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Antigenic Characterization of a Turkey Coronavirus Identified in Poult Enteritis- and Mortality Syndrome-Affected Turkeys

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SUMMARY. A turkey coronavirus (TCV [NC95]) was characterized by antigenic comparison with other avian and mammalian coronaviruses using immunofluorescence (FA) and immunoperoxidase (IP) procedures. Based on FA and IP procedures, TCV (NC95) was determined to be antigenically indistinguishable from turkey enteric (bluecomb) coronavirus (TECV). In addition, TCV (NC95) and TECV were found to be closely related to infectious bronchitis virus (IBV); a one-way antigenic relationship was demonstrated. Polyclonal antibodies specific for TECV and IBV reacted strongly against TCV (NC95), as determined by FA procedures. Monoclonal antibodies (MAbs) specific for IBV matrix protein (MAb 919) reacted strongly against TCV (NC95) and TECV as determined by FA and IP procedures; an IBV peplomer protein-specific MAb (MAb 94) did not recognize the two viruses. These studies suggest an identification of TCV (NC95) as a strain of TECV, and provide evidence of a close antigenic relationship between these viruses and IBV.

RESUMEN. Characterización antigénica de un coronavirus de pavos identificado en la enteritis de pavitos y en el síndrome de mortalidad que afecta a los pavos.

Se caracterizó un coronavirus de pavos identificado como NC95 mediante la comparación antigénica con otros coronavirus aviares y de mamíferos, utilizando las pruebas de inmunofluorescencia e inmunoperoxidasa. Basados en estas dos pruebas, se determinó que el coronavirus de pavos NC95 es antigénicamente indiferenciable del coronavirus entérico de pavos que produce la cresta azul. Además, se encontró que el coronavirus de pavo NC95 y el coronavirus entérico de pavos tienen una relación antigénicamente estrecha en una dirección con el virus de bronquitis infecciosa. Por medio de la inmunofluorescencia se determinó que anticuerpos policlonales específicos contra el coronavirus entérico de pavos y el virus de bronquitis infecciosa reaccionan fuertemente contra el coronavirus de pavos NC95. Por medio de las pruebas de inmunofluorescencia e inmunoperoxidasa se determinó que el anticuerpo monoclonal 919 específico contra la proteína matriz del virus de bronquitis infecciosa reaccionó fuertemente contra el coronavirus de pavos NC95 y contra el coronavirus entérico de pavos. El anticuerpo monoclonal 94 específico contra un peplómero del virus de bronquitis infecciosa no reconoció los dos virus. Estos resultados sugieren que el coronavirus de pavos NC95 es una cepa del coronavirus entérico de pavos y suministran evidencia de que existe una relación antigénica muy cercana entre estos dos virus y el virus de bronquitis.

Key words: coronavirus, infectious bronchitis virus, turkey coronavirus

Abbreviations: BCV = bovine coronavirus; CK = chicken kidney; EM = electron microscopy; FA = immunofluorescence; FITC = fluorescein isothiocyanate; HI = hemagglutination inhibition; HRT = human rectal tumor; IBV = infectious bronchitis virus; IEM = immunoelectron microscopy; IP = immunoperoxidase; M = matrix; MAb = monoclonal antibody; N = nucleocapsid; PBS = phosphate-buffered saline; PEMS = poult enteritis and mortality syndrome; PI = postinoculation; PK = pig kidney; S = peplomer; S1 = S1 peplomer subunit; S2 = S2 peplomer subunit; SPF = specific-pathogen free; TCV = turkey

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coronavirus; TECV = turkey enteric coronavirus; TGEV = transmissible gastroenteritis virus; TK = turkey kidney; VN = virus neutralization

The Coronaviridae is a large family of RNA-containing viruses that infect a wide variety of avian and mammalian species (14,18). Coronaviruses are characterized on the basis of their distinctive morphology. They are pleomorphic, enveloped particles with diameters of 60–220 nm, having long (12–24-nm), widely spaced, petal-shaped surface projections (14,18). Coronaviruses have at least three major structural proteins including the peplomer (S) protein (90–180 kD), a matrix (M) protein (20–35 kDa), and a nucleocapsid (N) protein (50–60 kD) (18). Infectious bronchitis virus (IBV) has been shown to be comprised of an S protein (180 kD) that is post-translationally cleaved into S1 (90 kD) and S2 (84 kD) subunits, an M protein (23–35 kD), and a N protein (approximately 50 kD) (1).

