Detection of Astrovirus, Coronavirus and Haemorrhagic Enteritis Virus in Turkeys with Poult Enteritis Mortality Syndrome in Turkey

Hasan Ongor¹, Hakan Bulut², Burhan Cetinkaya¹, Mehmet Akan³, Sukru Tonbak², Sunil K. Mor⁴ and Sagar M. Goyal⁴

¹ Department of Microbiology, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, Turkey
² Department of Virology, Faculty of Veterinary Medicine, Firat University, 23119 Elazig, Turkey
³ Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, 06110 Ankara, Turkey
⁴ Department of Veterinary Population Medicine and Veterinary Diagnostic Laboratory, University of Minnesota, 1333 Gortner Ave, St. Paul, MN 55108, USA

This study was carried out to investigate the presence of turkey astrovirus 2 (TAstV-2), turkey coronavirus (TCoV) and haemorrhagic enteritis virus (HEV) by molecular methods in cloacal swabs collected from both clinically healthy turkey flocks and those associated with poult enteritis mortality syndrome (PEMS) in Turkey. In the reverse transcriptase polymerase chain reaction (RT-PCR) examination of 230 cloacal swabs collected from 23 turkey flocks associated with PEMS, TAstV-2 was detected in 13.4% (31/230) of the animals and in 43.4% (10/23) of the flocks. In addition, this virus was found in two turkeys originating from one of the four clinically healthy flocks. On the other hand, neither TCoV nor HEV were detected in any of the turkey samples examined in this study. In the partial sequence analysis of four randomly selected DNA samples, 96% nucleotide identity was observed between our strains and reference Turkey astrovirus isolated from turkeys in Italy between 2000 and 2004 (sequence accession number DQ381378.1).

Key words: enteritis viruses, poult enteritis mortality syndrome, PEMS, turkey

J. Poult. Sci., 52: 232–237, 2015

Introduction

Poults enteritis complex (PEC) is a common term that includes all infectious intestinal diseases of young turkeys such as coronaviral enteritis of turkeys, maldigestion syndrome, runting and stunting syndrome of turkeys, poult malabsorption syndrome, poult enteritis and mortality syndrome (PEMS), spiking mortality of turkeys, poult enteritis syndrome (PES) and turkey viral enteritis. (Barnes et al., 2000; Jindal et al., 2014). The complex is characterized by enteritis, moderate to marked growth depression, retarded development, impaired feed utilization; poor feed conversion efficiency, and sometimes increased mortality (Jindal et al., 2014).

A number of viruses (Astrovirus, Calicivirus, Coronavirus, Haemorrhagic Enteritis Virus, Reovirus, Picornavirus, Pico-birnavirus, Enterovirus and Rotavirus), bacteria (Escherichia coli and species of Salmonella, Clostridium, Campylobacter and Enterococcus) and protozoa (coccidia, cryptosporidium) have been detected in PEMS-affected flocks (Barnes et al., 2000; Jindal et al., 2014). Astrovirus is an important cause of enteritis in humans and animals. Turkey astrovirus (TAstV) was first identified in 1980 in the United Kingdom from diarrheic turkey poults (McNulty et al., 1980) and subsequently reported from the United States (where it was named as TAstV-1) (Saif et al., 1985; Reynolds and Saif, 1986). Later, another turkey astrovirus (TAstV-2), genetically distinct from TAstV-1, was identified and characterized (Schultz-Cherry et al., 2000). TAstV-2 isolate (N/96) has been reported to bear many similar features to Human Astrovirus (Koci and Schultz-Cherry, 2002).

The most important infectious disease caused by TAstV is the enteric disease affecting the digestive tract of commercial poults which are reported to result in more economical losses than those affecting any other systems worldwide (Schultz-Cherry et al., 2000; Pantin-Jackwood et al., 2008).

