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Transmissible Enteritis of Turkeys: Experimental Inoculation Studies with Tissue-Culture-Adapted Turkey and Bovine Coronaviruses

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SUMMARY. Four Quebec isolates of turkey enteric coronaviruses (TCVs) and three isolates of bovine enteric coronaviruses (BCVs) were serially propagated in HRT-18 and compared for their pathogenicity in turkey embryos and turkey poults. By immunoelectron microscopy, hemagglutination-inhibition, and Western immunoblotting assays, tissue-culture-adapted Quebec TCV isolates were found to be closely related to the reference Minnesota strain of TCV and the Mebus strain of BCV. Genomic relationships between TCV isolates and the reference BCV strain were confirmed by hybridization assays with BCV-specific radiolabeled recombinant plasmids containing sequences of the N and M genes. Only TCV isolates could be propagated by inoculation in the amniotic cavity of chicken and turkey embryonating eggs, and induced clinical disease in turkey poults. Nevertheless, coronavirus particles or antigens were detected by electron microscopy or indirect enzyme-linked immunosorbent assay in the clarified intestinal contents of BCV-infected poults up to day 14 PI, and genomic viral RNA was detected by slot-blot hybridization using BCV cDNA probes.

RESUMEN. Enteritis transmisible de los pavos: Estudios de inoculación experimental con coronavirus de bovino y de pavo adaptados a cultivo celular.

Cuatro cepas de coronavirus entéricos de pavos aisladas en Quebec y tres cepas de coronavirus entéricos de bovinos fueron propagadas en la línea celular HRT-18 y su patogenicidad comparada en embriones de pavo y en pavitos. Mediante la prueba inmune con el microscopio electrónico, la inhibición de la hemoaglutinación, pruebas de transferencia puntual, se encontró que las cepas de Quebec adaptadas a cultivo celular tenían una estrecha relación con la cepa de referencia (Minnesota) de coronavirus de pavo y con la cepa Mebus de coronavirus bovino. Las relaciones genómicas entre los coronavirus de pavo y la cepa de referencia de coronavirus bovino fueron confirmadas por pruebas de hibridación con plásmidos recombinantes de coronavirus bovinos marcados radioactivamente, que contenían secuencias de los genes N y M. Sólo las cepas de coronavirus de pavo pudieron propagarse por inoculación en la cavidad amniótica de embriones de pollo y pavo, y produjeron enfermedad clínica en pavitos. Sin embargo, por microscopía electrónica o por el inmunoensayo con enzimas asociadas, se detectaron partículas de coronavirus o antígenos en el contenido intestinal clarificado obtenido de pavitos infectados con el coronavirus bovino hasta 14 días después de la inoculación. También se detectó RNA genómico viral mediante hibridación usando sondas de DNA complementario del coronavirus bovino.

Turkey enteric coronavirus (TCV) is one of the major causative agents of epidemic diarrhea in turkey poults (2,18,21). In its natural host, it infects and destroys the mature absorptive epithelial cells of the small and large intestines, leading to diarrhea that is often severe in poults under 6 weeks of age but leading only to mild clinical signs in adults (7,9,18,19).

The virus was first described in the early 1970s, but studies on its characterization were delayed due to the lack of a suitable tissue-culture system (16,21). Recently, it has been demonstrated that TCV can be propagated efficiently in the human rectal tumor cell line HRT-18 (23), provided trypsin is added to the culture medium (6). Previous findings in this laboratory on the morphological, biological, and molecular properties of egg-adapted and tissue-culture-adapt-

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ed TCV isolates indicated that TCV shares features reported thus far only for mammalian hemagglutinating coronaviruses, such as the presence of additional short granular projections on the surface of the virion (3,4). These projections are associated with the hemagglutinating activity of the virus and are made of a 140-kilodalton glycoprotein, a disulfide-linked dimer of two 65-kilodalton glycoproteins (4,12). Moreover, close antigenic and genomic relationships have been demonstrated among reference strains of TCV and bovine enteric coronavirus (BCV) to the point that only a few monoclonal antibodies could distinguish the two viruses (5,8). Molecular hybridization studies, using BCV cDNA probes corresponding to the genes coding for the N or M proteins, confirmed their genomic relatedness (8,26).

The current study was undertaken to further define the pathogenicity of TCV and BCV for turkeys. It was observed that although both viruses could replicate in the intestines and the bursa of Fabricius of turkey poults, only TCV could reproduce the clinical signs and lesions of transmissible enteritis.

MATERIALS AND METHODS

Viruses and cells. The prototype egg-adapted Minnesota strain (21) of TCV was supplied to us by Dr. B. S. Pomeroy of the University of Minnesota. The origin of the Quebec TCV isolates (Q.15, Q.17, Q.23, and Q.1713) has been described (4). The Mebus strain of BCV was obtained from the American Type Culture Collection (ATCC VR874), Rockville, Md. The Quebec BCV isolates (Q.6 and Q.12) were recovered from clinical cases of epidemic diarrhea in neonatal calves in Quebec dairy herds. Both TCV and BCV isolates were propagated in HRT-18 cells in the presence of bovine trypsin, as described (6,8).

