AVIAN DISEASES 44:624–631, 2000

Comparison of Virus Isolation, Immunohistochemistry, and Reverse Transcriptase–Polymerase Chain Reaction Procedures for Detection of Turkey Coronavirus

Jamie J. Breslin,[A] Lynda G. Smith,[A] H. John Barnes,[B] and James S. Guy[AC]

[A]Department of Microbiology, Pathology, and Parasitology
[B]Department of Farm Animal Health and Resource Management
North Carolina State University, Raleigh, NC 27606

Received 17 November 1999

SUMMARY. A reverse transcriptase–polymerase chain reaction (RT-PCR) procedure and two monoclonal antibody (MAb)-based immunohistochemical procedures were developed for detection of turkey coronavirus (TCV) in tissues and intestinal contents/dropping samples. The RT-PCR, MAb-based fluorescent antibody (FA), and MAb-based immunoperoxidase (IP) procedures were compared with virus isolation (VI) for detection of TCV in experimentally infected turkeys.

TCV was detected in experimentally infected turkeys as early as day 1 postexposure (PE) by each of the four detection procedures. TCV was detected as late as day 35 PE by FA or IP and days 42 and 49 PE by VI and RT-PCR, respectively. With VI as a reference, sensitivity and specificity of RT-PCR were 93% and 92%, respectively; specificity of both FA and IP was 96%, and sensitivities were 69% and 61%, respectively. Each of the examined procedures was highly specific, but the RT-PCR procedure was also highly sensitive. These findings demonstrate the utility of both immunohistochemistry and RT-PCR for detection of TCV. In addition, the findings indicate that RT-PCR is a highly sensitive and specific alternative to conventional diagnostic procedures.

RESUMEN. Comparación de los procedimientos de aislamiento viral, inmunohistoquímica y reacción en cadena por la polimerasa transcriptasa reversa, para la detección de coronavirus de pavo.

Se desarrollaron una prueba de reacción en cadena por la polimerasa transcriptasa reversa (de las siglas en inglés RT-PCR) y dos procedimientos inmunohistoquímicos basados en anticuerpos monoclonales para la detección del coronavirus de pavos en muestras de tejidos y de materia fecal. La prueba RT-PCR, la inmunofluorescencia con anticuerpos monoclonales y la prueba de la inmunoperoxidasa con anticuerpos monoclonales fueron comparados con el aislamiento viral para la detección del coronavirus en pavos infectados experimentalmente. Se pudo detectar el coronavirus de pavos desde el primer día después de la infección por medio de cada uno de los cuatro procedimientos. El coronavirus de pavos se pudo detectar incluso hasta el día 35 por medio de la prueba de la inmunofluorescencia y la prueba de la inmunoperoxidasa y hasta el día 42 y el día 49 por medio del aislamiento viral y el RT-PCR, respectivamente. Con el aislamiento viral como referencia, la sensibilidad y la especificidad de la reacción en cadena por la polimerasa fue de 93% y 92%, respectivamente. La especificidad de la inmunofluorescencia y de la inmunoperoxidasa fue de 96% y la sensibilidad fue de 69% y 61%, respectivamente. Cada uno de los procedimientos examinados fue altamente específico pero la prueba RT-PCR fue también altamente sensible. Estos hallazgos demostraron la utilidad de la inmunohistoquímica y el RT-PCR para la detección del coronavirus de pavo. Además, los hallazgos inidican que el RT-PCR es una alternativa altamente sensible y específica a los procedimientos convencionales de diagnóstico.

Key words: turkey coronavirus, reverse transcriptase–polymerase chain reaction

[C]Corresponding author.

Resources used to support this research were provided by United States Poultry and Egg Association.

Abbreviations: CK = chicken kidney; DMEM = Dulbecco's minimal essential medium; EID_{50} = 50% embryo infectious doses; FA = fluorescent antibody; IBV = infectious bronchitis virus; IP = immunoperoxidase; MAb = monoclonal antibody; NDV = Newcastle disease virus; PCR = polymerase chain reaction; PE = postexposure; PI = postinoculation; RT = reverse transcriptase; RT-PCR = reverse transcriptase–polymerase chain reaction; TCV = turkey coronavirus; VI = virus isolation

Turkey coronavirus (TCV) is the cause of an acute, highly contagious enteric disease of turkeys referred to as bluecomb disease or mud fever (11). Bluecomb disease was first identified in turkeys in 1951, and a coronavirus was determined to be the cause of the disease in 1973 (11). TCV produces enteric disease that is characterized by diarrhea, depression, weight loss, and increased mortality (11).

