Poult Enteritis and Mortality Syndrome Final Report

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Edited by Andrea M. Miles and Linnea Newman

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Forward

Many investigators have contributed to research efforts related to poult enteritis and mortality syndrome (PEMS) since it was first recognized in 1991. PEMS devastated the North Carolina poultry industry in the early 1990s. In 1995 a PEMS task force was convened to coordinate PEMS research. The State of North Carolina and several commercial turkey companies contributed funds. The task force reviewed research proposals and provided funds to support research at several different universities.

In 2001 the Dean of the North Carolina State University College of Veterinary Medicine, Dr. Oscar Fletcher, invited several PEMS researchers and other interested people to attend a meeting to discuss the results of PEMS research and to determine strategies for future research. The meeting was held on April 10th, from 9:00 am to 2:00 pm at the Ramada Inn on Blueridge Road in Raleigh North Carolina. Prior to meeting each investigator was asked to prepare a 1-page summary of each major research project supported by the PEMS research fund. The meeting was conducted as a roundtable discussion and each investigator was asked to give a 10-minute presentation. John Barnes served as a moderator.

This publication contains a summary of the research results and future research needs, written reports provided by the investigators who were funded by the PEMS task force, and the resulting abstracts and publications. A great deal of information was generated from the PEMS task force funds:

51 Peer Reviewed Articles
10 Trade Journal Articles
3 Book Chapters
130 Abstracts Presented at Scientific Meetings
2 PhD Dissertations
1 Masters Thesis

Linnea Newman, who was not present at the meeting, reviewed the written reports as well as the recorded sessions and meeting notes. She wrote a summary of the research results and future research needs *Poult Enteritis and Mortality Syndrome: Recent Clinical and Laboratory Research* (the first paper in this publication) and *Summary Comments* (the last paper in this publication)..

Andrea M. Miles
Linnea Newman
Editors

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Executive Summary

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Poult Enteritis and Mortality Syndrome is not a single disease entity, but a syndrome of clinical signs induced by one of several combinations of enteric viruses and bacteria. Researchers have begun to characterize some of the pathogens involved, and the pathophysiology associated with the syndrome. PEMS clinical signs and lesions have been reproduced to some extent in challenge models involving turkey coronavirus (Barnes and Guy), astrovirus (Saif and Schultz-Cherry), reovirus (Qureshi and Schat), and a torovirus (Reynolds). A secondary stressor, such as a bacterial infection is required to fully reproduce the disease.

Diagnostic tests have been developed to detect the most common isolates, but are still not widely available to the commercial turkey industry. Stacey Schultz-Cherry's lab has developed a polymerase chain reaction (PCR) test for turkey coronavirus, and reverse-transcriptase PCR (RT-PCR) tests for astrovirus, torovirus and reovirus; as well as a multiplex RT-PCR test designed to detect both astrovirus and coronavirus from the same field sample. These tests are currently being conducted in Holly Seller's Laboratory at the University of Georgia Poultry Diagnostic Research Center. Tom Hooper's Laboratory at the Animal Disease Diagnostic Lab, in Dubois Indiana can do fluorescent antibody tests for coronavirus, enterovirus (also detects some astroviruses) and rotavirus from field samples. Jim Guy's laboratory at the North Carolina State University College of Veterinary Medicine can test for turkey corona virus by PCR, a monoclonal antibody-based immunoperoxidase procedure, and a monoclonal antibody-based immunofluorescence test or for antibodies to turkey corona virus by ELISA.

Treatment of the bacterial component of the infection with fluoroquinolone antibiotics will reduce mortality, but there is no current treatment that effectively reduces the stunting and other clinical signs associated with the syndrome. Control of PEMS has focused on eradication through depopulation and single-age housing rather than vaccination. These control methods have been effective in reducing the incidence of PEMS in North Carolina. Further research is needed to fully define the combinations of pathogens capable of inducing this syndrome, and their interaction with field environmental conditions. The development of cost-effective control methods will depend upon the results of this future research.

Poult Enteritis and Mortality Syndrome:
Recent Clinical and Laboratory Research

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Introduction. Poult enteritis and mortality syndrome (PEMS) is a clinical disease of young turkeys 7 to 28 days of age. Diarrhea, stunting, mortality, and immune dysfunction characterize the syndrome, formerly known as "spiking mortality of turkeys". First recognized in North Carolina in August 1991, PEMS devastated the state's turkey industry, resulting in a decline in turkey production from 62 million turkeys in 1992 to 53 million turkeys in 1997. Union County, NC experienced the greatest turkey production loss, dropping from over 14 million turkeys in 1992 to just 5 million in 1997. PEMS has been catastrophic to the growers and the overall economy of the region, effectively eliminating most turkey producers in the area.

Depopulation of affected farms, strict clean out and biosecurity measures and a move toward single-age farms has been implemented with some success. Although PEMS appears to have peaked in North Carolina in 1996, with fewer flocks reported in subsequent years, the disease continues to produce widespread significant performance losses in the turkey industry.

Clinical Signs. Typical clinical signs of vocalization, diarrhea, growth depression, immune dysfunction and significant mortality affect turkey poults from 7 to 28 days of age. Older flocks appear to be resistant to clinical signs; these flocks exhibit only mild symptoms or remain asymptomatic.

There appear to be two forms of the syndrome: a severe form, originally identified as "spiking morality of turkeys" (SMT), which is defined as mortality that equals or exceeds 1% on 3 consecutive days or 9% between 8 and 28 days of age; and a mild form, which is defined as mortality which is greater than 2% but less than 9% during the same time period, but which does not equal or exceed 1% for three consecutive days.

Gross and Microscopic Lesions. PEMS is grossly characterized by fluid accumulation in the intestine and atrophy of the bursa of Fabricius. Atrophy of the thymus and liver and variable atrophy or enlargement of the spleen has also been observed.

Microscopically, PEMS is characterized by atrophy of bursal epithelium, attaching and effacing lesions of the intestinal epithelium, necrosis of the microvilli with destruction of the terminal web of microvilli, acute inflammation of the large and small intestine characterized by eosinophilic granulocytes, and by eosinophilia associated with tissue degeneration in the thymus, bursa and pancreas. Mitochondria are ruptured and intracellular organelles in general are damaged.

Physiological and Immunological Profile. Affected poults demonstrate severely compromised intestinal maltase activity, hypoglycemia, hypophosphatemia, hypocalcemia, decreased blood proteins and hemoconcentration.

The immune system is compromised, as characterized by atrophy of the lymphoid organs (thymus, bursa and spleen) and by the reduction of *E. coli* clearance from circulation and compromised macrophage phagocytic activity. CD4+ T lymphocytes and tumor necrosis factor α are significantly lower in PEMS poults, while interleukin-1 and interleukin-6 and nitric acid are increased.

While the immune system is compromised by some measures, enhanced production of proinflammatory cytokines and metabolites by activated macrophages have been demonstrated. Enhanced production of proinflammatory cytokines and macrophage metabolites may contribute to intestinal inflammation, gut motility and anorexia in PEMS poults, analogous to human inflammatory bowel syndrome.