Other coronaviruses used for this study—the 67N strain of hemagglutinating encephalomyelitis virus (HEV) of swine, the Purdue strain of porcine transmissible gastroenteritis virus (TGEV), and serotype 3 of the mouse hepatitis virus (MHV-3)—also were obtained from the ATCC. These viruses were grown respectively in HRT-18, ST (swine testicle), and L2 (mouse fibroblast) cells (5). The Beaudette, Holland, and Connecticut strains of avian infectious bronchitis virus (IBV) were propagated by inoculation into embryonating chicken eggs (5). The tissue-culture-adapted NCDV strain of bovine rotavirus and the Colorado strain of bovine herpesvirus type I (IBR) were supplied to us by R. Assaf of the Institute Armand-

Frappier, Laval, Quebec, Canada. The viruses were grown respectively in African green monkey (BSC-1) and bovine (MDBK) kidney cells, as described (1).

The production of tissue-culture-adapted viruses was determined by titration of clarified tissue-culture medium using an endpoint dilution procedure and the calculation of 50% tissue-culture infective doses (TCID₅₀) per ml (6).

Viral purification. The extracellular virions were purified from the supernatants of infected cell cultures by differential and isopycnic ultracentrifugation on sucrose gradients, as described (3).

Antisera. Rabbit and guinea pig hyperimmune sera to purified egg-adapted or tissue-culture-adapted Minnesota strain of TCV were prepared following described immunization protocols (5). The animals were tested before and after immunization for the presence of specific anti-TCV antibodies by immunoelectron microscopy (IEM) and hemagglutination inhibition (3,5). The sources of hyperimmune sera to BCV, IBV, TGEV, MHV-3, HEV, and human coronavirus HCV-229E have been described also (5).

Electron microscopy (EM) and protein A-gold immunolabeling. The purified viral preparation was adjusted to approximately 10⁷ particles/ml, as determined by EM calibration with latex spheres. Aliquots (100 μl) were poured into nitrocellulose microtubes and concentrated by airfuge ultracentrifugation at 120,000 × g (Beckman Airfuge, rotor model A-100, 30 lb./in.²) for 10 minutes onto 400-mesh naked-nickel grids, as described (4).

For immunogold labeling, virus-coated grids were first washed three times in 0.05 M Tris-buffered saline (TBS [pH 8]), partly dried, and floated for 5 minutes on a drop of TBS containing 0.05% Tween 20 (TBS-T). The grids were then incubated for 10 minutes at room temperature on a drop of rabbit anti-TCV or anti-BCV hyperimmune serum (diluted 1:250) in TBS-T. Following another washing step, grids were incubated on a drop of TBS-diluted protein A-gold (PAG) complex (4). The grids were finally washed with TBS, rinsed three times in distilled water, and counterstained with 2% sodium phosphotungstate (pH 7.0) (2). Specificity of the labeling was demonstrated by controls including non-immune sera and incubation with PAG complex alone.

Polyacrylamide gel electrophoresis (PAGE) and Western immunoblotting. For PAGE, sucrose gradient-purified viruses were disrupted in an equal volume of double-strength Laemmli sample buffer containing 5% 2-mercaptoethanol, boiled for 3 minutes, and clarified at 10,000 × g for 15 minutes before electrophoresis in 12.5% sodium dodecyl sulfate (SDS)-polyacrylamide slab gels, as described (3). Western immunoblotting assays were also conducted as described (4).

Enzyme-linked immunosorbent assay (ELISA). The double-antibody sandwich ELISA used for the

detection of viruses in fecal samples has been described (5).

Bovine cDNA probes and hybridization assays.

BCV genomic RNA was extracted from sucrose gradient-purified virions and copied into cDNA for molecular cloning as described (24). BCV-specific recombinant plasmids holding sequences of the N and M genes were used in hybridization assays (25). Aliquots of 10 μ l or 100 μ l of purified viral preparations diluted in 1 \times SSC (0.15 M NaCl, 0.015 M sodium citrate [pH 7.2]) were directly applied to nitrocellulose membranes (0.20- μ m pore size) using a slot-blot apparatus (Schleicher & Schuell Inc., Keene, N.H.). Denaturation and RNA fixation were achieved by baking the membranes under vacuum for 90 minutes at 80 C. Membranes were then rehydrated in a 6 \times SSC solution, prehybridized overnight at 42 C, and hybridized for 24 hours in a standard hybridization solution containing 65% formamide, 5 \times Denhardt's components, 5 \times SSPE salts, 0.1% SDS and 100 μ g of denatured, sheared calf thymus DNA per ml (24). Several radiolabeled probes containing non-overlapping viral sequences (as established by Southern blot analysis) were used alone or in combination to amplify the detection signal as described (24,25).

Inoculation of embryonating eggs. Clarified supernatant fluids from TCV- or BCV-infected HRT-18 cells, adjusted to 10^{6.0} TCID₅₀/0.1 ml, were inoculated (0.3 ml) into the amniotic cavity of 22-to-24-day-old embryonating turkey eggs or 16-to-18-day-old embryonating chicken eggs obtained from a source known to be free from the usual specific pathogens of turkeys and chickens (Couvoir Unik, Dorval, Quebec, Canada). After inoculation, the eggs were incubated at 37 C for 3 to 4 days. Embryo intestines were then harvested and homogenized in 10 volumes of TBS using a Waring blender (Sorvall omnimixer, Ivan Sorvall Inc., Newton, Conn.). The homogenates were clarified by centrifugation at 10,000 \times g for 20 minutes, and supernatants were used for subsequent inoculations.

