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Baculovirus Expression of Turkey Coronavirus Nucleocapsid Protein

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SUMMARY. The nucleocapsid (N) gene of turkey coronavirus (TCV) was amplified by reverse transcriptase–polymerase chain reaction, cloned, and expressed in the baculovirus expression system. A recombinant baculovirus containing the TCV N gene (rBTCV/N) was identified by polymerase chain reaction and expression of TCV N protein as determined by western immunoblot analysis. Two TCV-specific proteins, 52 and 43 kDa, were expressed by rBTCV/N; one of these proteins, p52, was comparable in size to native TCV N protein. Baculovirus-expressed N proteins were used as antigen in an indirect enzyme-linked immunosorbent assay (ELISA) for detection of TCV-specific antibodies. The ELISA detected antibodies specific for TCV and infectious bronchitis virus, a closely related avian coronavirus, but did not detect antibodies specific for other avian viruses (avian influenza, avian reovirus, avian paramyxovirus 3, avian adenovirus 1, or Newcastle disease virus). These findings indicate that baculovirus-expressed TCV N protein is a suitable source of antigen for ELISA-based detection of TCV-specific antibodies in turkeys.

RESUMEN. Expresión de la proteína del nucleocápsido del coronavirus de pavo en un Baculovirus.

Se amplificó el gen del nucleocápsido del coronavirus de pavo por medio de la prueba transcriptasa reversa - reacción en cadena por la polimerasa (de las siglas en inglés RT-PCR), se clonó y se expresó en el sistema de expresión del baculovirus. Se identificó un baculovirus recombinante que contenía el gen nucleocápsido del coronavirus de pavo por medio de la prueba PCR y se determinó la expresión de la proteína del nucleocápsido del coronavirus de pavo por medio de la inmunotransferencia puntual Western. El baculovirus recombinante que contenía la proteína N del coronavirus de pavo expresó dos proteínas específicas del coronavirus de pavos de 52 y 43 kilodaltons. Una de ellas, la p52 fue similar en tamaño a la proteína N original del coronavirus de pavo. Las proteínas N expresadas en el baculovirus fueron utilizadas como antígeno en una prueba indirecta de inmunoensayo con enzimas asociadas (ELISA) para la detección de anticuerpos específicos contra el coronavirus de pavo. La prueba ELISA detectó anticuerpos específicos contra el coronavirus de pavo y contra el virus de bronquitis infecciosa, un coronavirus aviar estrechamente relacionado, pero no se detectaron anticuerpos específicos para otros virus aviares (influenza aviar, reovirus aviar, paramixovirus 3, adenovirus aviar serotipo 1 o virus de la enfermedad de Newcastle). Estos hallazgos indican que la proteína N del coronavirus de pavo expresada en el baculovirus es una fuente apropiada de antígeno para la prueba ELISA para la detección de anticuerpos específicos contra el coronavirus de pavos en esta especie.

Key words: turkey coronavirus, baculovirus, enzyme-linked immunosorbent assay

Abbreviations: AcMNPV = *Autographa californica* multiple nuclear polyhedrosis virus; ELISA = enzyme-linked immunosorbent assay; FBS = fetal bovine serum; IBV = infectious bronchitis virus; IFAT = indirect fluorescent antibody; M = membrane; MOI = multiplicity of infection; N = nucleocapsid; PAGE = polyacrylamide gel electrophoresis; PBS = phosphate-buffered saline; PBST = phosphate-buffered saline plus 0.05% Tween 20; PCR = polymerase chain reaction; PI = postinfection; rBTCV/N = recombinant baculovirus containing TCV N gene; RT = reverse transcriptase; S = surface; SDS = sodium dodecyl

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sulfate; SF-9 = *Spodoptera frugiperda*; SPF = specific-pathogen free; TCV = turkey coronavirus; TNT = 10 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.05% Tween 20

Turkey coronavirus (TCV) causes an acute, highly contagious enteric disease of turkeys referred to as bluecomb disease (17). Bluecomb disease was first identified in turkeys in 1951, and a coronavirus was determined to be the cause of the disease in 1973 (17). In recent years, TCV has been increasingly recognized as an important cause of enteric disease in turkeys, resulting in economic loss because of impaired growth and poor feed conversion. The virus also has been associated as a cause of poult enteritis and mortality syndrome, a disease of unknown etiology characterized by high mortality, severe growth depression, and immune dysfunction (2).