Turkey coronavirus (TCoV) (mud fever, bluecomb diseases, coronaviral enteritis of turkeys) infects turkeys of any age, morbidity being close to %100, and mortality varying from 10 to 50% or more, being the highest in young birds. The virus has been detected only in epithelium of the in-
testinal tract. It was first described in the state of Washington in the 1940s and rose to prominence following outbreaks in Minnesota in 1951. Some 20 years later, it was demonstrated that the causative agent was a coronavirus (Cavanagh et al., 2001). TCoV is also among the pathogens associated with PEMS, and had significant economic importance in the US during the 1990s (Goodwin et al., 1995, Barnes and Guy, 1997). Two manifestations of the syndrome have been described as “spiking mortality of turkeys”, and less severe “excess mortality of turkeys” (Cavanagh et al., 2001).

Only group II avian adenovirus of haemorrhagic enteritis virus (HEV) has economic impact in turkeys. It has been identified in the majority of the countries where turkeys are raised intensively (Arbuckle et al., 1979). HEV is ubiquitous, and most commercial turkeys acquire infection with the virus from the environment. Haemorrhagic Enteritis (HE) is a disease of turkeys 4 weeks of age and older (Pierson and Donermuth, 1997). Clinical outbreaks are characterized by intestinal haemorrhages accompanied with immunosuppression.

When compared with most of the countries worldwide, turkey production in Turkey can be regarded as not very widespread but this is growing sector in Turkey. However, there is little quantitative information about infectious diseases that have significant effect on turkey breeding. In particular, no data are available about the significance of PEMS associated viral enteritis in turkey population of the country. This study was therefore carried out to investigate the presence of TAstV, TCoV and HEV by generic polymerase chain reaction (PCR) assays in cloacal swabs collected from both clinically healthy turkey flocks and those associated with PEMS.

Materials and Methods

Sample Collection

A total number of 270 cloacal swab samples were collected from different turkey flocks belonging a commercial turkey company located in the north of Turkey between April and July 2014. The turkeys were white feathered Californian breeds at the ages ranging from 11 to 119 days.

Of the cloacal swab samples, 230 were from 23 different turkey flocks associated with PEMS and the remaining 40 samples were collected from 4 turkey flocks of clinically healthy turkeys. The samples were originated from a total of 27 different turkey flocks, each flock being represented with 10 samples. Each flock sampled in this study had the capacity of approximately 7000 turkeys.

In the clinical examination of the animals within the flocks, various symptoms such as severe diarrhea, moderate to marked growth depression, retarded development, impaired feed utilization; poor feed conversion efficiency were recorded in 23 flocks which were linked with PEMS. The turkeys in the remaining four flocks seemed to be apparently healthy.

Cloacal swab samples within Stuart Transport Medium were stored in cool boxes and transported to the laboratory within two days. Each swab sample was processed separately.

RNA Extraction

Cloacal swabs were homogenized in one ml phosphate buffered saline (0.01M PBS). Following spinning at 800 g for 10 min, the supernatant was transferred to another microtube and was recentrifuged at the above conditions. Then, the upper aliquot of the suspension was used for RNA and DNA extraction.

Total RNA was extracted directly from 150 μl of the supernatant using EZ-10 Spin Column (Bio Basic Inc., Canada) according to the manufacturers’ instructions. The extracted RNA was resuspended in 50 μl of distilled water containing 0.1 % diethyl pyrocarbonate (DEPC).

DNA Extraction

300 μl of the supernatant was transferred into a microtube and routine procedures were followed to extract the DNA (Cetinkaya et al., 2002).

Reverse transcription- polymerase chain reaction (RT-PCR) Astrovirus

The primers used for the detection of TAstV in this study were evaluated previously (Koci et al., 2000) (Table 1). The screening of the flocks for TAstV was performed by employing a one-step RT-PCR kit (Qiagen, Hilden, Germany) combined with the primer pair of MKPol 10 /MK Pol 11 which produces an approximately 802-bp fragment in positive samples.

