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Short Paper

Diagnosis of poult enteritis complex (PEC) and molecular detection of avian coronaviruses in some commercial turkey flocks in Iran

Kashi, F.[1]; Madani, S. A.[2*]; Ghalyanchilangeroudi, A.[3] and Najafi, H.[3]

[1]DVM Student, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [2]Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; [3]Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

[*]***Correspondence:*** *S. A. Madani, Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: madani@ut.ac.ir*

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Abstract

Background: Enteritis syndromes, also known as poult enteritis complex (PEC) with diverse etiologies, can affect turkey production. An avian coronavirus (AvCoV), turkey coronavirus (TCoV), is one of the most important viral causes of PEC in turkeys. **Aims:** In the present study, the occurrence of PEC and the presence of AvCoV in some commercial turkey flocks were investigated. **Methods:** PEC was diagnosed based on the history, clinical, and necropsy findings. A reverse transcriptase-polymerase chain reaction (RT-PCR) targeting the AvCoV nucleoprotein (N) gene was applied to detect the virus in the tissue samples. Cloacal swabs were collected from 11 flocks without a known history of PEC. **Results:** PEC was diagnosed in six (16.2%) out of 37 investigated turkey flocks. The daily mortality rate in affected flocks ranged from 0.2 to 1.2%. Samples from 8 flocks out of 18 (44.4%) were positive for AvCoV. Four PEC affected flocks were positive for AvCoV. Seven positive samples were sequenced and phylogenetic analysis revealed the close relationship with previously characterized avian infectious bronchitis viruses (IBV). **Conclusion:** The results suggested that PEC should be considered as a significant syndrome in the Iranian turkey industry. According to this preliminary study, it was shown that avian coronavirus infection is prevalent in commercial turkey farms of Iran. However, no causative association could be concluded between PEC occurrence and AvCoV infection in turkey flocks.

Key words: Avian coronavirus, Poult enteritis, Turkey

Introduction

Multifactorial transmissible infectious enteric diseases of young turkey poults are entitled as poult enteritis complex (PEC) (Barnes *et al*., 2000; Johnson and Day, 2020). Several different agents have been identified as the causes of PEC, including turkey coronaviruses (TCoV), turkey astroviruses (TAstV), adenoviruses, rotaviruses, reoviruses, and some bacteria (Barnes *et al*., 2000; Jindal *et al*., 2009; Lojkić *et al*., 2010; Mor *et al*., 2013; Jindal *et al*., 2014; Moura-Alvarez *et al*., 2014).

The most economically important avian coronaviruses (AvCoVs) are infectious bronchitis virus (IBV) and turkey coronavirus (TCoV) (Brown *et al*., 2016; de Wit and Cook, 2020). Turkey coronavirus (TCoV) is closely related to the avian infectious bronchitis virus (IBV) (Cavanagh *et al*., 2001; Brown *et al*., 2016). Formerly, it was known that turkey coronavirus (TCoVs) was the cause of enteritis in young turkey poults and decreased egg production in turkey breeders (Lin *et al*., 2002; Guy, 2020).

Poult enteritis complex (PEC) was noticed anecdotally by poultry veterinarians, but there is no published evidence of the syndromes in their regions. In the present study, the occurrence of PEC in Iranian turkey flocks was observed. Furthermore, using tissue and/or cloacal samples, the presence of avian coronaviruses in turkey farms was also investigated for the first time in the country.

Materials and Methods

Dead birds from 37 different commercial turkey flocks were submitted for necropsy three years, leading to September 2015. The flocks with the history of increased daily mortality (more than 0.1%) and the sign of diarrhea along with characteristic gross pathology of non-specific enteritis at the age of 1-7 weeks was diagnosed as PEC. Cases with characteristic lesions of necrotic enteritis and histomonosis or microscopic findings of any parasitism were excluded.

Fourteen intestinal and cloacal bursa pooled tissue samples were collected from six flocks in compliance with PEC diagnosis. Tissues from two or three carcasses were pooled together. Only a single pooled tissue sample belonged to a 45-day-old flock without PEC. Cloacal swabs were also collected from 285 turkeys belonging to 11 flocks with no known history of PEC. Every 5 swabs were pooled together. Consequently, 57 pooled cloacal specimens and 15 pooled tissue samples were subjected to RNA extraction.