TCV is a member of the family Coronaviridae, which consists of a large group of RNA viruses that infect a wide variety of both avian and mammalian species (9,15). Coronaviruses are characterized on the basis of their distinctive morphology. They are pleomorphic, enveloped viruses, 80–160 nm in diameter, with long (20 nm), widely spaced club-shaped surface projections (9,15). Coronaviruses consist of at least three major structural proteins, the surface glycoprotein, an integral membrane protein, and a nucleocapsid protein (9,15).

Diagnosis of TCV infection most often is accomplished by virus isolation (VI) and/or detection of antigen in tissues by direct and indirect fluorescent antibody (FA) procedures. Isolation of TCV is accomplished by amniotic inoculation of embryonated turkey eggs and subsequent FA testing of embryo intestines (7). VI is slow and labor intensive and requires virus-specific antisera. Detection of TCV antigens in tissues by FA procedures is a rapid, less cumbersome procedure but requires a specialized microscope having an ultraviolet light source as well as a source of virus-specific antisera.

Immunoperoxidase (IP) procedures have been successfully applied to the diagnosis of several viral infections (1,6). These procedures, in common with FA, provide a simple, rapid means for detecting viral antigens in tissues; however, several advantages of IP make it a particularly valuable diagnostic technique. IP uses a conventional light microscope, provides a more permanent record, and preserves histologic detail. Polymerase chain reaction (PCR) procedures for detecting viral nucleic acids in clinical specimens generally provide the distinct advantages of high sensitivity and specificity compared with other conventional diagnostic methods. Reverse transcriptase (RT)-PCR procedures have been described for detection of human coronaviruses from nasal aspirates and bovine coronavirus from fecal samples (10,17).

Recent studies have demonstrated a close antigenic and genomic relationship between TCV and infectious bronchitis virus (IBV) (3,7,16). On the basis of this relationship, a RT-PCR procedure for detection of TCV in infected turkeys was developed with DNA primers previously utilized for RT-PCR detection of IBV (2). These DNA primers amplify a 1100-base pair region spanning portions of the matrix and nucleocapsid genes of TCV (3).

The present study compared a RT-PCR procedure, two monoclonal antibody (MAb)-based immunohistochemical procedures (FA and IP), and VI for detection of TCV infection in experimentally infected turkeys.

MATERIALS AND METHODS

Viruses. TCV (NC95) was isolated from poult enteritis and mortality syndrome–affected turkeys as described (7). TCV (Minnesota) was obtained from the American Type Culture Collection (Rockville, MD), and three field isolates were obtained from Tom Hooper, Purdue University, Dubois, IN. Field isolates originated from turkeys in Indiana, Virginia, and Arkansas. TCV strains were propagated in embryonated turkey eggs as described (7).

IBV (Arkansas), IBV (Massachusetts), reovirus, and fowl adenovirus (serotype 3) were obtained from SPAFAS, Inc. (Norwich, CT) and propagated in chicken kidney (CK) cells (14). Newcastle disease virus (NDV) (Roakin) was obtained from Dr. D. J. King, Southeast Poultry Research Laboratory, Athens, GA, and propagated in CK cells. Transmissible gastroenteritis virus, Purdue strain, and bovine coronavirus, Nebraska strain, were obtained from the National Veterinary Services Laboratory (Ames, IA) and propagated in porcine kidney (PK15) cells and human rectal adenocarcinoma (HRT) cells, respectively.

An inoculum containing TCV (NC95) was prepared by amniotic inoculation of 18-day-old embryonated turkey eggs with TCV (NC95) at the 12th embryo passage. At 4 days postinoculation (PI), embryo intestines were harvested and prepared as a 10% suspension in Dulbecco's minimal essential medium (DMEM) (Sigma Chemical Co., St. Louis, MO). The suspension was homogenized, clarified by centrifugation for 10 min at 1200 × *g* and sequentially filtered through 0.8-, 0.45-, and 0.22-μm filters (Gelman Sciences, Ann Arbor, MI). Virus was titered by inoculation of 10-fold dilutions into each of three 23-day-old embryonated turkey eggs with examination of individual embryo intestines on day 3 PI by indirect FA; virus titer was calculated by the method of Reed and Muench (12). An inoculum was prepared to contain approximately 1.6×10^4 50% embryo infectious doses (EID_{50})/0.1 ml and stored at −70 C.