TCV is a member of the family Coronaviridae. The Coronaviridae are a large group of RNA viruses that infect a wide variety of avian and mammalian species (22,27). Coronaviruses have a distinctive morphology; they are pleomorphic, enveloped particles, 80–220 nm in diameter, with long club-shaped surface projections approximately 20 nm in length (22,27). The coronavirus genome consists of a positive-sense, single-stranded RNA molecule, 27–30 kb in size. Coronavirus virions are composed of three structural proteins: surface (S) glycoprotein (90–180 kD), integral membrane (M) protein (20–35 kD), and nucleocapsid (N) protein (50–60 kD). Additionally, some coronaviruses also contain a fourth major structural protein, the hemagglutinin esterase protein (120–140 kD) (22).

Coronaviruses have been subdivided into three major antigenic groups on the basis of differences identified by serologic analyses, and these findings have been substantiated by nucleotide sequence analyses (22,27). Human coronavirus 229E, transmissible gastroenteritis virus, canine coronavirus, and feline infectious peritonitis virus are members of group I; HCV OC43, murine hepatitis virus, and bovine coronavirus are members of group II; and infectious bronchitis virus (IBV) belongs to group III (22,27). Recent antigenic studies and nucleotide sequence analyses indicate that TCV belongs to group III (3,4,11,24). Whereas all coronaviruses possess S, M, and N structural proteins, only members of group II have been shown to possess the hemagglutinin esterase protein (22).

The coronavirus N protein binds to virion RNA and provides the structural basis for the helical nucleocapsid (14). It is the most abundant viral polypeptide in coronavirus-infected cells and it is immunodominant (13,14). The complete nucleotide sequences of TCV N gene and a portion of TCV M gene recently were reported; the deduced amino acid sequences of TCV M and N proteins were determined to be very similar (>90% identity) to those of IBV M and N proteins (3,4). Sequence analysis of the TCV S protein has not been reported.

Serologic diagnosis of TCV infection currently is accomplished by indirect fluorescent antibody (IFAT) procedures (10,19). These IFAT procedures are labor intensive and time consuming. In addition, they require expensive equipment, highly trained personnel, and an antigen obtained from frozen sections of TCV-infected turkey embryo intestines or epithelial cells exfoliated from bursae of Fabricius of TCV-infected turkeys (10,19). A TCV-specific enzyme-linked immunosorbent assay (ELISA) would be an improved method for serologic diagnosis; however, the production of large quantities of high-quality antigen for this procedure has been hampered by the inability to propagate TCV in cell culture. The purpose of the present study was to express TCV N protein in a baculovirus expression system and utilize the recombinant DNA-derived protein in an ELISA for detection of TCV-specific antibodies.

MATERIALS AND METHODS

Viruses, cells, and antisera. TCV (NC95) was isolated from enteritis-affected turkeys in North Carolina (10). TCV (Minnesota) was obtained from the American Type Culture Collection (Rockville, MD). TCV strains were propagated in embryonated turkey eggs (11). IBV (Massachusetts) was obtained from SPAFAS, Inc. (Norwich, CT) and propagated in 9- to-11-day-old embryonated chicken eggs (21).

Wild-type baculovirus, *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV), and recombinant viruses were propagated in *Spodoptera frugiperda* (SF-9) cells (Invitrogen, San Diego, CA) in

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supplemented Grace's insect cell culture medium (Gibco BRL, Grand Island, NY) with 10% fetal bovine serum (FBS) and gentamicin (10 μg/ml). Recombinant viruses also were propagated in *Trichoplusia ni* (High Five™) cells (Invitrogen) in serum-free High Five cell culture media (Invitrogen) containing gentamicin (10 μg/ml).

Antisera against TCV strains (NC95, Minnesota) were prepared in 4-wk-old specific-pathogen-free (SPF) chickens (SPAFAS, Inc.) as previously described (11). Antiserum prepared in SPF turkeys against TCV (Minnesota) was obtained from Dr. Y. M. Saif (Ohio State University, Wooster, OH). This antiserum was prepared by oral inoculation of SPF turkeys with turkey embryo-propagated TCV (Minnesota) followed by two intramuscular inoculations at 3-wk intervals with sucrose-gradient-purified virus mixed with incomplete Freund's adjuvant.

Antisera prepared in SPF chickens against IBV (Massachusetts, Arkansas, Connecticut, JMK), avian reovirus, avian influenza virus, avian adenovirus 1, avian paramyxovirus 3, and Newcastle disease virus were obtained from SPAFAS, Inc. Negative control serum from unimmunized SPF chickens was obtained from SPAFAS, Inc.