One-step RT-PCR was performed in a TC 512 Temperature Cycling System (Techné, Staffordshire, UK) in a

| Gene                      | Primer          | Oligonucleotide sequence (5’-3’ )                       | Fragment Length (bp) | Reference         |
|--------------------------|-----------------|---------------------------------------------------------|----------------------|-------------------|
| Polymerase               | MKPOL10         | TGGCGGCGAACTCTCCAACA                                      | 802                  | Koci et al., 2000 |
|                          | MKPOL11         | AATAAGGTCCTGCACAGGTG                                      |                      |                   |
| Hexon                    | HEV 1F          | TACTGCTGCTATTGTTGTC                                      | 1647                 | Hess et al., 1999 |
|                          | HEV 1R          | TCATTAACCTCCAGCAATTGG                                     |                      |                   |
| UTR                      | UTR 41          | ATGTCTATCGCAGAGGAAATGTC                                    | 266                  | Cavanagh et al., 2001 |
|                          | UTR 11          | GCTCTAACTCTATCTAGGCT                                      |                      |                   |
|                          | UTR 41          | ATGTCTATCGCAGAGGAAATGTC                                    |                      |                   |
|                          | UTR 31          | GGGCGTCAAATGCTGTACCC                                     |                      |                   |

Table 1. Primer sequences and lengths of PCR amplification products
reaction volume of 25 μl. RT-PCR was carried out as one RT cycle at 42 °C for 1 h, followed by enzyme inactivation at 94 °C for 3 min, then 35 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 1 min. and extension at 72 °C for 2 min. The final extension cycle was done at 72 °C for 10 min. The amplified products were detected by staining with ethidium bromide (0.5 mg/ml) after electrophoresis at 80 V for 2 h (7 V/cm) in 1.5% agarose gels.

Coronavirus
A nested PCR was performed for TCoV. Firstly a one-step RT-PCR kit (Qiagen, Hilden, Germany) combined with the primer pair of UTR-41/UTR-11 (Cavanagh et al., 2001) which produces an approximately 266-bp fragment in positive samples was employed. Then, the nested PCR using primer pair of UTR-41/UTR-31 (Cavanagh et al., 2002) which produces an approximately 179-bp fragment in positive samples was conducted.

RT-PCR was carried out as one RT cycle at 45 °C for 1 h, followed by 10 minutes at 72 °C, 94 °C for 3 min for initial denaturation, then repeated 35 cycles of denaturation at 94 °C for 30 s, annealing at 48 °C for 1 min. and extension at 72 °C for 2 min. with a final extension cycle at 72 °C for 10 min. The nested PCR was performed at the above conditions.

Hemorrhagic Enteritis Virus
The primers used for the detection of HEV in this study were also evaluated previously (Hess et al., 1999) (Table 1). The screening of the flocks for HEV was performed by employing PCR combined with the primer pair of HEV 1F/HEV 1R (Hexon gene) which produces an approximately 1647 bp fragment in positive samples. PCR reaction conditions were described by Hess et al., 1999.

Positive and Negative Controls
Infectious bronchitis vaccine H120 (supplied from Bornova Veterinary Control and Research Institute, Izmir) and HE Vaccine (Merial, France) were used as positive controls for TCoV and HEV assays, respectively and distilled water was used as negative controls in all the assays. As we were unable to find a reference strain for Astrovirus at the beginning of the study, four field samples which were detected to be positive for TAstV by both RT-PCR and sequence analysis were used as positive controls during the assays.

Sequence and Phylogenetic Analysis
Four randomly selected RT-PCR products amplified with MKPol 10/MK Pol 11 primers for TAstV-2 were submitted to a sequencing company (Pendik Veterinary Control and Research Institute, Istanbul, Turkey) for partial DNA sequence analysis. The phylogenetic tree of the sample determined as TAstV-2 (2014/TAstV/BL.1) was constructed by using the neighbour-joining method in the PHYLIP sequence program.

Statistical Analysis
A chi-squared test was used to compare the results obtained from PEMS associated flocks and healthy flocks, and at different age groups; P<0.05 was considered statistically significant.

