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Antigenic and Genomic Relatedness of Turkey-Origin Coronaviruses, Bovine Coronaviruses, and Infectious Bronchitis Virus of Chickens

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SUMMARY. In earlier studies in our laboratory, we found that bovine coronavirus (BCV) was pathogenic for 1-day-old turkey poults. This finding prompted us to study the antigenic and genomic relatedness of turkey origin coronaviruses (TOCVs) to BCV. A one-step reverse transcription (RT)–polymerase chain reaction (PCR) targeting a 730-base pair fragment of the nucleocapsid (N) gene of BCV and a nested PCR targeting a 407-base pair fragment of the N gene were used in an attempt to detect TOCV from North Carolina, Indiana, and a prototype turkey coronavirus (TCV) obtained from the American Type Culture Collection. Both the one-step RT-PCR and the nested PCR amplified cell culture–passaged isolates of calf diarrhea strains of BCV but none of the 15 tested TOCVs or transmissible gastroenteritis coronavirus of swine. TOCVs also did not cross-react in a BCV antigen-capture (AC) enzyme-linked immunosorbent assay (ELISA) system with monoclonal antibodies (MAbs) against N, spike glycoprotein, and hemagglutinin esterase glycoprotein proteins of BCV as coating antibodies. The same TOCVs could be detected with primers designed from the genome of infectious bronchitis virus (IBV) of chickens. These primers amplified a 1082-base pair region spanning portions of the membrane glycoprotein (M) and N protein genes of IBV and TCV. The TOCVs also cross-reacted in an AC-ELISA with MAbs against the M and subunit 2 of spike glycoprotein of IBV.

RESUMEN. Relación antigénica y genómica de coronavirus procedentes de pavos, el coronavirus bovino y el virus de la bronquitis infecciosa aviar.

En estudios previos realizados en nuestro laboratorio concluimos que el coronavirus bovino es patógeno para pavos de un dia de edad. Estos hallazgos nos llevaron a estudiar la relación antigénica y genómica entre coronavirus aislados de pavos y el coronavirus bovino. Para alcanzar este objetivo se utilizó una reacción de transcripción reversa seguida por una reacción en cadena por la polimerasa con la cual se amplificó un segmento genómico de 730 pares de bases a partir del gen que codifica por la proteína N del coronavirus bovino. Usando este primer producto de 730 pares de bases como blanco, se realizó una segunda reacción en cadena por la polimerasa de tipo anidada con la cual se trató de amplificar un segmento de 407 pares de bases a partir del gen que codifica por la proteína N en aislados de coronavirus de pavos procedentes de los estados de Carolina del Norte e Indiana, y un aislado de coronavirus de pavos obtenido de la American Type Culture Collection. Ambos tipos de reacciones pudieron amplificar productos a partir del genoma de los coronavirus bovino aislados en cultivo celular, pero ningún producto pudo ser amplificado a partir de los 15 aislados de coronavirus de pavo ni de los aislados del virus de la gastroenteritis transmisible de los cerdos

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usados en el estudio. Los antígenos de coronavirus de pavo no presentaron reacción cruzada cuando se usó una prueba de inmunoabsorción ligada a enzimas mediante captura de antígeno (AC-ELISA) específica para coronavirus bovino, en la cual se utilizaron anticuerpos monoclonales específicos contra las glicoproteínas N, S y hemaglutinina-esterasa como anticuerpos primarios. Estos aislados de coronavirus de pavos no pudieron ser detectados mediante la reacción en cadena por la polimerasa cuando se utilizaron iniciadores de reacción diseñados para amplificar productos a partir del genoma del virus de bronquitis infecciosa aviar. Estos iniciadores amplificaron un producto de 1082 pares de bases a partir del fragmento del genoma que codifica por las proteínas N y M del virus de la bronquitis infecciosa aviar y el coronavirus de pavo obtenido del ATCC. Los aislados de coronavirus de pavo procedentes de Carolina del Norte e Indiana presentaron reactividad cruzada en una prueba de AC-ELISA con anticuerpos monoclonales específicos contra la proteína M y la subunidad 2 de la glicoproteína S.

