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High Mortality and Growth Depression Experimentally Produced in Young Turkeys by Dual Infection with Enteropathogenic Escherichia coli and Turkey Coronavirus

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SUMMARY. Six-day-old turkeys were inoculated with turkey coronavirus (TCV) and an enteropathogenic *Escherichia coli* (EPEC) (isolate R98/5) that were isolated from poult enteritis and mortality syndrome (PEMS)-affected turkeys. Turkeys inoculated with only R98/5 did not develop clinically apparent disease, and only mild disease and moderate growth depression were observed in turkeys inoculated with only TCV. Turkeys dually inoculated with TCV and R98/5 developed severe enteritis with high mortality (38/48, 79%) and marked growth depression. R98/5 infection resulted in attaching/effacing (AE) intestinal lesions characteristic of EPEC: adherence of bacterial microcolonies to intestinal epithelium with degeneration and necrosis of epithelium at sites of bacterial attachment. AE lesions were more extensive and were detected for a prolonged duration in dually inoculated turkeys compared with turkeys inoculated with only R98/5.

An apparent synergistic effect in dually inoculated turkeys was indicated by increased mortality, enhanced growth depression, and enhanced AE lesion development. The results suggest that TCV promoted intestinal colonization by R98/5; however, R98/5 did not appear to alter TCV infection. The present study provides a possible etiologic explanation for PEMS.

RESUMEN. Producción experimental de mortalidad elevada y depresión del crecimiento en pavos jóvenes por medio de la infección dual con Escherichia coli enteropatógena y coronavirus de pavos.

Se inocularon pavos de seis días de edad con coronavirus de pavos y con la cepa enteropatógena de *Escherichia coli* R98/ aislada de un pavito con el síndrome de enteritis y mortalidad en pavos. Los pavos inoculados únicamente con la cepa R98/5 de *E. coli* no desarrollaron enfermedad clínica aparente y se observó sólamente una enfermedad suave y depresión moderada en el crecimiento en los pavos inoculados con el coronavirus. Los pavos inoculados con el coronavirus y con la cepa R98/5 desarrollaron severa enteritis con mortalidad alta (34/48, 79%) y una depresión marcada en el crecimiento. La infección con R98/5 resultó en lesiones intestinales características de las cepas enteropatógenas de *E. coli:* adherencia de microcolonias bacterianas al epitelio intestinal con degeneración y necrosis de epitelio en los sitios de unión. Estas lesiones fueron más extensas y fueron detectadas por un período más prolongado en los pavos inoculados dualmente comparados con los pavos inoculados sólo con la R98/5 de *E. coli.* Se observó un efecto sinérgico aparente en los pavos inoculados con los dos microorganismos en el aumento de la mortalidad y desarrollo más severo de las lesiones de adherencia de microcolonias bacterianas al epitelio intestinal con degeneración y necrosis del epitelio en los sitios de unión. Los resultados sugieren que el coronavirus promueve la colonización intestinal por la cepa R98/5. Sin embargo, no parecen indicar que la cepa R98/5 de *E. coli* altera la infección por coronavirus. El presente estudio suministra una explicación etiológica para el síndrome de la enteritis y mortalidad en pavos.

Key words: turkey coronavirus, *Escherichia coli,* enteritis

Resources used to support this research were provided by the United States Poultry and Egg Association.

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Abbreviations: AE = attaching and effacing; CFU = colony-forming units; DMEM = Dulbecco's minimal essential medium; eae = *E. coli* attaching and effacing; EID_{50} = 50% embryo infectious doses; EPEC = enteropathogenic *Escherichia coli*; ETEC = enterotoxigenic *Escherichia coli*; FA = immunofluorescence; PCR = polymerase chain reaction; PE = post-exposure; PEMS = poult enteritis and mortality syndrome; PI = postinoculation; TCV = turkey coronavirus

Poult enteritis and mortality syndrome (PEMS) is a recently described enteric disease of young turkeys of unknown etiology (1). The disease affects turkeys during the brooding period and is characterized by diarrhea, dehydration, growth depression, and high mortality (greater than 1% per day for three or more consecutive days). Microscopic lesions include an acute enterotyphlitis with villous atrophy and lymphoid depletion in the bursa of Fabricius and thymus. A variety of infectious agents including turkey coronavirus (TCV) and *Escherichia coli* have been identified in PEMS-affected turkeys and suggested as potential etiologic agents; however, the role of these agents in the disease has not been determined (2,3,4,10).

