were compared, TK/IL/04 (H3N2); TK/OH/03 (H3N2); TK/NC/03 (H3N2) and SW/NC/03 (H3N2). In this experiment, the TK/OH/03 was also the only virus that efficiently transmitted from turkeys to pigs. Both species seroconverted at HI titers of 1:344 and 1:320 respectively. None of the other viruses were transmitted. The TK/OH/03 influenza virus was also inoculated into chickens, and ducks. The virus replicated in the inoculated chicken for 5 days but was not detected in the contact chicken. Infected chickens seroconverted but the contact ones did not. The virus could not be detected in either inoculated nor contact ducks. (Ohio) (2006)

The intraspecies transmissibility of the A/turkey/OH/ 313053 in turkeys was examined. The virus replicated in 90% of the inoculated turkeys and transmitted to more than 70% of the contact turkeys. The A/turkey/OH/313053 virus is highly infectious and transmissible in turkeys. (Ohio) (2007)

Three phenotypically different TR H3N2 viruses using the reverse genetics technique were rescued:A/turkey/Ohio/313053/04: Transmissible strain (both ways) between swine and turkeys A/turkey/North Carolina/03: Transmissible strain (one way) from swine to turkeys A/swine/North Carolina/03: Non-transmissible strain between swine and turkeys. Directed reassortments were made by changing a single gene of one virus with a similar gene from another virus of the above rescued strains. Reassortments with changes/replacements in the HA and NA genes were tested for their replication and transmissibility between turkeys. It was found that the HA gene plays a major role in determining transmission of the virus. (Ohio) (2008)

Research to determine the resistance and susceptibility of various poultry lines against AIV infection was conducted with an emphasis on the MX gene. Mice that express high levels of the MX gene are resistant to AIV and VSV challenge. Chickens have an MX gene, but it is unknown the gene provides similar resistance to chickens. It was determined that there is polymorphism in the expression of the Mx gene in various chicken breeds. The amino acid residue 631, at which, Asn determines antiviral activity, and Ser renders the MX protein inactive. Most of the year has been spent developing reagents (an expression vector for chicken interferon-alpha from another scientist, transfecting it, and characterizing the titer of the interferon). Also, assays have been refined for CEF preparation, developing faster methods of Mx typing, developing quantitative PCR for Mx expression, and evaluating replication by ELISA. The plan is to correlate MX expression of various breeds with susceptibility of CEFs from their eggs to AIV replication. (Alabama) (2006)

Lines of chickens were selected for high and low frequency of Mx gene expression. Chickens from each line were sent to the South Eastern Poultry Research Laboratory (SEPRL) for challenge with a highly pathogenic (HP) H5 isolate. Birds from Mx Asn 631 allele line showed significantly delayed mortality compared to birds from the M x Ser611 allele line. This study paves the way for selection of lines of chickens resistant to AIV. (SEPRL) (2007)

The wild bird low pathogenic H5 subtypes of AIV were found to replicate and transmit among poultry without clinical disease. (SEPRL) (2007)

A pathogenesis experiment was performed to examine the interactions of different immunosuppressive agents and infection with a low pathogenic avian influenza virus. Two experiments were performed to evaluate the effects of exposure of chickens with and without maternal antibodies to chicken anemia virus (CAV) and infectious bursal disease virus (IBDV) to low path AIV challenge. In the first experiment, the infection of commercial broiler chickens at different ages with CAV and IBDV did not affect (enhance or decrease) the pattern of low path

H7N2 AIV (A/chicken/Maryland/Minh Ma/2004) detection (virus isolation and real time RT-PCR) in tracheal swabs following challenge on day 21 days of age or in the serum antibody responses (AGID. ELISA, and HI). The second experiment was set up similarly to the first experiment except that specific pathogen free leghorn type chickens were used instead of commercial broilers. Because of the lack of maternal antibody, a 60% mortality occurred in the SPF leghorns concurrently infected with IBD and CAV prior to the planned 21 day challenge with the LP H7N2 AIV, so this treatment group was not used in the trial. Remaining CAV only and IBDV infected only birds and controls were challenged intraocularly with the H7N2 virus at 21 days of age. No affect (enhance or decrease) on the pattern of low path H7N2 AIV detection (VI or RRT-PCR)) in tracheal swab was observed between treatment groups. Compared to AI challenge controls, SPF leghorns infected with IBDV had diminished AIV antibody titers determined by HI and ELISA, as well as fewer positive AGID responders. Infection with CAV reduced HI antibody titers but had no effect on ELISA and AGID antibody responses. The results of this experiment show that early infection with CAV or IBDV reduces AIV serological responses in response to a later challenge, but not likely enough to prevent detection by standard test. (Delaware) (2006)

The biological characterization of 20 low pathogenic avian influenza viruses (wild bird isolates, isolates from live bird markets and commercial poultry operations) were completed in three different bird species that included 2-week-old ducks, 3-week-old turkeys and 4-week-old chickens. In general, all the isolates tested infected and transmitted to the contact control birds of all the three species of birds, although showing differing degrees of viral shedding and seroconversion. Results indicated that turkeys support the replication and intraspecies transmission of these isolates better than chickens and ducks. Characterization data obtained with wild bird isolates showed that except for a mute swan isolate, the transmission of the other isolates was very low or transient in chickens, however, the wild bird isolates showed higher seroconversion among infected and contact control turkeys. Similarly, except for mute swan isolate, the viral titers in ducks were similar to chickens and turkeys. All the domestic bird isolates tested infected chickens, ducks and turkeys to comparable titers. However, serological data showed higher seroconversion with these isolates in contact control turkeys indicating turkeys to be better hosts for LPAI viruses. Further, turkeys showed lower 50 percent infectious dose for 3 selected isolates tested in our study in comparison to chickens. In addition, immunohistochemical staining for receptors showed an abundance of α2,3 linked receptors (which prefers binding of avian like viruses) along with positive staining for α2,6 linked receptors (preferentially binds human viruses) in the trachea, bronchi and kidney of turkeys with some differences in distribution being observed with age. (Ohio) (2008)

A comparative study examining replication and disease pathogenesis associated with low pathogenic H5N1, H5N2 or H5N3 avian influenza virus infection of chickens and ducks was performed. Chickens or ducks were inoculated with an isolate from a wild bird [A/Mute Swan/ MI/ 451072/06 (H5N1)] or isolates from chickens [A/Ck/PA/13609/93 (H5N2),

A/Ck/TX/167280-4/02 (H5N3)] and virus replication, induction of a serological response, disease pathogenesis, and HA and NA gene sequencing of the isolates determined. Virus isolated from tracheal and cloacal swabs showed that H5N1 replicated better in ducks whereas H5N2 and H5N3 replicated better in chickens. Comparison of the NA gene sequences showed that chicken-adapted H5N2 and H5N3 isolates both have a deletion of 20 amino acids in the NA stalk region which was absent in the H5N1 isolate. Histopathological examination of numerous organs showed that H5N2 and H5N3 isolates caused lesions in chickens in a variety of organs but to a greater extent in the respiratory and intestinal tracts, whereas H5N1 lesions in ducks were observed mainly in the respiratory tract. This study suggests that the H5N1 and H5N2 and H5N3 infections occurred at distinct sites between chicken and ducks, and that comparative studies in different model species are needed to better understand the factors influencing the evolution of these viruses. (PDRC) (2008)

The titer (EID_{50} and HA) was determined for 24 LPAI viruses. To date, 16 LPAI viruses in 4-week old SPF chickens and 3-week old commercial turkeys have been tested. Virus shedding, clinical signs, macroscopic and microscopic lesions, and seroconversion were examined. Virus recovery was detected by real time RT-PCR for 11 of 16 viruses given to chickens and 8 out of 16 viruses given to turkeys. No clinical signs were observed except for one virus that caused mild upper-respiratory signs in turkeys and resulted in one death and one moribund bird (MN 99-263). Histopathological lesions, consistent with viral infection, observed in chickens were confined to the upper-respiratory and gastrointestinal tracts. The histopathological data for turkeys are pending. Seroconversion was detected by ELISA and HI tests in chickens for 4 of 16 viruses and in turkeys for 13 of 16 viruses. (PDRC) (2008)

An in-depth analysis was provided, including sequencing and animal studies, of the first highly pathogenic avian influenza virus (HPAIV) in the U.S. in the last 20 years. An outbreak of avian influenza occurred in Texas in 2004 that had the sequence of a highly pathogenic avian influenza virus, but it was not highly pathogenic when it was used to infect chickens. This laboratory in collaboration with the National Veterinary Services Laboratories, APHIS provided in depth sequence analysis, animal studies, and mutational studies to try and understand why the sequence and animal studies did not match together. The data helped to provide evidence that the sequence definition of highly pathogenic avian influenza needs to be reconsidered based on this and other exceptions to the O.I.E. rules. It remains critical, because of the severe effects on trade, to accurately diagnosis and correctly report any HPAI outbreaks. (SEPRL) (2006)

The Asian H5N1 highly pathogenic avian influenza (HPAI) viruses have changed from producing mild respiratory infections in ducks, to some strains producing severe disease and mortality. Differences in host response to infection with H5N1 HPAI viruses with different pathogenicity were examined in ducks by determining differential gene expression in tissues of infected ducks using a chicken genome microarray and detected a large number of genes showing up or down-regulation. Semi-quantitative RT-PCR was used to confirm the regulated expression of several of the differentially expressed genes. The results obtained suggested that different mechanisms are

potentially induced by avian influenza viruses to modulate the host response to infection. The differentially expressed genes identified in this study are candidates for further hypothesis-driven investigation of the mechanisms involved in resistance to AI viruses in ducks. (SEPRL) (2008)

A highly pathogenic outbreak of H5N1 from poultry from South Korea was characterized for its relationship to other outbreaks in the region and its potential for crossing the species barrier. Sequence analysis and animal studies of the outbreak were performed in conjunction with the Centers for Disease Control in Atlanta and the Veterinary Research and Quarantine Service in South Korea. The results suggested a multiple point source of introduction which helped shape the regulatory response to the outbreaks. Reporting of low pathogenicity avian influenza outbreaks in poultry in the USA has resulted in trade embargos on chicken meat. (SEPRL) (2005)

