TURKEY CORONAVIRUS: AN UPDATED REVIEW

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ABSTRACT

Turkey coronavirus (TCoV) causes acute atrophic enteritis and uneven flock growth in turkey farms leading to economic loss. Since 1990’s, turkey flocks have kept experiencing coronaviral enteritis sporadically in the United States, Canada, Europe, and Brazil. Poult enteritis and mortality syndrome (PEMS) caused by the co-infection of TCoV, astrovirus, and other viruses or bacteria resulted in significantly high mortality. Diagnosis of TCoV depends on reverse transcription polymerase chain reaction (RT-PCR), quantitative real-time PCR, immunohistochemistry (IHC), immunofluorescent antibody assay and virus isolation (VI). Genomic organization of TCoV is as follows: 5' UTR-1a-1b-S-3a-3b-E-M-5a-5b-N-UTR 3'. Genomic analysis suggests the emergence of TCoV from infectious bronchitis virus (IBV) through the recombination of spike (S) gene. Both TCoV and IBV belong to species Avian coronavirus in genus Gammacoronavirus and have a single stranded RNA genome with a size about 27 kb. High similarity of S genes has been found between TCoV isolates in contrast to low similarity between IBV strains. TCoV infection induced strong humoral and cellular immune responses, characterized by high levels of antibody and interferon gamma. The fragment containing neutralizing epitopes in the S protein has been identified. Vaccines conferring protection against TCoV have not been developed and used in the fields but live attenuated, killed, DNA, and fowlpox virus vectored vaccines have been generated and their efficacies were evaluated. Molecular epidemiology of TCoV in recent outbreaks sheds more information on the evolution and transmission of TCoV, which will aid in developing effective vaccines or treatment to prevent, control, or eliminate TCoV infection.

Keywords: Turkey coronavirus; Genomic organization; Phylogeny; Pathology; Diagnosis; Immunology; Vaccinology.
INTRODUCTION

In 1951, the outbreak of an “unfamiliar turkey disease” occurred in muddy areas of Washington State, so the outbreak was called “mud fever” or “mud disease”. The disease was characterized by anorexia, wet droppings, ruffled feathers, decreased weight gain, sudden onset, and rapid spread. In 1953, the outbreak of the identical disease was reported in Minnesota. A term, “transmissible enteritis”, was introduced based on its highly transmissible nature. Retrospectively, turkey enteritis caused more than 23% of all turkey mortality in Minnesota between 1951 and 1971.1 In 1971, bacteria-free filtrate from intestinal contents was able to propagate in turkey embryos older than 15 days and reproduce the transmissible enteritis in turkeys.2 In 1973, coronavirus-like particles were observed from filtrated intestinal content by electron microscopy and given the name of turkey coronavirus, TCoV.3 The same particles were also found in the cytoplasms of turkey intestinal epithelial cells under transmission electron microscope (TEM).4 In the 1970s, eradication efforts were began in Minnesota by controlled depopulation and decontamination with a rest period before restocking, leading to successful elimination of the virus from Minnesota turkey flocks by 1976.5 In 1980s, the outbreaks of TCoV happened in Quebec, Canada and caused significant economic loss to the poultry industry.6

Major outbreaks of turkey coronavirus enteritis occurred in Indiana and North Carolina in early 1990s.7 TCoV is also one of several pathogens associated with poult enteritis and mortality syndrome (PEMS), characterized with spiking and excess mortality and identified in North Carolina during the 1990s. In 2000, another outbreak of turkey coronavirus enteritis happened in United Kingdom and TCoV was isolated from the intestinal contents of the infected turkeys.8 In 2004, 17 turkey flocks suffered acute enteritis in Brazil. Of which, 14 flocks were tested positive for TCoV and 7 were tested positive both for TCoV and turkey astrovirus by reverse transcription polymerase chain reaction (RT-PCR).9,10 TCoV have still continued to cause mild to severe enteritis in the turkey flocks in Missouri, Arkansas, Indiana, North Carolina, USA and Ontario, Canada nowadays.11,12

