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Isolation of Coronaviruses Antigenically Indistinguishable from Bovine Coronavirus from Wild Ruminants with Diarrhea†

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Diarrheal feces from three sambar deer and one waterbuck in a wild animal habitat and one white-tailed deer on a wildlife farm in Ohio contained coronavirus particles which were agglutinated by antiserum to bovine coronavirus (BCV) in immune electron microscopy. Three coronavirus strains were isolated in human rectal tumor cells from the feces of the sambar and white-tailed deer and the waterbuck, respectively. Hemagglutination, receptor-destroying enzyme activity, indirect immunofluorescence, hemagglutination inhibition, virus neutralization, and Western blot (immunoblot) tests showed close biological and antigenic relationships among the isolates and with selected BCV strains. Gnotobiotic and colostrum-deprived calves inoculated with each of these isolates developed diarrhea and shed coronavirus in their feces and from their nasal passages. In a serological survey of coronavirus infections among wild deer, 8.7 and 6.6% of sera from mule deer in Wyoming and from white-tailed deer in Ohio, respectively, were seropositive against both of the isolates and selected BCV isolates by indirect immunofluorescence tests. These results confirm the existence of coronaviruses in wild ruminants and suggest that these species may harbor coronavirus strains transmissible to cattle.

Coronaviruses are divided into at least three antigenic groups, and antigenic cross-reactivity exists within an antigenic group (15, 33). Mouse hepatitis virus, murine enteric coronavirus, rat coronavirus, human respiratory coronavirus OC43, porcine hemagglutinating encephalomyelitis virus, and bovine coronavirus (BCV) belong to the same antigenic group. Turkey coronavirus may also belong to this group, although the data are conflicting (6, 17). However, these viruses usually can be distinguished from one another by virus neutralization (VN) or hemagglutination inhibition (HI) tests when homologous and heterologous antibody titers are compared (8, 11, 15, 22).

BCV is an important agent of neonatal calf diarrhea (CD) (4) and is also associated with acute diarrhea of adult cattle, referred to as winter dysentery (WD) (4, 18). Besides infecting the small and large intestines of calves, BCV also possesses a tissue tropism for the upper respiratory tract (14, 16, 20). Bovine coronavirus has four major structural proteins: the nucleocapsid (N), the transmembrane (M), the spike (S), and the hemagglutinin-esterase (HE) proteins (25). The S and HE proteins form fringes with longer and shorter surface projections, respectively (4). Both proteins can cause hemagglutination (HA) and contain neutralizing epitopes (7, 23). BCVs comprise a single serotype (4, 16), although there are minor antigenic and biological variations among BCV strains (5, 8, 13, 27). Coronaviruses have also been detected in diarrheic feces from other ruminants, e.g., sheep, sitatunga, and waterbuck (3, 31). However, the antigenic properties of these viruses are unclear because attempts to cultivate them in vitro were unsuccessful.

In this report, we describe the isolation of coronaviruses from the feces of sambar and white-tailed deer and a waterbuck with epizootic or sporadic diarrhea. The antigenic and biological properties of the isolates were compared with those of selected BCV strains causing CD and WD, and the seroepidemiology of coronavirus infections in wild populations of white-tailed and mule deer was investigated.