The coronaviruses have been subdivided into four antigenic groups based on antigenic differences determined primarily by immunofluorescent (FA), enzyme-linked immunosorbent assay, and immunoelectron microscopic (IEM) studies (11,14,15,18). Mammalian and avian coronaviruses have been shown to fall into four distinct and unrelated groups; mammalian coronaviruses comprise antigenic groups 1 and 2 whereas avian coronaviruses, IBV, and turkey enteric coronavirus (TECV) comprise groups 3 and 4, respectively. Infectious bronchitis virus and TECV were determined to be antigenically distinct from each other and mammalian coronaviruses based on IEM, hemagglutination inhibition (HI), and virus-neutralization (VN) studies (2,13). More recent antigenic studies by Dea *et al.* (3) have suggested that TECV is closely related to bovine coronavirus (BCV), a mammalian group 2 coronavirus.

The avian coronaviruses, IBV and TECV, cause several different diseases of economic importance. Infectious bronchitis virus is the cause of an acute, highly contagious respiratory disease in chickens. Infectious bronchitis virus also may infect the kidney and reproductive tract of chickens resulting in kidney lesions and decreased egg production, respectively (8). Turkey enteric coronavirus is the cause of an acute, highly contagious enteric disease of turkeys referred to as bluecomb disease or mud fever (12). The disease caused by TECV is characterized by diarrhea, depression, inappetence, weight loss, and high mortality. Like most other coronaviruses, IBV and TECV exhibit host-specificity; they have not been identified as causes of disease in heterologous species (8,12).

Poult enteritis and mortality syndrome (PEMS), otherwise referred to as spiking mortality of turkeys, is a recently described enteric disease of young turkeys of unknown etiology (5). A variety of different infectious agents, including a coronavirus, have been identified in PEMS-affected turkeys; however, the role of the coronavirus in the disease has not been determined. The present report describes the propagation and antigenic characterization of a turkey coronavirus (TCV [NC95]) identified in PEMS-affected turkeys.

MATERIALS AND METHODS

Cell culture. Chicken kidney (CK) and turkey kidney (TK) cells were prepared as described (16) using 1-day-old specific-pathogen-free (SPF) chickens (SPAFAS, Norwich, CT) and 1-day-old commercial turkeys. Human rectal tumor (HRT) cells were obtained from Dr. D. A. Brian (University of Tennessee, Knoxville, TN) and pig kidney (PK15) cells were obtained from the American Type Culture Collection (Rockville, MD). Cells were grown at 37 C in a 5% CO_2 incubator in a growth medium consisting of RPMI-1640 and 10% fetal bovine serum.

Viruses. Turkey coronavirus (NC95) was isolated in embryonated turkey eggs following inoculation of eggs with homogenates of bursae of Fabricius from turkeys with experimentally induced PEMS. Bursae of Fabricius were prepared as 10% (w/v) suspensions in RPMI-1640, homogenized, clarified by centrifugation at 2000 × *g* for 20 min, and filtered sequentially through 0.45- and 0.22-μm filters (Millipore Products Division, Bedford, MA). Fertile, 20-day-old embryonated turkey eggs were inoculated with 0.2 ml of homogenized bursal tissue into the amniotic sac (17). Eggs were returned to the incubator and examined daily for mortality. After a second passage using embryo intestines collected at 6 days postinoculation (PI) and processed as described above, embryo intestinal fluids were collected at 5 days PI and examined by negative-stain electron microscopy (EM) as described (4).