Virus purification. TCV and IBV were partially purified from infected turkey embryo intestines or virus-laden allantoic fluids, respectively, as described (3).

Construction of baculovirus transfer vectors. TCV (NC95) N gene was amplified by reverse transcriptase (RT)–polymerase chain reaction (PCR) with two custom primers (Gibco BRL) designed from previously reported nucleotide sequence of TCV N gene (4): N5′-TTCTGCAGATGGCAAGCGGTAAGGCAACT and N3′-TGAATTCCTACAA GTGATTCTCTCCTAG. Primers N5′ and N3′ contained *Pst*I and *Eco*RI restriction sites, respectively, to facilitate cloning. The amplification product (1.2 kb) was cloned into *Pst*I and *Eco*RI sites of plasmid pUC19 (Gibco BRL) and transformed into competent *Escherichia coli* strain DH5α cells (Gibco BRL). This plasmid was designated pTCVN (Fig. 1). pTCVN was digested with *Pst*I and *Eco*RI, DNA fragments were analyzed by gel electrophoresis, and the TCV N gene was excised from the gel and purified with a commercial kit (Gel Extraction Kit; Qiagen, Valencia, CA). Purified DNA was cloned into the *Pst*I and *Eco*RI sites of baculovirus transfer vectors pMelBac A, B, and C (Invitrogen), which are designed to direct expression of recombinant proteins through the secretory pathway to the extracellular medium. The pMelBac vector is supplied in three versions to allow correct in-frame fusion of the foreign gene with the melittin secretion signal in one of the vectors. Clones were amplified in competent *Escherichia coli* strain DH5α cells (Gibco BRL), and plasmids were purified with a commercially available kit (S.N.A.P.™ Miniprep Kit; Invitrogen). The three transfer vector constructs were sequenced at the University of North Carolina (Chapel Hill) Automated DNA Sequencing Facility on a Model 373A DNA Sequencer (Applied Biosystems, Foster City, CA) with the Taq DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems). Forward and reverse sequencing primers were provided by Invitrogen with the transfer vectors. The pMelBac C construct contained the TCV N gene in frame and is referred to as pBacTCV.

Viral RNA extraction, RT-PCR, and cloning procedures were performed according to methods previously described (3,20).

Transfection. SF-9 cells were cotransfected with linearized AcMNPV DNA (Bac-N-Blue™ DNA; Invitrogen) and pBacTCV DNA (18). Briefly, a 60-mm tissue culture plate was seeded with 4×10^6 SF-9 cells; cells were allowed to attach for 1 hr at room temperature. In a sterile 1.5-ml microcentrifuge tube, 0.5 μg linearized AcMNPV DNA was incubated with 4 μg purified pBacTCV DNA, 1 ml Grace's insect medium (Invitrogen) without supplements or FBS, and 20 μl of Insectin-Plus™ liposomes (Invitrogen) for 15 min at room temperature. Medium was removed and cells were carefully rinsed with Grace's insect medium without supplements or FBS. The entire transfection mixture was added dropwise to the cells and incubated for 4 hr at room temperature. After incubation, 1 ml of supplemented Grace's insect medium with 10% FBS and gentamicin (10 μg/ml) was added to the plate, and cells were incubated for 4 days at 27 C.

Screening of baculovirus recombinants. Baculovirus recombinants were identified in SF-9 cells as nonrefractive, occlusion-negative plaques under a dissecting microscope (17). Recombinants were purified three times by plaque purification (18).

PCR was used to determine the presence of TCV N gene and absence of contaminating wild-type DNA in the recombinants. Single recombinant plaques were picked with a pipette from agar-overlaid monolayers of infected cells, placed in 1 ml of supplemented Grace's insect medium, vortexed briefly, and incubated at room temperature for 30 min to allow the virus to diffuse from the plug. An aliquot of the plaque suspension was mixed with an equal volume of cold 20% polyethylene glycol (Sigma Chemical Co., St. Louis, MO) in 1 M NaCl and incubated at room temperature for 30 min. Virus particles were collected by centrifugation (80,000 × *g*, 2 hr), and the pellet was resuspended in water containing 1 mg/ml proteinase K (Gibco BRL) and incubated at 50 C for 1 hr. DNA was extracted with phenol chloroform and resuspended in 10 μl sterile water. Purified DNA was amplified by PCR with recombinant baculovirus primers (Invitrogen). The PCR reaction mixture consisted of 10 μl of PCR buffer (100 mM Tris-HCl, pH 8.85, 250 mM KCl,

