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Review

Infection with Avian Coronaviruses: A recurring problem in turkeys

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Abstract

Turkey coronavirus (TCoV) is a *Gammacoronavirus* causing acute contagious enteritis in young turkeys, leading to impaired growth, low feed conversion, and increased mortality. The TCoV infections, in association/combination with other enteropathogenic viruses, bacteria and protozoa, are associated with poult enteritis-mortality syndrome (PEMS) in turkeys of 1-4 weeks age. In this review, classification and genotyping of TCoV, the implications of its recombination, and challenges to develop efficient vaccines against are discussed. Though TCoV is monophyletic with infectious bronchitis virus (IBV) with a sequence similarity of ⩾86, however a classification scheme gathering all avian coronaviruses (ACoVs) is not established. Based on the nucleocapsid gene, ACoVs are classified into five clades. Clades 1 and 2 (chickens), clade 3 (pigeon) clade 4 (duck), and clade 5 (goose). The Spike (S) gene of ACoVs has shown exceptional lability of being easily switched with multiple recombination events suggesting that TCoV maybe an IBV recombinant. Recombination events altered the pathogenicity, host specificity, and tissue tropism of TCoVs. Attempts to develop attenuated, inactivated, DNA, and virus-vectored vaccines are ongoing. Experimentally, the attenuated TCoV strains induced strong humoral and cellular immune responses and completely protected against the homologous challenge but not heterologous TCoV challenge. Meanwhile, genetically engineered vaccines, either DNA or virus vectored vaccines, are limited with either late induction of a protective immune response and/or inability of the elicited antibody to neutralize virus infection and protect against virus challenge. Future research should focus on improving vaccine efficiency against TCoVs by developing more immunogenic vaccines, determining the appropriate dosing regimens, and include potent adjuvants.

Keywords: Turkey coronavirus, TCoV, Turkeys, PEMS, Immunity, Vaccines

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Introduction

Turkey Coronaviruses (TCoVs) causes acute contagious enteritis in young turkeys and is associated with impairment in growth, poor feed conversion, and high mortality rates (Guy et al., 2000; Saif et al., 2020). TCoVs were first isolated in the USA in 1951, causing a disease known as "Mud Fever". Twenty years later, TCoVs were identified as coronaviruses causing Bluecomb disease (Adams and Hofstad, 1971). Recently the disease was described as coronaviral enteritis of turkeys (Cavanagh et al., 2001; Saif et al., 2020).

TCoVs belong to the family *Coronaviridae*, genus *Gammacoronavirus* and subgenus *Igacovirus* that also include infectious bronchitis (IBV), TCoVs, and guinea fowl coronavirus (GfCoV). Other ACoVs within the subgenus *Igacovirus* include duck coronavirus (DCoV) and pheasant coronavirus (PhCoV) (Chen et al., 2013a; de Wit and Cook, 2020) (Figure 1). The ACoV species (IBV, TCoV, and GfCoV) have shown high identities of the replicase (a and b), envelope (E), membrane (M), and nucleocapsid (N) genes (90% nucleotide sequence identity). However, the spike gene 1 (S1) of the ACoV is divergent, with only ⩾57% differences in nucleotide sequence identity suggesting that they may have a common ancestor (Gomaa et al., 2008; Miłek and Blicharz-Domańska, 2018; de Wit and Cook, 2020). The whole-genome alignments of ACoV with other *Gammacoronaviruses* revealed that TCoV is monophyletic with IBV with a sequence similarity of ⩾86 (Woo et al., 2009). This current review was directed to discuss

Family	*Coronaviridae*					
Genus	*Gammacoronavirus*					
Subgenus	*Igcavirus*					
Species	*Avian Coronaviruses* (ACoV)					
Subspecies*	IBV	TCoV	GfCoV	DCoV	PhCoV	PCoV
Host range						

* Infectious Bronchitis (IBV), Turkey Coronavirus (TCoV), Guinea fowl Coronavirus (GfCoV), Duck Coronavirus (DCoV), Pheasant Coronavirus (PhCoV), Pigeon Coronavirus (PCoV).

Figure 1: Turkey coronaviruses (TCoVs) in domestic birds.