Innate immunity influences host susceptibility to avian influenza viruses (AIV), an important worldwide cause of disease in birds, reptiles, and mammals. Comparison of the innate immune response in chickens and ducks to H5N1 avian influenza showed a markedly different response between species. These studies emphasize the importance of innate immunity in birds and correlate increased pathogenicity of recent H5N1 viruses for wild waterfowl with an enhanced suppression of the host immune response. (SEPRL) (2007)

Quail have previously been suggested as host that can support replication of a number of avian influenza viruses. Quail and other species were examined for the ability of viruses to attach to different tissue types. Quail trachea and intestine appeared capable of binding both avian and human lineage viruses. Human trachea/bronchial epithelial (HTBE) cells were also evaluated for the ability to support influenza replication. These cultured cells included a variety of cell types that were polarized. This cell line allowed avian viruses with human virus receptor specificity to replicate, but avian viruses with normal receptor specificity did not. This appears to be a useful model to examine virus replication and host specificity. (Maryland) (2005)

SPF chickens were exposed to virulent IBDV and bursal adherent cells were examined by immunohistochemisrty and RT-PCR for virus infection and by real-time quantitative RT-PCR (qRT-PCR) for mRNA transcripts of proinflammatory cytokines and iNOS. Viral genome was detected in bursal macrophages at 3, 5 and 7 days post-infection (dpi). Immunohistochemical staining revealed double positive cells for KUL01 (macrophage marker) and intracellular viral proteins, showing viral replication in bursal macrophages of infected chickens. A significant decrease in the total number of bursal macrophages in infected chickens was noted, probably due to the lysis of infected cells. Inflammatory cytokines (IL-6, IL-1b and IL-18) were upregulated. These data suggested that B cells may not be the sole targets for the virus; macrophages and possibly other cells may serve as host for IBDV. (Minnesota) (2006)

Infection with infectious bursal disease virus (IBDV) causes activation of macrophages, the key cells involved in inflammatory and immune-regulatory functions. Exposure of cells of avian macrophage line, NCSU and cultured spleen macrophages (SM) from SPF chickens to IBDV

resulted in the production of nitric oxide (NO). In addition, there was upregulation of gene expression of inducible nitric oxide synthase (iNOS), IL-8 and cyclooxygenase-2 (COX-2). The signal transduction pathways involved in macrophage activation were examined. The role of mitogen- activated protein kinases (MAPKs) and nuclear factor-ºB (NF-ºB) was tested by using specific pharmacological inhibitors. Addition of p38 MAPK inhibitor, SB-203580, and NF-ºB inhibitor Bay 11-7082, suppressed IBDV-induced NO production and mRNA expression of iNOS, IL-8 and COX-2. The results suggest that IBDV uses cellular signal transduction machinery, in particular the p38 MAPK and NF-ºB pathways, to elicit macrophage activation. The increased production of NO, IL-8 and COX-2 by macrophages may contribute to bursa inflammatory responses commonly seen during the acute IBDV infection. (Minnesota) (2006)

Molecular characteristic of IBDV were done using viruses from four continents. Some IBDVs associated with high mortality did not have the typical molecular characteristics of very virulent (vv) IBDV. (Ohio) (2007)

Infectious bursal disease was observed in layer flocks and four viruses were isolates from these flocks. These viruses were genetically similar to classic vaccine viruses, but these viruses were pathogenic in SPF birds. (Ohio) (2007)

VP4 sequences of different IBDVs were compared. Amino acid (aa) mutations in the VP4 region of the attenuated and pathogenic strains were not consistently associated with virulence. Four aa were observed to be consistent with the vv viruses. (Indiana) (2007)

Nucleotide sequences of 15 IBDV isolates were generated by the University of Delaware (Dr. Gelb) and sent to The Ohio State University (Dr. Jackwood) for analysis. The sequences included the hypervariable region of the VP2 gene. The predicted amino acid translations and phylogenic analysis suggested that all 15 viruses are non-vaccine strains. The amino acids 253Q, 279N and 284A were found in all 15 viruses and suggest they would be pathogenic in SPF chickens. The phylogenic analysis further indicates that 6 viruses were very closely related to the variant Del-E strain. The other viruses were separated into 2 clades with some minor branching. (Ohio, Delaware) (2008)

Two classic infectious bursal disease virus (IBDV) strains GA-1 and H-30 caused significant gross and microscopic lesions in specific-pathogen-free chicks. The lesions and bursa/body weight ratios were consistent with classic pathogenic strains of IBDV. The viral genomes of these two classic strains were sequenced in their entirety and compared to sequences of attenuated strains of IBDV. All the attenuated strains examined had identical genome segment B sequences and compared to these viruses, the GA-1 and H-30 isolates each had one silent mutation in the gene that encodes VP1. When genome segment A sequences of the GA-1 and H-30 viruses were compared to the attenuated viruses, three nucleotide mutations in GA-1 and four in H-30 were observed. These nucleotide mutations caused one amino acid (H253N) change in the GA-1 virus and two amino acids (H253Q and G259D) were different in the H-30 virus. The data suggest that

GA-1 and H-30 are genetically related and have a common ancestor even though they were isolated from geographically distant flocks in Ohio and Iowa. Molecular and pathogenicity study data indicate that a single amino acid mutation from Histidine (H) to Glutamine (Q) or Asparagine (N) at position 253 in VP2 was responsible for the increased *v*irulence of GA-1 and H-30 compared to the attenuated classic viruses examined. (Ohio) (2008)

Infectious bursal disease viruses (IBDV) from commercial broiler chickens were determined to be unique based on VP2 sequencing and monoclonal antibody testing. IBDV isolates were obtained from three- or four- week-old commercial broiler chickens raised on the Delmarva peninsula in 2007. The VP2 genes of sixteen isolates recovered from broilers were sequenced to determine their relatedness to reference strains. Isolates representing five different phylogenetic clades were analyzed by virus-neutralization and monoclonal antibody typing to determine their relatedness to known IBDV strains. The isolates produced gross and microscopic lesions consistent with IBDV infection in the commercial broilers and experimentally in specific-pathogen-free leghorn chickens. (Delaware, Ohio, Georgia) (2008)

Positive sense RNA transcripts of infectious bursal disease virus (IBDV) genome segments A and B have previously been shown to be infectious. We demonstrated that recovery of IBDV from the transfection of Vero cells with positive sense RNA transcripts of genome segments A and B was enhanced by expression of the viral structural proteins VP2 with VP3 or by expression of viral polyprotein VP243 from DNA plasmids in trans. Expression of individual viral proteins VP2, VP3, or VP4 alone from DNA plasmids did not enhance IBDV recovery. Earliest virus recovery from transfection of positive sense RNA transcripts of genomic segments A and B was at 36 h and mean titers were $10^{1.8}$ pfu/ml. IBDV was recovered 6 hours after transfection in cells concurrently expressing either VP2 with VP3 or VP243 and mean titers were $10^{8.5}$ pfu/ml or $10^{9.2}$ pfu/ml, respectively. Likewise, expression of the viral polyprotein from DNA plasmid increased the permissiveness of Vero cells for infection with non-culture adapted IBDV. The titer of recovered non-culture adapted virus from $10^{3.3}$pfu/ml to $10^{10.3}$pfu/ml with expression of the viral polyprotein. (Indiana) (2005)

A comparative study was conducted to determine the pathogenicity and immunogenicity of wild bird isolates of avian paramyxovirus 2, 4, and 6 in chickens. Based on hemagglutination inhibition (HI) assay, nine antigenically distinct serotypes of avian paramyxoviruses (APMV) are described. Isolates from APMV 2, 3, 6 and 7 can cause respiratory symptoms and/or problems of the reproductive tract that may produce complications if secondary infections occur while isolates from APMV 4, 5, 8 and 9 rarely produce clinical signs in species that they are isolated from. Isolates belonging to the APMV 1 subtype induce a wide range of disease symptoms varying from mild symptoms to a disease with devastating consequences as caused by velogenic Newcastle disease virus (NDV). In this report, one isolate each of APMV 2, 4, and 6 were isolated from wild birds and subsequently characterized in SPF chickens. All three isolates caused no clinical symptoms but showed microscopic lesions in the trachea, lungs, gut, and pancreas characteristic for a viral infection. Interestingly, only APMV 2 induced HI antibodies, while HI

antibodies of chickens infected with APMV 4 and 6 were not detected. The replication of the virus in the birds was confirmed by isolation of the virus in embryonated eggs. (PDRC) (2008)

Last year the unusual Mycoplasma gallisepticum -685 highly attenuated vaccine strain was reported, in which MG-685 percent invasion rate was 18.8 percent in to Chick embryo Fibroblast cells. In contrast MG-S6, MG-PG31, MG-IOIO and MG-r strain ranged from 0.45-5.6%. In order to more carefully document these phenomena, confocal microscopy using propidium iodide and cytodye 119 will be used to gain more accurate information on M. gallisepticum 685 invasion of Chick embryo fibroblast cells. (Delaware) (2006)

An MG isolate of particular interest from broilers in Georgia was isolated because of our inability to distinguish the isolate from the MG ts-11 vaccine that had been administered to the breeders. Presently, bird trials are being conducted to compare the pathogenicity of this isolate to the ts-11 vaccine and a virulent MG strain. (PDRC) (2008)

Gene targeted sequencing and random amplified polymorphic DNA (RAPD) analysis have been used to determine the source and spread of MG and MS isolates in the field. The circulation of field strains within complexes and companies has been identified. (PDRC) (2008)

A total of 50 Infectious laryngotracheitis isolates from four regions, the US, Europe, Mexico and Colombia had been genotyped by multiple gene sequence analysis in our laboratory. The most prevalent genotype in the US poultry regions (40 samples) tested in this study is Group V virus. For the limited number of samples tested from Europe, Mexico and Colombia, these were genotyped as either Group IV (CEO vaccine) or Group II (TCO vaccine). A field isolate coinfecting broiler breeders along with Avibacterium paragallinarum was characterized and determined to be similar to IBV DE072. Although this strain of IBV is commonly found in Georgia, the relevance of its finding resides in the severity of the clinical case in which IBV DE072 and Avibacterium paragallinarum warranted the elimination of the affected breeder flock. (PDRC) (2008)