MATERIALS AND METHODS

Clinical specimens. An epizootic outbreak of diarrhea occurred at three areas about 1 km distant from each other in a wild animal habitat in southern Ohio during the winter of 1993 to 1994. The first outbreak was observed in a barn of antelope. This outbreak spread to barns of sambar deer (*Cervus unicolor*) and Cape buffalo (*Syncerus caffer caffer*) at intervals of about 1 week. The disease was characterized by an acute onset of diarrhea with bloody feces and high morbidity, with the diarrhea persisting for several days. Three of 10 affected sambar deer died several days after the onset of diarrhea. Episodes of sporadic diarrhea were observed in adult white-tailed deer (*Odocoileus virginianus*) on a wildlife farm in north central Ohio throughout 1994. The disease was characterized by an acute onset of diarrhea with liquid feces that persisted for several days. Fecal samples were collected from one adult waterbuck (*Kobus ellipsiprymnus*) and three sambar deer affected with diarrhea in the habitat and from one white-tailed deer with diarrhea on the farm. These samples were diluted 1:10 for virus isolation and immune electron microscopy (IEM) and 1:25 for enzyme-linked immunosorbent assay (ELISA) in 10 mM phosphate-buffered saline (PBS; pH 7.4) and were clarified by low-speed centrifugation (3,000 × *g* for 10 min). The fecal samples from the waterbuck and white-tailed deer were tested for *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., *Coccidium* spp. and *Cryptosporidium* spp. by using standard techniques. Serum samples were collected from three affected sambar deer in the habitat at the acute phase or before the diarrhea outbreak (in 1991 or 1992) and at the convalescent phase. Thirty serum samples from free-ranging healthy adult white-tailed deer in a metropolitan park in Ohio were kindly supplied by R. Mohan, Ohio Department of Agriculture, Reynoldsburg. Twenty-three serum samples from free-ranging healthy adult mule deer (*Odocoileus hemionus*) in Wyoming were kindly supplied by B. Williams, University of Wyoming, Laramie.

Viruses and hyperimmune antisera. The Mebus and 216XF strains of BCV causing CD and the TS, SD, and DBA strains of BCV causing WD were propagated in HRT-18 cells (1, 30). Hyperimmune antisera against these strains were prepared in guinea pigs (30) and were used for indirect immunofluorescence (IF), VN, and HI tests.

Virus isolation. The HRT-18 cells derived from a human rectal adenocarcinoma (28) were grown in roller tubes and were used for virus isolation as described previously (1, 30). Briefly, 0.2-ml samples of fecal suspensions were inoculated onto HRT-18 cells that had been washed two times with Eagle minimal essential medium (MEM). Four roller tubes were used for each sample.

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After adsorption for 1 h at 37°C, the cells were washed once with MEM and received 2 ml of MEM supplemented with 10 μg of pancreatin (GIBCO Laboratories, Grand Island, N.Y.) per ml. The cultures were incubated in a roller drum for 3 to 4 days at 37°C and were examined for cytopathic effects (CPEs). After incubation, the cells of one tube were removed for direct IF tests. The cells of the remaining tubes were frozen and thawed once to harvest cell lysates, and subsequent passages were carried out in the same manner with 0.2 ml of cell lysates. Five passages were done if neither CPEs nor fluorescent cells were detected. After four passages, three isolates (from waterbuck and sambar and white-tailed deer) were cloned two times in HRT-18 cells by plaque assay (32).

IEM. Fecal suspensions and infected cell culture supernatants were processed and examined by IEM as described previously (19). Diluted and filtered samples were reacted with bovine anti-BCV (Mebus strain) serum, negatively stained, and examined by using an electron microscope.

IF tests. Direct IF tests were performed with inoculated HRT-18 cell cultures and nasal epithelial cells and mucosal impression smears from the duodenum, jejunum, ileum, colon, nasal turbinates, trachea, and lung of inoculated calves. The cell culture or nasal epithelial cells and mucosal impression smears were placed on glass slides, fixed with acetone, and stained with fluorescein isothiocyanate (FITC)-conjugated anti-BCV (Mebus strain) serum (20). The cells were considered positive for coronavirus antigen when they showed specific cytoplasmic fluorescence distinct from any background reactions in controls. The percentage of positive cells in each specimen was estimated after the examination of five different fields of view.