The tissue specimens were grinded and homogenized in 100 µL of phosphate buffered saline (PBS). Cloacal swabs were placed into 300 µL of PBS and vortexed. Total RNA was extracted using SinaPure™ ONE (Sinaclon, Iran).

The complementary DNA (cDNA) was synthesized using the random hexamer primers (Sinaclon, Iran). PCR was performed targeting a 357-bp fragment corresponding to nucleotides 445-801 within the conserved region of the N gene using N103F (CCT GAT GGT AAT TTC CGT TGG G) and N102R (ACG CCC ATC CTT AAT ACC TTC CTC) primers (Loa *et al*., 2005).

Seven positive PCR products were subjected to sequencing (Bioneer, Korea). All the sequences were aligned using the ClustalW with MEGA7 software (Kumar *et al*., 2016). Distance-based Neighbor-Joining trees were constructed using the Tamura-Nei model and 1000 bootstrap replicates.

Results

Six flocks (16.2%) out of 37 investigated cases fulfilled the criteria for PEC. The poult ages of the affected flocks were 5, 7, 21, 24, 30, and 32 days old at the time of presentation. As a long-term follow-up was not performed, it is not possible to determine the overall mortality. The daily mortality ranged from 0.2 to 1.2%. Diarrhea, dirty vent feathers, plantar contamination with fecal materials, cachexia, and dehydration were evident in the affected poults. Catarrhal enteritis characterized by ballooning and increased fluid content, thinning of the intestinal wall, and frothy fluid content of the ceca was eminent in postmortem findings (Figs. 1A-F).

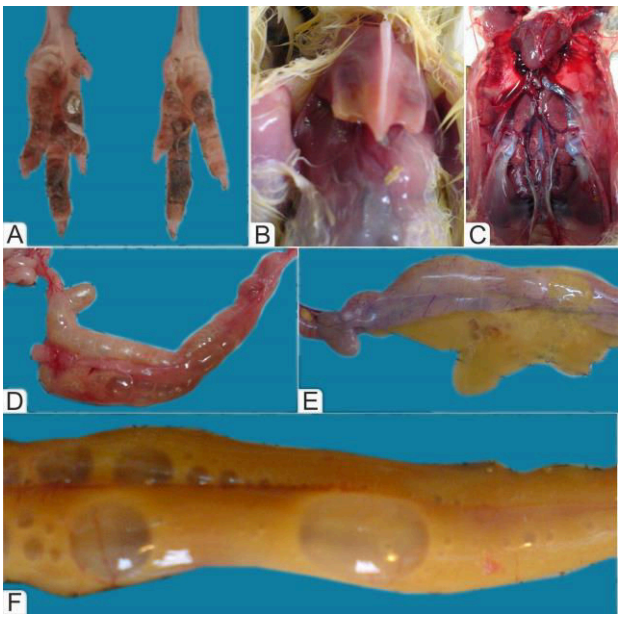

Fig. 1: Pathologic findings of poult enteritis complex (PEC) in young commercial turkeys. **A**: Dirty plantar region in a young turkey due to wet litter caused by diarrhea in the flock, **B**: Pectoral muscle atrophy and cachexia in the affected poult, **C**: Loss of coronary adipose tissue and dehydration associated with nephrosis, and **D-F**: Voluminous frothy fluid content in the thin-walled ceca of the affected poults

Table 1: Commercial turkey flocks sampled to detect avian coronaviruses (AvCoV) in Iran using PCR targeting the nucleoprotein (N) gene. The NCBI accession numbers of sequenced PCR products are shown in this table.

Location (province)	Sample type	Flock age (days)	PEC**	PCR result	Sequenced strain code	Accession No.
Tehran	Tissue*	21	+	+	TCoV9	MN902176
Tehran	Tissue	30	+	+	TCoV10	MN902177
Isfahan	Tissue	5	+	+	TCoV11	MN902178
Tehran	Tissue	32	+	+	Not performed	-
Tehran	Tissue	45	-	-	-	-
Qom	Tissue	7	+	-	-	-
Tehran	Tissue	24	+	-	-	-
Isfahan	Cloacal swab	165	NK	+	TCoV25	MN896935
Isfahan	Cloacal swab	160	NK	+	TCoV44	MN896936
Isfahan	Cloacal swab	65	NK	+	TCoV52	MN896934
Isfahan	Cloacal swab	130	NK	+	TCoV93	MN896937
Isfahan	Cloacal swab	50	NK	-	-	-
Isfahan	Cloacal swab	140	NK	-	-	-
Isfahan	Cloacal swab	26	NK	-	-	-
Isfahan	Cloacal swab	112	NK	-	-	-
Isfahan	Cloacal swab	60	NK	-	-	-
Isfahan	Cloacal swab	60	NK	-	-	-
Isfahan	Cloacal swab	127	NK	-	-	-