Etiology. Researchers have been able to reproduce PEMS-like clinical signs and lesions with a number of different viruses and bacteria isolated from PEMS outbreaks. It would appear that PEMS is analogous to broiler respiratory disease, bovine respiratory disease complex, or the common human cold, where any number of viruses may cause initial damage, predisposing the birds to, and exacerbated by, coinfection with other viruses or bacteria and perhaps enhanced by excessive immune system response.

Researchers have demonstrated PEMS clinical signs and lesions in challenge models using turkey coronavirus (TCV) (Barnes and Guy) astrovirus (Saif and Schultz-Cherry), reovirus (Qureshi and Schat), and to some degree, a torovirus (Reynolds). The viruses appear to damage the intestine, bursa, liver and thymus, but often do not induce the severe clinical signs and mortality associated with PEMS, and may not yield all of the specific histopathological lesions associated with the field cases. The researchers have attempted to characterize the virus isolates and to develop diagnostic tools to identify these viruses in both affected and unaffected flocks.

Turkey coronavirus in combination with enteropathogenic *Escherichia coli* and torovirus in combination with *Campylobacter jejuni* have both shown enhanced disease signs, with turkey coronavirus and enteropathogenic *E. coli* R98/5 (Guy) appearing to duplicate the full pattern of gross and microscopic lesions and clinical signs, including mortality, described as PEMS.

Although the TCV / enteropathogenic *E. coli* model is capable of reproducing PEMS, not all PEMS flocks are TCV-positive or *E. coli* R98/5 positive. In fact, eastern North Carolina flocks in 2 major clinical studies (1996 and 1998) showed a relatively low incidence of TCV-positive flocks, and commonly yielded cryptosporidia and salmonella instead of enteropathogenic *E. coli*. This has led the North Carolina researchers to distinguish two types of PEMS: TCV-positive PEMS and TCV-negative PEMS. In addition, TCV-positive flocks (as well as flocks positive for astrovirus, reovirus and torovirus) can be asymptomatic.

Diagnosis. A variety of diagnostic tests have been developed to help clinicians monitor flocks for the viruses that may be associated with PEMS.

Tests which may be used to detect virus in samples of fecal material or lower intestinal content include: a polymerase chain reaction (PCR) test for turkey coronavirus, reverse-transcriptase PCR (RT-PCR) tests for the astrovirus, torovirus and reovirus, and a multiplex RT-PCR test designed to detect both astrovirus and coronavirus from the same field sample. Monoclonal antibody-based immunofluorescence and immunoperoxidase procedures can be used to detect TCV antigens in tissues, with the immunofluorescence demonstrating the greatest sensitivity and specificity. An antigen-capture enzyme-linked immunosorbent assay (ELISA) has been developed for detection of astrovirus. Serological tests for antibodies include ELISA tests for TCV and astrovirus, and a fluorescent antibody assay (FA) and indirect FA for torovirus.

While the diagnostic tests have been developed, they are not currently widely available at regional diagnostic laboratories. PCR based tests are currently being conducted in Holly Seller's Laboratory at the University of Georgia Poultry Diagnostic Research Center (PDRC). Tom Hooper's Laboratory at the Animal Disease Diagnostic Lab, in Dubois Indiana, can do fluorescent antibody tests for coronavirus, rotavirus and enterovirus (the same antibody can also detect some astroviruses). Jim Guy's laboratory at the North Carolina State University College of Veterinary Medicine can test for turkey corona virus by PCR, a monoclonal antibody-based immunoperoxidase procedure, and a monoclonal antibody-based immunofluorescence test or for antibodies to turkey corona virus by ELISA.

Epidemiology. Preliminary epidemiological studies have indicated that no significant risk appeared to be associated with the integrated company with whom the growers had a contract, proximity to cattle or hogs, distance of the turkey houses from roads or trees or the methods of dead bird disposal. There was no significant association between PEMS flocks and fly or darkling beetle infestation levels. Biosecurity and compliance with biosecurity programs was highly variable on both PEMS-affected and unaffected farms. There appeared to be some correlation between PEMS-affected flocks and the hatchery of origin, the removal of used litter by a contractor and rodent control measures.

Clinical signs of PEMS are strongly associated with young poult flocks, but older flocks may actively shed astrovirus and TCV despite being asymptomatic. Transmission of virus from older birds to young poults on the same farm may be a key factor in the development of PEMS in young flocks.

Studies have shown that TCV is distinct from bovine coronavirus, although laboratory infection with the bovine virus will cause bursal atrophy. Cattle do not appear to be the infection source.

The TCV virus is distinct from infectious bronchitis of chickens, another coronavirus. Chickens can be infected with TCV under laboratory conditions, and even after several chicken passages of the TCV, contact control studies indicate that the chickens will transmit TCV to turkeys. Field surveys using sentinel birds, however, have not demonstrated any

naturally occurring TCV-positive chicken flocks. Chickens do not appear to be a significant source of TCV infection under field conditions.

Treatment. Fluoroquinolone antibiotics appear to significantly reduce mortality associated with PEMS, although flocks may still experience weight loss and stunting.

Recent studies indicate that over-stimulation of the immune system may be part of the pathophysiology of PEMS. Similar to the human cold, which is symptomatically treated with antihistamines, clinical signs of PEMS may potentially be reduced by prednisone or other treatments designed to moderate the immune response. No clinical studies have been conducted to date.

Prevention and Control. Depopulation, single-age housing and strict biosecurity appear to be reasonably effective in controlling PEMS. PEMS-associated astrovirus is very stable and resistant to disinfection efforts, so clean out without additional measures is ineffective.

Depopulation and single-age housing efforts can be focused by the use of monitoring programs for TCV and astrovirus. Unfortunately, the significant number of asymptomatic virus-positive flocks makes depopulation and single-age housing based upon a positive laboratory test an economically costly and controversial control method.

Studies using anti-stunting syndrome agent (SSA) antibody demonstrate that antiserum can be used to reduce the weight loss associated with PEMS. This indicates that a breeder vaccine, which would confer passive immunity to the progeny, may be possible. A large amount of antibody would be necessary to adequately protect the intestinal tract, however, and it would have to remain at protective levels through 28 days of age to be successful. No breeder vaccine is under development at this time.

Turkey poults injected with embryo-adapted TCV at one day of age demonstrated resistance to challenge with TCV-positive PEMS, indicating that there is a potential to produce a TCV vaccine to reduce signs associated with TCV-positive PEMS. Since the industry has rejected the idea of seeding turkey houses with live TCV no further research into the production of a vaccine has been undertaken.

Astroviruses are poor antigens, and are therefore, poor vaccine candidates. Antiviral treatments may hold some promise for astrovirus infection.