Indirect IF tests were done for antigenic comparisons between the isolates and BCV strains (Mebus, 216XF, DBA, TS, and SD) and for serological surveys with serum samples from deer and waterbuck. Infected and fixed HRT-18 cells were prepared in 96-well microplates, and FITC-conjugated anti-bovine and anti-guinea pig sera (ZYMED Laboratory, San Francisco, Calif.) were used for deer and waterbuck sera and hyperimmune guinea pig antisera, respectively. Preimmune gnotobiotic calf and guinea pig sera and PBS were used as serum controls.

ELISA. An ELISA with monoclonal antibodies for the detection of BCV was conducted by using the fecal samples and the isolates (24). Three monoclonal antibodies recognizing the N, S, and HE proteins of the DB2 strain of BCV causing CD were pooled and used as capture antibodies in the ELISA.

VN tests. VN tests were conducted with HRT-18 cells as described previously (30). Serial twofold dilutions of sera were mixed with an equal volume of virus suspensions containing 200 median tissue culture infective doses per 0.1 ml, and the mixtures were incubated for 1 h at 37°C. HRT-18 cells grown in microplates were inoculated with 0.1 ml of each virus-serum mixture, and the mixtures were incubated for 5 days at 37°C. The VN antibody titers were expressed as the reciprocal of the highest serum dilution that completely inhibited the CPE.

HA, RDE, and HI tests. HA tests were conducted by the microtiter method (21). The isolates and BCV strains were purified from infected cell culture supernatants, which were concentrated approximately 100- to 200-fold. The HA titers were expressed as the reciprocal of the highest dilution of virus showing complete HA of 0.4 and 0.2% suspensions of mouse and chicken erythrocytes, respectively, after 1 h of incubation at 4 or 37°C. The plates incubated at 4°C were moved to 37°C for 2 h to measure inactivation of receptors reflected by the disaggregation of the BCV-erythrocyte complexes mediated by the receptor-destroying enzyme (RDE) activity (26). The HI tests were done by standard techniques with mouse erythrocytes and sera treated with kaolin (21).

Western blot assay. Western blot (immunoblot) assays were performed as described previously (12), except that 10% polyacrylamide separating gels were used. The blotted membranes were cut and incubated with gnotobiotic calf hyperimmune anti-BCV (DBA) serum diluted 1:200. Bound antibodies were detected with horseradish peroxidase-conjugated anti-bovine immunoglobulin G (ICN Biomedicals Inc. Costa Mesa, Calif.) diluted 1:3,000 and developed with ECL reagents (Amersham Life Science Inc., Arlington Heights, Ill.).

Experimental inoculation of calves. Two 4- and 47-day-old gnotobiotic calves and two 24- and 30-day-old colostrum-deprived calves were used for viral challenge tests. These calves were procured and maintained as described previously (20). The older gnotobiotic calf and the colostrum-deprived calves had recovered from a prior experimental oral challenge with bovine group A rotavirus at 2 days of age.

The 4-day-old gnotobiotic calf was inoculated orally (20 ml) and nasally (5 ml) with a 20% suspension of diarrheic feces of the waterbuck from which coronavirus was isolated in HRT-18 cells. Another 47-day-old gnotobiotic calf and the two colostrum-deprived calves were inoculated orally (15 ml) and nasally (5 ml) with the white-tailed deer, waterbuck, and sambar deer coronavirus isolates, respectively, with titers of $10^{7.5}$ to $10^{8.2}$ median tissue culture infective doses per ml. Feces and nasal swab specimens were collected daily, and clinical signs were noted. Feces were processed and examined for coronavirus by ELISA and IEM. Nasal epithelial cells were collected from nasal swab specimens and were used for the direct IF tests. Calves were euthanized at 24 to 36 h after the onset of diarrhea, and the duodenum, jejunum, ileum, colon, nasal turbinates, trachea, and lung were collected and used to prepare mucosal impression smears for the direct IF tests.

