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Antigenic and Genomic Relationships among Turkey and Bovine Enteric Coronaviruses

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Antigenic and genomic relationships among tissue culture-adapted turkey enteric coronavirus (TCV) isolates, three strains of avian infectious bronchitis virus (IBV), and mammalian coronaviruses were investigated. Immunoblotting and immunoprecipitation experiments using polyclonal antisera showed that the four major structural proteins of TCV cross-reacted with the four homologous proteins of bovine enteric coronavirus (BCV), the N and M proteins of mouse hepatitis virus serotype 3, and the N protein of IBV. Close antigenic relationships between TCV and BCV were also established by seroneutralization and hemagglutination-inhibition. Of 49 monoclonal antibodies produced against either TCV or BCV, 11 differentiated the two viruses. Five of these monoclonal antibodies had neutralizing activities and were directed to either the peplomeric S (gp200-gp100) or hemagglutinin HE (gp140-gp65) glycoproteins. BCV cDNA probes tested on purified viral preparations and coronavirus-positive (by electron microscopy) fecal samples from diarrheic turkey poults confirmed the relatedness of TCV and BCV. The two viruses produced distinct cytopathic changes in HRT-18 cells in the presence of trypsin, whereas only TCV isolates were able to reproduce the clinical symptoms in turkey poults. Their matrix (M) proteins undergo different glycosylation processes.

Serological studies on coronaviruses demonstrated the existence of at least four distinct subgroups within the family (24, 31). Viruses within each subgroup show partial antigenic cross-reactivity, but they are readily distinguished by their host specificities and clinical syndromes (29). Avian and nonavian coronaviruses each appear to fall into two distinct and unrelated subgroups. Hemagglutinating mammalian coronaviruses, including hemagglutinating encephalomyelitis virus of swine, bovine enteric coronavirus (BCV), respiratory human coronavirus (HCV) OC43, and coronavirus DVIM of mice, belong to the same antigenic subgroup and are antigenically related to the nonhemagglutinating mouse hepatitis virus (MHV) strains (12, 18, 32). Except for HCV OC43 and BCV (11, 30), the viruses from this subgroup do not seem to cross-react by seroneutralization (SN) and hemagglutination (HA) inhibition (HI), but all share analogous antigenic determinants on their structural proteins (12). Among the viruses that belong to the other subgroup of mammalian coronaviruses, porcine transmissible gastroenteritis virus (TGEV), feline infectious peritonitis virus, and canine coronavirus also share antigenic determinants involved in virus neutralization and cross-protection (13, 24, 35). The sequences of the peplomer proteins of TGEV and feline infectious peritonitis virus have a high degree of homology (14). Other data available on the nucleic acid sequences of coronaviruses are in agreement with the antigenic classification and suggest evolutionary divergence between avian and mammalian coronaviruses (1, 15, 18). Cloned cDNAs corresponding to the N and the M protein genes of TGEV and BCV were used as hybridization probes to identify coronaviruses antigenically closely related to the parental virus but not the coronaviruses belonging to an antigenically unrelated subgroup (28).

Turkey enteric coronavirus (TCV) is one of the major causative agents of epidemic diarrhea in turkey poults (6, 25). The virus shares morphological and physicochemical characteristics with other members of the *Coronaviridae* family, but little is known concerning the molecular and antigenic structures of the TCV virion (22, 27). The antigenic classification of TCV in a subgroup distinct from infectious bronchitis virus (IBV) and mammalian coronaviruses was established in 1973 on the basis of results obtained by immunoelectron microscopy and indirect immunofluorescence (26, 27). This classification has not been revised, probably because of failure to obtain large amounts of viruses and, consequently, to obtain highly specific immunological probes. Our recent findings on the morphological, biological, and molecular properties of egg-adapted TCV isolates (4, 5, 7) indicated that TCV shares features reported only for mammalian hemagglutinating coronaviruses, such as the presence of additional short granular projections (hemagglutinin glycoprotein) on the surface of the virion (16, 32). Antigenic relatedness between TCV and BCV and between TCV and IBV demonstrated by enzyme-linked immunosorbent assay (ELISA) appears to contradict the established classification of coronaviruses (6).