Future Research Needs. PEMS has yet to be fully defined. Matrix studies to characterize interactions of various potential pathogens in specific pathogen free (SPF) birds are needed. Additional fieldwork to characterize the interaction of the pathogens or combinations of pathogens and environmental conditions is necessary to develop effective management methods to control the disease. Fieldwork in addition to laboratory work is also needed to define the potential interaction of the pathogens and vectors such as rodents or insects. Improvement in diagnostic tests and improvement in the availability of these diagnostic tests to the commercial turkey industry is urgently required to focus effective control measures.

Clinical Studies on Poult Enteritis Mortality Syndrome

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Introduction. Poult enteritis mortality syndrome (PEMS) is a clinical disease of young turkeys characterized by mortality, stunting, diarrhea disease, and immune dysfunction that occur in mild and severe forms. The severe form, originally referred to as 'spiking mortality of turkeys" (SMT) is defined as a mortality that equals or exceeds 1% on three consecutive days or 9% between 8-28 days of age. It is clearly distinct from other types of enteric disease of young turkeys. The mild form has been called "excess mortality of turkeys" (EMT) and is defined as a mortality >2%<9% during this same time period that does not equal or exceed 1% for three consecutive days. The definition of the mild form of PEMS overlaps with clinical features of other poult intestinal diseases.

Clinical Investigations. Major clinical studies were conducted during the summers of 1996 and 1998. Most of the flocks in the first study were from farms in western NC. Approximately two-thirds of the diseased flocks were infected with turkey coronavirus (TCV). This was unexpected and unknown at the time. Diagnostics were developed and monitoring programs were put into place. By the end of 1998, no TCV-positive flocks remained in western NC. This was accompanied by a substantial improvement in performance but not complete elimination of intestinal disease. Flocks from eastern NC had a much lower occurrence of TCV infection. Cryptosporidia and salmonella were commonly found in PEMS flocks in eastern NC. The results of these and correlative studies led to the realization that, based on etiology, there were two types of PEMS: TCV-positive PEMS and TCV-negative PEMS.

Sentinel Studies. A sentinel method was developed to: a) confirm the occurrence of PEMS in a clinically affected flock, b) determine if a flock might be harboring agents that could cause PEMS but not be clinically affected, and c) to study the disease under controlled conditions. The clinical and pathological manifestations of both TCV-positive and TCV-negative PEMS have been determined. TCV-negative PEMS typically does not withstand freezing whereas TCV-positive PEMS can be stored frozen. Stunting occurs regardless of how material is handled but mortality is variable and may take a few passages to be restored. Changes in the bursal epithelium distinguish the two types of PEMS. TCV in TCV-positive PEMS was shown to be the cause of these bursal lesions. Material from these flocks was provided without restriction to other task force members for experimental studies. New agents and combinations of agents were discovered; other investigators will present these.

Treatment & Prevention. Early on, it became clear that PEMS was multi-factorial. A putative pathogenesis, which still appears to be largely correct, was proposed. In essence, one or more viruses cause the stunting and predispose to one or more bacteria, which cause the mortality. Studies done in cooperation with Dennis Wages showed treatment with fluoroquinolones effectively stopped mortality but had only a limited impact on stunting.

Similarly, controlled early exposure to TCV followed by later exposure to TCV-positive PEMS infected birds indicated this approach could be useful although it would not be appropriate if eradication were being contemplated.

Chickens. Because the areas where PEMS was affecting turkeys were also areas of intense broiler production there was concern about the possibility of PEMS affecting chickens or chickens potentially being a source for PEMS. Experimental and field studies showed that chickens become infected but remain asymptomatic and can transmit PEMS to turkeys by contact. However, in limited sentinel studies natural infection of chickens was not found.

Education/Other. Numerous presentations to industry and scientific groups have been given. An overview of PEMS is available on our Poultry Health Management website (see below).

This research emphasized the significance of the disease, this aided in funds being appropriated for USDA-ARS investigators to study PEMS.

Poultry Health Management Website http://www.cvm.ncsu.edu/info/departs/fae/PHM/

Poult Enteritis Mortality Syndrome:
Characterization and Diagnosis of Turkey Coronavirus and
Characterization of PEMS-Associated *E. coli*

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PEMS Research Funded by: PEMS Task Force, FRA, US Poultry and Egg Association

Characterization of PEMS-associated coronavirus. Antigenic analyses of coronaviruses isolated from PEMS affected turkeys, herein referred to as turkey coronavirus (TCV), demonstrated a close antigenic relationship between these viruses and infectious bronchitis virus (IBV) (29). Subsequent nucleotide sequence studies substantiated these findings (8, 9). The nucleocapsid protein genes, 3' untranslated region and a portion of the matrix protein genes of three epidemiologically-distinct isolates of TCV (NC95, Indiana and Minnesota "bluecomb" strains) were sequenced and compared with published nucleotide sequences of other avian and mammalian coronaviruses. Based on these comparisons, a high degree of similarity (>90%) was observed between the matrix, nucleocapsid genes, and 3' untranslated regions of TCV, and infectious bronchitis virus (IBV). The TCV matrix and nucleocapsid proteins had limited homology (<30%) with mammalian coronaviruses. Phylogenetic analyses were done based on TCV matrix and nucleocapsid protein sequences and published sequences of other coronaviruses. These analyses divided the avian and mammalian coronaviruses into three distinct genotypes with the avian coronaviruses, IBV and TCV, comprising one distinct genotype (28).

Development of improved methods for TCV diagnosis. A polymerase chain reaction (PCR) procedure and two monoclonal antibody-based immunohistochemical procedures were developed and evaluated for TCV diagnosis (11). A Mab-based immunoperoxidase (IP) procedure, Mab-based immunofluorescence (IF) procedure and the PCR procedure were compared with virus isolation for detection of the virus in experimentally infected turkeys. PCR was used to detect the presence of the virus in intestinal contents and dropping samples, IP and IF procedures were used to detect viral antigens in intestinal tissues. The IP and IF procedures were highly sensitive and highly specific compared with virus isolation. TCV was detected in experimentally infected turkeys on days 1-16 PE, but not day 22 post exposure, by the IP and IF procedures. The IF procedure was determined to have the greatest sensitivity and specificity; in addition, this procedure was the fastest and easiest procedure to perform. The PCR was highly specific, but less sensitive than either IP or IF procedures; however, antemortem diagnosis of TCV was possible with PCR but not IP and IF procedures. Additionally, a TCV specific enzyme-linked immunosorbent assay for detection of TCV-specific antibodies has been developed using a baculovirus-expressed TCV nucleocapsid protein (10).

Identification and characterization of PEMS-associated *E. coli*. An enteropathogenic *E. coli* designated R98/5 was isolated from dropping samples of PEMS-affected turkeys (31).

R98/5 was shown to possess the *E. coli* attaching/effacing (eae) gene and was shown to produce attaching/effacing lesions in experimentally infected turkeys. Attaching/effacing lesions, similar to those produced by R98/5, previously have been identified in turkeys with naturally occurring PEMS. Attaching/effacing lesions were characterized by adherence of bacterial microcolonies to intestinal epithelium with degeneration/necrosis of epithelium at sites of bacterial attachment. While *E. coli* previously have been identified in PEMS-affected turkeys and associated as a cause of the disease, this is the first time that enteropathogenic *E. coli* has been identified in this disease.