Diarrheagenic strains of *E. coli* are divided into four main categories: enterotoxigenic, enteroinvasive, enteropathogenic, and enterohemorrhagic (19, 22). Enterotoxigenic strains elaborate heat-labile or heat-stable toxins that induce diarrhea due to potentiation of intestinal secretion. Enteroinvasive strains invade intestinal cells and produce diarrhea in a manner similar to *Shigella* spp. Enteropathogenic *E. coli* (EPEC) strains are characterized by intimate adherence between the bacterium and intestinal epithelial cell membrane and production of intestinal disease without elaboration of heat-labile and heat-stable toxins, and they are not invasive. Enterohemorrhagic strains produce intestinal disease by intimate adherence to intestinal epithelium and elaboration of shigalike toxins.

A hallmark of EPEC strains is the production of characteristic intestinal lesions referred to as "attaching and effacing" (AE) by Moon *et al.* (15). AE lesions are characterized by intimate attachment of bacteria to epithelial surfaces, microcolony formation, effacement of microvilli, and dense accumulations of actin filaments in cytoplasm beneath adherent bacterial cells (15). A chromosomal gene, termed eae for *E. coli* attaching and effacing, was identified by Jerse *et al.* (12) to be necessary for development of AE lesions. EPEC have been identified as causes of intestinal disease in several different animal species including chickens, calves, pigs, lambs, goats, rabbits, dogs, cats, and human beings (5,6,15,16,19,26). Recently, EPEC have been identified in turkey poults with enteritis (23).

The purpose of the present study was to examine the pathogenesis of an EPEC (isolate R98/5) isolated from PEMS-affected turkeys and to determine whether the interaction of this EPEC and TCV contributed to the severity of the disease.

MATERIALS AND METHODS

Bacteria. *Escherichia coli* R98/5 was isolated from PEMS-affected turkeys. Serotyping and examination for virulence properties were done at Pennsylvania State University, *E. coli* Reference Center, University Park, PA. Serotyping of R98/5 was done as described (28). R98/5 was tested for heat-labile enterotoxin, heat-stable enterotoxins a and b, shigalike toxins I and II, and the eae gene by polymerase chain reaction (PCR) procedures with DNA extracts of the bacteria (7,8,21).

R98/5 was propagated in Luria broth at 37 C with aeration to an optical density of approximately 0.4 at 600 μM. On the basis of previous growth curve experiments, R98/5 grown to this density contained approximately 5×10^8 colony-forming units (CFU)/ml. An inoculum was prepared to contain approximately 2×10^6 CFU/ml by diluting bacteria in Luria broth. The inoculum was used immediately after preparation, and an exact titer then was determined by preparing 10-fold dilutions of inoculum in Luria broth and streaking a 0.1-ml volume of each dilution over the surface of MacConkey agar plates. Inoculated agar plates were incubated overnight at 37 C, and colonies were counted to determine CFU/ml.

Virus. TCV (NC95) was isolated from PEMS-affected turkeys as described (10). An inoculum was prepared by amniotic inoculation of 20-day-old embryonated turkey eggs with TCV (NC95). Embryos were inoculated with 0.1 ml of TCV (NC95) at the 11th embryo passage (titer undetermined); embryo intestines were harvested 4 days postinoculation (PI) and prepared as a 10% suspension in Dulbecco's

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minimal essential medium (DMEM). These suspensions were homogenized, clarified by centifugation (1200 × *g* for 10 min), and sequentially filtered through 0.8-, 0.45-, and 0.22-μM filters. Virus was titered by inoculation of 10-fold dilutions into each of five 23-day-old embryonated turkey eggs and examination of intestines from individual embryos on day 3 PI by direct immunofluorescence (FA) (10). An inoculum was prepared to contain approximately 4000 50% embryo infectious doses (EID_{50})/0.1 ml and stored at −70 C.

The TCV (NC95) inoculum was examined for presence of extraneous viruses by virus isolation and indirect FA. Chicken kidney cells were inoculated with TCV (NC95) inoculum as described (10). Embryo intestines from TCV (NC95)-inoculated embryonated turkey eggs were examined for presence of turkey astrovirus, group A rotavirus, group D rotavirus, and turkey enterovirus by indirect FA (9). Antisera specific for turkey astrovirus and group D rotavirus were obtained from D. Reynolds, Iowa State University; antisera specific for group A rotavirus were obtained from T. Hooper, Purdue University.