Experimental inoculation of turkey poults.

One-day-old turkey poults were obtained from local breeding flocks with no history of "bluecomb disease." The birds were placed in groups of six to 10 in wire-floored brooders kept in isolation units. An antibiotic-free commercial turkey starter ration and water were supplied *ad libitum*. During the first 2 days, fecal samples were collected and examined by EM to confirm the absence of coronaviruses and other enteric viruses. The poults were then inoculated orally with 10^{6.0} TCID₅₀ of either purified egg- and tissue-culture-adapted TCV isolates or purified tissue-culture-adapted BCV isolates, 0.5 ml of EM coronavirus-positive clarified intestinal contents from TCV-infected poults or TCV-infected embryos, 0.5 ml of EM coronavirus-positive clarified intestinal contents from diarrheic calves, or phosphate-buffered saline

(PBS [pH 7.2]). Four separate experiments were conducted, and poults were kept for up to 2 weeks. Inoculated birds were observed daily for clinical signs and weighed at regular intervals. Fecal samples were collected and processed for examination by EM, ELISA, and by virus isolation in cell cultures, as described above. For histopathological investigations, intestines of inoculated poults were fixed in 10% neutral buffered formalin and processed for paraffin tissue sectioning according to conventional methods. Sections were stained with hematoxylin-phloxin-safran (HPS) (2). Frozen intestinal sections were also fixed in cold acetone (-20 C), air dried, and processed for indirect immunofluorescence staining (IIF) using hyperimmune rabbit anti-TCV or anti-BCV serum and fluorescein-conjugated anti-rabbit IgG (Nordic Immunology, Tilburg, The Netherlands) (6,19).

Statistical analysis. The mean (\bar{X}), standard deviation (SD) and the coefficient of variation (CV) were computed for the values. Unpaired Student's *t*-tests were used to determine statistically significant differences between groups (20). The level of significance (*P*) was set at 0.05 in each statistical procedure.

RESULTS

Characteristics of TCV and BCV isolates.

As reported for the reference Minnesota strain of TCV (6), the four Quebec TCV isolates chosen for the present study (Q.15, Q.17, Q.23, and Q.1713) induced HRT-18 cells to form syncytia within 48 to 72 hours postinoculation (PI) in the presence of 10 U/ml of bovine trypsin. Under these conditions, the cytopathic effect led to complete destruction of the cell monolayers within 96 to 120 hours PI upon the third to fifth passage, and infectivity titers ranged from 10^{6.0} to 10^{7.5} TCID₅₀/ml. Most purified extracellular viral particles possessed surface projections of two distinct sizes (Fig. 1A), and specific immunogold labeling was obtained using anti-TCV hyperimmune serum (Fig. 1B). Similar results were obtained with the anti-BCV hyperimmune serum. Gold granules appeared closely associated with the virus particles, with minimal gold background staining. Control experiments, in which pre-immune serum was used or in which the anti-TCV or anti-BCV serum was omitted, showed that the viral particles in the test were specifically labeled. No labeling was obtained using hyperimmune serum to IBV (strains Beaudette, Connecticut, and Holland), TGEV strain Purdue, HEV strain 67N, MHV-3, and HCV-229E (data not shown).