This study was undertaken to further define the antigenic relationships among tissue culture-adapted TCV isolates, IBV strains, and mammalian coronaviruses. It was found that TCV and BCV are antigenically and genomically closely related to the point that only a few monoclonal antibodies (MAbs) could distinguish the two viruses. Furthermore, they could be distinguished by their cytopathogenicity in HRT-18 cells, by their pathogenicity for turkey poults, and by the glycosylation process of their matrix (M) protein. This is the first report on a close antigenic relatedness between avian and mammalian coronaviruses.

(This report was taken in part from a dissertation to be submitted by S.D. to the Department of Virology, Institut Armand-Frappier, University of Quebec, in partial fulfillment of the requirements for the Ph.D. degree.)

Monolayer cultures of HRT-18 human rectal tumor cells

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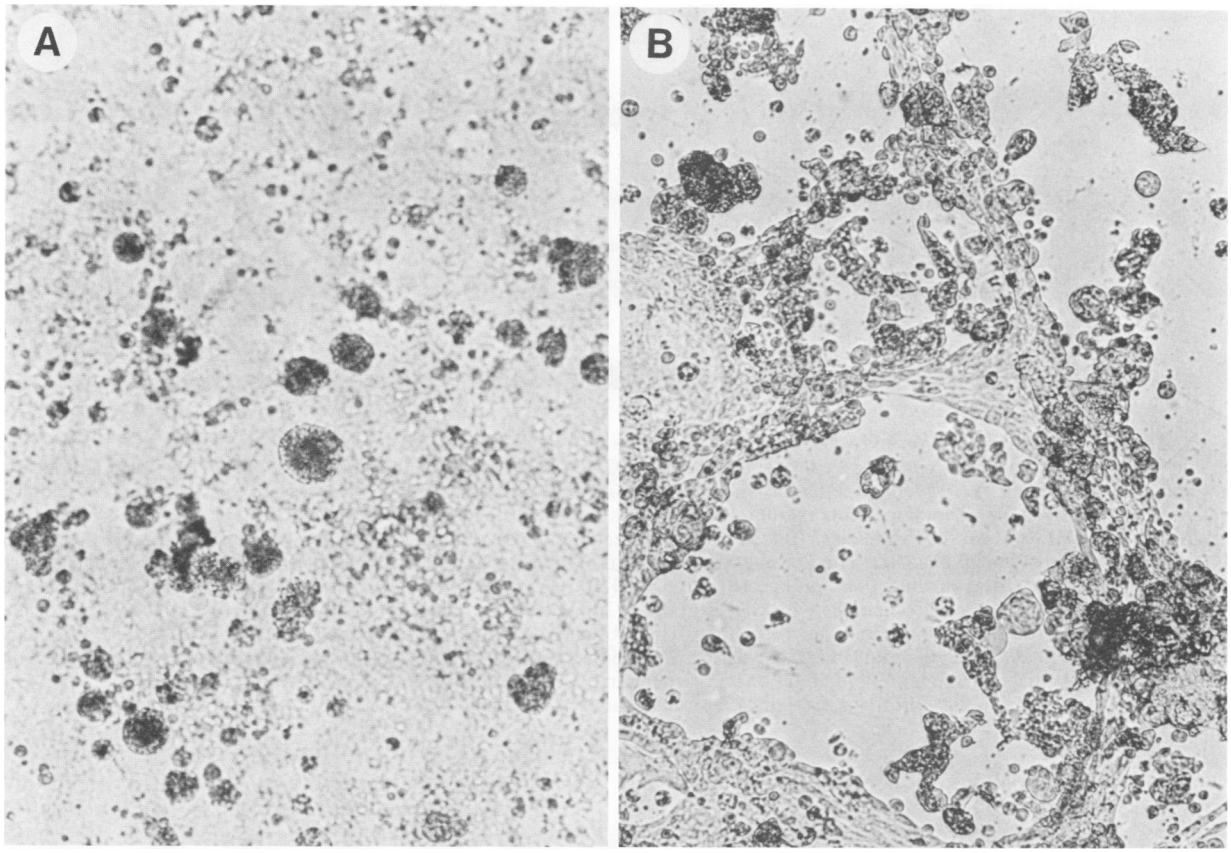