Turkeys. Commercial medium white turkeys were obtained at 1 day of age from a primary breeder company (British United Turkeys of America, Lewisburg, WV). The turkeys originated from a flock that was monitored by the primary breeder company for *Mycoplasma gallisepticum, Mycoplasma synoviae, Mycoplasma meleagridis, Mycoplasma iowae, Salmonella pullorum, Salmonella typhimurium, Salmonella enteritidis, Salmonella arizona,* avian influenza virus, TCV, and reticuloendotheliosis virus. Turkeys were housed in wire-floored, electrically heated brooders in an isolation room with controlled access until turkeys were 6 days of age. Turkeys were fed nonmedicated game bird starter (Granville Milling, Creedmoor, NC). Feed and water were provided *ad libitum.*

Experimental design. At 6 days of age, 192 turkeys were individually identified by wing bands, weighed, and randomly allocated to four groups having approximately the same mean weight. Forty-eight birds were allotted to each group and distributed to electrically heated brooders in four separate isolation rooms. Turkeys were inoculated by placing a no. 10 French catheter (Monoject, St. Louis, MO) into the crop. Inoculations were done as follows: group 1 (sham-inoculated controls), 0.5 ml DMEM and 0.5 ml Luria broth; group 2, 1 ml containing 4000 EID_{50} TCV (NC95); group 3, 1 ml containing 7×10^5 CFU *E. coli* R98/5; group 4, 1 ml containing 4000 EID_{50} TCV (NC95) and 7×10^5 CFU *E. coli* R98/5.

Turkeys were examined daily for signs of illness and mortality and were weighed on days 7 and 14 postexposure (PE). Three birds were randomly selected from each group on days 2, 4, 6, 8, and 10 PE and necropsied. At necropsy, liver, spleen, bursa of Fabricius, thymus, pancreas, duodenum, jejunum, ileum, and cecum were collected for histopathology. Ileum, cecum, and bursa of Fabricius were collected and immediately frozen in O.C.T. (Tissue-Tek, O.C.T. Compound; Miles Laboratories, Elkhart, IN) for immunohistochemistry. Only live birds were selected on sampling days for postmortem examination, histopathology, and immunohistochemistry; birds that died were not necropsied.

Immunohistochemistry. Frozen tissues (ileum, cecum, bursa of Fabricius) were sectioned with a cryostat, fixed in cold (4 C) absolute acetone for 10 min, and stored at 4 C until stained. TCV antigens were detected in frozen tissue sections by direct FA staining as described (10).

Histopathology. Tissues were placed in neutral buffered formalin and processed routinely for histopathology. Slides were stained by both Giemsa and hematoxylin and eosin methods. Microscopic lesions in the intestines of inoculated turkeys were identified as AE if each of the following characteristics was present: microcolony formation, intimate adherence of bacteria to intestinal epithelium, and degeneration and/or necrosis of epithelium at sites of bacterial adherence (Fig. 1).

Statistical evaluation. Inoculation groups were compared for weight gain and average daily gain by one-way analysis of variance (ANOVA) (24).

RESULTS

Bacteria. *Escherichia coli* R98/5, a lactose nonfermenter, could not be serotyped with known O- and H-specific antisera. R98/5 did not produce heat-labile or heat-stable enterotoxins or shigalike toxins; however, R98/5 was determined to possess the eae gene.

TCV inoculum. No extraneous viruses were detected in the TCV (NC95) inoculum. No cytopathic effects were observed in cell cultures inoculated with TCV inoculum during two passages, and virus was not detected in cell culture supernatant fluids by electron microscopy. Turkey astrovirus, turkey enterovirus, group A rotavirus, and group D rotavirus were not detected by indirect FA staining of inoculated turkey embryo intestines.