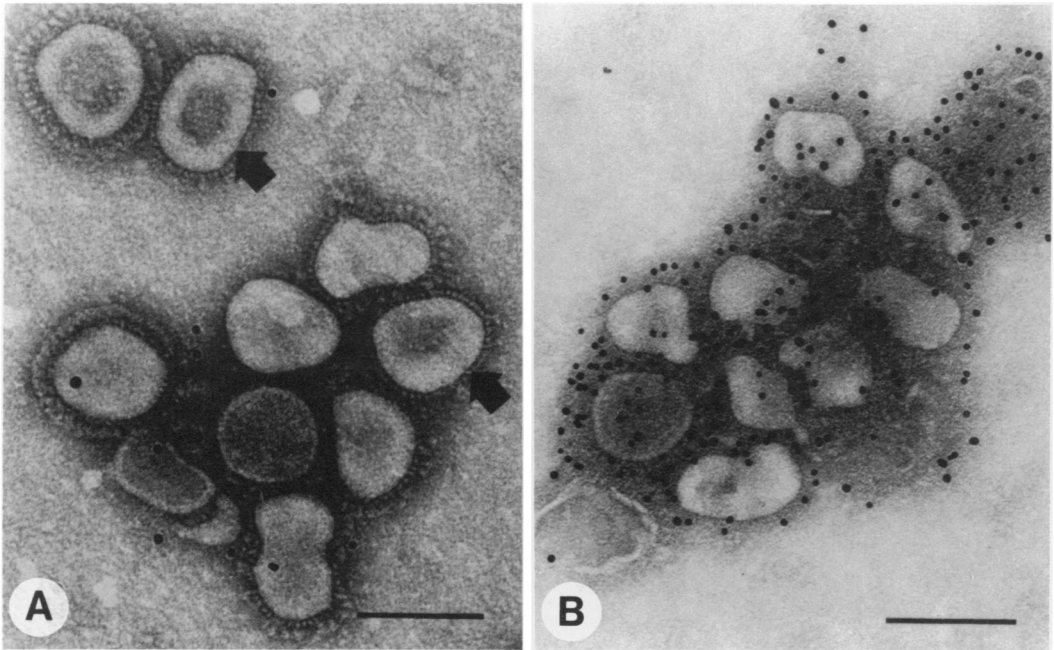


Fig. 1. A) Electron micrograph of sucrose gradient-purified tissue-culture-adapted TCV (Minnesota strain) viral particles with a double fringe of surface peplomers. The arrows indicate the additional fringe of short granular projections. B) Indirect protein A-immunogold labeling of tissue-culture-adapted Minnesota strain of TCV after incubation with rabbit anti-TCV hyperimmune serum. Bars = 100 nm.

Under the same conditions, infection of HRT-18 cells with the Mebus strain of BCV and two Quebec BCV isolates (Q.6 and Q.12) resulted in barely noticeable cytopathic changes; upon their fifth passage in the presence of trypsin, cell monolayers remained almost unchanged until 5 to 7 days PI. Nevertheless, hemagglutinating titers obtained with supernatant fluids from BCV-infected cell cultures were similar to those obtained with the various TCV isolates (range: 1:128 to 1:512), and EM also suggested that comparable amounts of extracellular virus were produced. The hemagglutinating activity of both BCV- and TCV-infected cell-culture supernatants was inhibited after incubation with anti-TCV hyperimmune serum (dilution up to 1:640).

SDS-PAGE and Western immunoblotting analyses of sucrose gradient-purified TCV and BCV isolates revealed similar polypeptide profiles. Four major polypeptide species—the peplomer glycoprotein gp200/100 (S), the hemagglutinin protein gp140 (HE), the nucleocapsid protein p52 (N), and the matrix protein gp24/p20 (M)—were identified by using homologous

hyperimmune rabbit serum. When purified TCV and BCV isolates were electrophoresed on the same gel and immunoblotted with hyperimmune rabbit serum to either virus, cross-antigenic relationships were revealed among their homologous proteins (Fig. 2).

Specificity and cross-reactivity of BCV cDNA probes. To determine the specificity of BCV cDNA probes, RNA or DNA extracted from purified bovine rotavirus, bovine herpesvirus 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 for hybridization with a probe-pool consisting of six radiolabeled recombinant plasmids, largely containing non-overlapping sequences, and covering about one-fourth of the BCV genome (24). Under relatively stringent conditions (e.g., 65% formamide), the BCV probes exhibited no reactivity toward these viral or cellular nucleic acid preparations (data not shown). However, a strong signal was obtained when BCV probes were hybridized to tissue-culture-adapted Mebus strain of BCV, as well as to tissue-culture-adapted BCV Q.6 and BCV Q.12 isolates (Fig.

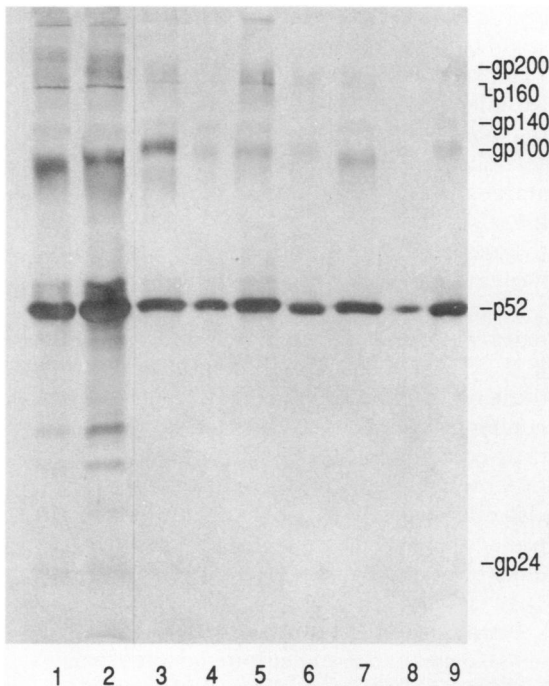


Fig. 2. Western immunoblots of TCV and BCV isolates as revealed by anti-TCV hyperimmune serum. Purified tissue-culture-adapted TCV and BCV isolates were electrophoresed in 12.5% polyacrylamide gels in the presence of 5% 2-mercaptoethanol. The viral proteins were electrophoretically transferred to nitrocellulose membranes and incubated with hyperimmune rabbit serum to the Minnesota strain of TCV. The immune reaction was revealed as described in the text. Lanes 1 to 3 correspond to BCV isolates Mebus (lane 1), BCV Q.6 (lane 2), and BCV Q.12 (lane 3). Lanes 4 to 9 correspond to TCV isolates Minnesota (lanes 4 and 5), TCV Q.1713 (lane 6), Q.23 (lane 7), Q.15 (lane 8), and Q.17 (lane 9).

