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Experimental Bovine Coronavirus in Turkey Poults and Young Chickens

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SUMMARY. The DB2 calf strain of bovine coronavirus (BCV) was used to inoculate 1-day-old specific-pathogen-free (SPF) turkey poults in three trials. In all trials, the birds developed clinical signs of enteritis at 48–72 hr postinoculation. Birds euthanatized at 3, 5, and 7 days postinoculation (DPI) had flaccid, pale intestines with watery contents, and the ceca were markedly enlarged with frothy contents. Coronavirus particles were detected by immune electron microscopy with BCV antibodies from the intestinal contents of birds killed at 3, 5, 7, and 12 DPI. Body weights of inoculated poults killed at 3, 5, and 7 DPI were significantly reduced as compared with controls. Hemagglutinating antibodies were detected in sera of convalescent birds at 12 DPI. However, experimental inoculation of 1-day-old SPF chicks in two trials with the same virus resulted in no clinical signs or macroscopic or microscopic lesions. No coronaviruses were detected from intestinal contents, and there were no significant differences in body weights of inoculated and noninoculated control chicks.

RESUMEN. Infección experimental con el coronavirus bovino en pavitos y pollitos jóvenes.

Se utilizó la cepa DB2 del coronavirus bovino para inocular experimentalmente pavitos libres de patógenos específicos de un día de edad. En tres experimentos realizados, las aves desarrollaron signos clínicos de enteritis de las 48 a las 72 horas después de la inoculación. Las aves sacrificadas a los 3, 5 y 7 días después de la inoculación tenían intestinos pálidos, flácidos con contenido acuoso y el ciego estaba severamente agrandado con contenido espumoso. Por medio de la inmunomicroscopía electrónica utilizando anticuerpos de coronavirus bovino, se detectaron partículas de coronavirus en el contenido intestinal de las aves sacrificadas a los 3, 5, 7 y 12 días después de la inoculación. El peso corporal de los pavitos inoculados sacrificados a los 3, 5 y 7 días después de la inoculación estuvo bastante reducido en comparación con el de los controles. En el suero de aves convalescentes se detectaron anticuerpos hemaglutinantes a los 12 días después de la inoculación. Sin embargo, en dos experimentos la inoculación de pollitos libres de patógenos específicos de un día de edad con el mismo virus no produjo signos clínicos ni lesiones macroscópicas ó microscópicas. No se observó el coronavirus en el contenido intestinal y no hubo diferencias significantes en el peso corporal de los pollitos inoculados y los controles no inoculados.

Key words: turkey coronavirus, bovine coronavirus, bluecomb, poult enteritis and mortality syndrome, diarrhea, enteritis, chickens

Abbreviations: BCV = bovine coronavirus; DPI = days postinfection; GI = gastrointestinal; GMT = geometric mean titer; H&E = hematoxylin and eosin; HI = hemagglutination inhibition; HRT-18 = human rectal adenocarcinoma; IBV = infectious bronchitis virus; IEM = immune electron microscopy; PEMS = poult enteritis and mortality syndrome; PTA = phosphotungstic acid; SPF = specific-pathogen free; TCV = turkey coronavirus

Enteric diseases are an important cause of morbidity and mortality in turkey poults. Co-

ronaviruses (3,5,6), enteroviruses (9,10,17), parvoviruses (20), astroviruses (16), adenoviruses (17), rotaviruses (12,13), a small round virus (21), and reovirus (7,17) have been associated with field outbreaks of diarrhea, increased mortality, and decreased body weight in turkeys. Turkey coronavirus (TCV) is a major cause of an acute, highly contagious enteric disease of

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turkey poults referred to as bluecomb disease or mud fever (15). Coronaviruses comprise four antigenic groups: group 1 includes transmissible gastroenteritis virus of swine, canine coronavirus, and feline enteric coronavirus; group 2 includes bovine coronavirus (BCV), mouse hepatitis virus, and diarrhea virus of infant mice; group 3 includes infectious bronchitis virus (IBV) of chickens; and group 4 includes TCV, whose antigenic relationship to either BCV or IBV is controversial (18). Dea *et al.* (2) reported the propagation of a TCV with an established cell line derived from human rectal adenocarcinoma (HRT-18), which has been used successfully to propagate BCV. BCV is widely recognized as an important cause of neonatal calf diarrhea (14) and it was reported to be antigenically related to TCV (4). This reported antigenic similarity of BCV to TCV and the ability of BCV to replicate in HRT-18 cells prompted us to initiate this study to investigate a possible role for BCV in enteric disease of turkey poults and young chickens.