Results
In the RT-PCR examination of 230 cloacal swab samples collected from turkey flocks associated with PEMS, TAstV-2 was detected in 13.4% (31/230) cloacal swabs representing 43.4% (10/23) turkey flocks. In addition, this virus was found in two turkeys originating from one of the four clinically healthy flocks, representing the proportion of 25% and 5% at flock and animal bases (Table 2). The difference between the detection rates obtained from PEMS associated and healthy flocks was not significant (p>0.05). The overall positivity for TAstV-2 was calculated as 12.2% (33/270) and 40.7% (11/27) at animal and flock bases, respectively. When the results were considered according to different age groups, 15 positive samples were detected at the ages ranging from 11 to 28 days (growing phase) in the examination of 50 samples from five flocks. The positivity was obtained from 18 samples at the ages ranging from 29 to 119 days (finishing phase) in the examination of 220 samples from 22 flocks. The differences by age was found to be statistically significant (p<0.05) (Table 2). On the other hand, neither TCoV nor HEV were detected in any of the turkeys examined in this study.

Four randomly selected TAstV-2 positive samples were

| Flock Status | Number of flocks | Number of samples | Age Status (days) |
|--------------|------------------|------------------|------------------|
|              | + N %            | + N %            | 11–28 15 30 16 180 8.9 |
|              |                  |                  | 29–119 29 18 220 8.2 |
| PEMS         | 10 23 43.4       | 31 230 13.4      |                  |
| Healthy      | 1 4 25           | 2 40 5           |                  |
| TOTAL        | 11 27 40.7       | 33 270 12.2      |                  |

*All the samples were negative for TCoV and HEV.
 Each flock had the capacity of approximately 7000 turkeys.
 \( p > 0.05 \)
 \( p < 0.05 \)
† Number of positives
N: Total number
PEMS: Poulteries mortality syndrome
investigated further by partial sequence analysis using the amplicons yielding a specific product in the RT-PCR combined with primers specific for polymerase gene. PCR products were sequenced on both forward and reverse strands, using the ABI 310 Genetic Analysis System. The sequences of four products amplified with RT-PCR in this study revealed 100% nucleotide identity. The sequence identity was between 90% and 96% with the same gene region of TAstV-2 obtained from the GenBank database. The highest nucleotide identity was detected with Turkey astrovirus 2654/04 polymerase gene (sequence accession number DQ381378.1) which was isolated from turkeys in Italy between 2000 and 2004 (Cattoli et al., 2007). The phylogenetic analysis of one sample (2014/TAstV/BL.1, Accession number: KM875548) by the neighbour-joining method in the PHYLIP sequence program confirmed it as TAstV-2 (Fig. 1).

Discussion

There is a paucity of information about the aetiology of PEMS in turkey population of Turkey. Although many studies toward the investigation of PEMS have been reported elsewhere particularly in countries with intensive turkey breeding such as the USA and Brazil (Pantin-Jackwood et al., 2006; da Silva et al., 2009; Jindal et al., 2010; Mor et al., 2013; Moura-Alvarez et al., 2014), to the best of author’s knowledge no study has been conducted on this subject in Turkey so far. The aim of this study was therefore to determine the presence and frequency of TAstV-2, TCoV and HEV in both PEMS-associated and clinically healthy meat-type turkeys.

High proportions ranging from 59% to 100% have been reported for the prevalence of astrovirus in various European countries (Domanska-Blicharz et al., 2014). When com-
pared to the above mentioned proportions, the frequency of TAstV-2 in PEMS-associated turkey flocks was lower with 43.4% in this study. This difference might be due to sample type, age, sampling time or weather conditions. In the present study, the positivity rate obtained from turkeys at growing phase (11–28 days) was significantly higher than that obtained at finishing phase (29–101 days). This is in agreement with the results of previous studies (Pantin-Jackwood et al., 2006). On the other hand, in a study carried out by Domanska-Blicharz et al. (2014) which reported 94.1% for the prevalence of TAstV-2, the highest positivity was reported in turkey flocks at the ages of 5 to 9 weeks.

