Isolation of a Reovirus from Poult Enteritis and Mortality Syndrome and Its Pathogenicity in Turkey Poults


Source: Avian Diseases, 46(1) : 32-47

Published By: American Association of Avian Pathologists

Isolation of a Reovirus from Poult Enteritis and Mortality Syndrome and Its Pathogenicity in Turkey Poults

C. L. Heggen-Peay, A M. A. Qureshi, A E. F. W. Edens, A B. Sherry, B P. S. Wakenell, C P. H. O'Connell, D and K. A. Schat D

A Department of Poultry Science, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC 27695
B Department of Microbiology, Pathology, and Parasitology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC 27606
C Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, CA 95616
D Unit of Avian Medicine, Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853

Received 2 May 2001

SUMMARY. Poult enteritis and mortality syndrome (PEMS) is an acute, infectious intestinal disease of turkey poults, characterized by high mortality and 100% morbidity, that decimated the turkey industry in the mid-1990s. The etiology of PEMS is not completely understood. This report describes the testing of various filtrates of fecal material from control and PEMS-affected poults by oral inoculation into poults under experimental conditions, the subsequent isolation of a reovirus, ARV-CU98, from one of the PEMS fecal filtrates, and in vivo and in vitro studies conducted to determine the pathogenicity of ARV-CU98 in turkey poults. In order to identify a filtrate fraction of fecal material containing a putative etiologic agent, poults were challenged in two independent experiments with 220- and 100-nm filtrates of fecal material from PEMS-negative and PEMS-positive poults. The 100-nm filtrate was chosen for further evaluation because poults inoculated with this filtrate exhibited mortality and significantly lower (P < 0.05) body weight and relative bursa weight, three clinical signs associated with PEMS. These results were confirmed in a third experiment with 100-nm fecal filtrates from a separate batch of PEMS fecal material. In Experiment 3, body weight and relative bursa and thymus weights were significantly lower (P < 0.05) in poults inoculated with 100-nm filtrate of PEMS fecal material as compared with poults inoculated with 100-nm filtrate of control fecal material. Subsequently, a virus was isolated from the 100-nm PEMS fecal filtrate and propagated in liver cells. This virus was identified as a reovirus on the basis of cross-reaction with antisera against avian reovirus (FDO strain) as well as by electrophoretic analysis and was designated ARV-CU98. When inoculated orally into poult reared under controlled environmental conditions in isolators, ARV-CU98 was associated with a higher incidence of thymic hemorrhaging and gaseous intestines. In addition, relative bursa and liver weights were significantly lower (P < 0.05) in virus-inoculated poult as compared with controls. Virus was successfully reisolated from virus-challenged poults but not from control birds. Furthermore, viral antigen was detected by immunofluorescence in liver sections from virus-challenged poult at 3 and 6 days postinfection and virus was isolated from liver at 6 days postinfection, suggesting that ARV-CU98 replicates in the liver. In addition to a decrease in liver weight, there was a functional degeneration as indicated by altered plasma alanine aminotransferase and aspartate aminotransferase activities in virus poult as compared with controls. Although this reovirus does not induce fulminating PEMS, our results demonstrated that ARV-CU98 does cause some of the clinical signs in...
PEMS-associated reovirus

PEMS, including intestinal alterations and significantly lower relative bursa and liver weights. ARV-CU98 may contribute directly to PEMS by affecting the intestine, bursa, and liver and may contribute indirectly by increasing susceptibility to opportunistic pathogens that facilitate development of clinical PEMS.

RESUMEN. Aislamiento de un reovirus a partir de pavos con síndrome de enteritis y mortalidad y determinación de su patogenicidad para pavos jóvenes.

El síndrome de enteritis y mortalidad de los pavos es una enfermedad infecciosa aguda de los pavos jóvenes caracterizada por alta mortalidad y hasta un 100% de morbilidad que afectó y diezmó la industria del pavo comercial a mediados de la década de 1990. La etiología de la enfermedad todavía no ha podido ser determinada claramente. Este reporte describe el efecto de varios filtrados, obtenidos a partir de heces de aves controles y aves enfermas, inoculados por la vía oral bajo condiciones experimentales y el subsecuente aislamiento de un reovirus, denominado ARV-CU98, a partir de uno de los filtrados obtenido de las aves enfermas. Se realizaron estudios in vivo e in vitro para determinar la patogenicidad del ARV-CU98 en pavos jóvenes. Para identificar la fracción del filtrado que contenía el agente etiológico, se desafiaron pavos jóvenes con diferentes filtrados de heces obtenidos de aves controles y aves enfermas, mediante el uso de filtros con poros de 220 y 100 nm en diámetro. Se eligió para una evaluación más exhaustiva el filtrado obtenido mediante el uso del filtro de 100 nm debido a que los pavos inoculados con el mismo presentaron alta mortalidad y una disminución significativa (P ≤ 0.05) del peso corporal y peso relativo de la bolsa de Fabricio (tres de los signos clínicos asociados con el síndrome). Estos resultados fueron confirmados en un tercer experimento en el cual se utilizó el filtrado obtenido con el filtro de 100 nm a partir de muestras de heces obtenidas de otro grupo de aves enfermas. En este experimento el peso corporal y los pesos relativos de la bolsa y timo fueron significativamente más bajos (P ≤ 0.05) en los pavos inoculados con el filtrado obtenido de las aves enfermas, que en los pavos inoculados con filtrado obtenido de aves del grupo control. Subsecuentemente se aisló un virus a partir del filtrado obtenido mediante el uso del filtro de 100 nm en muestras de heces de aves enfermas, el cual se propagó en cultivo de células hepáticas. Este virus fue identificado como reovirus basado en su reactividad cruzada con anticuerpos específicos contra la cepa FDO del reovirus aviar y por el patrón obtenido a partir del análisis de electroforesis, denominándose ARC-CU98. Cuando se inoculó oralmente el ARC-CU98 en pavos criados bajo condiciones de medio ambiente controladas, este virus fue asociado con una mayor incidencia de hemorragias en el timo y presencia de gas en los intestinos. También los pesos relativos de la bolsa y el hígado fueron significativamente (P ≤ 0.05) más bajos en los pavos inoculados con el virus, que en los pavos usados como control. Fue posible reaislar el virus a partir de los pavos desafiados, pero no a partir de los aves control. Se detectaron los antígenos virales mediante la técnica de inmunofluorescencia en secciones histológicas de hígado en las aves desafiadas a los 3 y 6 días después de la inoculación y se pudo reaislar el virus a partir del hígado a los 6 días después de la inoculación, lo cual sugiere que el virus ARV-CU98 es capaz de replicarse en este órgano. Además de disminución en el peso relativo del hígado, los niveles plasmáticos de aminotransferasa de alanina y aminotransferasa de aspartato indicaron una degeneración en la función del órgano en las aves inoculadas con el virus en comparación con las aves usadas como control. Aunque este reovirus no induce un síndrome de enteritis y alta mortalidad fulminante, nuestros resultados demostraron que el ARS-CU98 es capaz de causar algunos de los signos clínicos atribuidos a la enfermedad, incluyendo las alteraciones intestinales y disminución de los pesos relativos de la bolsa de Fabricio y el hígado. El virus ARS-CU98 puede contribuir directamente al síndrome debido a su capacidad de afectar los intestinos, bolsa e hígado y puede contribuir indirectamente mediante el aumento de la susceptibilidad de las aves afectadas a patógenos oportunistas, lo cual facilita el desarrollo del síndrome clínico de la enfermedad.