TCoV classification and the recently introduced proposals for classification and genotyping. Additionally, we will shed light on the implications of the recombination events of TCoV on TCoV pathogenicity. Finally, challenges related to the development of efficient vaccines against TCoV are highlighted.

TCoV morphology and structure

Coronaviruses are roughly spherical and pleomorphic enveloped viral particles with a diameter of approximately 50 to 200 nm. They have characteristic petal-shaped spikes on their surface responsible for their crown-shaped morphologic appearance observed under the electron microscope (Dea et al., 1989).

Genome organization and viral proteins of TCoV

The TCoV genome is a positive sense linear single-stranded RNA with a 5' cap and poly (A) tail at the 3' end. Two large open reading frames (ORFs) occupy the proximal 2/3 of the genome. They are involved in polyprotein processing, genome replication, and subgenomic RNA synthesis, while the remaining one-third of the genome codes for structural proteins. The two large ORFs encode polyproteins 1a (pp1a) and 1b (pp1b), then ORF S, 3a, 3b, E, M, 4b, 4c, 5a, 5b, N, and 6b. ORF1 consists of two overlapping ORFs (ORF1a and ORF1b) translated into 1a and 1a/1b polyproteins by a ribosomal frame-shifting mechanism. ORF1a encodes two proteases: papain-like cysteine protease (PLP) and picornavirus 3C-like chymotrypsin protease (3CLP). Both proteases cleave the polyproteins into at least 16 cleavage products (Gomaa et al., 2008; Saif et al., 2020). The virus particle comprises four major structural proteins, including the highly variable S glycoprotein, the conserved M, N, and E proteins. The S protein demonstrates higher variability among coronaviruses, while M and N proteins are more conserved among coronaviruses of different antigenic groups. The S protein has distinctive peplomers on the viral surface that contains neutralizing and group-specific epitopes and is involved in viral entry by receptor-mediated virion attachment to the host cell (Casais et al., 2003) and tissue tropism of ACoVs (Wickramasinghe et al., 2014).

The S protein consists of two subunits, S1 and S2; S1 is responsible for virus attachment, while S2 is responsible for initiating the fusion and internalization of the virus with the host cell. The binding capability of respiratory coronaviruses is dependent on 2,3-linked sialic acids on host tissues (Wickramasinghe et al., 2015). A novel glycan-binding receptor (poly-LacNAc) was found in TCoV, GfCoV and quail coronavirus (QCoV) for binding of the S protein to alimentary tissues and is expressed predominantly on the intestinal epithelium of various avian species such as chicken, turkey, guineafowl, quail, Canada goose, graylag goose, partridge, pheasant, teal and pigeon (Ambepitiya Wickramasinghe et al., 2015). However, the TCoV S1 protein has shown a higher affinity for LacNAc than the GfCoV and QCoV S1 proteins. This difference in avidity is not clear; however, it was previously linked to the virulence and the persistence of TCoV in the field. Meanwhile, phenotypic differences of ACoVs are observed in terms of causing enteritis with relatively low mortalities compared to higher mortalities in GfCoV and QCoV infections (Ambepitiya Wickramasinghe et al., 2015).

TCoV infection, disease and immunity

The TCoVs have the potential to affect turkeys of all ages. According to the severity of the infection, morbidity can exceed 100%, and mortality can range from 10% to 50% or more. Young poults are the most susceptible. The virus is epitheliotropic, and the main sites for replication are intestinal villi and bursa of Fabricius (Adams et al., 1970; Nagaraja and Pomeroy, 1997). Pheasants, seagulls, Coturnix quail, and hamsters are refractory to the infection (Saif et al., 2020).

Up to date, reports of related TCoVs in chickens are next to none, but this may be due to diagnostic flaws that prevent accurate detection. Experimentally, TCoV/Brazil/2006 (acc.no.FJ188401) was detected in the respiratory tissue of infected chickens (Gomes et al., 2010). The first report of natural infection with TCoVs described the isolation of two TCoV strains in Trinidad and Tobago. The two viruses (18RS/1461-9- acc.no.