FIG. 1. Cytopathic effects of TCV and BCV in HRT-18 cells in 10 U trypsin per ml. At 72 h p.i. with the Mebus strain of BCV (A) and the Minnesota stain of TCV (B) at passage 5 in HRT-18 cells, the extents of cytopathic changes varied markedly for both viruses.

(33) were grown in RPMI 1640 medium (GIBCO Laboratories, Grand Island, N.Y.) containing 50 μg of gentamicin, 60 μg of tylosin, and 25 μg of lincomycin per ml and 15% heat-inactivated fetal bovine serum. The prototype egg-adapted Minnesota strain (10) and the Quebec isolate TCQ.1713 (7) of TCV and the Mebus strain of BCV were serially propagated in these cells in 10 U of crystallized bovine trypsin (grade XIII; Sigma Chemical Co., St. Louis, Mo.) per ml as previously described (8). Infectivity titers were determined by titration of clarified tissue culture medium using an endpoint dilution procedure and calculation of 50% tissue culture-infective doses per milliliter (8). HA assays for TCV and BCV were also performed as already described (4, 16).

TGEV (Purdue strain) and MHV serotype 3 (MHV-3) were grown, respectively, in swine testicle and mouse fibroblast L2 cells (6). The Beaudette, Holland, and Connecticut strains of avian IBV were propagated by inoculation in embryonating chicken eggs (5). The extracellular virions were purified either from the supernatants of infected cell cultures or from the clarified allantoic fluids of IBV-infected embryonating eggs by differential and isopycnic ultracentrifugation on sucrose gradients as previously described (5). Purified respiratory HCV 229E was a gift from P. Talbot (Department of Virology, Institut Armand-Frappier).

Rabbit and guinea pig hyperimmune sera to purified egg- or tissue culture-adapted TCV strains were prepared by immunization protocols described elsewhere (5). The animals were tested for the presence of specific anti-TCV antibodies by immunoelectron microscopy and HI (4, 6). Rabbit antisera to BCV, IBV, TGEV, and MHV-3 were also produced in our laboratories by the same methods. The source of antisera to HCV 229E, hemagglutinating encephalomyelitis virus, and rabbit enteric coronavirus has been mentioned in a previous report (6). Hybridoma cell lines producing MAbs to TCV and BCV major structural polypeptides were established following fusion experiments with spleen cells from mice inoculated with either the Minnesota strain of TCV or the Mebus strain of BCV. Immunoblotting experiments with purified immunizing virus allowed assessment of the polypeptide specificities of 29 anti-TCV (7) and 20 anti-BCV MAbs (unpublished data).

A set of radioisotopically labeled recombinant plasmids containing the BCV matrix (gp24 or M) and nucleocapsid (p52 or N) genes and other nonoverlapping sequences obtained by cloning randomly primed cDNA were used individually in hybridization assays (34).

Growth and cytopathic effects of TCV and BCV in HRT-18 cells. The cytopathic effects of TCV and BCV in HTR-18 cells in the presence of trypsin are shown in Fig. 1. Upon passages 3 to 5 of the Minnesota strain of TCV, formation of syncytia was seen from 24 to 36 h postinoculation (p.i.). The syncytia progressively increased in number and size, disintegrated, and sloughed off the growth surface, leading to complete destruction of the cell sheets within 72 to 96 h p.i. Viral yields increased from $10^{4.5}$ to $10^{9.5}$ 50% tissue culture-infective doses per ml during five passages, after which the TCV infectivity titers remained stable. Infection with the Mebus strain of BCV resulted in less-cytopathic changes; upon passages 3 to 5, little or no fusion was observed 72 h

