VirusDis. (April–June 2014) 25(2):173–185
DOI 10.1007/s13337-014-0198-8

ORIGINAL ARTICLE

Enteric viruses in turkey enteritis

Naresh Jindal · Sunil K. Mor · Sagar M. Goyal

Received: 2 October 2013 / Accepted: 27 January 2014 / Published online: 19 February 2014

Abstract Gut health is very important to get maximum returns in terms of weight gain and egg production. Enteric diseases such as poult enteritis complex (PEC) in turkeys do not allow their production potential to be achieved to its maximum. A number of viruses, bacteria, and protozoa have been implicated but the primary etiology has not been definitively established. Previously, electron microscopy was used to detect the presence of enteric viruses, which were identified solely on the basis of their morphology. With the advent of rapid molecular diagnostic methods and next generation nucleic acid sequencing, researchers have made long strides in identification and characterization of viruses associated with PEC. The molecular techniques have also helped us in identification of pathogens which were previously not known. Regional and national surveys have revealed the presence of several different enteric viruses in PEC including rotavirus, astrovirus, reovirus and coronavirus either alone or in combination. There may still be unknown pathogens that may directly or indirectly play a role in enteritis in turkeys. This review will focus on the role of turkey coronavirus, rotavirus, reovirus, and astrovirus in turkey enteritis.

Keywords Enteritis · Turkeys · Coronavirus · Rotavirus · Reovirus · Astrovirus

N. Jindal (✉)
Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar 125 004, India
e-mail: nareshjindal1@gmail.com

S. K. Mor · S. M. Goyal
Department of Veterinary Population Medicine College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108, USA

Introduction

Several intestinal diseases have been described in turkeys over the years namely, 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, and turkey viral enteritis. All these syndromes have been included in poult enteritis complex (PEC), a general term that describes infectious intestinal diseases of young turkeys [6]. The PEC is an economically important disease of young turkeys characterized by enteritis, diarrhoea, moderate to marked growth depression, impaired feed utilization, poor weight gain, and in some cases, high mortality as in PEMS [6]. A number of viruses (coronavirus, calicivirus, reovirus, astrovirus, rotavirus, picornavirus, picobirnavirus, parvovirus, and adenovirus), bacteria (*Escherichia coli*, *Salmonella* spp., Clostridia, *Campylobacter*, and *Enterococcus*) and protozoa (coccidia and cryptosporidium) have been implicated in PEC [6, 18, 47, 73, 105, 109]. Recently, two new syndromes have been described in Minnesota turkeys namely, the 'poult enteritis syndrome' or PES and 'light turkey syndrome' or LTS. The PES is an infectious intestinal disease of young turkeys between 1 day and 7 weeks of age and is characterized by diarrhoea, depression, and lethargy with pale intestines and/or excessively fluid cecal contents [47]. The LTS is a problem of market age turkeys having lower body weight as compared to their standard breed character [68].We believe that enteritis in poults at a young age sets up conditions for future development of LTS in older birds. In addition, we consider both PES and LTS as a part of PEC.

Enteric viruses are a common cause of primary damage to the gastro-intestinal (GI) tract of young poults thereby providing a conducive environment to bacteria and/or

protozoa to grow, and cause further damage to the gut. It is possible that damage caused to the gut initially by enteric viruses and subsequently by secondary pathogens may lead to irreversible changes to the host, which may lead to decreased body weight at marketing. The purpose of this review is to discuss in detail the four most important viruses causing turkey enteritis namely, coronavirus, reovirus, rotavirus, and astrovirus.

Coronavirus

Coronaviral enteritis of turkey is an acute, contagious disease characterized by dullness, depression, diarrhoea and decreased body weight gain. The disease was first described in the 1940s in Washington State, USA. The causative agent was not identified and the disease was named as 'mud fever'. However, with the identification of turkey coronavirus (TCoV) from these cases, the name was changed to 'coronaviral enteritis of turkeys'. This disease had the most devastating effects in Minnesota turkeys in 1970s. Steps were initiated for disease eradication which led to elimination of this virus from Minnesota. Presently, TCoV is reported sporadically from turkey-producing regions of North America and other countries. The TCoV is also associated with PEMS, which is an acute, highly contagious enteric disease characterized by depression, anorexia, diarrhoea, and high mortality in turkeys.

Genome

The coronaviruses are pleomorphic enveloped particles, roughly spherical, with diameters ranging from 50 to 200 nm. According to the latest proposal of the International Committee on Taxonomy of Viruses (ICTV), the family *Coronaviridae* includes two sub-families, *Coronavirinae* and *Torovirinae*. The *Coronavirinae* comprises of three genera, *Alphacoronavirus*, *Betacoronavirus* and *Gammacoronavirus* [54] of which the latter includes coronaviruses of birds. The coronavirus genome consists of positive sense single stranded RNA. The full-length genome is 27,632 nucleotides plus the 3′ poly (A) tail. There are two open reading frames (ORFs), ORFs 1a and 1b, which reside in the first two-thirds of the genome with nine additional ORFs downstream. The region between the membrane (M) and nucleocapsid (N) protein genes contains three potential small ORFs: (1) ORF-X, (2) a previously uncharacterized ORF with an associated putative TRS within the M gene (apparently shared among all group III coronaviruses), and (3) previously described ORFs 5a and 5b. The TCoV does not contain the gene for hemagglutinin–esterase [29].

Variability in different gene segments of TCoV has been reported. Comparison of sequences of entire 3′-end structural protein encoding region of four TCoV with the corresponding sequences of infectious bronchitis virus (IBV) revealed that TCoV probably shared the same origin with that of IBV and acquired S gene sequences for turkey intestine tropism during the process of evolution [61]. Lin et al. [60] reported that S protein sequence of TCoV had only 33.8–33.9 % of identity with IBV even though both viruses clustered on the same genomic lineage. The full-length spike (S) gene sequences of French origin TCoV shared 98 % identity at both the nucleotide and amino acid levels among themselves [65]. However, these viruses showed 65 and 60 % similarity with North American TCoVs and 50 and 37 % similarity with IBV at the nucleotide and amino acid levels, respectively.