MN699606 and 18RS/1461-10- acc. no. MN699607) showed 96, and 97% identity, respectively, with TCoV isolate PA/640/02 (acc.no. KF652235) (Brown Jordan et al., 2020). However, the authors declared that the infection is likely to be an incidental finding. Additionally, a *Gammacoronavirus* strain (ahysx-1 strain, acc. no. MK142676) with S gene like North American TCoVs was isolated from apparently healthy commercial chickens in Anhui Province, China (Wang et al., 2020).

Epidemiology

The virus was firstly isolated from North America, and then it spread to South America, Europe, and Australia (Jindal et al., 2014; Saif et al., 2020). The TCoVs are transmitted horizontally, and there is no evidence for vertical transmission through embryonated eggs from infected turkey breeders. Insects such as Adult *Alphitobius diaperinus* and Domestic houseflies such as *Musca domestica Linnaeaus* may harbor the TCoVs infection mechanically (Calibeo-Hayes et al., 2003). Compared to IBV, which may show clinical signs after 18-36 hours, the incubation period for TCoV ranges from 2 to 15 days, with the most common being 2-3 days. This delay may be because IBV and TCoV have different tissue or host tropism (Jackwood and Wit, 2020; Saif et al., 2020). A recent study discovered that TCoV could spread rapidly in specific pathogen-free (SPF) birds where infected birds could transmit the virus within 2.5 hours to a new susceptible host and within 24 hours to contact birds with an extended shedding for 6 days up to weeks. However, airborne transmission of the virus was limited, within 2-3 meters (Brown et al., 2019).

Pathogenesis

Affected turkeys usually show depression, ruffled feathers, watery diarrhea, dehydration, and decreased weight gain due to reduced water and feed consumption (Saif et al., 2020). The primary gross lesions are pale, flaccid, and thin-walled intestines with watery contents. Microscopically, villous atrophy, cryptic cell amplification, epithelial desquamation, and catarrhal enteritis with hemorrhage can be observed. 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 immunohistochemistry and Immunofluorescence assays (IF) (Guy et al., 1997). Stunted growth is observed in TCoV-infected turkeys with diarrhea, malabsorption, and maldigestion resulting from destruction of villous epithelium and alterations in the normal intestinal flora (Naqi et al., 1971; Pomeroy et al., 1978).

Poult enteritis and mortality syndrome

TCoV infections are mainly associated with astrovirus, small round virus, *E. coli*, and probably other unconfirmed infectious agents in producing poult enteritis-mortality syndrome (PEMS) in turkeys of 1-4 weeks age (Barnes and Guy, 1997; Guy et al., 2000; Yu et al., 2000; Ismail et al., 2003). The syndrome is characterized by diarrhea, growth depression, immune dysfunction, and significantly high mortality (Yu et al., 2000). The "spiking mortality of turkeys" and the less severe "excess mortality of turkeys" are the two manifestations of PEMS (Cavanagh et al., 2001; Hafez and Shehata, 2021). The major debate is whether TCoV alone can cause mortality and clinical responses similar to natural PEMS.

Experimental studies of the TCoV ATCC AR-911 and TCoV MG10 isolates had shown similar symptoms like PEMS (Yu et al., 2000; Gomaa et al., 2009b), while TCoV NC-95 produced severe disease with high mortality (79%) when poults were co-infected with an enteropathogenic *E. coli* (EPEC) (Guy et al., 2000). The TCoV infection predisposes young turkeys to secondary EPEC infection. No or mild disease is observed in turkeys infected with a high concentration of EPEC only. In contrast, the most severe cases in turkeys were exposed to TCoV (Pakpinyo et al., 2003). Thus, it has been proposed that TCoV is the leading cause of TCoV enteritis, and secondary infections are induced by other opportunistic microorganisms exacerbating the PEMS (Saif et al., 2020).