Key words: turkey coronavirus, infectious bronchitis virus, bovine coronavirus, bluecomb, poult enteritis and mortality syndrome

Abbreviations: AC = antigen capture; AMV RT = avian myeloblastosis virus reverse transcriptase; ATCC = American Type Culture Collection; BCV = bovine coronavirus; CD = calf diarrhea; DEPC = diethylpyrocarbonate; dNTP = deoxynueleoside triphosphate; ELISA = enzyme-linked immunosorbant assay; EMEM = Eagle minimum essential medium; FA = immunofluorescence; HE = hemagglutinin esterase glycoprotein; HI = hemagglutination inhibition; IBV = infectious bronchitis virus; IEM = immune electron microscopy; M = membrane; MAb = monoclonal antibody; N = nucleocapsid; PBS = phosphate-buffered saline; PBST = phosphate-buffered saline/Tween 20; PCR = polymerase chain reaction; PEMS = poult enteritis and mortality syndrome; RT = reverse transcription; S = spike glycoprotein; SPF = specific-pathogen free; TCV = turkey coronavirus; TOCV = turkey origin coronavirus

The Coronaviridae is a large family of RNA-containing viruses that infect a variety of mammalian and avian species (13). Until recently, coronaviruses were divided into four distinct antigenic groups. Group 1 included transmissible gastroenteritis virus of swine, canine coronavirus, and feline enteric coronavirus; group 2 included bovine coronavirus (BCV), mouse hepatitis virus, and diarrhea virus of infant mice; group 3 included infectious bronchitis virus (IBV) of chickens; and group 4 included turkey coronavirus (TCV), whose antigenic relationship to either BCV or IBV is controversial (8). Studies on antigenic relationships among several coronaviruses with polyclonal antibodies (6) and monoclonal antibodies (MAbs) (5) indicated that the TCV was closely related to group 2 of mammalian coronaviruses, which includes BCV. Subsequent sequence analysis of genes encoding the nucleocapsid (N) and membrane (M) proteins revealed a 99% or greater identity between TCV and BCV gene sequences (16). These studies suggested that TCV should be included with group 2 coronaviruses. In later studies by Guy *et al.* (7) based on immunofluorescence (FA) and immunoperoxidase studies, TCVs isolated from turkey flocks with either bluecomb or poult enteritis and mortality syndrome (PEMS) were found to be closely related to IBV of chickens, a member of coronavirus serogroup 3. The BCV is widely recognized as an important cause of neonatal calf diarrhea (15) and was reported to be antigenically related to TCV (5). In a previous study, we found that BCV infects 1-day-old turkey poults, resulting in diarrhea similar to that induced by turkey-origin coronaviruses (TOCVs) (10). The present study was designed to investigate the antigenic and genomic relationships between TOCVs, BCV, and IBV by antigen-capture (AC) enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase (RT)–polymerase chain reaction (PCR).

MATERIALS AND METHODS

Viruses. The TCV used in this study was the American Type Culture Collection (ATCC) turkey coronavirus (ATCC VR-911) supplied as intestines from diarrheic turkeys. Three TOCVs were isolated in our laboratory from PEMS-affected turkeys, and 12 isolates were generously supplied by Mr. Tom Hooper, Animal Disease Diagnostic Laboratory, Pur-

due University, Dubois, IN. Coronaviruses obtained from turkeys will be referred to in this manuscript as TOCVs. All samples were confirmed positive for coronaviruses by immune electron microscopy (IEM) and FA with turkey hyperimmune serum. The Arkansas and Massachusetts IBVs were generously provided by Dr. Mark Jackwood, University of Georgia, and were propagated in our laboratory. BCV strains included the calf diarrhea (CD) strains, Mebus and DB2 (3).

Propagation of viruses. Turkey and chicken embryos used for propagation of different coronaviruses originated from specific pathogen-free (SPF) flocks maintained at the Food Animal Health Research Program. These flocks were routinely monitored for different enteric viruses including coronaviruses. The RT-PCR was used for testing fecal material for coronaviruses, and the hemagglutination inhibition (HI) test was used for monitoring coronavirus antibodies. Both ATCC TCV and TOCVs were propagated in turkey embryos. The fecal samples containing TOCVs and the negative control samples were clarified at 3000 × *g* for 30 min, and the supernatants were filtered through 0.8-μm filters and then through 0.45-μm filters (Corning Glassware, New York, NY). A volume of 0.2 ml of filtrate was inoculated into 22-day-old turkey embryos via the amniotic cavity route. The intestinal tracts of inoculated embryos were harvested at 48 hr postinoculation. Arkansas and Massachusetts strains of IBV were inoculated into the chorioallantoic sac of 10-day-old SPF chicken embryos, and the virus-containing allantoic fluids were collected 24–30 hr postinoculation. Negative control embryos were inoculated with phosphate-buffered saline (PBS), pH 7.4. The Mebus and DB2 strains of BCV were passaged as previously described (3) in human rectal adenocarcinoma (HRT-18) cells.