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50 mM $[NH_4]SO_4$), 2.5 mM $MgCl_2$, 1 mM each deoxynucleoside triphosphate, 100 ng/μl each forward and reverse primer, 1.5 units Taq polymerase, 5 μl viral DNA, and water to 50 μl. PCR reagents were obtained from Promega (Madison, WI). Samples were placed in a hot (94 C) programmable thermal cycler and amplified as follows: 2 min initial denature at 94 C, 30 cycles of 1 min at 94 C, 2 min at 55 C, 3 min at 72 C, and a final extension of 7 min at 72 C. PCR products were analyzed on a 1% agarose gel.

Recombinant positive plaques were amplified for further analysis (18). Briefly, 50% confluent monolayers of SF-9 cells were infected with recombinant virus at a multiplicity of infection (MOI) of 1 and harvested 4 days postinfection (PI). Cell supernatant was collected and cellular debris removed by centrifugation at 1000 × *g* for 10 min. An aliquot of supernatant (10 μl) was analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE) and western immunoblot analysis; recombinant baculovirus containing the TCV N gene was referred to as rBTCV/N.

SDS-PAGE and western immunoblot analysis. Proteins were analyzed on a 4% stacking, 10% resolving gel by a discontinuous SDS-PAGE system (20). Electrophoresis was performed with the Mini-Protean®II Dual Slab Cell apparatus (BioRad Laboratories, Richmond, CA). Electrophoretic separation of proteins was performed for 1 hr at 200 volts. After SDS-PAGE, proteins were transferred onto a 0.45-μm Trans-Blot® nitrocellulose membrane (BioRad Laboratories) with a Mini Trans-Blot® Electrophoretic Transfer Cell (BioRad Laboratories). Transfer was accomplished in 1 hr at 100 volts in transfer buffer (25 mM Tris, pH 7.5, 192 mM glycine, 20% methanol). Membranes were air dried and incubated for 3 hr at room temperature in block solution consisting of 1% nonfat dried milk in phosphate-buffered saline (PBS). Block solution was decanted and membranes were incubated for 2 hr at room temperature with a 1:125 dilution of TCV (NC95) antiserum in TNT (10 mM Tris-HCl, pH 7.5, 0.5 M NaCl, 0.05% Tween 20) with 1% bovine serum albumin (Sigma Chemical Co.). Membranes were washed for 15 min with four changes of TNT and incubated for 1.5 hr at room temperature with a 1:2000 dilution of peroxidase-labeled goat anti-chicken immunoglobulin G (Kirkegaard and Perry Laboratories, Inc., Gaithersburg, MD) in block solution. Membranes were washed as above and reacted with a solution of 3,3′-diaminobenzidine (Vector Laboratories, Burlingame, CA) for 5–10 min. The reaction was stopped by washing membrane in dH_2O for 10 min.

ELISA. High Five™ cells were infected with rBTCV/N at an MOI of 5 and harvested at 4 days PI. Cell supernatant containing secreted TCV N protein was collected and stored at 4 C after removal of cells and cellular debris by centrifugation at 1000 × *g* for 10 min. Negative control antigen was similarly prepared from mock-infected High Five™ cells.

Optimal concentrations of antigen, positive serum, and peroxidase-labeled goat anti-chicken immunoglobulin G were determined by checkerboard titration as described (5). The rBTCV/N antigen was diluted 1/80 in 0.2 M carbonate/0.2 M bicarbonate buffer, pH 9.6, added to 96-well ELISA plates (ProBind™ Assay Plate, Falcon®; Becton Dickinson and Co., Lincoln Park, NJ), and incubated overnight at 4 C. ELISA plates coated with negative control antigen were similarly prepared. Antigen-coated plates were washed three times with PBS plus 0.05% Tween 20 (PBST), then 200 μl of block buffer (PBST containing 1% nonfat dried milk) was added to each well and incubated for 1 hr at room temperature. Plates were washed three times with PBST, then 75 μl of serum, diluted 1:40 in block buffer, was added to each well and incubated for 30 min at 37 C. Plates were washed three times with PBST, once for 10 min with block buffer and three additional times with PBST. Peroxidase-labeled goat anti-chicken immunoglobulin G was diluted 1:50 in block buffer and 75 μl was added to each well. Plates were incubated for 30 min at 37 C, then plates were washed three times with PBST, once with block buffer, and three additional times with PBST. ABTS (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate (Kirkegaard and Perry Laboratories, Inc.), 100 μl, was added to each well; color development was stopped after 15 min with 1% (w/v) SDS in water. Plates were read on an ELISA reader (BT 2000 MicroKinetics Reader; Fisher Scientific, Norcross, GA) at 405 nm.

