ABSTRACT

MACALINTAL, LIZZA MAGSOMBOL. Comparative Pathogenicity Studies on Avian Reoviruses. (Under the direction of Dr. Frank W. Edens)

Poul enteritis and mortality syndrome (PEMS), a condition with multifactorial etiology is characterized by an acute, contagious enteric disease of turkey poults between the ages of 2-4 weeks. The current study was conducted to define the role of PEMS-associated agents on poult performance. In the first study, the “novel” Cornell virus, defined as the reovirus ARVCU98, a small round virus (SRV or ARVCU98) and a turkey astrovirus, Ohio State University isolate (TastOSU), were gavaged orally into the crop of turkey poults. Reduced body weights and reduced relative weights of the bursa of Fabricius, thymus, and liver were observed in virus-challenged poults. The reduced body weight gain and tissue atrophy was exacerbated by the presence of E. coli. In study number two, the possibility of vertical transmission of reovirus via the egg was tested. In ovo inoculation resulted in pathogenic and metabolic alterations in broilers challenged in ovo at day 9 of embryonation with ARVCU98 and the field isolated S1733 (1:100 and 1:500 dilution). In a third study, hyperimmunization of turkey breeder hens against the ARVCU98 reovirus provided limited protection to progeny as indicated by decreased weight gain and loss of lymphoid organ integrity in post hatch ARVCU98-challenged poults. Overall these studies demonstrated that PEMS-associated astrovirus and reovirus affected poult performance by decreasing body weight and altering lymphoid organ integrity, and the addition of E. coli further exacerbated these signs under a controlled environment. Additionally, ARVCU98 reovirus is a turkey
isolate, and the evidence presented herein clearly demonstrated that it can infect broilers and that vertical transmission via the egg is a strong possibility.
COMPARATIVE PATHOGENICITY STUDIES ON AVIAN REOVIRUSES

by

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<th>Full Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>AST</td>
<td>Aspartate aminotransferase</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>AVT</td>
<td>Arginine vasotocin</td>
</tr>
<tr>
<td>BW</td>
<td>Bodyweight</td>
</tr>
<tr>
<td>C</td>
<td>Cortex</td>
</tr>
<tr>
<td>CB</td>
<td>Chromophobe</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CL</td>
<td>Chromophil</td>
</tr>
<tr>
<td>COR</td>
<td>Cisternae of reticulum</td>
</tr>
<tr>
<td>CPE</td>
<td>Cytopathogenic effect</td>
</tr>
<tr>
<td>DAB</td>
<td>Days after boost</td>
</tr>
<tr>
<td>DPI</td>
<td>Days post inoculation</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediamine tetra acetate</td>
</tr>
<tr>
<td>EID</td>
<td>Embryo infective dose</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>F</td>
<td>Follicle</td>
</tr>
<tr>
<td>FDO</td>
<td>Avian reovirus strain</td>
</tr>
<tr>
<td>FITC</td>
<td>Fluorescein isothiocyanate</td>
</tr>
<tr>
<td>HPA</td>
<td>Hypothalamo-pituitary-adrenocortical axis</td>
</tr>
<tr>
<td>IGF</td>
<td>Insulin-like growth factor</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IL-1</td>
<td>Interleukin 1</td>
</tr>
<tr>
<td>IL-6</td>
<td>Interleukin 6</td>
</tr>
<tr>
<td>IZ</td>
<td>Inner zone</td>
</tr>
<tr>
<td>LHM</td>
<td>Chicken hepatoma cell line</td>
</tr>
<tr>
<td>M</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>MT</td>
<td>Mesotocin</td>
</tr>
<tr>
<td>N</td>
<td>Nucleus</td>
</tr>
<tr>
<td>NCARS</td>
<td>North Carolina Agricultural Research Services</td>
</tr>
<tr>
<td>OCT</td>
<td>Optimal cutting temperature</td>
</tr>
<tr>
<td>PAGE</td>
<td>Polyacrilamide gel electrophoresis</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffered saline</td>
</tr>
<tr>
<td>PEMS</td>
<td>Poult enteritis and mortality syndrome</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>PE</td>
<td>Pseudostratified epithelium</td>
</tr>
<tr>
<td>PP</td>
<td>Pancreatic polypeptide</td>
</tr>
<tr>
<td>RER</td>
<td>Rough endoplasmic reticulum</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Reverse transcriptase-polymerase chain reaction</td>
</tr>
<tr>
<td>SCZ</td>
<td>Subscapular zone</td>
</tr>
<tr>
<td>SG</td>
<td>Secretory granules</td>
</tr>
<tr>
<td>SN</td>
<td>Serum neutralization</td>
</tr>
<tr>
<td>SRV</td>
<td>Small round virus</td>
</tr>
<tr>
<td>TastOSU</td>
<td>Turkey astrovirus – Ohio State University</td>
</tr>
<tr>
<td>TCID</td>
<td>Tissue culture infective dose</td>
</tr>
<tr>
<td>TCV</td>
<td>Turkey coronavirus</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TG</td>
<td>Thyroglobulin</td>
</tr>
<tr>
<td>TSH</td>
<td>Thyroid stimulating hormone</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor necrosis factor</td>
</tr>
<tr>
<td>T3</td>
<td>Triiodothyronine</td>
</tr>
<tr>
<td>T4</td>
<td>Thyroxine</td>
</tr>
<tr>
<td>UC</td>
<td>Undifferentiated cells</td>
</tr>
<tr>
<td>VN</td>
<td>Virus neutralization</td>
</tr>
<tr>
<td>ZG</td>
<td>Zymogen granules</td>
</tr>
</tbody>
</table>
Viral Enteric Infections in Turkeys and Broilers

Enteric diseases in turkeys and chickens have been linked to various viral organisms that are enteropathogens, which are capable of mounting enteric infection or conditions in turkeys (Reynolds, 1991). In 2000 (Barnes et al., 2000), the term poult enteritis complex (PEC) was used to describe the syndrome encompassing enteric diseases in the turkey poult. Enteric diseases in poultry are of prime importance because it can cause large economic losses in the poultry industry. Reynolds (1991) emphasized that 1) enteric diseases may not necessarily be caused by only one agent and that detection of virus particle(s) does not necessarily mean that it is the causative agent, 2) it is likely that pathogenesis of the viral enteritis is complex one, and 3) the potential for detecting new viral agent(s) is difficult even if the etiologic virus has been identified or implicated.

Because multiple viruses are associated with enteric diseases (Table 1), this study dealt mainly with the avian reovirus field isolate (S1733), the poult enteritis and mortality syndrome (PEMS)-associated reovirus (ARVCU98) and astrovirus (TastOSU), and atypical Escherichia coli (Types I and II).

Avian reovirus (respiratory enteric orphan virus) infection in commercial poultry industry has been considered a disease with major economic impact and occurs worldwide. This includes economic losses due to poor hatchability, mortality, vaccination expenses, cost of medication, culling and processing related activities. The persistence of reovirus does not necessarily imply infection since it can be isolated from normal, healthy birds, (Rosenberger
and Olson, 1991, Robertson, 1984). Furthermore, depending on the origin, reovirus infection is not only limited to avian species but to mammalian species as well.

In turkeys, reovirus is one of the organisms implicated in PEMS (Heggen-Peay et al., 2002) and tenosynovitis (Jones, 2000). Reoviruses are considered to be age-linked and dose dependent for infection to take place. The occurrence of runting and stunting (malabsorption) in chickens and turkeys is a major concern of poultry raisers. Affected birds fail to reach desirable market weight at the end of the grow-out period. In 1997 (Montgomery et al., 1997) isolated a variety of organisms from intestinal homogenates of infected birds, which primarily included twelve aerobic, two anaerobic bacteria, IBV (infectious bursal disease virus), a reovirus, and two bacteriophages.

Table 1.1 Enteric viral infections (from Reynolds, 1991).

<table>
<thead>
<tr>
<th>Virus Type</th>
<th>Species affected</th>
<th>associated condition/disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus (group II)</td>
<td>Turkey</td>
<td>Hemorrhagic enteritis</td>
</tr>
<tr>
<td>Astrovirus</td>
<td>Turkey</td>
<td>Turkey viral enteritis</td>
</tr>
<tr>
<td>Corona virus</td>
<td>Turkey</td>
<td>Blue comb disease</td>
</tr>
<tr>
<td>Enter virus</td>
<td>Turkey</td>
<td>Diarrhea/enteritis</td>
</tr>
<tr>
<td>Orthoreovirus</td>
<td>Turkey</td>
<td>Turkey viral enteritis</td>
</tr>
<tr>
<td>Parvovirus-like virus</td>
<td>Turkey</td>
<td>Enteropathy, stunting, diarrhea</td>
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Enteritis in Turkeys

Poult Enteritis and mortality Syndrome (PEMS)

History

The PEMS condition emerged as an important disease in the mid-90’s and nearly destroyed the turkey industry in the Southeastern United States. At the time of its appearance, there was no known etiology for the disease. It was reported initially in a flock of turkeys in western North Carolina (Barnes and Guy, 1997). Within two years after its emergence, USDA-ARS reported that PEMS-losses amounted to more than $35 million (ARS Annual Report, 2003). Recent surveys have suggested that since 1991, PEMS has cost the turkey industry approximately $15 million per year or about $0.05 per turkey poult placed and grown to market age. To date, PEMS is classified as a disease with a multifactorial etiology owing to the fact that several other organisms were found to be associated with the disease process (Heggen-Peay, 2002b). Viral agents such as coronavirus (Lin, et al., 2002; Guy and Barnes, 2000; 1989 Yu et al., 2000), adenovirus (Yu et al., 2000), astrovirus (Yu et al., 1998, 2000; Qureshi et al., 2000; Koci et al., 2000) and reovirus (Schat et al., 1998) were implicated as well as bacterial agents like; salmonella, campylobacter, clostridia and *E. coli* types I and II (Edens, et al., 1997abc).

Description of the Disease

In the fall of 1991, acute enteritis, thymus and bursal atrophy in commercial turkey poults from North Carolina was reported as turkey spiking mortality (Brown et al., 1997). It had a rapid onset with mortality of 1% of the flock per day for 1-5 days in 5-25 days old
poults. During its emergence PEMS was turkey spiking mortality (Barnes & Guy 1997), but the description was later called PEMS due to its less severe clinical signs (Barnes, et al., 2000). Mortality averaged about 12% of all the poults placed in a brooder house and peaked around 19 days of age. Feed refusal, vocalization, enteritis, diarrhea, decreased growth, high mortality and flock unevenness were commonly found in poults affected by PEMS. The spike in PEMS-attributed mortality was correlated with low feed intake, poor nutrient absorption, hypothermia and hyypo-phosphatemia (Edens, 1997; Edens et al., 1997abc; Qureshi et al., 1997). Poults that survived the infection were unable to compensate for the stunting associated with the disease (Odetallah, 2001) because there was impaired nutrient absorption and energy utilization (Doerfler et al., 2001ab). Further characterization of PEMS infection revealed that challenged poults had severe malabsorption (Doerfler et al., 2000a). Depressed blood levels of insulin, thyroxine (T_4) and triiodothyronine (T_3) were characteristic for PEMS infected poults (Doerfler et al., 2000b).

**Disease Transmission**

PEMS can be readily transmitted through direct contact exposure to seeder poults (Odetallah et al., 2001; Doerfler et al., 1998; Heggen et al., 1998; Qureshi et al., 1997). Co-housing normal, healthy poults with suspected PEMS-infected poults resulted in decreased body weight at 6 days post-exposure (Qureshi et al., 1997). As early as 2 days post-exposure (Doerfler et al., 1998), huddling and clinical signs of PEMS have been reported. Brown et al. (1997) used litter from previously infected flocks and reproduced spiking mortality within five days in poults raised on the litter.
Accordingly, infection through oral transmission has also been reported. Experimental oral inoculations of filtered fecal and intestinal contents as well as thymic materials from infected poults were shown to induce PEMS-like signs (Heggen-Peay et al., 2002b; Doerfler et al., 2000ab; Qureshi et al., 1999; Schultz-Cherry et al., 2000). Egg transmission of PEMS has not been reported but that possibility does exist.

Description of PEMS – Associated Agents

Turkey Coronavirus

Turkey corona virus (TCV) was first isolated and associated with poult enteritis (Lin et al., 1996). Later PEMS infection was found to be induced by TCV negative fecal material (Carver et al. 2001; Barnes et al., 1997). In fact all the works published by Doerfler, Edens and their colleagues were the result of studies conducted with TCV negative fecal inocula. When *E. coli* was challenged into TCV-positive poults, it took about 60 minutes to clear the bacteria in the bloodstream (Heggen et al., 1998). PEMS positive, TCV-negative poults had a lower CD4+/CD8+ ratio compared to the PEMS positive, TCV-positive and control poults at 14 days post infection. TCV-positive poults did not diminish the number of cells in the mononuclear phagocytic system (MPS) but showed an increased macrophage recruiting ability (Heggen, et al., 1998). These observations suggest that PEMS caused impaired the MPS and altered lymphocytic populations.

Small round virus (SRV)

A novel small round virus (SRV) was isolated at the Ohio State Agricultural Research and Development Center from PEMS-positive, TCV-negative fecal material (Yu et al.,
2000). It was reported that when the SRV was given orally to turkey poults (Qureshi et al., 2000), it induced clinical signs similar to those in poults exhibiting a mild form of PEMS and was characterized by diarrhea, weight loss, lymphoid organ atrophy, and alteration in the lymphocyte subpopulation as well as reduction in the lymphoproliferative response to concanavalin A (ConA). When SRV was given in combination with TCV, mortality was exacerbated (Yu et al., 2000ab). Later studies (Qureshi et al., 2001) revealed that the virus capsid of the Ohio SRV had a 100% homology with an astrovirus implicated in PEMS (Koci et al., 2000). The Ohio State University SRV would later be designated as Tast-OSU (Qureshi et al., 2001)

**Turkey Astrovirus**

Astrovirus has been found routinely in poults exhibiting diarrhea and enteritis with unknown etiology (Reynolds et al., 1986). In turkey hatchlings, astrovirus has been associated with diarrhea (Thouve nelle et al., 1995). It has been suggested that turkey astrovirus is one of the many etiologic agents linked with development of PEMS. Qureshi et al. (2001) reported that Tast-OSU challenge induced defects in macrophage effector functions, implying that PEMS Tast-OSU can potentially impair the immune responsiveness of turkeys by reducing macrophage viability and decreasing phagocytosis and intracytoplasmic killing of *E. coli*. Another astrovirus, TastV (Schultz-Cherry et al., 2000) was isolated from PEMS-positive, TCV-negative turkey poults. Astrovirus replication can be inhibited by cellular nitric oxide production (Koci, et al., 2004).
Reovirus – ARVCU98

Fecal filtrates (100nm) from PEMS-positive, TCV-negative infected turkey poults were studied by Heggen-Peay and co workers (2002a). The ARVCU98 reovirus was a novel virus isolated and described by Heggen-Peay et al. (2002). Oral challenge with this isolate caused intestinal and cecal inflammation as well as excessive flatulence (Heggen-Peay et al., 2002a) similar to signs in poults suffering from spiking mortality (Barnes and Guy, 1997). Lymphoid organ integrity was compromised as evidenced by atrophy of the bursa (75%), thymus (99%) and spleen (75%), which was similar to the signs of experimentally induced PEMS (Qureshi et al., 1997). Heggen-Peay et al. (2002) also reported that ARVCU98 decreased relative weights of bursa and thymus.

Thymus has been shown to be an important target in the PEMS pathogenesis. Poults challenged with thymus filtrate from PEMS-infected birds manifested diarrhea, growth depression, mortality, pathology, and immunosuppression similar to poults exposed to the intestinal filtrate (Schultz-Cherry et al., 2000). Liver weight on the other hand was found to be decreased as a result of oral inoculation with the ARVCU98 (Heggen-Peay, 2002a). The ARVCU98 virus can be isolated easily at 3-6 days post infection but thereafter virus shedding diminishes.

The ARVCU98 virus isolate was found to be susceptible to heat. At 80°C the virus was completely inactivated, but it was only partially inactivated at 60°C. Through polyacrilamide gel electrophoresis (PAGE) analysis, it determined that ARVCU98 contains 10 segments of double stranded RNA in which the pattern of migration is not similar to that of the avian reovirus strain (FDO) and the mammalian Dearing strain (Heggen-Peay et al., 2002a). Using different cell lines, it was observed that ARVCU98 had cytopathic effects
(CPE) on a chicken hepatoma cell line (LMH) and primary turkey liver cells. The CPE includes syncytia formation, cell death and sloughing off of the adherent infected cells. The liver appeared to be the targeted site of replication because ARVCU98 did not induce the same CPE on either the macrophage or B cell lines (Heggen-Peay et al., 2002b). This can explain liver atrophy in PEMS infected pouls (Heggen-Peay et al., 2002b).

PEMS induces atrophy of the primary and secondary lymphoid organs leading to altered immune response (Qureshi et al., 1997). There is enhancement of interleukin-1 (IL-1) and IL-6 activity and nitrite production and lowered tumor necrosis factor (TNF) (Heggen et al., 2000; Qureshi et al., 2001). Up-regulation of different macrophage-produced cytokines appears to have contributed in the inflammatory process in the intestines leading to diarrhea, increased mucosal permeability, and nitrite production, which has been linked to inhibition of virus replication (Heggen et al., 2000; Koci et al., 2004).

**Escherichia coli**

Edens et al., (1997 a, b) isolated two aggressive and one time considered as atypical *E. coli* (BBL: 36570 and 34560 for colony types 1 and 2, respectively; API-20E: 5144572 and 5144512 for colony types 1 and 2, respectively) in PEMS infected pouls. When given orally, these isolates caused mortality, diarrhea, weight depression, and cyclophosphamide treatment further enhanced the response (Edens, et al., 1997a). Additionally, these *E. coli* isolates caused the ileal epithelium cell microvilli and subcellular organelles to be damaged, which contributed to malabsorption of nutrients associated with PEMS infection (Edens et al., 1997a). These *E. coli* isolates were very important in determining that PEMS had a
multifactorial etiology, and that *E. coli* might be the ultimate cause of mortality in pouls that had been compromised by either reovirus or astrovirus infection.

**Detection**

Aside from monitoring clinicopathological findings, surveillance of on-farm activity is a useful methods of detecting PEMS. Other methods used to detect agents associated with PEMS infection are immunofluorescent testing for the presence of viral antigen (Heggen-Peay et al., 2002a; Qureshi et al., 1999) and demonstration of the virus through isolation and eventual electron microscopic examination. Recently, Koci et al. (2000) developed an RT-PCR for detection of PEMS-associated astrovirus.

**Prevention**

The implementation of strict biosecurity, which includes effective cleaning and disinfection as forms of prevention, has been considered. Since 1.0% formaldehyde was found to be effective *in vitro* to inactivate spiking mortality organisms (Brown et al., 1997), then it is possible to use this as a cleaning /disinfecting agent. No vaccines have yet been developed for PEMS, but limited use of some antibiotics can be used to avert possible bacterial infection that complicated the disease. Nutritional intervention resulted in limited improvement in flock performance (Doerfler et al., 2000a; Roy et al., 2002). The addition of electrolytes, glucose and citric acid to drinking water improved the humoral immune responses of pouls with PEMS (El Hadri et al., 2004).
Enteritis in Chickens

Avian Reovirus

History

The history of avian reovirus was reviewed by Van der Heide (2000). Briefly, reovirus was first reported by Olson and co-workers in 1957 as the synovitis-inducing agent and Kerr and Olson (1964) found this agent to be pathogenic in young broilers chickens. Hence, it was called the viral arthritis agent after determining that the synovitis-inducing agent was a virus (Olson et al., 1966). This was similar to the Fahey-Crawley virus (Fahey et al., 1954) originally isolated in chickens infected with chronic respiratory disease and later confirmed by Petek et al. (1967). It was not until 1972 that this virus was found to be a reovirus when it was confirmed through electron microscopic analysis (Walker et al., 1972).