Key words: poult enteritis and mortality syndrome, reovirus, turkey, fecal filtrate

Abbreviations: ALT = alanine aminotransferase; ARV = avian reovirus; AST = aspartate aminotransferase; CEF = chicken embryo fibroblast; CEL = chicken embryo liver; CKC = chick kidney cell; CPE = cytopathic effects; DPI = days postinfection; FITC = fluorescein isothiocyanate; MD = Marek’s disease; OCT = optimal cutting temperature; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate-buffered saline; PEMS = poult enteritis
Poult enteritis and mortality syndrome (PEMS) is an acute, infectious intestinal disease of turkey poults, characterized by diarrhea, dehydration, weight loss, anorexia, growth depression, and high mortality (3). In addition, immune dysfunction has been associated with this disease. Specifically, previous studies have demonstrated significantly lower relative bursa of Fabricius, thymus, and spleen weights in PEMS poults as compared with control poults as early as 6 days and as late as 23 days after exposure to PEMS agents (25). In addition, both humoral and cellular immunity are negatively affected by PEMS (25). Reduced phagocytic potential of macrophages and abnormal cytokine profiles in abdominal exudates macrophages also have been associated with PEMS (14,15). Furthermore, alterations in CD4:CD8 T-lymphocyte ratios occur in the blood and spleen in poults with PEMS as compared with unchallenged controls (15).

The emergence of PEMS in 1991 led to a major depression in the turkey industry in North Carolina and other states during the remainder of the 1990s. Barnes and Guy (3) divided PEMS into two clinically distinct forms. “Excess mortality,” the milder form of PEMS, was characterized by flock mortality that was >2% from 7 to 28 days of age, with daily mortality not exceeding 1% for three consecutive days. The more severe form of PEMS, “spiking mortality,” was characterized in flocks with mortality >9% from 7 to 28 days of age, including at least three consecutive days with daily mortality of more than 1%.

The etiology of PEMS is not yet completely understood, although evidence suggests that it is a multifactorial disease because viruses, bacteria, protozoa, insects, young age (3), temperature, and wet litter (10) all have been associated with this syndrome. Experimentally, PEMS can be reproduced by contact exposure to infected birds (15) or by inoculation with fecal material or organ homogenates of infected birds (5). A variety of infectious agents have been identified in birds with PEMS, including coronavirus (13), enteroviruslike viruses, rotavirus (group D), adenovirus, Cryptosporidium, Cochlosoma, Salmonella, Clostridium (2), and atypical Escherichia coli strains with unique biochemical profiles (11,12). It is believed that a virus may be the initiating infectious factor in PEMS and that this infection compromises the immune system of turkey poults. The primary virus infection ultimately leads to infection by opportunistic bacterial pathogens such as E. coli strains (11,12).

Initially, turkey coronavirus (TCV) was proposed as a putative causative factor (20). However, further studies revealed that not all birds with PEMS were also TCV positive (4). Subsequently, small viruses were isolated from fecal material of PEMS poults in three independent laboratories. The first agent, “small round virus” reported by Yu et al. (37), is an ssRNA virus approximately 30 nm in diameter propagated successfully in specific-pathogen-free (SPF) turkey embryos (38). In 2000, Koci et al. (18) reported the isolation of an astrovirus (TAstV) from thymus and intestines of poults with PEMS. Molecular sequence comparison has revealed that these two viruses demonstrate 100% homology in the sequence encoding capsid protein (Y. M. Saif, pers. comm.). Therefore, these isolates likely represent the same viral agent but are referred to as TAstV-OSU and TAstV, respectively.

In 1998, Schat et al. (30) reported the isolation of a virus from 100-nm filtrate of PEMS fecal material that caused cytopathic effects (CPE) in chicken embryo fibroblasts. This virus was not identified at that time but has been identified in this paper as a reovirus, ARV-CU98. The isolation of a reovirus from PEMS poults, PEMS ARV, was also reported in 2000 by Simmons et al. (32).

This report describes experimental poult challenge studies with 220- and 100-nm filtrates of PEMS fecal material and the subsequent isolation of ARV-CU98 from 100-nm PEMS fecal filtrates. The recent identification of ARV-CU98 as a reovirus, its propagation in cell culture, and the effects of ARV-CU98 on liver and lymphoid organ growth and development when inoculated into turkey poults are also demonstrated.
MATERIALS AND METHODS

Experimental birds and animal welfare. One-day-old female hybrid turkey pouls were received from Sleepy Creek Hatchery (Goldsboro, NC). Depending on the experiment, pouls were housed in separate but identically controlled isolation rooms or in four HEPA-filtered, bubble-type isolation units located at the North Carolina State University Dearstone Avian Research Center. Turkey starter diet (North Carolina Agricultural Research Service, Raleigh, NC) and water were provided ad libitum in the experiments in the isolation rooms, whereas sterile irradiated Mazuri pheasant grower feed (Purina Mills, St. Louis, MO) and sterile water were provided ad libitum for the experiments in the HEPA-filtered isolators in order to maintain the clean conditions in the isolators. All experimental animal studies were conducted in compliance with the guidelines established by the North Carolina State University Institutional Animal Care and Use Committee.