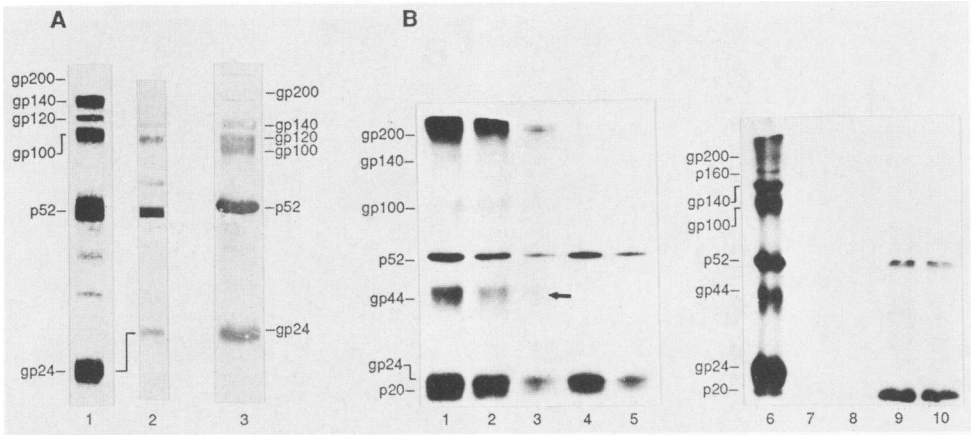

FIG. 2. Direct comparison of TCV and BCV structural polypeptides and effect of tunicamycin. (A) The purified tissue culture-adapted Minnesota strain of TCV (lanes 1 and 2) and the Mebus strain of BCV (lane 3) were electrophoresed in 9.5% polyacrylamide gels without (lanes 1 and 3) or with (lane 2) 5% 2-mercaptoethanol. The viral proteins were electrophoretically transferred to nitrocellulose membranes and incubated with homologous hyperimmune rabbit serum. Immunoblots were revealed as described previously (7). The results were taken from three different gels. (B) BCV- or TCV-infected HRT-18 cells were incubated in maintenance medium containing 10 U of trypsin per ml and 0 to 1 μg of tunicamycin per ml and labeled with [^{35}S]methionine at 6 to 24 h postinfection. Viral particles were isolated from the culture media and purified by isopycnic ultracentrifugation on sucrose gradients. Labeled virions were incubated with homologous hyperimmune rabbit serum, and immunoprecipitates were analyzed by SDS-PAGE in 10% polyacrylamide gels without 2-mercaptoethanol. Lanes: 1 to 5, autoradiographs of BCV structural proteins after incubation without (lanes 1 and 2) or with 0.1 (lane 3), 0.5 (lane 4), or 1.0 (lane 5) μg of tunicamycin per ml; 6 to 10, autoradiographs of TCV structural proteins after incubation without (lane 6) or with 0.5 (lane 9) or 1.0 (lane 10) μg of tunicamycin per ml; 7 and 8, immunoprecipitation with preimmune rabbit serum and mock-infected cell lysates, respectively. Estimated molecular weights of viral structural proteins are indicated in thousands. The results were taken from two different experiments.

p.i., and the cell monolayers remained almost unchanged until 5 to 6 days p.i.

Large amounts of coronavirus particles were observed by electron microscopy (EM) in the supernatant fluids of TCV- and BCV-infected cell cultures. Most extracellular virions possessed surface projections of two distinct sizes (data not shown). HA titers obtained with supernatant fluids from passages 1 to 3 of both BCV- and TCV-infected cell cultures rose from 1:128 to 1:2,048 and suggested that comparable amounts of virus were produced.

Direct comparison of TCV and BCV virion polypeptides and effect of tunicamycin. The structural polypeptide profiles of sucrose gradient-purified TCV and BCV were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and by Western immunoblotting (5). The five major proteins described previously for BCV isolates (16, 18) were identified by using homologous hyperimmune rabbit serum (Fig. 2a, lane 3). Analysis by SDS-PAGE under nonreducing conditions of virions purified from supernatant fluids of TCV-infected HRT-18 cell cultures also consistently revealed five major polypeptide species with molecular weights similar to those of the polypeptides of BCV. These polypeptides were identified in immunoblotting experiments using hyperimmune rabbit sera produced against either the egg-adapted or the tissue culture-adapted Minnesota strain of TCV (Fig. 2a, lanes 1 and 2). As described for BCV (16), the 140,000-molecular-weight hemagglutinin polypeptide of TCV was also reduced to a major 65,000-molecular-weight polypeptide species in the presence of 2-mercaptoethanol (Fig. 2a, lane 2).