Newcastle disease virus is a negative sense single stranded virus infection of poultry. The virus encodes for multiple proteins that are produced as viral constructs separated by short intergenic sequences. In an effort to evaluate the role these intergenic sequences have on viral virulence, reverse genetics was used to modify the length of the sequences. The viruses were rescued and little difference was seen in growth in cell culture, but in general a decrease in virulence was seen when chickens were challenged with the virus. The use of reverse genetics was also evaluated for the examination of the virulence role of the polymerase gene, which is important for virus replication. The polymerase gene was swapped from a virulent virus to a non-virulent virus. These studies suggest a role for the polymerase gene as a possible virulence factor. (U Maryland) (2005)

Infection and disease of turkeys with NDV strains of low to high virulence were characterized. In general, disease among NDV infected turkeys was found to be less severe than in similarly

infected chickens, and turkeys infected with virulent strains shed virus for a longer time and appeared to be subclinical carriers for some of the isolates. (SEPRL) (2005)

A system to make Newcastle disease viruses with specific genetic changes was established to study Newcastle disease virus (NDV) infection in chickens. Different NDV strains cause variable clinical disease in chickens that ranges from severe to mild or in some cases inapparent infections. A full-length copy of the genome of the NDV anhinga strain has been constructed by combining each of the virus genes in an artificial system that allows virus replication to generate a virus that can be propagated and infect chickens just like the original field isolate. This system is being utilized to replace NDV anhinga genes with genes from other NDV strains that cause different clinical outcomes to identify which genes are important in controlling the severity and form of the clinical disease resulting from an NDV infection. The findings from application of this system will impact vaccine development and the identification of the role of the genes controlling the different clinical disease forms may impact other control strategies. (SEPRL) (2006)

From 1997 to 2002, the ARK type of IBV was the most dominant strain isolated from birds with respiratory disease submitted to the AL State Lab in Auburn. S1 gene analysis and challenge studies of these isolates indicated that they were closely related to vaccine strains. Experimental IBV vaccine and challenge studies with CAV and IBDV infections indicated that these 2 pathogens compromised IBV vaccine efficacy. (Alabama) (2006)

Infectious bronchitis virus field isolate, DMV/5642/06, obtained from Delmarva broilers, produced respiratory disease but not renal lesions in commercial broiler type chickens. (Delaware) (2007)

The genetic relatedness of vaccine viruses and field isolates of IBV were determined and most field isolates were found to be reisolated vaccine viruses. In addition, genetic selection and mutations were observed in the reisolated vaccines. (PDRC) (2008)

Studies were initiated to examine the ability of avian metapneumovirus (AMV) to induce mucosal cellular and humoral immunity in the upper respiratory tract (URT). Avian metapneumovirus in turkeys revealed that the attenuated and virulent strains of the virus induced IgA+ cells in the respiratory mucosa of the upper respiratory tract (URT). Turkey macrophages were also shown to be susceptible to in vitro infection and activation by AMV. (Minnesota) (2005)

Adherent cells from spleen, bone marrow and the circulation of normal turkeys were cultured in vitro. After 7 days, the cells were inoculated with the 63rd Vero cell passage of subtype C aMPV. At 96 hours following exposure, viral genome was detected by RT-PCR in the RNA extracts of virus exposed cells. Immunohistochemistry staining of the cells revealed the presence of intracellular viral proteins. Virus-exposed adherent cells had upregulation of nitric oxide production, iNOS gene and genes of several proinflammatory cytokines and chemokines. These results indicated that turkey macrophages were susceptible to infection and activation by aMPV63. (Minnesota) (2006)

Turkeys exposed to aMPV showed extensive lymphoid cell infiltrations in the upper respiratory tract (URT). The cellular infiltration occurred after the first virus exposure but not after reexposure. Quantitation of the relative proportions of mucosal IgA+, IgG+ and IgM+ cells in controls and virus-exposed turkeys revealed that at 7 days following the first virus exposure, when mucosal infiltration was well pronounced, there was a significant increase ($P<0.05$) in the numbers of infiltrating IgA+ but not of IgG+ and IgM+ cells. Following the second virus exposure, although the overall numbers of mucosal lymphoid cells were similar in the virus-exposed and control turkeys, the relative proportions of IgA+ and IgG+ cells were significantly higher in the virus-exposed turkeys ($P<0.05$) than in controls. Further, elevated levels of aMPV-specific IgA were detected in the nasal secretions and the bile of virus-exposed birds after the second but not after the first virus exposure. This result suggested, for the first time, the possible involvement of local mucosal immunoglobulins in the pathogenesis of aMPV in turkeys. (U Minnesota) Two respiratory adjuvants at three dose levels (10¼g, 20 ¼g , 40 ¼g or 60¼g) were tested: poly(I:C) and holotoxin-containing cholera toxin B (hCTB). One-day-old turkeys were given daily intranasal injections of each adjuvant for 6 days. Neither of the adjuvants caused detectable gross or microscopic lesions in the URT or lasting loss in body weight gain. This result indicated that two of the most commonly used respiratory adjuvants were safe for turkeys. Poly(I:C) and hCTB were given intranasally or oculonasally alone or in combination with inactivated aMPV. IgA cells were enumerated in the turbinate tissue of treated and untreated turkeys. Poly(I:C)+inactivated aMPV group had higher numbers of IgA+ cells than the untreated group or the groups given Poly(I:C) or inactivated aMPV alone. (Minnesota) (2006)

The aMPV G protein is a major determinant for distinguishing virus subtypes and different lengths (and resulting changes in the predicted amino acid and nucleotide sequences) have been reported. Sequence analysis revealed that the complete 1.8kb G gene was found when aMPV was propagated in our immortalized turkey turbinate (TT-1) cells. In contrast, Vero cell propagated aMPV revealed an essentially deleted G gene in the viral genome, resulting in no G gene mRNA expression. The lack of expression was confirmed by Northern blot hybridization. As expected, viral G gene mRNA was not detected in the Vero-aMPV at any time post infection (p.i.), while the TT-1-aMPV showed increased levels of G gene mRNA in a time-dependent manner post infection. Both the TT-1-aMPV and Vero-aMPV templates were examined for the existence of splicing mRNA variants containing any partial G gene fragments using an RNase protection assay. After RNase digestion, the TT-1-aMPV showed a single mRNA transcript of approximately 1.8kb, while the Vero-aMPV did not show any detectable G gene fragments. The functional role of viral genes may be different depending on the species of the cellular host substrate. While the G protein may function as a key attachment protein in TT-1 cells, it appears not to be required for Vero cell infection. (Minnesota) (2006)

The sequence of the Colorado 96 AMV isolate was completed. The virus was similar to a human metapneumovirus and to other type C viruses from Minnesota. The virus was more divergent with

the types A and B AMV isolates found in other countries. The g protein was highly variable with up to 19 % sequence divergence with the Minnesota viruses. (Minnesota) (2005)

Using the cloned genes in a reverse genetic system, a complete genome was rescued of the type C AMV virus. The rescued virus biological was the same as the parent virus. (Maryland) (2005)

The availability of the complete genome information is essential for development of a reverse genetics system to study the molecular biology and rescue infectious aMPV from cloned cdna. Therefore, we determined the nucleotide (nt) sequence of the complete genome of ampv-c colorado strain (ampv-c-co) propagated in vero cells in our laboratory (here designated as seprl variant). The full-length genome is comprised of 13,136 nt encoding eight genes, a 40 nt leader at its 3end and a 45 nt trailer at its 5 end. It is two nt longer than the ampv-c-co strain propagated in the university of minnesota (umn variant, lwamba et al., 2005), and 1,014 nt shorter than the same strain of virus propagated in the university of maryland (umd variant, govindarajan and samal, 2005). The significant difference in length between these variants was found in the coding region of the g gene, where the seprl and umn variants were 1,015 nt or 333 amino acids (aa) shorter when compared with the umd variant. In addition, there were 23 nt differences scattered along the genome of the variants. Nine of them resulted in eight aa coding changes in five genes, three of which were located in the l gene. Based on the genomic sequence of the seprl variant, we developed a reverse genetics minireplicon system using a green fluorescence protein (gfp) gene as a reporter, which allowed us to assess the effects of coding differences in the l gene on viral gene expression. It was found that one of the coding differences (position 1371 leu vs phe) in the rna-dependent polymerase l gene was critical for the polymerase functionality. (SEPRL) (2006)

To study the public health implications of MPV, it is important to examine if the avian MPV and the human MPV cross species barriers. Turkeys developed clinical disease when exposed to the human virus. (Minnesota) (2007)

Three hundred fifty E. coli isolates were characterized. E. coli are an important cause of disease in poultry. Specific virulence factors include tsh, iss, iucC, Intll and TraT. Tsh encodes for an autotransporter protein adhesin. Iss encodes for increased serum survival and is commonly seen in chickens with colibacillosis. This gene allows the bacteria to evade the host complement system by preventing the deposition of host proteins on the bacterial surface. Trat and Iss proteins prevent the formation of the membrane attack complex of the complement system that may contribute to serum resistance. iucC resides on the aerobactin operon and is involved in the iron transport system and lntll encodes for a class 1 integrase which may link with antibiotic resistance genes. (Minnesota) (2007)

The first step in the infection process by a pathogen is to establish a foot-hold in the mucosal tissues of the host. It was demonstrated that ORT expresses an adhesin-like molecule that may aid the pathogen in attachment and subsequent colonization of the upper respiratory tract tissues. This is the first report on adhesin-like molecules expressed by ORT. (NADC) (2008)

All pathogens contain two types of inducible iron acquisition mechanisms. The mechanism that ORT uses is unknown. It was demonstrated that the mechanism of iron acquisition by ORT cultures depends upon the utilization of iron from host iron-containing proteins including hemoglobin and transferrin. This is the first report on the iron acquisition mechanism of this respiratory pathogen of poultry. (NADC) (2008)

To investigate the role of sialic acid uptake in pathogenesis, a sialic acid uptake mutant of an avian strain of *P. multocida* P-1059 (A:3) was constructed and assessed for virulence in turkeys. Inactivation of sialic acid uptake resulted in a high degree of attenuation when turkeys were challenged either intranasally or intravenously. Resistance of the sialic acid uptake mutant to killing by turkey serum complement was similar to that of the parent, suggesting other mechanisms are responsible for attenuation of virulence in turkeys such as evasion of detection by the reticuloendothelial system. (NADC) (2008)

Objective 4. Develop new prevention and control strategies for poultry respiratory diseases.