Experimental reproduction of PEMS with TCV and *E. coli* R98/5. Clinical disease identical to the naturally occurring PEMS was experimentally reproduced in young turkeys by coinfection with TCV and *E. coli* R98/5 (31). Six-day-old turkeys inoculated with only R98/5 did not develop clinically apparent disease, and only mild disease and moderate growth depression were observed in turkeys inoculated with only TCV. However, turkeys dually inoculated with TCV and R98/5 developed severe enteritis with high mortality and marked growth depression. R98/5 infection resulted in attaching/effacing lesions in turkeys inoculated with only R98/5, and turkeys infected with both TCV and R98/5. Attaching/effacing lesions were more extensive and were detected for a prolonged duration in dually inoculated turkeys. Turkeys inoculated with both TCV and R98/5 exhibited the salient features observed in PEMS-affected turkeys: high mortality, severe growth depression, and lymphoid depletion in thymus and bursa of Fabricius. These studies represent the first successful reproduction of PEMS at NCSU College of Veterinary Medicine using laboratory-propagated infectious agents. The interaction of TCV and enteropathogenic *E. coli* provides a possible explanation for the pathogenesis of PEMS; however, we anticipate that other virus-bacterial combinations also may produce this disease. Additional studies are underway to further investigate the interaction of TCV and R98/5 in the pathogenesis of PEMS; the interaction of other viruses and bacteria identified in PEMS-affected turkeys is also being investigated.

Studies on Poult Enteritis and Mortality Syndrome

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Immune alterations associated with PEMS. Our studies have demonstrated a variety of immune alterations associated with poult enteritis and mortality syndrome (PEMS). Affected poults exhibit atrophy of lymphoid organs, such as thymus, bursa and spleen. A functionally decreased proliferative response to phytohemagglutinin mitogen and a reduction in primary antibody response has been observed (42). *E. coli* clearance from circulation is reduced and phagocytic ability of macrophages is adversely compromised. Expression of CD4+ T lymphocytes was significantly lower in PEMS poults at 9 days post-infection. In addition, CD4:CD8 lymphocyte ratios in the blood and spleen were altered in the coronavirus-positive (spleen) and coronavirus-negative (blood) forms of PEMS (33). Interleukin-1, interleukin-6, and nitric oxide were increased in PEMS poults, whereas tumor necrosis factor-α was decreased. Enhanced production of proinflammatory cytokines/metabolites by activated macrophages in PEMS poults may contribute to the intestinal inflammation, gut motility, and anorexia that characterize this disease (32).

***In vivo* studies.** Oral inoculation of 0.2, 0.1, and 0.05 μm fecal filtrates into poults demonstrated that the 0.1 μm filtrate of PEMS fecal material resulted in higher mortality and significantly lower body weight and relative bursa weight, three clinical signs associated with PEMS (35). Subsequently**,** a reovirus, ARV-CU98, was isolated from the 0.1 μm PEMS fecal filtrate (169). Oral inoculation of ARV-CU98 into poults resulted in a higher incidence of thymic hemorrhaging and gaseous intestines as well as a significant decrease in relative bursa and liver weights. Viral antigen was detected in the liver by immunofluorescence, suggesting replication of virus in the liver. Furthermore, functional hepatic degeneration was indicated by altered plasma ALT and AST activities in virus-challenged poults**.** (34).

***In vitro* studies.** ARV-CU98 causes cytopathic effects; most notably syncytia formation followed by sloughing and cell death, in LMH (a chicken hepatocyte cell line) as well as primary cell culture from day-old poult liver but not in MQ-NCSU (a macrophage cell line), or RP-9 and DT-40 (B lymphocyte cell lines). Metabolic labeling with ^{35}S-methionine demonstrated viral replication in LMH but not MQ-NCSU cells as indicated by the presence of viral bands in ARV-CU98 infected LMH cell lysates, but not MQ-NCSU lysates. Furthermore, via transmission electron microscopy, ARV-CU98 was found in abundance in the cytoplasm of LMH-infected cells, but was localized outside MQ-NCSU cells near the cell membrane. These results suggest that although MQ-NCSU cells do not appear to be susceptible to infection by ARV-CU98, an interaction between the virus and macrophages may still occur. In fact, interleukin-1 (IL-1) mRNA was up regulated in MQ-NCSU cells two hours after exposure to ARV-CU98 and seemed to decrease, in a time-dependent manner, up to ten hours of exposure as measured by RT-PCR (167). In contrast, IL-1 mRNA was present in both sham-exposed and ARV-CU98 exposed LMH cells and appeared to decrease following 8-10 hours of exposure, suggesting a possible downregulation of IL-1 by CU98 in

liver cells and signaling the beginning of cytopathic effects. Overall, these data suggest a potential role of ARV-CU98 in PEMS, particularly in immunological and metabolic alterations associated with the disease.

Challenge studies. Poults challenged with PEMS-associated turkey astrovirus (TAst-OSU) exhibit reduced *E. coli* clearance upon challenge. Macrophage-mediated cytokines (IL-1, IL-6) are reduced after *in vivo* or *in vitro* challenge whereas nitrite levels are higher in TAst-OSU exposed macrophages (44). Both PEMS TAst-OSU and Classical Astrovirus (isolated in the 80's) cause reduction in thymic weight in SPF poults. Lymphoblastogenic response to Con-A was lower in TAst-OSU challenged poults where as poults in the Classical Astrovirus exposed group had reduced antibody response (43, 166).

Progress Report on Enteric Disease of Young Turkeys (1997-2001)

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Studies on a Torovirus Involved in Stunting Syndrome

Etiologic agent. The etiologic agent of stunting syndrome (SS) was isolated and identified as a virus in experiments using poults (4). Methods for the *in vitro* propagation of the viral agent were explored and developed (3). The SS virus was characterized with respect to its physicochemical properties (2), and it was found that the SS virus was an enveloped, RNA virus, with morphology and physical characteristics suggestive of a torovirus. Other studies indicated that the SS virus was not related to a number of other viruses including turkey coronavirus, infectious bronchitis virus, bovine coronaviruses, bovine bredaviruses, Newcastle diseases virus and avian influenza virus (1). More recently, we were able to amplify a small portion of the polymerase protein gene of SS virus. Primers were designed based on sequence information from the polymerase protein gene of Bernevirus, a member of the genus torovirus. The sequence of the amplified product was 95% related with other toroviruses. This information, coupled with previous characterization information, suggests that the SS virus is a torovirus.

Diagnostic aids. A fluorescent antibody assay (FA) and an indirect FA (IFA) for the detection of SS virus and serologic antibodies to SS virus, respectively were developed (46). Using these assays we determined that the SS virus exists in other countries (Israel). In addition, survey studies of US turkeys indicated that SS virus infected between 25-30% of the flocks evaluated (168).