Incidence and distribution

Unlike the generally undefined viral enteric disease syndromes, TCoV-enteritis can be directly attributed to an infection with TCoV [35, 38, 41]. TCoV has worldwide distribution and has been detected in enteritis-affected turkeys from North America, South America and Europe [34, 59, 65, 70, 98]. As much as 37 % of intestinal samples from diseased turkey flocks in Europe were found positive for TCoV [65]. In a recent study from Brazil, most of the intestinal samples obtained from both enteritis-affected and non-affected turkeys were found positive for TCoV [70]. In this study, 55.3 % of TCoV-positive samples were also positive for other enteric viruses. Recently, outbreaks of coronaviral enteritis have been reported from Arkansas and North Carolina in the USA with severe diarrhoea and mortality [19, 83, 100, 106] indicating the importance of continuous epidemiological surveillance for this virus. TCoV appears to be absent from non-PEMS syndromes of PEC [44, 45, 62, 68, 75, 105]. For example, no coronavirus was detected when 2,400 samples from poults showing clinical signs of enteritis were tested in California [105]. Similarly, in a study of 33 enteritis-affected turkey flocks in the US, Pantin-Jackwood et al. [75] did not find any TCoV. We also did not find any TCoV in cases of PES and LTS [44, 45, 68].

The TCoV can affect birds of any age group but causes high mortality in young turkey poults of 1–4 weeks of age. Mortality ranges from less than 10 to 50 %. Turkeys appear to be the only natural host of TCoV; on experimental inoculation, TCoV caused disease in poults but not in chickens [42]. Replication of TCoV occurs in enterocytes lining the apical portion of intestinal villi. Destruction of intestinal villus epithelium leads to diarrhoea, maldigestion, and malabsorption, which may lead to decreased weight gain in turkey poults.

Transmission

The virus is present mainly in the intestine and faeces of affected poults, hence the virus transmission is via the faecal oral route. Direct transmission occurs through direct contact with infected birds while indirect transmission occurs by mechanical means via workers and/or contaminated equipment. However, in an experimental study TCoV antigen could also be detected in the paranasal sinus and lachrymal accessory gland (Harderian gland) of infected poults for up to 14 days post-inoculation [33]. The virus is shed in the faeces of turkeys for many weeks after recovery from clinical disease. Most infections occur during summer (May–August) with sporadic occurrences in autumn. TCoV can survive for a prolonged period and can be transmitted easily between poultry farms in a cool environment.

Clinical signs and pathology

Turkey poults affected with TCoV huddle together and show ruffled feathers, decreased feed and water intake, wet droppings, and loss of body weight. Older birds may exhibit depression, diarrhoea and stunting [34]. Droppings are watery and frothy, may contain mucus, and are green to brown in color. The clinical signs may persist for up to 2 weeks and recovery of weight, if achieved, may take several weeks. Laying birds experience a drop in egg production [3] with abnormal pigmentation of eggs. Birds between 1 and 4 weeks of age that show diarrhoea, dehydration and growth depression of 40 % or more are considered to be experiencing PEMS. There are two forms of PEMS: (1) a severe form i.e. spiking morality of turkeys (SMT), which is defined as mortality that equals or exceeds 1 % on 3 consecutive days or 9 % between 8 and 28 days of age; and (2) a mild form, which is defined as mortality greater than 2 % but less than 9 % during the same time period but does not equal or exceed 1 % for three consecutive days. In an experimental study, both morbidity and mortality were recorded in poults experimentally inoculated with TCoV at 2 days of age, however, birds infected at 28 days of age had significant growth retardation but exhibited no mortality [32].

The TCoV may act synergistically with other pathogens to increase the severity of disease or may predispose the birds to secondary infections. Poults inoculated with only TCoV developed moderate growth depression without mortality and those inoculated with only enteropathogenic *Escherichia coli* (EPEC) did not develop clinical disease. However, inoculation of poults with TCoV and EPEC simultaneously caused increases in mortality, growth depression, and attaching/effacing lesions [38].

The affected birds are stunted and dehydrated. Intestines are markedly enlarged and filled with loose yellow contents. Intestinal walls are flaccid and pale. Caeca are also distended with frothy and watery contents. Gross changes such as atrophy of pancreas, spleen and bursa of Fabricius may also be observed along with distended gall bladder and ureters. Infected hens have more pronounced ovarian lesions with misshapen, mal-developed ovaries and accumulation of caseous exudates in the peritoneum. Peritoneal adhesions of the oviduct are also observed.

Depending on the severity of infection, mild multifocal to severe enteritis with villous atrophy is observed. There is sloughing of epithelial cells with congestion and widespread infiltration of lymphocytes and heterophils into the lamina propria of the villi. In addition, bursal follicular lymphoid depletion and thymic cortical atrophy may also occur.

Diagnosis

Clinical signs such as diarrhoea, depression, huddling and stunted growth are indicative of enteric infection. High mortality in young poults may be indicative of PEMS.

Virus isolation, electron microscopy (EM), detection of viral RNA, and serology can help in diagnosis. The virus can be grown in embryonated turkey or chicken eggs but is difficult to grow in cell cultures. Negative contrast EM may reveal virus particles of $\sim$50–200 nm size with typical crown-shaped morphology in the intestinal contents. Direct and indirect fluorescent antibody tests and ELISA have been used for the detection of TCoV antigen and antibodies, respectively [30, 31, 37]. High seroprevalence of TCoV has been reported from breeders (71.1 %) and meat turkeys (56.7 %) in Ontario, Canada [31]. In Arkansas, 64.2 % of the serum samples tested positive for antibodies [30].

Molecular tests to detect and differentiate TCoV from other enteric viruses are available. Different reverse transcription-polymerase chain reaction (RT-PCR) tests are available targeting the 3′ untranslated region (UTR), S, N and/or M gene of TCoV [11, 12, 16, 89, 92, 104]. Real time RT-PCR has also been developed, which is the most sensitive and specific test in which the viral load can be quantitated in turkey tissues as well as in fecal samples [15]. Direct immunohistochemistry has been advocated as an alternate strategy to RT-PCR to detect TCoV in field samples [14].

Prevention and control

There is no specific treatment or vaccine available. Biosecurity emphasizing litter management and proper disposal of dead birds and used litter appear to be the only

way to prevent the spread of infection. In addition, general measures such as decreased litter moisture, use of antibiotics to combat secondary bacterial infections, and improvement of hygiene may minimize the effects of TCoV enteritis.