MAb to TCV. MAbs specific for TCV were prepared by the procedure described by Carter *et al.* (4). Briefly, BALB/c mice were immunized with partially purified TCV (NC95), and splenocytes collected from immunized mice were fused with murine myeloma cells. Hybridoma colonies secreting antibodies specific for TCV virus were detected by assay of culture supernatant fluids by indirect FA with frozen sections of TCV-infected embryo intestines (7). Each positive hybridoma colony was cloned twice by limiting dilution; antibody was produced from positive hybridomas in the form of exhausted cell culture supernatants. The immunoglobulin subclass of TCV-specific MAbs was determined with commercial enzyme-linked immunosorbent assay test system (MonoAb ID EIA kit; Zymed Laboratories, San Francisco, CA) by the manufacturer's instructions.

Turkeys. Commercial turkeys were obtained at 1 day of age from Cuddy Farms (Aurora, MO). Turkeys were housed in wire-floored, electrically heated brooders in an isolation room with controlled access until they were 2 wk of age. Turkeys were fed nonmedicated game bird starter (Granville Milling, Creedmoor, NC). Feed and water were provided *ad libitum.*

Experimental design. *Expt. 1.* At 2 wk of age, turkeys were wing banded and inoculated with TCV (NC95); after exposure, birds were maintained in electrically heated brooders. Turkeys were inoculated at 2 wk of age by placing a no. 10 French catheter (Monoject, St. Louis, MO) into the crop. Each bird received approximately 1.6×10^4 EID_{50} TCV (NC95).

Six birds were randomly selected on days 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, and 22 postexposure (PE) and necropsied. At necropsy, dropping samples/intestinal contents and intestinal tissues were collected from each bird. Droppings and/or intestinal contents (approximately 3 g) were collected for RT-PCR and stored at 4 C. Intestinal contents were collected from the ceca with cotton-tipped swabs for virus isolation; these swabs were immediately placed in 1 ml DMEM and stored at −70 C. Intestinal tissue at the ileocecal junction was collected for FA and IP and immediately frozen in Tissue-Tek O.C.T. Compound (Miles Laboratories, Elkhart, IN).

Expt. 2. At 2 wk of age, turkeys were wing banded and inoculated with TCV (NC95); after exposure, birds were maintained in an isolation room with controlled access on sawdust bedding. Turkeys were inoculated as described in Expt. 1. Six birds were randomly selected on days 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 PE and necropsied. At necropsy, samples were collected as described in Expt. 1.

Immunohistochemistry. Tissues (ileocecal junction) were sectioned with a cryostat, fixed in cold (4 C) absolute acetone for 10 min, and stored at 4 C until stained. TCV antigens were detected in frozen sections by indirect FA staining and IP staining as described with TCV MAb 4.24 exhausted supernatant at a dilution of 1:5 (6,7). Tissue sections from uninfected turkeys, embryonated turkey eggs, and cell cultures were used as negative controls for both FA and IP staining. Antibody controls also were included for each section; a MAb specific for infectious laryngotracheitis virus was used in place of MAb 4.24.

VI. Intestinal contents were collected with cotton-tipped swabs, immediately immersed in 1 ml DMEM, clarified by centrifugation at 1500 × *g* for 20 min at 4 C, and filtered through a 0.45-μm filter (Gelman Sciences). Two 21-to-24-day-old embryonated turkey eggs were each inoculated with 0.2 ml of sample. Three days PI, embryo intestines were collected for indirect FA staining.