Physical Properties and Morphology of TCoV

TCoV can be purified from the intestinal content, intestinal tissue, or bursa of Fabricus of TCoV-infected turkeys by using sucrose density gradient centrifugation.13,14 From Naqi’s study, two very closely placed opalescent bands with a density of 1.16 to 1.17 g/mL with maximum light absorption at 254 nm were yielded after ultracentrifugation.15 From Lin’s study, two opalescent bands corresponding to slightly different buoyant densities of 1.14 to 1.15 g/mL and 1.18 to 1.20 g/mL were yielded, respectively.14 Electron microscopy revealed the particles of various sizes and shapes, mainly spherical shape, surrounded by a fringe of regularly spaced petal or pear-shaped surface projections about 12 nm long, like a solar corona. The diameter, including the spikes of the smallest and largest particles, ranged from 55 to 220 nm.13,14 The internal component appeared as a helix with a diameter of 7 nm. Under electron microscopy, the band of higher density are composed of intact virus particles.14 By using Sephacryl S-1000 chromatography, the integrity of the virus can be kept but the method is not suitable for processing of large number of samples.15 An improved or new method to purify virus with intact structure, particularly S protein, is still desired.

Classification of TCoV

TCoV is the second Avian coronavirus recognized other than infectious bronchitis virus (IBV) but the classification of TCoV has been controversial. The studies using immune electron microscopy, hemagglutination inhibition (HI) and virus neutralization (VN) assays showed that IBV and TCoV were antigenically distinct from each other and the mammalian CoV, so IBV and TCoV comprised group 3 and 4 CoV, respectively.3,6 In 1990s, the findings of immunoblotting, immunoprecipitation, VN, HI assays, DNA hybridization, and genome sequence analyses on human colorectal adenocarcinoma cell (HRT-18)-adapted TCoV suggested a close antigenic and genomic relationship between TCoV and bovine coronavirus (BCoV). Thus, TCoV was reclassified into group 2 CoV.16,17 However, similar results could not be repeated by using non-HRT cell-adapted TCoV. Contamination of cell culture system with a group 2 CoV by laboratory error was implied.18 Recent studies showed that IBV and TCoV shared a close antigenic and genomic relationship. Immunofluorescence antibody (IFA) assay showed that polyclonal antibodies against IBV and monoclonal antibody to membrane (M) protein of IBV could react strongly against TCoV while polyclonal antibodies against IBV and TCoV could not recognize porcine transmissible gastroenteritis virus (TGEV, group 1 CoV) or BCoV (group 2 CoV) and vice versa.7,19 Although Guy’s group failed to recognize IBV by using polyclonal antibodies specific for TCoV, Lin’s group detected antibody to TCoV by using IBV antigens coated antibody-capture enzyme-linked immunosorbent
assay (ELISA). Phylogenetic analyses of TCoV and IBV based on whole genome sequence, polymerase gene, M gene and nucleocapsid (N) gene demonstrated that TCoV is closely related to IBV genetically and both TCoV and IBV are located in the same phylogenetic lineage or cluster. Only in phylogenetic tree constructed by spike (S) gene, the IBV isolates clearly fell outside the cluster of TCoV isolates. In addition to genomic evidence, monoclonal antibodies specific for S protein of IBV group failed to recognize TCoV antigens by IFA assay indicating that TCoV is a unique group 3 coronavirus but not a variant of IBV. The most updated taxonomy of coronaviruses according to the International Committee of Taxonomy of Viruses (ICTV) has classified previous groups into four genera of alpha-, beta, gamma-, and deltacoronavirus. Now TCoV and IBV belong to species Avian coronavirus in genus Gammacoronavirus.