Turkey enteric coronavirus (Minnesota) was obtained from the American Type Culture Collection

(Rockville, MD) and propagated in embryonated turkey eggs as described above. Infectious bronchitis virus (Arkansas) was obtained from SPAFAS and propagated in CK cells (8). Transmissible gastroenteritis virus (TGEV) (Purdue strain) and BCV (Nebraska strain) were obtained from the National Veterinary Services Laboratory (Ames, IA) and propagated in PK15 cells and HRT cells, respectively.

Propagation of TCV (NC95) and TECV (Minnesota) in HRT cells was attempted using methods similar to those described by Dea *et al.* (3) for propagation of BCV and TECV. Briefly, embryo-propagated virus was inoculated onto drained, confluent monolayers of HRT cells in 25-cm^2 tissue-culture flasks and incubated 1 hr at 37 C to allow virus adsorption. The inoculum then was replaced with serum-free RPMI-1640 medium containing 2 μg/ml trypsin (Type IX; Sigma Chemical Co., St. Louis, MO) and incubation was continued at 37 C. Inoculated cell cultures were examined daily for cytopathic effects and passaged at 5–7-day intervals for a total of six cell culture passages. Cell culture supernatant fluids were examined at each passage by negative-stain EM (4). Bovine coronavirus (Nebraska) was propagated in HRT cells in media without added trypsin.

Antibodies, fluorescein isothiocyanate (FITC) conjugates, and monoclonal antibodies (MAbs). Four-week-old SPF chickens (SPAFAS) were inoculated with 2.0 ml of a 20% suspension of turkey embryo intestines containing TCV (NC95) by passing a no. 5 French catheter (Monoject, St. Louis, MO) into the crop. Four weeks later chickens were inoculated intravenously with 0.5 ml of TCV (NC95), partially purified from infected embryo intestines by sucrose-gradient ultracentrifugation. Serum was collected 10 days after the last inoculation.

Antiserum prepared in SPF chickens against IBV (Massachusetts) was obtained from SPAFAS. Infectious bronchitis virus-specific antisera was produced by aerosol exposure of SPF chickens to egg allantoic fluid containing low embryo-passage IBV (Massachusetts), followed 2 wk later by intramuscular inoculation of allantoic fluid containing high embryo-passage IBV (Massachusetts) mixed with Freund's complete adjuvant. Serum was collected 7–10 days later.

Antiserum prepared in SPF turkeys against TECV (Minnesota) was obtained from Dr. Y. M. Saif (Ohio State University, Wooster, OH) (SPF turkeys used for antiserum preparation were free of known avian pathogens including TECV). Turkey enteric coronavirus-specific antisera was produced by oral inoculation of SPF turkeys with turkey embryo-propagated TECV (Minnesota), followed by two intramuscular inoculations at 3-wk intervals with sucrose-gradient-purified virus mixed with incomplete Freund's adjuvant.

Monoclonal antibodies specific for IBV (MAb 94 and MAb 919) were obtained from Dr. S. Naqi (Cornell University, Ithaca, NY) (7).

Fluorescein isothiocyanate–antibody conjugates were prepared with polyclonal antibodies specific for IBV (Massachusetts) and TCV (NC95) by the procedure described by McNulty and Allan (9). Fluorescein isothiocyanate–antibody conjugates were absorbed with a tissue homogenate prepared from intestinal mucosa of normal 4-wk-old turkeys (9). Fluorescein isothiocyanate–antibody conjugates specific for TGEV and BCV were obtained from VMRD (Pullman, WA).

Antigen preparations. Tissues were immediately frozen in Tissue-Tek O.C.T. Compound (Miles Laboratories, Elkhart, IN), sectioned with a cryostat, fixed in cold (−20 C) absolute acetone for 10 min, and stored at 4 C until staining. Just prior to staining, sections were washed in phosphate-buffered saline (PBS) to remove O.C.T. Compound.