Intestinal homogenates processing for ELISA and virus purification. The inoculated turkey embryo intestinal homogenates were diluted 1:10 in PBS and then clarified by centrifugation (1000 × *g* for 30 min at 4 C); the supernatants were used for the AC-ELISA. For virus purification, supernatants were purified on 20%–50% sucrose density gradient by ultracentrifugation (112,000 × *g* for 2 hr at 4 C). The band at the 20%/50% interface was collected, pelleted by ultracentrifugation (112,000 × *g* for 2 hr at 4 C), resuspended in Eagle minimum essential medium (EMEM) and stored at −70 C (9).

Detection of BCV antigen by BCV AC-ELISA. Three MAbs (BC 22 F8.3C for nemagglutin esterase glycoprotein [HE] protein, BC 28 H1.2C for N protein, and BC 29 G7.2C for spike [S] protein) previously produced against the CD DB2 strain of BCV and hyperimmune antisera previously produced against the CD Mebus strain of BCV in guinea pigs were used for the BCV AC-ELISA as described previously (3,9,14). The ELISA was done on the cell culture–passaged BCV and all the TOCVs. The absorbance values of duplicate samples were read (414 nm) and averaged, and samples with absorbance three times the standard deviation (3SD) above the mean absorbance of the samples in the BCV antibody negative control wells were considered positive (3).

Detection of IBV antigen by IBV AC-ELISA. Two MAbs (for M and S2 protein) produced against IBV were obtained from Dr. Syed Naqi, Cornell University, and pooled hyperimmune antisera produced in our laboratory against the Arkansas and Massachusetts strains of IBV in guinea pigs were used for the IBV AC-ELISA. The IBV AC-ELISA was developed in our laboratory and standardized with IBV-containing allantoic fluids. Briefly, 96-well microtitration plates were coated with a mixture of MAbs against the IBV structural proteins (M and S2 proteins) or antibody-negative mouse ascetic fluids as negative coating. After overnight incubation at 4 C, 5% (w/v) skim milk in PBS/Tween 20 (PBST), pH 7.4, was applied as a blocking reagent for 1 hr at 20–22 C. Infected and negative allantoic fluids (1:25 dilution) were added to duplicate wells coated with the IBV-capture MAbs or IBV antibody-negative mouse ascetic fluid. Turkey fecal specimen supernatants (1:10 dilution) positive and negative for TCV were also added to duplicate wells containing antibody-positive or -negative coating and incubated for 1 hr at 20–22 C. After the plates were washed with PBST, the secondary antibody, optimally diluted guinea pig anti-IBV hyperimmune serum (1:400), was added. The plates were incubated for 1 hr at 25 C, and the indicator antibody, consisting of diluted goat anti-guinea pig immunoglobulin G conjugated to horseradish peroxidase (1:6000), was added. The substrate used was O-phenylenediamine dihydrochloride with a final concentration of 0.03% hydrogen peroxide. The absorbance values of duplicate dilutions were read (490 nm) and averaged, and the samples with an absorbance of 3SD above the mean absorbance of the samples in the IBV antibody-negative control wells were considered positive.

Extraction of viral RNA for BCV RT-PCR. The Mebus and DB2 strains of CD BCV (3,9) were diluted in EMEM. For TCV RNA extraction, 15 TOCVs-containing fecal samples, TOCVs-infected turkey embryo intestinal homogenates, and sucrose gradient–purified TCV were used. Mock-infected turkey embryos and negative fecal samples obtained from our SPF turkey flocks were also used. RNA was extracted based on the acid guanidinium-phenol-chloroform RNA extraction method (4). Briefly, 500 μl solution D (4 M guanidinium thiocyanate, 25 mM sodium citrate, pH 7, 0.5% sarcosyl, 0.1 M 2-mercaptoethanol) and 50 μl 2 M sodium acetate, pH 4.0, were added into tubes containing 200 μl of BCV specimens, ATCC TCV, fecal suspensions (1:25 di-