MATERIALS AND METHODS

Birds. Specific-pathogen-free (SPF) 1-day-old chicks and turkey poults used in this experiment were obtained from SPF flocks maintained by the Food Animal Health Research Program. The flocks are free of detectable chicken and turkey pathogens, including enteric viruses, as indicated by periodic testing. All the birds used in the different experimental groups were kept in wire cages inside high-security isolation rooms provided with HEPA-filtered intake and exhaust air and provided with feed and water *ad libitum*. Different experimental groups were kept in separate rooms.

Virus. Large intestinal contents used for inoculation of birds were obtained from diarrheic gnotobiotic calves experimentally inoculated with the BCV DB2 strain and tested positive for BCV by immune electron microscopy (IEM) and enzyme-linked immunosorbent assay (11,19). The virus was originally isolated from a calf with naturally occurring diarrhea and was serially passaged in gnotobiotic calves to maintain virulence (19). The virus used in trials 1 and 2 was passaged five times and that used in trial 3 was passaged four times in gnotobiotic calves (19). The fecal material was diluted 1:5 in phosphate-buffered saline, filtered through 0.45- μ m syringe filters (Corning Glass Work, Corning, NY), and stored in aliquots at -70 C.

IEM. Samples from the gastrointestinal (GI) tract were collected at necropsy. The GI tracts of birds

from the same treatment at each necropsy period were pooled and homogenized 1/10 (w/v) in 0.05 M Tris-HCl buffer, pH 7.5, then clarified by low-speed centrifugation at $3000 \times g$ for 30 min at 4 C. The supernatants were filtered through 0.45- μ m disposable syringe filters. Two hundred microliters of the filtrate was incubated overnight at 4 C with 200 μ l of convalescent gnotobiotic calf anti-BCV serum diluted 1/10 in 0.05 M Tris-HCl buffer, pH 7.5. After incubation, the mixtures were centrifuged for 15 min at $160,000 \times g$ through a 50- μ l cushion of 40% sucrose with a Beckman Tabletop Airfuge[®]. Pellets were suspended in 400 μ l of filtered distilled water and centrifuged again without the sucrose cushion as previously described. The pellets obtained were re-suspended in 25 μ l of sterile distilled water. One drop of the resuspended solution was placed on carbon-coated 300-mesh Formvar[®] copper grids and stained with a drop of phosphotungstic acid (PTA) solution (3% PTA, 0.4% sucrose, pH 7.0). The grids were examined for the presence of virus at 80 kV with a transmission electron microscope (Philips 201; Philips Norelco, Eindhoven, The Netherlands).

Histopathology. Samples from the GI tracts were collected at 3 days postinfection (DPI), preserved in Prefer[®] (Nalge Co., Rochester, NY) and processed for histopathology by routine procedures. Slides were stained by hematoxylin and eosin (H&E) methods.

Hemagglutination inhibition (HI). A HI test was performed using 96-well microtiter plates. Eight units of concentrated BCV hemagglutination antigen in 0.025 ml and 0.025 ml of serial twofold dilutions of the test sera were mixed and then incubated at 37 C for 1 hr before adding 0.025 ml of 0.5% mouse erythrocytes. The microplates were incubated at 4 C for 1 hr. The HI antibody titer is the reciprocal of the highest dilution of serum causing HI and is expressed as the geometric mean titer (GMT). The diluent used throughout was veronal buffered saline, pH 7.2.

Statistical analysis. Statistical comparison of body weights and body weight gain between challenged and control birds was performed with one-way analysis of variance (ANOVA) (22).

Experimental inoculation of turkey poults.

Trial 1. Fifty 1-day-old SPF turkey poults were each orally inoculated with 0.2 ml of the BCV material described previously. The titer of the BCV in the inoculum used in this trial was not determined because the virulent strain of DB2 BCV is unadapted to cell culture. Another group of the same number of poults was kept in a separate room and served as unexposed controls.