Immunity

Turkeys that survived TCoV infection at an early age are resistant to subsequent challenges with TCoV, and no clinical signs in older previously exposed turkeys were observed. Moreover, TCoV has not been detected in the intestine and feces by IFA or RT-PCR (Pomeroy et al., 1975). Measurable antibodies to the N or S1 proteins of TCoV were found in serum of infected turkeys. Therefore, it was hypothesized that TCoV infection might trigger defensive antibody responses (Gomaa et al., 2009c). The TCoV N or S1 protein-based ELISA observed TCoV S1 or N protein-specific antibody as early as seven days post-infection (dpi) and continued to rise until 42 dpi (Gomaa et al., 2009a). TCoV specific secretory IgA antibodies in the intestine and bile were detectable by immunodiffusion assay or IFA (Nagaraja and Pomeroy, 1978, 1980a) up to 6 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 1-week post-infection (PI), reached the peak at 3- or 4-weeks PI, and declined afterward but was still measurable at 9-weeks PI (Gomaa et al., 2009a).

Cellular immune responses to TCoV infection were evidenced by positive antigen-specific turkey lymphocyte proliferation and turkey IFN interferon-gamma (IFN-γ) bioassay. Lymphocyte proliferation responses to stimulation of T-cell specific mitogen, concanavalin A (ConA), or TCoV were significantly higher in TCoV-infected turkeys' peripheral blood and spleen lymphocytes than in non-infected turkeys up to 63 dpi (Loa et al., 2001). Increased lymphocyte stimulation index was detected six months after infection with TCoV (Nagaraja and Pomeroy, 1980b). Using the IFN-γ bioassay, recombinant TCoV N protein stimulated

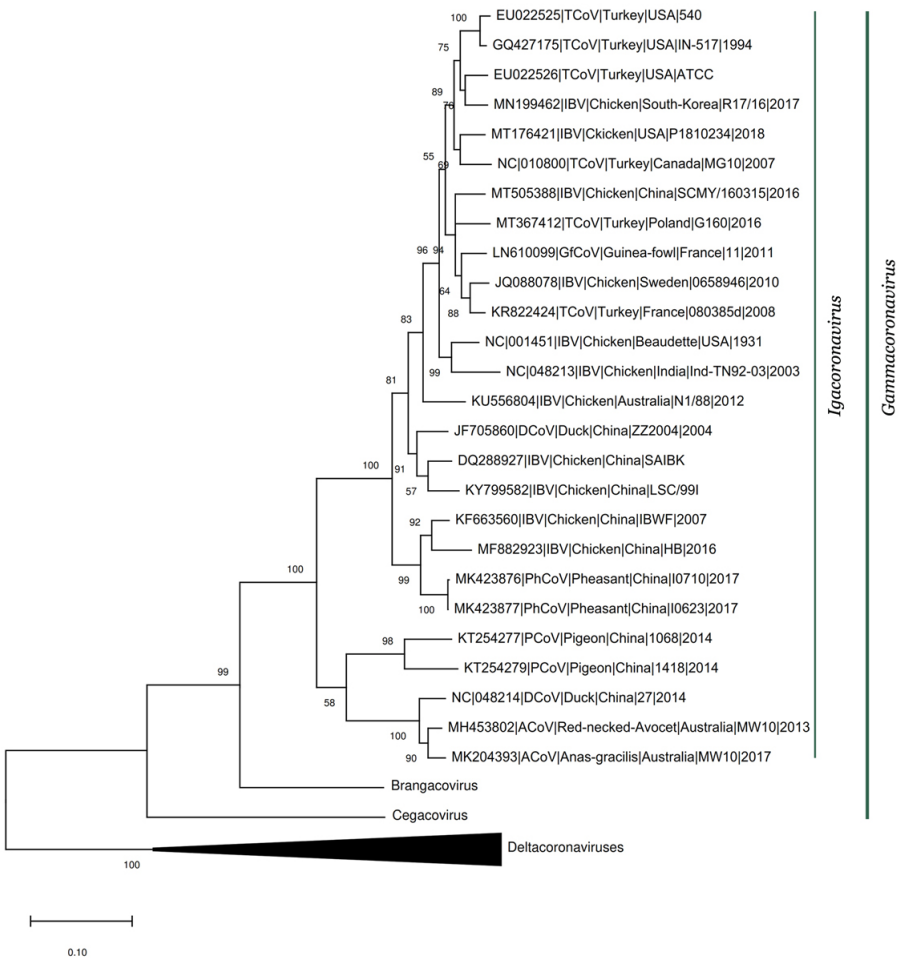

Figure 2: Phylogenetic analysis of the S1 gene of *Gamma-* and *Betacoronaviruses* involved 41 nucleotide sequences. The sequence name comprises accession number, subspecies, host, strain name, and year of isolation. The available S1 sequences in the GenBank were aligned using MAFFT software. Then, the evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model. The evolutionary distances were computed using the Maximum Composite Likelihood method. And the bootstrap test is 1000 replicates, Using the MEGAX software.