Preparation of fecal filtrates. Experimentally infected turkeys maintained by Dr. H. J. Barnes (College of Veterinary Medicine, North Carolina State University, Raleigh) served as the original source of PEMS material. Fecal samples for the original preparation of filtrates were obtained from pouls that were contact exposed to the pouls maintained by Dr. H. J. Barnes (batch A, used as inocula in Expts. 1 and 2). Subsequent fecal samples were obtained directly from pouls maintained by Dr. H. J. Barnes (batch B, used as inocula in Expt. 3). All materials were frozen immediately and shipped to Cornell University, Frozen material was thawed, and each tube was diluted to 50 ml with sterile phosphate-buffered saline (PBS), pH 7.2. After centrifugation at 1000 rpm for 10 min, supernatant was harvested and centrifuged again at 1000 rpm for 10 min. Supernatants were harvested and filtered initially through a 450-nm filter and then through 220- and 100-nm filters. Filtrates were stored at −80°C until used.

Pathogenicity studies with fecal filtrates. Seven-day-old pouls were inoculated orally with either 220- or 100-nm fecal filtrates (Expts. 1 and 2, respectively). Three treatment groups were included in each experiment: 1) C-PBS, a control group inoculated with PBS only, 2) C-E filtrate, a control group inoculated with filtered fecal material from uninfected control pouls, and 3) PEMS filtrate, a test group inoculated with filtered fecal material from PEMS pouls. The C-PBS group consisted of 20 pouls in Expts. 1 and 2, whereas the C-E filtrate and the PEMS filtrate groups each consisted of 120 pouls. Because of the large number of birds, some endpoints were measured over 2 days. Total mortality and body weights were determined for all birds at 28–29 days of age (21–22 DPI, Expt. 1) or 27–28 days of age (20–21 DPI, Expt. 2). Relative lymphoid organ weights were determined for 10 (C-PBS) or 60 (C-E filtrate and PEMS filtrate) pouls per treatment group at 28–29 days of age (21–22 DPI, Expt. 1) or 27–28 days of age (20–21 DPI, Expt. 2). Humoral immunity, cell-mediated immunity, and lymphocyte subpopulations in the blood were also examined as described in the appropriate sections. Expt. 3 was conducted to confirm the effect of the 100-nm filtrate of PEMS fecal material on turkey pouls and consisted of five treatment groups (30 pouls/treatment group) defined by inocula: 1) control group inoculated with PBS (C-PBS); 2) control group inoculated with 100-nm filtrate of fecal material from uninfected pouls (C-E filtrate); 3) control group inoculated with crude fecal material from uninfected pouls (C-crude); 4) PEMS group inoculated with 100-nm filtrate of fecal material from PEMS pouls (PEMS filtrate), and 5) PEMS group inoculated with crude fecal material from PEMS pouls (PEMS crude). Pouls in Expts. 1–3 were housed in heated batteries in separate isolation rooms at North Carolina State University Dearstone Avian Research Center as described in the Experimental birds and animal welfare section. Mortality, body weight, and relative lymphoid organ weights were determined for all birds in each treatment group in Expt. 3 at 28 days of age (21 DPI).

Body and organ weights. At the termination of each trial, total body weight of live birds and the weights of the bursae of Fabricius, thymi (all lobes from the left side of the neck), spleens, and livers were determined. In studies in which blood samples were taken, body weights were measured prior to bleeding. All organ weights were expressed as the percentage of body weight and were reported as relative organ weights.

Humoral immunity. Approximately 9–11 DPI, 1 ml of a 7% suspension of sheep red blood cells (SRBC) in 0.85% saline was injected i.v. into 10 (C-PBS) or 60 (C-E filtrate and PEMS filtrate) pouls per treatment group (Expts. 1 and 2). Serum samples were collected at 4, 8, and 11 days postinjection, heat inactivated at 56°C for 30 min, and stored at −20°C until analysis; antibody titers were analyzed by a microhemagglutination technique (35) with a 2% suspension of SRBC. Anti-SRBC antibodies were expressed as log, of the reciprocal of the last agglutinating dilution.

Cell-mediated immunity. In order to elicit delayed-type hypersensitivity responses, 100 μg of phytohemagglutinin-P (PHA-P) (Sigma Chemical Co., St. Louis, MO) dissolved in 100 μl of sterile saline was injected into the toe web of the left foot of 10 (C-PBS) or 60 (C-E filtrate and PEMS filtrate) pouls per treatment group between 11 and 13 DPI (Expts. 1 and 2). Sterile saline (100 μl) was injected intradermally into the corresponding toe web on the right foot of each turkey poult as a control. Measurements were performed 24 and 48 h after injection.
of toe web thickness were taken before injection and at 24 and 48 hr after injection with micrometer calipers. For each treatment group, data were expressed as PHA-P-mediated swelling minus saline-injected swelling and reported in millimeters.

**Lymphocyte subpopulation analysis.** CD4:CD8 ratio in blood was determined as reported previously (15). Briefly, 1 ml of whole blood was collected in a heparinized syringe between 6 and 11 DPI from the same poult samples used for assessment of humoral immunity but prior to being challenged with SRBC (Expts. 1 and 2). Peripheral blood leukocytes were separated by Ficoll/LymphoH density gradient (Atlanta Biologicals, Norcross, GA). Peripheral blood leukocytes were labeled with 1:100 mouse anti-chicken CD4 (fluorescein isothiocyanate [FITC]) plus mouse anti-chicken CD8 phycoerythrin (R-PE) (Southern Biotechnology Associates, Birmingham, AL) for 30 min and analyzed by flow cytometry (Becton-Dickinson Immunocytometry, San Jose, CA).