Genomics of TCoV

The full-length genomic sequences of several TCoV isolates, including TCoV/IN-540/94 (EU022525; 27,749 nt), TCoV/MN-ATCC (EU022526, 27,816 nt), TCoV/Canada-MG10 (EU095850; 27,632 nt), TCoV/VA-74/03 (GQ427173; 27,771 nt), TCoV/TX-GL/01 (GQ427174; 27,619 nt), TCoV/IN-517/94 (GQ427175; 27,665 nt), and TCoV/TX-1038/98 (GQ427176; 27,782 nt), have been determined. All TCoV isolates shared the similar genome organization starting from one untranslated region (UTR) at the 5' end, followed by two open reading frames (ORFs), ORF 1a and 1b, resided in the first two thirds of the genome. The rest one-third of genome at the 3' end was composed of genes encoding for major structure proteins: S, envelope (E), M, and N proteins, and genes encoding for accessory or hypothetical proteins, like gene 3 and gene 5. The order was S-3a-3b-E-M-5a-5b-N from 5' to 3' end and followed by 3' UTR and poly (A) tail. The consensus transcription-regulating sequence (TRS) for S (CTGAACAA), gene 3 (ATGAA- CAA), M (CCTAACAA), gene 5 (CCTAACAA), and N genes (ATTAACAA) was found in all sequenced TCoV isolates and highly conserved at the levels of nucleotide sequence and the distance between TRS and the initiation codon of individual genes.

The presence of gene 3 and gene 5 was unique for Gammacoronavirus because neither of them was found in Alpha- or Betacoronavirus. Three more putative ORFs were predicted in the genome of TCoV/IN-540/94 and TCoV/MN-ATCC by the ORF finder at NCBI. ORFs 4b and 4c came immediately after M gene and ORF 6b followed N gene with 8 nucleotides apart. ORF 4b, designated as ORF-X by Gomaa, contained 282 nt and was predicted to code for a protein of 94 amino acids that had no significant homology to any protein sequence available in the databases. However, the nucleotide sequence of ORF 4b (X) was highly conserved among TCoV isolates and IBV strains. While a conserved sequence, GUCAACAA, found 288 nt upstream of the initial codon for ORF X within the M gene, was suggested to be possible TRS for ORF X by Gomaa. Cao predicted ORF 4b and another small ORF 4c shared the same TRS with M gene. ORF 6b can be found in TCoV/TX-1038/98, TCoV/TX-GL/01, and TCoV/VA-74/03, but not in TCoV/Canada-MG10 and TCoV/IN-517/94. Moreover, the predicted size of ORF 6b was 74 aa in TCoV/IN-540/94, MN-ATCC, and TX-GL/01 but the predicted size of ORF 6b was only 32 aa in TCoV/TX-1038/98 and VA-74/03. There were no ORF 4c and 6b found in TCoV/Canada-MG10. Northern blotting with 32P-labeled PCR probe specific to the N gene on the total RNA isolated from the small intestine of turkey infected with TCoV/IN-517/94 revealed 7 RNA bands can be assigned to genomic RNA, defect interfering (DI) RNA, subgenomic RNA (sgRNA) for expression of S, E (3), M, 5, and N proteins based on the location of TRS on the genome and the predicted sizes of genomic and sgRNA. It implied that ORF 4b (X) shared the same sgRNA with M gene instead of using its own sgRNA for translation. Further experimental confirmation is required to determine whether there are proteins produced from ORF 4b (X), 4c and 6b, and their biochemical and biological functions.

TCoV polyprotein (pp) 1a and pp1ab via -1 frame shift translation from ORF 1 were predicted to be processed to 15 non-structure proteins (nsp2 to nsp16), with nsp1 missing. Comparison of amino acid sequences of each nsp of TCoV with those of other coronaviruses implied the enzymatic activity of nsp as follows: nsp2, replicase; nsp3, papain-like proteinase (PLpro); nsp5, 3C-like proteinase (3CLpro); nsp8, nsp9, and nsp10, RNA binding activity; nsp12, RNA-dependent RNA polymerase (RdRP); nsp13, helicase; nsp14, exoribonuclease; nsp15, endoribonuclease; and nsp16, 2'-O-methyltransferase. According to Cao's experiments in vitro, TCoV nsp15 is a U-species Nidovirales endonuclease (NendoU), which can cleave single-stranded RNA, hairpin RNA, pRNA, and double-stranded RNA but slowly. No divalent metal ion was required and disulfide bond was essential for in vitro enzymatic activity of the TCoV nsp15. More studies are needed for the characterization of other non-structural proteins of TCoV.