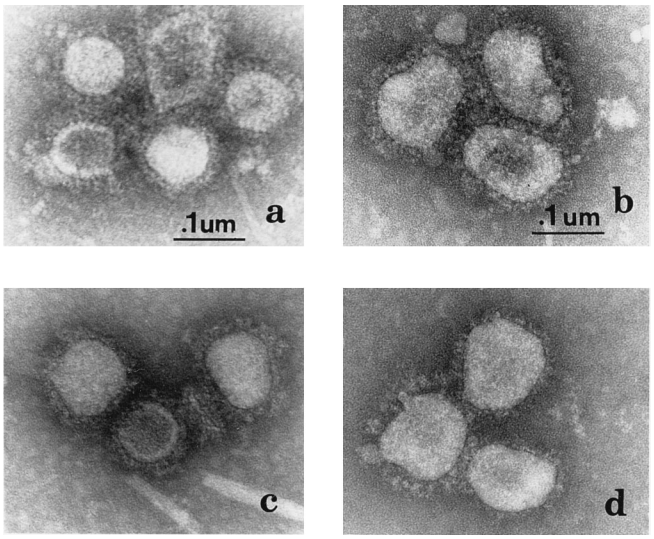

FIG. 1. Immune electron micrograph of the sambar deer coronavirus KI-D2 strain and the white-tailed coronavirus WTD strain incubated with gnotobiotic calf anti-bovine coronavirus (Mebus strain) serum. (a) Aggregate of coronavirus particles observed in the feces of a sambar deer; (b) aggregate of coronavirus particles observed in the cell culture fluid of HRT-18 cells infected with the KI-D2 isolate (passage 10); (c) aggregate of coronavirus particles observed in the feces of a white-tailed deer; (d) aggregate of coronavirus particles observed in the cell culture fluid of HRT-18 cells infected with the WTD isolate (passage 9).

RESULTS

Fecal examination. Coronavirus particles, which were 80 to 150 nm in diameter and had typical short and long surface spikes or projections, were observed in all of the fecal samples from three adult sambar deer and one waterbuck in the habitat and from one white-tailed deer in the farm. The coronavirus particles were agglutinated by hyperimmune anti-BCV serum in IEM (Fig. 1a and c). Other viruslike particles were not observed. These five fecal samples also reacted in the ELISA for BCV antigen detection. The fecal samples from the waterbuck and white-tailed deer were negative for *Salmonella* spp., *Campylobacter* spp., *Yersinia* spp., *Coccidium* spp., and *Cryptosporidium* spp.

Virus isolation in HRT-18 cells. Coronaviruses from the fecal samples of the waterbuck, sambar deer, and white-tailed deer were isolated in HRT-18 cells. Coronavirus antigens were observed by direct IF tests as specific cytoplasmic fluorescence in HRT-18 cells inoculated with each of these samples at the second passage. The number of fluorescing cells increased after further serial passages. After the second passage, CPEs were observed in the cultures inoculated with each isolate, which were characterized by enlarged, rounded, and densely granular cells that occurred in clusters at postinoculation days 2 to 3. These clustered cells resembled syncytia. No differences in CPEs were observed among the isolates and the selected BCV strains. Coronavirus particles were seen in the culture supernatant of HRT-18 cells infected with each isolate; these particles were similar in morphology to the particles observed in the original feces and were agglutinated by hyperimmune anti-BCV serum in IEM (Fig. 1b and d). We designated these isolates the KI-WB strain of waterbuck coronavirus, the KI-D2 strain of sambar deer coronavirus, and the WTD strain of white-tailed deer coronavirus, respectively.