Trial 2. Sixty-four 1-day-old SPF turkey poults were allotted into two groups of 32 birds each. Fifteen of the 32 poults from the first group were each orally inoculated with 0.2 ml of the BCV material described. The inoculated birds were wing banded

Table 1. Mean body weights of turkey poults inoculated with CB2 strain of BCV in trials 1 and 2.

Treatment	Mean body weight (g) ± standard deviation ^a											
	3 DPI			5 DPI			7 DPI			12 DPI		
	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Control	60.83 ± 2.58	59.18 ± 3.41	99.55 ± 1.30	99.11 ± 2.11	115.76 ± 3.20	122.18 ± 5.87	168.01 ± 4.76	169.54 ± 5.27	168.01 ± 4.76	169.54 ± 5.27	168.01 ± 4.76	169.54 ± 5.27
Contact	ND ^b	57.82 ± 3.04	ND	95.25* ± 2.08	ND	106.19*	ND	106.19*	ND	106.19*	ND	106.19*
Inoculated	57.86* ± 2.19	49.48* ± 8.25	95.66* ± 2.63	91.17* ± 2.36	110.87* ± 2.92	107.09* ± 4.29	166.92 ± 4.71	168.94 ± 5.82	166.92 ± 4.71	168.94 ± 5.82	166.92 ± 4.71	168.94 ± 5.82

^a* = Significantly different from controls ($P < 0.05$).

^bND = not done.

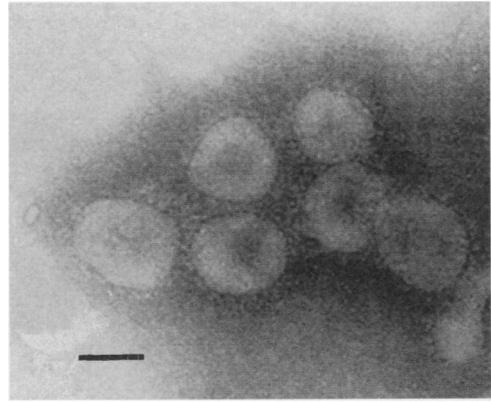


Fig. 1. Electron micrograph of the BCV particles detected from pooled intestinal contents of inoculated turkey poults at 3 DPI. Bar = 100 nm.

and mixed with the remaining uninoculated birds, which served as contact-exposed birds. The other group of 32 birds was kept separately to serve as unexposed controls.

In trials 1 and 2, poults were observed daily for clinical signs. Groups of poults from each treatment were weighed and euthanatized at 3, 5, 7, and 12 DPI in both trials. Birds were examined for lesions and intestinal contents and intestines were collected for IEM and histopathologic examination, respectively. Blood samples were collected from birds at 12 DPI for HI tests.

Trial 3. Forty-four 1-day old turkey poults were allotted into two groups of 22 birds each. Each individual bird in the first group was orally inoculated with 0.2 ml of the BCV material described previously. The other group of 22 birds was kept separately to serve as unexposed controls. In both groups, body weight of all birds was recorded at hatch, and the birds were observed daily for clinical signs. From each group, five to seven poults were weighed, euthanatized, and examined for lesions at 3, 5, 7, and 12 DPI. Intestines were collected for IEM and histopathologic examinations. Blood samples were collected at 12 DPI for HI tests.

Experimental inoculation of chickens. Trial 1. Fifty 1-day-old SPF chicks were orally inoculated with 0.2 ml of the BCV material described previously. Another group of the same number was kept in a separate room to serve as controls. Chicks were observed daily for clinical signs, and 12–14 chicks from each treatment were weighed and euthanatized at 3, 5, 7, and 12 DPI. Birds were examined for lesions, and intestinal samples were collected for IEM and histopathologic examination. Blood samples were collected from birds at 12 DPI for HI tests.

Trial 2. Thirty-two 1-day-old SPF chicks were orally inoculated with the BCV material described

Table 2. Mean body weight gain of turkey poults inoculated with DB2 strain of BCV in the third trial.

Treatment	Mean body weight gain (g) \pm standard deviation			
	3 DPI	5 DPI	7 DPI	12 DPI
Control ^A	20.72 \pm 6.66	44.99 \pm 6.16	70.89 \pm 12.86	117.98 \pm 6.12
Inoculated ^A	20.24 \pm 2.02	49.75 \pm 6.03	82.39 \pm 2.66	107.29* \pm 8.22

^A* = Significantly different from controls ($P < 0.05$).

previously. Another group of the same number was kept in a separate room as unexposed controls. Chicks in both challenged and control groups were wing banded and weighed at hatch and observed for clinical signs throughout the experiment. Eight chicks from each group were weighed, euthanized and necropsized at 3, 5, 7, and 12 DPI. Samples from intestines were collected for IEM examination and histopathologic evaluation. Blood samples were collected from birds at 12 DPI for HI tests.