The alignments of the whole genome sequences of TCoV isolates and other Gammacoronavirus showed...
three major clades. TCoV isolates form a monophyletic group in the same clade with group of IBV strains supported by the fact that the percent similarity between the TCoV and IBV strains for the full-length genomes are greater than 86%. A single virus SW1 from a beluga whale separates into a second clade. Munia CoV (MuCoV HKU13), thrush CoV (ThCoV HKU12), and bulbul CoV (BuCoV HKU11) make up a third distinct lineage, which was assigned to genus Deltacoronavirus now. Phylogenetic analysis of the protein sequences for S, M, RdRp, N, 3CLpro, and helicase indicated that SW1 from the beluga whale and thrush CoV do not cluster with the other viruses or with each other for all of the proteins except S protein. For the S protein, the IBV strains clearly fall outside the cluster of TCoV viruses. It is supported by the 90% similarities for the S proteins among TCoV isolates whereas less than 36% between TCoV and IBV. The amino acid identities of the S1, S2, and N proteins between TCoV and IBV were around 21%, 50%, and 88%. The nucleotide sequence similarity of the entire 3’-end structural protein-encoding region between TCoV and IBV was about 70% and the differences were mainly contributed by S gene. Comparative genomics of TCoV isolates and IBV strains by SimPlot program revealed two major recombination events, one in the 3’-end structural protein-encoding region between TCoV and IBV, and another one in the 3’-end of gene 1ab and one in the 3’ end of S, suggesting the possible emergence of TCoV from IBV from the recombination of S gene.

**TCoV Serotype**

A total of 18 TCoV isolates collected from the outbreaks of TCoV enteritis between 1994 and 1999 from different geographic regions of the United States shared the positive antigenic reactivity pattern to the polyclonal antibodies to the TCoV Indiana (517), Virginia (1002), Minnesota ATCC isolate or the IBV Massachusetts strain as well as the monoclonal antibodies to the TCoV North Carolina isolate or the M protein of IBV. The close antigenic relationship is consistent with the high similarity of the deduced amino acid sequence, 92.8% to 99.3%, found in the S gene of 22 TCoV isolates collected from different geographic regions (unpublished data). Four genetic groups were observed in the sub-branch of the full genomic sequence of TCoV isolates with TCoV/TX-GL/01 and TCoV/TX-1038/98 grouping together, TCoV/Canada-MG10 and TCoV/VA-74/03 forming a second group, TCoV/IN-517/94 and TCoV/IN-540/94 making up a third group, and TCoV/MN-ATCC alone in a fourth group. All viral genomes share at least 92.4% similarity and within group similarity ranges from 96.0% to 97.0% at the nucleotide level. Based on the results of cross-detection and cross-protection studies, isolates TCoV/MN-ATCC, TCoV/IN-517/94, and TCoV/VA-1002/97 could not be distinguished and were considered as the same serotype. However, real-time RT-PCR-based serum neutralization tests revealed that isolates TCoV/VA-74/03, TCoV/TX-1038/98, and TCoV/IN-517/94 could not neutralize each other and should be recognized as three different serotypes of TCoV, consistent with three antigenic groups based on the genomic sequences. Considering many serotypes found in IBV, there should be more than one serotype in TCoV. However, more than 90% of nucleotide homology in S genes shared among TCoV isolates, indicate TCoV isolates have high genetic and antigenic relatedness and they are possible to be in the same serotypes. Further studies are needed to clarify and identify the serotypes within TCoV isolates and elucidate the correlation among the different isolates and serotypes, their geographic locations, time of isolation, and disease outcomes.