HA and RDE activities of the isolates. The KI-WB, KI-D2, and WTD strains agglutinated both mouse and chicken erythrocytes at 4°C, but the HA titers with chicken erythrocytes

TABLE 1. HA and RDE activities of coronaviruses from waterbuck (KI-WB), sambar deer (KI-D2), and white-tailed deer (WTD) and of BCVs and HI and VN antibody titers against these coronaviruses

Coronavirus strain (derivation)	HA titer[a]				RDE titer[b]		HI antibody titer[c] of hyperimmune guinea pig sera to:					VN antibody titer[d] of hyperimmune guinea pig sera to:				
	4°C		37°C													
	Mouse	Chicken	Mouse	Chicken	Mouse	Chicken	TS	216XF	DBA	Mebus	SD	TS	216XF	DBA	Mebus	SD
KI-WB	12,800	100	12,800	<12.5	<12.5	≥100	10,240	1,280	80	80	80	10,240	5,120	1,280	320	640
KI-D2	51,200	400	51,200	<12.5	<12.5	≥400	10,240	1,280	80	160	160	10,240	10,240	640	320	320
WTD	25,600	100	25,600	<12.5	<12.5	≥100	20,480	2,560	40	160	40	10,240	10,240	640	320	640
BCV[e]																
TS (WD)	51,200	100	51,200	<12.5	<12.5	≥100	**20,480**	1,280	160	80	160	**20,480**	20,480	1,280	640	640
216XF (CD)	25,600	1,600	25,600	<12.5	<12.5	≥1,600	10,240	**2,560**	80	160	80	10,240	**20,480**	1,280	640	320
DBA (WD)	51,200	3,200	51,200	3,200	<12.5	<12.5	≥20,480	1,280	**160**	160	160	20,480	20,480	**2,560**	320	640
Mebus (CD)	102,400	25,600	102,400	25,600	<12.5	<12.5	5,120	2,560	640	**160**	320	5,120	20,480	2,560	**10,240**	5,120
SD (WD)	51,200	3,200	51,200	3,200	<12.5	<12.5	20,480	2,560	160	80	**≥20,480**	10,240	10,240	640	5,120	**10,240**

[a] Expressed as the reciprocal of the highest dilution of virus showing complete HA of 0.4 and 0.2% suspensions of mouse and chicken erythrocytes, respectively, after 1 h of incubation at 4 or 37°C.
[b] Expressed as the reciprocal of the highest dilution of virus causing complete disappearance of HA patterns at 4°C after 2 h of incubation at 37°C.
[c] Expressed as the reciprocal of the highest dilution of serum inhibiting 4 HA units of virus. Homologous titers are in boldface type. Titers which differed by 16-fold or greater from the homologous titers are underlined.
[d] Expressed as the reciprocal of the highest dilution of serum inhibiting 100 median tissue culture infective doses of virus. Homologous titers are in boldface type. Titers which differed by 16-fold or greater from the homologous titers are underlined.
[e] Data for activity against BCV were extracted from a previously published article (29).

were very low compared with those with mouse erythrocytes (Table 1). At 37°C, these strains agglutinated mouse erythrocytes with the same HA titers as those with which they agglutinated mouse erythrocytes at 4°C, but no agglutination of chicken erythrocytes (HA titers, <12.5) occurred. These strains displayed RDE activity against chicken erythrocytes but not against mouse erythrocytes. These reactivities were similar to that of the TS strain of BCV (Table 1).

Antigenic relationships between the isolates and BCV. In indirect IF tests, all five anti-BCV sera reacted to the isolates (KI-WB, KI-D2, and WTD) with high titers (102,400 to 409,600), and each antiserum showed no significant difference in reactivity with the homologous and heterologous BCV strains and the isolates (not greater than a twofold difference) (data not shown).

The isolates and the BCV strains were neutralized by all of the anti-BCV sera, but antisera to the Mebus and SD strains of BCV showed 16-fold or lower VN antibody titers against the isolates than against the homologous strains (Table 1). However, these antisera also distinguished the TS, DBA, and 216XF strains of BCV from the homologous strains with 16-fold or greater differences in the VN antibody titers (Table 1).