Reovirus

Avian reovirus was first isolated from chickens suffering from chronic respiratory disease and was named as the Fahey–Crawley (FC) agent. Later, the FC agent was characterized as a reovirus. In chickens, however, avian reovirus causes many syndromes including viral arthritis/tenosynovitis. Recently, reoviruses have been isolated from turkey arthritis/tenosynovitis and have been named as TARV (turkey arthritis reovirus) [69]. Reoviruses are mainly of two types: fusogenic and non-fusogenic. Fusogenic viruses have the ability to cause fusion of infected cells, resulting in the formation of multinucleated syncytia whiles non-fusogenic viruses do not cause fusion of infected cells. Non-fusogenic types are mainly mammalian reovirus (MRV) while fusogenic reoviruses affect mammals, birds, and reptiles.

Genome

Reovirus belongs to genus *Orthoreovirus* in the family *Orthoreoviridae*. The virus is non-enveloped having icosahedral symmetry and a particle size of 70–80 nm. It contains a double stranded (ds) RNA genome of ten segments, which are divided into three classes named as large (L), medium (M), and small (S) depending on their migration pattern on polyacrylamide gel electrophoresis. The L and M genes are further subdivided into three segments each (L1, L2, L3 and M1, M2, M3, respectively) while the S gene has four segments (S1, S2, S3, S4) [7].

The reovirus genome has 12 open reading frames, which encode for eight structural and four non-structural proteins. The structural proteins are an important part of progeny virions while non-structural proteins are not present in the mature virion and are only expressed in infected cells. The structural proteins encoded by L, M and S genes are lambda (λ), mu (μ) and sigma (σ), respectively. Three structural proteins λA, λB and λC are encoded by L gene segments L1, L2, and L3, respectively. M1 and M2 segments encode two structural proteins (μA and μB), respectively, while M3 segment encodes a non-structural protein (μNS). The three σ proteins σC, σA, and σB are encoded by the S1, S2, and S3 segments, respectively, while S4 segment encodes for non-structural protein σNS. S1 segment encodes two additional non-structural proteins namely, p10 and p17 [7, 10].

Based on sequence analysis of different genes, reoviruses of turkey origin have been reported to differ from chicken reoviruses [20, 44, 45, 52, 88]. For example, the nucleotide and amino acid sequence similarity of S3 segment encoding the sigma B protein of turkey reovirus (TRV) was found to be about 61 and 78–80 %, respectively, when compared to the chicken reovirus isolates [88]. Day et al. [20] reported that sigma C protein of S1 gene of TRV shared 57 % amino acid identity with that of chicken reovirus (reference strain S1133), while p10 and p17 proteins shared 72 and 61 % identity, respectively, with the corresponding S1133 proteins.

The sigma C protein of mammalian reovirus and the fusogenic Nelson Bay reovirus share 25–28 % amino acid identity with the TRV sigma C protein. We have previously reported that S4 gene sequence similarity at the nucleotide level of TRVs was 71.1–74.2 % when compared to reoviruses of chicken origin [45]. These studies indicate that TRVs form a distinct, separate group relative to chicken and other avian reovirus isolates. As a result, TRVs could potentially be considered a separate virus species within subgroup 2 of the *Orthoreovirus* genus. This view is further supported by experimental studies in which TRVs were unable to produce disease signs in chickens, although they may replicate at low levels in the intestinal tract of chicken. Furthermore, a highly pathogenic reovirus strain of chicken-origin (ARV-1733) causing high mortality in chickens produced no clinical signs in commercial turkeys and only mild clinical signs in specific-pathogen-free turkeys [93].

Incidence and distribution

About 85–90 % of reovirus isolates are non-pathogenic types. Pathogenic strains of reoviruses have been associated with various disease conditions in chickens and turkeys. In turkeys, this virus has been detected in several different enteric disease conditions namely LTS, PEC, PES, myocarditis, and recently in arthritis/tenosynovitis [6, 28, 39, 44, 47, 68, 69, 90, 91]. In a study of 33 turkey flocks, 46 % of the flocks were positive for reoviruses [75]. Jindal et al. [44] tested intestinal contents of 43 PES-affected flocks during 2007–2008 and reported the presence of reovirus in 40 % flocks. Enteritis caused by reovirus mainly occurs in young turkey poults of 1–7 weeks of age and the incidence is higher in birds of 1–3 weeks of age, which decreases with the advancement of age.

Reoviruses have also been detected in apparently healthy chickens and turkeys [45, 68, 75]. In a study of five healthy turkey breeder flocks, 10.4 % of the 193 samples tested (from age 1 to 9 weeks) were reovirus positive [45]. Testing of intestinal samples of 33 commercial turkey flocks from all regions of the United States during 2005

and 2006 revealed the presence of reovirus in 45.5 % flocks [75].

Transmission

Horizontal transmission through the fecal oral route is common. Due to stability of virus in the environment mechanical transmission may also occur. Different strains of reovirus vary in their ability to spread horizontally. Vertical transmission of enteric reovirus infection is suspected but not proven.

Clinical signs and pathology

Affected birds have diarrhoea, depression, ruffled feathers, and reduced weight gain [93] and mortality. The ARV infection may also predispose birds to secondary complications or may act synergistically along with other infections. It has been reported that ARV infection may potentiate coccidial infection in broiler chickens [85]. The possibility of synergistic action of TRV with other pathogens in turkeys also exists.

In most cases the intestinal contents are frothy and watery. Atrophy of bursa of Fabricius has been observed in some cases [22, 93]. Intestinal lesions consist of mild crypt hyperplasia due to infiltration of lymphocytes, heterophils and eosinophils in lamina propria and submucosa at 11–14 days post-infection. Moderate to severe follicular lymphocyte depletion is seen in bursa of Fabricius [22, 93]. In addition to bursal atrophy, there can be mild to moderate lymphoid depletion in the spleen and lymphocytic infiltration in the liver, pancreas, heart, and proventriculus.