Data analysis. ELISA data are presented as the sample absorbance values minus the negative control absorbance values; each data point is the mean of six separate assays. Absorbance of 0.1 was selected as the cutoff for a positive assay; this value is greater than four standard deviations above absorbance values for negative control antisera.

IFAT. TCV antibody titers were determined for each antiserum by an IFAT. Antigen for the IFAT procedure consisted of epithelial cells exfoliated from the bursae of Fabricius of experimentally infected turkeys (10). TCV-infected epithelial cells were spotted onto glass microscope slides, air-dried, and fixed in cold (4 C) absolute acetone for 10 min. Twofold dilutions of antisera were prepared in PBS starting at a 1:20 dilution. Diluted antisera were overlaid onto cells and incubated at 37 C for 15 min. Slides were washed briefly in two changes of PBS, and cells were overlaid with a 1:40 dilution of fluorescein isothiocyanate–labeled rabbit anti-chicken immunoglobulin G (ICN Biomedicals, Inc., Costa Mesa, CA). Slides were incubated at 37 C for 15 min, washed twice with PBS, and examined by epifluorescence.

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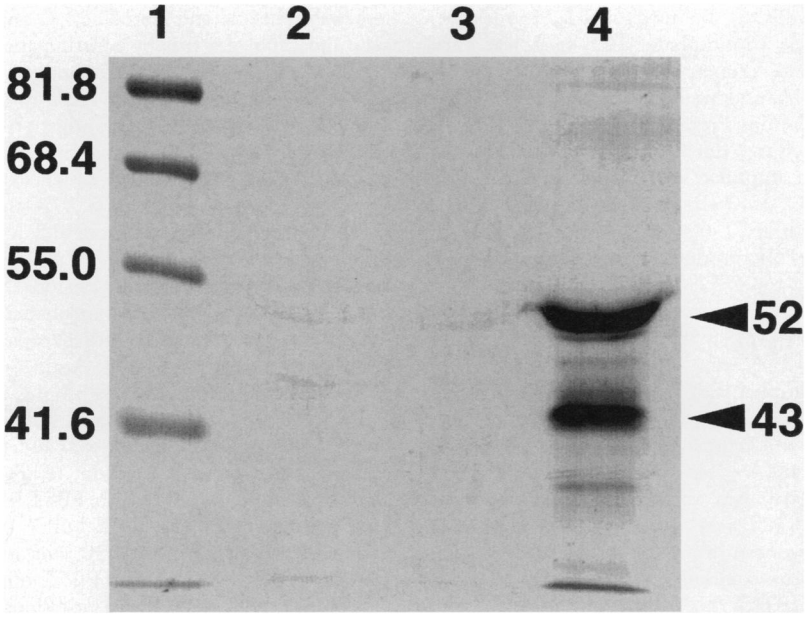

Fig. 1. Western immunoblot of rBTCV/N. Proteins were separated by SDS-PAGE and analyzed by immunoblotting. Lane 1, molecular weight marker expressed in kilodaltons; lane 2, mock-infected SF-9 cell supernatant; lane 3, wild-type baculovirus-infected SF-9 cell supernatant; lane 4, rBTCV/N-infected SF-9 cell supernatant. Recombinant proteins are indicated by arrowheads: 52 kD, 43 kD.

RESULTS

SDS-PAGE and western immunoblot analysis. SF-9 insect cells were infected with rBTCV/N, and proteins expressed by rBTCV/N-infected cells were identified by western immunoblot analysis (Fig. 1). Baculovirus-expressed proteins were detected with antiserum specific for TCV (NC95). Recombinant proteins, 52 kD and 43 kD (Fig. 2, lane 4), were identified. No proteins were observed in samples of mock-infected SF-9 cells or wild-type baculovirus-infected SF-9 cells (Fig. 1, lanes 2 and 3). Additionally, no proteins were observed when staining was performed with negative control (nonimmune) chicken serum (data not shown).