The HI antibody titers of the antisera are summarized. The isolates showed cross-reactivity against anti-BCV sera, but antiserum to the BCV SD strain had 128-fold or lower HI antibody titers against the isolates than against the homologous strain (Table 1). However, this antiserum sample also distinguished other BCV strains with 64-fold or greater differences in the HI antibody titers (Table 1).

In Western blots, anti-BCV (DBA) serum reacted with the structural HE (124-kDa), S (100-kDa), N (53-kDa), and M (25-kDa) proteins of the homologous and the Mebus strains of BCV (Fig. 2). The antiserum also reacted with structural proteins from the KI-D2 and WTD strains with similar molecular masses (Fig. 2). Similar results were obtained with KI-WB strains (data not shown).

Experimental inoculation of the calves with the coronavirus isolates. The gnotobiotic calf inoculated with feces of the affected waterbuck from which the KI-WB strain was isolated developed profuse watery diarrhea at postinoculation day (PID) 3, and diarrhea persisted until euthanasia at PID 4 (Table 2). Coronavirus antigens were detected in nasal epithelial cells by direct IF and from fecal specimens by both ELISA and IEM at PID 2. Virus shedding persisted until euthanasia. The colostrum-deprived calves and another gnotobiotic calf inoculated with the tissue culture-adapted KI-WB, KI-D2, and WTD strains also developed severe diarrhea at PIDs 2 to 3, and diarrhea persisted until euthanasia at PIDs 3 to 4 (Table 2). Virus shedding from their feces and nasal passages was detected at PIDs 1 to 3 and persisted until euthanasia.

The calves were euthanized at 24 to 36 h after the onset of diarrhea, and tissue smears were examined for coronavirus antigens by direct IF tests (Table 2). Coronavirus antigens were detected in the small and large intestines (duodenum and colon, respectively) and also in the upper respiratory tract

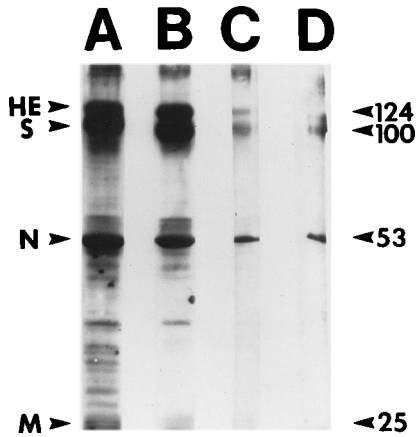

FIG. 2. Western blotting of BCV and the isolates with antiserum to BCV. Viral proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were electrotransferred onto nitrocellulose membranes. Lanes A, B, C, and D, BCV Mebus, BCV DBA, KI-D2 isolate, and WTD isolate, respectively. Blotted strips were reacted with gnotobiotic calf anti-BCV (DBA) serum; this was followed by reaction with horseradish peroxidase-conjugated anti-bovine immunoglobulin G and ECL reagents. The right margin indicates the molecular mass (in kilodaltons), and the left margin shows the positions of the four BCV structural proteins.

TABLE 2. Occurrence of diarrhea and distribution of coronavirus antigens in tissue smears from calves inoculated with waterbuck (KI-WB), sambar deer (KI-D2), or white-tailed deer (WTD) coronaviruses

Calf[a]	Inoculum	PID[b]	Coronavirus antigen[c] (% IF positive cells)						
			Respiratory tract			Intestine[d]			
			Turbinate	Trachea	Lung	D	J	I	C
Gn 1	Fecal KI-WB	4	+ (<1)	+ (2)	−	+ (1)	+ (3)	+ (10)	+ (40)
Gn 2	TC[e] WTD	4	+ (<1)	+ (1)	−	+ (<1)	+ (5)	+ (<1)	+ (30)
C-D 1	TC KI-WB	4	+ (<1)	−	−	+ (<1)	−	−	+ (10)
C-D 2	TC KI-D2	3	+ (<1)	+ (1)	+ (<1)	+ (1)	+ (3)	+ (40)	+ (10)

[a] Gn, gnotobiotic. C-D, colostrum deprived.
[b] All calves had diarrhea at necropsy.
[c] Coronavirus antigen was detected by direct IF tests with FITC-conjugated anti-BCV serum.
[d] D, duodenum; J, jejunum; I, ileum; C, colon.
[e] TC, tissue culture adapted.