Chicken kidney, PK15, and HRT cells were grown to confluency in 25-cm^2 tissue-culture flasks, inoculated with IBV (Arkansas), TGEV (Purdue), and BCV (Nebraska), respectively, and incubation was continued for 1–3 days. Antigen for FA staining was prepared by scraping infected cells off growth surfaces, pelleting cells by centrifugation (800 × *g* for 5 min), and resuspending the cell suspension in PBS to one-tenth the original volume. Drops of cell suspension were placed onto glass slides, air dried, and then fixed in acetone for 10 min at room temperature.

Immunohistochemical procedures. Direct and indirect FA staining procedures were carried out as described (4,9). Direct FA staining was done using FITC-conjugated antisera specific for TGEV, BCV, IBV (Massachusetts), and TCV (NC95). Fluorescein isothiocyanate conjugates specific for TGEV and BCV were used undiluted according to manufacturer's recommendations; FITC conjugates prepared against IBV (Massachusetts) and TCV (NC95) were diluted 1:2 in PBS. Indirect FA staining was done using antisera specific for TECV (Minnesota) and MAbs specific for IBV; these were used at a dilution of 1:50 in PBS. Antigen preparations were examined with an epifluorescence microscope. The intensity of fluorescence was arbitrarily rated on a scale of − (undetectable) to +++ (maximal fluorescence).

Tissue sections were stained by an indirect immunoperoxidase (IP) procedure as described (6). Monoclonal antibodies specific for IBV were used at a dilution of 1:50 in PBS.

Tissue sections from uninfected turkeys, embryonated turkey eggs, and cell cultures were used as negative controls for both FA and IP staining. Antibody controls also were included for each section; a FITC–antibody conjugate specific for Newcastle disease vi-

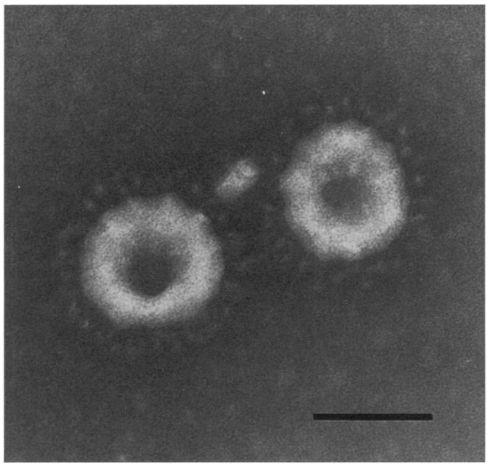

Fig. 1. Coronaviruses identified in intestinal contents of turkey embryos, 5 days after yolk-sac inoculation on day 20 of embryonation. Bar = 100 nm.

rus and a MAb specific for feline herpesvirus 1 were used in place of coronavirus-specific antibodies.

Field studies. Intestines and bursae of Fabricius were collected from 12-day-old turkeys from one enteritis-affected flock in which coronaviruses were identified in droppings by negative-stain EM. Tissues were processed and stained as described above using IBV-specific MAbs and polyclonal antibodies specific for IBV, TECV, and TCV (NC95). Control tissues were obtained from uninoculated, age-matched turkeys raised in confinement at the North Carolina State University College of Veterinary Medicine.

RESULTS

Virus propagation. Attempts to cultivate the coronavirus TCV (NC95) in TK and HRT cell cultures were unsuccessful. No cytopathic effects were observed in cell cultures during six passages, and virus was not detected in cell culture supernatant fluids by negative-stain EM.

Turkey coronavirus (TCV [NC95]) was isolated in embryonated turkey eggs after one blind passage. Coronaviruses were identified in embryo intestinal fluids by negative-stain EM; virus particles were approximately 80–130 nm in diameter and had approximately 20-nm petal-shaped projections on their surfaces (Fig. 1). No virus particles were observed in intestines of age-matched uninoculated embryos.