**Cell cultures.** Primary chicken embryo fibroblasts (CEF) and chicken embryo liver (CEL) cells were prepared from 10- to 11-day-old embryos from departmental SPF flocks by standard procedures (31). Chick kidney cell (CKC) cultures were prepared from 2-wk-old SPF chicks as described (31). The cells were pretreated overnight with 0.1% gelatine at 4 C, and grown on glass coverslips and inoculated with the FDO-1 strain of avian reovirus (22). Coverslips were harvested when CPE started to develop, fixed in acetone, air-dried, and stained at −20 C until used. For the direct immunofluorescence assay, coverslips were incubated for 30 min in a humidified chamber with FITC-conjugated, convalescent chicken anti-reovirus (anti-FDO-1 serum) (22), washed in PBS, and mounted on a microscope slides. For the indirect immunofluorescence assays, coverslips were first incubated with dilutions of convalescent sera from the experimentally infected turkeys used in these studies, washed and incubated with goat anti-turkey immunoglobulin G(H+L) serum (Southern Biotechnology Associates), washed in PBS, incubated for 30 min with convalescent sera from the experimentally infected turkeys used in these studies, washed and incubated with goat anti-turkey immunoglobulin G(H+L) serum (Southern Biotechnology Associates), washed in PBS, and mounted on microscope slides. MDCT-CU45 cells were harvested, washed in PBS, counted, and readdressed to 100 cells/ml. Ten microliters of cell suspensions were dropped on 12-well slides, air-dried, fixed in acetone, and stained as described for CCK cells.

Livers from poult in trial 5 were snap frozen in optimal cutting temperature (OCT) compound (Tissue Tek; Miles, Inc., Elkhart, IN), cut into 5-μm sections, and stored at −20 C. Tissue sections from control and virus-infected poult cells were incubated for 30 min in a humidified chamber in the absence of light with 1:5 dilution of chicken anti-serum against the FDO-1 strain of avian reovirus conjugated with FITC (22). After two 10-min washes in PBS and a final wash in sterile water, the sections were briefly dried and mounted with coverslips with Immumount (Shandon, Inc., Pittsburgh, PA). Slides were examined under a fluorescent microscope at various magnifications for positive fluorescent staining.

**Susceptibility of ARV-CU98 to heat treatment.** Virus suspensions were incubated for 1 hr at 80, 60,
leting a fraction from the supernatants at 41,000 × g for 5 min and 10,800 × g for 30 min followed by pelleting a fraction from the supernatants at 41,000 × g for 60 min. One drop of pellet suspension was added to a Formvar carbon-coated copper grid and incubated for 3 min for adherence. The grid was stained with a 2% aqueous solution of phosphotungstic acid, pH 7.2, for 30 sec. Preparations were examined on a Philips/FEI 208S microscope at 80 kV.

**Polyacrylamide gel electrophoresis (PAGE).** Three virus strains were used for the analysis: ARV-CU98, the chicken FDO strain (22), and the marmalian Dearing isolate (33). Infected cell cultures exhibiting CPE were pelleted by centrifugation at 1000 rpm for 10 min, and pellets were stored at −20°C. Thawed pellets were resuspended in 3 ml minimum essential medium (GIBCO, Rockville, MD) and incubated with 200 µg proteinase K for 30 min at 37°C. After addition of 150 µl 10% Nonidet-P40 (NP40), samples were incubated on ice for 30 min. Samples were centrifuged at room temperature for 10 min at 1900 × g. After addition of 150 µl 20% sodium dodecyl sulfate (SDS), samples were mixed and warmed to 37°C; 3 ml phenol (preequilibrated with 100 mM NaCl, 50 mM Tris, pH 7.4, 1 mM ethylenediaminetetraacetic acid) was added, and samples were vortexed. Samples were centrifuged at room temperature for 5 min at 2400 × g. The aqueous (upper) phase was removed to a fresh tube, vortexed with 7.5 ml 20% ethanol, and incubated at −70°C for 1 hr. After centrifugation for 10 min at 12,000 × g, pellets were resuspended in 100 µl deionized distilled H₂O, and then 100 µl 2× sample buffer (20% glycerol, 10% beta-mercaptoethanol, 4% SDS, 125 mM Tris, 0.01% bromophenol blue) was added. Samples were stored at −20°C until electrophoresis. One quarter of each sample (50 µl) was loaded on a 10% Laemmli polyacrylamide gel (19) and was electrophoresed at a constant 30 mA for 16 hr. Gels were stained with ethidium bromide and photographed under ultraviolet light.

**In vivo pathogenicity study with PEMS reovirus.** Two independent experiments (Expts. 4 and 5) were conducted with 80 female British United Turkey of America poults. At 7 days of age, poults in two isolators were orally inoculated with 1 ml of 10³ median tissue culture infected dose (TCID₅₀) of ARV-CU98 (p4 in LMH cell culture for Expt. 4 and p5 in LMH cell culture for Expt. 5), whereas control poults in the other two isolators were sham inoculated with 1 ml of cell culture supernatant from non-infected LMH cells. At 0, 3, 6, 10 (both experiments) and 20 DPI (Expt. 4 only), body weights of all poults in the isolation units were measured. At 3, 6, 10 (both experiments) and 20 DPI (Expt. 4 only), five poults were removed from each isolator, yielding a total of 10 control birds and 10 virus-infected birds at each time point. In Expt. 5, blood was collected from 10 poults per treatment in sterile tubes containing sodium citrate as an anticoagulant at 3, 6, and 10 DPI for quantitation of plasma alanine aminotransferase (ALT) and aspartate aminotransferase (AST). These poults were euthanatized via CO₂ exposure and necropsied. Relative liver, bursa, thymus, and spleen weights were determined as described. In addition, either small tissue samples were frozen in OCT compound with liquid nitrogen for detection of viral antigen by immunofluorescence and stored at −20°C or tissues from three different birds per treatment were fixed in 10% neutral buffered formalin (Sigma) for histologic examination. Finally, intestines (Expt. 4) or expressed fecal material (Expt. 5) was collected for virus isolation and stored at −70°C.

**Histology.** After 72 hr in 10% neutral buffered formalin, tissues were transferred to 70% ethanol, embedded in paraffin, serially sectioned at 5 µm, and stained with hematoxylin and eosin.

**Quantification of plasma ALT and AST.** Plasma was tested for the liver enzymes ALT and AST with enzyme-linked immunosorbent assay kits (Sigma) following manufacturer’s protocols.

**Statistical analysis.** Data in which only two treatment groups were involved were analyzed by one-tailed t-test procedure (28). In Expts. 4 and 5, replicate isolators were combined for data analysis after t-test analysis of the replicates demonstrated that no statistical difference existed. However, one virus replicate was excluded from the analysis in Expt. 5 because body weight was significantly different from the other virus replicate and the two control replicates prior to virus challenge. Data involving multiple treatment groups in which birds were placed and sampled randomly were analyzed by analysis of variance with the general linear model procedure of SAS (28). Means were separated by using Duncan multiple range test. Significance was based on P ≤ 0.05.