Diagnosis

Diarrhoea in a flock is an indication of enteric infection. It is difficult to pin point the virus/agent involved on the basis of diarrhoea alone and laboratory tests are needed for confirmation. Electron microscopy may reveal the presence of ~70–80 nm size virus particles in the intestines of infected birds. The virus grows well in the embryonated chicken egg via yolk sac or chorioallantoic membrane routes and has also been isolated in primary chicken embryo liver cells (CEL), chicken embryo kidney cell (CEK), and Japanese Quail fibrosarcoma (QT-35) cells. Due to the fusogenic nature of TRVs, a characteristic cytopathic effect (CPE) is observed in inoculated cell cultures. ELISA kits are commercially available for the detection of antibodies against chicken reovirus but no such specific kits are presently available for turkey reovirus. Recently, we have developed an ELISA test for the detection of antibodies against turkey reovirus. In this test, we cloned and expressed S1 sigma C protein and used it as an antigen for the detection of antibody against TRV (Mor et al. unpublished data).

Several RT-PCR tests targeting different gene segments of reovirus are available [4, 20, 44, 45, 52, 88]. Real time RT-PCR is the most sensitive and specific test in which the viral load in clinical samples can be quantitated. A robust, ultrasensitive, and accurate quantitative assay for ARV with the Light Cycler SYBR Green-based real-time RT-PCR was developed which could detect 39 copies/microl of ARV genomic RNA [53]. Recently, a highly sensitive and specific real time RT-PCR for the detection of TRVs has been developed in our laboratory. We designed primers and probes from the S4 genome segment. The test is specific for TRVs (turkey enteric and arthritis reoviruses) and can detect up to 10 copies of in vitro transcribed standard viral RNA per reaction (Mor et al. unpublished data).

Prevention and control

There is no effective treatment against reoviral infections. The virus is ubiquitous in nature and due to its high stability in the environment, it is difficult to make birds and farms free from reovirus. Currently there is no vaccine available for turkey reoviruses and hence biosecurity remains the only way to control infection.

Rotavirus

Rotaviruses are important pathogens that are associated with neonatal diarrhoea in humans and in a wide range of animals and poultry. This virus has also been detected from commercial broiler chickens affected with runting and stunting syndrome [72].

Genome

Rotaviruses are RNA viruses belonging to the genus *Rotavirus* and family *Reoviridae*. The genome of rotavirus consists of 11 segments of double-stranded RNA; the RNA is surrounded by a triple layer of icosahedral protein capsid. Each segment codes for at least one protein. The outer protein layer is composed of two major neutralizing antigens—viral protein 4 (VP4) and viral protein 7 (VP7). Viral protein 6 (VP6) of the second layer of the capsid is called the group antigen, which is used for detection and classification of rotavirus into distinct groups. In addition, at least five nonstructural proteins are encoded by the rotavirus genome.

Based on the antigenic properties of VP6, the ICTV divided rotaviruses into five serological species (A–E) and two additional species (F and G) [81], which are also called rotavirus groups. Groups A, B and C are known to infect

humans and animals, while groups D, E, F and G infect only animals, mainly birds [50, 63, 102]. Recently, a potentially new RV species, rotavirus H, has been reported [64]. These authors maintain that an amino acid sequence cutoff value of 53 % permits differentiation of various rotavirus species.

In contrast to group A avian rotaviruses, group D rotaviruses cannot be propagated in MA104 cells [25] and are mainly identified by the 5:2:2:2 electrophoretic migration pattern of their genome segments, which is considerably different from the 5:1:3:2 pattern of avian group A rotaviruses. However, classification of a rotavirus strain only on the basis of its RNA migration profile is problematic due to the possible occurrence of genome rearrangements. Unlike group A rotaviruses, which preferentially infect duodenal cells, group D viruses have a predilection for the jejunum and ileum.

The rotavirus of turkey origin differs from rotavirus of chicken origin [58]. For example, amino acid sequence homology of VP7 gene of the Ty-1strain (turkey) to that of the avian Ch-2 VP7 was reported to be only 70 % [58]. The A, B, and C variable epitope regions of Ty-1 were unique compared to those of Ch-2 and other strains representing the 14G serotypes. The low homology (53 %) of the A and C regions of Ty-1 and Ch-2 suggested that Ty-1 strain may be of a different serotype than the G7 reference strain Ch-2. Comparison of the sequences of different genes (VP4, VP6, VP7, NSP5) of turkey and chicken rotaviruses revealed that interspecies transmission and reassortment among avian group A rotaviruses may occur [87].

Incidence and distribution

Rotaviruses have worldwide distribution and have been reported from healthy as well as enteritis-affected turkeys [44, 45, 69, 71, 75, 77, 99]. Periodic monitoring of commercial turkey flocks revealed the circulation of four groups (on basis of NSP4 gene sequence analysis) of rotaviruses. Among the four rotavirus groups, there were 96.1–97.5 and 97.5–99.55 % similarities at the nucleotide and amino acid levels, respectively [77]. The rotaviruses were present before placement as well as after placement until 12 weeks of age [77]. In another study of 33 turkey and 43 chicken flocks, rotaviruses were detected in 46 % of chicken flocks and 70 % of turkey flocks [75]. We have also reported the presence of rotaviruses in apparently healthy turkey breeder flocks with maximum occurrence until 5 weeks of age [45]. In another study, 93 % of PES-affected flocks were positive for rotaviruses [44]. In a surveillance study, Mor et al. [68] reported six (7.5 %) fecal sample pools to be positive for rotavirus in LTS affected flocks while 13 (33 %) sample pools were positive from non-LTS flocks.

Rotavirus detection is greater in young poults and the detection rate decreases as the age advances. In PES and LTS surveillance, maximum number of poults was positive for rotavirus at 2–5 weeks of age followed by a decrease in the number of positive samples at 8–9 weeks of age [44, 45, 68]. It has been shown that primers based on a highly conserved region of rotavirus genome (specifically, the non-structural proteins NSP3 or NSP4) are suitable for the identification of rotavirus in different clinical, animal or environmental samples via conventional or real-time RT-PCR [51, 84]. In a study from Europe, rotaviruses in chickens and turkeys were identified using two different methods i.e. polyacrylamide gel electrophoresis with RT-PCR and real time RT-PCR assays specific for group A and D rotaviruses [71]. These results indicate that further studies are needed to accurately determine the types of rotaviruses circulating in turkey flocks in different geographic locations.