TCV and IBV proteins from preparations of partially purified virus were electrophoretically separated in parallel with rBTCV/N proteins and detected by immunoblot analysis with antiserum specific for TCV (NC95) (Fig. 2). Recombinant p52 was comparable in molecular mass to native TCV and IBV N proteins (Fig. 2). No proteins were observed when immunoblot analysis was performed with negative control (nonimmune) chicken serum (data not shown).

ELISA. Baculovirus-expressed TCV N protein was used as antigen in an indirect ELISA. Antisera prepared against TCV strains (NC95, Minnesota), IBV strains (Connecticut, Massachusetts, Arkansas, JMK), and various other avian viruses were analyzed by the ELISA (Table 1). The TCV N ELISA detected antibodies in all antisera prepared against TCV and IBV strains. Samples were considered positive if their absorbance was ≥ 0.1. Absorbance values for TCV and IBV antisera were all ≥ 0.3. No antibodies (absorbance < 0.1) were detected in antisera prepared against avian reovirus, avian influenza virus, avian adenovirus 1, avian paramyxovirus 3, Newcastle disease virus, and negative control serum.

ELISA titers were compared with titers determined by IFAT (Table 1). IFAT detected antibodies in antisera prepared against TCV strains (NC95, Minnesota) and IBV strains (Connecticut, Massachusetts, Arkansas, JMK). Each IBV- or TCV-specific antiserum had IFAT titers $\geq 1:320$. Antibodies specific for TCV

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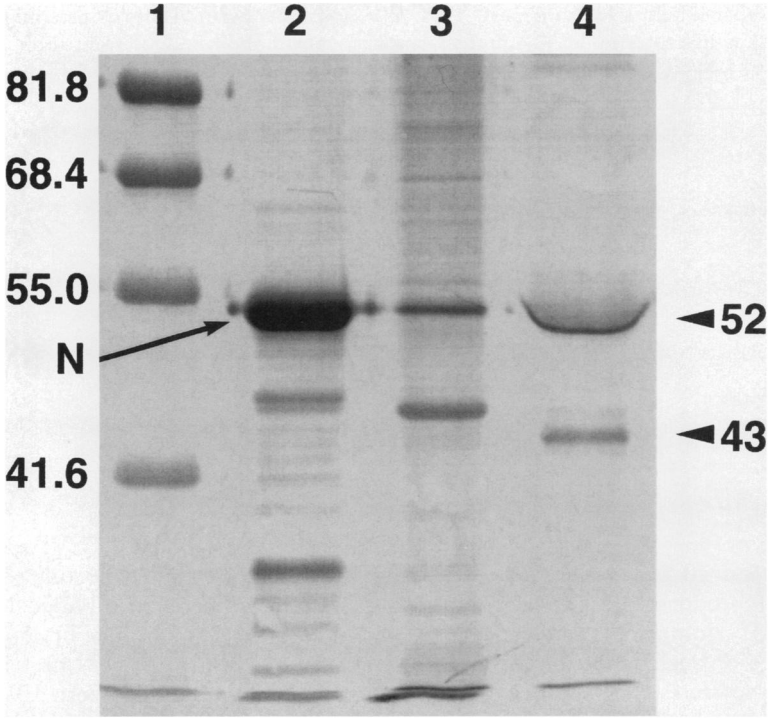

Fig. 2. Western immuoblot of partially purified viral and rBTCV/N proteins. Proteins were separated by SDS-PAGE and analyzed by immunoblotting. Lane 1, molecular weight marker expressed in kilodaltons; lane 2, partially purified infectious bronchitis virus; lane 3, partially purified turkey coronavirus; lane 4, rBTCV/N-infected SF-9 cell supernatant. Recombinant proteins are indicated by arrowheads: 52 kD, 43 kD; location of IBV and TCV N proteins is indicated by arrow.

were not detected in negative control serum or antisera prepared against avian reovirus, avian influenza virus, avian adenovirus 1, avian paramyxovirus 3, and Newcastle disease virus.

DISCUSSION

Baculovirus expression systems have been used extensively to express large quantities of proteins that are antigenically similar to native proteins. Baculovirus-expressed nucleocapsid proteins have been used as antigens in ELISA-based assays for detection of antibodies specific for measles virus, vesicular stomatitis virus, and Newcastle disease virus (1,8,12). In the present study, TCV N gene was expressed in baculovirus and the antigenicity of the expressed protein was evaluated by an indirect ELISA.