* Intestinal and cloacal bursa pooled tissue samples of submitted dead birds, and ** Diagnosis of poult enteritis complex (PEC) based on flock history, daily mortality, clinical and postmortem findings. NK: Not known about previous histories of PEC in flocks with cloacal swab samples

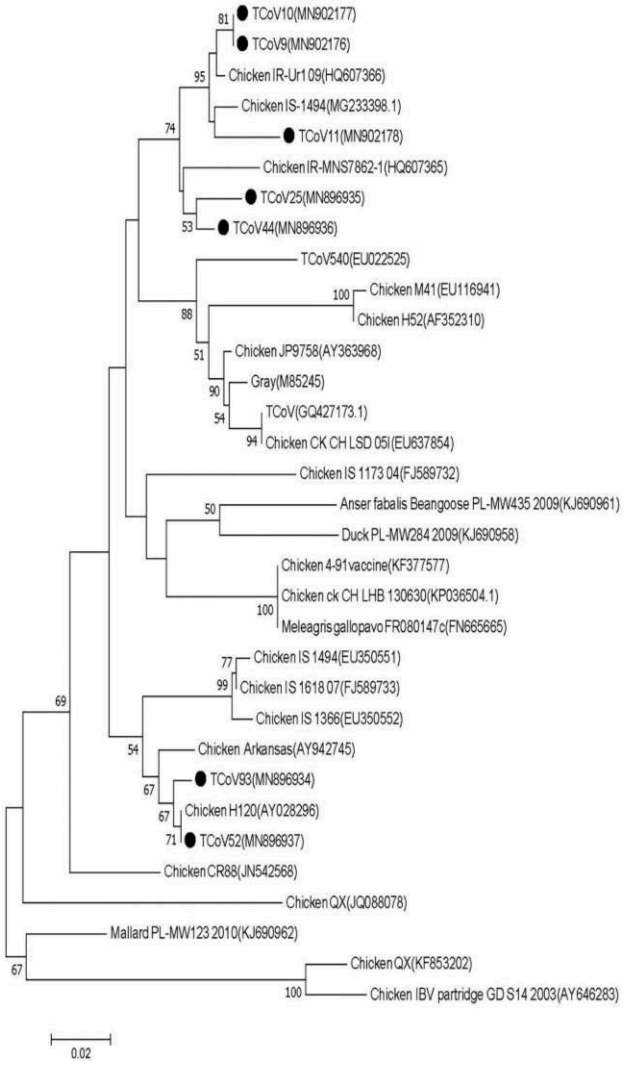

Fig. 2: Phylogenetic tree of the nucleotide sequences of N gene of some avian coronaviruses (AvCoVs) detected from commercial turkey flocks in Iran. Values at the branches and clusters are bootstrap values, and the bar indicates a distance scale from the roots. Viruses from the present study are marked with black circles

Four PEC-affected flocks were positive for AvCoV. The only PEC affected 7-day-old flock from Qom was negative for AvCoV and the other 24-day-old PEC affected and 45-day-old flocks from Tehran province. No signs of PEC have been seen in the latter. Regarding the cloacal swabs, the AvCoV N gene was detected in 4 out of 11 flocks (36.3%). Altogether, 8 out of 18 investigated turkey flocks (44.4%) were positive for the presence of AvCoV.