Diseases Associated with Reovirus

Aside from viral arthritis and leg weakness-related problems (Goodwin, et al., 1993; Robertson and Wilcox, 1986; Rosenberger and Olson, 1991), numerous other disease conditions such as myocarditis, pericarditis (Rosenberger et al., 1988), hepatitis (Mandelli, 1978), splenitis (Hieronymus et al., 1983), bursal atrophy (Kibenge, 1987), enteric problems and malabsorption syndrome (Kibenge and Wilcox, 1983), respiratory disease (Fahey and Crawley, 1954), and immunosuppression (Sharma et al., 1994) have been reported in avian reovirus-infected chickens. Although, avian reovirus mainly affects chickens and turkeys (Page et al., 1982), it was also found to infect geese (Palya et al., 2003), pheasants (Mutlu et al., 1998), and quail (Magee et al., 1993).
**Virus Description**

Reovirus (respiratory enteric orphan virus) belongs to the family *Reoviridae* genus *Orthoreovirus* (Urbano, et al., 1994). Avian reovirus differs from mammalian isolates in that it lacks hemagglutination activity (Glass et al., 1973), lacks ability to induce cell fusion (Wilcox, 1982), and is associated with naturally occurring pathological condition (Robertson and Wilcox, 1986). Additionally, if one is using the virus neutralization test, no cross-reaction between avian and mammalian viruses has been observed (Spandidos and Graham, 1976). Similarities between avian and mammalian viruses do exist- both are RNA viruses, they are heat, ether and chloroform resistant, and particle size is between 50-100nm in diameter (Desmukh and Pomeroy, 1969b). The structure and function of reovirus has been reviewed (Joklik, 1981). Reovirus is a double stranded, non-enveloped, RNA virus surrounded by double concentric icosahedral capsid shell which contains transcriptase and methyl transferase as an integral part of the viral core (Spandidos and Graham, 1976). The migration pattern through polyacrylamide gel during electrophoresis revealed that reovirus is made up of 10 genomic segments, namely large (L), medium (M) and small (S) numbered 2, 3, and 4, respectively (Gouvea and Schnitzer, 1982; Hrdy et al., 1979; Spandidos and Graham, 1976; Shatkin et al., 1968) and 4 non-structural proteins (microNS, sigma NS, p17, and p10) (Bodellon et al., 2001). Additionally, there are at least 10 viral proteins (Ni and Kemp, 1995; Varela, 1994) and each of which shows different genomic segments (Varela, 1994). Similarly, the protein encoded by avian reovirus has three classes namely; λ (large), μ (medium) and δ (small). The S1 segment contributes to the infective nature of reovirus (Ni and Kemp, 1995; Weiner et al., 1977). Subsequently, a polymorphism was observed among
reovirus isolates within the same serotype (Wu, et al., 1994; Rekik 1990; Gouvea and Schnitzer, 1982b; Hrdy et al., 1979).

![Diagram of Avian Reovirus particle](from Connolly, J. L. and T. S. Dermody. 2002).

**Mode of infection**

Horizontal and vertical transmissions are two possible routes of infection for avian reovirus. It has been well established that the horizontal mode of reovirus transmission occurs through direct exposure to feces (Kerr and Olson, 1964; Jones, 1972 and 1978; Robertson, et al., 1984). Another mechanism is through vertical transmission, whereby, the progeny acquire virus from the dam while the ova are *in situ* (Hussain et al., 1981; Menendez et al., 1975; Van der Heide and Kalbac, 1975; Desmukh and Pomeroy, 1969b). Glass (1973) showed the possibility of egg transmission. This form of disease transmission occurred at a level much lower than that horizontal. Menendez and co-workers (1975) were able to show egg transmission of reovirus by inoculating 20 breeders with FDO-1 isolates through nasal, oral, and esophageal routes, and subsequently, three birds tested positive for reovirus (Menendez et al., 1975). Van der Heide (1975) challenged a low dose of reovirus
subcutaneously into breeders and later detected infection in eggs laid between 8 and 12 days post inoculation. In a related study (Al-Muffarej, et al., 1996) showed that both the trypsin resistant and non-resistant strain of reovirus was transmitted to eggs although at a different rate, the former having the higher (19.2%) rate.

**Pathogenesis**

It has been reported that in reovirus pathogenesis, both the intestine and bursa (Jones, 1989; Kibenge, et al., 1985) serve as the portal of entry and site of initial virus replication. At 12-24 hours after oral inoculation, virus particles can be detected in these organs. Afterwards, it spreads to other tissues/organs through the circulatory system. Two days after infection regardless of site of experimental inoculation (Ni, et al., 1995), virus can be detected in the liver and spleen, and the number of virus particles peaks at 4-6 days after challenge. Ni, et al. (1995) noted that reovirus replicates primarily in the intestines and can spread to other organs. Avian reovirus causes enteritis or malabsorption, but when it spreads to other organs, it induces viral arthritis. Both malabsorption and viral arthritis can be found in the same bird. According to several studies, the liver appears to be the primary target organ for reovirus replication (Jones, 1989; Kibenge, et al., 1985; Mandelli, 1978). However, there appears to be greater virus expression in the duodenum than in the liver, and it is likely that much of the virus load is shed through the feces (Kibenge et al., 1985).

It has been implied that mobile cells, *i.e.*, macrophages facilitate the spread of reovirus (Kibenge et al., 1985; Tang et al., 1987). Additionally, macrophages from reovirus-infected chickens are primed to produce nitric oxide in response to T cell cytokines and bacterial lipopolysaccharides (Pertile et al., 1996; Neelima et al., 2003).
It has been reported that reovirus infection is deemed to be age-linked and dose-dependent (Jones, 1985; Roessler and Rosenberger, 1989; Meanger, et al., 1997). It was pointed out that age-associated infection (Roessler and Rosenberger, 1989) might be due to improved ability of the bird’s immune system to prevent virus dissemination and in lower cellular virus content in all of the affected tissues. Clinical disease tends to be more pronounced in birds challenged at 1 day of age compared to challenge at two weeks of age (Rosenberger et al., 1989). Repeated oral exposures to the virus can lead to virus persistence in the intestines compared to a transitory influence associated with a single oral inoculation (Jones, 1994). Highly pathogenic isolates (2408, S1733) cause extensive cellular damage compared to a low pathogenic isolate (2177) (Rosenberger, et al., 1989). Meanger et al (1997) confirmed that high challenge doses of the three reovirus isolates resulted in more severe lesions. Olson (1959) suggested that the inflammatory process during reovirus infection persists even when no virus can be detected.

**Virus replication**

For reovirus replication to occur, cellular apoptosis is important, and in order for this to happen, virus attachment of the protein sigma1 to a cell surface receptor is necessary (Tyler et al., 1995). Apoptosis is the cellular host response to infection (O’Brien, 1998), and it is directly induced by reovirus infection both *in vivo* and *in vitro* (Labrada et al., 2002; Connolly et al. 2002). *In vitro*, the cytopathic effect (CPE) induced by reovirus is manifested by cell shrinkage, rounding, detachment from plate, nuclear damage, chromatin condensation (Fig. 2), which usually occurs 8 hours post infection and plateaus around 16 hours (Labrada et al. 2002). Thus, the reovirus CPE involves both apoptosis and syncitium formation (Bodelon et al., 2002). The S1 genome segment of reovirus is responsible for the syncytium-
inducing property of the virus (O'Hara et al., 2001; Duncan et al., 1988) through its encoded 10kDa (p10) fusion protein (Shmulevitz et al., 2000; Bodelon et al., 2000).

Fig. 1.2 Electron microscopic appearance of uninfected cell (A) Infected cells undergo various stages of chromatin condensation (B), margination of chromatin at the nuclear membrane (C) and complete condensation of the nucleus (D). Courtesy of Tyler et al., 1995; Journal of Virology.

Following infection, reovirus particles specifically protein δ1 (Fig. 1.2) (Labrada et al., 2002; Frazier et al., 1990, Matinez-Costas et al., 1997), attaches to the cell surface sialic acid (Barton, et al., 2001) and JAM (Connolly et al., 2002). Protein δ1 is a filamentous lollipop-shaped molecule about 48 nm in length, with a flexible "tail," approximately 40 nm long by 4 to 6 nm wide, and terminates at its distal end in a globular "head", which consists of the carboxy-terminal domains containing the receptor-binding sites folded into compact globular conformations (Frazier et al., 1990). Apoptosis is characterized by virus-receptor engagement, fusion with or penetration of cellular membranes, and disruption of host cell transcriptional and translational machinery (Everett, et al., 1999). Reovirus receptors do not initiate the signaling events that elicit apoptosis from the cell surface, but rather from endocytic vesicles (Connolly et al., 2002). Viral uncoating is a major requirement for
programmed cell death induction (Labrada et al., 2002). This takes place at the endolysosomes in the cytoplasm. For example, when researchers treated the cultured cell with ammonium chloride at the earlier onset of infection, viral protein replication was inhibited (Connolly et al., 2002). During viral uncoating the outer capsid protein $\sigma_3$ is removed, followed by proteolytic cleavage of the inner capsid proteins $\mu_1$ and $\mu_{1C}$ to form $\delta$ and $\phi$, and conformational changes in $\sigma_1$. Consequently, this leads to the formation of infectious subviral particles (Connolly et al., 2002). Early transcription of the double stranded RNA is encoded by viral polymerase. Transcription and translation of genome segments takes place, and RNAs are then conservatively transcribed leading to synthesis of (+) sense mRNAs.

These transcripts leave the core particle and are translated in the cytoplasm. The capsid is assembled and the particles are released (Fig. 1.3) (Connolly et al., 2002).
Virus Detection

Several diagnostic tests have been developed to detect the presence of reovirus in infected birds. In cultured cells, the CPE is evident at 72 hours post-challenge. For the standard serological testing employed in the detection of reovirus, serum neutralization (SN) is a commonly used technique (Giambrone, 1988; Olson, 1975). SN can detect specific long lasting antibodies, but the method requires the use of live virus and cell culture. For large sample sizes, the ELISA assay for detection of reovirus can be used. The ELISA results closely correlate with the serum neutralization results (Liu et al., 2002). A variation of the ELISA assay is the monoclonal capture ELISA test (Pai, 2003; Chen et al., 2004; Liu, 2000). This technique is more sensitive than conventional ELISA since it detects the target viral RNA. For example, the ELISA results obtained when using the expressed sigmaC and sigmaB proteins were found to be 100% correlated with serum neutralization and that between serum neutralization and conventional ELISA, it showed about 89% correlation (Shien et al., 2000; Liu et al 2002). In this thesis, the expressed sigma proteins were used as coating antigens in inducing the neutralization of antibodies against reovirus. Sigma proteins are the targets for type specific neutralizing antibodies (Wickramasinghe et al., 1993). The sigmaB protein is the major component of the outer capsid while the sigmaC is the part that attaches to the cell (Schnitzer, 1982). Therefore, specificity is apparent. Additionally, molecular techniques were developed (Liu et al., 1999) such as in situ hybridization (ISH) and reverse transcriptase in situ polymerase chain reaction (RT-in situ-PCR), although the RT in situ PCR test was more sensitive and provided the rapid, sensitive, and specific detection of avian reovirus infections compared with the ISH. Earlier, indirect fluorescent
antibody testing (IFA) was applied to tissue sections or impression smears to determine the presence of viral antigens (Adair, et al., 1987).

**Prevention**

Avian reovirus is naturally infective in domestic fowls, but the mere presence of the virus is not indicative of an infectious condition. Induction of an infectious condition is dependent upon numerous events in the bird responding to the alteration of the host environment. To assure prevention of infectious bouts of reovirus infection, poultry producers must adhere to strict biosecurity practices such as thorough cleaning and disinfection of poultry houses after each grow out cycle. Since reovirus can survive for at least 10 days on feathers, wood shavings, chicken feed and eggshells (Savage et al., 2003), it is important that these materials be removed from the poultry houses when clean-up and disinfection is done.

Vaccination is one of the most important tools for prevention. This has led to development and usage of vaccines, live and inactivated (Van der Heide, 1983). It is aimed at providing direct immunity to chickens through active immunization either by administering the vaccine at a young age or to breeders, who pass antibodies to their chicks via the egg. Passive immunity facilitated by maternal vaccination allows for the transfer of mainly immunoglobulin G (IgG) antibody via the eggs. These IgGs are sequestered from the maternal blood and transported into the yolk mass and can cross the embryonic yolk sac membrane into the embryonic circulation (Dohms et al., 1978; Roth et al., 1976; Kramer et al., 1970). Therefore, by vaccinating the breeders, egg transmitted diseases such as those caused by reovirus may be blocked (Van Loon et al., 2001). Breeder vaccination against
tenosynovitis resulted in immunity of progeny against experimental reovirus challenge compared to unvaccinated control (Van der Heide et al., 1976). Recently, in ovo administration of an antibody complex vaccine was studied (Guo et al., 2002). Given at day 18 of incubation, the antibody-complex vaccine provided at least 70% protection and apparently did not affect hatchability. With regard to available breeder pullet vaccination programs, it was reported that, day old progeny from hyperimmunized pullets receiving one dose of live and 2 doses of inactivated reovirus vaccine provided the highest numerical antibody titer and best resistance to clinical infection following experimental challenge (Giambrone, 1986). However, in one study, multiple vaccinations of chickens with reovirus strain RAM-1 produced a broadly specific neutralizing antibody response, but the protective immunity against challenge seemed to be type-specific (Meanger et al., 1997)

**Organs Associated with the Digestive Tract**

**Liver**

Avian liver is subdivided into a left and a right lobe that is connected cranially by a bridge dorsal to the heart (Dyce et al., 1996). The right lobe is larger than the left lobe. The chicken liver weighs about 35-51g representing 1.7-2.3 % (relative weight) of the body weight (Nickel et al., 1977). The largest part of the liver is located in the part of the body enclosed by ribs while the remainder lies in the sternum. It lies against the heart, proventriculus, gizzard and cranial end of the duodenal loop, the spleen and the gall bladder. Hepatocytes and mesenchymal cells comprise the largest cellular mass in the liver.

The importance of the liver to the metabolic activity of the body can be seen in large quantities of nutrients and other substances it receives via the portal vein from the gut
The body cannot directly utilize many of the substances absorbed from the intestinal tract, and therefore, those substances must be converted or otherwise stored in the liver before being released into the circulation. The liver is involved in numerous important functions in the metabolism of protein and lipid (Geraert et al., 1996; Belloir et al., 1997; Griffin et al., 1992; Leveille et al., 1975), and carbohydrates and vitamins in addition to its vital role in the detoxification of body wastes and other toxins (Nickel et al., 1977). The endocrine function of the liver is to synthesize proteins for gradual release into the bloodstream instead of storing them in the cytoplasm as secretory granules. These protein granules are produced by the hepatocytes.

Another type of cell present in the liver consists of the phagocytic cells of the reticuloendothelial system and is known as Kupffer cells, which are liver resident macrophage cells (Chang et al., 1996). These cells secrete cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor α (TNF) (Kaiser, 1996), which are involved in synthesis of acute phase reactants. In pouls exhibiting PEMS, IL-6 and IL-1 production were increased as a result of the ability of macrophages to produce these cytokines despite the impaired ability of the cells to phagocytized foreign antigens (Heggen-Peay, 2002). In addition, during hepatic injury the extent of the cellular damage can be determined by the blood alanine aminotransferase (ALT) and blood aspartate aminotransferase (AST) activities (Chang et al., 1996).

While the liver is the principal organ where insulin-like growth factor I (IGF-I) and IGF-II are derived (McNabb, 2000), their production by a variety of extrahepatic tissues suggests both autocrine and paracrine modes of action in addition to typical endocrine mechanisms (Le Roith et al., 1992). In agreement with this concept, IGF-I gene expression is
not detected in the liver until hatch (Kikuchi et al. 1991), which may mean the circulating IGF-I is extrahepatic in origin in the developing avian embryo (McMurtry, 1998). Avian IGF-I has been purified and sequenced, and it was found to contain 8 amino acid substitutions in comparison with human IGF-I (Ballard et al., 1990). On the other hand IGF-II was found to contain 13 amino acid residues that differed from human IGF-II, and six of these 13 differences occurred in the C-domain sequence (Kallincos et al. 1990). Insulin regulates the transcription of these related growth factors (Houston and O’Neil, 1991) that are structurally and functionally similar with that of the IGFs (Lewitt, 1994). In addition, IGFs actions are mediated by surface receptors which in turn are mediated by the IGF binding proteins (IGFBP). These IGFs were found to be important in growth, development and metabolism in birds (McMurtry et al., 1997). In both chickens and turkeys, IGF-II is relatively higher than IGF-I during embryo development and declines with time (McMurtry et al., 1998). IGF-I is important during posthatch skeletal muscle development (Conlon et al., 2002). In PEMS infected poults, depression of both of these factors was reported (Doerfler et al., 2000). Similarly, in a study by Leili et al. (1997), prolonged feed restriction led to a progressive growth retardation, wherein body-weight gain and bone growth were reduced. The plasma concentrations IGF-I and IGF-II were decreased, and the degree of reduction in the plasma concentrations of these growth factors seemed to be directly proportional to the magnitude of feed restriction.

Likewise, fasting, protein deprivation, and insulin deficiency depressed the levels of both IGF-I and IGF-II (McMurtry, 1998; McMurtry, et al 1997; 1996). According to Kita (1996), IGF-I and not IGF-II is affected by plane of nutrition in chickens. But then, it was later suggested that plasma IGF-I dramatically increases after withdrawal of feed and return
to normal level after refeeding (McMurtry, et al., 1998). And that IGF-II responds differently to nutritional state in chicken either it is feed withdrawal against feed restriction. The action of IGFs at cellular levels depended on their concentration in the circulation.

Endocrine Glands

Pituitary Gland

The pituitary glands or hypophysis lies at the base of the brain below the hypothalamus, and is subdivided into two parts, the adenohypophysis (glandular part) and neurohypophysis (neural part). Unlike mammals there is no pars intermedia in birds (Scanes, 1986). In chickens, differentiation of pituitary glands from Ratkhe’s pouch occurs at day 10 of embryonation (Sturkie, 2001). This reddish brown organ weighs about 10-22mg in hens and 11-25mg in cockerels and about 13-36mg in capons (Nickel et al., 1977). It is the major endocrine gland as it influences the secretion of hormones from several other glands. Typically, cells in the pituitary gland are classified according to the type of hormones they secrete, e.g., a) somatotrophic b) mammotrophic/lactotrophic c) gonadotrophic d) thyrotrophic and e) corticotrophic cells (Bossis et al., 2004; Mikami et al., 1984). The secreted hormones act on target organs, tissues, or cells by interacting with specific receptors found cell surfaces, within the cell’s cytoplasm, or nucleus (Sturkie, 2001).

The posterior pituitary gland on the other hand, is made up of the neurosecretory terminals for the secretion of mesotocin (MT) or arginine vasotocin (AVT) (Scanes, 2000). AVT is an antiuretic hormone and is the counter part of arginine vasopressin in mammals while MT is the oxytocin principle. AVT modulates different aspects of reproductive
behavior including courtship vocalization and copulation (Jurkevich et al., 2003) in addition to its role in osmoregulation and adaptation processes during the perihatch period (Takahashi et al., 2003; Grossman et al., 1995). MT is decreased during acute heat stress (Wang et al., 1989). Infusion of MT has no effect on cardiovascular functions, plasma ions and osmolality (Robinson et al., 1988), but it does participate in some regulatory effects on the blood flow to the kidneys as renal perfusion during hemorrhage is positively correlated with plasma MT levels (Bottje et al., 1989).

**Pancreas**

The pancreas is a long, narrow, grey-white gland that lies in the mesentery connecting the duodenal loop (Hodges, 1974), it consists of dorsal and ventral lobes which are connected distally (Dyce et al., 1999). In chickens, the notochord is the source of chemical signals required for pancreatic development (Seung et al., 1997). The ablation of the notochord at stage 11 of embryonation leads to the absence of gene expression needed for pancreatic differentiation (Seung et al., 1997). The avian pancreas is divided into two major and functionally distinct areas; the endocrine pancreas, which consists of hormone producing cells and exocrine cells which secrete enzyme involved in digestion (Chang et al., 1996; Hodges, 1974). The exocrine pancreas, which is the larger part of the pancreatic tissue, secretes the pancreatic juice through the zymogen granules in acinar cells. The enzymes are carried into the duodenum via the ducts (Nickel et al., 1977; Hodges, 1974). On the other hand, the pancreatic islets or the islets of Langerhans perform the endocrine role through the alpha, beta, delta and pancreatic polypeptide producing cells (PP cells) (McNabb, 2000; Sitbo et al., 1980).
Alpha cells secrete the hormone glucagon whereas the beta cells are involved in the production of insulin; these two hormones are antagonistic to each other (Figs. 1.4-1.5). Insulin is a protein, which is made up of two chains (alpha & beta) of amino acids connected by a disulfide bond (Hazelwood, 1986). It mainly increases transfer of plasma glucose and other monosaccharides across the cell membrane. Additionally, insulin plays a role in glucose oxidation, glycogenesis, proteogenesis (Teruel et al., 1998), lipogenesis (Dupont et al., 1999), and ketogenesis. Insulin is formed first as production proinsulin. Insulin has the ability to reduce glycogenolysis with accompanying incorporation of glucose molecules into de novo production of glycogen in the liver (Hazelwood, 1986).