**RESULTS**

**Pathogenicity studies with fecal filtrates.** Poults inoculated with 220 nm of PEMS fecal filtrate had significantly lower body weights and relative spleen weights compared with the C-PBS and C-E filtrate treatment groups (Table 1, Expt. 1). However, relative bursa weights...
Table 1. Body weights and relative organ weights in poults challenged at 7 days of age with crude PEMS inoculum or 200-nm and 100-nm filtrates of PEMS inoculum.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Inoculuma</th>
<th>Materialb</th>
<th>Filter</th>
<th>No. dead/total (%)</th>
<th>Body weight in gramsc</th>
<th>% Relative organ weightd</th>
<th>Thymus</th>
<th>Bursa</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C-PBS</td>
<td>—</td>
<td>0/20</td>
<td>0.070 ± 0.03c</td>
<td>0.110 ± 0.004</td>
<td>0.100 ± 0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-E filtrate</td>
<td>220 nm</td>
<td>43/120</td>
<td>0.058 ± 0.002b</td>
<td>0.121 ± 0.002</td>
<td>0.107 ± 0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEMS filtrate</td>
<td>220 nm</td>
<td>15/120</td>
<td>0.052 ± 0.001b</td>
<td>0.116 ± 0.002</td>
<td>0.087 ± 0.002</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>C-PBS</td>
<td>—</td>
<td>0/20</td>
<td>0.069 ± 0.007b</td>
<td>0.150 ± 0.008c</td>
<td>0.101 ± 0.007</td>
<td>808.2</td>
<td>631.6</td>
<td>416.1</td>
</tr>
<tr>
<td></td>
<td>C-E filtrate</td>
<td>100 nm</td>
<td>2/120</td>
<td>0.102 ± 0.003b</td>
<td>0.159 ± 0.003c</td>
<td>0.097 ± 0.003</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEMS filtrate</td>
<td>100 nm</td>
<td>24/120</td>
<td>0.056 ± 0.003b</td>
<td>0.111 ± 0.003c</td>
<td>0.101 ± 0.003</td>
<td>618.5</td>
<td>430.0</td>
<td>369.7</td>
</tr>
<tr>
<td>3</td>
<td>C-PBS</td>
<td>—</td>
<td>3/30</td>
<td>0.108 ± 0.005c</td>
<td>0.149 ± 0.006c</td>
<td>0.113 ± 0.004</td>
<td>672.9</td>
<td>747.6</td>
<td>602.1</td>
</tr>
<tr>
<td></td>
<td>C-E filtrate</td>
<td>100 nm</td>
<td>0/30</td>
<td>0.110 ± 0.004c</td>
<td>0.167 ± 0.005c</td>
<td>0.118 ± 0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PEMS filtrate</td>
<td>100 nm</td>
<td>1/30</td>
<td>0.090 ± 0.004c</td>
<td>0.141 ± 0.006c</td>
<td>0.111 ± 0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>C-crude</td>
<td>—</td>
<td>0/30</td>
<td>0.111 ± 0.004c</td>
<td>0.157 ± 0.005c</td>
<td>0.116 ± 0.004</td>
<td>668.8</td>
<td>747.6</td>
<td>602.1</td>
</tr>
<tr>
<td></td>
<td>PEMS crude</td>
<td>—</td>
<td>4/30</td>
<td>0.058 ± 0.004c</td>
<td>0.116 ± 0.006c</td>
<td>0.111 ± 0.004</td>
<td>407.1</td>
<td>597.6</td>
<td>416.1</td>
</tr>
</tbody>
</table>

aInocula for Expts. 1 and 2 were prepared from the same batch of PEMS-positive and -negative material; inocula for Expt. 3 was prepared from a different batch.

bPoults were challenged at 7 days of age with PBS (C-PBS), filtered or crude fecal material from control poults (C-E filtrate and C-crude, respectively), or filtered or crude material from PEMS-positive poults (PEMS filtrate and PEMS crude, respectively).

cMean body weight was determined at 28–29 days of age (Expt. 1), 27–28 days of age (Expt. 2), or 28 days of age (Expt. 3).

dMeans with different lowercase superscripts in a column within an experiment are statistically significantly different at $P \leq 0.05$.

eMean relative organ weights were determined at the termination of each experiment and are expressed as percentage of body weight with the formula (organ weight/body weight) × 100.
were not significantly different among the three groups. Relative thymus weight was significantly lower in both C-E and PEMS filtrate groups as compared with the C-PBS control group. The C-E filtrate group had 36% mortality, whereas only 13% mortality was observed in the PEMS filtrate group, and mortality did not occur in the C-PBS group.

In Expt. 2, body weight and relative bursa weights were significantly lower in the PEMS 100-nm filtrate group compared with both the C-PBS and C-E 100-nm filtrate groups (Table 1, Expt. 2). Although relative thymus weight was lowest in the PEMS filtrate group, it was significantly different only from the C-E filtrate group and not from the C-PBS group. There were no differences in relative spleen weight among the three groups. The PEMS filtrate group had 20% mortality vs. no mortality in the C-PBS group and 1.6% mortality in the C-E filtrate group.

When humoral and cellular immunity were assessed by SRBC challenge and toe web PHA-P injection, respectively, a few significant differences were found among treatment groups, but no clear pattern emerged as observed in clinical PEMS (data not shown). Furthermore, no significant differences were observed in CD4:CD8 ratio in the blood among the treatment groups in Expts. 1 and 2 (data not shown). The fecal materials used to prepare the filtrates for Expts. 1 and 2 were tested retrospectively for the presence of ARV-CU98. The C-E filtrate was negative for ARV-CU98, whereas the PEMS filtrates were positive.

After Expt. 2 revealed intriguing differences in mortality, body weight, and relative bursa weight in pouls inoculated with the 100-nm filtrate of PEMS fecal material, a third experiment was performed with an independent batch of PEMS material to confirm the results obtained in Expt. 2. In this experiment, body weight was significantly less in the PEMS 100-nm filtrate group as compared with all three control groups, C-PBS, C-E 100-nm filtrate, and C-crude groups (Table 1, Expt. 3). However, the body weight for the pouls infected with the PEMS crude material was significantly lower than in the pouls infected with the 100-nm PEMS filtrate. Similar significant trends were observed in relative thymus weight, whereas no significant differences were observed in relative spleen weight among the various groups. Retrospective virus isolation showed that the 100-nm PEMS filtrate but not the C-E filtrate was positive for ARV-CU98.