RESULTS

Experimental inoculation of turkey poults. In the three trials, inoculated poults had diarrhea with frothy droppings starting at 2–3 DPI and lasting for about 4 days. The morbidity was 100% but no mortality occurred. On postmortem examination, the ceca were markedly enlarged and filled with frothy yellowish contents. The intestines were flaccid, with pale coloration and thin walls. In trial 1, there was a significant reduction in body weight of inoculated poults as compared with controls at 3, 5, and 7 DPI (Table 1). In the second trial, the inoculated and contact-exposed birds had significant reductions in body weight as compared with controls that started as early as 3 DPI in inoculated and 5 DPI in contact-exposed poults (Table 1). In the third trial, the body weight gain was significantly reduced in the challenged group as compared with the control group only at 12 DPI (Table 2). BCV was detected from pooled intestinal contents of challenged poults by IEM at 3, 5, 7, and 12 DPI (Fig. 1). The HI GMTs of convalescent sera collected at 12 DPI were 16.81, 19.33, and 14.49 for the three trials, respectively. No HI

antibodies were detected in convalescent sera of control birds. Histopathologic examination of intestines from inoculated poults revealed mild to moderate nonsuppurative ileitis characterized by villous atrophy and crypt hyperplasia and lymphocytic infiltration into the lamina propria at 3, 5, 7, and 12 DPI. Mild to moderate typhilitis with loss of mucosal glands and mucosal gland hyperplasia was also observed at 3, 5, 7, and 12 DPI (Fig. 2).

Experimental inoculation of chickens.

In both trials, there was no diarrhea or any other clinical sign. The body weights of challenged chicks were not significantly different from their corresponding controls in trial 1 (Table 3). There were also no significant differences in body weight gain between challenged and control chicks in trial 2 (Table 4). BCV was not detected by IEM in intestinal contents of challenged chicks throughout the experimental period. No HI antibodies were detected in convalescent sera of inoculated birds. Histopathologic examination of sections from intestines of challenged birds revealed normal tissues.

DISCUSSION

Studies on antigenic relationships among several coronaviruses with polyclonal antibodies (5) and monoclonal antibodies (4) indicated that TCV was closely related to serogroup 2 of mammalian coronaviruses, which includes BCV. Subsequent sequence analysis of genes encoding the nucleocapsid and membrane proteins revealed a 99% or greater identity between TCV and BCV gene sequences (23). Dea *et al.* (2) reported the propagation of TCV on a hu-

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Fig. 2. Intestinal sections from poults at 5 DPI. Control (A) and infected (B) duodenum. Arrows depict villous atrophy and crypt hyperplasia (50 \times ; H&E). Bar = 100 μ m. Control (C) and infected (D) duodenum at higher magnification (100 \times ; H&E). Bar = 50 μ m. Control (E) and infected (F) ceca showing mucosal gland hyperplasia and lymphocytic infiltration in the lamina propria (50 \times ; H&E). Bar = 100 μ m.