The recombinant baculovirus containing TCV N gene, rBTCV/N, produced two TCV-specific proteins (52 and 43 kD). The 52-kD protein was comparable in molecular mass to native TCV and IBV N proteins as determined by coelectrophoresis of recombinant N protein and virion proteins of IBV and TCV. The predicted molecular mass of the TCV N protein was determined to be 45 kD on the basis of sequence information (4); however, the size of native TCV N protein was determined by SDS-PAGE and western immunoblot analysis in this study to be approximately 52 kD. Studies examining the structural proteins of IBV have reported N proteins that range from 51 to 54 kD (6,25,26). The nature of the 43-kD protein has not been determined; however, because this protein is a product of rBTCV/N and reacts specifically with TCV-specific antibody, it likely is a cleavage product of p52. A 1981 study, which examined viral proteins of mouse hepatitis virus, demonstrated a precursor–product relationship between nucleocapsid proteins p56 and p50; it was determined in this study that p50 was a cleavage product of p56 (7). A similar study, which examined viral proteins of

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Table 1. Absorbance values for antisera to TCV, IBV, and other avian viruses as determined by indirect ELISA with baculovirus-expressed TCV N protein; comparison with indirect fluorescent antibody (IFA) titers.

Antiserum	Absorbance	IFA titer
TCV (NC95)	0.300 ± 0.021	1:640
TCV (Minnesota)	0.421 ± 0.115	1:320
TCV (Minnesota)	0.653 ± 0.082	1:320
IBV (Massachusetts)	1.393 ± 0.108	1:640
IBV (Connecticut)	1.270 ± 0.109	1:320
IBV (Arkansas)	1.035 ± 0.117	1:320
IBV (JMK)	1.402 ± 0.124	1:320
Reovirus	0.020 ± 0.060	<1:20
Adenovirus 1	−0.005 ± 0.019	<1:20
Paramyxovirus 3	0.008 ± 0.006	<1:20
Avian influenza virus	0.026 ± 0.048	<1:20
Newcastle disease virus	0.043 ± 0.028	<1:20
Negative serum	0.016 ± 0.011	<1:20

IBV, reported polypeptides with molecular weights of 42 and 40 kD, which appeared to be degradation products of the nucleocapsid protein p51 (25). Comparable findings of proteolytic cleavage or degradation of the N proteins of paramyxoviruses have also been reported (15,16).

The baculovirus-expressed N protein was successfully used as antigen in an indirect ELISA for detection of TCV-specific antibodies. The ELISA procedure detected N protein-reactive antibodies in antisera prepared against TCV strains (NC95, Minnesota) as well as IBV strains (Connecticut, Massachusetts, Arkansas, JMK). Absorbance values for IBV-specific antisera (1.035–1.402) were substantially greater than those for TCV-specific antisera (0.300–0.653); however, this likely is due to different immunization methods used to produce these different antisera and/or greater antigenic mass with IBV, a virus readily propagated to high titer *in vitro*. Positive IFAT titers correlated with positive ELISA absorbances, but differences in IFAT titers for IBV- and TCV-specific antisera, as observed with absorbance values, were not apparent. This likely is attributable to the relative insensitivity of the IFAT procedure compared with ELISA.

Recent antigenic and nucleotide sequence analyses have demonstrated that TCV and IBV are closely related viruses (3,4,11,24). Antigenic analyses indicated that IBV and TCV were antigenically related on the basis of cross-immunofluorescent studies with both polyclonal and monoclonal antibodies (11). Subsequent studies indicated that IBV and TCV were closely related on the basis of nucleotide sequence analyses of the integral membrane, N, and polymerase genes (3,4,24). The findings of the present study, in which TCV N protein was shown to specifically react with both IBV- and TCV-specific antibodies, provide additional evidence supporting a close antigenic relationship between these viruses.

The detection of both IBV- and TCV-specific antibodies by the TCV N ELISA would appear to be an obstacle to specific detection of TCV infection in turkeys, in that the TCV N ELISA would not discriminate between TCV and IBV infection. However, on the basis of our current understanding of the host range of IBV, this is not an impediment because turkeys are not susceptible to this virus. IBV, like TCV and other coronaviruses, has a limited host range. Chickens and pheasants are the only known natural hosts for IBV; experimental attempts to infect a variety of other avian species, including turkeys, have not been successful (9,23).

The findings of the present study indicate that TCV N protein was successfully cloned and expressed with the baculovirus system. The baculovirus-expressed TCV N protein was shown to be a suitable antigen for ELISA-based detection of TCV-specific antibodies.