(nasal turbinates) in all of the calves. The colon especially showed a high percentage of IF-positive cells. Except for the calf inoculated with the tissue culture-adapted KI-D2 strain, coronavirus antigens were also detected in the trachea or lung of the respiratory tract and in the jejunum and ileum of the small intestine.

Antibody responses in affected sambar deer. Sera from three affected sambar deer were seronegative against both BCV strains (Mebus and DBA) and the isolates from the acute phase or before the outbreaks by both indirect IF and VN tests (indirect IF antibody titer, <10; VN antibody titer, <20) but were seropositive in both tests against both BCV strains and the isolates in the convalescent phase, with VN antibody titers ranging from 160 to ≥2,560 (Table 3).

Serological survey of coronavirus infections in wild mule and white-tailed deer. Two of 23 serum samples (8.7%) from wild adult mule deer in Wyoming were seropositive against both BCV strains (Mebus and DBA) and the isolates by both indirect IF and VN tests, with indirect IF antibody titers of 160 and VN antibody titers of 40 and 80 (Table 3). On the other hand, 2 of 30 serum samples (6.6%) from adult white-tailed deer in Ohio were seropositive against both BCV strains and

TABLE 3. Indirect IF and VN antibody titers against bovine (Mebus, DBA), waterbuck (KI-WB), and sambar deer (KI-D2) coronaviruses in sera from the affected sambar deer and from wild white-tailed deer and mule deer in Ohio and Wyoming, respectively

Serum specimen	Antibody titer to indicated coronavirus by:							
	Indirect IF				VN			
	Mebus	DBA	KI-WB	KI-D2	Mebus	DBA	KI-WB	KI-D2
Affected sambar deer								
No. 1								
Before outbreak[a]	<10	<10	<10	<10	<20	<20	<20	<20
Acute phase	<10	<10	<10	<10	<20	<20	<20	<20
Convalescent phase	≥1,280	≥1,280	≥1,280	≥1,280	320	640	640	640
No. 2								
Before outbreak	<10	<10	<10	<10	<20	<20	<20	<20
Acute phase	NA[b]	NA	NA	NA	NA	NA	NA	NA
Convalescent phase	80	80	80	80	160	160	160	160
No. 3								
Before outbreak	NA	NA	NA	NA	NA	NA	NA	NA
Acute phase	<10	<10	<10	<10	<20	<20	<20	<20
Convalescent phase	≥1,280	≥1,280	≥1,280	≥1,280	≥2,560	≥2,560	≥2,560	≥2,560
White-tailed deer								
No. 1 through 28	<10	<10	<10	<10	<20	<20	<20	<20
No. 29	320	320	320	320	<20	<20	<20	<20
No. 30	320	320	320	320	<20	<20	<20	<20
Mule deer								
No. 1 through 21	<10	<10	<10	<10	<20	<20	<20	<20
No. 22	160	160	160	160	40	40	40	40
No. 23	160	160	160	160	80	80	80	80

[a] Collected from deer 1 in 1991 and from deer 2 in 1992.
[b] NA, not available.

the isolates by indirect IF tests with antibody titers of 320, but these 2 serum samples neutralized neither BCV strains nor the isolates (VN antibody titers, <20) (Table 3).