Live-virus vaccines have distinct advantages over inactivated vaccines such as triggering mucosal immune responses and inducing a cell-mediated immunity, which may give the animal a more cross-protective and longer-lasting immunity. From the TK/OR/71-del (H7N3) virus, it was previously found that several variants with different sizes of the NS gene can be generated by serial passage of the virus in embryonating chicken eggs. To create a H5 vaccine strain (since the selected variants are H7 subtype) that contains the selected NS gene, a traditional reassortment method was used. Briefly, D-del var1 and TK/WI/68 (H5N9) viruses were co-infected into 10-day-old embryonating eggs for reassortment. After 48 hrs of co-infection, infectious allantoic fluid was harvested, followed by intensive plaque purification of derivatives in CEF cells. Individual clones were examined for their gene composition by RT-PCR and sequencing. A H5-D-del-v1 variant was obtained which has the NS gene of D-del var1 and other remaining genes of TK/WI/68 virus. (Ohio) (2006)

A microsphere-based multiplex assay was developed as an alternative to RRT-PCR for the detection and subtyping of H5 and H7 subtype avian influenza virus. To accomplish this, we utilized branched DNA (bDNA) signal amplification technology (a sandwich nucleic acid hybridization assay) and microsphere-based assay for the detection of influenza viral RNA. The microshpere-based array system is a newly emerging technology that provides the multiplexing of up to 100 different assays within a single sample. In this study, this system was utilized, coupled with branched DNA (bDNA) signal amplification technology (a sandwich nucleic acid hybridization assay) to detect and subtype H5 and H7 influenza virus. In the 3-plex assay, different HA subtype of influenza virus was detected and differentiated H5 and H7 HA subtype at the same time based on capture probes specific for the M, H5, and H7 gene. In addition to

multiplex capacity, this system does not require an RNA extraction step and samples can simply be treated with lysis buffer for the assay. (Ohio) (2006)

A multiplex real time RT-PCR for avian influenza and subtypes H5, H7 and H9 was developed at the Guangxi Veterinary Research Institute Nanning. Multiplex real time RT-PCR is being tested on the North American AI isolates to confirm its sensitivity and specificity for improved AIV detection. (SEPEL) (2007)

Reverse genetic studies of AIVs were conducted for improved vaccines. One strain of Hemagglutinin (H) 3 Neuraminidase (N) 2 turkey viruses was rescued using reverse genetics techniques. All the genes of two other strains were cloned into the transcriptional vector PHH21 and the rescue experiments are underway. Six genes of the A/turkey/IL/04 were cloned into PHH21 and the work is continuing to clone 2 more genes. (SEPEL) (2007)

A commercial H3 vaccine strain of influenza virus was found to be antigenically and genetically very different from H3N2 viruses currently circulating in commercial turkeys. Field evidence has indicated lack of protection from egg production drops in flocks vaccinated with that vaccine when the birds were naturally exposed to field challenge with the H3N2 virus. Results point to the importance of using live virus strains that are antigenically similar to circulating field strains. (SEPEL) (2007)

There are several different ways to attenuate influenza A virus for developing live attenuated vaccines including modification of HA cleavage site, M2 mutants, NS variants, temperature sensitive (ts) mutants, and much more. Previous studies demonstrated that two NS variants are more attenuated and immunogenic in chickens, which can be potential live vaccine candidates. However, in ovo vaccination studies showed that both variants with NS deletion strategy alone or combination of ts mutation were not attenuated enough in embryonating eggs, indicating a need for further attenuation. Recently, it was shown that single or two mutations introduced at the non-coding regions (NCR) of PB1 and PA gene could alter the protein expression levels. Studies confirmed the importance of NCRs in influenza virus replication and the potential role of NCR in determining the virulence or attenuation. (Ohio) (2008)

In the last two years, an avian influenza DNA microarray has been developed and successfully evaluated. This array contains 21 elements representing various avian influenza hemagglutinin (HA) and neuraminidase (NA) subtypes, as well as a pan-influenza probe, based on the matrix (M) gene sequence. These 21 elements are spotted in duplicate (42 spots) creating a "subarray". As a result of the subarray being spotted four times on each slide, each element is represented by 8 individual spots. Each subarray consists of a number of hermagglutinin, matrix, and neuraminidase genes). The three matrix elements were derived from AIV strains containing three different HA subtypes. Six elements on the array represent three neuraminidase subtypes (N1, N2, and N3). A DNA product of the Newcastle disease virus (NDV) fusion (F) gene is also included as a negative control. The majority of the array elements (9) correspond to hemagglutinin

subtypes (HAS, HA7, HA9). The microarray was evaluated with a panel of 10 coded samples provided by Dr. Suarez. The results of the unknown panel test indicated 80% of the HA and NA subtypes were correctly identified, and all of the isolates were correctly identified as type A influenza. All of the neuraminidase subtypes were correctly identified with the exception of a N7. No N7 gene elements are present on the array. The H1 strain (A) was also incorrectly identified, and was also not represented on the array. (Delaware) (2006)

Avian influenza (AI) vaccines protect chickens from morbidity and mortality and reduce, but do not completely prevent replication of AI viruses in the field. For accurate surveillance programs, infected birds must be identified within the vaccinated populations and eliminated. In this study, chickens were immunized with a commercial biotechnologically advanced fowlpox virus vaccine containing an H5 hemagglutinin gene (rFP-H5) from an avian influenza (AI) virus. Chickens immunized with the rFP-H5 vaccine did not develop agar gel immunodiffusion (AGID) antibodies because the vaccine lacked AI nucleoprotein and matrix genes (NP/M), but H5 hemagglutination inhibition (HI) antibodies were present indicating the birds were vaccinated and not infected with the live AI virus. The vaccinated chickens survived high pathogenicity AI virus challenge and antibodies were detected by both AGID and HI tests. This indicates that the rFP-H5 vaccine allowed easy serological differentiation of infected from non-infected birds in vaccinated populations of chickens when using standard AGID and HI tests and could be used as an aid in AI eradication efforts. (SEPRL) (2008)

An adenovirus-vectored vaccine, originally developed for humans against H5N1, was given to SPF leghorns in ovo and was shown to produce measurable antibody titers. The birds were challenged with a highly pathogenic avian influenza virus and most birds were clinically protected from disease. This vector system is replication restricted virus and therefore it has the safety of a killed vaccine, but the immune response of a live vaccine. The use of in ovo vaccination offers the possibility of mass vaccination of poultry during an avian influenza outbreak. (Alabama and SEPRL) (2005)

A replication-defective adenovirus recombinant vaccine to protect chickens against avian influenza virus was developed that encoded the hemagglutinin gene from the low pathogenic avian influenza virus Turkey/Wisconsin/68 H5N9. The vaccine was given to SPF leghorns in ovo or at day of age by SQ route. The AdTW68.H5 vectored vaccine induced measurable HI (log29) titers against the LP turkey H5N9, but no titers using IDEXX ELISA, when given in ovo at 18 days or SQ at day of age. Thirty one day old vaccinated birds were challenged at the USDA SEPRL lab in Athens, GA with either the Mongolian HP H5N1 (89% hemagglutinin sequence homology) or Mexican H5N2 (94% hemagglutinin sequence homology) AIVs. The vaccine induced 68 % protection against the Asian and 100% against the Mexican virus. (Alabama) (2006)

Protective immunity against avian influenza (AI) virus has been elicited in chickens by single-dose *in ovo* or intramuscular vaccination with a replication-competent adenovirus (RCA)-free

human adenovirus vector (Ad) encoding the AI virus H5 (AdTW68.H5) or H7 (AdChNY94.H7) hemagglutinins. AdTW68.H5-vaccinated chickens were protected against both H5N1 and H5N2 highly pathogenic (HP) AI virus challenges. AdChNY94.H7-vaccinated chickens were protected against an H7N3 HPAI virus challenge. Chickens vaccinated *in ovo* with AdTW68.H5 followed by post-hatch intramuscular vaccination with AdChNY94.H7 responded to both vectors with robust antibody titers against both the H5 and H7 AI proteins. The use of a synthetic AI H5 gene codon optimized to match the tRNA pool found in chicken cells is more potent than the cognate H5 gene. Mass-administration of this AI vaccine can be streamlined with available robotic *in ovo* injectors. In addition, Ad5-vectored vaccines can be produced rapidly and the safety margin of the non-replicating vector is superior to that of a replicating counterpart. Furthermore, this mode of vaccination is compatible with epidemiological surveys of natural AI infections. (Alabama) (2008)

A transgenic AIV vaccine in yeast was developed. The HA gene, from an H10 virus from wild ducks, was cloned and expressed in *Schizasaccharomyes pombe.* The expression was confirmed by western blotting. We will assess the immunogenicity of this vaccine, given orally in poultry, using the HA test. An efficacious vaccine in yeast could be propagated and given in mass by drinking water to poultry of all ages. Yeast are routinely given in the drinking water as a probiotic, in place of antibiotics, to kill bacteria in commercial poultry. (Alabama) (2008)

The use of DNA vaccines for use in the production of reference diagnostic reagents were improved with the addition of the cytokine adjuvants, IL-2 and interferon. DNA vaccines can produce high quality antibodies to specific proteins that are extremely valuable for diagnostic reagents, but the response to DNA vaccines are variable. Alternative methods for adjuvanting or increasing the immune response were conducted for both the H5 and H7 hemagglutinin protein of avian influenza, and it was found that both the inclusion of plasmids with the cytokines IL-2 and type 1 interferon improved the response for H7 vaccines. This work will allow the improved production of reference antibodies in the future that are safer and easier to produce than the current methods. (SEPRL) (2005)

Stem cells were examined for their susceptibility to infectious bursal disease virus (IBDV) and a non-pathogenic in ovo vaccine was developed that stimulates humoral and cell-mediated immunity. Bone marrow mesenchymal stem cells were identified as new targets for IBDV replication. Susceptibility of these cells may contribute to immunosuppression caused by the virus. (Minnesota) (2007)

A real-time RT-PCR assay was developed utilizing dual-labeled fluorescent probes binding to VP4 sequence that are specific to the classical, variant and very virulent strains of Infectious Bursal Disease Virus (IBDV). The assay was highly sensitive and could detect as little as 3 ´ 102 to 3 ´ 103 copies of viral template. The variant sequence-specific probe was found to be highly specific in detecting isolates classified as variant A, D, E, G and GLS-5, and did not react with classical strains. The classical sequence-specific probe also demonstrated high sensitivity and

specificity and differentiated between isolates that were variant and classical strains. The very virulent sequence-specific probe positively detected the Holland vvIBDV isolate and did not react with classical or variant strains. (Indiana) (2005)