**Detection and Differentiation of TCoV**

The diagnostic methods for detecting TCoV include virus isolation (VI), immunohistochemistry (IHC), indirect IFA, RT-PCR, multiplex RT-PCR and quantitative real-time RT-PCR. Without proper cell culture system for TCoV, the VI has to use 22-day-old turkey embryonated eggs for propagation of TCoV, which is time consuming and labor intensive. Therefore, antibody-based methods, IFA for frozen tissues and IHC for formalin fixed embedded-paraffin tissues, are most efficient and economic tools to diagnose the infection of TCoV by detecting the TCoV antigen. With the available sequences of TCoV, RT-PCR and multiplex RT-PCR can be used to detect and differentiate TCoV and other pathogens associated with PEMS. In addition, real-time RT-PCR can detect and quantify TCoV RNA in tissues and feces rapidly and specifically.

**Pathology and Pathogenesis of TCoV Enteritis**

Turkey coronavirus enteritis caused by TCoV can occur at different ages but the severity of clinical disease varies by different TCoV isolates and the age of turkey. Severe clinical signs and gross lesions were observed in one-day-old but not four-week-old turkeys infected with
TCoV VR-911 isolate. For TCoV/Canada-MG10 Canada isolate, both two-day-old and four-week-old turkeys showed clinical signs and gross lesions. The incubation time of disease may vary from 1–5 days, but typically is 2–3 days. The spread of disease within the flock can cause high morbidity close to 100% with various mortality rates depending on the age of the birds, concurrent infection, management practices, and weather conditions. The major economic losses resulting from TCoV infection are primarily due to poor feed conversion rate, uneven flock growth, rapid drop of egg production in laying hens, and poor quality of chalky eggs lacking normal pigmentation. Affected turkeys usually show depression, ruffled feathers, watery diarrhea, dehydration and decreased weight gain due to the decreased consumption of water and feed. The major gross lesions are flaccid, pale, and thin-walled intestines with watery contents. Villous atrophy, villous epithelial desquamation, cryptic epithelial cells amplification, and catarrhal enteritis with hemorrhage can be seen microscopically. The villus-to-crypt ratios remained depressed for 10 days after infection due to the shortened length of villous and the increased depth of crypt. The apical portions of intestinal villi and the epithelium of the bursa of fabricius are the most common areas where TCoV antigen can be detected by IFA or IHC. Stunted and uneven growth among TCoV-infected turkeys mainly result from diarrhea, malabsorption, and maldigestion due to TCoV-induced destruction of villous epithelium, the damage and disturbance in the physiology of the infected cells, and the alterations of the intestinal normal flora.

Poult Enteritis and Mortality Syndrome

Turkey coronavirus was associated with astrovirus, small round virus, E. coli, and probably other unconfirmed infectious agents in producing PEMS in turkeys between 1 and 4 weeks of age. The syndrome was characterized by diarrhea, growth depression, immune dysfunction, and significantly high mortality. Two manifestations of PEMS were “spiking mortality of turkeys” and the less severe “excess mortality of turkeys.” The major debate was whether TCoV alone can cause mortality and clinical responses similar to those of natural PEMS. The experimental studies of TCoV VR-911 and TCoV/Canada-MG10 isolates showed that TCoV itself can cause similar symptoms to PEMS while the TCoV NC-95 only caused mild disease but produced severe disease and high mortality (79%) when poulets were co-infected with TCoV NC95 and an enteropathogenic E. coli (EPEC). TCoV infection predisposed young turkeys to secondary EPEC infection and enhanced the pathogenicity of EPEC because no or mild disease was observed with the single infection of high concentration of EPEC and the most severe diseases occurred when the turkeys exposed to TCoV seven days before the infection of EPEC compared to the simultaneous infection of TCoV and EPEC. It was suggested that TCoV was the major causative pathogen for TCoV enteritis and the secondary infection of other opportunity microorganisms will enhance the severity of TCoV enteritis and PEMS.