Virus that was purified from the supernatant fluids of TCV- or BCV-infected cell cultures and cultivated in the presence of 1.0 μg of tunicamycin per ml lacked both infectivity and HA activity (data not shown). SDS-PAGE and immunoblotting with homologous hyperimmune sera confirmed loss of their peplomeric and hemagglutinin glycoproteins (Fig. 2b). Synthesis of the M protein of BCV was unaffected by tunicamycin, in agreement with a glycosylation process involving O-linked oligosaccharides (Fig. 2b, lanes 2 to 5). In contrast, treatment with tunicamycin resulted in a slightly higher mobility of the M of TCV concomitant with loss of the gp44 species, possibly corresponding to a dimer of M (Fig. 2b, lanes 9 and 10), as described for BCV (9).

Cross-antigenic relationships of TCV proteins with homologous proteins of other coronaviruses. When purified TCV and BCV were electrophoresed on the same gel and immunoblotted with hyperimmune rabbit serum to each virus, cross-antigenic relationships were revealed among their homologous proteins. Antisera to both the tissue culture- and egg-adapted Minnesota strains of TCV identified all major polypeptides of BCV but reacted only moderately with the 120K glycoprotein (Fig. 3a).

Cross-antigenic relationships were also found between the p52 nucleocapsid (N) protein of TCV and the homologous proteins of MHV-3 and IBV strains M41, Holland, and Connecticut (data not shown). However, cross-antigenic relatedness among the structural polypeptides of IBV and MHV-3 was absent. Cross-reactivity was shown between N proteins of BCV and MHV-3, whereas anti-BCV serum failed to react with the homologous protein of IBV. Reciprocal cross-reactivity was also found between the M protein of MHV-3, BCV, and TCV. Hyperimmune sera produced against TGEV and HCV 229E failed to react with the structural proteins of TCV, BCV, MHV-3, and IBV but reacted with the major structural proteins of their homologous viruses. Similar results were obtained by immunoblotting with extracts prepared from cells infected with the various coronaviruses (Fig. 3b).

Cross-antigenic relationships of TCV with BCV and other coronaviruses as defined by MAbs. By SN and HI tests, close antigenic relationships were demonstrated between TCV and BCV, whereas none of the antisera to other coronaviruses reacted against these two viruses (data not shown).