3). When tested against purified preparations of various coronaviruses, strong detection signals were also obtained with tissue-culture-adapted Minnesota strain and the four Quebec TCV isolates. No detection signal was obtained with purified TGEV, HCV-229E, MHV-3, and the three strains of IBV. The BCV probes exhibited no reactivity toward undiluted supernatants or cell extracts prepared after two successive passages in HRT-18 of the coronaviruses tested, except for BCV and TCV isolates.

Experimental inoculation of poults. Four separate experiments were done using as inoculum egg-adapted TCV isolates, tissue-cul-

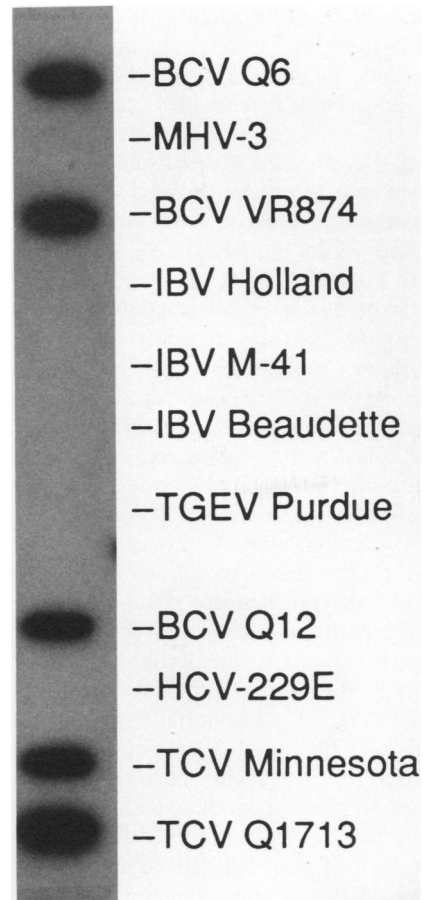


Fig. 3. Specificity and cross-reactivity of BCV cDNA probes. To examine the reactivity of BCV probes with other coronaviruses, the various cell-culture-grown coronaviruses were first purified by isopycnic ultracentrifugation into sucrose gradients, dialyzed, and adjusted to 10^{11} to 10^{12} viral particles per ml, as determined by electron microscopy by admixture with known amounts of latex spheres. Aliquots of 100 μ l of purified coronaviral preparations were applied to nitrocellulose membranes and reacted with a pool of six non-overlapping BCV cDNA probes holding sequences of the M and N genes of the virus.

ture-adapted TCV or BCV isolates, and EM coronavirus-positive intestinal contents from diarrheic poults or diarrheic calves. As summarized in Table 1, turkey poults inoculated with 10^6 TCID₅₀ of purified egg-adapted and tissue-culture-adapted Minnesota and Quebec Q.1713 isolates of TCV developed clinical signs of transmissible enteritis. The birds appeared

depressed by the second or third day PI, and their feed and water consumption decreased markedly. The majority of TCV-infected birds produced soft feces with orange-tinged mucus by day 3 to 5; the diarrhea was of short duration (2 to 3 days). Single deaths usually occurred by day 4 to 6. Survivors showed significant growth retardation: Average body weights of TCV-infected poulters increased 40% to 60%, compared with 110% to 120% for the mock-infected poulters over the 12- or 14-day observation period. Loss of body weight was generally less severe in cases of poulters inoculated with Quebec tissue-culture-adapted TCV isolates (Q.15, Q.17, and Q.23). In all cases, gross lesions included flaccid intestines with watery and greenish contents, and markedly distended ceca. Atrophy of the spleen was occasionally observed in poulters that were inoculated with egg-adapted isolates of the virus. Microscopic lesions consisted of a mild-to-severe atrophy of the villi, replacement of the columnar absorptive cells by cuboidal or simple squamous epithelial cells, and infiltration of the lamina propria with mononuclear cells (Fig. 4). Occasionally, fusion of adjacent villi was observed. Viral replication was confirmed by IIF staining of frozen intestinal sections prepared from day 3 to 14 PI. TCV antigens were first detected in the cells covering the upper half of the villi, but as the infection progressed, fluorescence became more apparent in scattered cells randomly located in the lamina propria (data not shown). TCV antigens were also detected in the stroma of the bursa of Fabricius of poulters inoculated with the egg-adapted or tissue-culture-adapted Minnesota strain; no cells fluoresced in the follicles themselves (data not shown). The fluorescence was observed using both anti-TCV and anti-BCV hyperimmune serum. Coronavirus particles in the clarified intestinal contents of infected poulters were observed by EM from day 4 to day 14 PI and could be reisolated in HRT-18 cells. Slot-blot hybridization with BCV probes did not permit the detection of viral RNA in the intestinal contents of TCV-infected birds, but positive signals were obtained after amplification by one passage in HRT-18 cells.