**Virus isolation.** MDCT-CU45 was inoculated with several dilutions of 100-nm filtrates prepared from batch B, and after 2 days, some enlarged cells were observed. CU45 inoculated with filtrates from controls did not show enlarged cells after 48 hr. Supernatant fluids and cells were harvested, frozen/thawed three times, and centrifuged, and supernatant fluids were frozen until used (cell culture passage [p]1). Primary CEFs were inoculated with the supernatant fluids. The cultures infected with the PEMS-derived inoculum showed some degeneration, and a few detached enlarged cells were observed. The second passage in CEFs was harvested (p3) and used to inoculate primary CEL cells. CPE consisting of syncytia developed rapidly in these cells, and supernatant fluids were harvested and stored as p4 and used in challenge Expt. 4. Similar CPE, consisting of syncytia, cell death, and sloughing, were detected when LMH cells (Fig. 1A,B) and CKCs were infected with p4. The LMH-propagated virus was stored as p5 and used in challenge Expt. 5. CEL and LMH cells were equally susceptible to infection. However, no CPE were evident when LSCC-RP9 and MDCC-MSB1 cells were incubated with ARV-CU98.

Immunofluorescence staining of virus-infected CKC or LMH cells with the FDO conjugate was positive for both cell types (Fig. 1C,D), identifying the virus as a reovirus, which was named avian reovirus (ARV)-CU98. Fluorescence in infected cultures appeared to have a perinuclear distribution. Uninfected control cultures or cells infected with control filtrates remained negative for reovirus. Subsequently, 100-nm filtrates of batch A were inoculated directly on CEL cells and found to be positive for ARV-CU98.

**Susceptibility of ARV-CU98 to heat treatment.** Titer after incubation at 37 °C was 6.2 TCID₅₀/ml. Virus was completely inactivated by incubation at 80 °C but only partially inactivated by treatment at 60 °C (4.2 TCID₅₀/ml). In a second experiment, treatment at 50 °C did not inactivate the virus, but treatment at 60 °C reduced the titer from 6.2 TCID₅₀/ml at 37 °C to 2.2 TCID₅₀/ml at 60 °C.

**Negative staining electron microscopy.** Particles with the typical morphology of reovi-
Fig. 1. Interactions of ARV-CU98 with different cell types. Uninfected (A) and ARV-CU98–infected LMH cells (B) at 5 DPI. CPE such as cell death and sloughing are evident in ARV-CU98–infected LMH cells. Immunofluorescence staining of ARV-CU98–infected chicken embryo liver cells (C) and chick kidney cells (D) with FITC-labeled anti-reovirus chicken serum (FDO strain).

Virus were detected in purified materials from infected cultures (Fig. 2). The average size of the particles was estimated at 75 nm.

Electropherotyping. PAGE analysis of ARV-CU98 demonstrated the presence of 10 segments of double-stranded RNA (Fig. 3). The patterns of migration for the chicken FDO isolate and the mammalian Dearing isolate were clearly distinct from that of ARV-CU98.

In vivo pathogenicity study with PEMS reovirus. After we demonstrated that ARV-CU98 could be isolated and propagated successfully in cell culture, the experimental effort was focused on examining the pathogenicity of this isolate. In two independent experiments, 10 poults per treatment group were sampled at DPI 3, 6, 10 (Expts. 4 and 5), and 20 (Expt. 4 only). Upon necropsy, the most common lesions associated with ARV-CU98 infection were increased incidence of thymic hemorrhaging and gaseous intestines. Body weights (Table 2) and relative thymus weights (data not shown) were not significantly different between treatments. Relative spleen weights were not affected except for a significant increase in the virus-infected group at 6 DPI in Expt. 4. In contrast, relative bursa and liver weights were significantly lower in the virus-infected groups compared with the control groups. Relative bursa weight was less in the virus-infected group as compared with the control group at 3, 6, and 10 DPI in Expt. 4 and at 6 and 10 DPI in Expt. 5. These differences were significant at 6 DPI in both trials. Similarly, relative liver weight was less in the virus-infected group at all time points in Expt. 4, with significant differences at 6 and 10 DPI, and less at two out of the three time points in Expt. 5, with significant differences at 6 DPI. Virus was successfully reisolated from the intestinal contents from the virus-infected poults but not from control poults in both trials (Table 2). It is evident that virus is easily reisolated at 3 and 6 DPI, but at later times, virus shedding in feces appeared to be diminished. No mortality was observed in any of the treatment groups.

Viral replication in livers. When sections of liver tissue were examined by immunofluo-
Fig. 2. Purified ARV-CU98 from infected LMH cell cultures was examined by electron microscopy with negative staining. Average particle size was estimated at 75 nm. Bar = 100 nm.

rescence, scattered cells with cytoplasmic staining were evident in sections from virus-inoculated poults but not from control poults at 3 and 6 DPI (data not shown). Virus isolations were performed only at 6 DPI in Expt. 4. Reovirus was isolated from livers obtained from infected but not control poults.

Histology. Histologic analysis of bursa, thymus, and intestine in Expt. 4 revealed minor differences between control and virus-inoculated poults. In intestinal sections, crypt depth was moderately increased in ARV-CU98–challenged poults, and there was extensive crypt formation compared with control birds. In two sections of bursa, there were focal lesions containing numerous eosinophils similar to the condition found in early PEMS infections, but one of the control bursa sections demonstrated similar focal lesions. Mild pyknosis of medul-}

lary cells accompanied by mild eosinophilia, numerous infiltrating monocytic cells, mild cellular disruption, and loss of cellularity were observed in two sections of thymus from virus-challenged poults. In a third thymic section, there were numerous eosinophils in association with Hassell corpuscles, but cells in the vicinity were not degenerate as found in fulminating PEMS. Control thymic sections exhibited occasional eosinophilic granulocytes and a few monocytic cells but were otherwise unremarkable.