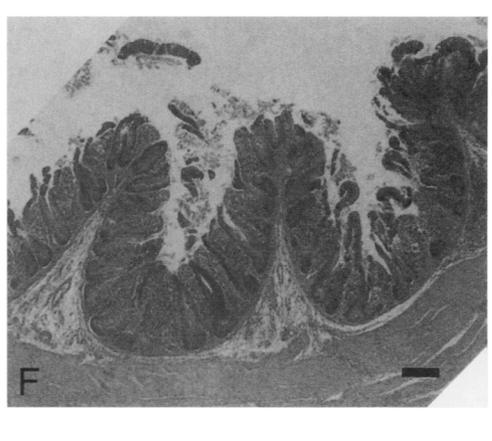
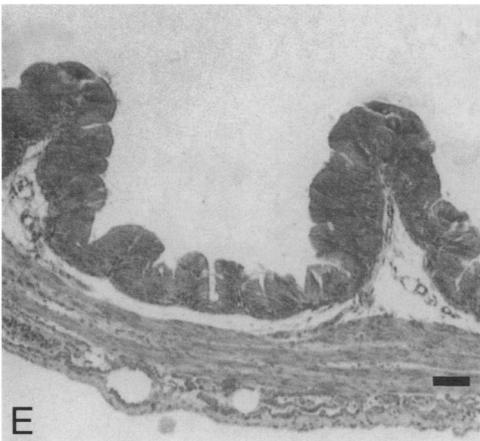
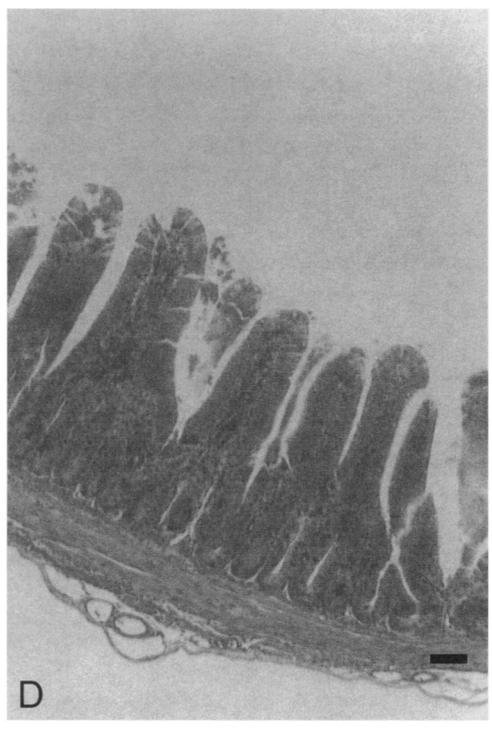
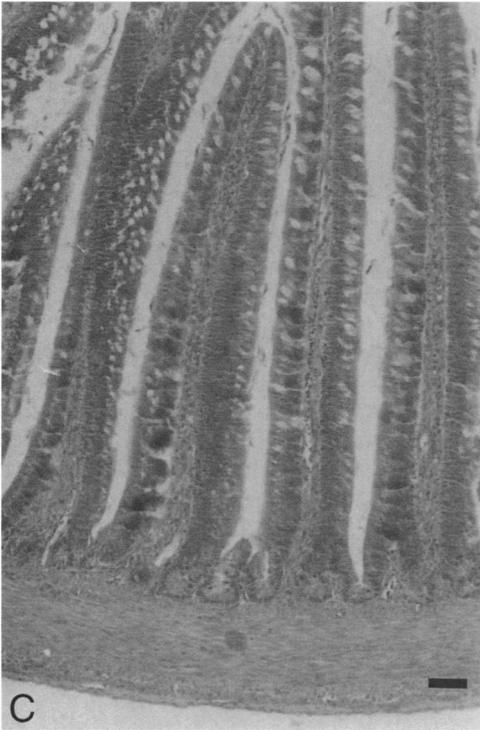
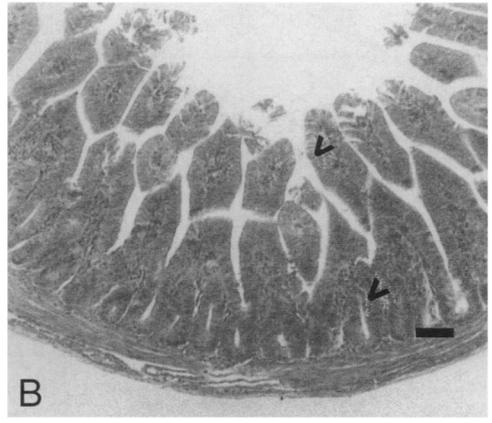
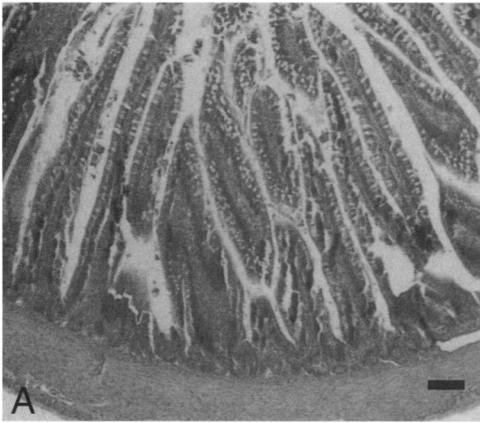


Table 3. Mean body weights of chickens inoculated with DB2 strain of BCV in trial 1.