Immunohistochemistry. *Polyclonal antibodies.* Turkey coronavirus (NC95)-infected embryo intestines were used as antigen to determine whether antibodies specific for four different coronaviruses, representing the four coronavirus antigenic groups (11,14,15,18), would recognize the virus. Immunofluorescence results are shown in Table 1. Antibodies specific for IBV (Massachusetts) and TECV (Minnesota) reacted strongly against TCV (NC95) (Table 1, Fig. 2), whereas fluorescence was not detected using antibodies specific for TGEV and BCV. Each of the coronavirus-specific antibodies reacted strongly against its homologous antigen; no fluorescence was observed when antibodies specific for TGEV and BCV were applied to TECV-infected embryo intestines (results not shown).

Table 1. Antigenic relationship of turkey coronavirus (TCV [NC95]) to mammalian (antigenic groups 1 and 2) and avian (antigenic groups 3 and 4) coronaviruses as determined by direct and indirect immunofluorescent antibody tests. Antigenic groups are represented by: group 1, transmissible gastroenteritis virus (TGEV); group 2, bovine coronavirus (BCV); group 3, infectious bronchitis virus (IBV); group 4, turkey enteric coronavirus (TECV).[A]

	Antibody			
Antigen	Group 1: TGEV	Group 2: BCV	Group 3: IBV	Group 4: TECV
TCV (NC95)	−	−	+++	++

[A]Antigen for immunofluorescence staining consisted of TCV (NC95)-infected turkey embryo intestines. Fluorescein isothiocyanate–antibody conjugates specific for TGEV, BCV, and IBV (Massachusetts) were used in a direct immunofluorescence procedure. Indirect immunfluorescence was used for TECV (Minnesota). Each antibody reacted strongly to the homologous antigen. The intensity of fluorescence was arbitrarily rated on a scale of − (undetectable) to +++ (maximal fluorescence).

Cross-immunofluorescence studies subsequently were done in order to examine antigenic relationships between IBV (Arkansas), TCV (NC95), and TECV (Minnesota). Infectious bronchitis virus (Arkansas), TCV (NC95), and TECV (Minnesota) were examined by FA staining using homologous and heterologous antibodies (Table 2). Turkey coronavirus (NC95) and TECV (Minnesota) were indistinguishable from each other, and a one-way antigenic relationship between these two viruses and IBV (Arkansas) was demonstrated. Strong fluorescence was observed in TCV (NC95)- and TECV (Minnesota)-infected turkey em-

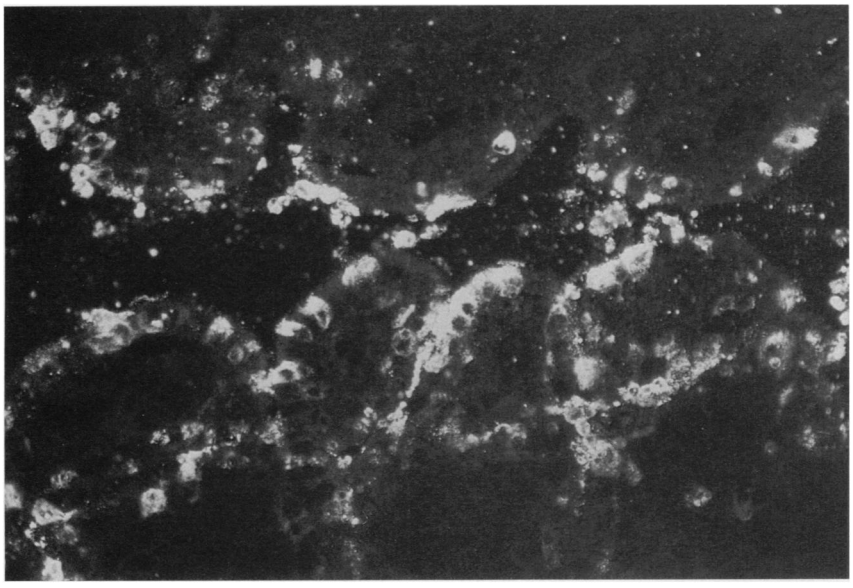

Fig. 2. Immunofluorescent staining of cryostat sections of turkey coronavirus (NC95)-infected turkey embryo intestines using fluorescein isothiocyanate-conjugated antibodies specific for infectious bronchitis virus (Massachusetts). 384×.