lutions), purified TOCVs, and TOCVs-inoculated embryos intestinal homogenate supernatants (1:10 dilutions). After mixing, 500 μl water-saturated phenol, pH 4.5, and 100 μl chloroform and isoamylalcohol (49:1) were added, vortexed briefly, and placed on ice for 15 min. The mixtures were centrifuged at 20,800 × *g* for 20 min at 4C, and the supernatants were transferred into a new tube. To remove nonspecific inhibitors from the extracted samples, the supernatants were purified with the Rnaid kit according to the manufacturer's instructions (BIO 101, Inc., La Jolla, CA). As negative controls, RNA was extracted from mock-infected HRT-18 cells, swine testicular cells infected with the Miller strain of transmissible gastroenteritis coronavirus, the mock-infected turkey embryos, and SPF turkey negative feces.

Extraction of viral RNA for IBV RT-PCR. The IBV-laden allantoic fluid and TOCVs-inoculated embryo intestinal homogenate supernatants (1:10 dilutions) were concentrated by ultracentrifugation at 100,000 × *g* for 3 hr and then used for RNA extraction by the guanidium-isothiocyanate-phenol-chloroform method with a commercial kit (Trizol LS reagent; Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Briefly, 0.25 ml of purified IBV was mixed with 0.75 ml of Trizol LS reagent. After 5 min at room temperature, 0.2 ml of chloroform was added for phase separation. The mixture was centrifuged at 12,000 × *g* for 15 min at 4 C, and the aqueous phase was collected. Isopropyl alcohol was added to the aqueous phase to precipitate the RNA. After centrifugation at 12,000 × *g* for 10 min, the pelleted RNA was washed with 75% ethanol, dried for 10 min at room temperature, and dissolved in 20 μl of diethylpyrocarbonate (DEPC)-treated water (18). Allantoic fluids and intestinal homogenates from mock-infected SPF embryos were used as negative control.

Preparation of oligonucleotide primers. The oligonucleotide primers used in the RT-PCR and nested PCR were designed from the published sequence of the N gene of the Mebus strain of BCV (GenBank accession no. M16620) as described previously (3). The expected RT-PCR and nested PCR products were 730 and 407 base pairs, respectively. The genome of IBV was amplified with primers described earlier that amplified a 1082-base pair region spanning portions of the M and N protein genes of IBV (1).

RT-PCR procedure with BCV primers. A one-step RT-PCR assay was performed with modifications as described previously (3,17). The tube containing 5 μl of the RNA samples (BCV, TCV, TOCV) and 1 μl of dimethyl sulfoxide was incubated at 70 C for 10 min and then quenched on ice. Subsequently, 44 μl of the RT-PCR mixture was added. The RT-PCR mixture consisted of 5 μl of 10 × buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 5 μl of $MgCl_2$ (25 mM), 1 μl of 10 mM deoxynucleoside triphosphates (dNTPs), 1 μl of the upstream primer (50 pmol), 1 μl of the downstream primer (50 pmol), 0.5 μl of RNAsin (Promega Corp., Madison, WI), and 0.5 μl of avian myeloblastosis virus reverse transcriptase (AMV RT) (Promega), 0.5 μl of Taq polymerase (5 U/μl; Promega). The mixture was incubated for 60 min at 42 C, preheated for 5 min at 94 C, and subjected to 35 cycles of 1 min at 94 C, 1 min at 58 C, .2 min at 72 C, and a final 7-min incubation at 72 C. The PCR products were visualized on 1.5% agarose gels stained with ethidium bromide.

Nested PCR with BCV primers. To increase the sensitivity of the RT-PCR with the BCV primers, 5 μl of diluted RT-PCR products (1:100) was added to a tube containing 45 μl of the PCR mixture as described previously (3) and subjected directly to nested PCR. The PCR mixture consisted of 5 μl of 10 × buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 5 μl of $MgCl_2$ (25 mM), 1 μl of 10 mM dNTPs, 1 μl of the nested PCR upstream primer (50 pmol), 1 μl of the nested PCR downstream primer (50 pmol), 0.5 μl of Taq polymerase (5 U/μl; Promega), and 31.5 μl water. The mixture was preheated for 5 min at 94 C and subjected to 30 cycles of 1 min at 94 C, 1 min at 58 C, .2 min at 72 C, and a final 7-min incubation at 72 C. The PCR products were visualized on 1.5% agarose gels stained with ethidium bromide. As a negative control, RT-PCR products with RNA extracted from mock-infected HRT-18 cells were used.