RT-PCR. Intestinal contents/dropping samples (approximately 3 g) were prepared as 20% (w/v) suspensions in TNE buffer (0.01 M Tris-hydrochloride, pH7.4, 0.1 M NaCl, 1 mM ethylenediaminetetraacetic acid) and sonicated for 30 sec. These suspensions were clarified by centrifugation at 1000 × *g* for 10 min at 4 C and then at 8000 × *g* for 30 min at 4 C. The supernatant was layered onto a 20% (w/v) sucrose cushion and centrifuged at 80,000 × *g* for 2 hr at 4 C. Nucleic acid was harvested from the resultant pellets by incubation in 0.5% sodium dodecyl sulfate for 5 min at room temperature followed by two phenol-chloroform extractions. Nucleic acid was precipitated in cold (−20 C) ethanol, and pellets were resuspended in RNAse-free water.

The RT-PCR procedure was performed as described (3). To confirm the identity of the RT-PCR products, direct nucleotide sequencing was performed on selected samples. DNA was sequenced at the University of North Carolina (Chapel Hill) Automated DNA Sequencing Facility on a Model 373 A, DNA sequencer (Applied Biosystems, Foster City, CA) with the Taq DyeDeoxy™ Terminator Cycle Se-

quencing Kit (Applied Biosystems). Nucleotide sequencing was carried out with the EcoM (5′-TGAATTCTCAGTGGCTTGCTAAGTGTGAACC-3′) primer (3).

Sensitivity of the RT-PCR procedure was determined by RT-PCR of nucleic acid extracted from serial dilutions of a known concentration of TCV (NC95). Briefly, TCV (NC95) inoculum (titer = 1.6×10^4 EID_{50}/0.1 ml) was serially diluted in water, then 0.1 ml of each dilution was incubated in 0.5% sodium dodecyl sulfate (Gibco BRL, Grand Island, NY) for 5 min at room temperature, followed by a phenol-chloroform extraction and precipitation of nucleic acid in cold (−20 C) ethanol. Resultant pellets were resuspended in RNase-free water and assayed by RT-PCR.

Specificity of the RT-PCR was determined by use of nucleic acid harvested from other viruses. Two strains of IBV, Massachusetts and Arkansas, were propagated in embryonated chicken eggs as described (14). NDV, reovirus, and fowl adenovirus (serotype 3) were propagated in CK cells (13). Virus-laden allantoic fluids and infected CK cells were freeze–thawed two times and centrifuged at 8000 × *g* for 10 min at 4 C; viral particles were pelleted from the supernatant by centrifugation through 20% (w/w) sucrose at 80,000 × *g* for 2 hr. Nucleic acid was extracted and RT-PCR was performed as described above.

Statistics. Sensitivity and specificity were calculated by standard formulas (5).

RESULTS

MAb production and characterization. A single hybridoma cell line was identified that secreted antibodies (MAb 4.24) specific for TCV. The cell line was selected on the basis of a strong reaction of antibody to TCV antigens as determined by indirect FA and absence of specific reaction when FA was performed with uninfected cells. MAb 4.24 reacted strongly by FA to turkey embryo intestines infected with five epidemiologically distinct isolates of TCV: NC95, Minnesota, and field isolates from Indiana, Arkansas, and Virginia. No FA reaction was observed with uninfected CK cells, uninfected turkey embryo intestines, or cell cultures infected with IBV (Arkansas), NDV, reovirus, transmissible gastroenteritis, or bovine coronavirus. MAb 4.24 was determined to be an immunoglobulin IgG2a isotype.

RT-PCR. The RT-PCR yielded a distinct band of approximately 1100 bp when performed with RNA extracted from partially purified, embryo-propagated TCV (NC95) (Fig. 1). In contrast, no bands were observed when the assay was performed with RNA extracted from uninfected embryos or when RT-PCR was run without RT (data not shown). Direct nucleotide sequencing of the 1100-bp RT-PCR product revealed that the nucleotide sequence corresponded to a region spanning the matrix and nucleocapsid genes of TCV (3).

Sensitivity of the RT-PCR procedure was determined by RT-PCR of RNA extracted from 0.1 ml of each 10-fold serial dilution of the TCV inoculum (titer = 1.6×10^4 EID_{50}/0.1 ml). TCV amplification products were seen in the undiluted (16,000 EID_{50}), 10^{-1} (1600 EID_{50}), and 10^{-2} samples (160 EID_{50}); amplification products were not detected at higher dilutions (Fig. 1). Therefore, the detection limit of the RT-PCR was calculated to be 160 EID_{50}.