Host of TCoV

Turkeys are believed to be the only natural host for TCoV. Pheasants, sea gulls, coturnix quail, and hamsters were resistant to TCoV infection. A coronavirus sharing 79% to 81% amino acid identity with TCoV isolates was discovered in the intestinal content of European quail (Coturnix coturnix) reared in a farm in Italy in 2005. It was unclear whether the isolated quail coronavirus (QCoV) was a variant of TCoV or quail was a natural host to TCoV, but QCoV appeared to be “TCoV-like” virus because of the relatively high sequence conservation in the S1 protein between QCoV and TCoV. Chickens experimentally inoculated with TCoV did not show clinical signs or gross lesions but susceptibility to TCoV infection. TCoV was detected by RT-PCR in the intestinal content and pooled gastrointestinal tract of the infected chickens. In Gomes’s study, TCoV can be detected by IHC and RT-PCR in the pooled tissue specimens of nasal concha, Harderian gland, and paranasal sinus. However, the horizontal transmission of TCoV did not occur among chickens. In addition, TCoV could not be maintained by consecutive passages of TCoV in chickens for more than three times.

TCoV Shedding

By using IFA, TCoV antigen was detected in the intestinal epithelium for up to 28 days after experimental infection of 10-day-old turkey poult with TCoV/IN-540/94. Strong IFA responses to TCoV were observed from one to seven days post-infection (dpi). The intensity of IFA signals to TCoV declined from 14 to 28 dpi. According to the experimental infection study with TCoV VR-911 isolate, the TCoV antigen was detected by IFA and immuno-peroxidase assay in the intestines of the infected turkeys up to 35 dpi, by VI
assay in the intestinal content up to 42 dpi, and by RT-PCR in the fecal dropping up to 49 dpi. Shorter period of shedding time up to 14 dpi for the TCoV/Canada-MG10 and TCoV VR-911 isolates were observed as determined by RT-PCR. The relatively brief period of clinical signs, TCoV antigen in the intestines, and viral shedding in most TCoV-infected turkeys may explain why TCoV was not detected in the fecal samples collected from commercial turkey farms showing no signs of diseases.

**Epidemiology of TCoV**

TCoV has been isolated from turkey farms in Minnesota, Indiana, North Carolina, South Carolina, Georgia, Missouri, Arkansas, Texas, Virginia, and Pennsylvania in the USA, as well as Canada, Brazil and Europe, but little is known about the seroprevalence of TCoV in the field. ELISA-based method is more suitable for high-throughput field survey than IFA and VN assay for detecting serum antibody level to TCoV. Several ELISAs have been developed by using whole IBV, recombinant N protein from *Escherichia coli* or baculovirus, or recombinant S1 protein (amino acid residue 54 to 395 or 482 to 678) from *E. coli*. Shown in Table 1, the seroprevalence of TCoV determined by TCoV N-based ELISA was 79% of breeder and 60% of meat turkeys in Ontario of Canada and 64.2% in turkey farms in Arkansas. By TCoV S1-based ELISA, positive prevalence of TCoV antibodies was 71% of breeder and 57% of meat turkeys in Ontario of Canada. From the survey in Indiana in 1999, the positive prevalence of TCoV antibodies in field serum samples was 53.8%.

**Table 1. Seroprevalence of TCoV by Different Detection Methods.**

<table>
<thead>
<tr>
<th>Methods</th>
<th>Breeder Turkeys</th>
<th>Meat Turkeys</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCoV-N-based ELISA</td>
<td>79%</td>
<td>60%</td>
<td>Ontario, Canada</td>
</tr>
<tr>
<td>TCoV-S1-based ELISA</td>
<td>71%</td>
<td>57%</td>
<td>Ontario, Canada</td>
</tr>
<tr>
<td>TCoV-N-based ELISA</td>
<td>NA</td>
<td>64.2%</td>
<td>Arkansas, USA</td>
</tr>
<tr>
<td>IBV-based ELISA</td>
<td>NA</td>
<td>50.2%</td>
<td>Indiana, USA</td>
</tr>
<tr>
<td>TCoV-based IFA</td>
<td>NA</td>
<td>53.8%</td>
<td>Indiana, USA</td>
</tr>
</tbody>
</table>

Note: N: nucleocapsid protein; S1: amino-terminal spike protein; ELISA: enzyme-linked immunosorbent assay; IBV: infectious bronchitis virus; IFA: immunofluorescent antibody assay; NA: data not available.