DISCUSSION

The diarrheal symptoms observed in adult wild ruminants in a wild animal habitat in Ohio during the winter of 1993 to 1994 were similar to WD of adult cattle, which is characterized by an acute onset of bloody diarrhea and high morbidity during winter (2). Recently, many reports have suggested that BCV is associated with WD worldwide (4, 18). Also, BCV might be related to sporadic diarrhea in adult cattle (10). In the present study, coronaviruses antigenically related to BCV were detected in the feces of these affected waterbuck and sambar and white-tailed deer by IEM and ELISA. Seroconversion to BCV was observed in the affected sambar deer. Other enteric pathogens were not detected in the feces of the affected waterbuck and white-tailed deer. These data suggest that coronaviruses antigenically related to BCV might be an agent of epizootic and sporadic diarrhea in wild ruminants.

BCVs belong to a single serotype, although minor antigenic and biological variations have been reported (4, 5, 8, 13, 27). Recently, we compared antigenic and biological properties between BCV strains causing CD and WD (29). Antigenic diversity by the VN and HI tests and variations in HA and RDE activities to chicken erythrocytes were observed among the BCV strains, but they were unrelated to the clinical source of the strains, i.e., CD or WD. In the present study, three coronavirus strains designated KI-WB, KI-D2, and WTD from the feces of the affected waterbuck, sambar deer, and white-tailed deer, respectively, were isolated in HRT-18 cells. To our knowledge, this is the first report of the isolation of coronavirus in wild ruminants. These isolates were similar to one another in their antigenic and biological properties. Especially, the KI-WB and KI-D2 strains might belong to the same strain because these strains were isolated from animals in the same habitat. The antigenic and biological properties of all isolates were indistinguishable from those of certain strains of BCV causing WD and CD. The structural proteins of all isolates also had molecular masses similar to those of BCV.

In addition to infecting the intestines of calves, BCV also causes respiratory tract infections (14, 16, 20). In a previous report by Saif et al. (20), gnotobiotic and colostrum-deprived calves inoculated orally and intranasally with BCV causing CD developed severe diarrhea and shed BCV rectally and nasally with incubation periods of 1 to 3 days. These calves had coronavirus antigens in the intestine and respiratory tract, with most infected cells being in the colon and nasal turbinates. On the basis of our recent data (9), a strain of BCV causing WD also caused diarrhea in gnotobiotic and colostrum-deprived calves, and the clinical signs and disease syndrome were indistinguishable from those caused by BCV causing CD. In the present study, the fecal KI-WB strain and the tissue culture-adapted KI-WB, KI-D2, and WTD strains caused severe diarrhea in gnotobiotic or colostrum-deprived calves, with incubation periods of 1 to 3 days. Virus shedding was observed in their feces and from nasal passages after inoculation, and coronavirus antigens related to BCV were detected in the small and large intestines, especially the colon, and in the upper respiratory tracts of all calves. The results of these challenge exposure tests and antigenic and biological comparisons between BCV and the KI-WB, KI-D2, and WTD strains suggest that BCV might have been transmitted to these wild ruminants (waterbuck and sambar and white-tailed deer). These wild ruminants could constitute a reservoir for BCV strains infectious for cattle. Further studies are in progress to compare the antigenic and genetic properties between BCV and the isolates by using monoclonal antibodies and nucleotide sequencing analysis.

Coronaviruses were detected from diarrheic feces of sitatunga and waterbuck in England by electron microscopy and a BCV-specific ELISA (3). Further characterization was not done because attempts to cultivate these viruses in cell cultures were unsuccessful. Also, attempts at experimental transmission of these viruses to gnotobiotic calves by using infected feces were unsuccessful. Thus, the antigenic relationship of these viruses to BCV could not be conclusively confirmed.

The results of the serological survey for coronavirus infections in wild mule and white-tailed deer suggest that at least two kinds of coronaviruses may exist among deer in the United States: a coronavirus antigenically similar to BCV, represented by the isolates in the present study, and another coronavirus that is reactive with BCV group antigens in IF tests but that is distinguishable from BCV in VN tests. The latter coronavirus might be specific to deer species. However, further study is needed to isolate and characterize the latter coronavirus from deer for final confirmation.

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