Specificity was determined by performing the RT-PCR procedure with nucleic acid harvested from other viruses (data not shown). Positive PCR amplification products were observed for both IBV strains (Massachusetts and Arkansas). This result was expected because the primers used in the reaction are IBV derived (3). No amplification products were observed with nucleic acid harvested from CK cells infected with NDV, reovirus, and fowl adenovirus.

Detection of TCV in experimentally infected turkeys. TCV antigens were identified in intestinal sections of infected turkeys by both FA and IP staining with TCV MAb 4.24. In the IP procedure, the specific reaction was seen as a distinct red deposit in the cytoplasm of intestinal epithelial cells, primarily in the apical areas of the cells (Fig. 2). Early in infection (days 1–6 PE), intense staining of villous epithelial cells was observed with both FA and IP, and large numbers of positive-staining cells were detected in both ileum and cecum (Fig. 2). Later (days 8–35 PE), antigens were detected in relatively few scattered villous epithelial cells, primarily in the ileum (Fig. 2). The specificity of FA and IP was verified by observing no staining when an unrelated MAb was used in place of TCV MAb 4.24 in either procedure. In addition, TCV antigens were not detected in samples collected from turkeys prior to TCV inoculation (day 0 PE).

VI detected TCV beginning on day 1 PE; no virus was detected by VI in samples collected before experimental infection (day 0 PE). RT-PCR also detected the virus in infected turkeys

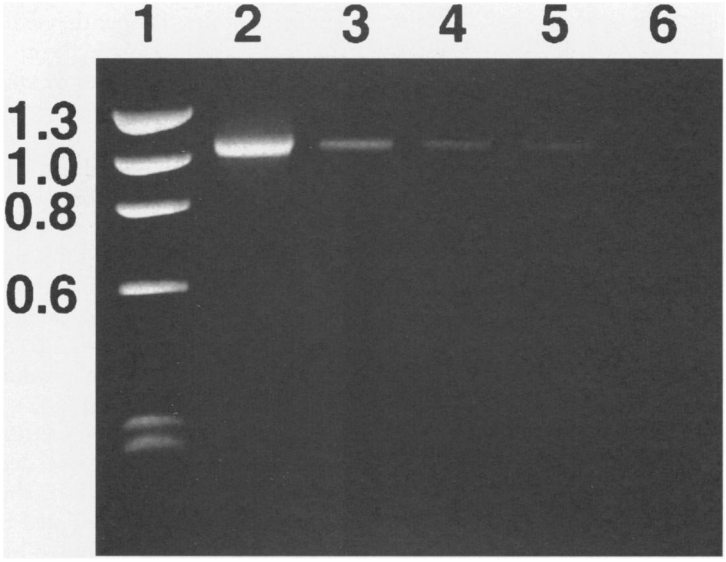

Fig. 1. Sensitivity of RT-PCR as determined by assay of 10-fold dilutions of a known concentration of TCV (NC95). Lane 1, molecular weight marker ϕX174/*Hae*III (numbers on vertical axis indicate molecular weight in kilobase pairs); lane 2, partially purified, embryo-propagated TCV (NC95); lanes 3–6, serial dilutions of TCV (NC95) inoculum. Lane 3, undiluted (16,000 EID_{50}); lane 4, 10^{-1} dilution (1600 EID_{50}); lane 5, 10^{-2} dilution (160 EID_{50}); lane 6, 10^{-3} dilution (16 EID_{50}).

beginning on day 1 PE; no virus was detected by RT-PCR in turkeys examined on day 0 PE. The nucleotide sequence of four RT-PCR products obtained from Expts. 1 and 2 was determined. Direct sequencing was done with two products from Expt. 1 representing days 4 and 16 and two products from Expt. 2 representing days 7 and 35. The nucleotide sequence data