Clinical signs, mortality, and weight gain. Clinical signs consisting of depression, inappetance, and decreased water consumption were observed beginning on day 3 PE in turkeys inoculated with TCV + R98/5. No clinical signs were observed in sham-inoculated turkeys or in turkeys inoculated with only TCV or R98/5. High mortality was observed only in

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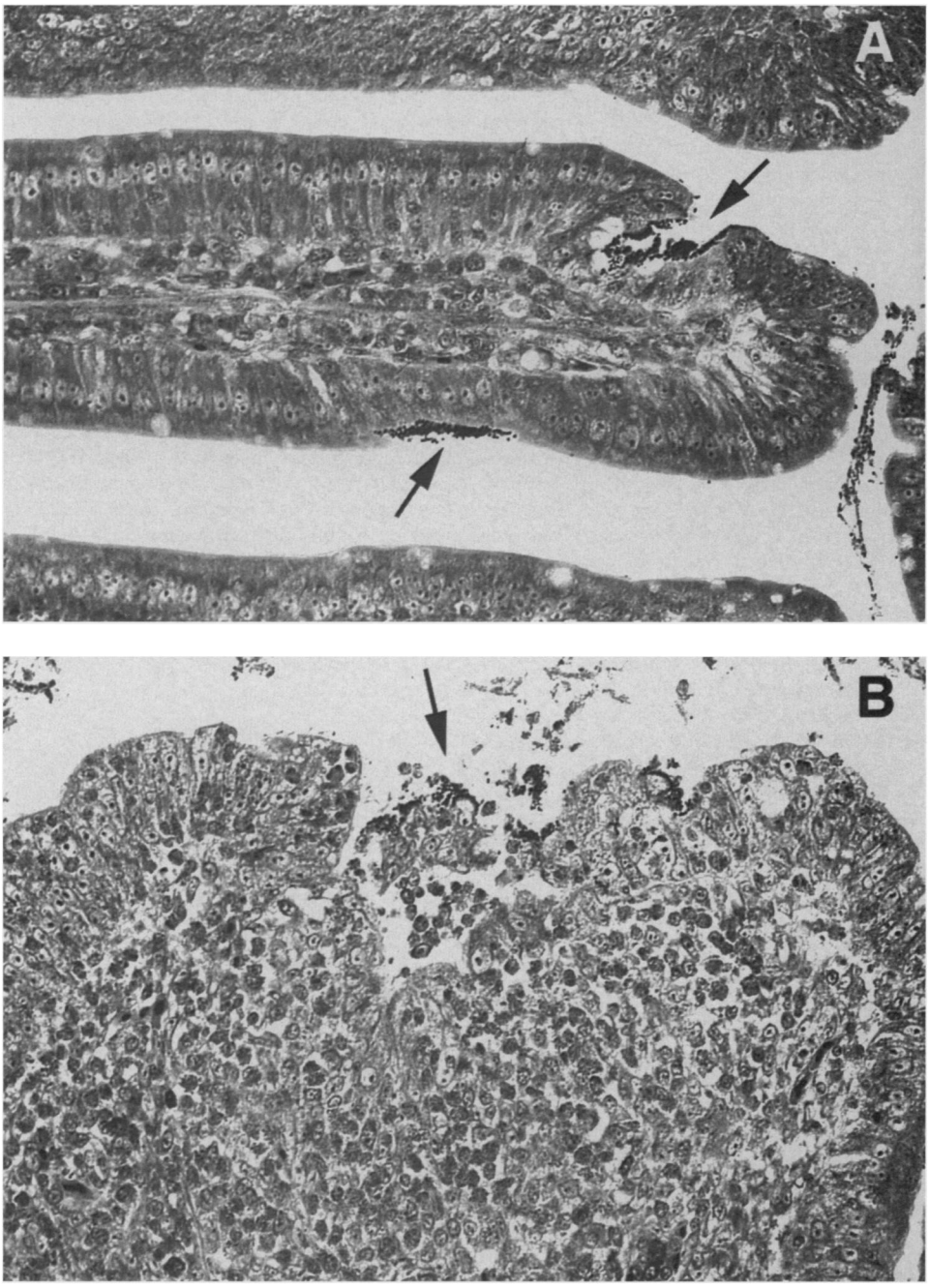

Fig. 1. Attaching and effacing lesions in intestines of turkey dually inoculated with TCV and *E. coli* R98/5, 4 days postexposure. Giemsa stain. 406×. (A) Jejunum. Bacterial microcolonies (arrows) are adhering to enterocytes with degeneration and necrosis at sites of attachment. Near the villous tip is a pitlike erosion containing numerous adhering bacteria. (B) Cecum. Adherent bacterial microcolonies are found in association with degenerated and necrotic enterocytes with focal erosion (arrow). Infiltration of lamina propria with lymphocytes and heterophils has resulted in expansion of the villous width.