No clinical signs were observed in poulters that were either inoculated with diarrheic fecal samples from a calf that was experimentally infected with the Mebus strain of BCV or inoculated with 10^6 to 10^7 TCID₅₀ of tissue-culture-adapted Que-

bec BCV isolates (Q.6 and Q.12) and Mebus strain (VR874) of the virus. Poulters killed at days 7 and 14 PI showed no clinical signs of enteritis. No microscopic lesions were demonstrated in the intestines or other organs. Frozen intestinal sections were negative for BCV antigens by IIF, although desquamated cells in the lumen of the intestine generally appeared slightly IIF-positive in poulters inoculated with the EM-positive fecal samples or the tissue-culture-adapted Mebus strain. Furthermore, coronavirus-like particles were observed by EM or indirect ELISA in the clarified intestinal contents of poulters from these two different groups up to day 14 PI, and genomic viral RNA was detected in their intestinal contents by slot-blot hybridization using BCV probes. A positive hybridization signal was also obtained with supernatant fluids of the first passage in HRT-18 cells of the intestinal contents of poulters that were inoculated with BCV Q.12 isolate.

Propagation in embryonating eggs. Both field isolates and tissue-culture-adapted isolates of TCV and BCV were also tested for their capacity to propagate in embryonating chicken and turkey eggs inoculated via the amniotic cavity. Following two successive passages in eggs, only TCV isolates could be recovered by EM, indirect ELISA, and isolation in cell cultures from the intestinal contents of infected embryos (Table 2). The two BCV isolates that were tested failed to serially propagate in embryonating eggs, although residual virus could still be detected by molecular hybridization and ELISA 4 days PI upon their first passage. No detection signals were obtained following a second passage in eggs, and both BCV isolates failed to induce macroscopic and microscopic lesions in the embryos.

In contrast, the five TCV isolates tested were able to induce macroscopic and microscopic lesions compatible with transmissible enteritis or bluecomb disease. Upon the second passage in chicken embryonating eggs, few embryos died within 3 days following inoculation with egg-adapted reference Minnesota strain of TCV and the original and tissue-culture-adapted Quebec Q.1713 strain (Table 2). No deaths occurred in cases of other tissue-culture-adapted isolates. Nevertheless, gross lesions were evident upon the fourth day of infection and were confined to the intestinal tract. The contents of the duodenum, jejunum, and ceca were watery and

Table 1. Experimental inoculation of turkey poults with egg-adapted and tissue-culture-adapted turkey enteric coronavirus (TCV) and bovine enteric coronavirus (BCV) strains.

Group (inoculum) ^a	No. poults	Average body weight of live birds (g ± SD) ^b				CD ^c	Lesions		IIF ^d of intestine	EM/ELISA ^e of feces	cDNA hybridization ^f	
		Day 0 PI	Day 6 PI	Day 12 PI	Day 18 PI		Macro.	Micro.			Feces	HRT-18
Minnesota-egg	9	48.4 ± 2.5	60.2 ± 9.3*	73.0 ± 13.1*	1	+	+	+	+	-	+	
Minnesota-HRT	10	54.3 ± 2.8	68.1 ± 10.5*	88.8 ± 12.5*	2	+	+	+	+	+	+	
TCV Q.1713-egg	10	52.6 ± 2.4	72.5 ± 9.5*	88.1 ± 11.9*	0	+	+	+	-	-	+	
TCV Q.1713-HRT	10	48.6 ± 2.4	79.5 ± 14.5	104.6 ± 15.7	0	+	+	+	+	+	+	
PBS	8	50.8 ± 1.6	85.1 ± 10.2	106.1 ± 11.4	0	-	-	-	-	-	-	
Minnesota-egg	9	66.3 ± 5.1	72.1 ± 9.9*	92.4 ± 12.8*	0	+	+	+	+	ND ^g	+	
Minnesota-HRT	9	69.0 ± 4.0	87.1 ± 10.1*	99.7 ± 21.8*	2	+	+	+	+	ND	+	
TCV Q.1713-egg	9	65.2 ± 5.9	69.0 ± 10.5*	101.0 ± 15.0*	5	+	+	+	+	ND	+	
TCV Q.1713-HRT	8	66.8 ± 2.5	69.5 ± 9.9*	99.8 ± 3.1*	4	+	+	+	+	ND	+	
PBS	6	73.1 ± 3.6	116.1 ± 11.4	153.5 ± 24.0*	0	-	-	-	-	ND	-	
Minnesota-HRT	8	76.6 ± 1.2	115.6 ± 11.6	164.2 ± 18.3*	2	+	ND	ND	+	+	+	
TCV Q.23-HRT	8	79.0 ± 0.93	121.1 ± 3.4	172.6 ± 14.5*	4	+	ND	ND	+	-	+	
TCV Q.15-HRT	9	68.2 ± 1.0	112.4 ± 16.1	163.7 ± 19.7*	1	+	ND	ND	+	-	+	
TCV Q.17-HRT	9	72.0 ± 1.0	112.4 ± 16.1	163.7 ± 23.8*	2	+	ND	ND	+	+	+	
PBS	6	79.5 ± 3.0	129.6 ± 15.8	195.5 ± 16.3	0	-	ND	ND	-	-	-	
BCV Q.6-HRT	10	53.4 ± 3.5	86.2 ± 6.4	124.5 ± 16.2	0	-	-	-	-	-	-	
BCV Q.12-HRT	10	64.4 ± 2.9	94.7 ± 10.5	146.5 ± 17.7	0	-	-	-	-	+	+	
BCV VR874-HRT	10	57.4 ± 3.6	87.0 ± 9.8	128.2 ± 16.9	0	-	-	+/	+	+	+	
BCV VR874-calf	10	63.5 ± 3.4	93.7 ± 6.3	135.7 ± 15.2	0	-	-	+/	+	-	+	
PBS	8	59.6 ± 3.6	91.3 ± 12.0	130.8 ± 17.6	0	-	-	-	-	-	-	

^aThe inoculum was a 100-fold dilution of intestinal contents from TCV-infected turkey embryos (-egg) or BCV-infected calf (-calf), or supernatant fluids from TCV-infected or BCV-infected HRT-18 cells (-HRT) adjusted to 10⁶ TCID₅₀/ml.