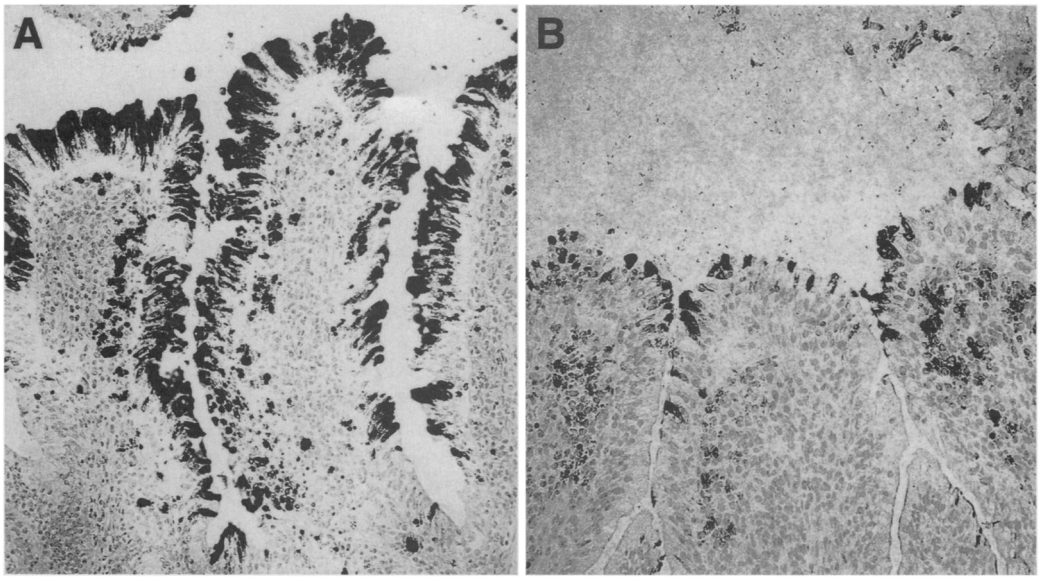

Fig. 2. Immunoperoxidase staining of intestinal tissues from TCV (NC95)-inoculated turkeys. (A) Cecum, day 1 postexposure. (B) Ileum, day 14 postexposure. 350×.

Table 1. Experiment 1. Comparison of indirect fluorescent antibody (FA) procedure, indirect immunoperoxidase (IP) procedure, virus isolation (VI), and reverse transcriptase–polymerase chain reaction (RT-PCR) for detection of TCV in experimentally infected turkeys.[A]

Days postexposure	No. turkeys positive/no. tested			
	FA	IP	VI	RT-PCR
1	4/6	4/6	4/6	3/6
2	6/6	6/6	5/6	4/6
4	6/6	6/6	6/6	6/6
6	6/6	6/6	6/6	6/6
8	5/6	5/6	6/6	6/6
10	5/6	2/6	6/6	5/6
12	2/6	1/6	6/6	6/6
14	3/6	2/6	6/6	6/6
16	2/6	2/6	6/6	6/6
22	0/6	0/6	4/6	4/6

[A]Virus was not detected by FA, IP, VI, or RT-PCR on day 0 postinoculation.

for all RT-PCR products corresponded to the matrix/nucleocapsid gene region of TCV (3).

Table 1 compares FA, IP, VI, and RT-PCR for detection of TCV in infected turkeys during a 1-to-22-day PE period (Expt. 1). Detection by both FA and IP began on day 1 PE and continued until day 16 PE. VI and RT-PCR both detected infection on days 1–22 PE, slightly longer than FA and IP. VI and RT-PCR also detected more infected birds at days 12, 14, and 16 PE than did FA and IP. However, FA and IP detected more infected birds early in the course of infection, days 1 and 2 PE (Table 1).

Table 2 compares FA, IP, VI, and RT-PCR for detection of TCV in infected turkeys during a 7-to-63-day PE period (Expt. 2). Samples for Expt. 2 were taken at weekly intervals. FA and IP both detected TCV infection on days 7, 14, 21, and 35 PE; however, neither procedure detected virus on day 28 PE or after day 35 PE. VI and RT-PCR detected TCV infection beginning on day 7 PE and ending on days 42 and 49 PE, respectively.

Sensitivity and specificity of FA, IP, and RT-PCR relative to VI were calculated by combining results of Expts. 1 and 2 (Table 3). With VI as a reference, the sensitivities of FA, IP, and RT-PCR were 69%, 61%, and 93%, respectively. The specificity of both FA and IP when compared with VI was 96%, and RT-PCR had a specificity of 92%.