RT-PCR with IBV primers. To perform the RT-PCR assay with RNA extracted from the IBV, TOCVs, and negative control samples, the RT-PCR procedure was done as described previously (1) but with modifications. In addition, a RT step was added as follows. The tube containing 5 μl of RNA sample, 2 μl of downstream IBV primer (25 pmol), and 4 μl of DEPC-treated water was incubated in a boiling water bath for 2 min, then quenched on ice for 5 min. Subsequently, 9 μl of the RT-PCR mixture was added. The RT-PCR mixture consisted of 4 μl of 5 × RT buffer, 2 μl of 0.1 dithiothretol, 2 μl of 10 mM dNTPs (Promega), 0.5 μl RNAsin (Promega), and 0.5 μl AMV RT (Promega). The mixture was incubated at 42 C for 90 min. After incubation, 10 μl of the resulting template was mixed with 50 μl of PCR mixture. The PCR mixture consisted of 5 μl of 10 × buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), 5 μl of $MgCl_2$ (25 mM) (Promega), 1 μl 10 mM dNTP (Promega), 1 μl of upstream primer (25 pmol), 1 μl of downstream primer (25 pmol), 0.5 μl of Taq polymerase (Promega), and 26.5 μl DEPC-treated water. The mixture was preheated at 94 C for 5 min, then subjected to 35 cycles of 1 min at 94 C, 2 min at 37 C, 5 min at 72 C, and a final 15-min incubation at

72 C. The PCR products were visualized on 1.2% agarose gels stained with ethidium bromide.

RESULTS

None of the TOCVs including the ATCC strain cross-reacted with BCV antibody reagents in the BCV AC-ELISA. However, ATCC TCV and all TOCVs cross-reacted with the IBV antibody reagents in the IBV AC-ELISA. For all TCV and TOCVs, the average absorbance readings were greater than the cut-off value.

A specific 730-base pair band was detected after RT-PCR with Mebus and DB2 strains of BCV. To increase sensitivity, the nested PCR was performed with the BCV RT-PCR products. The PCR products of 407 base pairs were detected by the nested PCR when performed with RT-PCR products from both Mebus and DB2 strains of BCV. To study the reactivity of the primers, RT-PCR was also performed with RNA extracted from transmissible gastroenteritis coronavirus and mock-infected HRT-18 cells. With the BCV primers, the RT-PCR and nested PCR produced no bands with the above RNA samples. When RT-PCR was performed on the extracted TOCVs RNA from the ATCC strain, three TOCVs from PEMS-affected turkey flocks, and 12 TOCVs from Indiana and from the same viruses after purification with BCV primers, no detectable bands were produced with any TOCVs. Further detection with RT-PCR products to run the nested PCR also yielded no product (Table 1).

On the other hand, when primers from IBV were used, bands of predicted size (1082 base pairs) were detected when RT-PCR was performed on RNA extracted from both Arkansas and Massachusetts strains of IBV. The same size bands were detected when performing the RT-PCR on RNA extracted from all concentrated isolates of TOCVs including the ATCC strain (Fig. 1).