Transmission and Control of TCoV

It is believed that TCoV is transmitted mainly by the fecal–oral route because of the shedding of TCoV in feces. The materials or vehicles contacting turkeys or their feces are potential sources of TCoV to infect other susceptible turkeys, including insects like Panzer beetle (*Alphitobius diaperinus*) and houseflies (*Musca domestica*). The strict biosafety protocols and pest control are the most basic methods to prevent TCoV infection. There is no specific treatment for TCoV enteritis but antibiotics are used to control the secondary infection or co-infection of bacteria. Vaccine is also not available for the prevention and control of turkey coronaviral enteritis. Depopulation followed by thorough cleaning and disinfection of house and equipment is still the preferred method used to eliminate TCoV infection from the infected farms.

Adaptive Immunity

Turkeys that survived the infection of TCoV in the early age were resistant to subsequent challenge with TCoV at the older age because no clinical signs were observed and no TCoV were detected in the intestine and feces by IFA or RT-PCR. Since the measurable antibody to N or S1 proteins of TCoV was detected in the serum of the infected turkeys, it was suggested that the infection of TCoV could induce protective antibody responses. The TCoV S1 or N protein-specific antibody can be detected by TCoV N or S1 protein-based ELISA as early as 7 dpi and kept increasing till 42 dpi, the end of experimental infection study of TCoV/Canada-MG10. The serum IgG to TCoV was markedly increased from 21 dpi and remained high till 63 dpi. The level of serum IgM peaked at 7 dpi, declined at 14 dpi, and became non-measurable after 21 dpi. The level of IgA in serum to TCoV was very low but detectable from 7 to 21 dpi. The serum antibodies to TCoV can be detected from 9 to 160 dpi by IFA but only up to 30 dpi by immunodiffusion assay. The TCoV-specific secretory antibodies IgA in the intestine and bile were detectable by immunodiffusion assay or IFA up to six months after the infection. The kinetics of TCoV-specific IgA antibody responses determined by ELISA in duodenum, jejunum, and ileum were similar: gradually increased from one week post-infection (pi), reached the peak at three or four week pi, and declined afterward but
still measurable at nine week pi. While the IgA responses in feces reached the peak at three week pi and no measurable level of IgA was detected in feces after four week pi, the serum IgA were initially detected at one week pi, reached the peak at three or four week pi, and were still measurable at nine week pi.\textsuperscript{63}

Cellular immune responses to the infection of TCoV were evidenced by positive antigen-specific turkey lymphocyte proliferation and turkey IFN-\textgamma bioassay. The significantly higher lymphocyte proliferation responses to the stimulation of T cell specific mitogen, concanavalin A (ConA), or TCoV were detected in the peripheral blood and spleen lymphocytes from TCoV-infected turkeys as compared to those from non-infected turkeys at 3, 14, 28, and 63 dpi, respectively.\textsuperscript{60} The increased lymphocyte stimulation index was detected six months after infection with TCoV.\textsuperscript{64} By IFN-\textgamma bioassay, recombinant TCoV N protein stimulated spleen lymphocytes from TCoV-infected turkeys to secrete significantly (\(p < 0.01\)) elevated turkey IFN-\textgamma determined by significantly (\(p < 0.01\)) higher concentration of nitrite released from activated chicken macrophages as compared to those from non-infected turkeys at 3 and 7 dpi.\textsuperscript{65}

### Passive Immunity

Although there are some success to treat or prevent the coronaviral infection, like SARS-CoV or TGEV,\textsuperscript{66,67} by passive inoculation of protective antibodies, the turkeys received antiserum against TCoV by subcutaneous or intraperitoneal route and were not protected from sequential challenge of TCoV.\textsuperscript{1} The poults hatched from the eggs lay by the layers recovered from TCoV infection did not process the maternal antibodies that could provide protection against TCoV.\textsuperscript{68}