DISCUSSION

VI, FA, IP, and RT-PCR were shown to be highly specific methods for detection of TCV infection; however, sensitivities of FA and IP were poor compared with those of VI and RT-PCR. The findings of the present study indicate that the RT-PCR is a sensitive and specific alternative to conventional diagnostic procedures.

RT-PCR was more sensitive than either FA or IP for detection of TCV infection but less sensitive than VI. As determined by comparison with VI, sensitivities of RT-PCR, FA, and IP were 93%, 69%, and 62%, respectively. Although VI and RT-PCR were able to detect TCV for a longer period of time than were FA and IP, these immunohistochemical procedures appeared to have greater sensitivity early in the course of infection (Table 1, days 1 and 2 PE), likely due to an abundance of viral proteins in cells at that time (Fig. 2). Increased sensitivity of RT-PCR, compared with FA and IP, is attributed to enzymatic amplification of minute quantities of virus-specific nucleic acid.

The RT-PCR procedure was slightly less sensitive than VI; however, RT-PCR was able to detect TCV 1 wk longer than was VI. Five samples that were positive by VI during Expts. 1 and 2 were negative by RT-PCR. Perhaps TCV RNA was lost from samples during the extensive processing of intestinal contents/dropping samples or during the RNA extraction procedure. Alternatively, RNAses or inhibitory substances in intestinal contents/dropping samples may have resulted in RNA degradation or inhibition of PCR amplification, respectively. False-negative results have been shown to be a particular problem for PCR detection of mammalian viruses in fecal samples (8,18). The

Table 2. Experiment 2. Comparison of indirect fluorescent antibody (FA) procedure, indirect immunoperoxidase (IP) procedure, virus isolation (VI), and reverse transcriptase–polymerase chain reaction (RT-PCR) for detection of TCV in experimentally infected turkeys.[A]

Days post-exposure	No. turkeys positive/no. tested			
	FA	IP	VI	RT-PCR
7	6/6	6/6	5/6	5/6
14	4/6	3/6	6/6	4/6
21	4/6	4/6	5/6	6/6
28	0/6	0/6	2/6	4/6
35	1/6	1/6	1/6	1/6
42	0/6	0/6	1/6	1/6
49	0/6	0/6	0/6	1/6
56	0/6	0/6	0/6	0/6
63	0/6	0/6	0/6	0/6

[A]Virus was not detected by FA, IP, VI, or RT-PCR on day 0 postinoculation.

presence of inhibitory substances in feces, primarily polymerase inhibitors, is largely responsible for these false-negative reactions (8,18). During the present study, relatively extensive processing of intestinal contents/dropping samples was necessary for successful application of the RT-PCR procedure for TCV detection. Such extensive processing of intestinal contents/dropping samples likely was necessary in order to eliminate substances inhibitory to the enzymatic reactions that are used for amplification and, possibly, nucleases.

Specificity of RT-PCR was high, 92%, but less than FA and IP, which were both 96%. Two samples in this study were positive by IP and/or FA but negative by VI and, therefore, were considered false positives in calculations of specificity. Similarly, four samples in this study were positive by RT-PCR but negative by VI and were considered false positives. However, these samples possibly were true positives that were undetectable by VI because of limitations in this procedure. RT-PCR, FA, and IP procedures have a distinct advantage over VI in that they do not require infectious virus, only noninfectious subcomponents of the virus. In addition, VI of enveloped viruses, such as coronaviruses, often is unsuccessful because of the lability of these viruses.

TCV was detected for a prolonged duration in TCV-inoculated turkeys. The virus was detectable for up to 42 days PE by VI and 49

Table 3. Detection of TCV in experimentally infected turkeys: evaluation of indirect fluorescent antibody (FA) procedure, indirect immunoperoxidase (IP) procedure, and reverse transcriptase–polymerase chain reaction (RT-PCR) relative to virus isolation.

	Virus isolation				
	Positive	Negative	Agreement (%)	Sensitivity[A]	Specificity[B]
FA					
Positive	52	2			
Negative	23	49	101/126 (80%)	69%	96%
IP					
Positive	46	2			
Negative	29	49	95/126 (75%)	61%	96%
RT-PCR					
Positive	70	4			
Negative	5	47	117/126 (93%)	93%	92%

[A]Sensitivity = true-positive results/(true-positive + false-negative results).
[B]Specificity = true-negative results/(false-positive + true-negative results).

days PE by RT-PCR. These findings support previous studies that demonstrated prolonged shedding of TCV in dropping samples of turkeys after recovery from clinical disease (C. T. Larsen, unpubl.).