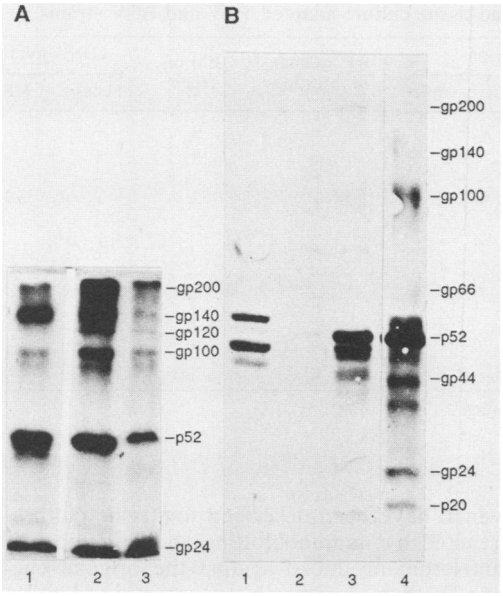

FIG. 3. Immunoprecipitation and immunoblotting of structural polypeptides of various coronaviruses by hyperimmune anti-TCV sera. (A) Purified BCV (lane 1) or TCV (lanes 2 and 3) virions labeled with [^{35}S]methionine were disrupted in Nonidet P-40 and incubated with hyperimmune rabbit serum to the tissue culture-adapted (lanes 1 and 2) or egg-adapted (lane 3) Minnesota strain of TCV. Immunoprecipitates were solubilized with electrophoresis buffer without 2-mercaptoethanol and analyzed by SDS-PAGE and fluorography. (B) Extracts of cells infected with MHV-3 (lane 1), the Purdue strain of TGEV (lane 2), the Mebus strain of BCV (lane 3), or the Minnesota strain of TCV (lane 4) were prepared at 24 h postinfection and analyzed by SDS-PAGE and immunoblotting with hyperimmune rabbit serum to TCV. Proteins were solubilized with electrophoresis sample buffer without 2-mercaptoethanol. Estimated molecular weights of TCV structural proteins are indicated in thousands.

The high degree of serological relatedness between TCV and BCV was further confirmed by comparison of the heterologous activities of anti-BCV and anti-TCV MAbs. Most of these MAbs reacted similarly against both viruses by ELISA and by SN and HI tests. Nevertheless, at least four anti-TCV and seven anti-BCV MAbs reacted more specifically with their homologous virus (Table 1). Particularly interesting were anti-TCV MAbs M33 and M31, which strongly neutralized or inhibited the HA activity of only the homologous virus, thus suggesting the presence of TCV-specific antigenic determinants on both peplomeric and hemagglutinin glycoproteins. Three anti-BCV MAbs to the peplomeric glycoprotein neutralized BCV to a greater extent than TCV. Two anti-BCV MAbs (V27 and 314c) and one anti-TCV MAb (M23) to the N proteins reacted against only the homologous virus by ELISA.

Specificity and cross-reactivity of BCV cDNA probes. To determine the specificity of BCV cDNA probes, nucleic acid extracted from purified bovine rotavirus, bovine herpes virus type I strain Colorado, and HRT-18 cells was applied to nitrocellulose in amounts ranging from 0.5 to 10 ng per slot and tested in hybridization assays with six nonoverlapping BCV probes that would hybridize to about one-fourth of the total length of the viral RNA (A. J. Verbeek, S. Dea, and P. Tijssen, Mol. Cell. Probes, in press). No reactivity of the BCV probes towards these viral or cellular nucleic acid preparations was observed. However, strong signals were obtained when BCV probes were hybridized to the purified tissue culture-adapted Mebus strain of BCV and the Minnesota and Quebec TCQ.1713 isolates of TCV. No detection signal was obtained with purified TGEV, HCV 229E, and the three strains of IBV. For these tests, purified coronavirus suspensions were adjusted to approximately 10^{11} to 10^{12} viral particles per ml, as determined by EM by admixture with known amounts of latex spheres.

Pathogenicity of TCV and BCV isolates for turkey poults. Turkey poults (1 to 3 days old) infected orally with 10^6 50% tissue culture-infective doses of purified egg- and tissue culture-adapted TCV isolates developed typical symptoms of enteritis. Mucoid diarrhea occurred by days 3 to 5 and was of short duration (2 to 3 days). Although the birds did not die from the infection, they showed significant growth retardation; averaged body weights of TCV-infected poults increased about 50 to 60% compared with 110 to 120% for mock-infected poults over the 12-day observation period (Table 2). Gross lesions included flaccid intestines with watery and greenish contents, markedly distended ceca, and atrophy of the spleen. Microscopic lesions consisted of mild to severe atrophy of the villi, replacement of columnar absorptive cells by cuboid or simple squamous epithelial cells, and infiltration of the lamina propria with mononuclear

TABLE 1. Anti-TCV and anti-BCV MAbs with different reactivities toward TCV and BCV