DISCUSSION

In the present study, TOCVs isolated from turkeys experiencing PEMS and TOCVs isolated from bluecomb cases from Indiana and the ATCC TCV were tested for their antigenic and genomic relationships to both BCV and IBV. A BCV AC-ELISA with MAbs to HE, N, and S proteins of BCV was used for testing the antigenic cross-reactivity of these TOCVs with BCV. The TOCVs did not cross-react with the BCV MAbs in the ELISA. Moreover, attempts were unsuccessful to amplify a part of TOCVs genome with primers from BCV in a RT-PCR followed by the more sensitive nested PCR, which could amplify BCV N gene. On the other hand, the same TOCVs cross-reacted in an IBV AC-ELISA with pooled MAbs to the M and S2 proteins of IBV. These MAbs are used to detect IBV (11). When we used primers from IBV to amplify the M and N genes of TOCVs, a predicted size band was detected. These primers are successfully used to amplify the same gene region of IBV (1). In earlier studies of TOCVs in 1973 (12) with the use of IEM and HI test, the virus was shown to be unrelated to either group 2 BCV or group 3 IBV. These procedures detect common antigenic similarities or differences among viruses only on the virion surface (8). In later studies (5,6,16), a close antigenic relationship between TOCVs and BCV was illustrated. The discrepancy between our current results and their findings could have resulted from use of turkey-origin BCV in their studies because they used an HRT-18 cell–propagated virus (TOCV will not replicate on HRT-18 cells) and perhaps only turkey-origin BCV grew in the cell line tested. It is also possible that two coronaviruses were present in their original specimens and only BCV was grown selectively in the HRT cell line. Guy (8) and Ismail *et al.* (10) reported the failure to adapt TOCVs to HRT cells or a variety of primary and established cell lines. In addition, it is commonly recognized that BCV is adaptable to replication in HRT-18 cells (3,9,14). We also reported recently (10) that gnotobiotic calf–passaged BCV was pathogenic to turkey poults and produced signs similar to those produced by TOCVs. Hence, it is conceivable that the viruses used in early investigations (5,6,16) were turkey-origin BCVs that were circulating in turkey flocks or isolated in laboratory settings. Our findings are consistent with the more recent findings of Guy *et al.* (7) and Breslin *et al.* (2), who reported that recent TCVs isolated from PEMS cases were closely related to IBV on the basis of immunofluorescence, immunoperoxidase procedures, and nucleocapsid gene sequencing. In conclusion, on the basis of our current results, TOCVs and

Table 1. Results of tests to detect different bovine, turkey, and chicken coronaviruses by different tests.[A]

Coronavirus sample and origin	BCV ELISA	IBV ELISA	BCV RT-PCR/nested PCR	IBV RT-PCR[B]
BCV				
Mebus (tissue culture)	+	−	+/+	−
DB2 (tissue culture)	+	−	+/+	−
TCV				
ATCC	−	+	−/−	+[a]
TOCVs				
PEMS				
1	−	+	−/−	+[a]
2	−	+	−/−	+[a]
3	−	+	−/−	+[a]
Indiana				
1	−	+	−/−	+[a]
2	−	+	−/−	+[a]
3	−	+	−/−	+[a]
4	−	+	−/−	+[a]
5	−	+	−/−	+[a]
6	−	+	−/−	+[a]
7	−	+	−/−	+[a]
8	−	+	−/−	+[a]
9	−	+	−/−	+[a]
10	−	+	−/−	+[a]
11	−	+	−/−	+[a]
12	−	+	−/−	+[a]
IBV				
Arkansas	−	+	−/−	+[a]
Massachusetts	−	+	−/−	+[a]

[A] + = positive; − = negative.

[B] + with the superscript letter a indicates that RT-PCR was useful only with viruses concentrated by ultracentrifugation.

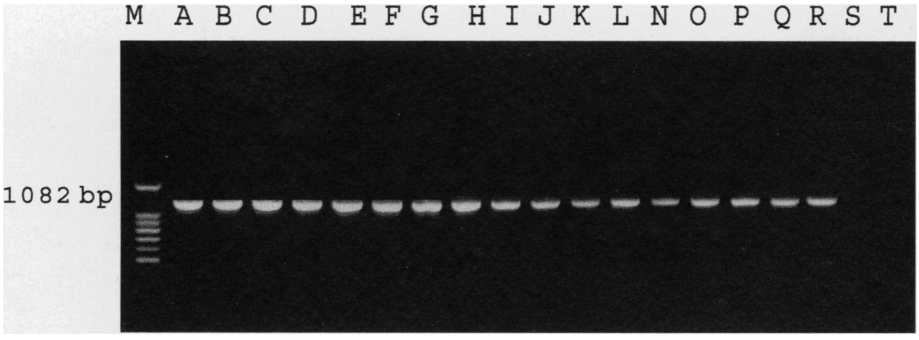

Fig. 1. The RT-PCR products of the Massachusetts strain of IBV (lane A), ATCC TCV (lane B), TOCVs from PEMS (lanes C–E), and TOCVs from Indiana samples (lanes F–R). M, marker; S and T, negative controls. The primer was designed from the Beaudette strain of IBV.