There is a lack of information about the pathogenicity of astrovirus. Likewise other studies (Pantin-Jackwood et al., 2006; Moura-Alvarez et al., 2013, Domanska-Blicharz et al., 2014), TAstV-2 was detected in a healthy flock in this study, and the difference between the detection rates of this virus from cloacal samples obtained in PEMS associated and healthy flocks was not statistically significant. Also, a weak correlation has been reported between presence of astrovirus and health status of turkey flocks (Domanska-Blicharz et al., 2014). The detection of TAstV-2 in healthy birds has arisen strong suspicion about the presence of strains with different virulence factors (Jindal et al., 2010; Domanska-Blicharz et al., 2011). In a comparative pathogenicity of TAstV-2 obtained from turkey flocks affected with PES and from clinically normal birds, Mor et al. (2011) reported that TAstV-2 from PES birds may be more pathogenic than those from apparently healthy poults. The overall growth depression by TAstV-2 from PES and clinically healthy as compared to control was 16% and 2%, respectively. There is therefore an urgent need to develop effective typing methods in order both to have a better understanding of the pathogenic features and to characterize serotypes of astrovirus circulating in poultry (Guix et al., 2005).

There are two antigenically and genetically different types of astrovirus and, TAstV-2 has been reported at higher proportions in turkey flocks when compared to TAstV-1, with the exception of a few studies such as one carried out in Brazil which reported TAstV-1 at a higher rate (Moura-Alvarez et al., 2013). Therefore, only MKPOL10/MKPOL11 primer pair specific to TAstV-2 was employed to investigate the presence of astrovirus in turkey samples in the current study.

In previous studies, the most frequently detected agent in turkeys with PEMS has been reported as TAstV-2 which was usually coincided in the format of co-infection with other agents rather than individually. It can therefore be commented that the severity of astrovirus infection by only itself would be limited when compared to co-infection with other agents (Saif et al., 1985; Pantin-Jackwood et al., 2008).

A 100% identity was obtained between the four randomly selected positive samples in the phylogenetic analysis which confirmed the isolates of the present study as TAstV-2. In the comparison of the results with the sequence data obtained from GenBank database for polymerase gene revealed the highest nucleotide identity with the strains (sequence accession number DQ381378.1) isolated in Italy between 200 and 2004 (Cattoli et al., 2007). This cluster could be explained by geographical proximity.

Other PEMS associated agents examined here, namely TCoV and HEV, could not be detected in any of cloacal swab samples. There may be a number of plausible explanations for this. Symptoms in turkeys with PEMS could be associated with other agents such as reovirus, rotavirus, small round viruses, parasites or bacteria. Alternatively, the number of samples examined in this study was limited or these agents might have little significance in the turkey population of the country. In a study conducted by Pantin-Jackwood et al., 2008, although the presence of TAstV-2 was reported as 100%, no positivity was detected for TCoV and HEV, similar to our study. On the other hand, the presence of rotavirus and reovirus were reported at the proportions of 69.7% and 45.5%, respectively in the same study. In another study, while TCoV and Group1 adenovirus were not reported in turkeys with PEMS, various rates ranging from 15% to 70% were noted for the presence of astrovirus, reovirus and rotavirus (Jindal et al., 2012). The fact that the investigation of rotavirus and reovirus in PEMS-associated turkeys was beyond the scope of the current study can be interpreted as a deficiency. On the other hand, the presence of TCoV and HEV has been reported at different rates in both PEMS-associated and clinically healthy turkey flocks in various studies (Carver et al., 2001, Culver et al., 2006; Villarreal et al., 2006; Palya et al., 2007; Teixeira et al., 2007; Domanska-Blicharz et al., 2010; Lojkic et al., 2010). It is known that viral enteritis due to the agents associated with PEMS can cause important disorders such as poor feed conversion rate and marked growth depression which result in great economical losses in meat-type turkeys. The absence of effective vaccines for the important agents like TAstV-2 and TCoV is one of the major constraints against the control of PEMS associated viral enteritis. The detection of TAstV-2 in clinically healthy turkeys, as reported in the current study and by other researchers, needs to be clarified. Large-scaled studies are therefore required to obtain comprehensive data which will help us to better understand the etiology of PEMS-cases, to develop effective vaccines and improve biosecurity procedures.