The primary activity of the pancreatic alpha cells, which produce glucagon, a 29-amino acid peptide, is to counter the effects of insulin and promote glycogenolysis and gluconeogenesis (Hazelwood, 2000; Sitbon et al., 1980). Pancreatic glucagon circulates in the plasma at about 2-4ng/ml plasma normally and increases in the presence of fasting (Hazelwood, 2000). In broilers suffering from spiking mortality syndrome and exhibiting hypoglycemia, it was found that the mean pancreatic glucagon content was depressed 98.75% when compared to normoglycemic control birds (Davis, 1995). Both insulin and glucagon are secreted mainly from the pancreas, but with a 99% surgical removal of the pancreas both hormones could be produced from extrapancreatic sources (Hazelwood, et al., 1989), i.e., the splenic remnant tissue. Additionally, only the PPA was eliminated in the circulation when the pancreas was removed (Cocla et al., 1982). Glucagon is reported to be a stress hormone (Freeman, 1980), and consequently, it is responsible for activating the hypothalamo-hypophyseal-adrenocortical axis (Freeman, 1980). This was concluded after glucagon was injected intra-abdominally into day old chicks, which responded after 7 days
with increased adrenal weights and adrenal cholesterol stores. The chicks then became hypoglycemic and hypercholesteremic (Freeman, 1980). Nonetheless, the glucagon/insulin ratio is essential to the regulation of plasma glucose level in birds since increases in the ratio leads to diabetes (Sitbon et al., 1980)

In birds suffering from malabsorption of nutrients, depressed glycogen storage is evident (Davis, 1995). During stress responses glycogen is converted to glucose by glucagon (glycogenolysis) to augment plasma glucose level. Therefore, chicks with S1133 infection are likely to become hypoglycemic, especially during the time when they suffer from the extreme effects of reovirus infection.

![Fig.1.4. Metabolic effects of glucagon](image-url)
In developing embryos, the thyroid gland arises as a single midventral evagination of the floor of the pharynx before dividing into two separate lobes by day 5 of incubation (Astier, 1980). Then between 10.5 and 12.5 day of embryonation, functionality of the thyroid axis occurs (Lui et al., 2003). In the adult, it is situated on either side of the trachea, very close to the carotid artery and the jugular vein. These two lobes appear as round to oval in shape and are enclosed in a thin connective tissue capsule and are dark red in color (Wentworth et al., 1986). This red color appearance is attributed to the dense vascular network of the parenchyma (Astier, 1980) that distinguishes thyroid from its neighboring but
similarly shaped organ, the last thymic lobe (Dyce et al., 1996). Furthermore thyroid gland comprises about 0.2% of the total body weight of chickens (Duke, 1997).

Cells within the thyroid glands are arranged in chord like rows (McNabb, 2000), whose height depends on the thyroid activity (Khan et al., 1999). Actively secreting cells are tall columnar cells with vacuolated colloid, but it can be reduced in height, i.e., cuboidal or low columnar and colloids can accumulate in quiescent thyroids (Astier, 1980). The colloid in follicles that are surrounded by the secretory cells of the thyroid is a gelatinous substance composed of an iodinated protein, the thyroglobulin (TG). Anatomically, avian thyroid does not have calcitonin cells or parafollicular cells in the interstitial cells, and this is due to the fact that the ultimobrachial gland which is the origin of calcitonin secreting cells is not connected with the thyroid gland throughout the life span of birds (Astier, 1980; Wentworth et al., 1986).

Dietary iodine is converted into iodide (I\(^-\)) before its absorption from the small intestines before its release into the circulation (Engelking, 2000). Iodine concentration in thyroid is proportionate to the amount of dietary iodine intake (Newcomer, 1978). In general, iodine metabolism involves 3 major steps leading to secretion of hormones, i.e., 1) iodide is actively transported into the thyroid by the follicular cells, also known as iodide trap, 2) a peroxidase system within the thyroid converts iodide to I\(_2\) and then to I\(^+\), and 3) a second enzyme system is responsible for combining the iodinated tyrosines with in the polypeptide chain of TG to eventually form triiodothyronine (T\(_3\)) and thyroxine (T\(_4\)) (Wentworth et al., 1986). The T\(_4\) and T\(_3\) are distributed throughout the body via the circulation and under the control of thyroid-stimulating hormone (TSH) and are maintained across a large range of
physiological concentrations (Reyns et al., 2001). Thyroid hormone secreted from the thyroid is mostly T4 (McNabb, 2000; Astier, 1980; Lam et al. 1986)).

Upon hatching the level of T₃ in chickens increases until 2 weeks of age and gradually decreases (Kuhn et al., 1993), but plasma T₄ levels start rising at around day 15 (Thommes et al., 1977). Kuhn et al. (1993) noted that a positive correlation exists between T₃ levels and growth. In young birds, this hormone is essential for yolk sac resorption, functional maturation of the lungs, pipping, and hatching (Van der Geyten, 1997). T₃ has been reported to directly stimulate growth and maturation of embryonic chick cartilage and enhance the in vitro action of somatomedins on cartilage growth (King, 1984). At the time when the chick embryo switches from chorioallantoic to lung respiration, as it penetrates the air sac, both plasma T₃ and T₄ reach their highest values (Lui et al., 2003).

Thereafter, during the growing period, thyroid hormones stimulate growth and maturation (Liu, et al., 2004; Kahn et al., 1998). In relation to this, as chickens grows older, the secretion of thyroid hormone is governed by the negative feed back mechanism involving the hypothalamic-pituitary-thyroid axis (Engelkin, 2000). This mechanism is also seen when exogenous T₃ is supplemented in the diet and hyperthyroidism develops as indicated by dramatic increases in plasma T₃ level and liver T₄ conversion declines (Collin et al., 2003). In addition, the plasma T₃ concentration in adult birds is lower whereas the growth hormone and T₄ are high depending on the strain of birds (Kuhn et al., 1993). In certain conditions such as the malabsorption syndrome, the thyroid is the earliest target organ (Rudas et al., 1986), wherein the T₃ and T₄ levels were decreased as a result of oral inoculation of infected intestinal homogenates. In the same manner, hypothyroidism greatly affects growth during the post hatch period whereby body weight gain is affected more than bone growth (King et
Meanwhile, it has been suggested that feed restriction in chickens resulted in high levels of circulating T4 and decreased T3 levels, which means that the availability of T4 from the liver and thyroid is not affected (Van der Geyten, 1999; Reyn et al., 2002; Geris et al., 1999; Rosebrough et al., 1989; Kuhn et al., 1987; Klandorf and Harvey, 1985). As a consequence, since liver is the main contributor of T3, decreased hepatic iodothyronine deiodinase type II (ID2), which converts T4 into T3, and increased hepatic iodothyronine deiodinase type III (ID3), which converts T3 into reverse T3 (rT3) and T2 will reduce the T3 supply to the whole body and decrease energy metabolism. It has long been known that thyroid hormones exert a profound effect on development, growth, and metabolism of skeletal tissues (Klaushofer et al., 1995). This is due to the interactions between T3, the most active form, and nuclear receptors that bind to thyroid hormone response elements, causing modification of the expression of specific genes associated with growth and development.

Apart from the effects of thyroid hormones in growth and maturation (Darras et al., 2000), it aids in thermogenesis specifically a T3-driven thermogenic effect (Collins et al., 2003; Silva, 1985). In fact, T3 plays a major role in metabolic heat production that is necessary for maintenance of high and constant body temperature (McNabb, 2000; Darras et al., 2000). Thus, defeathering results in increase thyroxine level in plasma and experimental chickens that are defeathered have higher metabolic rates as well as increased thyroid mass (Pietras, 1981; Tulett et al., 1980).
Adrenals

Adrenal glands are paired yellowish-brown, oval to triangular shaped structures situated at the cranial pole of the corresponding kidney. They are related ventrally to the ovary or epididymis (Dyce et al., 1996). Similar to mammals, the adrenal gland cells arise from the neural crest and mesoderm (King et al., 1984). In chickens, there is no clear distinction between outer cortex or inner medulla unlike the arrangement in mammalian adrenals, which is distinctly separated. In birds, the chromaffin cells are intermingled with cortical cells (Harvey et al., 1986). In the adrenals, there are two types of chromaffin cells, which secrete epinephrine and norepinephrine (Astier, 1986). Functional activation of the adrenal gland in chick embryo is regulated at three different levels- 1) the adrenal gland itself, 2) the anterior pituitary, and 3) the hypothalamus or what is commonly termed as hypothalamo-pituitary-adrenocortical (HPA) axis. From day 8 until shortly after day 14 of embryonic development, adrenal gland appears capable of secreting glucocorticoids independently, at which point the pituitary influences adrenocortical activity and at the same age, the hypothalamic level of control also starts (Jenkins et al., 2004; Bossis et al., 2004).

Avian adrenals account for two regions of steroidogenic (cortical) tissue; the subcapsular zone (SCZ), which secretes aldosterone and the inner zone (IZ) producing the corticosterone (Holmes et al., 1984; Klingbeil et al., 1979). These cells from the SCZ consisted of irregular nuclei, mitochondria with shelf-like cristae and a moderate abundance of smooth endoplasmic reticulum. The IZ cells contain more vascular space and contain large round nuclei surrounded by an abundance of smooth endoplasmic reticulum and the mitochondria have tubular rather than shelf-like cristae (Klingbeil et al., 1979; Pearce et al.,
Adrenal size is influenced by factors such as response to seasonal and environmental change, onset of reproduction, population densities and social interaction and in some species habitat (Holmes et al., 1980). Adrenals are essential for the increase in free fatty acids induced by insulin (Veiga et al., 1983). The adrenal relative weights of feed restricted birds tend to be increased but decrease with time (Carsia, 1998; Freeman, 1981, Nir, 1975). Water deprivation can cause adrenal corticosterone to increase (Tome, 1985). Stressors that induced corticosterone secretion include hyperthermia (Edens and Siegel, 1975), anesthesia (Scanes et al., 1980), infections (Edens et al., 1991; Curtis et al., 1980), exertion (Rees et al., 1984), immobilization (Beuving and Vonder 1979) and frustration (Harvey et al., 1985). Davis et al. (1989) observed that feed deprivation for 3 days on newly hatched pouls resulted in a low corticosterone level, and eventually, mortality was observed. This was in contrast to findings in chickens (Edens et al., 1991c) that infection coupled with 24 hour fasting lead to increase plasma CS. Adrenocortical hormone secretions are stimulated by adrenocorticotrophic hormone (ACTH) (Koscis et al., 1989; Carsia et al., 1987), angiotensins and other putative regulators (McNabb, 2000).

**Current Study**

Avian reovirus infection causes significant physiological and pathological problems in chickens and turkeys. Understanding the mode of disease transmission can help in the design of managerial programs that may alleviate or even prevent the disease process. It is important to evaluate the physiological impact or changes that underlie the disease. Thus, this
investigation dealt with the pathogenicity of PEMS associated agents, passive antibody transfer from hyperimmunized breeders, and experimental in ovo disease transmission. In addition, for the very first time, this study dealt with the potential vertical transmission of PEMS-associated turkey reovirus ARCU98 using an in ovo approach in broiler chickens.
CHAPTER 2

Comparative Pathogenicity Study of PEMS Associated Astrovirus and Reovirus in the Presence or Absence of *E. coli* in Turkey Poults

**ABSTRACT.** Poult enteritis and mortality syndrome (PEMS) is an acute, contagious enteric disease characterized by high morbidity and mortality of poult between 2-4 weeks of age. The current study was conducted to compare the relative pathogenicity of various organisms (viruses and bacteria) associated with PEMS, given alone or in combination through oral gavage to turkey poult under a controlled environment. Determinants for clinical pathologic changes were body weight, liver, bursa, spleen and thymus relative weights. In one experiment, poult were challenged with the PEMS astrovirus (Tast-OSU, 0.2mL, 10^3 EID_{50}), Tast-OSU + *E. coli* (0.2mL, 1 x 10^8cfu), and *E. coli* alone. The results of this experiment showed that the 7 day post-challenge body weight gain was significantly less in all treatment groups with the Tast-OSU group exhibiting the poorest weight gain. Thymic (P≤0.05) and bursal atrophy were observed in Tast-OSU group. In another experiment, poult were challenged with the reovirus ARVCU98 (0.2mL, 10^3 EID_{50}), Tast-OSU (0.2mL, 10^3 EID_{50}), or ARVCU98 +Tast-OSU given at day one post hatch followed three days later with the *E. coli* challenge (0.2mL, 1 x 10^8cfu) and a sham-exposed control group. The results of this experiment showed a progressive and significant decrease in body weight at six days after infection. The poult exhibited severe watery, foul smelling, foamy diarrhea with thin-walled, gaseous distended intestines. Bursal and thymic atrophy were observed at nine days after infection with no difference in the liver and spleen weight. Taken together, these experiments showed that while no mortalities occurred, the astrovirus and reovirus reduced
body weight and induced bursal and thymic atrophy. The addition of *E. coli* with these viruses increased the severity of the weight loss and associated signs of PEMS.

**Keywords**: Poult enteritis and mortality syndrome, Reovirus, Astrovirus, *E. coli*, Pouls

**INTRODUCTION**

Poult enteritis and mortality syndrome (PEMS) emerged as a disease with an unknown etiology in 1991 that caused a highly significant economic loss in the poultry industry in the US, particularly the southeastern region. The disease affected poult between two and four weeks of age, but some resistance developed as maturity approached. PEMS primarily affected the digestive tract as poult exhibited severe diarrhea with associated immune dysfunction as evidenced by its effect on the bursa of Fabricius and thymus. The fecal-oral route appeared to be the main mode of transmission of the disease.

Several organisms have been linked to PEMS, but to date it is classified as a disease with multifactorial etiology. Certain viruses, bacteria, insects, protozoans as well as some environmental factors contributed to the ontogeny of the disease. One of the viruses implicated in PEMS was the reovirus ARVCU98. ARVCU98, which was isolated from fecal filtrates, caused increased incidence of thymic hemorrhaging, gaseous intestines, decreased liver size, and altered liver AST and ALT activities (Heggen-Peay et al., 2002a). Earlier reports also implicated astrovirus in enteric diseases in turkey poult (Koci et al., 2000; Reynolds, 1986). Turkey astrovirus can induce body weight loss, thymic atrophy, and diarrhea (Koci et al., 2003; Qureshi et al., 2001; Yu et al., 2000). Additionally, it has been established that a small round virus (SRV) isolated from the same fecal pool as the reovirus
ARVCU98 was actually a turkey astrovirus (Tast-OSU) and was the same as the SRV that had been described earlier (Qureshi et al., 1999, 2001; Yu et al 2000).

In this investigation, the turkey astrovirus- Tast-OSU (Qureshi et al., 1999, 2000, 2001) and turkey reovirus- ARVCU98 (Heggen-Peay et al., 2001), which were isolated from a fecal pool from PEMS-positive, turkey coronavirus-negative poults, were examined to compare their pathogenicity in the development of PEMS. The viruses were given by oral gavage either alone or followed in combination with *E. coli* (Edens et al., 1997a, b) three days post infection (DPI). The pathogenicity of the viruses either alone or in combination with *E. coli* was evaluated by monitoring their effects on body weight, lymphoid organ and liver weights, and over all performance of poults at different time points after infection.

**MATERIALS AND METHODS**

**Animal Welfare.** This study was conducted following the guidelines established by the North Carolina State University (NCSU) Institutional Animal Care and Use Committee.

**Poults.** Specific pathogen free poult embryos\(^1\) were used in these experiments to provide intestinal homogenates for control poults. Commercial poults were the source of experimental animals. Hybrid strain poults were used in trial 1 of experiment 1, and Nicholas poults were used in trial 2 of experiment 1. Within six hours after the poults hatched, they were transported to North Carolina State University, chosen randomly for wing-bandung, weighed and placed into treatment groups for each experiment. In each of the two experiments, initial ambient temperature was started at 35°C and was decreased by 4°C at intervals of 7 days. Continuous incandescent lighting was provided in each experiment. Feed and water were provided ad libitum. In experiment 1, the standard North Carolina
Agriculture Research Service (NCARS) turkey starter diet fed. In experiment two, a special irradiated feed\(^2\), equivalent to the standard NCARS turkey starter diet, was used to prevent bacterium or viral contamination. Feed and water for the poults in experiment 2 were placed in bulk into the isolators and weighing scales were also placed in the isolators until the end of the investigation. Poults removed from the isolators were first caught using the glove ports in the isolators, placed into an antechamber that was opened and resealed before a second seal was removed from the isolator to allow collection of the sample poults. After the sample poults had been removed from the antechamber, it was resealed and fumigated with a chlorine compound.

**Experiment 1.** For experiment 1, there were two trials consisting of four treatment groups that are listed as follows: 1) control poults received an intestinal homogenate from normal specific pathogen free (SPF) poult embryos, 2) turkey astrovirus isolated at Ohio State University (Tast-OSU; \(10^3\) EID\(_{50}\)), 3) \(E.\ coli\) (1x10\(^8\) cfu/poult), 4) Tast-OSU + \(E.\ coli\). Forty-one poults per group were raised in Petersime\(^3\) brooder batteries. At seven days post hatch, the poults were challenged orally with 0.2mL of the virus challenge material. Three days post virus challenge when the poults were 10 days of age; group 3 and 4 poults were challenged orally with \(E.\ coli\).

**Experiment 2.** For experiment 2, three treatment groups were established as follows: 1) Cornell University avian reovirus isolate (ARVCU98, \(10^3\) EID\(_{50}\)) + Tast-OSU (\(10^3\) EID\(_{50}\)), 2) ARVCU98 + Tast-OSU + \(E.\ coli\), 3) control challenged with phosphate buffered saline, pH

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\(^1\) SPF poults were obtained from Ohio Agriculture Research and Development Center, Wooster, OH.

\(^2\) Feed irradiated
7.0 (PBS). There were 25 pouls per group, and the pouls were maintained in bubble isolators\(^4\) with HEPA-filtered, heated air. On the day of hatching, each poult was gavaged orally with virus challenge inoculum and treatment 2 was given \textit{E. coli} at 3 days post virus challenge.

**Body weights.** All pouls were weighed before placement into their treatment groups. In experiment 1, body weights were determined 7, 10, 13, 15 and 17 days after virus challenge. In experiment 2, body weights were determined at 4, 6, 9 and 11 days after virus challenge.

**Lymphoid organs.** The following organs, bursa of Fabricius, spleen and thymus (all lobes from the left side of the neck) were removed and weighed at each time point listed above. A total of three to ten pouls per treatment were sampled each time body weights were determined as shown above. The organ weights were converted to percent of body weight and reported as lymphoid organ relative weights. For study 2, the same protocol was followed with the addition of the determination of the liver relative weight.

**Statistical Analysis.** A completely randomized experimental design was utilized for both experiments. The data gathered were analyzed using the General Linear Models procedures of SAS (SAS Institute, 1999). There were treatment and time main effects, and the treatment X time interaction was included in the statistical model. If the interactions were not significant, their degrees of freedom were incorporated in the residual error term. If there were significant main effects, treatment means were separated using the least significant difference procedure of SAS (1999). Significance was set at \(P \leq 0.05\).

\(^3\) Petersime Equipment Co., Gettysburg, OH 45328
\(^4\) Standard Safety, Chicago, IL.
RESULTS

Experiment 1

**Trial 1.** Tables 2.1 and 2.2 depict the BW of conventional poults at 7, 10, 13, 15 and 17DPI that were challenged either with Tast-OSU, Tast-OSU + *E. coli*, *E. coli* alone, or SPF turkey embryo intestinal homogenate (control). In trial 1, at 17DPI a significant BW depression (P≤0.056) was observed in Tast-OSU challenged poults. *E. coli* poults were comparable to the control. The BW of the virus challenged poults was consistently lower in comparison with the control. When the 7 day weight gain was calculated, Tast-OSU challenged birds had lower weight gain than the control, but the Tast-OSU + *E. coli* group was not significantly different from the control.

**Trial 2.** In trial 2 (Table 2.2), at 10 days after virus challenge, Tast-OSU + *E. coli* and *E. coli* had significantly (P = 0.002) lower body weights than control and Tast-OSU groups. The seven day body weight gain of Tast-OSU- and *E. coli*-challenged groups differed from control and Tast-OSU + *E. coli* (P = 0.068).

The lymphoid organ integrity results for trial 1 are shown in Table 2.3. At 3 days post *E. coli* challenge, the bursa of Fabricius relative weights of poults in the control and both Tast-OSU-challenged groups were significantly lower than *E. coli* alone. At five days after *E. coli* challenge, only the Tast-OSU challenged groups were showing depressed relative weights of the bursa of Fabricius. At seven days after *E. coli* challenge, there were no differences among treatment groups for bursa of Fabricius relative weights. The relative weights of the thymus from Tast-OSU challenged groups approached significance (P = 0.07).
at five days after *E. coli* challenge but not at any other time. The relative weights of the spleen were not affected by either virus or bacterial challenge.

In trial 2 of experiment 1, the relative weights of neither the bursa of Fabricius nor the spleen were affected by either the virus or bacterial challenges (Table 2.4). The low thymus relative weights of Tast-OSU-challenged poults approached significance at three days after *E. coli* challenge, and at seven days after *E. coli* challenge, the thymus relative weights of Tast-OSU virus challenged poults and *E. coli* challenged poults were significantly (P< 0.05) lower than controls.