Treatment	Mean body weight gain (g) \pm standard deviation			
	3 DPI	5 DPI	7 DPI	12 DPI
Control ^A	40.49 \pm 2.16	55.81 \pm 3.41	65.23 \pm 3.54	102.81 \pm 3.66
Inoculated ^A	39.78 \pm 1.76	54.80 \pm 3.58	64.64 \pm 4.70	101.72 \pm 3.81

^AThere were no significant differences between inoculated and control groups.

man rectal tumor cell line (HRT-18) that has been used successfully to grow BCV. In other studies by Guy *et al.* (8), based on immunofluorescence and immunoperoxidase studies, TCVs isolated from turkey flocks with either bluecomb or poult enteritis and mortality syndrome (PEMS) were found to be closely related to IBV of chickens, a member of coronavirus serogroup 3. In the same study, TCV was not adapted to grow on the HRT-18 cell line. In our laboratory, extensive efforts to adapt the TCV originating from either bluecomb- or PEMS-affected turkey flocks to the HRT-18 cells were unsuccessful. These findings led us to hypothesize that BCV might be associated with field cases of enteritis and diarrhea in turkeys. In the present study, poult challenged with a virulent (DB2) calf strain of BCV had diarrhea and growth depression in all three trials. The DB2 strain used in our studies is a virulent strain of BCV not adapted to tissue culture that was chosen to avoid the possibility of loss of pathogenicity during adaptation. Nevertheless, that strain has been adapted to HRT-18 cells in our laboratory. In trial 1, the growth depression started as early as 3 DPI and continued to 7 DPI. In trial 2, the growth depression started at 3 DPI in inoculated birds and at 5 DPI in contact birds, whereas in trial 3, the difference in body weight gain was significant only at 12 DPI. These variations may be due to differences in virus origin and dosage and the use of body weight gain rather than reduction in body weight as used in the other experiments. Body weight gain could not be used to assess growth

rate in the first two trials in turkey poult and in the first trial in chickens because the birds' body weight was not recorded at hatch. The propagation of the Kakegawa strain of BCV in suckling mice, rats, and hamsters (1) could raise the question of the potential role of these animals as reservoirs for BCV and subsequent transmission to turkeys.

In the two trials with chickens, there were no clinical signs or gross or microscopic gut lesions, and no virus was detected by IEM in intestinal contents. The sera of convalescent birds had no HI antibodies, and there was no significant difference in growth rate between control and inoculated groups. We conclude that chickens are resistant to infection with the DB2 strain of BCV. The findings of consistent diarrhea, gross and microscopic intestinal lesions, BCV detection from challenged turkey poult, and HI antibody response indicate that the DB2 strain of BCV is pathogenic to turkey poult and can initiate enteric disease and lesions similar to that produced by TCV infection.

REFERENCES

1. Akashi, H., Y. Inaba, Y. Miurai, K. Sato, S. Tokuhisa, M. Asagi, and Y. Havashi. Propagation of the Kakegawa strain of bovine coronavirus in suckling mice, rats and hamsters. *Arch. Virol.* 67:367-370. 1981.
2. Dea, S., S. Garzon, and P. Tijssen. Isolation and trypsin-enhanced propagation of turkey enteric (bluecomb) coronaviruses in a continuous human

Table 4. Mean body weight gain of chickens inoculated with DB2 strain of BCV in trial 2.

Treatment	Mean body weight gain (g) \pm standard deviation			
	3 DPI	5 DPI	7 DPI	12 DPI
Control ^A	13.90 \pm 1.11	32.79 \pm 1.78	40.94 \pm 6.20	63.99 \pm 6.11
Inoculated ^A	12.86 \pm 2.83	29.79 \pm 4.53	46.54 \pm 6.18	58.90 \pm 3.46

^AThere were no significant differences between inoculated and control groups.