Transmission

Fecal oral route is the main route of horizontal transmission. Virus can survive in the environment for a long time and hence mechanical transmission may play a role in its transmission. Vertical transmission of rotavirus has been suspected but has not been confirmed so far. For example, Pantin-Jackwood et al. [77] detected rotaviruses in 50 % of the samples tested prior to bird placement. Based on this information, the authors opined that rotavirus may be transmitted vertically. We have also detected rotaviruses in 2-day-old commercial poults and in breeder poults [45, 47] indicating that vertical transmission of rotavirus may occur.

Interspecies transmission of rotavirus has also been described [87, 103]. In one study, group A bovine-origin rotaviruses ($n = 6$) were detected in enteric contents of turkeys [2]. The electropherotypes showed a migration pattern identical to the Nebraska calf diarrhoea virus, and the complete NSP4 gene phylogeny showed that all six strains segregated in genotype E2 thereby confirming them to be bovine rotavirus. Avian rotaviruses have not been detected in humans and human rotaviruses have not been found to contain avian rotavirus sequences [87] indicating low probability of human infection with avian rotaviruses.

Clinical signs and pathology

Dullness, depression, watery droppings, loss of appetite, pasting of vent and reduced weight gain are consistent findings in affected birds. Mortality may or may not be present and morbidity may vary. Rotavirus infection in laying hens may result in a drop in egg production.

In most cases frothy and watery intestinal contents are observed. Pallor of the intestinal tract and distension of the

cecum with frothy or non-frothy fluid contents may be observed. Other gross pathological findings are dehydration, stunted growth, and pasty vents. Multifocal and superficial erosions in duodenum and jejunum are also noticed. Most common microscopic changes are hypercellularity of lamina propria due to infiltration of heterophils, eosinophils and mononuclear cells and villus atrophy. Scanning electron microscopy indicate roughened villus surfaces, distortion of the normal morphologic features of the villi and loss of microvilli in cells located on the tips of the villi. In chickens, different rotavirus strains may have different preferential sites of replication. For example, McNulty et al. [66] reported that in experimentally infected chickens, group A rotavirus had affinity for duodenum while group D rotavirus preferred jejunum and ileum.

Diagnosis

Diarrhoea, dehydration, and decreased weight gain in a flock is indicative of enteric infection. The EM of intestinal contents and faeces reveals the presence of ~70 nm (approximately 50–70 nm) size viral particles with distinctive "wheel like" appearance. Although difficult to isolate, the virus does grow in MA104 cells with trypsin treatment. A coagglutination test has been used for the detection of rotaviruses in turkeys and has been found to be a simple and rapid screening test. Polyacrylamide gel electrophoresis is commonly used for detection of characteristic migration pattern of the 11 genome segments of rotavirus and is almost as sensitive as EM.

Several RT-PCR and real time RT-PCR targeting different genome segments of rotavirus are available for virus detection [8, 21, 77]. Recently, a highly sensitive and specific one step- real time RT-PCR targeting the nonstructural protein 4 (NSP4) with internal control system for detection of turkey rotaviruses has been reported [1].

Prevention and control

There is no specific control for rotavirus infections. Obtaining poults from rotavirus-free sources and the implementation of proper biosecurity measures are the ways to minimize rotavirus infection in a flock.

Astrovirus

Turkey astrovirus (TAstV) was first detected in turkey poults of 6–11 days of age with diarrhoea and increased mortality in UK. In the US, TAstV was detected from turkey poults and was later named as TAstV-1. Subsequently, a TAstV associated with PEMS was isolated and characterized. This virus was genetically and immunologically distinct from the previously described US isolate, TAstV-1, and was named as TAstV-2 [56, 86]. In addition to TAstV-1 and TAstV-2, an astrovirus of chicken origin i.e. avian nephritis virus (ANV) has also been detected in turkeys [26, 75]. The ANV was first described as a small round virus, then as a picornavirus, and was later determined to be an astrovirus [40]. Thus, three astrovirus types (TAstV-1, TAstV-2, and ANV) have been detected in turkeys. However, sequence data suggest that many more types maybe present in nature [94]. In the 1980s, all detected astroviruses were strictly confined to the GI tract of poults and there was no evidence of systemic disease. However, TAstV was also detected in thymus and bursa, suggesting a possible effect on the immune system [86]. Detection of astroviruses in chicken and turkey flocks affected with enteric and/or locomotive disorders (tenosynovitis) has also been reported [26, 82].

Genome

Astroviruses are non-enveloped RNA viruses of 25–35 nm diameter belonging to the genus *Avastrovirus* of the *Astroviridae* family. The astrovirus genome consists of RNA of 6.8–7.9 kb and has a 5′ UTR followed by three ORFs, a 3′ UTR, and a poly-A tail. ORF-1a encodes for the serine protease and other nonstructural proteins, ORF-1b for the RNA-dependent RNA polymerase, and ORF-2 for the capsid protein. The overall length of the genomes of three astroviruses viz. TAstV-1 (7,003 nt), TAstV-2 (7,325 nt) and ANV (6,927 nt) varies; this length excludes the poly-A tail [55].

Most studies on detection and characterization of TAstVs have been carried out by molecular methods using primers specific for the capsid or polymerase gene. A number of studies have indicated variations at the nucleotide and amino acid levels in the capsid gene and/or polymerase gene of TAstVs [13, 44–46, 78, 79, 97]. A high level of genetic variation has been reported among turkey astroviruses from US turkey flocks [78]. The nucleotide sequence identity in the capsid gene, associated with serotype and viral pathogenesis, was as low as 69 % but the polymerase gene was more conserved with 86–99 % nucleotide identity. Recently, phylogenetic analysis of the capsid precursor protein revealed extensive variations in the amino acid sequences of TAstV-2 (81.5–100 %) but not in TAstV-1 (96.2–100 %) [79]. We have also detected variations at the amino acid level in the capsid gene of TAstV-2 [44, 46]. These changes suggest that different serotypes of turkey astrovirus may exist in nature. Studies on complete capsid gene sequences from apparently healthy and PEC-affected flocks from different geographic areas are indicated for better understanding of TAstV

serotypes circulating in turkeys. Changes in the capsid gene due to mutations or recombination may have an effect on the antigenicity and pathogenicity of the viruses, and consequently have practical implications for virus detection methods, epidemiological studies and development of potential vaccines against astrovirus infections.