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Table 1. Effect of TCV (NC95) and *E. coli* R98/5 on mortality and weight gain. Total mortality is shown for the duration of the experiment (0–14 days PE); weight gain and average daily gain are shown for the 0–7-day PE period.[A]

TCV	*E. coli* R98/5	Mortality	Mean weight gain 0–7 days PE (g)	Average daily gain 0–7 days PE (g)
−	−	2/48 (4%)	114[a]	16.3[a]
+	−	3/48 (6%)	78[b]	11.1[b]
−	+	2/48 (4%)	113[a]	16.3[a]
+	+	38/48 (79%)	10[c]	1.4[c]

[A]Excessive mortality in turkeys dually infected with TCV and *E. coli* R98/5 prevented an evaluation of weight gain in this group at later time intervals. Values within a column followed by the same lowercase superscript letter are not significantly different ($P > 0.05$).

turkeys inoculated with TCV + R98/5 (38/48, 79%; Table 1). Mortality in TCV + R98/5-inoculated turkeys began on day 4 PE (six deaths) and peaked on day 7 (15 deaths). Mortality ranged from 4% to 6% in sham-inoculated turkeys and turkeys inoculated with only R98/5 or TCV; mortality in these groups was attributed to vent picking/cannibalism.

Table 1 shows the mean body weight gains observed during the 0–7-day PE period. Excessive mortality in birds inoculated with TCV + R98/5 prevented assessment of weight gain at times later than day 7 PE. Weight gain of turkeys inoculated with only R98/5 was not different from that of sham-inoculated controls. Weight gain of turkeys inoculated with only TCV was significantly depressed compared with that of sham-inoculated controls; however, weight gain of turkeys dually inoculated with TCV + R98/5 was significantly depressed compared with both sham-inoculated controls and turkeys inoculated with only TCV (Table 1). In the 0–7-day PE period, sham-inoculated turkeys and turkeys inoculated with only R98/5 averaged a weight gain of 16.3 g/day. Turkeys inoculated with only TCV averaged a weight gain of 11.1 g/day, and turkeys inoculated with TCV + R98/5 averaged a weight gain of 1.4 g/day.

Gross pathology. Gross lesions were observed in turkeys inoculated with TCV and TCV + R98/5 beginning on day 2 PE. The small intestines and ceca of turkeys inoculated with TCV and TCV + R98/5 were pale, thin walled, and distended with gas and brown, watery fluid; however, these effects were more pronounced in dually inoculated turkeys.

Turkeys inoculated with TCV + R98/5 exhibited signs of dehydration beginning on day 4 PE. By day 8 PE, thymus and bursa of Fabricius were atrophied in dually inoculated turkeys. These effects were not observed in TCV-inoculated turkeys. No gross lesions were observed in sham-inoculated turkeys or turkeys inoculated with only R98/5.

Histopathology. AE lesions characterized by adherence of bacterial microcolonies to intestinal epithelium (Fig. 1) were observed in turkeys inoculated with R98/5 and TCV + R98/5 beginning on day 2 PE (Table 2). AE lesions were detected in duodenum, jejunum, ileum, and cecum but most often in ileum and cecum. AE lesions were identified in the jejunum of turkeys inoculated with TCV + R98/5 but not in turkeys inoculated with only R98/5. In turkeys inoculated with TCV + R98/5,

Table 2. Attaching and effacing lesions identified in turkeys inoculated with only *E. coli* R98/5 or dually inoculated with TCV and *E. coli* R98/5 at different times postexposure.[A]

Days PE	R98/5 only[B]				TCV + R98/5[B]			
	Duodenum	Jejunum	Ileum	Cecum	Duodenum	Jejunum	Ileum	Cecum
2	0/3	0/2	2/3	2/3	0/3	2/3	2/3	2/3
4	1/3	0/3	1/3	0/3	1/3	2/3	3/3	3/3
6	0/2	0/3	0/3	0/3	2/3	1/3	3/3	3/3
8	0/3	0/3	0/3	0/3	0/3	1/3	1/3	2/3
10	0/3	0/3	0/3	0/3	0/3	0/3	0/3	1/3

[A]Attaching and effacing lesions were not identified in sham-inoculated turkeys or turkeys inoculated with only TCV.

[B]Number with lesions/number examined.