The effect of prime-boost on protection of chickens against infectious bursal disease by DNA vaccination was examined. Multiple intramuscular injections with a large dose of DNA carrying a large segment gene of the infectious bursal disease virus (IBDV) have been shown to provide effective protection to chickens against infectious bursal disease (IBD). The present study was conducted to determine if priming with DNA carrying a large segment gene of the IBDV and boosting with killed IBD vaccine could adequately confer protection of specific pathogen free (SPF) chickens against IBD. One-day-old chickens were intramuscularly injected with DNA plasmid coding for a large segment gene of the IBDV strain variant E (VE) (P/VP243/E) followed by an intramuscular injection of killed IBD vaccine containing both standard and variant IBDV at 1 or 2 weeks of age. Chickens were orally challenged with IBDV strain VE or standard challenge strain (STC) at 3 weeks of age and observed for 10 days. Bursal lesion scores, bursa weight/body weight (B/B) ratios, protection efficacy, IBDV antigen in bursae, enzyme-linked immunosorbent assay (ELISA) titers to IBDV, and virus neutralization (VN) titers to IBDV were determined. Chickens primed with 50, 100, 200, or 400 mg of P/VP243/E at 1 day of age and boosted with 0.5 ml of killed IBD vaccine at 1 or 2 weeks of age had 80 to 100% protection against challenge by IBDV strain VE or 71 to 100% protection against challenge by IBDV strain STC. Chickens in the groups primed with P/VP243/E and boosted with killed vaccine had significantly higher ($P<0.05$) B/B ratios and significantly lower ($P<0.05$) bursal lesion scores than chickens in the challenge control (CC) groups and groups primed with vector plasmid and boosted with killed IBD vaccine or only primed with P/VP243/E. No IBDV antigen was detected by immunofluorescent antibody assay (IFA) in bursae of chickens protected by the DNA vaccine prime and killed vaccine boost vaccination. Prior to challenge, chickens (21 days of age) in the groups primed with P/VP243/E and boosted with killed IBD vaccine had significantly higher ($P<0.05$) ELISA and VN titers to IBDV. These results indicate that SPF chickens at 1 day of age primed with a DNA vaccine and boosted with killed IBD vaccine can be adequately protected against challenge by homologous variant or heterologous classical IBDV. A prime-boost strategy may be useful in enhancing immunity and protection of chickens against IBD by DNA vaccination. (Indiana) (2006-2007)

Studies were carried out to determine whether an infectious bursal disease (IBD) virus (IBDV) large segment gene-based DNA fused with avian influenza virus (AIV) hemagglutinin (HA) gene could trigger immune response to both IBDV and AIV. Hemagglutinin gene of AIV was amplified from cDNA of A/turkey/WI/68 (H5N9) strain by PCR and inserted into IBDV VP3 gene in a vector carrying IBDV VP243 gene. One-day-old specific pathogen free (SPF) chickens were intramuscularly injected with plasmid DNA carrying VP243 (VP243/pcDNA), H5 (H5/pcDNA), or VP243-H5 (VP243-H5)/pcDNA weekly for three times, followed by a two-week interval for the fourth injection. The virus neutralization (VN) titers to IBDV were significantly higher ($p<0.05$) in chickens inoculated with VP243/pcDNA than those with VP243-H5/pcDNA 2

to 6 weeks after the first inoculation. The hemagglutination inhibition (HI) titers to AIV were significantly higher ($p<0.05$) in chickens inoculated with H5/pcDNA than those with VP243-H5/pcDNA 2 to 6 weeks after the first inoculation. The findings indicated that IBDV large segment gene-based DNA fused with AIV HA gene in DNA vaccination can elicit specific neutralizing antibody response to both IBDV and AIV. (Indiana, SEPRL) (2008)

IBDV exists as two antigenically different groups, and a RT-PCR test was developed that could rapidly differentiate serotype 1 viruses, serotype 2 viruses, and the vv strains of IBDV. These tools allow the rapid differentiation of the different serotypes to provide rapid characterization of the viruses. (Ohio) (2005)

A mild form of IBDV is found in the U.S., but very virulent forms are found in many other countries. A sequence comparison of the virulent and non-virulent forms showed sequence differences that were used as a differential RT-PCR test. A diagnostic assay was developed that reliably differentiated very virulent infectious bursal disease virus (vvIBDV) from non-vvIBDV strains. The availability of of rapid diagnostic tests facilitate identification of field isolates, and allow a rapid response for control of highly virulent IBDV viruses are introduced in the U.S. (Ohio) (2005)

The persistence of infectious bursal disease virus (IBDV) infections in chickens is dependent on the pathogenesis of the virus. The disease usually lasts 5-10 days but virus can be found in the bursa for several weeks in convalescent birds. Maternal immunity can delay infectious bursal disease virus (IBDV) infection until broilers are several weeks of age. We have observed gross and microscopic lesions in the bursa and identified IBDV in broilers 3 – 4 weeks of age. Late field virus infections suggest IBDV may still be present when the birds are processed. We have detected IBDV in the bursa tissue of broilers at processing plants. The export of U.S. broiler products to some foreign countries has been restricted because of the potential for IBDV contamination. Until now, the presence of IBDV in broilers at the processing plant was unknown. (Ohio) (2008)

The broiler industry is using both recombinant products off-label, via *in ovo* application achieving variable results in the field. We evaluated the efficacy of both recombinant vaccines to protect broilers against the standard USDA challenge strain after *in ovo* application at different embryo incubation ages under controlled-experimental conditions. The FPV-LT vaccinated groups, at 17, 18 and 19 days of embryo age, showed a 45%, 61%, and 55% percentage of protection, respectively. The HVT-LT vaccinated groups, at 17, 18, 19 days of embryo age, showed a 48%, 78%, 84% protection, respectively. Viral DNA reaching 10^4 to 10^6 genome copy numbers (GCN) was detected in the tracheas of FPV-LT and HVT-LT vaccinated birds, between days 3 to 5 post-inoculation. These viral DNA levels were similar to the levels detected in the non vaccinated-challenge groups. The peak of viral DNA coincided with the peak of clinical signs indicating active challenge virus replication in both vaccinated as well as non-vaccinated birds. From this study we concluded that *in ovo* vaccination at 18 days of embryo age did not affected hatchability

or body weight gain after challenge, reduced overall clinical sign severity, but did not reduced challenge virus replication. Similar studies has been conducted utilizing challenge viruses from genotypes Groups V and VI where the HVT-LT recombinant vaccine reduced the severity of clinical signs but did not reduced challenge virus replication in the trachea. (PDRC) (2008)

Three commercial chicken embryo origin vaccines provided protection against recent infectious laryngotracheitis virus field isolates representing RFLP genotypes 5 and 6. (PDRC) (2007)

At least fourteen genes have been successfully deleted from the genome of virulent European and Australian ILTV strains. Some of these mutants grew efficiently in vitro and showed different degrees of attenuation in vivo indicative of their potential as live attenuated marker vaccines. The immediate goal of this study is to develop the system to genetically manipulate currently circulating US isolates. The first specific objective of this proposal is to delete ILTV genes from US isolates. The second objective of this proposal is to evaluate the *in vitro* stability and growth characteristics of ILTV gene deleted mutants. (PDRC) (2008)

Effort has been devoted to study the development of a new genetically modified infectious laryngotracheitis (ILT) vaccine based on a continuously growing chicken cell line. The research findings indicate that the potentially immortalized chicken cell lines are being established in cultures. One lung cell line, which was co-transfected originally with chicken telomerase catalytic subunit (cTert) and viral telomerase RNA component (vTR), continues to grow for more than 8 month at current passage of 35. The growth rate of this cell line currently shows 0.6 - 0.7 population doublings per day (PD/day). Two live cell lines, which were transfected originally with either small interfering RNA (siRNA) for p53 or pcDNA 3.1-hygro control vector respectively, continue to grow for more than 8 or 6 months, respectively. A cell line derived from the siRNA-p53 transfectant shows relatively slow growth rate (less than 0.1 PD/day), while a cell line derived from the control vector transfectant shows 0.7-0.8 PD/day. One spontaneously immortalized live cell line without transfection continues to grow in culture showing 0.7-0.8 PD/day of its growth rate. Thus, the potential immortalized cell lines can be utilized either to propagate ILT virus or to attenuate virus for cell culture based vaccine production. (Arkansas) (2008)

ILTV is highly contagious pathogen of poultry that is often controlled by vaccination. For broilers, mass vaccination techniques results in environmental contamination that can result in persistence in the house leading to back passage (bird to bird transfer) of the virus resulting in increased virulence. With reduced down time between lots, 5 days or less, and the use of built up litter, this condition is causing serious losses in the broiler belt in SE USA. In addition, there is not sufficient vaccine produced in the US on a yearly basis to vaccinate all the broilers in affected areas. Therefore, management practices to reduce the ILTV concentrations in chicken houses are needed. A natural challenge method was developed, using sentinel chickens reared in isolation units on reused litter contaminated with ILT back passed vaccine virus and a nested polymerase chain reaction (PCR) to determine the presence of ILT vaccine virus in the feces and tracheas of

the chickens. Using these methods, it was determined that several commercially available poultry litter treatments (Poultry GuardTM, Al+Clear TM, PLTTM), heating the litter to 38C0 (1000 F) for 24 h, and in house composting for 5 days inactivated ILT vaccine virus. This information is of immediate use to the poultry industry for controlling ILT vaccine virus induced disease in broilers and may reduce other important viral pathogens as well. (Alabama) (2006)