IBV share antigenic and genomic properties, and we propose considering including them as group 3 avian coronaviruses. In addition, the BCV was antigenically and genetically dissimilar to TOCVs and IBV. Sequencing studies of the N and M genes of the TOCVs will be helpful in extending our studies.

REFERENCES

1. Andreasen, J. R., M. W. Jackwood, and D. A. Hilt. Polymerase chain reaction amplification of the genome of infectious bronchitis virus. Avian Dis. 35: 216–220. 1991.

2. Breslin, J. J., L. G. Smith, F. G. Fuller, and J. S. Guy. Sequence analysis of the turkey coronavirus nucleocapsid gene region of turkey coronavirus. Intervirology 4:22–29. 1999.

3. Cho, K.-O., M. Hasoksuz, P. R. Nielson, K. O. Chang, S. Lathrop, and L. J. Saif. Cross-protection studies between respiratory and calf diarrhea or winter dysentery coronavirus strains in calves and RT-PCR and nested PCR for their detection. Arch. Virol. In press. 2001.

4. Chomczynski, P., and N. Sacchi. Single step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform extraction. Anal. Biochem. 162:156–159. 1987.

5. Dea, S., A. J. Verbeek, and P. Tijssen. Antigenic and genomic relationships among turkey and bovine coronaviruses. J. Virol. 64:3112–3118. 1990.

6. Dea, S., A. J. Verbeek, and P. Tijssen. Transmissible enteritis of turkeys: experimental inoculation studies with tissue-culture-adapted turkey and bovine coronaviruses. Avian Dis. 35:767–777. 1991.

7. Guy, J., H. J. Barnes, L. J. Smith, and J. Breslin. Antigenic characterization of a turkey coronavirus identified in poult enteritis and mortality syndrome–affected turkeys. Avian Dis. 41:583–590. 1997.

8. Guy, S. J. Turkey coronavirus is more closely related to avian infectious bronchitis virus than to mammalian coronaviruses: a review. Avian Pathol. 29:207–212. 2000.

9. Hasoksuz, M. H., S. L. Lathrop, K. L. Gadfield, and L. J. Saif. Isolation of bovine respiratory coronaviruses from feedlot cattle and comparison of their biological and antigenic properties with bovine enteric coronaviruses. Am. J. Vet. Res. 60:1227–1233. 1999.

10. Ismail, M. M., K.-O. Cho, L. A. Ward, L. J. Saif, and Y. M. Saif. Experimental bovine coronavirus in turkey poults and young chickens. Avian Dis. 45: 157–163. 2001.

11. Karaca, K., S. Naqi, and J. Gelb, Jr. Production and characterization of monoclonal antibodies to three infectious bronchitis virus serotypes. Avian Dis. 36:903–915. 1992.

12. Ritchie, A. E., D. R. Desmukh, C. T. Larsen, and B. S. Pomeroy. Electron microscopy of coronavirus-like particles characteristic of turkey bluecomb disease. Avian Dis. 17:546–558. 1973.

13. Saif, L. J., and R. A. Heckert. Enteropathogenic coronaviruses. In: Viral diarrhea of man and animals. L. J. Saif and K. W. Theil, eds. CRC Press, Inc., Boca Raton, FL. pp. 185–236. 1990.

14. Smith, D. R., H. Tsunemitsu, R. A. Heckert, and L. J. Saif. Evaluation of two antigen-capture ELISAs using polyclonal or monoclonal antibodies for the detection of bovine coronavirus. J. Vet. Diagn. Invest. 8:99–105. 1996.

15. Tsunemitsu, H., and L. J. Saif. Antigenic and biological comparisons of bovine coronaviruses derived from neonatal calf diarrhea and winter dysentery of adult cattle. Arch. Virol. 140:1303–1311. 1995.

16. Verbeek, A., and P. Tijssen. Sequence analysis of the turkey enteric coronavirus nucleocapsid and membrane protein genes: a close genomic relationship with bovine coronavirus. J. Gen. Virol. 72: 1659–1666. 1991.

17. Xu, L., D. Harbour, and M. A. McCrae. The application of polymerase chain reaction to the detection of rotaviruses in feces. J. Virol. Methods 27: 29–38. 1990.

18. Yu, M., Y. Tang, M. Guo, Q. Zhang, and Y. M. Saif. Characterization of a small round virus associated with the poult enteritis and mortality syndrome. Avian Dis. 44:600–610. 2000.

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