^bValues followed by asterisks are significantly different from controls ($P < 0.05$).

^cCD = cumulative deaths.

^dIIF = Indirect immunofluorescence using anti-TCV hyperimmune serum; + = detection of fluorescent enterocytes or fluorescent cells randomly located in the lamina propria of TCV- or BCV-infected poults.

^eDetection of coronavirus particles or antigens by negative electron microscopy or indirect enzyme-linked immunosorbent assay using anti-TCV hyperimmune serum.

^fHybridization signals (+) obtained with BCV cDNA probes specific to the N and M genes.

^gND = not done.

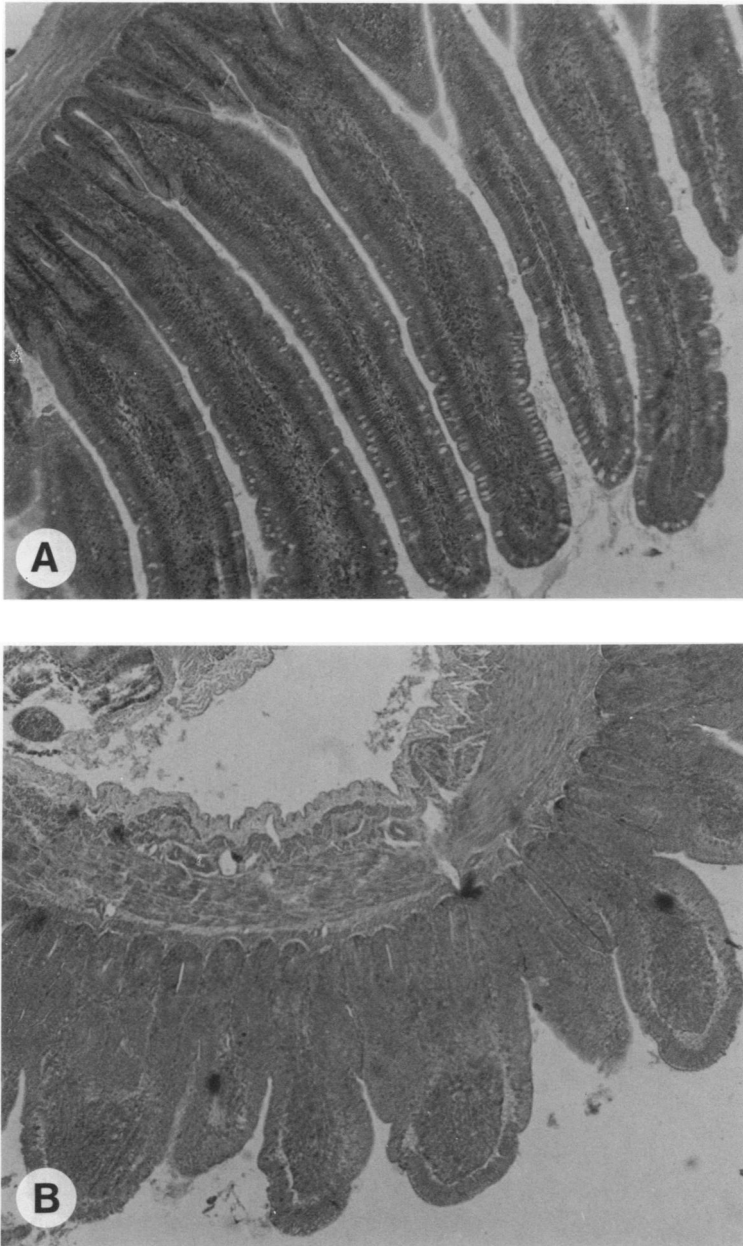


Fig. 4. Section from the middle jejunum of mock-infected (A) or TCV-infected (B) turkey poults that were killed 10 days after inoculation. Long, regularly spaced intestinal villi covered by cylindrical epithelial cells were observed in mock-infected poults (A), whereas atrophy and fusion of the intestinal villi were evident in poults inoculated with the tissue-culture-adapted Minnesota strain of TCV. HPS stain, 100 \times .

gaseous. The ceca were markedly distended and filled with watery greenish contents. Histopathological lesions were most distinct in the jejunum and were similar to those observed in naturally and experimentally infected poults.

DISCUSSION

The successful propagation in HRT-18 cells of TCV isolates obtained from field cases of epidemic diarrhea affecting 2-to-6-week-old tur-

Table 2. Growth of turkey enteric coronavirus (TCV) and bovine enteric coronavirus (BCV) isolates in embryonating chicken eggs.