spleen lymphocytes from TCoV-infected turkeys to secrete significantly elevated turkey IFN from activated chicken macrophages as compared to those from non-infected turkeys at 3 and 7 dpi (Ababneh, 2005).

TCoV genotyping and serotyping

The IBV ACoVs are divided into seven genotypes based on the complete S1 gene sequences (Valastro et al., 2016; Houta et al., 2021); however, due to the low S1 similarity (57% nt sequence) between TCoV and IBV, it may be difficult to classify them together. ACoVs do not have a classification scheme that can gather all of them into one genotypic relationship, despite some trials to harmonize the classification scheme. Due to the high similarities of the ACoVs N gene, a recent classification scheme was proposed to classify ACoVs depending on the N gene into five clades. Clades 1 & 2 (specific to chickens), Clade 3 (PCoV) Clade 4 (DCoV) and Clade 5 (GfCoV). Clade 1 is classified into five subclades according to region. Interestingly, the 1b3 region in the replicase b gene and their host range is found useful for classifying TCoVs into host-specific clusters (Chen et al., 2013a). It is worth noting that TCoV has never been categorized into clades or subclades based on the S1, N, or 1b3 genes (Chen et al., 2013b). The TCoV could be classified based on whole-genome sequence (WGS) alignments for TCoVs isolated from the USA as shown in (Figure 2).

Phylogenetic analysis revealed four groups as follows; group 1 contains TCoV/TX-GL/01 and TCoV/TX-1038/98, group 2 contains TCoV/MG10 and TCoV/VA-74/03, group 3 contains TCoV/IN-517/94 and TCoV/540/94, and finally, group 4 contains TCoV/ATCC and TCoV/VA-1002/97 (Jackwood et al., 2010). The latter group may be considered a single serotype based on cross-IF and cross-protection studies (Lin et al., 2002). Other studies for serotyping based on real-time RT-PCR and serum neutralization

tests revealed the relatedness values between the different groups to be 2.2 for TCoV/VA-74/03 (Group 2) and TCoV/TX-1038/98 (Group 1), 1.6 for TCoV/VA-74/03 (Group 2) and TCoV/IN-517/94 (Group 3), and 3.1 for TCoV/TX-1038/98 (Group 1) and TCoV/IN-517/94 (Group 3). The relatedness values suggest that the TCoV viruses are not serologically related (Jackwood et al., 2010). Like IBV, serotyping of TCoV is difficult due to the complexities of ACoV serotyping schemes standardization and the recombination events.

Recombination of ACoV

The S gene of ACoV has demonstrated a remarkable potential to be quickly replaced with an analog counterpart from other donors (Domanska-Blicharz and Sajewicz-Krukowska, 2021). In Europe, researchers found that European turkey and guinea fowl coronaviruses share a similar genetic backbone, indicating that they recombined in two different events with unknown ACoVs (an ancestor of Italian IBV/2005) from which they acquired their spike-3a genes (Brown et al., 2016; Wang et al., 2020). A pool of such genes is circulating widely in North and South America, Europe, and Asia (Jackwood et al., 2010; Moura-Alvarez et al., 2014; Ducatez et al., 2015; Wang et al., 2020). These S genes acquired from the recombination events act as a driving force for changes of pathogenicity, host specificity, and tissue tropism for better viral fitness for the new host.