Incidence and distribution

A number of studies have been undertaken to detect the presence of TAstVs in turkey flocks using different methods. Previously, the viruses in intestinal samples were detected by direct EM or immune EM. However, most of the recent studies use molecular tools such as RT-PCR and sequencing for detecting the presence of different astroviruses [36, 56, 57, 108]. In a US survey of eight commercial turkey operations and a research unit, 89.5 % of 96 intestinal content samples were positive for astrovirus by RT-PCR [77]. In a Minnesota study, 84 % of the 43 PES-affected turkey flocks were found positive for TAstV-2 by RT-PCR [44]. These samples were not tested for the presence of TAstV-1 and ANV, hence the presence of TAstV-1 and ANV in these samples cannot be ruled out. A number of other studies have also revealed high prevalence of TAstVs in US turkey flocks [75, 77, 78].

Studies on the prevalence of TAstVs have also been undertaken in other turkey-raising countries [17, 27, 62, 70, 74]. Da Silva et al. [17] reported TAsV in young poults affected with PEC in Brazil. In another study in Brazil, TAsV-1, TAstV-2, and ANV were identified in 64.5, 44.7 and 35.5 % of intestinal samples, respectively [70]. Of these, TAstV-1 and TAstV-2 were more prevalent in enteritis-affected turkey flocks. Domanska-Blicharz et al. [27] reported TAstV-2 to be the most prevalent virus in Poland; 30 of 77 turkey flocks (38.9 %) were positive. TAstV-1 was detected in only nine flocks while ANV in one flock. These three viruses were detected either as single or mixed infections. Testing of 23 intestinal samples from fattening turkeys showing clinical signs of enteritis revealed the presence of TAstV-2 in 17 samples in Croatia [62].

The TAstVs have not only been detected in enteritis-affected turkey flocks but have also been found in apparently healthy turkey flocks [45, 68, 78]. Jindal et al. [45] tested 193 pooled fecal samples of apparently healthy breeder turkey poults by RT-PCR and found 47.2 % to be positive for TAstV-2.

Transmission

Transmission of astroviruses is by the faecal-oral route. Healthy birds may be exposed to astrovirus either through direct contact with infected faeces or with materials contaminated with faeces. Astrovirus infections are thought to be species specific but recent reports indicate interspecies transmission [23, 101]. Thus, TAstV-2 and ANV have been detected in ducks (*Anas platyrhynchos domesticus*) [9]. Cross-species transmission and virus adaptation to new hosts or co-infection of the same host with different astroviruses may lead to the emergence of novel astroviruses that infect animals or that have a zoonotic potential [24].

Clinical findings and pathology

As in rota- and reovirus infections, astroviruses also cause diarrhoea, dehydration and retarded growth. Similar observations of diarrhoea and retarded growth in experimental poults inoculated with material containing TAstVs have been reported [48, 49, 76]. Experimental studies have been undertaken to ascertain the type of clinical disease produced by TAstVs having variant capsids or those that differed in their source of origin. For example, Pantin-Jackwood et al. [76] studied the pathogenicity of three different TAstV-2 viruses in specific-pathogen-free turkeys and found that TAstV-2 viruses with variant capsids produced a similar enteric disease in young turkeys. Mor et al. [67] found that TAstV-2 from PES birds was more pathogenic than that from apparently healthy poults. Astrovirus can potentially impair the immune response, thereby leading to enhanced susceptibility of turkeys to secondary bacterial infections [80].

Dehydration, distended intestines filled with watery contents and undigested feed, and dilated caeca with foamy contents are the necropsy findings. Mild to moderate bursal atrophy along with loss of intestinal tonicity may occur. Mild epithelial necrosis, lamina propria infiltrates and mild crypt hyperplasia are the histological findings.

Diagnosis

Diarrhoea, dehydration and decreased weight gain are indications of enteric infection. Negative contrast EM reveals virus particles having five or six projections on their surface, resembling a star. The star like morphology is not always maintained or clearly distinguishable. For this reason the AstVs by EM can be confused with picornavirus, picornavirus-like, enterovirus, and entero-like viruses. Secondly, poults may display clinical findings and pathology at a later stage in astrovirus infection but the intestinal contents may not have detectable astrovirus particles. In such a situation, even though the disease has occurred, the testing results may be negative. Molecular techniques have been used for detection and further characterization of the astroviruses. Most of the RT-PCR based assays have used primers specific to the polymerase or capsid gene of the TAstVs [56, 57, 96].

Mixed viral infections

Although individual viruses have been detected in enteritis-affected turkey flocks, most studies point to the presence of two or more enteric viruses [44, 45, 62, 68, 70, 75, 77, 105, 107]. Periodic monitoring of eight commercial turkey flocks in the US revealed continuous presence of TAStV and rotavirus [77]. Of the 96 samples tested, 89.5 % were positive for astrovirus and 67.7 % for rotavirus. All flocks were negative for TCoV, reovirus, and group 1 adenovirus at all sampling points. In another study, the presence of astroviruses was reported in 100 % of the turkey flocks tested [75]. TAstV-2 and TAstV-1 were found in 100 and 15.4 % of the turkey flocks, respectively. In addition, 12.5 % of turkey flocks were positive for ANV. Rotaviruses and reoviruses were present in 69.7 and 45.5 % of the flocks, respectively, while coronavirus and adenovirus were not detected.

Intestinal contents from 43 PES flocks in Minnesota were tested for the presence of enteric viruses by RT-PCR. All 43 flocks were positive for rotavirus or TAstV-2 or reovirus. The viruses in all cases were detected either alone or in combination of 2 or 3. Eight flocks (19 %) were positive for a single virus and the remaining 35 (81 %) had a combination of viruses. Fifteen flocks were positive for all three viruses. None of the flocks tested was found positive for TCoV [44]. Studies conducted in Brazil revealed the presence of 69.7 % of multiple viruses in intestinal samples of turkeys. The intestinal samples most often harbored 3–4 viruses per sample with viruses from the astrovirus family (TAstV-1, TAstV-2, or ANV, or a combination of these) being present in all of the samples that tested positive for viruses [70].