**Experiment 2**

Table 2.5 illustrates the mean body weights of poults oral challenges of combined pathogens [ARVCU98 + Tast-OSU, ARVCU98 + Tast-OSU + *E. coli* and or PBS (control)] associated with PEMS. At six days after challenge, poults receiving the combined challenges had mean body weights that were significantly less (P <0.0001) than control and persisted with significantly lower body weights than controls at nine (P = 0.08) and eleven (P = 0.0011) days after challenge.

In experiment 2, the relative weights of neither the spleen nor the liver were affected significantly by the combined pathogen challenges (Tables 2.6A-2.6D). Inconsistency marked the responses of the thymus and the bursa of Fabricius to the combined pathogen challenges (Tables 2.6A-2.6D). The relative weights of the bursa of Fabricius of poults in the ARVCU98 + Tast-OSU were reduced significantly at four days after the challenge. At eleven days after ARVCU98 + Tast-OSU and ARVCU98 + Tast-OSU + *E. coli*, the relative weights of the bursa of Fabricius were reduced significantly compared with control. The relative
weight of the thymus was reduced significantly ($P = 0.0049, 0.001, \text{and } 0.0301$) by ARVCU98 + Tast-OSU and ARVCU98 + Tast-OSU + *E. coli* challenges at six, nine and eleven days, respectively, after challenge compared with control.

## DISCUSSION

In an earlier report, Tast-OSU (formerly called SRV) given either orally or through contact exposure to seeder poults led to growth depression starting at 2-3 days post exposure and caused a severe foamy diarrhea (Qureshi et al., 1999). Tast-OSU caused alteration in the lymphocyte subpopulations and reduced lymphoblastogenic response to T cell mitogens. Likewise inoculation to young poults resulted in 11% mortality. When turkey coronavirus was given in combination with Tast-OSU, mortality rate was doubled. It was concluded that Tast-OSU when given alone or in combination with other viral agents can be detrimental to turkey poult under controlled conditions and possibly exacerbated in field exposures.

Therefore, because PEMS is considered to be a condition with a multifactorial etiology, experiments were undertaken to investigate the effects of combinations of putative etiologic agents implicated in the development of PEMS. In experiment 1 of this study, Tast-OSU was examined in combination with *E. coli* isolates found associated frequently with PEMS (Edens et al., 1997 abc). Results in Table 2.1 show that Tast-OSU and Tast-OSU + *E. coli* were able to depress the weight gain of poults. *E. coli* by itself did not cause a depression in weight gain, and this was in contrast with earlier findings (Edens, 1997 a). However, the conditions of the current investigation were different from the original study by Edens et al. (1997a) who used a more stressful protocol. All *E. coli*-challenged poults developed a severe diarrhea similar to PEMS and mortality was increased by both atypical *E. coli* colony types
when the poults were made immunodeficient (Edens et al., 1997a). Heggen-Peay (2000) reported that the atypical \textit{E. coli} by themselves did not cause any detrimental effect on poult performance. \textit{E. coli} in combination with Tast-OSU caused 7 day body weights to be lower than control (P=0.068) but not \textit{E. coli} alone. These observations suggest that each component etiology for PEMS must play a specific role and that independently, none are as potent as combinations.

Similar trends were seen in trial 2 of experiment 1 (Table 2.2). Generally, the body weights of the challenged poults were lower in comparison with the control. However, it is important to note that body weights of the treatment groups at the time of \textit{E. coli} challenge were uneven, suggesting that some of the groups might have been compromised by other unknown factors. Therefore, the 7 day weight gain differences of the challenged poults compared with controls might be more important than periodic weight gain information. The 7 day weight gain data showed that Tast-OSU and \textit{E coli} treatments alone were responsible for a significantly decreased weight gain (P=0.045) at 7 days after \textit{E. coli} challenge. Tast-OSU + \textit{E. coli} was not different from control but had a numerically lower weight gain.

Even though the poults given the \textit{E. coli} challenge in both trials 1 and 2 of this experiment did not show a dramatic depression in weight gain either alone or in combination with Tast-OSU, it must be considered that the challenge was given at 10 days post hatch. During the first 10 days of development, the enteric microbiota of the turkey poult is maturing and conceivably may have developed climax populations of commensal bacteria that have the potential to inhibit colonization of many different bacteria including the PEMS-associated \textit{E. coli}. The data presented here do not detract from the original PEMS-associated bacteria reports (Edens et al., 1997abc). In this study there was a clear depression in body
weight gain at 3dpi when E. coli was given alone in trial 1, and in trial 2, there was an overall depression in 7dpi weight gain that was as severe as the depression caused by Tast-OSU challenge. Instead, these observations point to the potential that the PEMS-associated E. coli might be able to exert if given during a time when the poult might be more susceptible, such as day of hatch rather than after several days of development after hatching.

In experiment 1, the E. coli challenge did not result in depression of either bursa of Fabricius or spleen relative weights. Tast-OSU challenge did cause a significant (P=0.02) depression in relative weight of the bursa of Fabricius at 5 days after E. coli challenge. In trial 1 at 5 days after E. coli challenge, thymus relative weights in the Tast-OSU and Tast-OSU + E coli challenged poults were reduced (P=0.07) by 31.3% and 25%, respectively, when compared to the control. In trial 2, thymic atrophy was observed at 3 (Tast-OSU only) and 7 days (Tast-OSU, Tast-OSU + E. coli, and E. coli) (P < 0.0001). Qureshi et al (1999) reported thymic atrophy (30.8%) in poults challenged with Tast-OSU between 4 and 8 DPI. The lymphoid organ relative weight data show that Tast-OSU either alone or in combination with E. coli can cause bursal and thymic atrophy in young turkey poults.

Heggen-Peay et al. (2001) reported that PEMS-associated reovirus ARVCU98, depressed the body weights of experimental poult between 22% and 28%. The lymphoid organ (bursa of Fabricius and thymus) relative weights were decreased in comparison with the control poults and that there was an increase in the incidence of thymic hemorrhaging. A similar trend was observed in experiment 2 of this investigation.

In experiment 2, body weights were depressed in turkey poults given combinations of viruses (ARVCU98 + Tast-OSU) and viruses plus E. coli (ARVCU98 + Tast-OSU + E. coli). Body weight depression evident as soon as 6DPI and persisted through 11DPI. These
observations clearly demonstrated the effect of *E. coli* when given together with reovirus because it exacerbated the body weight depression even in a controlled environment. Thus, it is possible that when combinations of several etiologic agents associated with PEMS are present, the possibility of development of fulminating PEMS is greatly increased.

In experiment 2, thymic atrophy in poults given both combinations of PEMS pathogens was observed at 6 DPI and persisted through 11DPI (Table 2.6A-2.6D). Thymic atrophy is a typical finding in PEMS (Qureshi et al., 1997). Schultz et al. (2000) reported that when poults were exposed to thymic filtrate, PEMS-like symptoms were exhibited by those poults. Therefore, it was concluded that filtrates of thymus tissues from PEMS-infected poults appear to harbor etiologic agents that can cause development of PEMS. An astrovirus was isolated from thymic filtrate (Schultz et al., 2000).

In this study, turkey astrovirus Tast-OSU was used in combination with the turkey reovirus ARVCU98. Bursal atrophy was evident as early as 4DPI in ARVCU98 + Tast-OSU poults and at and 11DPI in ARVCU98 + Tast-OSU and ARVCU98 + Tast-OSU + *E. coli* groups. Relative weights of both bursa of Fabricius and thymus are depressed significantly when poults are exposed to combinations of either reovirus, astrovirus or *E. coli*. The bursal and thymus are the primary lymphoid tissues in birds, and when their integrity becomes compromised, immunological functions likely suppressed in response to antigenic challenges. Ultimately the loss of cellularity in the thymus and bursa causes alterations in both T and B cell populations and in the mononuclear phagocytic system, which can result in immunosuppression or immunodysfunction found in PEMS (Qureshi et al., 1997).

The relative weights of neither the spleen nor the liver were affected significantly by the combinations of PEMS causing agents in experiment 2. When absolute liver weights of
the ARVCU98 + Tast-OSU + *E. coli*-challenged poults were measured, they were greatly reduced in comparison with controls. The absolute weight effects were due to the smaller body weights of the challenged poults.

In conclusion, it can be implied from the results of this investigation that exposure to Tast-OSU and ARVCU98 can lead to body weight reduction and that the addition of *E. coli* can further aggravate the situation. These etiologic agents, in combination appear to have the potential to disrupt the immune system and cause the immunodysfunction that has been reported for PEMS-infected poults (Qureshi et al., 1997). The results obtained in this investigation were obtained in highly controlled environmental conditions. It is assumed that under commercial conditions the responses to these etiologic agents would be exacerbated and possibly results in fulminating PEMS cases.
Table 2.1. The effect of small round virus (Tast-OSU) and E. coli oral challenge on growth of conventional poults\(^5\) (Trial 1)

<table>
<thead>
<tr>
<th>Challenge (^1)</th>
<th>7(^6)</th>
<th>10</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>7 day gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(^2)</td>
<td>114.3</td>
<td>139.50</td>
<td>208.3</td>
<td>248.0</td>
<td>303.5</td>
<td>164.8</td>
</tr>
<tr>
<td></td>
<td>±2.51(^a)</td>
<td>±4.02(^b)</td>
<td>±9.38(^a)</td>
<td>±15.6(^a)</td>
<td>±15.0(^a)</td>
<td>±11.1(^a)</td>
</tr>
<tr>
<td>Tast-OSU</td>
<td>105</td>
<td>139.60</td>
<td>198.7</td>
<td>226.7</td>
<td>261.3</td>
<td>121.7</td>
</tr>
<tr>
<td></td>
<td>±2.65(^a)</td>
<td>±4.23(^b)</td>
<td>±9.38(^a)</td>
<td>±15.6(^a)</td>
<td>±17.3(^b)</td>
<td>±12.8(^b)</td>
</tr>
<tr>
<td>Tast-OSU + E. coli</td>
<td>108.4</td>
<td>141.7</td>
<td>180.0</td>
<td>245.7</td>
<td>270.0</td>
<td>138.3</td>
</tr>
<tr>
<td></td>
<td>±2.65(^a)</td>
<td>±4.23(^b)</td>
<td>±9.38(^a)</td>
<td>±15.6(^a)</td>
<td>±17.3(^b)</td>
<td>±12.8(^ab)</td>
</tr>
<tr>
<td>E. coli</td>
<td>111.5</td>
<td>160.2</td>
<td>185.7</td>
<td>260.7</td>
<td>326.8</td>
<td>166.8</td>
</tr>
<tr>
<td></td>
<td>±2.51(^a)</td>
<td>±4.02(^a)</td>
<td>±9.38(^a)</td>
<td>±15.6(^a)</td>
<td>±15.0(^a)</td>
<td>±11.1(^a)</td>
</tr>
<tr>
<td>Pvalue(^4)</td>
<td>0.11</td>
<td>0.002</td>
<td>0.21</td>
<td>0.52</td>
<td>0.056</td>
<td>0.068</td>
</tr>
</tbody>
</table>

\(^{1}\) Poults orally challenged with 10\(^7\)EID\(_{50}\) Tast-OSU at 7 days of age followed by 1X10\(^8\)E. coli (turkey isolate Type I and II at 10 days of age.

\(^{2}\) Poults in control were challenged with 0.2ml of turkey embryo intestinal homogenate prepared from control (normal) SPF embryos.

\(^{3}\) Represent mean body weight (g)

\(^{4}\) Significance value as determined by General Linear Model in SAS

\(^{5}\) Poults were Hybrid strain.

\(^{6}\) Days in age
Table 2.2. The effect of small round virus (Tast-OSU) and *E. coli* oral challenge on growth of conventional poults\(^5\) (Trial 2)

<table>
<thead>
<tr>
<th>Challenge (^1)</th>
<th>7(^6)</th>
<th>10</th>
<th>13</th>
<th>15</th>
<th>17</th>
<th>7 day gain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control(^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>118.8</td>
<td>157.66</td>
<td>198.02</td>
<td>21.0</td>
<td>286.3</td>
<td>135.33(^a)</td>
<td></td>
</tr>
<tr>
<td>±3.92(^a)</td>
<td>±4.58(^ab)</td>
<td>±10.74(^a)</td>
<td>±12.0(^a)</td>
<td>±15.0(^a)</td>
<td>±10.50</td>
<td></td>
</tr>
<tr>
<td>Tast-OSU</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>124.2</td>
<td>153.60</td>
<td>181.33</td>
<td>235.0</td>
<td>260.0</td>
<td>100.0(^b)</td>
<td></td>
</tr>
<tr>
<td>±3.72(^a)</td>
<td>±4.34(^ab)</td>
<td>±10.74(^a)</td>
<td>±12.0(^a)</td>
<td>±17.3(^a)</td>
<td>±9.09</td>
<td></td>
</tr>
<tr>
<td>Tast-OSU + <em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>112.1</td>
<td>134.4</td>
<td>196.33</td>
<td>199.0</td>
<td>258.25</td>
<td>127.25(^a)</td>
<td></td>
</tr>
<tr>
<td>±3.72(^a)</td>
<td>±4.34(^c)</td>
<td>±10.74(^a)</td>
<td>±12.0(^a)</td>
<td>±17.3(^a)</td>
<td>±9.09</td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>116.0</td>
<td>146.9</td>
<td>191.3</td>
<td>229.3</td>
<td>240.0</td>
<td>99.50(^b)</td>
<td></td>
</tr>
<tr>
<td>±3.72(^a)</td>
<td>±4.34(^b)</td>
<td>±10.74(^a)</td>
<td>±12.0(^a)</td>
<td>±15.0(^a)</td>
<td>±9.09</td>
<td></td>
</tr>
<tr>
<td><strong>Pvalue</strong>(^4)</td>
<td>0.15</td>
<td>0.002</td>
<td>0.70</td>
<td>0.24</td>
<td>0.35</td>
<td>0.068</td>
</tr>
</tbody>
</table>

\(^1\) Poults orally challenged with 10\(^3\)EID\(_{50}\) Tast-OSU at 7 days of age followed by 1X10\(^8\)*E. coli (turkey isolate Type I and II at 10 days of age.

\(^2\) Poults in control were challenged with 0.2ml of turkey embryo intestinal homogenate prepared from control (normal) SPF embryos.

\(^3\) Represent mean body weight (g)

\(^4\) Significance value as determined by General Linear Model in SAS

\(^5\) Poults were Nicholas strain.

\(^6\) Days in age
Table 2.3. The effect of small round virus (Tast-OSU) and *E. coli* oral challenge\(^1\) on relative organ weight of conventional poults \(^5\) (Trial 1)

<table>
<thead>
<tr>
<th>Age(^6)</th>
<th>Organ</th>
<th>Control(^2)</th>
<th>SRV</th>
<th>SRV+ <em>E. coli</em></th>
<th><em>E. coli</em></th>
<th>(P) value(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Bursa</td>
<td>0.21 ± 0.02(^{b})</td>
<td>0.18 ± 0.02(^{b})</td>
<td>0.19 ± 0.02(^{b})</td>
<td>0.31 ± 0.02(^{a})</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.17 ± 0.02(^{a})</td>
<td>0.11 ± 0.02(^{a})</td>
<td>0.15 ± 0.02(^{a})</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.06 ± 0.01(^{a})</td>
<td>0.07 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.08 ± 0.01(^{a})</td>
<td>0.21</td>
</tr>
<tr>
<td>15</td>
<td>Bursa</td>
<td>0.24 ± 0.01(^{a})</td>
<td>0.17 ± 0.01(^{b})</td>
<td>0.20 ± 0.01(^{ab})</td>
<td>0.23 ± 0.01(^{a})</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.16 ± 0.01(^{a})</td>
<td>0.11 ± 0.01(^{b})</td>
<td>0.12 ± 0.01(^{ab})</td>
<td>0.15 ± 0.01(^{a})</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.09± 0.01(^{a})</td>
<td>0.07 ± 0.02(^{a})</td>
<td>0.33</td>
</tr>
<tr>
<td>17</td>
<td>Bursa</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.12 ± 0.02(^{a})</td>
<td>0.19 ± 0.02(^{a})</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.13 ± 0.02(^{a})</td>
<td>0.11 ± 0.02(^{a})</td>
<td>0.12 ± 0.02(^{a})</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.07 ± 0.01(^{a})</td>
<td>0.11 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.09 ± 0.02(^{a})</td>
<td>0.32</td>
</tr>
</tbody>
</table>

\(^1\)Poults orally challenged with 10\(^{7}\)EID\(_{50}\) ARVCU98 at 7 days of age followed by 1X10\(^{8}\)E. coli (turkey isolate Type I and II at 10 days of age.  
\(^2\)Poults in control were challenged with 0.2ml of turkey embryo intestinal homogenate prepared from control (normal) SPF embryos.  
\(^3\)Represent mean body weight (g)  
\(^4\)Significance value as determined by General Linear Model in SAS  
\(^5\)Poults were Hybrid strain  
\(^6\)Age in days
### Table 2.4. The effect of small round virus (SRV) and *E. coli* oral challenge on relative organ weights of conventional poults\(^5\) (Trial 2)

<table>
<thead>
<tr>
<th>Age (^6)</th>
<th>Organ</th>
<th>Control(^2)</th>
<th>SRV</th>
<th>SRV+ <em>E. coli</em></th>
<th><em>E. coli</em></th>
<th>Pvalue(^4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>Bursa</td>
<td>0.20 ± 0.02(^{a})</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.20 ± 0.02(^{a})</td>
<td>0.16 ± 0.02(^{a})</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.13 ± 0.01(^{a})</td>
<td>0.10 ± 0.01(^{a})</td>
<td>0.11 ± 0.01(^{a})</td>
<td>0.16 ± 0.01(^{a})</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.08 ± 0.01(^{a})</td>
<td>0.07 ± 0.01(^{a})</td>
<td>0.06 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.30</td>
</tr>
<tr>
<td>15</td>
<td>Bursa</td>
<td>0.20 ± 0.02(^{a})</td>
<td>0.21 ± 0.02(^{a})</td>
<td>0.19 ± 0.02(^{a})</td>
<td>0.18 ± 0.02(^{a})</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.08 ± 0.01(^{a})</td>
<td>0.06 ± 0.01(^{a})</td>
<td>0.10 ± 0.01(^{a})</td>
<td>0.10 ± 0.01(^{a})</td>
<td>0.35</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.07 ± 0.01(^{a})</td>
<td>0.76</td>
</tr>
<tr>
<td>17</td>
<td>Bursa</td>
<td>0.22 ± 0.02(^{a})</td>
<td>0.20 ± 0.01(^{a})</td>
<td>0.22 ± 0.02(^{a})</td>
<td>0.19 ± 0.01(^{a})</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Thymus</td>
<td>0.16 ± 0.01(^{a})</td>
<td>0.11 ± 0.01(^{b})</td>
<td>0.10 ± 0.01(^{b})</td>
<td>0.10 ± 0.01(^{b})</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>0.08 ± 0.01(^{a})</td>
<td>0.10 ± 0.01(^{a})</td>
<td>0.10 ± 0.01(^{a})</td>
<td>0.09 ± 0.01(^{a})</td>
<td>0.52</td>
</tr>
</tbody>
</table>

\(^1\) Poults orally inoculated with 10\(^{3}\)EID\(_{50}\) SRV at 7 days of age followed by 1X10\(^{8}\)E. coli (turkey isolate Type I and II, (Edens et al., 1997) at 10 days of age.

\(^2\) Poults in control were inoculated with 0.2ml of turkey embryo intestinal homogenate prepared from control (normal) SPF embryos.

\(^3\) Represent mean body weight (g)

\(^4\) Significance value as determined by General Linear Model in SAS

\(^5\) Poults were Nicholas strain.