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AE lesions were found in intestinal tissues of more birds and involved greater numbers of villi compared with turkeys inoculated with only R98/5. In addition, AE lesions were detected from days 2 to 10 PE in turkeys inoculated with TCV + R98/5 but only on days 2–4 PE in turkeys inoculated with only R98/5. AE lesions were not detected in sham-inoculated turkeys or turkeys inoculated with only TCV. AE lesions were more readily identified in Giemsa-stained sections and were easily missed in sections stained by the hematoxylin and eosin method. The presence of AE lesions was accompanied by 1) infiltration of the lamina propria with lymphocytes and heterophils, 2) increased villous width, 3) degeneration, necrosis, and detachment of epithelium at sites of bacterial attachment, and 4) focal erosion of epithelium, often with formation of pits (Fig. 1).

Microscopic lesions in turkeys inoculated with TCV (TCV only and TCV + R98/5) were detected in intestines, bursa of Fabricius, and thymus. In intestines, TCV infection resulted in villous atrophy, crypt cell hyperplasia, occasional sloughing of cells from villous tips, and infiltration of lamina propria with heterophils. These changes were observed primarily in jejunum, ileum, and cecum but were most consistently observed in jejunum. No significant change was observed in intestines of sham-inoculated controls.

In the bursa of Fabricius, TCV infection resulted in necrosis of follicular and interfollicular epithelium accompanied by infiltration of epithelium and adjacent lamina propria with heterophils. The epithelium of the bursa of Fabricius changed from a tall columnar, pseudostratified structure to a squamous epithelium. Microscopic lesions in epithelium of bursa of Fabricius did not differ between turkeys inoculated with only TCV and turkeys dually inoculated with TCV + R98/5. Lymphocyte depletion was observed in bursal follicles of TCV-infected turkeys; depletion was mild in turkeys inoculated with only TCV and moderate-to-severe in turkeys inoculated with both TCV and R98/5. No significant change was observed in bursa of Fabricius of sham-inoculated turkeys or turkeys inoculated with only R98/5.

Lymphoid depletion was evident in thymus of turkeys dually inoculated with TCV + R98/5. By day 4 PE, lymphocyte necrosis was observed in the cortex; lymphocyte depletion in the cortex was evident by day 6 PE. Lymphocyte depletion was not observed in the thymus of sham-inoculated turkeys or turkeys inoculated with only TCV or R98/5. No lesions were detected in the liver or pancreas that could be ascribed to either TCV or R98/5.

TCV antigens were detected in tissues (ileum, cecum, bursa of Fabricius) of turkeys inoculated with only TCV on days 2, 4, 6, 8, and 10 PE (other tissues were not examined). In turkeys inoculated with TCV + R98/5, TCV antigens were detected in these same tissues on days 2, 4, 6, and 8 PE. TCV antigens were not detected in ileum, cecum, or bursa of Fabricius of sham-inoculated turkeys or turkeys inoculated with only R98/5.

Escherichia coli having characteristics of isolate R98/5 (lactose nonfermenter, positive for eae by PCR) were recovered on days 2, 4, 6, and 8 PE from turkeys inoculated with R98/5 (R98/5 only, TCV + R98/5) but not from sham-inoculated turkeys or turkeys inoculated with only TCV.

DISCUSSION

Enteropathogenic *E. coli* isolate R98/5, by itself, failed to produce clinically apparent disease in experimentally infected turkeys. Similarly, an embryo-propagated strain of TCV resulted only in mild disease and moderate growth depression. However, dual infection of turkeys with both TCV and *E. coli* R98/5 produced severe disease characterized by high mortality, marked growth depression, enterotyphlitis, and lymphoid depletion in thymus and bursa of Fabricius. These clinical effects closely resemble those observed in naturally occurring cases of PEMS, thus the interaction of EPEC and TCV suggests a possible explanation for the pathogenesis of this disease.

Escherichia coli previously have been identified in PEMS-affected turkeys and suggested as the cause of this disease (3,4). Oral inoculation of young turkeys with these isolates at 1 day of age resulted in increased mortality and growth depression, and cyclophosphamide treatment enhanced these effects. The *E. coli* strains examined in these previous studies differed from *E. coli* R98/5 in that they did not possess eae genes and they did not produce AE lesions in experimentally infected turkeys. Taken together, these findings suggest that different types of *E.*

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coli may contribute to PEMS via different mechanisms.