We have recently developed a RT-PCR assay for the SS virus. This assay is based on primer sequences that were obtained from randomly primed portions of the SS virus genome, which were cloned and sequenced. Our preliminary data indicates that the primers, for the RT-PCR assay, are specific to SS virus and do not cross react with negative tissue or other known viruses (e.g. infectious bronchitis virus, bovine coronavirus, Newcastle disease virus, or enterovirus). To date, we have only evaluated this RT-PCR assay on purified virus (i.e. SS virus) that had been propagated in turkey embryos. We have not used this assay for detecting SS virus from intestinal tracts.

Prevention and control. Passive immunity was evaluated as a method of protecting poults from stunting syndrome. One-day-old poults were injected with tryptose phosphate broth, an anti-SS virus antibody preparation, or an anti-Newcastle disease virus antibody preparation before challenge. Poults that received anti-SS virus antibodies were significantly heavier ($P < 0.05$) and did not display clinical disease as severe as birds that did not receive the anti-SS virus antibodies. However, the birds that received anti-SS virus antibodies and were challenged were significantly lighter ($P < 0.05$) than birds that were not challenged. The

results of this trial demonstrate that the injection of anti-SS virus antibodies benefited poults undergoing stunting syndrome, but did not provide complete protection. The role of passive immunity, either through breeder hen vaccination or through providing antibodies to poults artificially (i.e., at the hatchery), may have future applications in alleviating stunting syndrome (45).

SS virus and other agents. Using an embryo-infection model, we recently found that when *Campylobacter jejuni* was inoculated into SS virus-infected embryos, fluid secretion into the intestinal tract of the embryo was greatly increased. This effect was not observed when other bacteria (i.e. *E. coli* 0157:H7 and *Strep. faecalis*) were inoculated into SS virus-infected embryos or when the bacteria were used as the sole inocula. Secretion of fluid within the intestinal tract of the embryo is a parameter we use to measure the response of the insulted intestine and the mount of secretion corresponds to the severity of intestinal disease. This potentiated increase in fluid was only detected when viable *Campylobacter jejuni* was used in infected SSA embryos. We hypothesize that *Campylobacter jejuni*, in combination with enteric viruses, causes a severe form of turkey viral enteritis.

Pathophysiologic mechanisms of enteric disease. Embryos that have been inoculated with torovirus via the amniotic route exhibit excessive fluid accumulation in their intestine and decreased intestinal maltase activity similar to poults experiencing SS. Since the alterations in the turkey embryo intestines are like those observed in torovirus infected poults, the embryo provides a model to study the pathophysiology of the torovirus. Histopathological evaluation of the intestinal tracts of either torovirus infected embryos or poults have been unremarkable. That is, we do not observe intestinal cell destruction and changes in the villi that often accompany some enteric viral infections. In fact, it is sometimes difficult to distinguish infected intestines from control intestines histopathologically. Therefore, we hypothesized that immune cells (or their products) may contribute to the pathophysiology of the enteric disease following torovirus infection. To test this hypothesis we injected turkey embryos with supernatants from cultures of turkey peripheral blood lymphocytes that had been stimulated with concanavalin A (Con A) or with sterile media (negative control). We also injected embryos with torovirus as a positive control. We evaluated intestinal fluid accumulation and intestinal maltase activity as parameters for enteric disease (as we have with SS). We found that embryos injected with the Con A stimulated PBL supernatants accumulated fluid in the intestinal tract and had decreased maltase activity, nearly identical to the results seen in torovirus infected embryos. This experiment was repeated with the same results, providing evidence that products of immune cells can cause a disease in embryos that is very much like SS. Thus, these results support our hypothesis that immune cell products may be involved in the pathophysiology of SS enteric disease.

To further test our hypothesis, we injected cyclophosphamide into the turkey embryos to render them immune deficient. We then inoculated immune competent (untreated) and immune deficient (cyclophosphamide treated) embryos with either torovirus or with sterile media. Again, we evaluated fluid accumulation and intestinal maltase activity as parameters of enteric disease. We found that immune competent embryos that had been injected with torovirus accumulated large amounts of fluid in their intestinal tracts and had decreased maltase activity as compared to noninfected control embryos. We also found that immune

deficient embryos infected with torovirus had little, if any, accumulation of fluid in their intestinal tracts and their maltase activities were not decreased. The immune deficient embryos that had been infected with torovirus did not display the clinical manifestations of disease (i.e. intestinal fluid accumulation and decreased maltase) as did the immune competent torovirus infected embryos. We confirmed virus infection in the embryos in both the immune competent and immune deficient groups by FA staining of the intestinal tracts. This provided more evidence that the SS enteric disease may be a manifestation of immune cell products (67).

We also developed a quantitative RT-PCR to measure the amounts of isomaltase mRNA produced in torovirus infected and noninfected control cells. We have found that the amount of isomaltase mRNA was not different between torovirus infected and noninfected cells. Thus, the effects of torovirus infection is not directly inhibiting or interfering with the transcription of isomaltase mRNA in the intestinal cell (66).

Studies on Astroviruses and Small Round Viruses

There have been reports of the presence of small round viruses (28-35 nm in size) in the intestinal/fecal material from turkeys. The identity of these small round viruses (SRVs) and their role in enteric disease is not clear at present. The objective of this study was to obtain genomic sequence information and to develop a reverse transcription - polymerase chain reaction (RT-PCR) assay for use as a diagnostic aid. A small round virus from turkeys (designated as IA isolate; SRV-IA) was propagated in turkey embryos and partially sequenced. A pair of primers was designed for use in the RT-PCR assay. The RT-PCR reaction using the specific primers amplified a product of predicted size (473-bp) from the purified SRV-IA. Similarly, the predicted size (473-bp) product was amplified from the total RNA isolated from the whole intestinal tissue, intestinal epithelial cells (IECs) and cecal fluid from turkey embryos inoculated with SRV-IA. However, total RNA isolated from the same tissues from control (PBS inoculated) embryos did not result in amplification. A positive RT-PCR reaction was also obtained from RNA isolated from the cecal contents of poults inoculated with SRV-IA but not from PBS inoculated poults. The ability of this RT-PCR assay to amplify the genome from other SRV isolates, enteroviruses and astroviruses was assessed. The SRVs evaluated were field isolates from Ohio, Indiana, and Georgia. Turkey astrovirus, enterovirus, enterovirus-like virus, avian encephalomyelitis virus (AEV), duck hepatitis virus - type 1 (DHV-1) and bovine enterovirus (BEV) type-1, 3, and 6 were also included in the study. The primers amplified the genomes of SRV isolates from Ohio, Indiana, and Georgia, turkey astrovirus, and turkey enterovirus-like virus. However, there was no amplification of predicted size product from the viral genome of AEV, DHV-1and BEV types-1, 3, and 6. The results of this study indicate that the SRVs isolates from various regions of the USA had some relatedness. The results also suggest that the SRVs of turkey origin are different from DHV-1, AEV, and BEV type-1, 3, and 6, and a turkey enterovirus isolate (5).