\(^6\) Age in days
Table 2.5. The effect of ARVCU98, Astrovirus and *E. coli* oral challenge on body weight (g) of conventional poults

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after challenge</th>
<th>0 (n=25)</th>
<th>4 (n=25)</th>
<th>6&lt;sup&gt;A&lt;/sup&gt; (n=6)</th>
<th>9 (n=6)</th>
<th>11 (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>59.76</td>
<td>140</td>
<td>192±6.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266±11.26&lt;sup&gt;c&lt;/sup&gt;</td>
<td>338±8.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astrovirus</td>
<td></td>
<td>60.72</td>
<td>126</td>
<td>159±6.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>227±11.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300±8.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astrovirus + <em>E. coli</em></td>
<td></td>
<td>60.20</td>
<td>120</td>
<td>137±6.31&lt;sup&gt;c&lt;/sup&gt;</td>
<td>246±11.26&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>280±8.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P* value: 0.0001 0.0805 0.0011

<sup>A</sup>Means ±SEM

<sup>ab</sup> In a column unlike letters differs significantly *P*≤0.05
### Table 2.6A. Relative organ weight (%) of challenged poults at 4 days post inoculation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Bursa</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.12±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.16±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.38±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astrovirus</td>
<td>0.14±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.08±0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.15±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astrovirus + <em>E. coli</em></td>
<td>0.09±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13±0.009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.01±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>A</sup>Means ±SEM

<sup>ab</sup> In a column unlike letters differs significantly P<0.05

| Pvalue                           | 0.1282               | 0.0002     | 0.7196     | 0.2042     |
Table 2.6B. Relative organ weight (%) of challenged poults at 6 days post challenge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus&lt;sup&gt;A&lt;/sup&gt;</th>
<th>Bursa</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.13±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.13±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.29±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astro</td>
<td>0.09±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.14±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.07±0.008&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.02±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARV+Astro + E. coli</td>
<td>0.10±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.09±0.008&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.20±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>P</sup>value | 0.0049 | 0.0979 | 0.0714 | 0.4403 |

<sup>A</sup>Means ±SEM
<sup>ab</sup>, In a column unlike letters differs significantly P<0.05
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus</th>
<th>Bursa</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15±0.008a</td>
<td>0.16±0.01a</td>
<td>0.07±0.007a</td>
<td>2.95±0.14a</td>
</tr>
<tr>
<td>ARV+Astrovirus</td>
<td>0.12±0.008b</td>
<td>0.14±0.01a</td>
<td>0.07±0.007a</td>
<td>3.10±0.14a</td>
</tr>
<tr>
<td>ARV+Astrovirus + E. coli</td>
<td>0.09±0.008c</td>
<td>0.14±0.01a</td>
<td>0.08±0.007a</td>
<td>2.86±0.14a</td>
</tr>
</tbody>
</table>

| P value           | 0.0010      | 0.4092      | 0.4693     | 0.5164      |

^A Means ±SEM

^ab In a column, means with unlike superscripts differ significantly P<0.05.
Table 2.6D. Relative organ weight (%) of challenged poults at 11 days post challenge.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Thymus</th>
<th>Bursa</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.15±0.01^a</td>
<td>0.31±0.01^a</td>
<td>0.10±0.01^a</td>
<td>3.01±0.14^a</td>
</tr>
<tr>
<td>ARV+Astro</td>
<td>0.11±0.01^b</td>
<td>0.13±0.01^b</td>
<td>0.10±0.01^a</td>
<td>3.21±0.14^a</td>
</tr>
<tr>
<td>ARV+Astro + E. coli</td>
<td>0.11±0.01^b</td>
<td>0.14±0.01^b</td>
<td>0.14±0.01^a</td>
<td>3.07±0.15^a</td>
</tr>
</tbody>
</table>

*Means ±SEM

^ab, In a column unlike letters differs significantly P<0.05
CHAPTER 3

Comparative Pathogenicity of PEMS-Associated ARVCU98 and the Field-Associated S1733 Reoviruses on Broilers Inoculated in ovo.

ABSTRACT. The effects of in ovo inoculation of two reoviruses- ARVCU98 (turkey) and S1733 (chicken) - in broiler chickens as shown by changes in body weights, relative weights of liver and lymphoid organs, and blood plasma chemistry were examined to characterize the pathogenicity of these isolates. Two independent trials were conducted using two separate groups of embryonated eggs. Eggs at day 9 of embryonation were challenged via the yolk sac with $100 \mu L/\text{egg}$ (ARVCU98 at $10^3 \text{ TCID}_{50}$; S1733 at $10^8 \text{ EID}_{50}$). Percent hatchability for the control group was 70%, but hatchability for ARVCU98 was 24% for S1733 (1:100 and 1:500) treatment groups hatchability was 59% and 67%, respectively. Body weights of virus-challenged chickens were significantly less ($P \leq 0.05$) than controls at 7 and 14 days of age. The bursa of Fabricius, thymus, and liver relative weights were decreased. Blood plasma glucagon and insulin decreased significantly ($P \leq 0.05$) in the virus-challenged groups. The $T_3/T_4$ ratio of virus-challenged chickens was slightly decreased compared with the control but were not significant. The lower levels of plasma insulin and glucagon coupled with a slight depression in the $T_3/T_4$ ratio suggested a metabolic dysfunction in virus-challenged chickens. The IGF-I and IGF-II levels in virus-challenged chickens were slightly but not significantly higher than the control. Cellular integrity tests, as indicated by aspartate amino transferase (AST) and amino alanine transferase (ALT) activities suggested that S1733 had an effect from 1 to 14 days of age but in the ARVCU98 challenged group, there was only a one day change in the activities of these enzymes. Immunohistochemical examination did not reveal viral antigen on either bursa or thymus at
14 and 28 days of age. Electron microscopic examination of the lymphoid organs and several endocrine glands revealed virus-related changes in cellular ultrastructure. Using electron microscopy, the reoviruses were found in the liver and pancreas. These results demonstrate that both the ARVCU98 and S1733 can be transmitted *in ovo* from day 9 of incubation. The PEMS-associated ARVCU98 can inhibit growth and development in broiler chicken body weight as seen in earlier studies with turkey poults and might be pathogenic in chickens under field conditions.

Keywords: Poult enteritis and mortality syndrome, reovirus, broiler, endocrine responses
INTRODUCTION

Avian reovirus infection is classified as an important economic disease of poultry worldwide. One of the agents implicated in poult enteritis and mortality syndrome (PEMS) that affects young turkey poult is an avian reovirus, ARVCU98. PEMS was first known as a disease with unknown etiology in the early 1990’s in North Carolina. It was widely characterized as an acute, highly transmissible, infectious enteric disease with high morbidity and mortality rate and affected poult failed to reach target market weight. Other signs of PEMS are diarrhea, dehydration, lethargy, and lymphoid organ and liver pathology. There is an increase in the pro-inflammatory cytokines by activated macrophages in poult infected with PEMS, which has been thought in part to be responsible for the intestinal inflammation, gut motility and anorexia (Qureshi et al., 1997; Heggen, et al., 2000). Therefore, is generally immunosuppressive in nature.

To date, no single organism has been found to be the causative agent for PEMS, and PEMS which is regarded as a disease with a multifactorial etiology. Reovirus, ARVCU98, was found to cause significant physiological and pathological changes turkey poult (Chapter 2), and when it was given in combination with a turkey astrovirus (Tast-OSU) and atypical E. coli (Chapter 2), significant physiological changes similar to PEMS were observed. In the area of North Carolina where PEMS developed, there is also a very high concentration of broiler and broiler breeder farms. Because reovirus can be introduced into a facility by mechanical means, one must be concerned that there might be some dissemination of virus from chickens to turkeys or from turkeys to chickens. Therefore, in this investigation, the potential for the turkey reovirus, ARVCU98, to be transmitted vertically to chickens was
examined. The objective of this investigation was to determine if *in ovo* administered reoviruses, ARVCU98 and S1733, had effects post hatch in broiler chickens.

**MATERIALS AND METHODS**

**Animal Care.** This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

**Virus.** The frozen (-20°C) stock S1733 reovirus isolate (supplied by Intervet USA, Inc., Millsboro, Delaware) had a $10^{5.8}$, 50% embryo infective dose (EID$_{50}$)/mL in a 1:100 dilution. The PEMS-associated ARVCU98 reovirus had a $10^3$ TCID$_{50}$ titer at 72 hr post inoculation at day 9 of incubation (Heggen-Peay, 2001).

**Embryo Inoculations and Incubation.** Two separate trials were conducted using virus-challenged embryonated eggs at day 9 of incubation. Embryonated broiler breeder eggs were obtained from the North Carolina Agricultural Research Service flock (Ross X Arbor Acres), which had not been vaccination against reovirus. Eggs were maintained under standard incubation and hatching conditions until they hatched. Eggs were candled to assure viability. Eggshells were thoroughly disinfected with a 70% ethyl alcohol wash followed by an antibiotic (Pen/Strep) wash. A small hole was then drilled on the small end of the egg and 100µL of virus inoculum/egg was injected into the yolk sac. The inoculation site was sealed with paraffin wax. Inoculated eggs were returned to the incubator until day 18 of incubation when they were segregated into their various treatment groups and transferred to pedigree hatching baskets. When the treatment groups hatched, they were wing banded and weighed.
On the hatching date, blood was collected from 5 birds/treatment group from which plasma was collected and stored at -80°C until used for plasma chemistry analysis. Day old chickens were placed into electrically-heated Petersime (Petersime Equipment Co., Gettysburg, OH 45328) brooder batteries. The control and virus-challenged chickens were maintained in different but identically-controlled isolation rooms. Body weights were recorded at days 1, 7, 9, 14 and 28, and randomly caught chickens were killed by carbon dioxide asphyxiation on days 14 and 28 for determination of the lymphoid organ (bursa, thymus and spleen) and liver relative weights. Tissue samples from the anterior pituitary, pancreas, thyroid and liver were collected for transmission electron microscopy. Tissue samples from the thymus and bursa were gathered for viral antigen detection and for histopathology (hematoxylin and eosin B).

**Animal Husbandry.** Hatched chickens were placed into heated brooder battery pens (15 chickens/pen) by treatment group. Pen temperature was maintained at 32°C for one week, reduced to 27°C at week two and then to room temperature (24°C) at week three through four weeks of age. Feed and water were provided for free consumption.

**Body and organ weights.** At 14 and 28 days of age, live body weights were determined and the chickens were killed by carbon dioxide asphyxiation. The chickens were then necropsied, and liver and lymphoid organs weights were recorded for calculation of relative weights \{(organ wt [g])/(BW [g]) \times 100\}.

**Feather scoring.** Feather tracks on the back, neck, wing, thigh, breast area and abdomen were graded subjectively and composite whole body feathering scores were calculated (Edens, 1998). Whole body feather scores ranged from 1 (poorest) to 5 (best).

**Serum AST and ALT.** Blood was collected in sterile serum separation tubes from five chickens per treatment group at 1, 14 and 28 days of age. Tubes were refrigerated overnight
for expression of serum. Quantitative determination for serum amino alanine transferase (ALT) and aspartate amino transferase (AST) activities were conducted following the manufacturer’s protocol (Stanbio Laboratories, Boerne, Texas)

**Blood Plasma Chemistry.** At 21 and 28 days of age, blood for plasma was collected via the jugular vein into sodium EDTA from 5 chickens per treatment group. Blood samples were centrifuged, and plasma was collected and stored at -20°C until assayed. Plasma for glucagon analysis was stored in vials containing aprotinin (Sigma-Aldrich, St. Louis, MO). Plasma radioimmunoassay for glucagon (Linco, Inc., St. Charles, MO), insulin (McMurtry, et al., 1983), thyroxine (T₄) and triiodothyronine (T₃) (McMurtry, et al. 1988), insulin-like growth factor (IGF-I) (McMurtry et al., 1994), IGF-II (McMurtry, et al., 1998) were sent to USDA-ARS, Beltsville, MD and assayed in the laboratory of Dr. J. P. McMurtry.

**Reovirus ELISA.** Detection of reovirus antibodies from the maternal serum was assayed using the FlockChek (IDEXX) reovirus ELISA test kit.

**Immunohistochemistry.** Bursa of Fabricius and thymus were collected from the three treatment groups at 14 and 28 days of age. Organs were embedded in optical cutting temperature compound (OCT) (Tissue Tek; Miles., Elkhart, IN), frozen and cut into 5µ sections. Tissue sections were deparaffinized and incubated for 30 minutes with convalescent serum made in PBS and heat inactivated at 56°C and was used as the primary antibody. Convalescent sera were obtained from chickens from each treatment group at 14 days of age. After a series of washes in phosphate buffered saline (PBS), the slides were incubated with 1:15 dilution of FITC–conjugated goat anti-chicken immunoglobulinG (IgG) heavy and light (H&L) chains (Southern Biotechnology Associates Inc., Birmingham, AL) for 30 minutes
and later washed with PBS. Slides were examined under fluorescent microscopy. Slides were scored as positive or negative for the presence of reovirus antigen in a given tissue.

**Transmission Electron Microscopy (TEM).** Adrenals, liver, anterior pituitary, pancreas and thyroid were cut into 1mm³ block and fixed in 3% glutaraldehyde and 0.1 M Na cacodylate buffer, pH 7.4 at 4°C. Samples were rinsed in 3 cold 30 minutes changes of 0.1M Na cacodylate buffer, pH 7.4. After which samples were post-fixed for 2 hours at 4°C in 1% osmium tetroxide in 0.1 M Na cacodylate buffer, pH 7.4. After samples were washed as described above, they were rehydrated with a graded ethanol series from 30-100% ethanol alcohol (EtOH). Tissues samples were embedded in BEEM capsules with fresh 100% Spurr’s solution overnight at 70°C after they were washed 3X with 100% Spurr’s before infiltrating with 1:3, 1:1, 3:1 Spurr’s:EtOH. Tissues were then thick-sectioned at 1µ and stained with Toluidine blue for preliminary examination under light microscopy before thin-sectioning at 75-100nm and affixed to 200-mesh uncoated grids. Thin sections of tissues were stained for 1 hour with 4% uranyl acetate and 4 minutes with Reynold’s lead citrate at room temperature prior to examination under TEM at 80kV (JEOL JEM-100S). The TEMs were digitized and processed using Adobe Photoshop 7.0 (Adobe Photo Systems, Inc. San Jose, CA 95110-2704).

**Histopathology.** Bursa of Fabricius and thymus were fixed in 10% buffered formalin. Tissues were dehydrated, embedded in paraffin, sectioned at 5µm and stained with hematoxylin and eosin B. Tissues sections were examined under light microscope, and photomicrographs were made with a Leitz photomicroscopy system using Kodak T160 slide film.
Statistics Analysis. A completely randomized experimental design was used. The data were analyzed using the General Linear Models procedures of SAS (SAS Institute, 1999). There were treatment and time main effects and treatment X time interaction in the statistical model. If the interactions were not significant, their degrees of freedom were incorporated in the residual error term. If there were significant main effects, treatment means were separated using the least significant difference procedure of SAS (1999). Significance was set at $P \leq 0.05$.

RESULTS

Hatchability and post-inoculation mortality. The overall hatchability of control eggs was 70%. Eggs challenged with ARVCU98 had 24% hatchability compared to the 59% and 67%, respectively, for the 1:100 and 1:500 dilutions for the S1733 treatment groups (Table 3.1). Embryo viability at 18d of incubation was 53% for the control, 37% for the ARVCU98, and 42% and 52%, respectively, for the two S1733 groups. Before 7d of age, only one chicken in the ARVCU98 group died, but there were no deaths in the other groups.

Body weight and growth. Body weights of the broiler chickens in Trials 1 and 2 (1, 7, 9, 14 and 28 d of age) are given in Tables 3.2 and 3.3, respectively. Even though there was a significant trial difference for body weights, trends within the two trials were similar. In trial 1, the hatching body weights were less than the hatching body weights in trial 2. In trial 1, there were no significant differences among treatment groups for hatching body weights (Table 3.2), but in trial 2 day of hatch body weights of S1733-challenged (1:500) chickens were heavier than all other groups (Table 3.3). At 7 d of age, the S1733-challenged chickens had significantly lower body weights than the controls in both Trials 1 and 2, and the
ARVCU98 chickens had significantly lower body weights in trial 2. At 14 d of age, the body weights of all virus-challenged groups were significantly less (P<0.05) than controls, and ARVCU98 chickens had the lowest body weights among all the groups (Tables 3.2 and 3.3). At 28 d of age, there were no significant differences among the treatment groups even though the virus-challenged groups had lower body weights (Table 3.2 and 3.3). Using average daily gain (Table 3.4) as an indicator of virus influence on growth, it was found that in ovo virus challenge caused a significant decrease in weight gain (P ≤0.05).

**Feed conversion.** Among treatment groups, feed conversion ratios (FCR) were not significantly different (Table 3.5).

**Lymphoid organ and liver weights.** Tables 3.5A-3.5B and 3.6A-3.6B show the 14 and 28d of age relative weights for liver, bursa of Fabricius, thymus, spleen and liver in trials 1 and 2, respectively. The relative weights of the liver from virus-challenged chickens were decreased significantly in trial 1 only (Table 3.5A). At 28d of age, liver relative weight from S1733 (1:100) challenged chickens was less than the liver relative weights of chickens given either ARVCU98 or S1733 (1:500), but all of the relative liver weights of the birds given virus challenges were greater that the relative weight of the controls (Table 3.5B). Among the relative weights for the bursa of Fabricius, thymus and spleen, there were no significant differences due to virus challenge (Tables 3.5AB and 3.6AB). In trial 1, the relative weight of the bursa of Fabricius was less than virus-challenged groups at 14d of age, but at 28d of age, the relative weight of the bursa of Fabricius of the S1733 (1:100) group was less than the relatives weights of the bursae from all other groups. In trial 2, relative weights of the bursa of Fabricius were not affected by any of the virus challenges (Tables 3.6AB). In trial 1, the thymus relative weights of the ARVCU98-challenged birds at 14d and 28 d of age were less
(P=0.067 and P=0.016, respectively) than relative weights from other challenged groups and control. In trial 2, virus challenge did not affect relative weight of thymus as compared with controls.

**Feather Scoring.** Subjective scoring of feathering was performed (Table 3.7). Control chickens were scored as 5 while virus challenged chickens, both ARVCU98 and S1733, were assigned a feather score of 3.

**Gross lesions.** Upon necropsy, the virus-challenged groups exhibited thin-walled, gas-filled, frothy intestines (Table 3.8). Noticeably, the S1733-challenged chickens had swollen, hyperemic kidneys and hearts were enlarged in association with pericarditis in comparison with the broiler chickens from ARVCU98 and control treatment groups.

**Histopathology.** Slides of bursa of Fabricius and thymus were prepared and examined under light microscopy to determine the histopathological changes attributed to *in ovo* infection. A summary of the histopathological signs in the bursa of Fabricius and thymus from reovirus-infected chickens at 14d and 28d of age are presented in Tables 3.9A and 3.9B for trials 1 and 2, respectively. Eosinophilia, based on the presence of eosinophil-like cells, containing spherical eosinophilic granules and bilobed nucleus, was a characteristic of virus infection in both the bursa of Fabricius and the thymus and was evident with ARVCU98 and S1733 infection at 14d and 28d of age (Tables 3.9 AB; Fig. 3.4 to 3.15). The presence of eosinophilia in the bursa and the thymus was also associated with loss of cellularity at both 14d and 28d of age (Tables 3.9 AB; Fig. 3.4 to 3.15). Macrophage infiltration into the thymus and bursa of Fabricius was obviously increased when the chickens were challenged with the reoviruses from both chicken (S1733) and turkey (ARVCU98) origin (Tables 3.9 AB; Fig. 3.4 to 3.15).
Blood Plasma Chemistry

**Plasma Glucagon, Insulin, and Glucose.** The blood plasma chemistry results are shown in Table 3.10. There were significant differences among treatment groups for the plasma glucagon (P ≤ 0.05) and plasma insulin (P = 0.0077) concentrations, but plasma glucose concentrations were not affected by infection (P = 0.1079). Virus-challenged chickens had significantly lower plasma glucagon than did control chickens. Plasma glucagon concentrations in ARVCU98-, S1733 (1:100)-, and S1733 (1:500)-challenged chickens were 45%, 41, and 32% lower, respectively, than control concentrations. Plasma insulin concentrations were not elevated as expected based upon the decreased plasma glucagon concentrations but were instead decreased in virus challenged chickens (Table 3.10). The plasma insulin concentrations in the ARVCU98- and S1733 (1:100)-challenged groups were significantly lower (P≤ 0.0077) than control and S1733 (1:500)-challenged groups.

**Insulin-like Growth Factors and Thyroid Hormones.** The plasma insulin-like growth factors (IGF-I and IGF-II) were not affected by virus challenge in these trials (Table 3.10). Likewise, the plasma T₄ and T₃ concentrations were also not affected significantly by virus challenge (Table 3.10). The T₃/T₄ ratio was also not affected significantly by virus infection (Table 3.10). However, the plasma T₃ and T₄ concentrations were very low in comparison with published values for these hormones.

**Cellular Integrity.** Cellular integrity of liver and muscle was also compromised by virus infection as indicated by activity of two enzymes, amino alanine transferase (ALT) and aspartate amino transferase (AST) that leak into plasma when there is cellular trauma due to such cellular events as oxidative stress. Results shown in Table 3.11 reflect the fact that both
ARVCU98 and S1733 in ovo challenge increased activities of these enzymes in the plasma. S1733 caused an increase in ALT at hatching and all virus treatments caused an increase in ALT at 14 d of age. There were time-dependent changes in ALT and AST activities (Table 3.11). The increased enzyme activities at 14 d of age corresponded to the time when the virus-challenged chickens showed a spike in post-hatch mortality. From 14 to 28 d of age, there is an apparent recovery as suggested by the decrease in the plasma activities of AST and ALT in the virus-challenged chickens.

**Immunohistochemistry.** Tissue samples of the bursa and thymus (14 and 28 days of age), treated with primary and secondary antibodies and examined microscopically did not reveal evidence for viral antigens in the virus-challenged groups. However, examination of the slides prepared for histopathology revealed numerous changes in the organization of the two primary lymphoid tissues (Fig. 3.4 to 3.15). Summaries of these pathological changes are presented in Tables 3.9A and 3.9B.