Acknowledgment

The authors wish to thank Mr. O. Bulut, DVM, Mr. I. Kesim DVM, for providing cloacal swabs, Dr. E. Atil for sequence analysis and Prof. Dr. H.M. Hafez for providing HE merial vaccine.

Conflict of Interests: The authors declare that there is no conflict of interest.

References

Aruckle JB, Parsons DG and Luff RP. Haemorrhagic enteritis syndrome of turkeys. Veterinary Record, 104: 435–436. 1979. Barnes HJ and Guy JS. Poult enteritis-mortality syndrome (“spiking mortality”) of turkeys. In: Diseases of Poultry 10th edn.
Carver DK, Vaillancourt JP, Stringham M, Guy JS and Barnes HJ. Mortality patterns associated with poult enteritis mortality syndrome (PEMS) and coronaviruses in turkey flocks raised in PEMS-affected regions. Avian Diseases, 45: 985–991. 2001.

Cattoli G, De Battisti C, Toffan A, Salviato A, Lavazza A, Cerioli A and Capua I. Co-circulation of distinct genetic lineages of astroviruses in turkeys and guinea fowl. Archives of Virology, 152: 595–602. 2007.

Cavanagh D, Mawditt K, Sharma M, Drury SE, Ainsworth HL, Britton P and Gough RE. Detection of a coronavirus from turkey poult in Europe genetically related to infectious bronchitis virus of chickens. Avian Pathology, 30: 355–368. 2001.

Cavanagh D, Mawditt K, Welchman D de B, Britton P and Gough RE. Coronaviruses from pheasants (Phasianus colchicus) are genetically closely related to coronaviruses of domestic fowl (infectious bronchitis virus) and turkeys. Avian Pathology, 31: 81–93. 2002.

Cetinkaya B, Karahan M, Atil E, Kalin R, De Baere T and Domanska-Blicharz K, Seroka A, Lisowska A, Tomczyk G and Minta Z. One-year molecular survey of astroviruses in turkey flocks in the South Eastern Region of Brazil. Brazilian Journal of Microbiology, 40: 248–253. 2009.

Da Silva SE, Bonetti AM, Petrocelli A, Ferrari HF, Luvizotto MC and Cardoso TC. Epidemiological aspects of astroviruses and coronavirus in poult in the South Eastern Region of Brazil. Brazilian Journal of Microbiology, 40: 248–253. 2009.

Domanska-Blicharz K, Seroka A, Lisowska A, Tomczyk G and Minta Z. Turkey coronavirus in Poland-Preliminary Results. The Bulletin of the Veterinary Institute in Pulawy. 54: 473–477. 2010.

Domanska-Blicharz K, Seroka A and Minta Z. One-year molecular survey of astrovirus infection in turkeys in Poland. Archives of Virology, 156: 1065–1072. 2011.

Domanska-Blicharz K, Jacukowicz A, Bocian L and Minta Z. Astroviruses in Polish commercial turkey farms in 2009–2012. Avian Diseases, 58: 158–164. 2014.

Goodwin MA, Brown J, Player EC, Steffens WL, Hermes D and Dekich MA. Fringed membranous particles and viruses in faeces from healthy turkey poult and from poult with putative poult enteritis complex/spiking mortality. Avian Pathology, 24: 497–505. 1995.

Guix S, Bosch A and Pintó RM. Human astrovirus diagnosis and typing: current and future prospects. Letters in Applied Microbiology, 41: 103–105. 2005.

Hess M, Raue R and Hafez HM. PCR for specific detection of haemorrhagic enteritis virus of turkeys, an avian adenovirus. Journal of Virological Methods, 81: 199–203. 1999.

Jindal N, Patnayak DP, Chander Y, Ziegler AF and Goyal SM. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poultry Science, 89: 217–226. 2010.

Jindal N, Chander Y, Patnayak DP, Mor SK, Ziegler AF and Goyal SM. A multiplex RT-PCR for the detection of astrovirus, rotavirus, and reovirus in turkeys. Avian Diseases, 56: 592–596. 2012.