Infectious laryngotracheitis virus (ILTV) vaccines can cause asymptomatic "silent" ILTV outbreaks in commercial chickens. These outbreaks can result in reduced body weight and increased processing plant condemnations. It was determined that live vaccine viruses were present in the drinking waterers and darkling beetles taken from commercial houses, which have recently experienced these ILTV outbreaks. Beetles and water lines and nipples drinkers contained ILTV DNA as determined by real time PCR and live virus as determined by infection of susceptible SPF chickens. Poultry producers need improved beetle control in their houses as well as remove biofilm in their drinking water lines. Biofilm produced by certain bacteria can harbor infectious organisms such as bacteria and viruses. Once the biofilm is removed for the lines these organisms will become susceptible to common water disinfectants such as chlorine. Results showed that common poultry industry practices of wind-row house composting did not kill all the beetles in the house. In addition, and common water sanitizers, Clorox, and citric acid, did not inactivate ILT vaccines from the water. Other methods are currently being assessed to kill beetles as well as studying other water sanitizers, which have been shown to remove biofilm from drinking waters. Our research will result in practical on farm methods to reduce ILT vaccine breaks, which continue to occur around the world. (Alabama) (2007-2008)

Current commercial ND vaccines when given correctly protect birds from dying or getting sick, but they do not protect vaccinated birds from being infected and shedding the outbreak virus to other birds. These vaccines are made with older strains of NDV that differ genetically from the strains that have caused recent outbreaks. The goal of our research was to assess the amount of antibodies produced by and the amount of challenge virus shed from birds vaccinated with ND vaccines formulated from viruses with different degrees of sequence similarities to the challenge virus. Antibody production was measured after vaccination with either an inactivated or a live ND vaccine and amount of virus shed after a challenge with either a recent outbreak virus or the current challenge virus used in the U.S. to test vaccine efficacy. The testing of live vaccines required the use of recombinant viruses that would be similar in sequence to the challenge virus without having the same virulence. The birds vaccinated with vaccines made from an isolates that were genetically similar to the challenge virus shed less virus in oral secretions and had fewer birds shedding virus than the birds vaccinated with vaccines made from viruses less similar to the challenge virus. A vaccine that effectively reduced virus shed should also reduce virus transmission to other birds. Matching the vaccine administered to the genotype of either the virulent viruses known to be circulating in that area or to the most likely outbreak virus for that area could be used to improve ND control. (SEPRL) (2007-2008)

Avian metapneumovirus (aMPV) causes a local immune response in the respiratory tract and that a non-infectious respiratory vaccine can be used to control the disease. This vaccine will reduce virus contamination of the environment and facilitate eradication. (Minnesota) (2007)

Efforts to develop live attenuated aMPV-C vaccines in the US have been made and various levels of protection against aMPV-C challenge were obtained. In this study, protection of mucosal vaccination with inactivated (killed) aMPV against virulent aMPV challenge was evaluated in turkeys. The results indicate mucosal vaccination with inactivated aMPV does not increase protection from viral infection and can increase histopathologic lesion severity following virus infection. (SEPRL) (2008)

Turkeys were immunized with adjuvanted rNP and recombinant M protein (rMP) administered intramuscularly and immunized and unimmunized controls were challenged with virulent avian metapneumovirus (AMV) by the respiratory route. At a dose of 40 ug/bird, rNP protected eight of nine birds. rMP at the same dosage level protected three out of seven birds. At a dose of 80 ug of each rNP and rMP, 100% protection was achieved. This recombinant vaccine shows promise as an improved control measure for AMV. Another study was designed to determine if in vivo passages of AMV would increase virus virulence leading to consistent clinical signs in turkeys. The results of this preliminary study indicate that in vivo passage of AMV in birds may increase virus virulence and the resulting virus could serve as a suitable challenge inoculum for use in vaccination trials. Currently Vero cells are commonly used for the isolation of AMV from clinical samples, but because Vero cells are a mammalian cell line, concern that the virus is changed by passage in this cell line is a concern. Sequence analysis supports this idea, since 11 amino acid differences in the F gene of AMV propagated in turkey cells and that propagated in Vero cells. In an attempt to find a better avian origin cell line for isolation of AMV, alternatives were tested. A non-tumorigenic immortal turkey turbinate cell line was developed that is susceptible to AMV, and may provide a valuable alternative to Vero cells. (Minnesota) (2005)

A sequencing project is almost complete to try and identify the genes related to virulence in aMPV. Once the genes are identified, a virus will be created by reverse genetics to remove that gene and create an improved vaccine based on reverse genetics. Infectious clones for aMPV have been developed by our collaborator Dr. Siba Samal at the University of Maryland. The viruses sequenced are: 1. MN-1a 9p: This virus was isolated from an outbreak of respiratory illness in turkeys in Minnesota in 1997. The virus was passaged in CEF for seven times and then twice in Vero cells; 2. MN-1a 41p: The virus MN-1a was passaged seven times in CEF cells and then 34 times in Vero cells; 3. MN-1a 63p: The virus MN-1a was passaged seven times in CEF cells and then 56 times in Vero cells; 4. MN-1a 65/Cp: The aMPV MN-1a after 41 passages in Vero cells was adapted to grow at cold temperature. It was passaged eight times each at 35ºC, 33ºC and 31ºC; and 5. MN-2a 7p: This virus was isolated from an outbreak of respiratory illness in turkeys in Minnesota in 1997 from a farm different from where MN-1a was isolated. This was passaged in CEF for seven times. (Minnesota) (2006)

Gene fragments encoding approximately the 5' one-third of *Pasteurella multocida* fhaB2 (filamentous hemagglutinin) were contained on three clones derived from *P. multocida* strain P-1059 (serotype A:3) and expressed in *Escherichia coli*. The protective immunity conferred by vaccination with the combined recombinant peptides on turkeys was evaluated. The results showed that turkeys immunized twice with the purified recombinant peptides were significantly protected against intranasal challenge with virulent P. multocida strain P1059. The FHAB2 proteins of a bovine (A:3) (14) an avian (A:3) and an avian (F:3) strain of *P. multocida* are highly conserved (>99% identity) suggesting that broad cross protection against different *P. multocida* capsular and serotypes may be achievable through immunization with specific recombinant FHAB2 peptides. (NADC) (2008)

Commercial vaccine containing modified live infectious bronchitis virus strains Massachusetts and Arkansas provided protection against a novel genotype, DMV/5642/06, recovered from broilers raised on the Delmarva Peninsula. (Delaware) (2007)

Eighteen days embryonating eggs were inoculated simultaneously with recombinant DNA plasmid containing IBV spike gene and with interferon alpha 1. Two weeks post hatched chicks were challenged with the field isolate of Mass 41. The study indicates production of spike protein specific antibody response prior to challenge. The various tissues were analyzed by RT-PCR and re-isolation of virus. In birds vaccinated with recombinant DNA vaccine along with interferon at 7 days, over 98% of the chicks were protected. (Connecticut) (2008)

Polymerase chain reaction (PCR) and immunoblotting are continuing to be used for differentiation of avianpox virus strains isolated from domestic poultry or wild birds. The presence of A-type inclusion (ATI) gene was detected in genomes of all strains. Common as well as different antigens were detected among various strains during immunoblotting analysis. (Illinois) (2006)

The application of a new chemical compound is being investigated as a disinfectant against an enveloped as well as a non-enveloped virus. As model viruses a low pathogenic avian influenza virus (lpAIV, H5N2) and a vaccine strain of infectious bursal disease virus (IBDV, D78) were used. Unlike many disinfectants currently in use, the biocide used in this process forms a gas, and therefore has a strong penetrating capability in sealed poultry houses. After treatment, once properly ventilated, the poultry houses are safe for subsequent use without the risk of further infection or contamination. Rendering the poultry litter free of virus and pathogens would allow the reuse of some or all of the litter safely. This would reduce the cost of poultry production, as litter has been increasing in cost rapidly. The results show that lpAIV in contaminated chicken litter is inactivated in less than one hour when the disinfectant is used at the recommended dose. This result was also obtained at one third the recommended dose. The impact of the disinfectant on chicken litter contaminated with IBDV was comparable. IBDV was inactivated in less than one hour with full and half doses. These results indicate that routine treatment of the facilities and litter at Broiler, Egg and Turkey production companies would greatly control or eliminate the risk

of transmission of virus between flocks which are raised on used litter. Use of this disinfectant after a disease outbreak to disinfect litter seems feasible. (PDRC) (2008)

Common cooking methods kill highly pathogenic avian influenza (HPAI) and Newcastle disease viruses in poultry. HPAI viruses can be present in the meat of infected poultry and a prior study demonstrated cooking was effective in killing an H5N1 HPAI virus. (PDRC) (2007)

Impacts

Objective 1

1) Starlings carry AIV that is genetically similar to aquatic wild birds in Ohio which may contribute to the spread of the virus.
2) Viruses circulating in swine are a possible threat to commercial turkeys and it is important to recognize and assess the genetic, antigenic, and pathogenic characteristics of recent isolates.
3) Poultry adapted viruses (A/Ck/CA/431/00(H6N2)) persists longer in contact with litter and feces than wild bird virus (A/Mallard/MN/355779/00(H5N2)).
4) Several low pathogenic H5N1 viruses were isolated in wild birds in collaboration with APHIS and USGS, and these viruses were sequenced and shown to be of North American lineage that are separate from the highly pathogenic H5N1 viruses found in Europe, Asia, and Africa. The biologic and sequence characterization of these viruses continue to provide evidence that highly pathogenic H5N1 viruses have not traveled to the Americas in wild birds, and clearly documents that low pathogenic H5N1 viruses are normally found at a low prevalence level in the Americas.
5) The first comprehensive biological characterization of H5N1 high pathogenicity avian influenza (HPAI) virus from wild birds was completed on viruses that came from Mongolia. Because the outbreak occurred where no poultry exist, this indicates the H5N1 HPAI virus spread into Mongolia by migrating wild birds, but is a virus that can infect poultry and cause severe disease.
6) An infectivity model for avian influenza viruses was developed that predicts transmissibility based on the intranasal bird mean infectious dose (BID50) test for chickens, turkeys, domestic ducks and geese, and Japanese quail. Chickens were not easily infected with most wild bird AIV, but domestic ducks and geese, Japanese quail and turkeys were easily infected and they could serve as key bridging species for waterfowl-origin AIV crossing into domestic poultry. Furthermore, these data suggest mixing of poultry species during rearing and using outdoor production systems is a major risk factor for transmission of AIVs from wild birds to domestic poultry.
7) Urban wild birds were assessed as reservoirs and transmission hosts for H5N1. Only some wild bird species could be involved with transmission of H5N1 HPAI viruses while others would be good sentinels for detection in wild bird populations.
8) The detection of aMPV antibodies and the presence of the virus in wild birds outside endemic areas demonstrate that wild birds can serve as a reservoir of subtype C aMPVs and may provide a potential mechanism to spread aMPV to poultry in other regions of the United States.
9) Mycoplasmas from more than 25 cases in Georgia have been isolated and characterized. Approximately 110 mycoplasmas were characterized by PCR and sequencing of target

genes (*mgc*2 and IGSR for *Mycoplasma gallisepticum* and vlhA for *Mycoplasma synoviae*).