**Transmission Electron Microscopy.** Transmission electromicrographs (TEM) of pancreas, anterior pituitary, and thyroid of control and reovirus-challenged broiler chickens at 14d of age were examined (Fig. 3.16-3.27). In the TEMs representing thin sections through the pancreas (Fig. 3.16-3.18), significant ultrastructural changes in acini cells in associated with virus infection were observed. In control pancreas (Fig. 3.16) the cisternae of the cellular reticulum was well developed and appeared to be actively involved in production of enzymes for secretion as zymogen granules into the digestive tract. The zymogen granules were not bounded by membranes. Mitochondria were well-formed and abundant. The endoplasmic reticulum was widely dispersed within the cytoplasm. In pancreas from broilers challenged with ARVCU98 reovirus, the cellular ultrastructure was radically different from the control
The mitochondria were enlarged and appeared to be in many instances in a degenerated condition. The endoplasmic reticulum in the acini cells was condensed with very small cisternae indicative of low activity, which was supported by the observation of reduced numbers of zymogen granules in the cytoplasm as compared with control chicken pancreas acini cells. The nuclei of the acini cells also appeared to be very irregular and possibly in a degenerative state. The pancreatic acini cells from S1733 reovirus-challenged presented another very different ultrastructural appearance (Fig. 3.18). Under light microscopic examination it was apparent that the cytoplasm of acini cells was literally packed with zymogen granules. In the TEMs, it was apparent that the zymogen granules were significantly smaller than the granules found in control and in ARVCU98-challenged acini cells. The mitochondria in these acini cells were also condensed and widely dispersed. The nuclei of the cells were generally large and diffuse as compared with those found in control and ARVCU98-challenged pancreatic acini cells. The representative TEMs for the anterior pituitary are presented in Figures 3.19 to 3.21. In thick sections and in the TEMs, it was possible to distinguish two cell types based on staining properties, but there was no method for distinguishing their true identities. One cell type was light staining and the other was dark staining, and based upon these observations, the designation of chromophobe-like (light staining) and chromophil-like (dark staining) was ascribed to these two cell types. In the representative TEMS (Fig. 3.19 to 3.21), chromophobe-like and chromophil-like cells are identified. In the control TEM (3.19) the cytoplasm of the chromophil-like cells had more secretory granules than the chromophobes. Mitochondria were numerous and well defined in both cell types, but occasionally in the chromophil-like cells mitochondria appeared to be swollen and disrupted in control. In the representative TEM from the ARVCU98-challenged
chicken, the density of the secretory granules in the chromophil-like cells (Fig. 3.20) was apparently less than in control (Fig. 3.19). Less secretory granule density was suggested in chromophobe-like cells also. Mitochondrial morphology was affected apparently by the ARVCU98-reovirus challenge. They were enlarged in all cell types and appeared to be in a degenerative state. The nuclei of the cells in the anterior pituitary of the ARVCU98 challenged chickens also were more irregular in shape than those found in control. The morphological appearance of the chromophobe-like and chromophil-like cells in the anterior pituitary of the S1733 reovirus-challenged chickens (Fig. 3.21) was different from both the control and ARVCU98 reovirus-challenged chickens (Fig. 3.19 and 3.20). The chromophobe-like cells and chromophil-like cells appeared to have very few secretory granules in comparison with similar cell types in control and ARVCU98 reovirus challenged birds. The secretory granules in the chromophobe-like cells appeared to be larger and with less dense staining properties. The mitochondria in the cell types in the S1733 reovirus-challenged pituitary cells were greatly enlarged and appeared to be quite distorted in comparison with control. Representative TEMs of the thyroid glands from control, ARVCU98 reovirus-challenged, and S1733 reovirus-challenged chickens are presented in Figures 3.22 to 3.24. Two features common to the reovirus challenged tissues were that the mitochondria in the thyroid follicle epithelial cells were enlarged and distorted (Fig. 3.23 and 3.24), and the nuclei of the follicular epithelial cells were more irregular (Fig. 3.23 and 3.24) than control (Fig. 3.22).

It was also possible to identify both the S1733 and ARVCU98 reoviruses in several tissues of the challenged chickens. Shown in Figure 3.25 are numerous virus S1733 particles in the liver of a challenged chicken. The virus appeared to be about 100nm in diameter. A
unique TEM depicting ARVCU98 shedding from centroacinar cells into the lumen of the acinus (Fig. 3.26). These virus particles appear to be around 80 nm in diameter. A comparison of ARVCU98 reovirus and S1733 reovirus (in cross section) is presented in Figure 3.27. Mitochondrial damage in these tissues was evident in the virus-challenged chickens at 14 days of age. Also there was a dense population of zymogen granules in the pancreas of S1733 challenged birds. In addition, in comparison to the control the follicular cells of both the virus-challenged groups were taller in height or thicker. ARVCU98 was demonstrated on the pancreas at 14 days of age, whereas S1733 was detected in the liver (Figs. 3.35 and 3.26).

**DISCUSSION**

Earlier studies (Qureshi et al., 1997, Heggen-Peay et al., 2000) demonstrated that oral inoculations of turkey poult with PEMS-associated reovirus (ARVCU98) caused lymphoid organ and liver atrophy and decreased growth rate in turkey poult. In this report pathogenicity of PEMS-associated ARVCU98 and a chicken reovirus isolate (S1733) were investigated after virus-challenge inocula were given *in ovo* into the yolk sac of broiler breeder eggs at 9 d of incubation. At 18 days of incubation (Table 3.1) percentage viability of the virus-challenged eggs was lower in comparison with the control, and those injected with ARVCU98 had the least viable eggs, which resulted in the lowest percent hatchability (24%) at day of hatch. The low level of hatch-rate for the virus-challenged chickens might be due to high virus replication in tissues that likely killed the embryos. These observations support the contention of Menendez et al. (1975) and Van der Heide et al. (1983) that vertical
transmission of reovirus was possible but at a low rate. The embryo lethality associated with reovirus in eggs helps to explain the low rate of vertical transmission.

*In ovo* inoculation of ARVCU98 and S1733 viruses resulted in growth depression from 7 d of age through 14 d of age with residual effects even through 28 d of age (Tables 3.2 and 3.3). In Trial 2, S1733 virus (1:500 dilution) at 1 d post hatch reduced body weight (Table 3.3). The fact that a large percentage of the S1733 virus (1:500 dilution) reovirus-challenged embryos developed and had hatch weights comparable with unchallenged controls is somewhat surprising. The data suggest that there is, perhaps, a threshold for lethality that was either inhibited by the embryo or that virus replication was more difficult than expected when low numbers of the virus were injected. Motha (1987) challenged six-day old embryonated eggs with a reticuloendotheliosis virus, and the hatchlings showed no weight difference at day of hatch when compared to the unchallenged chickens. Nevertheless, the results of this investigation show that reovirus given *in ovo* or by oral gavage at hatch, based on previous PEMS investigations from this laboratory, can induce a significant depression in post hatch body weight gain. Additionally, the average daily weight gain (Table 3.4) was significantly less in the virus-challenged chickens. Although there were no significant differences among treatment groups for feed conversion ratios (Table 3.5), virus-challenged chickens appeared to be less efficient in converting feed to body mass. Thus, impaired digestion, decreased uptake, and poor absorptive capacity in the intestinal tract of the reovirus-infected chickens and turkeys will contribute to decreased weight gain (Odetallah, 2001).

The relative weights of the bursa of Fabricius, thymus, and spleen were not affected consistently by *in ovo* virus challenge or by the virus type (Tables 3.6A-3.7B). Sporadic
effects were noted with both ARVCU98 and S1733 as they caused decreased relative weights of the thymus but not the bursa of Fabricius. The reason for these inconsistent results could be construed as an argument that other etiologic agents are required to allow for the fulminating enteritis that is associated with PEMS in turkeys and of other enteritis problems found in chickens. Liver relative weight of control chickens, as expected, was greater than liver relative weights of virus challenged chickens (Tables 3.6A-3.7B). The results for ARVCU98 reovirus challenge in chickens were comparable to those reported earlier in turkey pouls by Qureshi et. al. (1997) and Heggen-Peay (2001) who extended the hypothesis that a chicken reovirus virus might have been involved in the etiology of PEMS in the mid-1990’s. Support for this hypothesis comes from a report by Van der Heide (1980), who found that day old chickens experienced high rates of mortality and liver necrosis when challenged with a liver suspension from infected pouls and that the turkey reovirus isolate was neutralized \textit{in vitro} by antiserum from chickens with a reovirus infection. It is very possible that ARVCU98 reovirus might be a virus capable of infecting both chickens and turkeys. Thus, it is possible that a reovirus of chicken origin might have been the progenitor of the ARVCU98 that was involved in the index case for PEMS in 1991. The PEMS index case was in an area of North Carolina that had a very high concentration of both chicken and turkey farms in close proximity.

A plasma chemistry profile was established for chickens given an \textit{in ovo} virus challenged from either ARVCU98 or S1733 reoviruses. Plasma glucose levels were not different among the treatment groups and tended to be somewhat higher (337-382 mg/dL) than reported nonfasted (210-250 mg/dL) values for chickens (Hazelwood, 2000). This could be due to the fact that all the groups were full fed. Under a full feeding regimen
with elevated plasma glucose, plasma glucagon is down regulated (Hazelwood, 2000). In fact, plasma glucagon was at a level that was roughly 5-10% of chickens in a fasted state. Plasma glucagon and insulin concentrations in ARVCU98 and S1733 (1:100) were significantly lower in reovirus-challenged groups than in the control. Hazelwood (2000) contends that glucagon in the domestic fowl is the more potent of the two pancreatic hormones, but if that were to be the situation, the data would suggest that glucose levels should have been less in the reovirus-challenged chickens even with lower plasma insulin concentration (Table 3.11). Glucagon targets liver and muscle glycogen and converts it into glucose. Liver atrophy was exhibited in S1733 reovirus-challenged chickens but not in ARVCU98 which showed an increase in liver relative weight at 14 and 28 d post hatch.

Whether the virus challenge had affected uptake of plasma amino acids and glucose, used as hepatic and muscle gluconeogenic substrates, was not determined in this investigation. However, the decrease in insulin levels in reovirus-challenged chickens would suggest that tissue uptake might have been affected (Hazelwood, 2000). Insulin is a powerful anabolic hormone, and according to Hazelwood (2000) on a unit/unit basis even more potent than mammalian insulin. These observations lead one to conclude that reovirus challenge might have had a negative metabolic influence on infected chickens. Low concentrations of plasma in insulin are correlated with body weight depression such as that shown by ARVCU98- and S1733-challenged chickens. Doerfler et al. (2000), who worked with PEMS infected poults, reported lower level of plasma insulin and lower body weight gain in PEMS-affected poults. Sterner et al.(1988) used S1733-challenged chickens, which exhibited high plasma total protein and plasma albumin suggesting a dysfunctional liver. Those observations coupled with observations in the current investigation suggest that in ovo reovirus challenge resulted
in hepatic dysfunction possibly caused by decreased glucagon and insulin in virus-challenged chickens. The post hatch plasma IGF-I and IGF-II concentrations were not affected significantly by the *in ovo* reovirus challenges. IGF-II expression was reported to be greater than IGF-I in both chickens and turkeys (McMurtry, 1998), but in this investigation comparable levels of both were found. The fact that neither IGF-I nor IGF-II was affected by reovirus challenge in chickens was in contrast with observations reported by Doerfler et al. (2000) who used PEMS-infected poults.

The plasma concentrations of the thyroid hormones in the *in ovo* reovirus-challenged chickens was not affected significantly, which was in contrast to the depressions in T₄ and T₃ found in PEMS infected poults (Edens and Doefler, 1997a,b; Doefler et al., 2000). The T₃/T₄ ratio in the reovirus-challenged chickens was not significantly lower than the controls, but the numerical decrease in conversion of T₄ to T₃ supporting the hypothesis of a metabolic disorder in reovirus-challenged chickens.

Body weights were depressed as the result of virus challenge, but the virus-challenged chickens were not fat, but they were well fleshed. Thus, the lack of an IGF-I and IGF-II response would suggest that other factors might have been affected by the virus challenge. Decuypere et al. (1987) has suggested that lean birds have a low plasma insulin concentration but high plasma growth hormone concentration coupled with a low plasma T₃ concentration. In this investigation, plasma T₃ concentration in the control chickens was slightly, but not significantly higher compared to the plasma T₃ concentration in ARVCU98 and S1733 (1:100) reovirus-challenged groups. The plasma T₄ concentrations in all the virus-challenged broiler chickens were generally, but not significantly, higher as compared with controls. Doerfler *et al.* (1998) reported that turkey poults with a PEMS infection had
lower T₄ to T₃ conversion via deiodinases located in the liver, and this was demonstrated as a significant decrease in the T₃/T₄ ratio. The results obtained in this investigation suggest that there is a metabolic disorder associated with reovirus infection that appears to have been overlooked in the past.

Alterations in enzymes such as ALT and AST in PEMS-infected poults were consistent with the findings of Edens and Doerfler (1997) and Heggen et al., (2001). At 1 d of age, the ALT levels of the virus-challenged groups were significantly elevated suggesting a severe virus-related infective state in the liver. Elevated ALT activity was evident at 14 d of age in S1733 challenged chickens, but by 28 d of age there was no virus effect. AST activities were not affected significantly by virus challenge. These results were in contrast to observations made in PEMS-challenged poults. It should be noted that ALT is more indicative of liver damage than the AST, since the latter can be produced in tissues other than the liver (Chang et al., 1994). Sterner et al., (1988) also reported no difference in the AST activity between the negative control and S1733-challenged chickens. Elevated ALT levels were expected as liver pathology is attributed to reovirus infection.

Tissue sections of thymus and bursa from control, ARVCU98, and S1733 in ovo reovirus-challenged chickens were tested for the presence of viral antigen. At 14 and 21 days of age there was no viral antigens detected. It is possible that by these ages the chickens were recovering from the viremia. Heggen-Peay (2000) detected viral antigens in thymus and bursa of Fabricius at 2, 5, 7 days post inoculation. Therefore, viral antigen detection might be limited to a time of maximum virus expression at an earlier age.

A summary of the histopathology at 14 d post hatch revealed that eosinophilia-like changes were enhanced in ARVCU98 and S1733 reovirus-challenged chickens. Edens et al.
(1997c) have reported that the accumulation of eosinophils in lymphoid tissues such as the bursa of fabricius and the thymus precedes the eventual degeneration (decreased weight loss and decreased cellularity) of the tissue. By 28 d of age, loss of cellularity was detected in the thymus of S1733 and ARVCU98 reovirus-challenged chickens. The degenerative responses of the thymus of virus-challenged birds were also accompanied by increased numbers of macrophages in that tissue. These results suggest that virus-challenged chickens try to mount an immune response, but it is compromised during active infection leading to immunodysfunction. These observations were consistent with the results reported by Heggen et al., (1998).

When transmission electron micrographs of the pancreas, anterior pituitary, and thyroid glands from reovirus-challenged chickens were examined, they revealed high incidence of mitochondrial disruption similar to the findings liver cells from PEMS infected poults (Doerfler and Edens, 1997). Mitochondria are the cellular sites for oxidative metabolism. In the case of the virus-challenged chickens, this mechanism can become uncoupled and metabolic disruption can become manifest. The TEMs show mitochondrial abnormalities in thyroid, pancreas and pituitary of reovirus-challenged chickens. Thus, even though plasma glucose levels of virus-challenged chickens were not significantly different from the control, the ability of the virus-challenged chickens to utilize the primary energy source will be impaired and the birds will not be capable of maintaining body growth comparable with controls.

The TEMs show S1733 virus particles in the liver, which is believed to be the target organ in avian reovirus infection (Jones, 1989; Kibenge, 1985; Mandelli, 1978), and additionally ARVCU98 was found to be in the pancreas of ion ovo challenged chickens.
Body feathering of ARVCU98 and S1733 reovirus-challenged chickens was less than in control birds. Rosenberger et al., (1988) observed poor feathering in S1733-challenged broilers, and it has been observed in broilers suffering from runting and stunting syndrome, a condition linked to reovirus infection, poor feathering has been observed (Elmubarak et al., 1990).

Thus, it was concluded that reovirus infection from both the turkey isolate and the chicken isolate induce pathological signs that were comparable in chickens that were challenged in ovo. The results of the reovirus infection caused a metabolic disorder that was apparently of short duration but with extensive consequences. The metabolic hormones appear to have been compromised and several endocrine glands were also altered even down to ultrastructural level. The results from this investigation suggest that the ARVCU98, a turkey isolate, can cause pathogenic changes in chickens as well as in turkey poults. It is suggested without proof that ARVCU98 might have been a virus that might have been involved in the index case of PEMS.
Table 3.1: Percent hatchability and mortality of fertile eggs challenged with reovirus isolates.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Control</th>
<th>ARVCU98</th>
<th>S1733 (1:100)</th>
<th>S1733 (1:500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hatchability (%)</td>
<td>70</td>
<td>24</td>
<td>59</td>
<td>67</td>
</tr>
<tr>
<td>Viability at day 18 (%)</td>
<td>53</td>
<td>37</td>
<td>42</td>
<td>52</td>
</tr>
</tbody>
</table>

*A Eggs were challenged via the yolk sac at day 9 of incubation, 100ul/egg

Table 3.2. Body weights of broiler chicks challenged with reovirus isolates at day 9 of incubation, Trial 1.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weights (g) (LSMeans±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>37.32±0.55a</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>37.00±1.05a</td>
</tr>
<tr>
<td>S1733 (1:100)</td>
<td>36.94±0.63a</td>
</tr>
<tr>
<td>S1733 (1:500)</td>
<td>37.50±0.57a</td>
</tr>
</tbody>
</table>

*P value | 0.9169 | <0.0001 | <0.0001 | 0.1855 |

*A Mean body weights, at 1,7,14 and 28 days of age

*B Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation. 

*ab In a column, unlike superscript differs significantly (P<0.05)
Table 3.3. Body weights of broiler chicks challenged with reovirus isolates at day 9 of incubation, Trial 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Body weights (g) (LSMeans±SE) A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Control</td>
<td>44.2±0.68 b</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>45.4±0.79 b</td>
</tr>
<tr>
<td>S1733(1:100)</td>
<td>45.7±0.68 b</td>
</tr>
<tr>
<td>S1733(1:500)</td>
<td>48.5±0.68 a</td>
</tr>
</tbody>
</table>

Pvalue: 0.0004 < 0.0001 < 0.0001 0.0899

A Mean body weights, at 1,7,14 and 28 days of age
B Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.
ab In a column, unlike superscript differs significantly (P<0.05)

Table 3.4 Overall Mean Growth index A of broiler chicks from 1-28 days of age.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Growth Index A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>25.89 ±0.52 a</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>23.72 ±0.52 b</td>
</tr>
<tr>
<td>S1733 (1:100)</td>
<td>23.48 ±0.52 b</td>
</tr>
<tr>
<td>S1733 (1:500)</td>
<td>22.75 ±0.52 b</td>
</tr>
</tbody>
</table>

Pvalue 0.0478

A Calculated by division of body weight at termination by bodyweight at day 1 of age (d28/d1)
Table 3.5. Feed conversion ratio of broilers challenged with reovirus isolates at day 9 of incubation\(^1\). (Trial 1 and 2 combined.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>0-1 week</th>
<th>0-2 weeks</th>
<th>0-4 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.11±0.08(^a)</td>
<td>1.11±0.05(^a)</td>
<td>1.49±0.29(^a)</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>1.19±0.08(^a)</td>
<td>1.33±0.05(^a)</td>
<td>1.61±0.29(^a)</td>
</tr>
<tr>
<td>S1733 (1:100)</td>
<td>1.05±0.08(^a)</td>
<td>1.20±0.05(^a)</td>
<td>1.48±0.29(^a)</td>
</tr>
<tr>
<td>S1733 (1:500)</td>
<td>1.21±0.08(^a)</td>
<td>1.29±0.05(^a)</td>
<td>1.56±0.29(^a)</td>
</tr>
</tbody>
</table>

\(^P\) value 0.5641  0.0985  0.9881

\(^1\)Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.

Table 3.6A. Effects on relative lymphoid organ weights of broiler chicks challenged in ovo reovirus isolates at day 9 of incubation. 14 days of age. Trial 1.

<table>
<thead>
<tr>
<th>Relative lymphoid organ weights (%) (LSMeans±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment(^B)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>ARVCU98</td>
</tr>
<tr>
<td>S1733 (1:100)</td>
</tr>
<tr>
<td>S1733 (1:500)</td>
</tr>
</tbody>
</table>

\(^P\) value 0.0725  0.0674  0.0827  \(<0.0001\)

\(^B\)Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.