Studies on Poult Enteritis and Mortality Syndrome

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Identification and characterization of infectious agents associated with PEMS. In cooperation with Frank Edens we examined some *Escherichia coli* isolates from poults affected with PEMS. Some of these isolates crossed the mucosal barriers in very young birds and translocated to internal organs. Infectious bursal disease virus (serotypes 1 & 2) did not cause disease or immunosuppression in turkey poults. A variety of viral agents were detected in poults affected with PEMS, including 3 serogroups of rotavirus, reovirus, coronavirus, and a small round virus (SRV). All of the flocks we examined had more than one agent. SRV was further characterized as an astrovirus based on genomic and morphologic characteristics. A bovine coronavirus that caused disease in SPF poults was different from the turkey coronavirus, whereas the turkey coronavirus had genomic and antigenic similarities to infectious bronchitis virus (IBV).

Reproduction of PEMS. Gut contents from poults affected with PEMS induced the disease in SPF poults resulting in 67% mortality and significant decrease in body weight of survivors. The astrovirus alone was shown to induce an enteric disease and gross lesions in the thymus and bursa of Fabricius. Muquarrab Qureshi demonstrated the immunosuppressive potential of the virus. A combination of turkey coronavirus and the astrovirus induced a disease similar to PEMS. Bovine coronavirus was shown to induce a disease in SPF poults similar to that caused by turkey coronavirus. Neither virus caused disease in SPF chicks.

Development of reagents and diagnostic tests. Antisera were developed to 3 serogroups of rotavirus, reovirus, enterovirus, astrovirus, turkey coronavirus, bovine coronavirus and IBV. RT/PCR tests were developed for turkey coronavirus, bovine coronavirus, IBV, astrovirus and enterovirus. Antigen capture and antibody ELISAs were developed for astrovirus.

Usefulness of Findings. The multifactorial etiology of the disease was illustrated. The role of coronaviruses and astroviruses were delineated. Diagnostic reagents and tests were developed.

Isolation of a PEMS-Associated Reovirus Causing Liver Pathology

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Introduction. Muquarrab Qureshi and colleagues have shown that turkeys develop changes in the immune system shortly after inoculation with PEMS material. We speculated that these changes would be a key component in the development of the multifactorial PEMS. We inoculated turkeys with unfiltered material from PEMS-infected bird feces and intestinal contents inoculation and filtrates of the same material, obtained after filtration through 200, 100 and 50 nm filters, to determine which of the filtrates would cause immunomodulation. Feces and intestinal contents were saved from these turkeys and used for a second round of filtrations, followed by inoculation of turkeys to fulfill Koch's postulates. Filtrates were inoculated into MDCT-CU45 (a turkey lymphoblastoid cell line developed from a Marek's disease tumor obtained from experimentally infected turkeys) and this cell-culture propagated virus was used to inoculate turkeys.

Materials and Methods (Virology only, see Muquarrab Qureshi's report on experimental animal work).
Cells. The culture conditions for MDCC-CU45 (a Marek's disease virus induced cells line), LMH cells (a chicken hepatocellular carcinoma cell line), primary and secondary chicken embryo fibroblasts (CEF), chick kidney cells (CKC) and primary chicken embryo liver cells (CEL) have been described.
Antisera. Sera were obtained from experimentally infected turkeys and used in indirect immunofluorescence antibody assays. A commercial FITC-labeled goat anti-turkey Ig (H-L) was used as the secondary reagent. In addition, a FITC-labeled convalescent chicken anti-reovirus IgG was used. Mab 51.3 specific for VP3 of chicken infectious anemia virus (CIAV) was used to test for the presence of CIAV-like viruses.
Viruses and inocula. The FDO strain of reovirus was used. PEMS material was obtained from Muquarrab Qureshi and filtered as described.

Results:
Virus isolation attempts in lymphoblastoid cells. MDCC-CU45 cells inoculated with 200 and 100 nm filtrates occasionally showed enlarged cells. Indirect immunofluorescence showed bright staining using convalescent anti sera, but unfortunately cells stained also when PBS or SPF turkey sera were used. Uninfected control cells and MSB-1 cells did not stain using these reagents, suggesting that the goat sera contained antibodies cross-reacting with a pathogen infecting CU45.
Virus isolation attempts in CEF. We inoculated CEF when the monolayers were approximately 70 to 80 % confluent with supernatant fluids from control and infected CU45

cells and also with the filtrates. We would see thinning of the cultures and some swollen cells would become detached, but generalized or discrete cytopathic effects (CPE) were not observed.

Virus isolation attempts in CEL, LMH, and CKC cells. Supernatant fluids of CEF cultures were used to infect CEL. CPE consisting of syncytia formation, cell swelling, and detachment was observed within 24 to 48 hours. Similar effects were observed when LMH and CKC were inoculated. Virus infected cells stained positively with the anti-chicken reovirus conjugate. Subsequent PAGE analysis (performed by Barbara Sherry) confirmed that the genome contains 10 ds RNA fragments. Negative staining electron microscopy suggests that the particles are between 65 to 75 nm. Fecal samples obtained from the turkeys inoculated with the filtrates showing lesions were analyzed on CEL and found to be positive for reovirus. A new finding was the observation that this reovirus isolate affects the liver.

Discussion:

Several viruses have been obtained from PEMS material, notably the astroviruses and the reovirus. It will be of interest to determine the interactions between these pathogens and the atypical *E. coli* isolates described by Frank Edens.

Poult Enteritis Mortality Syndrome:
Characterization, Diagnosis and Control of a Molecularly Distinct PEMS-Associated Astrovirus and Updated Research

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Introduction. The PEMS research in my laboratory focuses on 1) identifying viral agents associated with PEMS; 2) developing diagnostic tools to identify isolated viruses; 3) developing control and therapeutic interventions strategies; and 4) understanding the role of isolated viruses in PEMS disease. To date, our research has primarily concentrated on a molecularly and serologically distinct strain of astrovirus isolated from the thymus of PEMS-infected turkeys.

Molecular Virology. We characterized the complete PEMS-associated astrovirus genome, and have expressed individual astrovirus proteins in a recombinant transcription/translation system, bacteria, and mammalian cells. Studies are underway to express the astrovirus proteins in a baculovirus system. These proteins can be used for diagnostic and vaccine studies.

Animal Studies. Naïve poults infected with purified astrovirus exhibit clinical signs of PEMS including growth suppression, enteritis, vocalization, atrophy of the lymphoid tissues, and immune system dysregulation. Astrovirus alone causes low levels of mortality. Older birds (> 28 days of age) with either PEMS or astrovirus have mild diarrhea and minor growth suppression. These animals do shed infectious astrovirus in their feces, serving as a potential source for new infections.

Pathogenesis. An astrovirus-specific probe was generated for in-situ hybridization. Using in-situ, we demonstrated that the astrovirus replicates primarily in the enterocytes on the wall of the small intestines and to a limited extent in the bursa. Replicating virus is observed within 24 hours postinfection. In spite of the thymic atrophy, to date we have not found replicating virus in the thymus. The birds are viremic.