10) Evaluation of the potential role of domestic pigeons and other hobby birds in the dissemination of Newcastle Disease has provided a basis for establishing regulations concerning the vaccination as well as the movement and flying of racing pigeons in a quarantine zone during an END outbreak.

Objective 2

1) With the multiplex capacity and feasibility of the assay, the multiplex branched DNA assay has a great potential in rapid diagnosis and subtyping of influenza virus. Currently, 20-plex assay are being developed that can differentiate all 16 HA subtypes of influenza virus.
2) Reverse genetic technology was developed for avian influenza subtype H7 and H5 viruses for vaccine development and pathogenicity studies.
3) An N1-ELISA was developed and shown to be an effective and rapid assay to identify exposure to challenge avian influenza virus during a DIVA vaccination strategy.
4) A species-independent ELISA was developed for the detection of H5 antibodies in several species.
5) Genetic characterization of avianpox viruses was done by isolation of DNA from formalin fixed paraffin-embedded infected tissue sections. In this regard, presence of specific genes e.g. A-type inclusion body protein gene and REV envelope gene was determined by PCR.
6) A comparative sequence analysis is being conducted of the unique short region of three ILTV field isolates isolated over a 20-year period in order to determine sequence variability and evolution and to identify targets for future molecular diagnostic development. Progress has been made on sequencing two of the strains (632 (1985) and 9030 (1995)), concentrating initially on the gI and gE gene region.
7) The palatine cleft is a suitable alternative sampling site for the detection of MG and MS.
8) A multiplex real time RT-PCR assay was developed that allows identification of a broad range of class I and II Newcastle disease viruses and works in conjunction with the existing M-gene assay.
9) A reverse genetics system for rescue of recombinant aMPV-C viruses from cloned DNAs was developed. A number of full-length cDNA clones containing the wild-type aMPV-V genome, gene deletions or chimeric genomes were constructed and recombinant infectious viruses were rescued from these clones. The successful development of the reverse genetic technology for aMPV-C provides a powerful tool for developing bivalent or multivalent recombinant vaccines and for identifying pathogenic determinants of the virus.

Objective 3

1) In a series of studies, two low pathogenicity avian influenza viruses given intranasally to chickens grew only in the respiratory and intestinal tracts, and no virus was found in the blood, meat, bone or bone marrow. By contrast, two high pathogenicity avian influenza viruses grew not only in respiratory and intestinal tracts but spread systemically with virus being found in blood, meat, bone or bone marrow. Killed vaccines or recombinant fowl

pox-avian influenza vaccines prevented the high pathogenicity avian influenza viruses from being in the meat.

2) Although 2–6-week-old turkeys are routinely used for pathogenicity and vaccine protection studies, the low levels of viral shedding and asymptomatic infections in this age group often pose difficulty in interpretation of results. Studies show that breeder turkeys should be used to assess the potential pathogenicity of TR H3N2 viruses and the viral titers and pathology of the oviduct as well as egg production data can be good measures of protection following in vivo challenge in vaccine efficacy studies.
3) A comparative study examining replication and disease pathogenesis associated with low pathogenic H5N1, H5N2 or H5N3 avian influenza virus infection of chickens and ducks was performed. The study suggests that the H5N1, H5N2 and H5N3 infections occurred at distinct sites between chicken and ducks.
4) The titer (EID_{50} and HA) was determined for 24 LPAI viruses.
5) H3N2 viruses were isolated from young commercial turkeys.
6) The TR H3N2 virus is highly infectious and transmissible in turkeys. Hence, producers should be aware of the possible impact of that virus. Using reverse genetics, a better understanding of genetic basis for the important biological activities of the virus will be gained and development of vaccines will be facilitated.
7) The wild bird H5 subtypes were found to replicate and transmit among poultry without clinical disease. These isolates will be useful in determining the molecular basis of interspecies transmission. Studies indicated that turkeys are more susceptible to influenza viruses than chickens and ducks and signify the role of turkeys as intermediate hosts in the transmission of influenza viruses from wild birds to land based domestic poultry.
8) The use of whole genome chicken microarray was used to detect genes differentially expressed in ducks after H5N1 AIV infection.
9) Innate immunity in birds is important and studies correlate increased pathogenicity of recent H5N1 viruses for wild waterfowl with an enhanced suppression of the host immune response.
10) Fifteen infectious bursal disease virus (IBDV) isolates were sequenced at the University of Delaware and sent to The Ohio State University for analysis. The sequences indicated that all 15 viruses were wild-type variant strains that should be pathogenic in SPF chickens. Phylogenic analysis separated the viruses into 3 clades with some minor branching.
11) A single amino acid mutation at position 253 in the infectious bursal disease virus protein VP2 increases the virulence of classic strain viruses.
12) Molecular epidemiology studies are providing valuable information on the presence and variability of infectious bursal disease virus (IBDV) strains in commercial poultry. This genetic drift has lead to antigenic variability among these viruses. Regular monitoring of these genetic mutations has helped in the development of new vaccines and control strategies.
13) Infectious bursal disease viruses (IBDV) from commercial broiler chickens were determined to be unique based on VP2 sequencing and monoclonal antibody testing.
14) A comparative sequence analysis is being conducted of the unique short region of three ILTV field isolates isolated over a 20-year period in order to determine sequence variability and evolution and to identify targets for future molecular diagnostic

development. Progress has been made on sequencing two of the strains (632 (1985) and 9030 (1995)), concentrating initially on the gI and gE gene region.

15) The iron acquisition mechanism of ORT and the colonization factors for ORT infection have been determined.
16) One of the virulence mechanisms for *P. multocida* was identified and deletion mutants lacking the uptake mechanism for sialic acid uptake were less virulent for turkeys. Also, recombinant filamentous hemagglutinin peptides protected turkeys against a challenge with wild type virulent *P. multocida*.
17) Fifty infectious laryngotracheitis isolates from the US, Europe, Mexico and Colombia have been genotyped by multiple gene sequence analysis.
18) The genetic relatedness of vaccine viruses and field isolates of IBV were determined and most field isolates were found to be reisolated vaccine viruses. In addition, genetic selection and mutations were observed in the reisolated vaccines.
19) One hundred forty E. coli isolates from Delmarva poultry flocks were Ciprofloxacin resistant whereas 2 were intermediate. Nalidixic acid showed 101 susceptible while 40 were resistant.

Objective 4

1) It is expected that a safe and efficacious live attenuated influenza in ovo vaccine can be developed for poultry by using combination of NS deletion and NCR modification strategies.
2) Protective immunity against avian influenza (AI) virus has been elicited in chickens by single-dose *in ovo* or intramuscular vaccination with a replication-competent adenovirus (RCA)-free human adenovirus vector (Ad) encoding the AI virus H5 (AdTW68.H5) or H7 (AdChNY94.H7) hemagglutinins.
3) The rFP-H5 vaccine allowed easy serological differentiation of infected from non-infected birds in vaccinated populations of chickens when using standard AGID and HI tests and could be used as an aid in AI eradication efforts.
4) A transgenic AIV vaccine in yeast was developed. An efficacious vaccine in yeast could be propagated and given in mass by drinking water to poultry of all ages.
5) The development of a reverse genetics model for infectious bursal disease virus with high efficiency of virus recovery will help delineate the pathogenesis of IBDV and that of polymicrobial interactions of IBDV and poultry respiratory diseases.
6) Infectious bursal disease viruses were identified in bursa tissue from broilers at processing plants. Identification of IBDV at processing plants is the first step in devising new control strategies to reduce the risk of contaminating poultry products earmarked for the export market.
7) IBDV large segment gene-based DNA fused with AIV HA gene in DNA vaccination can elicit specific neutralizing antibody response to both IBDV and AIV. Thus, IBDV large segment gene-based DNA vaccine carrying AIV HA gene has the potential to confer protection of chickens against IBD and AI in DNA vaccination.
8) The vaccination strategy by priming chickens with DNA vaccine encoding IBDV VP243 gene linked to chicken CRT gene has the potential to enhance immunity and protection of chickens against IBD for practical prevention and control of IBD in the field.
9) SPF chickens at 1 day of age primed with a DNA vaccine and boosted with killed IBD vaccine can be adequately protected against challenge by homologous variant or

heterologous classical IBDV. A prime-boost strategy may be useful in enhancing immunity and protection of chickens against IBD by DNA vaccination.

10) A DNA vaccine was developed using the infectious bronchitis virus-S gene in a plasmid expression system for vaccination of chickens.
11) In-ovo vaccination with the use of interferon has further enhanced the protection and efficacy of the specific vaccine for infectious bronchitis infection.
12) The potentially immortalized chicken cell lines are being established in cultures. Three potentially immortal cell lines derived from chicken embryo liver and lung tissues have been growing for more than 6 months. Those cell lines are expected to become fully immortalized. Immortal cell lines can be utilized as a stable substrate for ILTV vaccine production.
13) Using a nested polymerase chain reaction (PCR) to determine the presence of Infectious Laryngotracheitis vaccine virus in the feces and tracheas of the chickens, it was determined that several commercially available poultry litter treatments (Poultry GuardTM, Al+Clear TM, PLTTM), heating the litter to 38C0 (1000 F) for 24 h, and in house composting for 5 days inactivated ILT vaccine virus. This information is of immediate use to the poultry industry for controlling ILT vaccine virus induced disease in broilers and may reduce other important viral pathogens as well.
14) Antigenic differences among NDV strains of different genotypes used in vaccine formulation affects viral shedding after a virulent challenge.
15) Diagnosis of Newcastle disease in turkeys was dependent on virus isolation to detect infected birds, a factor that must be considered for ND control programs.
16) A non-infectious respiratory vaccine for aMPV will reduce virus contamination of the environment and facilitate eradication.
17) Protection of mucosal vaccination with inactivated (killed) aMPV against virulent aMPV challenge was evaluated in turkeys. The results indicate mucosal vaccination with inactivated aMPV does not increase protection from viral infection and can increase histopathologic lesion severity following virus infection.
18) A reverse genetics system for avian metapneumovirus allows for detailed experiments on pathogenesis to be conducted as well as the potential to make improved vaccines.
19) A diagnostic test was developed to differentiate avian poxviruses using seven sets of primers from fowlpox virus genome (39K, EGF, REV envelope, REV LTR, homolog of HA, A-type inclusion and TK). This new test provides a rapid way to characterize field strains of avian pox virus.
20) Gamma irradiation is not a practical intervention to reduce the risk of IBDV introduction via processed poultry.
21) Proper cooking of poultry using the Food Safety Inspection Service's salmonella standards would be effective in killing both AIVs and NDVs.
22) Poultry producers need improved beetle control in their houses and need to remove biofilm in their drinking water lines. Biofilm produced by certain bacteria can harbor infectious organisms such as bacteria and viruses. Once biofilm is removed from the lines, these organisms will become susceptible to common water disinfectants such as chlorine.
23) A new disinfectant process was tested for poultry viruses. The biocide used in this process forms a gas, and therefore has a strong penetrating capability in sealed poultry

houses. After treatment, once properly ventilated, the poultry houses are safe for subsequent use without the risk of further infection or contamination.