Virus and inoculum ^a	First passage						Second passage					
	Isolation in HRT-18						Isolation in HRT-18					
	Deaths ^b	Lesions ^c	EM ^d	CPE ^e	ELISA ^f	Hybridization ^g	Deaths	Lesions	EM	CPE	ELISA	Hybridization
TCV Minn.-egg	+	+	+	+	+	+	-	+	+	+	+	+
TCV Minn.-HRT	-	+	+	+	+	+	-	+	+	+	+	+
TCV Q.1713-egg	+	-	+	+	+	W ^h	+	+	+	+	+	+
TCV Q.1713-HRT	-	+	+	+	+	+	-	+	+	+	+	+
TCV Q.15-HRT	-	+	+	+	-	-	-	W	ND ⁱ	+	+	+
TCV Q.17-HRT	-	+	+	+	+	+	-	W	ND	+	+	+
TCV Q.23-HRT	-	W	+	-	+	-	-	W	ND	+	+	+
BCV VR874-HRT	-	-	-	-	+	+	-	-	-	-	-	-
BCV Q.6-HRT	-	-	-	-	+	W	-	-	-	-	-	-
PBS	-	-	-	-	-	-	-	-	-	-	-	-

^aThe inoculum was a 100-fold dilution of intestinal contents from TCV-infected turkey embryos (-egg) or supernatant fluids from TCV- or BCV-infected HRT-18 cells (-HRT) adjusted to 10⁶ TCID₅₀/0.1 ml.

^b+ = deaths occurred, - = no deaths occurred.

^c+ = microscopic lesions observed, - = no microscopic lesions observed.

^d+ = coronavirus particles observed by electron microscopy, - = no viral particles observed.

^e+ = cytopathic effect observed, - = no CPE observed.

^fEnzyme-linked immunosorbent assay using anti-TCV hyperimmune serum.

^gHybridization with BCV cDNA probes specific to the N and M genes; + = strong hybridization signal, - = no signal.

^hW = weakly positive.

ⁱND = not done.

key poult provided a satisfactory source of the viruses for studying their molecular, serological, and pathological characteristics. Four Quebec TCV isolates were found to be serologically indistinguishable from the reference Minnesota strain by PAG-IEM, hemagglutination-inhibition, and Western immunoblotting. Close antigenic relatedness also was demonstrated between the Quebec TCV isolates and the reference Mebus strain of BCV, which agreed with previous findings by ELISA with egg-adapted strains of TCV (5). Serological findings were further confirmed by molecular hybridization studies with BCV cDNA probes holding sequences for the M and N genes of the virus. The existence of genomic relationships between TCV and BCV also was recently demonstrated by hybridization with EM-positive fecal samples obtained from diarrheic poult (26).

Because HRT-18 cells were routinely used in our laboratory for cultivation of BCV isolates, the possibility was eliminated that cells used for propagating TCV isolates were already persistently infected with BCV. In fact, the mock-infected cell cultures were negative for BCV and TCV by immunofluorescence, and thin sections prepared from these cells were negative for intracellular coronavirus particles by negative EM and PAG-IEM. Furthermore, no hybridization signals were obtained with nucleic acid prepared from uninfected cells. However, field and tissue-culture-adapted TCV and BCV isolates could at least be distinguished by their cytopathogenicity in HRT-18 in the presence of trypsin, and by their virulence for turkey poult and turkey or chicken embryos.

Only TCV isolates caused lesions in embryos and could be propagated for more than one passage in embryonating turkey or chicken eggs. Similarly, only TCV isolates were able to reproduce the clinical symptoms in 3-day-old poult. Nevertheless, BCV could be detected in the intestinal contents of experimentally infected poult up to day 14 PI by indirect ELISA and hybridization assays. These results suggested that BCV could at least replicate at a low level in the intestinal tract of poult but lacked the virulence to induce pathological lesions. Turkey and chicken embryos were not susceptible to BCV infection.

The HRT-18 cells have been reported to be susceptible to enteropathogenic strains of bovine, canine, and human coronaviruses

(11,13,14), and for the isolation of the human respiratory coronavirus HCV-OC43 (11), all of which belong 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 have been reported. In the present study, three different strains of avian IBV failed to adapt in this cell line, as well as TGEV and MHV-3. As HRT-18 cells retain many properties of the normal intestinal epithelium cells (23), these observations are consistent with the hypothesis that there is no rigid species restriction for the hemagglutinating enteric coronaviruses. Studies are in progress in our laboratory to determine if a common receptor exists for these viruses on HRT-18 cells and if this receptor is present on enterocytes from different animal species. There are few direct and indirect observations on the transmission of enteropathogenic coronaviruses from cattle to humans (10,17,22), which suggests a possibly wide host range for enteric coronaviruses. This hypothetical receptor may be present only in mature epithelial cells, which could explain the resistance of turkey and chicken embryos to BCV.

Recently, it has been demonstrated that RNA genomes of different strains of murine coronaviruses can undergo RNA-RNA recombinations during mixed infection at an extremely high frequency (15). Based on the results of the present study, a mixed infection with BCV and TCV in turkey poult could hypothetically have occurred naturally, giving rise to recombinants with different pathological properties. Further studies are needed to confirm this hypothesis and to provide more information on the significance of the results obtained in the present study as related to the epidemiology of TCV and BCV.

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