Immune Dysfunction. We are examining the effect of astrovirus versus PEMS infection on the innate, humoral, and cellular immune response in commercial turkeys. Extensive studies were performed with the goal of understanding the humoral response to PEMS (and astrovirus) infection in order to develop a vaccine.

Molecular Epidemiology. Astrovirus isolates from different turkey growing states across the United States have been sequenced and compared to the North Carolina isolates. Comparisons are also being made to the 1980's astrovirus isolated in the UK and a chicken astrovirus recently isolated. These studies will allow the monitoring of emerging strains of astrovirus in commercial turkeys.

Diagnostics. A reverse transcriptase polymerase chain reaction (RT-PCR) test was developed to diagnose astrovirus infection in commercial turkeys. This test can be performed directly on

feces or lower intestinal samples from commercial flocks, or on samples first passed through turkey embryos. The initial test is specific to the PEMS-associated astrovirus. We expanded the test in two ways: 1) developed reagents to a very conserved region of the virus that will detect numerous strains of astrovirus, including a chicken astrovirus, the 1980's strain, and theoretically, ANV. 2) A multiplex RT-PCR test was designed that will detect both astrovirus and coronavirus from the same field sample. Antibodies have also been produced to the astrovirus; these are being used to develop a fluorescent-based test.

Control. Current control measures need to focus on biosecurity and proper cleaning of infected houses. The astrovirus is very stable and resistant to most disinfectants. We evaluated the inactivation of the PEMS-associated astrovirus in the laboratory and reported which disinfectants work. Current studies are focused on developing a vaccine or anti-viral against the astrovirus.

Other viruses. Although most of our studies focus on the astrovirus, we also isolated and characterized a novel reovirus strain from PEMS-affected turkeys. The studies described above were also performed for the reovirus. Diagnostic tools (both molecular and serologic) were produced and are available. Finally, limited work was done with turkey coronavirus.

Epidemiology of Poult Enteritis Mortality Syndrome

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Introduction. This project had three components: 1) investigation of environmental and management factors as potential risks associated with poult enteritis mortality syndrome (PEMS); 2) assessment of pest densities relative to PEMS status of the farm; 3) determination of biosecurity in PEMS affected regions.

Risk factors associated with PEMS. The investigation was conducted on 52 farms (26 in Eastern NC and 26 in Western NC). These farms were under contract with four different turkey companies. Using a survey and farm data obtained from the companies, we assessed 478 potential risk factors. It was determined that the hatchery of origin, the removal of used litter by a contractor and rodent control measures were associated with PEMS affected farms. Factors found not to be associated with PEMS included: the integrated company with whom the growers had a contract; proximity to cattle or hogs; distance of the turkey houses from roads or trees; and the method of dead bird disposal. We also demonstrated that mortality associated with PEMS only was limited to the brooding period. Excess mortality observed later in the grow-out period was essentially due to coronavirus infection.

Assessment of Pest densities. Tube traps were used to assess lesser mealworm (beetles) densities in brooder houses on the 52 farms mentioned above as well as on four additional farms located in Eastern NC. House fly populations were estimated using speck cards on the same farms. Twenty-four of the 56 farms had PEMS-positive flocks. No significant association could be demonstrated between fly density or beetle density and PEMS status of the farms. There was also no temporal relationship between increases in pest density and poult mortality rate. Finally, although we had made arrangements to assess rodent population densities, we could not conduct this portion of the project because our main industry partner decided early on in the study to prevent us (and any other non-company personnel) from entering the farms on a regular basis.

Biosecurity in PEMS affected regions. The biosecurity investigation was conducted on 52 farms. Biosecurity data were obtained via a survey instrument, by collecting disposable material (plastic boots and head gear), and by using 24-hour camera surveillance. Overall, biosecurity was quite variable depending on the farm. Only 30.8% of the farms had a gate to prevent entrance by unauthorized personnel, less than 50% had signs advising to stay off farm and only 21.2% of the growers had specific requirements before a vehicle could enter their

farm. Surprisingly, although each company had guidelines limiting access to visitors who had been in contact with other poultry prior to a farm visit, only about 54 % of the growers were aware of such guidelines. Finally, dead bird disposal facilities did not prevent access to rodents or wild birds in 23 % of the farms and less than 25 % used fly proof containers. Requirements for visitors (coveralls, boots, cap, etc.) varied significantly depending on the farm. Biosecurity compliance was also very variable. Twenty-one farms had a login system installed for the duration of the study. Each visitor was required to login his or her visit. Compliance was similar to data reported in the human field for long-term medications for preventative purposes. Indeed, on average, less than 50% of visitors registered.

Spiking Mortality of Turkeys

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Spiking mortality of Turkeys/Poult Enteritis Mortality Syndrome was frequently found associated with four new turkey coronaviruses, two of which were capable of reproducing the disease. These pathogens were cultivated *in vitro*, and were resistant to environmental variables but were sensitive to formalin inactivation. Oral antibiotics reduced post-exposure mortality but not growth reduction. Chickens and cattle were inapparent carriers of these turkey coronaviruses. Nucleic acid sequences coding for turkey coronaviral S-1 glycoproteins were identified, compared to those of bovine enteric coronaviruses, and found to be highly similar. Turkey coronavirus S-1 genes were cloned in a eucaryotic expression vector. Expressed S-1 and the complementary nucleic acid were studied as vaccines. They offered partial protection against turkey coronaviral enteritis. These findings are significant in that turkey coronaviral enteritis was found to be: 1) induced by multiple different coronaviruses, 2) carried inapparently by chickens and cows, 3) very similar to bovine coronaviral enteritis, and 4) partially prevented by S-1 subunit and nucleic acid vaccines. Separation of turkeys from cattle and chickens is suggested due to inapparent carrier states in the latter.

Summary of Accomplishments Associated with Poult Enteritis and Mortality Syndrome

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Introduction. My primary mission, while I was a member of the PEMS Task Force, was to develop a physiological profile of PEMS-afflicted poults. My objective was to determine the physiological effects of the disease and use that information as a basis for design of managerial and nutritional interventions to either alleviate the signs of the disease or prevent its development. My work was conducted with a team of poultry specialists, headed by Dr. M. A. Qureshi and included Drs. G. B. Havenstein, C. R. Parkhurst, J. D. Garlich, S. Pardue, P. R. Ferket, J. L. Grimes, within the NCSU Poultry Science Department and with Dr. Y. M. Saif at the Ohio Agricultural Research and Development Center and Dr. K. A. Schat at Cornell University.

National Turkey Federation Research Award. Due to my activities and publications dealing with poult enteritis and mortality syndrome from 1994 to 2000, the Poultry Science Association selected me to be the recipient of the National Turkey Federation Research Award for 2000. The award, based on published research over a six year period, was announced at the annual meeting of the Poultry Science Association, this time in Montreal, Quebec, Canada, and it is awarded only one time every two years.