MAb	Immunizing virus	Polypeptide specificity	ELISA[a]		Reactivity by[b]:			
					SN		HI	
			TCV	BCV	TCV	BCV	TCV	BCV
M23	TCV	gp200, gp100	5.6	3.8	<10	<10	<10	<10
43C3	TCV	p52	4.0	<2.0	<10	<10	<10	<10
M31	TCV	gp140	5.0	5.0	5,120	<10	1,280	<10
M33	TCV	gp200, gp100	6.2	6.2	10,240	<10	<10	<10
VIF4E6	BCV	gp200, gp100	3.8	>4.7	<10	320	<10	<10
VIF4E5	BCV	gp200, gp100	3.8	>4.7	40	640	<10	<10
VIA5	BCV	gp200, gp100	3.8	6.2	40	160	<10	<10
VIF3A	BCV	gp140	3.8	>4.7	<10	<10	<10	<10
VIF3B	BCV	gp140	<2.6	>4.7	<10	<10	<10	80
314C	BCV	p52	<2.6	5.3	<10	<10	<10	<10
V27	BCV	p52	<2.6	5.9	<10	<10	<10	<10

[a] Log_{10} of the highest dilution of ascitic fluid giving an A_{492} value of >2.5 times the A_{492} value of a buffer control.
[b] SN and HI titers are expressed as described in the text.

TABLE 2. Experimental inoculation of turkey poults with egg- and tissue culture-adapted TCV and BCV strains

Group (inoculum)	No. of poults	Relative wt gain (CV%)[a]		Presence of lesions		IIF[b] staining of intestines	EM of feces	cDNA hybridization	
		Day 6	Day 12	Macroscopic	Microscopic			Feces	HRT-18 cells
Minnesota (egg)	9	1.24 (15.4)	1.5 (17.8)	+	+	+	+	−	+
Minnesota (HRT)	10	1.26 (15.3)	1.6 (14.1)	+	+	+	+	+	+
TCQ.1713 (egg)	10	1.38 (13.1)	1.6 (13.5)	+	+	+	+	−	+
TCQ.1713 (HRT)	10	1.64 (18.2)	2.1 (14.9)	+	+	+	+	+	+
PBS[c]	8	1.68 (11.9)	2.1 (10.7)	−	−	−	−	−	−
BECQ.6 (HRT)	10	1.61 (7.4)	2.4 (12.9)	−	−	−	−	−	−
BECQ.12 (HRT)	10	1.47 (11.1)	2.3 (12.1)	−	−	−	−	+	+
BCV (HRT)	10	1.52 (11.3)	2.2 (13.2)	−	−	−	+	+	+
BCV (calf)	10	1.48 (6.7)	2.1 (11.2)	−	−	−	+	−	+
PBS[c]	8	1.68 (13.1)	2.2 (13.5)	−	−	−	−	−	−

[a] CV%, Coefficient of variation is standard deviation of the mean expressed as a percentage.
[b] IIF, Indirect immunofluorescence.
[c] PBS, Phosphate-buffered saline.

cells. Viral replication in the enterocytes was confirmed by indirect immunofluorescence staining of frozen intestinal sections prepared from days 7 to 14 p.i. Coronavirus particles in the clarified intestinal contents of infected poults were observed by EM from days 4 to 14 and were reisolated in HRT-18 cells. Slot blot hybridization with BCV cDNA probes permitted weak detection of viral RNA in the intestinal contents of TCV-infected birds, but strong positive signals were obtained after only one passage in HRT-18 cells.

In contrast, infection of poults with BCV (from either diarrheic fecal samples or tissue culture strains) did not cause diarrhea, and poults killed after 1 week p.i. showed no macroscopic or histologic lesions. However, coronavirus particles were detected by EM and by hybridization with BCV cDNA probes in the clarified intestinal contents from poults from two different groups up to day 14.

The results of the present studies demonstrated that TCV isolates are antigenically related to BCV to such a degree that only certain MAbs could distinguish the two viruses. Several explanations can be offered for the discrepancies between the results of this study and previous serological findings responsible for the current classification of TCV in a separate antigenic subgroup (24, 31). The polyclonal antisera selected and the techniques used for the comparative studies may be responsible. It has been demonstrated that turkey poults produce relatively weak humoral immune response to TCV (25). Since sera from convalescent turkey poults were originally used in studies with egg-adapted TCV isolates, the antigenic relationships may have escaped detection (27). Because of their low sensitivities, use of immunoelectron microscopy and indirect immunofluorescence staining techniques on frozen intestinal sections from TCV-infected poults (26, 27) was not ideal for examination of viral cross-reactivities.