It is possible that viruses in combination may cause more severe adverse effects in poults than caused by an individual virus. In one study, turkey poults inoculated with a combination of TAstV-2, turkey reovirus and rotavirus had considerably lower body weights than controls [91]. In experimental studies, we have also reported that oral inoculation of poults with PES material (positive for rotavirus, TAstV-2, and Salmonella) led to significant growth retardation for up to 50 days post-inoculation and at no point post-inoculation did any of the challenged birds' weight converge with that of controls. The intestinal contents were watery and foamy/frothy (Figs. 1, 2) of the poults inoculated with PES material [49]. Similarly, turkey poults inoculated with intestinal contents from LTS-affected birds had significantly lower body weights at 20 weeks of age compared to poults inoculated with intestinal contents from non-LTS birds [68]. This extent of growth depression due to poult enteritis may lead to considerable economic losses to turkey growers as the affected birds may not attain the anticipated weight at marketing. At the farm level, the role of agents other than these four common viruses (TAstV, rotavirus, reovirus and coronavirus) in causing and/or increasing the severity of the enteric viruses cannot be ruled out. In addition, nutritional factors and management may also play a role in causing enteritis in a flock. Steps such as improvement in management practices and antimicrobial therapy to minimize losses have met with limited success [5]. No vaccine against enteric viruses is currently available.

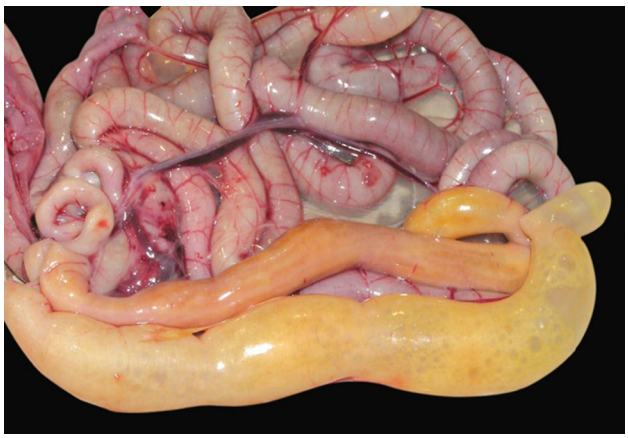

Fig. 1 Watery and frothy caecal contents of a poult inoculated orally with intestinal material of poult enteritis syndrome-affected birds

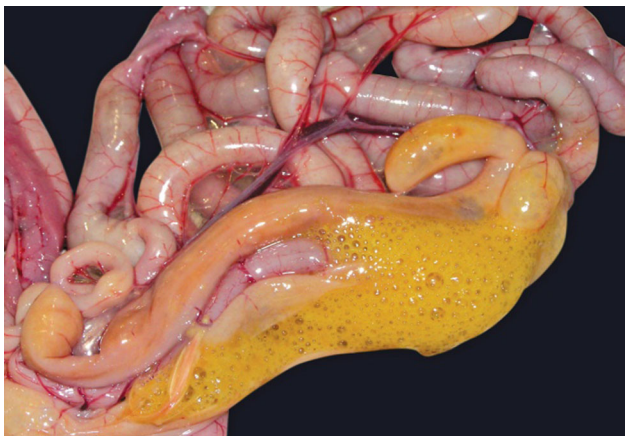

Fig. 2 Watery and frothy caecal contents of a poult inoculated orally with intestinal material of poult enteritis syndrome-affected birds. Caecum is cut open

Enteric viruses are usually detected by EM, serology, and PCR. Most of these viruses are difficult to grow in embryonating chicken/turkey eggs and cell cultures [95]. Hence, several single and multiplex RT-PCR (mRT-PCR) assays have been developed for the detection of turkey astrovirus, turkey rotavirus, turkey coronavirus, and turkey reovirus [21, 43, 56, 77, 89, 92]. Multiplex RT-PCR assays save time and money as two or more viruses can be detected in a single reaction.

General conclusions and future directions

Enteric viruses (TAstV, rotavirus, reovirus and coronavirus) are widespread in turkey flocks but their clinical importance is not clear. The full impact of these viruses on flock performance needs to be thoroughly investigated. Although virally induced enteric diseases are an economic burden on turkey farmers, no single virus has emerged as a likely causative agent so that appropriate prevention and control efforts can be formulated [18]. In several studies, two or more different viruses have been detected simultaneously indicating that multiple viruses may be involved in the pathogenesis of enteritis. The prevalence of viruses from the same genus in intestines of both healthy and poorly performing birds indicates the existence of different pathotypes of the virus. Further studies are needed to determine the validity of these statements.

With the advent of molecular diagnostic assays and sequencing, our understanding about enteric viruses has increased. There is also a possibility that our current methods cannot detect some yet unknown virus(es)/pathogens that may have implications in turkey enteritis. The availability and use of next generation high-throughput nucleic acid pyrosequencing techniques, such as Illumina sequencing, may reveal sequences of agents that have not been previously reported in turkeys. Metagenomic studies of the RNA viruses in the gut of turkeys experiencing enteric disease revealed the presence of sequences from dsRNA viruses (*Reoviridae* and Picobirnaviruses) and ssRNA viruses (*Caliciviridae*, *Leviviridae*, *Picornaviridae*, and *Astroviridae*) [18]. Detection of unknown viruses by the metagenomics approach would pave the way to develop diagnostic methods for these viruses. Further, there is a need to determine why the same virus types are found in enteritis-affected turkey flocks as well as in apparently healthy flocks. Complete understanding of the contribution of enteric viruses and other pathogens in enteric diseases of turkeys will go a long way in the development of preventive and control measures.