- rectal adenocarcinoma cell line. *Am. J. Vet. Res.* 50: 1310–1318. 1989.
3. Dea, S., and P. Tijssen. Viral agents associated with outbreaks of diarrhea in turkey flocks in Quebec. *Can. J. Vet. Res.* 52:53–57. 1988.
 4. Dea, S., A. J. Verbeek, and P. Tijssen. Antigenic and genomic relationships among turkey and bovine coronaviruses. *J. Virol.* 64:3112–3118. 1990.
 5. Dea, S., A. Verbeek, and P. Tijssen. Transmissible enteritis of turkeys: experimental inoculation studies with tissue-culture-adapted turkey and bovine coronaviruses. *Avian Dis.* 35:767–777. 1991.
 6. Deshmukh, D. R., and B. S. Pomeroy. Physicochemical characterization of a bluecomb coronavirus of turkeys. *Am. J. Vet. Res.* 35:1549–1552. 1974.
 7. Goodwin, M. A., K. S. Latimer, B. N. Nersessian, and O. J. Fletcher. Quantitation of intestinal D-xylose absorption in normal and reovirus-inoculated turkeys. *Avian Dis.* 28:959–967. 1984.
 8. Guy, J., H. J. Barnes, L. J. Smith, and J. Breslin. Antigenic characterization of a turkey coronavirus identified in poult enteritis and mortality syndrome-affected turkeys. *Avian Dis.* 41:583–590. 1997.
 9. Guy, J. S. Virus infections of the gastrointestinal tract of poultry. *Poult. Sci.* 77:1166–1175. 1998.
 10. Hayhow, C. S., and Y. M. Saif. Experimental infection of specific-pathogen-free turkey poults with single and combined enterovirus and group A rotavirus. *Avian Dis.* 37:546–557. 1993.
 11. Heckert, R. A., L. J. Saif, and G. W. Myers. Development of protein A-gold immunoelectron microscopy for detection of bovine coronavirus in calves: comparison with ELISA and direct immunofluorescence of nasal epithelial cells. *Vet. Microbiol.* 19:217–231. 1989.
 12. McNulty, M. S. Rotavirus infections. In: *Diseases of poultry*, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 692–701. 1997.
 13. McNulty, M. S., G. M. Allan, and J. C. Stuart. Rotavirus infection in avian species. *Vet. Rec.* 103:319–320. 1978.
 14. Mebus, C. A., E. L. Stair, M. B. Rhodes, and M. J. Twiehaus. Pathology of neonatal calf diarrhea induced by a coronavirus like agent. *Vet. Pathol.* 10: 45–64. 1973.
 15. Nagaraja, K. V., and B. S. Pomeroy. Coronaviral enteritis of turkeys (bluecomb disease). In: *Diseases of poultry*, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 686–692. 1997.
 16. Reynolds, D. L., and Y. M. Saif. Astrovirus: a cause of an enteric disease in turkey poults. *Avian Dis.* 30:728–735. 1986.
 17. Reynolds, D. L., Y. M. Saif, and K. W. Theil. Enteric viral infections of turkey poults: incidence of infection. *Avian Dis.* 31:272–276. 1987.
 18. Saif, L. J., and R. A. Heckert. Enteropathogenic coronaviruses. In: *Viral diarrhea of man and animals*. L. J. Saif and K. W. Theil, eds. CRC Press, Inc., Boca Raton, FL. pp. 185–236. 1990.
 19. Saif, L. J., D. R. Redman, P. D. Moorhead, and K. W. Theil. Experimentally induced coronavirus infections in calves: viral replication in the respiratory and intestinal tracts. *Am. J. Vet. Res.* 47:1426–1432. 1986.
 20. Saif, L. J., Y. M. Saif, and K. W. Theil. Enteric viruses of diarrheic turkey poults. *Avian Dis.* 29:798–811. 1985.
 21. Saif, Y. M., L. J. Saif, C. L. Hofacre, C. Hayhow, D. E. Swayne, and R. N. Dearth. A small round virus associated with enteritis in turkey poults. *Avian Dis.* 34:762–764. 1990.
 22. Snedecor, G. W., and W. G. Cochran. One way classifications; analysis of variance. In: *Statistical methods*, 7th ed. Iowa State University Press, Ames, IA. pp. 215–237. 1980.
 23. Verbeek, A., and P. Tijssen. Sequence analysis of the turkey enteric coronavirus nucleocapsid and membrane protein genes: a close genomic relationship with bovine coronavirus. *J. Gen. Virol.* 72: 1659–1666. 1991.

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