Table 2. Antigenic relationship of turkey coronavirus (TCV [NC95]) to infectious bronchitis virus (IBV) and turkey enteric coronavirus (TECV) [Minnesota]) as determined by cross-immunofluorescent antibody tests.^

	Antibody		
Antigen	Chicken anti-IBV (Massachusetts)	Chicken anti-TCV (NC95)	Turkey anti-TECV (Minnesota)
IBV (Arkansas)	+++	−	−
TCV (NC95)	+++	+++	+++
TECV (Minnesota)	++	+++	+++

^Antigen for immunofluorescence staining consisted of IBV (Arkansas)-infected chicken kidney cells, and TCV (NC95)- and TECV (Minnesota)-infected turkey embryo intestines. Fluorescein isothiocyanate–antibody conjugates specific for IBV (Massachusetts) and TCV (NC95) were used in a direct immunofluorescence procedure. Indirect immunofluorescence was used for TECV (Minnesota). Each antibody reacted strongly to the homologous antigen. The intensity of fluorescence was arbitrarily rated on a scale of − (undetectable) to +++ (maximal fluorescence).

bryo intestines using antibodies specific for TCV (NC95), TECV (Minnesota), or IBV (Massachusetts) (Table 2). No fluorescence was observed in IBV (Arkansas)-infected CK cells when stained with the antibodies specific for TCV (NC95) or TECV (Minnesota), whereas the homologous reaction with IBV-specific antibodies was very strong.

Monoclonal antibodies. Indirect FA and IP procedures were employed to examine the antigenic relationship of TCV (NC95) and TECV (Minnesota) to IBV using IBV-specific MAbs (Table 3). Monoclonal antibody 94, a group-specific MAb with antibody specificity to the S2 protein of IBV, reacted strongly against IBV-infected CK cells but not against TCV (NC95)- or TECV (Minnesota)-infected embryo intestines. Monoclonal antibody 919, a group-specific MAb with specificity to the M protein of IBV, reacted strongly against IBV (Arkansas), TCV (NC95), and TECV (Minnesota) (Fig. 3). No positive staining was observed when MAbs were used for FA or IP staining of TGEV- or BCV-infected cell cultures or uninfected embryo intestines (results not shown).

Field studies. Positive FA staining was observed in the apical one-half of intestinal enterocytes in the duodenum and jejeunum, and the

Table 3. Antigenic relationship of turkey coronavirus (TCV [NC95]) and turkey enteric coronavirus (TECV [Minnesota]) to infectious bronchitis virus (IBV) as determined by indirect immunofluorescence (FA) and immunoperoxidase (IP) staining procedures using monoclonal antibodies specific for IBV (MAb 94, MAb 919).[A]

Antigen	IBV MAb 94	IBV MAb 919
IBV (Arkansas)	+++	+++
TCV (NC95)	−	+++
TECV (Minnesota)	−	+++

[A]Antigen for FA and IP staining consisted of IBV (Arkansas)-infected chicken kidney cells and TCV (NC95)- and TECV (Minnesota)-infected turkey embryo intestines. No positive staining was observed when MAbs 94 and 919 were used for IP staining of transmissible gastroenteritis virus- or bovine coronavirus-infected cell cultures. The intensity of FA and IP staining was arbitrarily rated on a scale of − (undetectable) to +++ (maximal fluorescence).

epithelium of the bursa of Fabricius using polyclonal antibodies specific for IBV, TECV, and TCV (NC95). Similarly, positive IP staining was observed in these tissues using MAb 919 (Fig. 4). No staining was observed when these tissues were FA stained using polyclonal antibodies specific for BCV and IP stained using MAB 94. No FA or IP staining was observed using these antibody reagents on tissues collected from age-matched, uninoculated commercial turkeys.