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Grants

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Avian Influenza surveillance and control program. Source of support: USDA, NRI. CAP grant; Total amount: $1, 666, 666.00 ($ 26,000.00 CoPI); Start/end date: February 1, 2005 to January 31, 2007. Support type: Funded.

Avian Influenza surveillance and control program, Source of support: USDA, NRI. CAP grant ; Total amount: $1, 366, 666.00 ($33,000.00 CoPI), Start/end date: May 1, 2006 to June 30, 2007.) Support type: Funded.

Avian Mycoplasma epidemiological studies of isolates from various farms in Pakistan. Source of support Government of Pakistan Higher Education grant. Total amount: $ 15,000.00. December 1,2007 to May 10,2008. Funded.

Bio-information tools enabling large scale DNA bar-coding Source of support: National Science Foundation; Total amount: $ 399,800.00(Co-PI 30%). Start/end date: July 1, 2006 to June 30, 2009; Support type: Funded.

Development of a recombinant subunit vaccine for infectious bronchitis virus Source of support: USDA, Hatch ; Total amount: $18,000.00 Start/end date: October 1, 2005 to September 30, 2006; Support type: Funded.

Efficacy of classical and recombinant vaccine strains for infectious bronchitis (IB) and their relatedness to IB virus from deep pectoral myopathy cases in broilers at slaughter age. Source of support: Brazilian Government. Total amount: $12,000.00. February 1, 2008 to January 1. 2009. Funded.

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Inactivation of Salmonella and camplobacter in chicken meat and products using caprylic acid. Source of support: NRI. USDA; Total amount: $460,000.00 (CoPI 30%).; Start/end date: September 1, 2006 to August 31, 2009; Funded.

Inactivation of Salmonella and Campylobacter contamination of poultry meat by feeding caprylic acid to chickens. Source of support: USDA-NRI. Total amount: $ 366,000.00. Start/end date: September 1, 2006 to August 31, 2009. (CoPI 30%). Funded.

In ovo vaccination of IBV recombinant and DNA vaccine against IBV Source of support: Charles River-SPAFAS, Inc. Total amount: $ 10,000.00 Start/end date: January 2005 to December 2006; Support type: Funded.

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Lee, C.W. Development of DIVA vaccines for the control of trip reassortant H3N2 influenza in turkeys. U.S. Poultry & Egg Assoc. $74,693. 2007-2009.

Lee, C.W. and Saif, Y.M. Pathogenicity and antigenicity of different lineage of H3N2 viruses in turkeys. Iowa State University. $32,441. 2006-2008.

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Molecular characterization of Pakistani Infectious Bronchitis Virus strain. Variants and development of recombinant vaccine. Source of support: Pakistan-USDA- linkage Program; Total amount: Rs. 2.967 million ($55,000.00), Start/end date: March 1, 2002 to March 31, 2005. Support type: Funded.

Molecular diagnosis techniques for *Mycobacterium tuberculosis.* Source of support: Government of Egypt, Higher Education grant. Total amount: $ 80, 000.00. Start/end date: January 1, 2006 to June 30, 2008. Funded.

Molecular techniques for typing and susceptibility testing of Mycobacterium species. Source of support: Egyptian Culture and Education Bureau, Washington DC. Total amount: $ 44,000.00. Start/end date: January 1, 2006 to December 31, 2007. Support type: Funded.

Mundt , E.. Development of a species-independent ELISA for the detection of influenza A H5 antibodies, USDA/AI-CAP,$ 79,375.00, 2007-2008.

Mundt , E.. USDA/AI-CAP, Development of a species-independent ELISA for the detection of influenza A H6, H7, and H9 antibodies, $ 65,270.00 , 05/01/08-04/30/09.

Mundt, E.. Generation of monoclonal antibodies directed against subtypes of IBDV. Veterinary Medical Agricultural Research, Internal competitive grant, $16,004.00, 2007- 2008.

Mundt, E. (PI), and Seller, H.. Antigenic Characterization of Field Isolates Using the Reverse Genetics System of Infectious Bursal Disease Virus (IBDV), US Poultry and Egg**,** $ 94,278.00, 2007-2009.

Mundt, E. Characterization and control strategies of low pathogenic avian influenza virus H9N2. BINATL AGRI RSCH & DEV FUND, $157,000, 2008-2011.

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Saif, Y.M. Antigenic and molecular characterization of astrovirus from poult enteritis. U.S. Poultry and Egg Assoc. $30,000. 2001-2004.

Saif, Y.M. et al. Effects of nutrition and waste management technologies on pathogens in animal excreta. Initiative for Future Agricultural Food (IFAFS). $2,628,547, 2001-2005.

Saif, Y.M. Emergency detection and response research program on emerging animal and plant diseases at the OARDC. $2,534,746. 2004-2009.

Saif, Y.M., Lee, C.W. and Perez, D. Prevention and control of avian influenza in the U.S. USDA, University of Maryland. $197,603. 2005-2009.

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D. Tang, H. Toro, D. Suarez. In-ovo vaccination/avian influenza/bird/human. NIH-SBIR 2-R44-AI-068285-02. $1,000,000.00, 2009-2012.

H. Toro, F.J. Hoerr, V. van Santen, K. van Kampen**,** D. Tang. Control and Prevention of Avian Influenza in the U.S. Renewal proposal: Replication-Defective Adenovirus Recombinant Vaccine to Protect Chickens against Avian Influenza *USDA CSREES NRI CAP Award#2005-35605-15388,* $88,000.00, 2007-2008.

H. Toro, D. Tang D. Suarez, E. Collisson & F.W. van Ginkel. Protection of chickens against avian influenza by a non-replicating adenovirus vaccine. USDA AI CAP 2007-35203-18070, $498,000, 2008-2011.

Vakharia, V., and J. Gelb, Jr. Generation of infectious bronchitis virus from cloned cDNA: Potential for basic studies and vaccine development. USDA NRI. $360,000. 2004-2007.

V. van Santen, H. Toro, K. S. Joiner, L. Li. Significance of Minor Viral Subpopulations within Ark-type Infectious Bronchitis Vaccines, U.S. Poultry & Egg Association. $41,665.00, Sept. 2007- August 2010.

Wu, C.C., and Lin, T.L. Microalgal-based oral delivery system for poultry vaccines. Indiana 21st Century Research and Technology Grant. $785,413. 11/04 to 8/09.

Wu, C.C., and Lin, T.L. Infectious bursal disease virus vector. Purdue University School of Veterinary Medicine Internal Competitive Grant. $8,000. 7/05 to 6/07.

Significant collaboration/interaction

Ohio/UGA. Collaborating on influenza virus project by sharing technology and samples (2005-2009).

USDA-ARS Athens/UGA Sharing plasmid vectors for influenza research (2008-2009).

University of Delaware/UGA. Sharing reagents to study IBDV and Avian Influenza (2008-2009).

USDA/University of Minnesota collaborating on avian metapneumovirus project by sharing technology and samples (2003-2008).

USDA/CT sharing influenza low path viruses (2006-2008).

UGA/ICT sharing reagents to study Mycoplasma epidemiological (2008-2009).

DE/CT Sharing reagent to study IBV (2005-2006).

DE/USDA ARS SEPRL. Effects of concurrent respiratory and immunosuppressive viral infections on the pathogenesis, diagnosis and potential viral mutations of low-path avian influenza virus (H7N2) in chickens and turkeys (2005-2008).

DE/USDA ARS SEPRL- Biological characterization of low pathogenic H7 subtype avian influenza viruses and the effects of AI virus infection on gene expression (2006-2009).

DE/USDA ARS SEPRL/Ohio State University -Viral adaptation to host species-effect of AIV passage within a host on viral subpopulations and sequence changes (2008-2009).

DE/OH/GA – IBDV field isolate characterization by VP2 gene sequencing and monoclonal antibody analysis (2008-2009).

DE/GA – Provide AIV antisera for validation studies (2008).

DE/MD/GA – Provide ILTV isolates for characterization. (2007-2008).

Ohio/Delaware collaborating on IBDV project by sharing reagents and sequence information (2004–2009).

Ohio/USDA collaborating on influenza pathogenesis, diagnostic, and vaccine development project by sharing virus, antibodies, samples and technology (2005-2009).

Ohio/USDA collaborating on influenza diagnostic methods by sharing viruses and serum samples (2006-2009).

Ohio/IN collaborating on influenza project by sharing technology and samples (2005-2009).

Minnesota/IN collaborating in projects of avian pneumovirus and infectious laryngotracheitis virus (2006-2009).

Auburn U. received valuable AIV reagents from Dr. David Suarez of the SEPRL in Athens, Ga.

OH/IN; collaborating on influenza virus project by sharing technology and samples (2005-2009).

USDA (SEPRL)/IN; sharing plasmid vectors for influenza vaccine development (2006-2009).

GA (UGA)/IN; sharing reagents to study infectious laryngotracheitis pathogenesis and vaccine development (2007-2009).

OH/IN; sharing IBDV virus stock and reagents for the development of better molecular diagnostic methods (2005-2007).