TCV infection appeared to promote colonization of the intestines of turkeys by *E. coli* R98/5, and a synergistic effect on mortality, weight gain, and lesion development was observed. AE lesions characteristic of EPEC were identified in turkeys inoculated with only *E. coli* R98/5 and in turkeys inoculated with both TCV and *E. coli* R98/5; however, these lesions were found in greater numbers of birds and for longer duration in dually infected birds. In addition, concurrent TCV infection resulted in a wider distribution of AE lesions in the intestinal tract; AE lesions were detected in jejunum of dually infected turkeys but not in turkeys inoculated with only *E. coli* R98/5. Increased mortality and enhanced growth depression observed in dually infected birds likely were due to increased colonization of intestines by *E. coli* R98/5 and enhanced AE lesion development; however, this remains unproven.

The mechanism by which EPEC induce diarrhea is poorly understood; however, malabsorption due to loss of absorptive epithelium, signal transduction events leading to increased secretion, and increased epithelial permeability are believed to be important mechanisms (13). Additional studies are needed to elucidate the mechanisms responsible for severe disease leading to mortality in turkeys infected with both TCV and *E. coli* R98/5; however, mortality likely resulted from severe and prolonged diarrhea that led to dehydration and electrolyte imbalance. Similar clinical effects are observed in human beings infected with EPEC. EPEC have been shown to be responsible for severe outbreaks of human pediatric diarrhea; these outbreaks often are explosive and may result in mortality up to 50% (19,22).

TCV was identified in 1973 as the cause of a severe enteric disease of turkeys known variously as mud fever or bluecomb disease (17). In recent years, TCV has been associated as the cause of PEMS; however, the role of the virus in this disease remains undetermined. Recent epidemiologic studies demonstrated that TCV could be identified in a similar proportion of PEMS-affected and unaffected turkey flocks in North Carolina (2). In addition, our studies with TCV (NC95), as well as other embryo-propagated strains, have failed to produce significant disease in experimentally infected turkeys other than mild-to-moderate growth depression. Our inability to propagate TCV (NC95) in cell culture despite repeated attempts precluded purification of the virus by plaque purification or other similar techniques. Analysis of the TCV (NC95) inoculum used in the present study did not reveal the presence of other viruses; however, the possibility of an unidentified agent in the inoculum that may have contributed to the experimentally produced disease cannot be ruled out at the present time.

Previous investigations have demonstrated enhancement of enteric disease in animals as a consequence of mixed viral and bacterial infections. Synergistic interactions between rotavirus and enterotoxigenic *E. coli* (ETEC) have been observed in pigs, calves, lambs, and mice that were experimentally infected with these agents (10,14,20,25,27). Snodgrass *et al.* (25) demonstrated that rotavirus infection markedly enhanced intestinal colonization by ETEC in 6-day-old conventional calves. Hess *et al.* (11), with 1-day-old specific-pathogen-free calves, demonstrated that inoculation with only ETEC did not produce clinical disease, and calves inoculated with only rotavirus exhibited mild diarrhea; however, severe disease was produced in calves dually inoculated with both rotavirus and ETEC. Increased mortality has been observed as a consequence of combined rotavirus and ETEC infections in lambs and mice (20,27). These studies have provided experimental evidence that rotavirus enhances intestinal colonization by ETEC; however, the mechanism has not been determined. Whether rotavirus enhances ETEC colonization by impairing immune defenses such as mucus production or by damaging intestinal epithelium, thus exposing sites for bacterial attachment, is not known. Virus infection and epithelial degeneration may expose bacterial attachment sites not ordinarily present in healthy birds, or these may be preferentially expressed on immature epithelial cells that replace degenerating, mature epithelium. Whether TCV promotes EPEC R98/5 in a manner similar to that observed in rotavirus/ETEC infections remains to be determined; however, similar mechanisms likely are operative.

Dual infection of turkeys with TCV and *E. coli* R98/5 resulted in lymphoid depletion and atrophy of the bursa of Fabricius and thymus. Whether these effects are due to physiological

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factors such as stress or to more direct effects of *E. coli*/TCV infection remains to be determined. Experimental infection of chickens with *E. coli* previously has been shown to result in lymphoid depletion and atrophy of bursa of Fabricius and thymus (18).

These findings suggest an etiologic role for TCV and EPEC in the pathogenesis of PEMS; however, other viruses and bacteria likely interact in a similar manner to produce this severe enteric disease of turkeys. Additional studies examining other EPEC strains identified in PEMS-affected turkeys and the interaction of *E. coli* R98/5 with other enteric viruses of turkeys are warranted.

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