With an exception for a PEC affected-AvCoV positive flock aged 32 days old from Isfahan, a single PCR product from each positive flock was subjected to sequencing (Table 1). Phylogenetic analysis of the N sequences of seven positive samples revealed that 5 of them (TCoV9, TCoV10, TCoV11, TCoV25, and TCoV44) formed a distinct group from the other two samples (TCoV52 and TCoV93) (Fig. 2). TCoV9 and TCoV10 were identical. Also, the AvCoV strain from a PEC affected flock in Isfahan, TCoV11, shared more than 95% homology to IR-MNS7862-1 IBV strain. The second group comprising TCoV52 and TCoV93 shared 99% sequence similarity. TCoV52, detected in a 65-day-old flock in Isfahan, had 100% and 96% homology with H120 and IS/1494 IBV strains, respectively. The corresponding amounts for TCoV93 from a 130-day-old turkey flock in Isfahan were 99% and 95%, respectively.

Discussion

Poult enteritis complex (PEC) has been diagnosed in six (16.2%) out of 37 commercial turkey flocks from Tehran, Isfahan, and Qom provinces. In the present study, 300 specimens were collected from 18 commercial turkey flocks to evaluate the presence of AvCoV N gene. It was shown that AvCoV infection is prevalent in turkey flocks of Iran and eight flocks (44.4%) were positive. The presence of AvCoV was confirmed in Isfahan and Tehran, while the only investigated flock from Qom was negative.

Enteric diseases in turkey production have been reported in different countries (Carver *et al*., 2001; Barnes and Guy, 2003; Culvar *et al*., 2006; Jindal *et al*., 2009; Lojkić *et al*., 2010; Moura-Alvarez *et al*., 2014; Johnson and Day, 2020). Coronavirus infection is one of the multiple causes of PEC; Various viruses, including rotaviruses, toroviruses, astroviruses, enteroviruses, and picornaviruses might cause PEC (Barnes *et al*., 2000). Further studies are needed to elaborate on the role of pathogens in the incidence of PEC in Iran.

The association of AvCoV with PEC has been well documented in the United States (Barnes *et al*., 2000; Lin *et al*., 2002; Jindal *et al*., 2014), Canada (Dea and Tijssen 1988), England (Cavanagh *et al*., 2001; Culver *et al*., 2006), Brazil (Villarreal *et al*., 2006; Teixeira *et al*., 2007), and France (Maurel *et al*., 2011). To the best of the authors' knowledge, it is the first report of AvCoV infection in turkey flocks in the region. The specimens in the current survey were taken from different ages ranging from 5 to 165 days old and half of the positive flocks were under 32 days old. Six tissue samples in the present study were collected during necropsy of turkeys with typical signs of PEC. AvCoV was not detected in two of them, showing the possible role of other pathogens. There are reports of failure detection of the coronavirus infection in PEC cases in the United States (Pantin-Jackwood *et al*., 2008; Jindal *et al*., 2009; Mor *et al*., 2013), Croatia (Lojkić *et al*., 2010), and Turkey (Ongor *et al*., 2015).

TCoV is closely related to IBV (Guy, 2000; Cavanagh, 2005). TCoV is genetically homologous to IBV, sharing 90% nucleotide homology in the genes downstream of the spike gene (Cavanagh *et al*., 2001). High similarity of TCoV with IBV strains at the nucleocapsid was also reported (Akin *et al*., 2001). The TCoV N protein had more than 90% amino acid identity with the N protein of IBV (Breslin *et al*., 1999). The French TCoV was clustered with European and African IBVs (Brown *et al*., 2016). Such similarities between TCoVs and IBV strains were also observed in our study.

TCoV9, TCoV10, TCoV25, and TCoV44 shared more than 95% sequence identity with IR-MNS7862-1 and IR-UR1-09 (HQ607366), which are chicken IBVs isolated in 2009. TCoV52 and TCoV93 also had 96.1% and 95.2% homology with IS/1494 IBV strain, respectively.

Although the host can be distinguished for different AvCoV detected in various avian species, the differentiation of these coronaviruses from each other is difficult (Guy, 2020; Cavanagh, 2005). Turkeys once seemed insensitive to IBV infection and experimental attempts to infect turkeys with chicken IBV have been unsuccessful (Guy, 2000). Although no vaccine against coronaviruses is currently used in turkey flocks, the vaccine spillover from chicken flocks to turkey flocks could be a possible explanation for the detection of some AvCoV close to vaccine strains like H120.

In conclusion, the present study revealed that coronaviruses are circulating within Iranian turkey flocks. However, further studies are required to prove any causative relationship of this finding with the occurrence of poult enteritis syndromes.

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Conflict of interest

The authors declare that they have no conflict of interest.

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