Accomplishments

Developed an extensive physiological profile for PEMS

- Characterized PEMS-induced blood constituent alterations and demonstrated hypoglycemia, hypophosphatemia, hypocalcemia, decreased blood proteins, hemoconcentration, and much more
- Characterized PEMS-induced hypothermia during PEMS onset and peak severity and normothermia during recovery from PEMS
- Characterized PEMS-induced biochemical dysfunctions associated with glucose metabolism (relationship between glucose-6-phosphatase, blood phosphorus, blood insulin, blood glucagon, blood glucose, and hepatic glycogen); determined that PEMS afflicted poults had an inability to adequately metabolize glucose
- Characterized malabsorption of nutrients from the gut of PEMS-afflicted poults
- Characterized PEMS-induced alterations in several metabolically active hormones including tri-iodothyronine, thyroxine, IGF-I, IGF-II, glucagon, and insulin in an effort to isolate the cause of PEMS metabolic dysfunction
- Reported adrenal hypertrophy, hepatic atrophy, thyroid hypertrophy, pancreatic atrophy, splenic atrophy, bursal atrophy, and thymic atrophy (In cooperation with Dr. Qureshi, we reported PEMS-associated immune dysfunction based on many of these early observations.)
- Characterized decreasing activities of digestive enzymes with PEMS infection

Developed an extensive histopathology profile of PEMS-afflicted poults that focused on the gastrointestinal tract, the lymphoid organs, the liver, spleen and pancreas.

- Determined that intestinal epithelial cells were damaged by PEMS; microvilli were damaged; terminal web of microvilli was destroyed; mitochondria were ruptured; intracellular organelles in general were damaged
- There were attaching and effacing bacteria, but more importantly were found intense enteroinvasive bacteria associated with epithelial cells
- Acute inflammation of the large and small intestine was characterized by large numbers of eosinophilic granulocytes
- Eosinophilic granulocytes were not all heterophils or myelocytes; histochemical methods revealed than the majority of these cell types were eosinophils.
- Eosinophilia was found to be associated with tissue degeneration in thymus, bursa of Fabricius, necrosis in the large and small intestine, and in the pancreas
- Tissue eosinophilia associated with swollen / ruptured mitochondria within tissue cells

Extensive investigation of atypical *Escherichia coli* initiated.

- Atypical *E. coli* found in nearly all cases of PEMS infections
- Biochemical, serotyping, and infectivity studies revealed that our isolates were enteroinvasive
- Challenge studies with atypical *E. coli* induced conditions that were almost identical with PEMS
- The atypical *E. coli* resistance to commonly used antibiotics and developed resistance, characterized by a biochemical alteration, when exposed to two widely used fluoroquinolones, resistant to Gentian Violet and other antibacterials.
- Atypical *E. coli* found in wells on farms with a history of PEMS; chlorination was helpful
- Atypical *E. coli* also found in soil around barns that had contained PEMS-infected flocks
- Atypical *E. coli* infections resulted in accumulation of very large numbers of inflammatory cells in the large and small intestine, thymus, and bursa of Fabricius; infections were characterized by cellular degeneration where there were eosinophilic cells, tissue cells were disrupted with clear evidence of mitochondrial disruption
- Determined DNA finger prints for *E. coli* isolates, characterized plasmids contained in atypical *E. coli* isolates
- Atypical *E. coli* infection and severity of PEMS was decreased when brooding temperature was increased and litter moisture was decreased
- The atypical *E. coli* work was terminated by the task-force

Numerous intervention studies conducted.

- Feed supplements: MycoCurb, antibiotics, BioMos, BioChrome, MycoSorb and others
- Probiotic Interventions: Lactobacillus reuteri and other organisms
- Water Purification: chlorination, electrolysis of organisms in water supply lines
- Litter management: dryer litter with higher brooding temperatures and improved ventilation

- Water Additives: AquaTize, chloramine-T, and others
- Pre-feeding: Oasis

Summary Comments

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Upon review of the written presentations and taped record of the roundtable, I was struck by the similarity between broiler respiratory disease and PEMS. Broiler respiratory disease can be initiated by Newcastle Disease virus (ND), infectious bronchitis (IB) (a coronavirus), and *Mycoplasma gallisepticum* (MG), but the severity of the disease is often due to secondary infection with bacteria, usually *E. coli.* In addition to these major infectious agents, reovirus, *Mycoplasma synoviae*, *Bordetella avium*, and several others have been implicated in regional outbreaks of broiler airsacculitis. The list becomes even longer when a host of infectious agents not present in the U.S. are considered.

Like PEMS, the bacterial component of the disease can be treated with antibiotics, reducing the mortality associated with the disease. Unlike PEMS, the broiler industry has attenuated vaccines for Newcastle disease (ND) and many of the infectious bronchitis strains at their disposal. When the other infectious agents are involved, the broiler industry must still rely upon depopulation and clean out for control. Fortunately, those two viruses account for most of the field challenge in the U.S.

If we continue with the analogy, there may be lessons to be learned from the broiler industry. Respiratory disease forced the broiler industry to move to all-in, all-out production long ago. Many integrators even try to place chickens in production regions by age.

Vaccines have been very effective in controlling the frequency and severity of the outbreaks. ND vaccination in particular has been extremely effective in U.S. broiler flocks. Recent research, however, has demonstrated that the coronavirus (infectious bronchitis, IB) is capable of rapid genetic recombination, and the question has arisen whether our own heavy use of IB vaccination has promoted the development of variant IB strains. Regardless, IB vaccination is still highly effective in the control of IB outbreaks as long as the field virus and vaccine are similar strains.

Eradication was effectively used to remove *Mycoplasma gallisepticum* (MG) from the list of common broiler respiratory diseases. Great controversy has arisen over recent broiler integrator decisions to live with MG rather than costly depopulation of breeder flocks in a handful of recent outbreaks. Careful biosecurity and antibiotic treatment has permitted the industry to weather several MG outbreaks in the past few years without significant disease spread, and without the economic hardship of depopulation.

Another important aspect of broiler respiratory disease is the interaction of the disease, not only with environmental conditions, such as house ammonia levels or water contamination, but with production management factors. Field observations indicate that broiler growth rate, stocking density, and ration formulation can all have an impact upon the frequency and severity of respiratory disease outbreaks. The stress associated with rapid growth and high

stocking density creates a highly susceptible broiler population. Infectious agents that cause little or no disease in unstressed birds, such as breeder replacement pullets grown in the same area, can wreak havoc in commercial broiler flocks. As a result, broiler respiratory disease is often a "production disease" rather than simple infectious disease triggered by a combination of pathogens; it is often more effectively controlled by management than by vaccination alone.

PEMS may also be a "production disease", affected as much by turkey management practices as by the infectious agents that are involved in the pathophysiology of the disease. If this is true, a combination of vaccination and management tools may be necessary to control the disease in a cost-effective manner. Eradication of all of the potential pathogens may ultimately prove very difficult.

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Final Report

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