The recombinant Italy/Elvia/2005 strain (genetically related to the North American TCoVs) isolated from diseased quail flocks reared for restocking purposes in 2005 (Circella et al., 2007). Infected adult and young birds reported clinical signs of depression, extreme diarrhea, dehydration, stunted development, and mortality rates of 5-10 and 70%, respectively. However, in experimental studies with the recombinant TCoV strain, disease symptoms ranged from mild to moderate retardation in growth and negligible mortality (Circella et al., 2007). The French GfCoV strains with North American TCoV-like S gene in guinea fowl have been related to the fulminating disease. Infected guinea fowls had acute enteritis, severe prostration, substantial water and feed intake reduction, and up to 20% daily mortalities indicating the recombinant virus is well adapted to guinea fowls (Bouwman et al., 2019). The viruses also induced similar disease symptoms experimentally (Liais et al., 2014). The full-length S gene of some European TCoV revealed 98% nucleotide and 60% to 65% amino acid identities with North American TCoVs. Some IBV-related strains were found in both the North American and French TCoVs, implying that these TCoVs evolved through different recombination events (Maurel et al., 2011).

Advances in TCoV vaccine development

Live attenuated vaccines

Live attenuated vaccines for coronaviruses are developed by multiple serial passaging in susceptible laboratory systems (Ali et al., 2018). The TCoV has not adapted to any cell culture system; therefore, TCoV 540 strain (isolated from Indiana in 1994) was passaged 334 times in embryonated turkey eggs. The mutations in the attenuated strain (P334) genome were mainly in the S2 compared to the S1; meanwhile, 56% of them were silent mutations (Chen et al., 2018). These nucleotide changes were suggested to contribute to reduced infectivity of the P344 strain since minimal amino acid changes in S2 were enough to alter the membrane fusion ability of the S protein and thereby the infectivity of the virus (Fang et al., 2005). The attenuated P334 TCoV 540 completely protected (100%) against challenge with the homologous strain (P334) and partial protection (60%) against challenge with the heterologous strain (P3) (Chen et al., 2018).

Recombinant Vaccine

Fowlpox virus (FPV) is one of the largest double-stranded DNA animal viruses. Recombinant Fowlpox Virus (rFPV) has been used as a viral vector for many vaccines due to its high capacity to tolerate large insertions of foreign DNA, broad host range, ability to insert multiple transgenes, replication of the virus in cytoplasm reduces the risk of random insertions in host's DNA, the inability to produce infection in human, and induction of cell-mediated and/or humoral immunity (Chen et al., 2015). Though both lymphocyte proliferation and IFN- bioassays showed specific N protein stimulation, the positive antisera were not virus neutralizing, and subsequently, weak protection against TCoV infection was observed.

Similarly, an rFPV-expressing S1 gene of TCoV (rFPV-S1) induced a weak and late immune response 28 days after vaccination. There were no significant changes in either splenic lymphocyte proliferation or IFN-γ expression, and protective efficacy was weak against TCoV infection. The low levels of neutralizing antibodies induced by rFPV-S1 were attributed to a probable change in the physical properties of S1 protein and differences in the tissue tropism between FPV and TCoVs that may hinder the ability of rFPV-S1 to elicit complete protection against TCoV infection (Abdelwahab, 2007). The combined use of rFPV-N and rFPV-S1 recombinant vaccines produced weak protection against TCoV infection, evident by a slight decrease in virus staining in intestinal sections from vaccinated groups.

DNA Vaccines

DNA Vaccines are developed from S or N genes under the control of a eukaryotic cell promoter to induce humoral and cellular immune responses as DNA vaccines proven in other studies (Laddy and Weiner, 2006). DNA vaccines immunogenicity had been improved in many ways as co-administration with genes encoding immunostimulatory molecules, targeting DNA toward the proper antigen-presenting cells, applying prime (DNA)-booster (viral vector) strategy, or improving delivery using nanoparticles.

Nucleocapsid-based DNA vaccines

The efficacy of the pTriEX-N gene of the TCoV 540 strain was readily constructed and evaluated in turkeys. The specific (N protein) and non-specific (ConA) stimulation resulted in strong positive proliferation responses in blood lymphocytes and whole diluted blood after booster dose (Ababneh, 2005). The N-protein-based ELISA revealed a low level of antibodies. DNA vaccination based on the N gene generated only weak protection against TCoV infection as evaluated by IFA for TCoV antigen in the intestines of turkeys challenged with either homologous or heterologous isolates. Though DNA vaccination based on the TCoV N gene is insufficient to protect against infection, it may be of use in combination with other types of vaccines in an integrated program due to its capacity to induce cell-mediated immunity (Ababneh, 2005).