The RT-PCR procedure described in this study is a relatively simple, sensitive, and specific method for detection of TCV in intestinal contents/dropping samples of infected turkeys. In addition, it provides several advantages over conventional diagnostic methods; it is more sensitive than immunohistochemical techniques and less labor intensive and time consuming than VI. In contrast to VI, which requires several days to complete, RT-PCR could be accomplished within 24 hr. These findings indicate potential usefulness of the RT-PCR procedure for TCV diagnosis.

REFERENCES

1. Afshar, A., G. C. Dulac, and A. Bouffard. Application of peroxidase labeled antibody assays for detection of porcine IgG antibodies to hog cholera and bovine viral diarrhea viruses. J. Virol. Methods 23: 253–262. 1989.

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

3. Breslin, J. J., L. G. Smith, F. J. Fuller, and J. S. Guy. Sequence analysis of the matrix/nucleocapsid gene region of turkey coronavirus. Intervirology 42: 22–29. 1999.

4. Carter, P. B., K. H. Beegle, and D. H. Gebhard. Monoclonal antibodies: clinical use and potential. Vet. Clin. North Am. 16:1171–1179. 1986.

5. Fletcher, R. H., S. W. Fletcher, and E. H. Wagner. Clinical epidemiology—the essentials, 2nd ed. Williams and Wilkins, Baltimore, MD. 1988.

6. Guy, J. S., H. J. Barnes, and L. G. Smith. Rapid diagnosis of infectious laryngotracheitis using a monoclonal antibody-based immunoperoxidase procedure. Avian Pathol. 21:77–86. 1992.

7. Guy, J. S., H. J. Barnes, L. G. 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. Jiang, X., J. Wang, D. Y. Graham, and M. K. Estes. Detection of Norwalk virus in stool by polymerase chain reaction. J. Clin. Microbiol. 30:2529–2534. 1992.

9. Murphy, F. A. Virus taxonomy. In: Fundamental virology, 3rd ed., vol. 1. B. N. Fields, D. M. Knipe, and P. M. Howly, eds. Lippincott-Raven Publishers, Philadelphia, PA. pp. 15–57. 1996.

10. Myint, S., S. Johnston, and G. Sanderson. Evaluation of nested polymerase chain methods for the detection of human coronaviruses 229E and OC43. Mol. Cell. Probes 8:357–364. 1994.

11. Nagaraja, K. V., and B. S. Pomeroy. Coronaviral enteritis of turkeys (bluecomb disease). In: Diseases of poultry, 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, eds. Iowa State University Press, Ames, IA. pp. 686–692. 1997.

12. Reed, L. J., and H. Muench. A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27: 493–497. 1938.

13. Schat, K. A., and H. G. Purchase. Cell culture methods. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 167–175. 1989.

14. Senne, D. A. Virus propagation in embryonating eggs. In: A laboratory manual for the isolation and identification of avian pathogens, 3rd ed. H. G. Purchase, L. H. Arp, C. H. Domermuth, and J. E. Pearson, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 176–181. 1989.

15. Siddell, S. G. The Coronaviridae an introduction. In: Coronaviridae. S. G. Siddell, ed. Plenum Press, New York. pp. 1–9. 1995.

16. Stephensen, C. B., D. B. Casebolt, and N. N. Gangopadhyay. Phylogenetic analysis of a highly conserved region of the polymerase gene from 11 coronaviruses and development of a consensus polymerase chain reaction assay. Virus Res. 60:181–189. 1999.

17. Tsunemitsu, H., D. R. Smith, and L. J. Saif. Experimental inoculation of adult dairy cows with bovine coronavirus and detection of coronavirus in feces by RT-PCR. Arch. Virol. 144:167–175. 1999.

18. Wilde, J., J. Eiden, and R. Yolken. Removal of inhibitory substances from human fecal specimens for detection of group A rotaviruses by reverse transcriptase polymerase chain reactions. J. Clin. Microbiol. 28:1300–1307. 1990.