DISCUSSION

A TCV (NC95), isolated from turkeys in a flock experiencing enteric disease and high mortality (PEMS), was identified in the present study to be a strain of turkey enteric (bluecomb) coronavirus (TECV) based on antigenic analyses using FA and IP procedures. In addition, TECV and TCV (NC95) were determined to be closely related antigenically to IBV.

The findings of these studies strongly suggest that the avian coronaviruses, IBV and TECV, comprise a single antigenic group within the family Coronaviridae, and are not separate groups as previously indicated by IEM, HI, and VN studies (2,13). The discrepancies between the findings of the present studies and previous antigenic analyses are not surprising as antigenic comparisons using IEM, HI, and VN assays detect antigenic similarities that exist between viruses at the virion surface, primarily peplomer antigens, whereas FA and IP procedures potentially allow detection of antigenic similarities among all virion proteins. Based on FA and IP procedures used in this study, it is evident that common antigens are present in virion proteins of IBV, TECV, and TCV (NC95) other than those detected by IEM, HI, and VN. This was most clearly demonstrated in the present study using IBV-specific MAbs: an M protein-specific MAb (MAb 919) reacted strongly against IBV, TECV, and TCV (NC95) as determined by FA

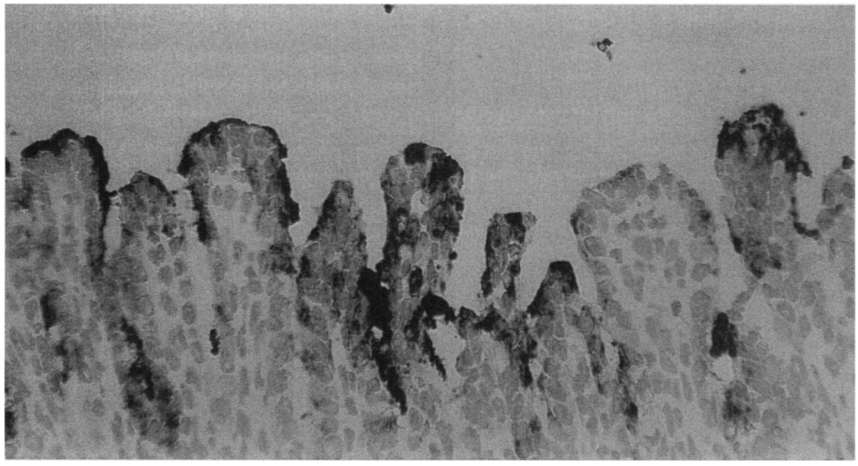

Fig. 3. Immunoperoxidase staining of turkey coronavirus (NC95)-infected turkey embryo intestines using monoclonal antibody 919. 384×.