\(^{ab}\)In a column, means with unlike superscripts differ significantly (\(P<0.05\))
Table 3.6B. Effects on relative lymphoid organ weights of broiler chicks challenged in ovo reovirus isolates at day 9 of incubation. 28 days of age. Trial 1.

<table>
<thead>
<tr>
<th>Treatment <em>B</em></th>
<th>Bursa</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.336±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.289±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.065±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.23±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>0.247±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.133±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.093±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.21±0.28&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1733(1:100)</td>
<td>0.212±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.233±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.090±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.68±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1733(1:500)</td>
<td>0.262±0.03&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.189±0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.079±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.12±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*P* value 0.0608 0.01634 0.4279 0.0452

<sup>B</sup>Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.

<sup>a,b</sup>In a column, unlike superscript differs significantly (*P*<0.05)
Table 3.7A. Effects on relative lymphoid organ weights of broiler chicks challenged \textit{in ovo} reovirus isolates at day 9 of incubation. 14 days of age. Trial 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bursa</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.205 ±0.02(^a)</td>
<td>0.175±0.02(^a)</td>
<td>0.064±0.01(^{ab})</td>
<td>3.971±0.27(^{ab})</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>0.203±0.02(^a)</td>
<td>0.171±0.02(^a)</td>
<td>0.089±0.01(^a)</td>
<td>4.751±0.30(^a)</td>
</tr>
<tr>
<td>S1733(1:100)</td>
<td>0.196±0.02(^a)</td>
<td>0.200±0.02(^a)</td>
<td>0.080±0.01(^{ab})</td>
<td>3.888±0.30(^{ab})</td>
</tr>
<tr>
<td>S1733(1:500)</td>
<td>0.259±0.02(^a)</td>
<td>0.201±0.02(^a)</td>
<td>0.077±0.01(^{ab})</td>
<td>3.761±0.27(^{ab})</td>
</tr>
</tbody>
</table>

\(P\) value 0.1555 0.7119 0.1418 0.1003

\(^a\)^ Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.
\(^{ab}\) In a column, means with unlike superscripts differs significantly (\(P<0.05\))

Table 3.7B. Effects on relative lymphoid organ weights of broiler chicks challenged \textit{in ovo} reovirus isolates at day 9 of incubation. 28 days of age. Trial 2.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bursa</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.237±0.03(^a)</td>
<td>0.198±0.02(^a)</td>
<td>0.092±0.01(^a)</td>
<td>3.400±0.19(^a)</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>0.262±0.03(^a)</td>
<td>0.186±0.02(^a)</td>
<td>0.088±0.01(^a)</td>
<td>3.429±0.19(^a)</td>
</tr>
<tr>
<td>S1733(1:100)</td>
<td>0.248±0.02(^a)</td>
<td>0.140±0.02(^a)</td>
<td>0.084±0.01(^a)</td>
<td>3.171±0.21(^a)</td>
</tr>
<tr>
<td>S1733(1:500)</td>
<td>0.266±0.02(^a)</td>
<td>0.160±0.02(^a)</td>
<td>0.087±0.01(^a)</td>
<td>3.261±0.17(^a)</td>
</tr>
</tbody>
</table>

\(P\) value 0.7145 0.1881 0.9479 0.7698

\(^b\) Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.
\(^{a,b}\) In a column, unlike superscript differs significantly (\(P<0.05\))
Table 3.8 Feather Scores of broilers challenged with reovirus isolates or PBS at day 9 of incubation\(^1\). (At 2 and 4 weeks of age.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feather Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>3</td>
</tr>
<tr>
<td>S1733</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^1\)Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.

Table 3.9 Necropsy observation of broilers inoculated or broilers challenged with reovirus isolates or PBS at day 9 of incubation\(^1\)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>ARVCU98</th>
<th>S1733</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestines</td>
<td>Unaffected</td>
<td>Thin-walled gaseous 16/16</td>
<td>Thin-walled gaseous 16/16</td>
</tr>
</tbody>
</table>

\(^1\)Broiler chicks were challenged with ARVCU98, S1733 (1:100 and 1:500) and or PBS at day 9 of incubation.
Table 3.10A. Histopathology of thymus from broiler chickens treated *in ovo* with reovirus.* Trial 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ARVCU98</th>
<th>S1733 (1:100)</th>
<th>S1733 (1:500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Eosinophilia” (14d)</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cell Loss (28d)</td>
<td>U</td>
<td>-</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Macrophage (28d)</td>
<td>U</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* U-unaffected
+ Occasional increase
++Obvious increase
+++Greatly increased
- Some loss of cells from medullary portion of follicle
-- Obvious loss of cells from medullary portion of follicle

Table 3.10B. Histopathology of bursa from broiler chickens treated *in ovo* with reovirus.* Trial 2.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>ARVCU98</th>
<th>S1733 (1:100)</th>
<th>S1733 (1:500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>“Eosinophilia” (14d)</td>
<td>U</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cell Loss (28d)</td>
<td>U</td>
<td>--</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Macrophage (28d)</td>
<td>U</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* U-unaffected
+ Occasional increase
++Obvious increase
+++Greatly increased
- Some loss of cells from medullary portion of follicle
-- Obvious loss of cells from medullary portion of follicle
Table 3.11. Overall mean plasma profile of broiler chicks challenged with reovirus isolates at day 9 of incubation.

<table>
<thead>
<tr>
<th>Plasma chemistry</th>
<th>Control</th>
<th>ARVCU98 (1:100)</th>
<th>S1733 (1:100)</th>
<th>S1733 (1:500)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>376±14a</td>
<td>337±14a</td>
<td>382±15a</td>
<td>377±14a</td>
<td>0.1079</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>216±45a</td>
<td>120±8b</td>
<td>128±16b</td>
<td>147±14ab</td>
<td>0.0502</td>
</tr>
<tr>
<td>Insulin (pg/ml)</td>
<td>3597±212a</td>
<td>1938±92b</td>
<td>2363±192b</td>
<td>3731±529a</td>
<td>0.0077</td>
</tr>
<tr>
<td>IGF-1 (ng/ml)</td>
<td>37±1.6a</td>
<td>42±2.2a</td>
<td>40±2.16a</td>
<td>41±2.4a</td>
<td>0.4562</td>
</tr>
<tr>
<td>IGF-2 (ng/ml)</td>
<td>43±2.9a</td>
<td>47±3.2a</td>
<td>51±6.2a</td>
<td>44±4.1a</td>
<td>0.6424</td>
</tr>
<tr>
<td>T3 (ng/l)</td>
<td>1.76±0.14a</td>
<td>1.60±0.16a</td>
<td>1.64±0.109a</td>
<td>1.79±0.62a</td>
<td>0.6577</td>
</tr>
<tr>
<td>T4 (ng/ml)</td>
<td>8.54±0.69a</td>
<td>9.04±0.46a</td>
<td>8.63±0.74a</td>
<td>9.3±0.77a</td>
<td>0.8153</td>
</tr>
<tr>
<td>T3/T4 ratio</td>
<td>0.207±0.04a</td>
<td>0.177±0.03a</td>
<td>0.210±0.03a</td>
<td>0.204±0.03a</td>
<td>0.7691</td>
</tr>
</tbody>
</table>

\(a,b\) In a row, means with different superscripts differ significantly \((P<0.05)\)
Table 3.12. Liver function assay (mg/μl) of broiler chicks challenged with reovirus isolates\(^1\) at day 9 of incubation overtime

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ALT</th>
<th>AST</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 14</td>
</tr>
<tr>
<td>Control</td>
<td>111±19(^b)</td>
<td>32±11(^b)</td>
</tr>
<tr>
<td>ARVCU98</td>
<td>178±26(^a)</td>
<td>28±11(^b)</td>
</tr>
<tr>
<td>S1733</td>
<td>177±22(^a)</td>
<td>64±8(^a)</td>
</tr>
</tbody>
</table>

\(P\) value 0.0562 0.0291 0.8465 0.2670 0.8122 0.9908

\(^1\) Broilers were inoculated with either ARVCU98, S1733 or control PBS
\(^ab\) In a column, unlike superscript differs significantly \(P\leq0.05\)
Figure 3.1. Comparison of wing feathers of control and S1733 challenged broiler chickens
Figure 3.2. Control broiler at four weeks of age.
Figure 3.3. S1733 challenged chicken at four weeks of age.
Figure 3.4. Control thymus at 14 days of age. Reticular structures (RS) are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.5. ARVCU98 challenged, thymus at 14 days of age. Reticular structures (RS) are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.6. S1733 challenged thymus at 14 days of age. Reticular structures (RS) are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.7. Control thymus at 28 days of age. Reticular structures are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.8. ARVCU98 challenged thymus at 28 days of age. Reticular structures are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.9. S1733 challenged thymus at 28 days of age. Reticular structures are the pale areas throughout the medullary (M) regions. The darker staining area is the cortex (C). 400X, Light Microscopy.
Figure 3.10. Control bursa of Fabricius at 14 days of age. LM is lamina propria, M is medulla; C is cortex.
Figure 3.11A. ARVCU98 challenged bursa of Fabricius at 14 days of age. LM is lamina propria; M is medulla; C is cortex; E is “eosinophilia”.
Figure 3.11B. ARVCU98 challenged bursa of Fabricius at 14 days of age. M is medulla; C is cortex.
Figure 3.12. S1733 challenged bursa of Fabricius at 14 days of age. M is medulla; C is cortex. E is “eosinophilia”. 400X. Light microscopy.
Figure 3.13. Control bursa of Fabricius at 28 days of age. F is follicle; C is cortex; M is medulla; UC is undifferentiated epithelial cells; PE is pseudostratified epithelium. 200X. Light microscopy.
Figure 3.14. ARVCU98 bursa of Fabricius at 28 days of age. F is follicle; C is cortex; M is medulla; UC is undifferentiated epithelial cells; 200X. Light microscopy.
Figure 3.15. S1733 challenged bursa of Fabricius at 28 days of age. C is cortex; M is medulla; UC is undifferentiated epithelial cells; 200X. Light microscopy.
Figure 3.16. This is a pancreatic acini cell from a control broiler chicken pancreas. The acini cell is distinguished from beta cell by the anatomy of the zymogen granule which is not bound by membrane in the acini cell. 5000X magnification.

N is nucleus
COR is cisternae of reticulum-develops as concentric and almost parallel waves of RER around nucleus after feeding
RER is rough endoplasmic reticulum
M is mitochondria
ZG is zymogen granule
Figure 3.17. This is a pancreatic acini cell from an ARVCU98 reovirus-challenged broiler chicken pancreas. The acini cell is distinguished from beta cell by the anatomy of the zymogen granule which is not bound by membrane in the acini cell. 5000X magnification.

N is nucleus
COR is cisternae of reticulum-develops as concentric and almost parallel waves of RER around nucleus after feeding
RER is rough endoplasmic reticulum
M is mitochondria
ZG is zymogen granule
Figure 3.18. This is a pancreatic acini cell from a S1733 reovirus-challenged broiler chicken pancreas. The acini cell is distinguished from beta cell by the anatomy of the zymogen granule which is not bound by membrane in the acini cell. 5000X magnification.

N is nucleus
COR is cisternae of reticulum-develops as concentric and almost parallel waves of RER around nucleus after feeding
RER is rough endoplasmic reticulum
M is mitochondria
ZG is zymogen granule
Figure 3.19. These are chromophobe and chromophil cells in the anterior pituitary of a control broiler chicken. 5000X magnification.

CB-is chromophobe
CL is chromophil
N is nucleus
RER is rough endoplasmic reticulum
M is mitochondria
SG is secretory granule
Figure 3.20. These are chromophobe and chromophil cells in the anterior pituitary of an ARVCU98 reovirus-challenged broiler chicken. 5000X magnification.

CB-is chromophobe
CL is chromophil
N is nucleus
RER is rough endoplasmic reticulum
M is mitochondria
SG is secretory granule
Figure 3.21. These are chromophobe and chromophil cells in the anterior pituitary of a S1733 reovirus-challenged broiler chicken. 5000X magnification.

CB is chromophobe
CL is chromophil
N is nucleus
RER is rough endoplasmic reticulum
M is mitochondria
SG is secretory granule
Figure 3.22. These are follicular epithelial cells in the thyroid gland from a control broiler chicken. 5000X magnification.

COR is cisternae of the reticulum
N is nucleus
RER is rough endoplasmic reticulum
M is mitochondria
SG is secretory granule
Figure 3.23. These are follicular epithelial cells in the thyroid gland from an ARVCU98 reovirus-challenged broiler chicken. 5000X magnification.

COR is cisternae of the reticulum
N is nucleus
RER is rough endoplasmic reticulum
M is mitochondria
SG is secretory granule
BC is blood cell in a capillary
Figure 3.24. These are follicular epithelial cells in the thyroid gland from a S1733 reovirus-challenged broiler chicken. 5000X magnification.

COR is cisternae of the reticulum  
G is Golgi  
N is nucleus  
RER is rough endoplasmic reticulum  
M is mitochondria  
SG is secretory granule  
BC is blood cell in a capillary
Figure 3.25. Reovirus particle in the liver of a S1733 reovirus-challenged broiler chicken. 70,000X magnification; bar represents 200nm.
Figure 3.26. ARVCU98 reovirus being shed from pancreatic cells from a broiler chicken. Magnification is 20,000X.
Figure 3.27. Electron micrographs comparing ARVCU98 reovirus isolated from turkey poultcs and S1733 reovirus isolated from chickens. Magnification: 89,600X.
CHAPTER 4

Efficacy of Hyperimmunizing Breeder Hens to Protect Progeny Against PEMS-Associated ARVCU98 Reovirus Challenge

ABSTRACT  A PEMS-associated ARVCU98 vaccine ($10^{5.7}$ TCID$_{50}$; 1mL/hen) in Freund’s adjuvant was injected subcutaneously on the back of the neck at the initiation of lay (30 weeks of age) into 8 breeder turkey hens, and these were called vaccinated hens. Another 8 hens were control injected with LMH cell supernatant. Booster vaccinations were given two times separated by intervals of 2 weeks. Egg collection was done for a period of two week intervals at 30, 45 and 60 days after booster vaccinations (DAB). At hatch, poults from the two parents stocks were challenged orally with PEMS-associated ARVCU98 reovirus (200ul/poult) and were designated as 1) vaccinated and challenged 2) vaccinated but not challenged 3) unvaccinated and challenged 4) unvaccinated but not challenged. At 6 days after homologous reovirus challenge, body weights of challenged poults in the 45 DAB group was depressed but not in the 30 and 60 DAB groups. At 10 days after reovirus challenge, body weights of reovirus-challenged poults in the 30 DAB group were decreased but this was not observed in poults from the 45 and 60 DAB groups. At both 6 and 10 days after reovirus challenge, bursa of Fabricius relative weights were decreased significantly in reovirus-challenged poults from both control and vaccinated breeders. Lymphoid organ relative weights of poults in the 45 DAB group were decreased after reovirus-challenge in both vaccinated and control parents stock. Thymus relative weights were decreased by reovirus challenge in poults from vaccinated control and breeder hens at 10 days after reovirus challenge. Spleen relative weights in poults from control and vaccinated breeders were increased significantly at 6 and 10 days after reovirus challenge in the 45 and 60 DAG
groups, respectively. At 6 days after reovirus challenge, liver relative weights in reovirus-challenged poult groups from control and vaccinated breeders were decreased significantly in the 60 DAB group. At 10 days after reovirus challenge, liver relative weights in reovirus-challenged poult groups from control and vaccinated breeders were decreased significantly in the 30 DAB group, but in the 45 DAB group, liver relative weights were increased significantly in reovirus-challenged poult groups from control and vaccinated breeder hens. These observations suggest that vaccination for ARVCU98 only had transitory effects in the progeny of breeder turkey hens subjected to reovirus challenge at hatch.

Key Words: poult enteritis and mortality syndrome, reovirus, vaccination
INTRODUCTION

The ARVCU98 reovirus has been implicated as one of the etiologic agents that cause PEMS infection in turkey poults (Heggen-Peay et al., 2001; Chapters 2 and 3). PEMS has been characterized as a transmissible enteric disease of young turkey poults with high rates of morbidity and mortality and has been classified as a disease with multifactorial etiology. PEMS infection results in reduced weight gain, lymphoid organ and liver atrophy, and immunosuppression/immunodysfunction. The immunodysfunction was characterized by enhanced proinflammatory cytokine production by activated macrophages and by intestinal inflammation, abnormal gut motility and anorexia (Heggen-Peay et al., 2000). Although poults may survive the infection, their body weights remained below market standards with no signs of compensatory weight gain after infection.

Since reovirus infection is age-related, efforts to control its spread have focused on hyperimmunizing breeder hens (Meanger et al., 1997). Immunity against certain infections can be achieved in two ways 1) vaccination or infection (active immunity) or 2) by transfer of antibodies or lymphocytes from actively immunized animals (Abbas et al., 2001). The transfer of passive antibodies from dam to progeny via the egg provides temporary resistance to infectious agents by providing immediate protection. Innate/passive immunity is time dependent as it declines to ineffectual levels by 10-20 days after hatch (Tizard, 1992). Innate/passive immunity results from transfer of maternal IgG to the developing yolk that ultimately transfers to the embryonic circulation (Saif et al., 1993).

Thus, it was hypothesized that vaccination of turkey breeder hens with ARVCU98 attenuated vaccine just before initiation of lay would confer immunity to progeny and protect
them against homologous reovirus challenge at day of hatch. The results of this investigation on the pathogenicity of ARVCU98 are reported in this paper.

**MATERIALS AND METHODS**

**Animal Care.** This project was approved and conducted under the supervision of the North Carolina State University Animal Care and Use Committee which has adopted Animal Care and Use Guidelines governing all animal use in experimental procedures.

**Animal husbandry.** This investigation was conducted with three separate hatches of pouls. Two turkey breeder hen groups of eight hens per group were designated as control or vaccinated/hyperimmunized. These hens were sourced from the North Carolina State University Flock (Nicholas strain). The turkey breeder hens were 30 weeks old when they were vaccinated (1ml/hen; subcutaneously at back of the neck) with the inactivated PEMS-associated ARVCU98 reovirus (10^{5.7} TCID_{50}) in incomplete Freund’s adjuvant, and a booster vaccination was administered after two weeks. Eggs were collected at two weeks interval commencing at 30, 45 and 60 days after the booster vaccination. Eggs were incubated under standard conditions for turkey eggs. At hatch, poults from the two breeder groups were wing-banded, weighed, challenged orally with homologous ARVCU98 reovirus (100ul/poult) and placed into plastic isolators (Standard Safety, Chicago, IL). There were four groups that were designated as 1) vaccinated and challenged 2) vaccinated but not challenged 3) unvaccinated and challenged 4) unvaccinated but not challenged. Poults in these four groups were placed in separate isolators. A total 20 poults per isolator were of started in an ambient temperature of 35°C that was decreased by 4°C at intervals of 7 days. There was continuous incandescent lighting provided in this investigation. Feed and water were provided for free access. A
special irradiated feed, sterilized Mazuri feed, (Purina Mills, St. Louis, MO), equivalent to the standard North Carolina Agriculture Research Service turkey starter diet, was used to assure that no extraneous bacterium or virus was unknowingly involved in the investigation. Feed and water for the poults in this investigation were placed in bulk into the isolators to prevent accidental loss of biosecurity, and scales for determination of body weight were also held in the isolators until the end of the investigation. Poults removed from the isolators were first caught using the glove ports in the isolators, placed into an antechamber that was opened and resealed before a second seal was removed from the isolator to allow collection of the sample poults. After the sample poults had been removed from the antechamber, it was resealed and fumigated with a chlorine compound.

Feed and water were given ad libitum. Body weight and lymphoid organ weights were measured to assess the effect of dam vaccination and post hatch ARVCU98 reovirus challenge.

**Vaccine production.** LMH cells were infected with ARVCU98 (10$^{5.7}$ TCID$_{50}$), frozen and thawed 3x and clarified by low speed centrifugation. Formaldehyde was added to inactivate the virus with a final concentration of 0.1%. Equal volume of incomplete Freund’s adjuvant was added to the mixture.

**Body weights and lymphoid organ weights.** Body weights were taken at day of hatch, and days 6 and 10 post hatch. Bursa of Fabricius, thymus (left side of the neck), spleen and liver were removed and weighed from 8 birds per treatment group. Lymphoid organs were expressed as percentage of body weight and reported as organ relative weights.

**Statistical Analysis.** The data from the completely randomized experimental design were subjected to analysis of variance testing using the General Linear Models procedures of SAS.
(SAS Institute, 1999). If the main effects of treatment or time were significant, treatment means were separated using the least significant difference procedure of SAS (1999). Significance was set at $P \leq 0.05$.