In Expt. 5, bursa, thymus, spleen, liver, and intestine were examined histologically. Overall, tissues from both control and ARV-CU98–inoculated poults were within normal limits, and no differences were found between the treatments. Slight cellular depletion was noted in some of the bursa sections from control and virus-inoculated poults at 3, 6, and 10 DPI. In one of the sections from virus-inoculated poults at 3 DPI and one of the sections from control poults at 6 DPI, depletion was accompanied by heterophilic interfollicular inflammation. Fatty change was observed in at least one of the three liver sections in both the control and virus-inoculated groups at each time point. In addition, ectopic foci were observed in two liver sections from the virus-inoculated group at 6 DPI and in one liver section from the control group at 10 DPI. Although no lesion was seen in any of the intestinal sections from control poults, one of the sections from the virus-inoculated group at 3 DPI demonstrated mild intraepithelial heterophilic inflammation. In addition, some of the spleen sections in both treatment groups were mildly reactive.

Liver functions. After we observed the dramatic effects on liver growth and development in Expt. 4, we measured ALT and AST levels in plasma in Expt. 5 to determine if there was hepatic dysfunction. Mean ALT activity in the plasma of virus-infected poults was decreased significantly compared with control poults at 3 DPI (Fig. 4A). Conversely, mean AST activity level in the plasma of virus-infected poults was increased significantly compared with controls at 10 DPI (Fig. 4B).

DISCUSSION

Enteric diseases in turkeys present a significant challenge to the maintenance of health and
cause significant revenue losses to the turkey industry. In earlier studies, a variety of viral agents, including rotaviruslike viruses, astroviruses, reoviruses, enteroviruses, and adenoviruses, were identified in turkey poultts exhibiting diarrhea (26,27). Similarly, many viral agents, including coronavirus (13), enteroviruslike viruses, rotaviruses (group D), and adenoviruses
Table 2. Body weight, relative organ weight, and virus isolation in poults after oral infection with 10⁵ TCID₅₀ of reovirus strain ARV-CU98 at 7 days of age.

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Virus</th>
<th>Days post-infection</th>
<th>Average body weight* (g)</th>
<th>Bursa</th>
<th>Spleen</th>
<th>Liver</th>
<th>Virus isolation(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No. birds</td>
<td>Mean ± SEM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>B</td>
<td>S</td>
<td>L</td>
<td></td>
</tr>
</tbody>
</table>

- **4**
  - - 3
    - 40
    - 141.6 ± 1.7
    - 0.173 ± 0.011
    - 0.059 ± 0.003
    - 3.72 ± 0.15
    - 0/10
  - +
    - 40
    - 140.8 ± 2.8
    - 0.151 ± 0.009
    - 0.065 ± 0.002
    - 3.39 ± 0.11
    - 10/10
  - - 6
    - 30
    - 197.4 ± 2.6
    - 0.187 ± 0.010
    - 0.065 ± 0.005
    - 3.60 ± 0.13
    - 0/10
  - +
    - 30
    - 193.4 ± 4.0
    - 0.146 ± 0.008\(^*\)
    - 0.084 ± 0.006\(^*\)
    - 3.23 ± 0.12\(^*\)
    - 10/10
  - - 10
    - 20
    - 271.6 ± 4.1
    - 0.191 ± 0.004
    - 0.079 ± 0.004
    - 3.17 ± 0.09
    - 0/10
  - +
    - 20
    - 271.8 ± 5.9
    - 0.177 ± 0.010
    - 0.086 ± 0.006
    - 2.84 ± 0.09\(^*\)
    - 5/10\(^e\)
  - - 20
    - 10
    - 514.8 ± 13.6
    - 0.159 ± 0.006
    - 0.070 ± 0.004
    - 2.80 ± 0.10
    - 0/10
  - +
    - 10
    - 540.2 ± 14.1
    - 0.162 ± 0.009
    - 0.071 ± 0.006
    - 2.75 ± 0.06
    - 5/10\(^e\)

- **5**
  - - 3
    - 24
    - 160.6 ± 2.8
    - 0.154 ± 0.009
    - 0.052 ± 0.002
    - 4.08 ± 0.05
    - 0/7
  - +
    - 28
    - 163.2 ± 4.9
    - 0.171 ± 0.018
    - 0.065 ± 0.018
    - 3.94 ± 0.01
    - 10/10
  - - 6
    - 17
    - 214.7 ± 4.3
    - 0.174 ± 0.009
    - 0.068 ± 0.003
    - 3.37 ± 0.06
    - 0/9
  - +
    - 18
    - 206.8 ± 5.7
    - 0.137 ± 0.009\(^*\)
    - 0.073 ± 0.008
    - 2.90 ± 0.10\(^*\)
    - 8/10
  - - 10
    - 8
    - 310.5 ± 7.8
    - 0.178 ± 0.019
    - 0.065 ± 0.003
    - 2.88 ± 0.06
    - 0/8
  - +
    - 8
    - 297.0 ± 12.3
    - 0.168 ± 0.025
    - 0.067 ± 0.003
    - 3.15 ± 0.23
    - 1/8\(^g\)

*Poults were used in isolation units immediately after hatch.

\(^a\)Body weights at 7 days of age were 107.4 ± 1.3 g, 106.0 ± 2.3 g, 124.0 ± 2.1 g, and 120.7 ± 3.5 g for control and virus-infected groups in Expts. 4 and 5, respectively.

\(^b\)Relative organ weights were expressed as percentage of body weight with the formula (organ weight/body weight) × 100. Means ± SEM within each control and virus-infected data set in a column followed by \(^*\) are significantly different (P ≤ 0.05).

\(^c\)Virus was isolated by inoculation of chicken embryo liver cells with 100-nm filtrates prepared from intestinal contents (Expt. 4) or fecal material (Expt. 5).

\(^d\)A blind passage was needed for some birds in this group.
Fig. 4. Alanine aminotransferase (ALT) (A) and aspartate aminotransferase (AST) (B) levels in plasma of control (striped bars) and virus-infected (solid bars) poults (n = 10/treatment) at 3, 6, and 10 DPI in trial 6. Poults were inoculated with 1 ml of $10^5$ TCID$_{50}$ PEMS reovirus at 7 days of age. ALT and AST levels were quantified by enzyme-linked immunosorbent assay. Data are represented as means ± SEM.

(3), have been associated with PEMS. Because a virus was believed to be most likely the initial causative agent of PEMS, the current study was conducted in an attempt to find a small virus(es) associated with PEMS and investigate its role in the etiology of PEMS. This search for a small virus resulted in the isolation of ARV-CU98, a reovirus, from the feces of PEMS-infected poults. This reovirus is clearly distinctive from the FDO virus; how this virus differs from other avian isolates is currently not known.