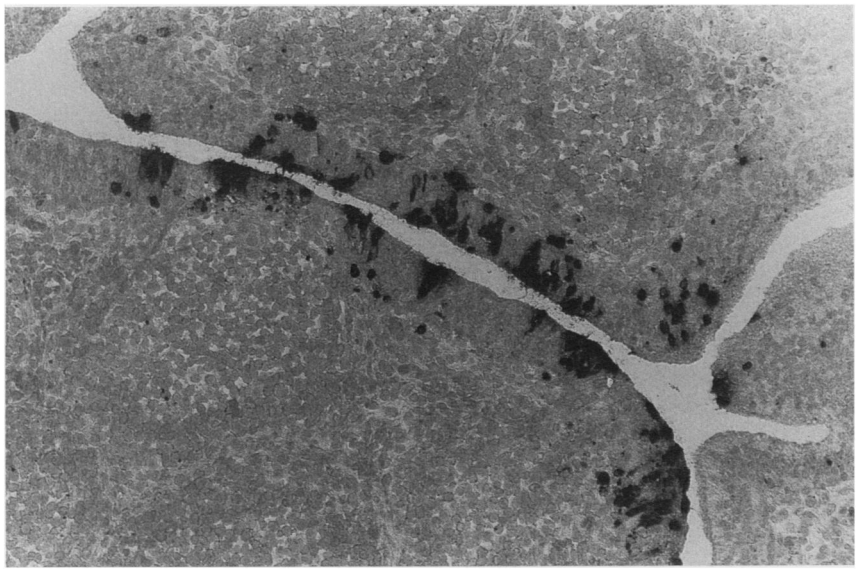

Fig. 4. Bursa of Fabricius from an 11-day-old turkey with naturally occurring poult enteritis and mortality syndrome. The tissue was stained by an indirect immunoperoxidase procedure using monoclonal antibody 919. 193×.

and IP procedures, but the S protein-specific MAb (MAb 94) reacted only against IBV.

The discrepancies between the present studies and those of Dea *et al.* (3) that indicate a close antigenic relationship between TECV and BCV are more difficult to explain. In the studies by Dea *et al.* (3), TECV was shown to replicate in HRT cells and to be closely related to BCV based on immunoblotting studies, HI, VN, and hybridization of BCV cDNA to TECV RNA. In contrast to the findings of Dea *et al.*, we failed to detect antigenic similarity between BCV and TECV based on cross-immunofluorescence, and our attempts to propagate TECV and TCV (NC95) in HRT cells were unsuccessful. Additional studies are needed to address the discrepancies between the present studies and those of Dea *et al.* (3).

Turkey enteric coronavirus and TCV (NC95) were distinguished from IBV by the failure of antibodies specific for these viruses to react with IBV antigens using FA and IP staining procedures. A similar one-way antigenic relationship between the mammalian coronaviruses, TGEV, feline infectious peritonitis virus, and canine coronavirus, was observed by Pedersen *et al.* (11) using FA procedures. Transmissible gastroenteritis virus- and feline infectious peritonitis virus-specific antibodies reacted strongly against canine coronavirus, but antibodies specific for canine coronavirus failed to recognize TGEV and feline infectious peritonitis virus antigens.

The identification of a close antigenic relationship between IBV, TECV, and TCV (NC95) has led to the development of diagnostic procedures for detection of TCV using IBV-specific antibodies. Infectious bronchitis virus-specific polyclonal antibodies and MAbs are commercially available, and these were used in the present study to detect TCV infection in a field case using fresh-frozen tissues. Intensive FA and IP staining were observed using these reagents in epithelium of the bursae of Fabricius and enterocytes lining the apical one-half of intestinal villi in the duodenum and jejunum of turkeys with coronavirus infection. Future studies will examine the possible use of IBV-specific MAbs to detect TCV in archived, formalin-fixed paraffin-embedded tissues. Retrospective studies using these tissues may provide a better understanding of the role of TCV in PEMS.

Turkey coronavirus (NC95) was identified in bursae of Fabricius of turkeys by virus isolation and immunohistochemical detection procedures. These findings are supportive of previous

studies suggesting the bursa of Fabricius as a possible site of TCV replication (10).

The present study also indicates that SPF chickens are susceptible to infection by TCV (NC95). The antisera collected from SPF chickens after oral and intravenous inoculation with embryo-propagated TCV (NC95) produced an excellent FA reagent when conjugated to FITC, and the staining pattern was identical to FITC-conjugated IBV-specific antibodies. One-day-old SPF chickens previously were shown to develop mild enteritis and growth depression when inoculated with fecal material from PEMS-affected turkeys (Guy and Barnes, unpubl. data), thus they were presupposed to be potentially suitable candidates for antibody production against infectious agents involved in this disease.

The findings of the present study suggest an identification of TCV (NC95) as a strain of TECV and demonstrate a close antigenic relationship among the avian coronaviruses IBV and TECV. Additional studies examining the relationship between these two viruses are warranted.

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