Jindal N, Mor SK and Goyal SM. Enteric viruses in turkey enteritis. Virusdisease, 25: 173–185. 2014.

Koci MD, Seal BS and Schultz-Cherry S. Development of an RT-PCR diagnostic test for an avian astrovirus. Journal of Virological Methods, 90: 79–83. 2000.

Koci MD and Schultz-Cherry S. Avian astroviruses. Avian Pathology, 31: 213–227. 2002.

Lokíč I, Bidin M, Bidin Z and Mikec M. Viral agents associated with poult enteritis in croatian commercial turkey flocks. Acta Veterinaria Brno, 79: 91–98. 2010.

McNulty MS, Curran WL and McFerran JB. Detection of astroviruses in turkey faeces by direct electron microscopy. Veterinary Record, 106: 561. 1980.

Mor SK, Abin M, Costa G, Durrani A, Jindal N, Goyal SM and Patnayak DP. The role of type-2 turkey astrovirus in poult enteritis syndrome. Poultry Science, 90: 2747–2752. 2011.

Mor SK, Sharafeldin TA, Abin M, Kromm M, Porter RE, Goyal SM and Patnayak DP. The occurrence of enteric viruses in Light Turkey Syndrome. Avian Pathology, 42: 497–501. 2013.

Moura-Alvarez J, Chacon JV, Scavnini LS, Nuñez LF, Astolfi-Ferreira CS, Jones RC and Ferreira AJ. Enteric viruses in Brazilian turkey flocks: single and multiple virus infection frequency according to age and clinical signs of intestinal disease. Poultry Science, 92: 945–955. 2013.

Moura-Alvarez J, Nuñez LF, Astolfi-Ferreira CS, Knöbl T, Chacón JL, Moreno AM., Jones RC and Ferreira AJ. Detection of enteric pathogens in Turkey flocks affected with severe enteritis, in Brazil. Tropical Animal Health and Production, 46: 1051–1058. 2014.

Palya V, Nagy M, Glávits R, Ivanics E, Szalay D, Dán A, Suveges T, Markos B and Harrach B. Investigation of field outbreaks of turkey haemorrhagic enteritis in Hungary. Acta Veterinaria Hungarica, 55: 135–149. 2007.

Pantin-Jackwood MJ, Spackman E and Woolcock PR. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Diseases, 50: 397–404. 2006.

Pantin-Jackwood MJ, Day JM, Jackwood MW and Spackman E. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Diseases, 52: 235–244. 2008.

Pierson FW and Domermuth CH. Hemorrhagic enteritis, marble spleen disease, and related infections. In Diseases of Poultry 10th edn. (Calnek BW, Barnes HJ, Beard CW, McDougald LR and Saif YM eds.), pp. 624–663. Ames: Iowa State Press. 1997.

Reynolds DL and Saif YM. Astrovirus: a cause of an enteric disease in turkey poult. Avian Diseases, 29: 728–735. 1986.

Saif LJ, Saif YM and Theil KW. Enteric viruses in diarrheic turkey poult. Avian Diseases, 29: 798–811. 1985.

Schultz-Cherry S, Kapczynski DR, Simmons VM, Koci MD, Brown C and Barnes HJ. Identifying agent(s) associated with poult enteritis mortality syndrome: importance of the thymus. Avian Diseases, 44: 256–265. 2000.

Teixeira MC, Luvizotto MC, Ferrari HF, Mendes AR, Da Silva SE and Cardoso TC. Detection of turkey coronavirus in commercial turkey poult in Brazil. Avian Pathology, 36: 29–33. 2007.

Villarreal LYB, Assayag MS, Brandão PE, Chacón JLV, Bunger AND, Astolfi-Ferreira CS, Gomes CR, Jones RC and Ferreira AJP. Identification of turkey astrovirus and turkey coronavirus in an outbreak of poult enteritis and mortality syndrome. Brazilian Journal of Poultry Science, 8: 131–135. 2006.