Higher mortality (36%) was associated with the 220-nm filtrate of control fecal material than with the 220-nm filtrate of PEMS fecal material (13%, Expt. 1). This unusual observation may be due to the presence of a filterable agent(s) in the fecal material from the control birds that was not present in the fecal material of the PEMS birds.

Among the two fecal filtrates inoculated into turkey poults (Expts. 1 and 2), the 100-nm PEMS fecal filtrate was selected for further study because it was the smallest filtrate associated with high mortality (20%) and significantly lower body weight and bursa of Fabricius weight. Both 220- and 100-nm filtrates were capable of containing a variety of agents. However, the 100-nm filter narrowed the number of potential agents in the filtrate as compared with the 220-nm filtrate so that a smaller size range of viral agents potentially containing a key viral agent could be focused upon and confounding agents could be filtered out. The data suggested that the 100-nm PEMS fecal filtrate contained a filterable agent capable of causing some of the clinical signs associated with PEMS. Attempts to associate previously noted immune alterations in PEMS, such as reduced antibody response, reduced T-lymphocyte response (25), and alterations in CD4:CD8 T-lymphocyte ratio in peripheral blood leukocytes (15), with a particular size filtrate of fecal material were unsuccessful. These results suggest that these immune alterations may be attributed to secondary agents, such as bacteria, or, alternatively, to the synergistic effect of a variety of agents involved in PEMS.

The results from Expts. 2 and 3 were similar with respect to the influence of the 100-nm PEMS fecal filtrate on body weight, bursa weights, and thymus weights. These results indicated that the 100-nm PEMS fecal filtrate contained a filterable agent important in the etiology of PEMS.

When inoculated into poults reared in isolation conditions, ARV-CU98 alone caused some of the signs commonly associated with PEMS; there was an increased incidence of inflammation and gas in the intestines and ceca in virus-infected poults as compared with control poults (3). Although body weight was not affected significantly by inoculation of ARV-CU98, bursa relative weights were 22% (Expt. 4) to 28% (Expt. 5) less than in controls at 6 DPI. This reduction in bursa weight is similar to earlier PEMS observations where bursa relative weight was 42% lower in PEMS poults as compared with control poults at 6 DPI (25). The results indicate that ARV-CU98 has a potential contributing role in the degradation of
bursal growth and development and may compromise the immune system, leading to infection by opportunistic pathogens.

Although increased spleen weight has not been noted previously in PEMS, this observation is common with other avian reoviruses (23). Increased spleen weight as a result of reovirus infection may not be evident in PEMS because it may be masked by the effect(s) of other agents involved in PEMS. As is the case with PEMS, ARV-CU98 infection seems to be an acute infection so that, by 10–20 DPI, relative organ weights in the virus pouls return to control levels and ARV-CU98 is more difficult to isolate from fecal material, suggesting that the pouls recover from the virus infection. The absence of an effect of ARV-CU98 on mortality, body weight, and thymus weight suggests that other pathogens may be responsible for those alterations in PEMS.

A significant reduction in liver weight in the ARV-CU98–challenged pouls was not unexpected because ARV-CU98 is propagated most successfully in hepatic cells in vitro. We have found that primary liver cells isolated from pouls at day of hatch developed CPE similar to those found in chicken liver cells when incubated with ARV-CU98 (unpubl. data). Edens and Doerfler (8,9) have shown that liver degeneration is a clearly defined part of PEMS. In addition to liver atrophy, the hepatocytes of PEMS-affected pouls show massive degeneration of mitochondria and biochemical lesions as well (8). Edens and Doerfler (8,9) did not report virus plaques in hepatic cells examined with transmission electron microscopy, but degenerative effects noted by them were consistent with the CPE noted in this study. The fact that Edens and Doerfler did not report virus infection in their study does not preclude the possibility that a reovirus was involved because the original PEMS fecal material from which the reovirus in this study was isolated was also the source of infective material for the Edens and Doerfler work. Liver lesions due to reovirus infection in pouls with PEMS infection have not been reported previously, but liver lesions similar to those we observed were reported in a publication describing sudden death in broilers with a reovirus infection (16).

Changes in ALT and AST plasma levels indicate that functional alterations accompany the physical changes noted in liver of ARV-CU98 pouls and are consistent with the report by Edens and Doerfler (8). Although ALT plasma activity was significantly lower in virus-challenged pouls as compared with controls at 3 DPI, AST activity, considered to be a more reliable indicator of liver injury (21), was significantly higher in virus-challenged pouls. Elevated plasma AST activity should be expected in pouls with significant liver pathology similar to the situation associated with PEMS reovirus infection in this study. The higher activity of plasma AST is due to leakage of enzyme from injured and compromised liver cells (7).

ARV-CU98 requires additional study to elucidate its role in the etiology of PEMS. Nevertheless, this study demonstrated that ARV-CU98, alone, was capable of inducing some of the clinical signs associated with PEMS, including intestinal lesions and suppression of bursal and liver growth and development. ARV-CU98 infection may facilitate infection by opportunistic pathogens that either alone or synergistically may be responsible for additional clinical signs associated with PEMS, including mortality, decreased body weight, and decreased thymus relative weight. Although the only diseases to which an avian reovirus has been linked directly are viral arthritis/tenosynovitis and sudden death in broilers (16), avian reoviruses have been implicated in a variety of other diseases, including malabsorption syndrome in broilers, bluecomb disease in turkeys, hepatitis, and various enteric disorders (34). Yersin and Edens (36) also reported on a viral enteritis in turkeys that was later determined to be due to a reovirus infection that caused high mortality rates and reduced growth. The recent association of a reovirus with sudden death in broilers and our association of ARV-CU98 with PEMS suggest that avian reoviruses may begin to play a more important role in terms of avian health and economics and warrant further study.

REFERENCES


**ACKNOWLEDGMENTS**

We acknowledge the technical assistance of R. Ali, L. Thomas, M. A. Blum, and S. Mann. In addition, we acknowledge the support from Dr. Michael J. Dykstra and the Laboratory for Advanced Electron and Light Optical Methods at the North Carolina State University College of Veterinary Medicine. We are grateful for the financial support of the Graduate Assistance in Areas of National Need Fellowship, the North Carolina Agricultural Foundation, and the PEMS Task Force.