Low potency has been recognized as a major constraint for DNA vaccine development. Attempts to address this problem include manipulating the vector backbone (e.g., optimizing gene regulatory elements, optimizing codon usage for the expressed genes, or using cytokines and co-stimulatory molecules as genetic adjuvants) to enhance and direct the immune response to the DNA vaccine antigen were carried out. Immune stimulating complexes (ISCOMs) adjuvants showed a higher capability to increase cell-mediated immune response by stimulating antigen-presenting cells that activate ThI and Th2 responses (Lövgren Bengtsson et al., 2011). Blood lymphocytes from turkeys boosted with the ISCOMs adjuvanted N protein vaccine had enhanced N protein-specific and non-specific (ConA) proliferation responses. The induction of strong cell-mediated immunity by ISCOMs adjuvanted N protein vaccine was also evident as a significant increase in IFN bioactivity for blood lymphocytes. The N-protein specific serum antibody response to the first booster produced detectable antibody levels only in turkeys receiving ISCOMs adjuvanted protein that rapidly increased during the second booster. The N-based subunit DNA vaccine formulated with ISCOMs with a boosting regime increased the protective efficacy, indicating a small but significant effect of the prime boosting strategy (Ababneh, 2005).

The marked enhancement of DNA vaccine potency achieved by coupling recombinant calreticulin (CRT) to antigen is a significant advance in DNA vaccination (Ahmed and Tait, 2020). Vaccination with N-based subunit DNA-CRT constructs significantly enhanced humoral and cell-mediated immune responses to N protein antigen (Kim et al., 2004; Ababneh, 2005); however, the elicited antibody responses were not virus neutralizing probably due to the intracellular location of the N-protein antigen.

Spike based DNA vaccines

The S-based DNAvaccine is constructed from TCoV 540 (EU022525) Strain. The DNA vaccine (pTriEX-4F/4R) is based on the gene fragment (4F/4R) to encode amino acid residues 482-678 of the S gene containing neutralizing epitopes. The vaccine was formulated from a naked DNA plasmid, the polymer nanoparticle disulfide-crosslinked polyethyleneimine (BEI), and polysaccharide-based polyanion sodium hyaluronate complex to improves the gene transfection efficiency of DNA-BEI complex by loosening up the complex (Chen, 2010).

The S protein was also produced, using the same gene fragment in *E. coli* and formulated with complete Freund's Adjuvant (CFA) to booster the DNA vaccine. The developed vaccine induces a robust immune response after priming turkeys with two successive doses at 1 and 7 days of age and boosting with one dose of S-protein and CFA at 21 days of age. Though the vaccine reduced clinical signs (20%) and viral loads in the ileum of infected turkeys, the S gene-based DNA vaccine could not fully protect against TCoV, even after enhancing the immunogenicity with nanoparticles and prime-boost strategy. Further improvements of the DNA vaccine efficacy against TCoV infection including, increased dosages concentration and/or frequency, replacing Freund's adjuvant with more Th1-oriented adjuvant, and co-administrating N and/or M proteins (Chen et al., 2011, 2013a) are being investigated.

Conclusions

TCoV infections remain a leading cause of massive economic losses in young turkeys in many countries worldwide. Classification trials of ACoV failed to establish a standard classification scheme that may gather all TCoVs into one genotypic relationship. Multiple recombination events of TCoV have occurred due to their remarkable ability to transform the S gene, resulting in major changes in pathogenicity, host specificity, and tissue tropism. However, tracing the changes in TCoVs genome is limited with the difficulty of virus propagation in *in-vitro* models. Efforts to develop effective TCoV vaccines using classical (attenuated and inactivated vaccines) and genetic engineering (DNA and FPV virus vectored vaccines) methods failed to induce early and protective humoral and cellular immune responses. The detrimental effects of TCoV on the turkey industry necessitate the development of effective vaccines to combat TCoVs infection in turkeys. Further modifications to improve the vaccine efficacy against TCoVs in turkeys, including increased dosages concentration and frequency, including more potent adjuvants, are recommended.

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