REFERENCES

1. Ahmad, S., M. Bassiri, A. K. Banerjee, and T. Yilma. Immunological characterization of the VSV

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nucleocapsid (N) protein expressed by recombinant baculovirus. Virology 192:207–216. 1993.

2. Barnes, H. J., and J. S. Guy. Poult enteritis–mortality syndrome ("spiking mortality") of turkeys. 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. 1025–1031. 1997.

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. Breslin, J. J., L. G. Smith, F. J. Fuller, and J. S. Guy. Sequence analysis of the turkey coronavirus nucleocapsid protein gene and 3′ untranslated region identifies the virus as a close relative of infectious bronchitis virus. Virus Res. 65:187–193. 1999.

5. Carpenter, A. B. Enzyme-linked immunoassays. In: Manual of clinical laboratory immunology, 4th ed. N. E. Rose, E. C. de Macario, J. L. Fahey, H. Friedman, and G. M. Penn, eds. American Society for Microbiology, Washington, DC. pp. 2–9. 1992.

6. Cavanagh, D. Structural polypeptides of coronavirus IBV. J. Gen. Virol. 53:93–103. 1981.

7. Cheley, S., and R. Anderson. Cellular synthesis and modification of murine hepatitis virus polypeptides. J. Gen. Virol. 54:301–311. 1981.

8. Errington, W., M. Steward, and P. T. Emmerson. A diagnostic immunoassay for Newcastle disease virus based on the nucleocapsid protein expressed by a recombinant baculovirus. J. Virol. Methods 55:357–365. 1995.

9. Gough, R. E., W. J. Cox, C. E. Winkler, M. W. Sharp, and D. Spackman. Isolation and identification of infectious bronchitis from pheasants. Vet. Rec. 138:208–209. 1996.

10. Guy, J. S. New methods for diagnosis of turkey coronavirus infections. In: Proc. 49th North Central Avian Disease Conference and Symposium on Enteric and Emerging Diseases, Indianapolis, IN. pp. 8–10. 1998.

11. 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.

12. Hummel, K. B., D. D. Erman, J. Heath, and W. J. Bellini. Baculovirus expression of the nucleocapsid protein gene of measles virus and utility of the recombinant protein in diagnostic enzyme immunoassays. J. Clin. Microbiol. 30:2874–2880. 1992.

13. Ignjatovic, J., and L. Galli. Structural proteins of avian infectious bronchitis virus: role in immunity and protection. Adv. Exp. Med. Biol. 342:449–453. 1993.

14. Laude, H., and P. S. Masters. The coronavirus nucleocapsid protein. In: The Coronaviridae. S. G. Siddell, ed. Plenum Press, New York. pp. 141–163. 1995.

15. Mountcastle, W. E., R. W. Compans, L. W. Caliguiri, and P. W. Choppin. Nucleocapsid protein subunits of simian virus 5, Newcastle disease virus, and Sendai virus. J. Virol. 6:677–684. 1970.

16. Mountcastle, W. E., R. W. Compans, H. Lackland, and P. W. Choppin. Proteolytic cleavage of subunits of the nucleocapsid of the paramyxovirus simian virus 5. J. Virol. 14:1253–1261. 1974.

17. 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.

18. O'Reilly, D. R., L. K. Miller, and V. A. Luckow. Baculovirus expression vectors: a laboratory manual. W. H. Freeman and Company, New York. 1992.

19. Patel, B. L., B. S. Pomeroy, E. Gonder, and C. E. Cronkite. Indirect fluorescent antibody test for the diagnosis of coronaviral enteritis of turkeys (bluecomb). Am. J. Vet. Res. 37:1111–1112. 1976.

20. Sambrook, J., E. F. Fritsch, and T. Maniatis, eds. Molecular cloning, a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. 1989.

21. 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.

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

23. Spackman, D., and I. D. R. Cameron. Isolation of infectious bronchitis virus from pheasants. Vet. Rec. 113:354–355. 1983.

24. 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.

25. Stern, D. F., L. Burgess, and B. F. Sefton. Structural analysis of virion proteins of the avian coronavirus infectious bronchitis virus. J. Virol. 42: 208–219. 1982.

26. Stern, D. F., and B. M. Sefton. Coronavirus proteins: biogenesis of avian infectious bronchitis virus virion proteins. J. Virol. 44:794–803. 1982.

27. Wege, H., S. Siddel, and V. ter Meulen. The biology and pathogenesis of coronaviruses. Curr. Top. Microbiol. Immunol. 99:165–200. 1982.

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