**RESULTS**

**In vivo studies.** Tables 4.1 and 4.2 present the treatment mean body weights of control and reovirus-challenged poults from control and hyperimmunized turkey breeder hens. At 6 days after homologous ARVCU98 challenge, body weights of reovirus-challenged poults in the 45 DAB group was depressed but not in the 30 and 60 DAB groups (Table 4.1). At 10 days after ARVCU98 challenge, body weights of ARVCU98-challenged poults in the 30 DAB group were decreased but this was not observed in poults from the 45 and 60 DAB groups (Table 4.2). At both 6 and 10 days after ARVCU98 challenge, bursa of Fabricius relative weights were decreased significantly in ARVCU98-challenged poults in the 45 and 60 DAB groups from both control and vaccinated breeders (Tables 4.3 and 4.4). Thymus relative weights at 6 and 10 days after ARVC98 challenge are presented in Table 4.5 and 4.6. At 10 days after ARVCU98 challenge, thymus relative weights of poults in the 45 DAB groups were decreased significantly in ARVCU98-challenged poults from both control and vaccinated breeders (Table 4.6). Spleen relative weights in ARVCU98-challenged poults from control and vaccinated breeders were increased significantly at 6 and 10 days after ARVCU98 challenge in the 45 and 60 DAB groups, respectively (Table 4.7 and 4.8). At 6 days after ARVCU98 challenge, liver relative weights in virus-challenged poults from control and vaccinated breeders were decreased significantly in the 60 DAB group (Table 4.9). At 10 days after virus challenge, liver relative weights in virus-challenged poults from
control and vaccinated breeders were decreased significantly in the 30 DAB group, but in the 45 DAB group, liver relative weights were increased significantly in virus-challenged poult from control and vaccinated breeder hens (Table 4.10).

**DISCUSSION**

The definitive etiologic agents that cause PEMS still elude researchers. Even though, several putative etiologic agents have been implicated in PEMS, research to identify veterinary procedures to prevent the disease are not available. The PEMS disease was very expensive and more than $161 million dollars in revenue were lost by the turkey industry in the Southeastern United States through 1997. In lieu of a vaccination program, the turkey industry initiated a very strict biosecurity program. The biosecurity program was helpful in breaking the disease cycle, but it is a process that can be easily by-passed.

One way to possibly deter PEMS infection is through a vaccination program that would immunize the poult against one or all of the potential etiologic agents implicated in the disease. The turkey reovirus ARVCU98 has been implicated as a virus that might compromise the intestinal tract of poult and set up the conditions leading to fulminating PEMS (Qureshi et al., 1997, Heggen-Peay et al., 2000). Post-hatch vaccination would, by necessity, have to be administered to newly hatched poult. However, the potential exists for maternal antibody interference against successful vaccination of newly hatched poult because maternal antibody persists in chicks and poult for 10-20 days post hatch (Tizard, 1992). Therefore, use of an inactivated ARVCU98 reovirus vaccine in an incomplete Freund’s adjuvant in turkey breeders before the initiation of lay was believed to be a means to increase protection against the ARVCU98 reovirus in poult from the vaccinated breeders.
The results were not as clear as one would hope, but there were indications that the vaccination program might have possibilities. Body weights of reovirus-challenged pouls from control and vaccinated breeders were reduced minimally at 6 days after reovirus challenge in the 45 DAB group and at 10 days in the 30 DAB group. Furthermore, the thymus weight was reduced only at 10 days after reovirus challenge in the 45 DAB group of PEMS-challenged pouls from control and vaccinated breeders. Thymus weight decrease is one of the primary signs of reovirus in pouls (Edens et al., 1997abc; Qureshi et al., 1997, Heggen-Peay et al., 2000). The observation that relative weights of thymus were decreased by reovirus challenge only one time in this investigation was very encouraging for the possible control by PEMS-associated reovirus vaccination. However, the route of administration of the pilot vaccine used in this investigation was via subcutaneous injection into the breeder hens. Van der Heide (1976), working with chickens, demonstrated that maternal immunization protected post hatch chicks from experimental reovirus challenge only when the vaccine was administered to the dam via the oral route. Nevertheless, the results from this investigation still strongly suggest that vaccination against reovirus in the breeder hen holds great potential, and it is suggested that additional work should be conducted to look at alternative means of vaccine administration that would generate the best transfer of maternal antibody to protect the poult from PEMS-associated reovirus or other viruses associated with PEMS.
Table 4.1. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on body weight of progeny at 6 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control Non-challenged</th>
<th>Control Challenged</th>
<th>Vaccinated Challenged</th>
<th>Vaccinated Non-challenged</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 DAB</td>
<td>65.85 (\pm 2.62^a)</td>
<td>70.00 (\pm 2.45^a)</td>
<td>66.12 (\pm 2.45^a)</td>
<td>63.25 (\pm 2.46^a)</td>
<td>0.3008</td>
</tr>
<tr>
<td>45 DAB</td>
<td>90.50^a (\pm 3.68)</td>
<td>81.33^b (\pm 3.47)</td>
<td>84.71^b (\pm 3.93)</td>
<td>95.37^a (\pm 3.67)</td>
<td>0.0494</td>
</tr>
<tr>
<td>60 DAB</td>
<td>90.62 (\pm 1.98^a)</td>
<td>90.29 (\pm 2.1^a)</td>
<td>93.0 (\pm 1.9^a)</td>
<td>86.87 (\pm 1.98^a)</td>
<td>0.1898</td>
</tr>
</tbody>
</table>

\(^{ab}\), in a row unlike superscript differs significantly \((P<0.05)\)

DAB- Day after boost
Table 4.2. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on body weight of progeny at 10 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control Vaccinated</th>
<th>Vaccinated</th>
<th>Vaccinated Non-challenged</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
<td></td>
</tr>
<tr>
<td>30 DAB</td>
<td>113.75 ± 3.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>89.25 ± 3.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>84.00 ± 3.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>114.00 ± 3.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>45 DAB</td>
<td>136.8 ± 6.74</td>
<td>120.25 ± 5.33</td>
<td>133.25 ± 5.33</td>
<td>139.78 ± 5.01</td>
<td>0.0737</td>
</tr>
<tr>
<td>60 DAB</td>
<td>149.11 ± 4.29</td>
<td>145.25 ± 4.56</td>
<td>140.89 ± 4.30</td>
<td>153.62 ± 4.56</td>
<td>0.2323</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.3. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on bursa weights (% of BW) of progeny at 6 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.094 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.094 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.090 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.082 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5986</td>
</tr>
<tr>
<td>30 DAB</td>
<td>0.147 ±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.125 ±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.122 ±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.159 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0474</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.209 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.154 ±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.130 ±0.016&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.161 ±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0243</td>
</tr>
</tbody>
</table>

<sup>a</sup>, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.4. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on bursa weights (% of BW) of progeny at 10 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
</tr>
<tr>
<td>30 DAB</td>
<td>0.089 ± 0.01a</td>
<td>0.081 ± 0.01a</td>
<td>0.087 ± 0.01a</td>
<td>0.086 ± 0.01a</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.150 ±0.10a</td>
<td>0.118 ±0.01b</td>
<td>0.101 ±0.01b</td>
<td>0.143 ±0.01a</td>
</tr>
<tr>
<td>60 DAB</td>
<td>0.209 ±0.01a</td>
<td>0.147 ±0.01ab</td>
<td>0.130 ±0.01b</td>
<td>0.182 ±0.01a</td>
</tr>
</tbody>
</table>

*ab, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.5. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on thymus weights (% of BW) of progeny at 6 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control Non-challenged</th>
<th>Control Challenged</th>
<th>Vaccinated Challenged</th>
<th>Vaccinated Non-challenged</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 DAB</td>
<td>0.122 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.122 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.111 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.122 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7732</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.104 ±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.110 ±0.09&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.093 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.097 ±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7895</td>
</tr>
<tr>
<td>60 DAB</td>
<td>0.120 ±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.108 ±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.115 ±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.120 ±0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.9499</td>
</tr>
</tbody>
</table>

<sup>a</sup>, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.6. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on thymus weights (% of BW) of progeny at 10 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control Vaccinated</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progeny</td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
</tr>
<tr>
<td>30 DAB</td>
<td>0.137 ± 0.01a</td>
<td>0.118 ± 0.01a</td>
<td>0.101 ± 0.01a</td>
<td>0.132 ± 0.01a</td>
<td>0.2044</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.138 ± 0.01a</td>
<td>0.096 ± 0.01b</td>
<td>0.104 ± 0.01b</td>
<td>0.114 ± 0.01ab</td>
<td>0.0218</td>
</tr>
<tr>
<td>60 PDB</td>
<td>0.134 ± 0.01a</td>
<td>0.110 ± 0.01ab</td>
<td>0.108 ± 0.01b</td>
<td>0.113 ± 0.01ab</td>
<td>0.1523</td>
</tr>
</tbody>
</table>

ab, In a row unlike superscript differs significantly (P<0.05)
DAB- Day after boost
Table 4.7. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on spleen weights (% of BW) of progeny at 6 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Non-challenged</th>
<th>Challenged</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Control</td>
<td></td>
<td>Control</td>
<td></td>
</tr>
<tr>
<td>Progeny</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 DBP</td>
<td>0.046 ± 0.005(^a)</td>
<td>0.048 ± 0.004(^a)</td>
<td>0.044 ± 0.004(^a)</td>
<td>0.038 ± 0.004(^a)</td>
<td>0.7732</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.055 ± 0.04(^b)</td>
<td>0.086 ± 0.04(^a)</td>
<td>0.079 ± 0.04(^a)</td>
<td>0.057 ± 0.04(^b)</td>
<td>0.0108</td>
</tr>
<tr>
<td>60 DAB</td>
<td>0.050 ± 0.03(^a)</td>
<td>0.075 ± 0.04(^a)</td>
<td>0.066 ± 0.03(^a)</td>
<td>0.101 ± 0.03(^a)</td>
<td>0.7506</td>
</tr>
</tbody>
</table>

\(^a\), In a row unlike superscript differs significantly (P≤0.05)

DAB- Day after boost
Table 4.8 Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on spleen weights (% of BW) of progeny at 10 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
</tr>
<tr>
<td>30 DAB</td>
<td>0.050</td>
<td>0.052</td>
<td>0.049</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>± 0.005^a</td>
<td>± 0.004^a</td>
<td>± 0.004^a</td>
<td>± 0.004^a</td>
</tr>
<tr>
<td>45 DAB</td>
<td>0.054</td>
<td>0.143</td>
<td>0.079</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>±0.01^a</td>
<td>±0.01^a</td>
<td>±0.01^a</td>
<td>±0.01^a</td>
</tr>
<tr>
<td>60 DAB</td>
<td>0.057</td>
<td>0.083</td>
<td>0.069</td>
<td>0.054</td>
</tr>
<tr>
<td></td>
<td>±0.01^b</td>
<td>±0.01^a</td>
<td>±0.01^ab</td>
<td>±0.01^b</td>
</tr>
</tbody>
</table>

^a, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.9. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on liver weights (% of BW) of progeny at 6 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control</th>
<th>Control Vaccinated</th>
<th>Vaccinated</th>
<th>Vaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-challenged</td>
<td>Challenged</td>
<td>Challenged</td>
<td>Non-challenged</td>
</tr>
<tr>
<td></td>
<td>30 DAB</td>
<td>3.349 ±0.136&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.631 ±0.127&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.364 ±0.127&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>45 DAB</td>
<td>4.29 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.04 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.83 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 DAB</td>
<td>3.301 ±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.893 ±0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.300 ±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a, b</sup>, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
Table 4.10. Effect of hyperimmunization of turkey breeder hens against ARVCU98 reovirus on post hatch ARVCU98 challenge on liver weights (% of BW) of progeny at 10 days after reovirus challenge.

<table>
<thead>
<tr>
<th>Breeder</th>
<th>Control Non-challenged</th>
<th>Control Challenged</th>
<th>Vaccinated Challenged</th>
<th>Vaccinated Non-challenged</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 DAB</td>
<td>4.176 ±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.329 ±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.071 ±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.139 ±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0019</td>
</tr>
<tr>
<td>45 DAB</td>
<td>3.413 ±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.215 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.437 ±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.194 ±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>60 DAB</td>
<td>3.436 ±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.988 ±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.399 ±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.469 ±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4178</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>, In a row unlike superscript differs significantly (P<0.05)

DAB- Day after boost
SUMMARY AND CONCLUSION

The purpose of this research was to further characterize PEMS in terms of pathogenicity. In earlier studies on PEMS, poult's were experimentally infected with either direct contact with seeder poult's or oral exposure to infected homogenates of either feces, intestinal tracts, or thymus. Since no single entity has been directly linked to the disease process, PEMS was classified as a multifactorial etiologic disease potentially caused by several viruses and bacteria. Results from these early studies established signs of PEMS as inhibition of weight gain, lymphoid organ (specifically thymus) degeneration, and liver atrophy. Lymphoid organ pathology led to an immunodyfunctional state characterized by several alterations in the immune system such as macrophage-induced cytokines and nitrite production and changes in the lymphocytic and mononuclear phagocytic system.

The first objective of this investigation was to compare the pathogenicity of PEMS-associated astrovirus (TastOSU, a turkey isolate), reovirus (ARVCU98, a turkey isolate), and ARVCU98 in the presence or absence of atypical *E. coli* frequently isolated from PEMS-infected poult's. This combination agent investigation reported in Chapter 2 was conducted to assess the effects of these organisms on poult body weight gain and lymphoid organ integrity. The ARVCU98 reovirus depressed body weight gain between 10-17 DPI. The presence of *E. coli* in the combination study did not result in significant differences compared with the control, but the presence of the *E. coli* was consistently associated with lower body weight gains. A decreased thymus relative weight in response to pathogen challenge approached significance (P=0.07) in comparison with the control poult's, and these results confirmed earlier reports of thymic atrophy due to astrovirus infection (Koci et al.,
2000; Schultz-Cherry et al., 1999; Qureshi et al., 1999). Although the lymphoid cell functions and population were not examined in this investigation, decreased organ relative weight and decreased body weight gain were in the same order of magnitude as results reported by Qureshi et al. (1999), wherein thymus degeneration was associated with lower lymphocytes counts and reduced lymphoproliferative abilities in TastOSU-challenged poults. Decreased immunologic status has been and is characteristic of PEMS infection. The presence of *E. coli* in the combination of pathogens (reovirus + astrovirus + *E. coli*) caused the lowest in weight gain and also had a negative influence on lymphoid organ relative weights.

Based on these results, PEMS infection with either ARVCU98 or TastOSU or their combination negatively influences body weight gain and lymphoid organ relative weights. This condition may be exacerbated by the presence of atypical *E. coli* frequently isolated in PEMS-susceptible poults. In poults exposed to these organisms in a natural setting, fulminating PEMS can be induced when there is interaction of many other factors that are common to the disease.

Vertical transmission of PEMS has been disputed ever since the disease was identified as a problem for the turkey industry in North Carolina. Nevertheless, it has been suggested that the possibility does exist. In Chapter 3, the second investigation in this thesis, instead of direct contact exposure to induce PEMS, an attempt was made to use the *in ovo* route as a means to infect embryos at 9 days of embryonation. Another innovative approach in this second study was to use the chicken embryo as the experimental model to test both turkey (ARVCU98 reovirus) and chicken (S1733 reovirus) isolates *in ovo* to evaluate the potential of zoonosis as well as vertical transmission of the reoviruses. Body weight gain,
lymphoid organ integrity, liver pathology, and pathophysiological alterations in the hatched chickens were assessed over a period of 28 days post hatch.

Body weight gains of chickens exposed \textit{in ovo} to the chicken and turkey reovirus were less than control chicken weight gains. This observation indicates that the ARVCU98 turkey isolate had the potential to be transmitted vertically in chicken embryos. From this observation, it was inferred that the index case of PEMS might have resulted from cross contamination of a chicken virus into turkey poultts and from that point onward, the virus may have mutated to become the causative agent that conditioned the intestinal tract to be susceptible to the other putative virus and bacterial agents associated with PEMS. However, the inferences are only limited to less weight gain because other signs of PEMS such as degeneration of bursa, thymus, spleen and liver were not immediately evident, but, during necropsy, it was noted that the virus infected broilers had thin-walled, gas-filled, frothy intestines. This intestinal pathology was earlier associated with increased activity of macrophage related inflammatory cytokines and nitrite production (Heggen-Peay, 2001). It was subjectively noted that S1733 (both 1:100 and 1:500) infected broilers had heart and kidney enlargement, which was absent in the ARVCU98 reovirus and control birds. The \textit{in ovo} virus challenges extended to decreased metabolic activity as indicated by decreased feather growth and development. When scoring was done, control birds scored 5 (best) while virus (ARVCU98 and S1733) infected chickens had a whole body feather score of 3.

More importantly, the results from this investigation showed that both the turkey and chicken reovirus isolates had significant negative effects on metabolically active hormones. Both glucagon and insulin were depressed in the virus challenged broiler chickens, but plasma glucose levels were not affected by virus challenge. The IGF-I and IGF-II levels were
not significantly affected by *in ovo* virus challenge. There was a slight decrease in the T₃/T₄ ratio for the all the virus challenged broilers suggesting that the conversion of T₄ to T₃ had been decreased. The hormonal imbalances found in this investigation were similar in the result reported by Doerfler et al (2000). Thus, it can be implied that the decrease in overall body weight gain in reovirus infected chickens/turkeys can be partially explained by metabolic disturbance that interferes with, muscle and skeleton development. An examination of transmission electron micrographs of the pancreas, anterior pituitary and thyroid revealed mitochondrial damage (degeneration) as a primary response to reovirus infection, but other cellular morphological changes also signaled virus-mediated disruption of function. Condensation of rough endoplasmic reticulum was commonly observed in cell from virus infected chickens, and this was associated with decreased production of secretory granules in the cell cytoplasm. Additionally, in the pancreas, it appeared that reovirus infection had inhibited zymogen granule secretion which would help to explain problems associated with increased feed conversions in field cases of reovirus and PEMS infection in the field. Mitochondrial degeneration in most of the tissues examined with transmission electron microscopy must be considered as an indicator of generally decreased metabolic activity, which would be consistent with the observation that muscle and organ wasting exhibited by chickens with reovirus infection and turkeys with PEMS is due to decreased mitochondria-based energy metabolism. The ARVCU98 was detected in the pancreas and S1733 was easily detected in the liver of infected chickens in this investigation.

The histopathological effect of *in ovo* virus challenge on bursa and thymus was evaluated, and between 14 and 28 days of age loss of cellularity eosinophilia was observed in tissues from all the virus-challenged broilers. In the ARVCU98-infected broilers,
macrophages were observed frequently in the thymus. The loss of cellularity in both the thymus and bursa of Fabricius was associated with accumulation of the eosinophil-like granulocytes.

In chapter 4, vaccination/hyperimmunization of turkey breeder hens against the reovirus ARVCU98 was done to assess its potential in preventing reovirus-mediated enteritis in pouls from the hyperimmunized breeders. It was believed that passive antibody transfer to progeny of the ARVCU98 vaccinated breeders would be sufficient to prevent enteritis resulting from ARVCU98 oral exposure of the pouls on the day of hatch. Protection was limited to day 6 post inoculation from the 30 days after boost treatment group progeny from either vaccinated or non-vaccinated control breeders. Those progeny from the 45 and 60 days after boost groups had sporadic signs of protection against the effects of exposure to reovirus after hatch. The route of administration of the pilot vaccine used in this investigation was via subcutaneous injection into the breeder hens. Van der Heide (1976), working with chickens, demonstrated that maternal immunization protected post hatch chicks from experimental reovirus challenge only when the vaccine was administered to the dam via the oral route. Nevertheless, the results from this investigation suggest that vaccination against reovirus in the turkey breeder hen holds great potential, and it is suggested that additional work be conducted to look at alternative means of vaccine administration that would generate the best transfer of maternal antibody to protect the poult from PEMS-associated reovirus or other viruses associated with PEMS.

In conclusion, important findings in this research program were found. First, PEMS-associated agents such as ARVCU98 and TastOSU cause depressed body weight gain and promote lymphoid organ and liver atrophy. The presence of *E. coli* exacerbates the pathology
associated with the reovirus and astrovirus infections. These results can even be more severe under field exposure where there are uncontrolled conditions that might facilitate more severe disease. Second, egg transmission was shown to be a possibility with avian reoviruses of both turkey and chicken origin. More importantly, this investigation showed that the turkey reovirus isolate, ARVCU98, can infect broilers challenged in ovo and cause significant pathology in the hatchling. There appeared to be parallel effects related to ARVCU98 and S1733 infections although severity of their induced pathologies was different. Reovirus challenges in ovo created pathological changes that resulted in conditions that were likened to development of metabolic disease in the infected hatchlings. This conclusion was drawn from observations on metabolically active plasma hormone concentrations and ultrastructural changes in the endocrine glands in which these hormones were secreted. Third, hyperimmunization of breeder turkey hens against the turkey reovirus isolate, ARVCU98, provided limited protection to progeny, which were given an ARVCU98 challenge at hatch. An alternative breeder hen immunization route, oral vaccination, might be more effective than subcutaneous inoculation. Additional work must be conducted to examine many of the questions arising from this research.
LIST OF REFERENCES


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