### Vaccine Development

Vaccines conferring protection against TCoV infection have not been developed for commercial use since depletion remains the major method to control TCoV infection. The protection provided by previous homologous viral infection showed the potential of live attenuated vaccine to prevent and control TCoV infection.\textsuperscript{59} Killed vaccine injected parentally did not produce a detectable immunity.\textsuperscript{59} DNA vaccine encoding TCoV N protein stimulated antigen-specific cellular immune responses revealed by lymphocyte proliferation and turkey IFN-\textgamma production and low level of antibody responses to TCoV N protein.\textsuperscript{65} By priming DNA and boosting with recombinant TCoV N protein encapsulated in immunostimulatory complexes (ISCOMs) or by the co-expression of TCoV N protein and turkey calreticulin (CRT) gene by DNA vaccine, the level of antibody to TCoV N protein was increased and vaccinated turkeys were partially protected against TCoV challenge.\textsuperscript{65} Recombinant fowlpox virus expressing N or S1 protein of TCoV (rFPV-N or rFPV-S1) was generated by the transfection of fowlpox virus-infected QT-35 cells with fowlpox virus vector carrying the TCoV N or S1 gene.\textsuperscript{55} The rFPV-N or rFPV-S1 was plaque-purified and the viral titer was determined by plaque titration in QT-35 cells. Significant higher antibody responses were elicited two weeks after two doses of rFPV-N, rFPV-S1, or the co-infection of both rFPVs with two week interval by wing web route. Spleen lymphocytes proliferation stimulated by TCoV S1 or N protein was observed 10, 14, and 28 days after the inoculation of rFPV-N. Significantly increased concentrations of nitrite were detected by IFN\textgamma bioassay in TCoV N protein stimulated spleen lymphocytes 28 days after the inoculation of rFPV-N but not rFPV-S1. Partial protection was produced by the reduced number and intensity of TCoV-specific fluorescent cells in the intestines of the vaccinated turkeys after the challenge of TCoV.\textsuperscript{55} In addition, the fragment containing neutralizing epitopes of S protein of TCoV identified in the carboxyl terminal S1 and amino terminal S2 subunits has been used for the development of a recombinant vaccine.\textsuperscript{69} By using DNA-prime protein-boost strategy, DNA vaccine encoding the identified fragment containing neutralizing epitopes of TCoV S protein, mixed with polyethyleneimine (PEI) and sodium hyaluronate (HA), induced humoral immune response and provided partial protection of turkeys against infectious challenge by TCoV.\textsuperscript{70} Nevertheless, development of efficient and efficacious vaccines to protect turkeys against TCoV infection is still critically warranted.

### CONCLUSION

Turkey coronavirus has been identified since 1950s and characterized in the last 40 years. There was not much knowledge gained about TCoV until the application of molecular biology and recombinant protein expression technology in the last 15 years because of the difficulty to grow TCoV in any cell culture system. Phylogenetic analysis places TCoV in species Avian coronavirus of genus Gammacoronavirus along with IBV. Turkey coronavirus infection can cause enteritis in all ages of turkeys, especially in young poults, and a spiking mortality and morbidity in the case of PMES often occur after co-infection of TCoV and other opportunistic...
pathogens, like astrovirus and E. coli. Strong humoral and cellular immune responses are induced by infection with TCoV. Many aspects of TCoV are still unclear and require further studies, including the discovery of a suitable cell culture system for the isolation and growth of TCoV, purification for TCoV with intact structure, elucidation of antigenic relationships among different TCoV isolates to define serotyping, classification of protective immunity upon exposure to TCoV, transmission of TCoV contributing to the outbreaks, correlation between the seroprevalence of TCoV in the field and the outbreak of turkey coronaviral enteritis, and most importantly, the development of effective and efficacious vaccines for complete protection of turkeys against TCoV infection. One of the approaches that can be considered for vaccine development is to induce mucosal immunity by using neutralizing epitope-based DNA vaccines. Another practical approach is to prime embryonic turkey eggs with DNA vaccine in ovo and boost young turkey pouls with the corresponding recombinant protein. It is important to keep performing surveillance and researching pathogenesis of TCoV and the knowledge gathered will be useful for the effective prevention and control of the outbreak of TCoV.

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