Successful propagation of TCV in HRT-18 cells provided a satisfactory source of virus for production of specific hyperimmune sera and study of its molecular biology and pathogenesis. This cell line, which has maintained many properties of normal intestinal epithelium cells (33), has been reported previously to be susceptible to enteropathogenic strains of bovine (16), canine, and human coronaviruses (17) and for isolation of respiratory HCV OC43 (11, 12), all belonging to the subgroup of hemagglutinating mammalian coronaviruses. TCV is the first avian coronavirus for which replication and propagation in cell cultures derived from human tissues has been reported (4, 8). All structural proteins which have been described for tissue culture-adapted TCV reacted by immunoblotting and immunoprecipitation with antiserum produced against the original egg-adapted virus (5, 7), thus confirming the antigenic identities of the viruses from both sources. These observations are consistent with the notion that there is no rigid species restriction for these coronaviruses and that they may have zoonotic potential. Natural infection of dogs by TGEV (13), infection of piglets with feline coronaviruses (35), and a few direct and indirect observations on the transmission of enteropathogenic coronaviruses from cattle to humans (11, 23, 30) also suggested possibly wide host ranges for enteric coronaviruses. However, infection of domestic animals by avian coronaviruses has not been described and the role of birds as reservoirs remains to be demonstrated.

Our studies established that TCV and BCV, although similar, are not identical. Under the conditions used for isolation and propagation of both viruses in HRT-18 cells, only TCV isolates induced syncytia. Also, the envelope glycoproteins of TCV, including the M protein, were found to be N glycosylated, as was also demonstrated for IBV (2, 3). These two avian coronaviruses thus differ from mammalian coronaviruses such as BCV and MHV, for which the M protein undergoes O-linked glycosylation (9, 18, 31). However, the N glycosylation of M is not peculiar to avian coronaviruses, since TGEV also possesses an N-glycosylated matrix protein (19). In addition, immunoblotting studies with heterologous antisera showed that common antigenic determinants appeared to be located on each of the structural polypeptides of TCV and BCV, but studies on the cross-reactivities of MAbs to TCV or BCV confirmed that epitopes specific for each virus were also present on these different structural proteins.

Furthermore, only TCV isolates reproduced diarrheic symptoms and microscopic lesions of transmissible enteritis in turkey poults. However, as BCV RNA could be detected by molecular hybridization in the feces of BCV-infected poults to at least 10 days p.i., whereas at the same times BCV from these intestinal contents was reisolated in HRT-18 cells, BCV was most probably able to replicate in turkey poults. These results were confirmed by EM and ELISA and suggested the possibility of recombinations between these two viruses during mixed infection. It has been demonstrated that the RNA genomes of different strains of murine coronaviruses can undergo RNA-RNA recombination during mixed infection at an extremely high frequency, reminiscent of the genetic reassortment of viruses with segmented

RNA genomes (20). Several mechanisms have been suggested to explain the emergence of recombinants among coronaviruses based on the model of leader-primed RNA transcription and evidence that replication of genomic RNA may proceed in a discontinuous rather than a continuous manner (21).

The genomic relationships between TCV and BCV were also demonstrated by hybridization tests with purified cell culture-adapted viruses or TCV-positive clinical samples from diarrheic poults. Preliminary sequence data showed that cloned cDNAs used as probes in this study corresponded to the gene for N or M (Verbeek et al., in press). Similar hybridization signals with the different BCV probes were obtained against both TCV and BCV, suggesting a close genetic analogy between these viruses. As previously described by Shockley et al. (28), BCV cDNA probes did not react with viruses belonging to unrelated antigenic subgroups. Cloning and sequencing of the RNA genome of the tissue culture-adapted Minnesota strain of TCV are in progress. Sequence comparisons of TCV, BCV, MHV-3, and HCV OC43 would be useful in determining the true extent of relatedness among these viruses.

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