References

1. Akimkin V, Bindel F, Hoferer M, Sting R, Polley B, Hanel A, Hafez HM. One-step RT-qPCR with an internal control system for the detection of turkey rotaviruses in faecal samples. J Virol Methods. 2011;177(1):112–7.
2. Asano KM, Gregori F, Souza SP, Rotava D, Oliveira RN, Villarreal LY, Richtzenhain LJ, Brandao PE. Bovine rotavirus in turkeys with enteritis. Avian Dis. 2011;55(4):697–700.
3. Awe OO, Ali A, Elaish M, Ibrahim M, Murgia M, Pantin-Jackwood M, Saif YM, Lee CW. Effect of coronavirus infection on reproductive performance of turkey hens. Avian Dis. 2013;57:650–6.
4. Banyai K, Dandar E, Dorsey KM, Mato T, Palya V. The genomic constellation of a novel avian orthoreovirus strain associated with runting–stunting syndrome in broilers. Virus Genes. 2011;42:82–9.
5. Barnes HJ, Guy JS. Poult enteritis-mortality syndrome. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, editors. Diseases of poultry. 11th ed. Ames: Iowa State University Press; 2003. pp. 1171–1180.
6. Barnes HJ, Guy JS, Vaillancourt JP. Poult enteritis complex. Rev Sci Tech. 2000;19:565–88.
7. Benavente J, Martınez-Costas J. Avian reovirus: structure and biology. Virus Res. 2007;123:105–19.
8. Bezerra DA, da Silva RR, Kaiano JH, Silvestre RV, de Souza Oliveira D, Linhares C, Gabbay YB, Mascarenhas JD. Detection of avian group D rotavirus using the polymerase chain reaction for the VP6 gene. J Virol Methods. 2012;185:189–92.
9. Bidin M, Bidin Z, Majnaric D, Tisljar M, Lojkic I. Circulation and phylogenetic relationship of chicken and turkey-origin astroviruses detected in domestic ducks (*Anas platyrhynchos domesticus*). Avian Pathol. 2012;41(6):555–62.
10. Bodelon G, Labrada L, Martınez-Costas J, Benavente J. The avian reovirus genome segment S1 is a functionally tricistronic gene that expresses one structural and two nonstructural proteins in infected cells. Virology. 2001;290:181–91.
11. Breslin JJ, Smith LG, Barnes HJ, Guy JS. Comparison of virus isolation, immunohistochemistry, and reverse transcriptase polymerase chain reaction procedures for detection of turkey coronavirus. Avian Dis. 2000;44:624–31.
12. Bunger AN, Chacon JL, Jones RC, Ferreira AJ. Detection and molecular characterization of gene 3 and 5 of turkey coronavirus from turkeys with severe enteritis in Brazil. Avian Dis. 2009;53:356–62.
13. Canelli E, Cordioli P, Barbieri I, Catella A, Pennelli D, Ceruti R, Moreno A, Lavazza A. Astroviruses as causative agents of poultry enteritis: genetic characterization and longitudinal studies on field conditions. Avian Dis. 2012;56:173–82.
14. Cardoso TC, Castanheira TL, Teixeira MC, Rosa AC, Hirata KY, Astolphi RD, Luvizotto MC. Validation of an immunohistochemistry assay to detect turkey coronavirus: a rapid and simple screening tool for limited resource settings. Poult Sci. 2008;87(7):1347–52.
15. Chen YN, Wu CC, Bryan T, Hooper T, Schrader D, Lin TL. Specific real-time reverse transcription-polymerase chain reaction for detection and quantitation of turkey coronavirus RNA in tissues and faeces from turkeys infected with turkey coronavirus. J Virol Methods. 2010;163:452–8.
16. Culver FA, Britton P, Cavanagh D. RT-PCR detection of avian coronaviruses of galliform birds (chicken, turkey, pheasant) and in a parrot. Methods Mol Biol. 2008;454:35–42.
17. Da Silva SEL, Bonetti AM, Petrocelli ATM, Ferrari HF, Luvizotto MCR, Cardoso TC. Detection of turkey astrovirus in young poults affected with poult enteritis complex in Brazil. J Vet Med Sci. 2008;70(6):629–31.
18. Day JM, Ballard LL, Duke MV, Scheffler BE, Zsak L. Metagenomic analysis of the turkey gut RNA virus community. Virol J. 2010;7:313. http://www.virologyj.com/content/7/1/313.
19. Day JM, Gonder E, Jennings S, Rives D, Tilley B, Wooming B. Molecular characterization of turkey enteric coronaviruses circulating in the United States in 2012. Paper presented in annual meeting of American Association of Avian Pathologist at Chicago, Illinois. 2013.
20. Day JM, Pantin-Jackwood MJ, Spackman E. Sequence and phylogenetic analysis of the S1 genome segment of turkey-origin reoviruses. Virus Genes. 2007;35(2):235–42.
21. Day JM, Spackman E, Pantin-Jackwood MJ. A multiplex RT-PCR test for the differential identification of turkey astrovirus type 1, turkey astrovirus type 2, chicken astrovirus, avian nephritis virus, and avian rotavirus. Avian Dis. 2007;51:681–4.

22. Day JM, Spackman E, Pantin-Jackwood MJ. Turkey origin reovirus-induced immune dysfunction in specific pathogen free and commercial turkey poults. Avian Dis. 2008;52(3):387–91.
23. De Battisti C, Salviato A, Jonassen CM, Toffan A, Capua I, Cattoli G. Genetic characterization of astroviruses detected in guinea fowl (*Numida meleagris*) reveals a distinct genotype and suggests cross-species transmission between turkey and guinea fowl. Arch Virol. 2012;157(7):1329–37.
24. De Benedictis P, Schultz-Cherry S, Burnham A, Cattoli G. Astrovirus infections in humans and animals—molecular biology, genetic diversity, and interspecies transmissions. Infect Genet Evol. 2011;11(7):1529–44.
25. Devitt CM, Reynolds DL. Characterization of a group D rotavirus. Avian Dis. 1993;37:749–55.
26. de Wit JJ, ten Dam GB, van de Laar JMAM, Biermann Y, Verstegen I, Edens F, Schrier CC. Detection and characterization of a new astrovirus in chicken and turkeys with enteric and locomotion disorders. Avian Pathol. 2011;40:453–61.
27. Domanska-Blicharz K, Seroka A, Minta Z. One-year molecular survey of astrovirus infection in turkeys in Poland. Arch Virol. 2011;156(6):1065–72.
28. Franca M, Crespo R, Chin R, Woolcock P, Shivaprasad HL. Retrospective study of myocarditis associated with reovirus in turkeys. Avian Dis. 2010;54(3):1026–31.
29. Gomaa MH, Barta JR, Ojkic D, Yoo D. Complete genomic sequence of turkey coronavirus. Virus Res. 2008;135(2):237–46.
30. Gomaa MH, Yoo D, Ojkic D, Barta JR. Seroprevalence of turkey coronavirus in North American turkeys determined by a newly developed enzyme-linked immunosorbent assay based on recombinant antigen. Clin Vaccine Immunol. 2008;15(12):1839–44.
31. Gomaa MH, Yoo D, Ojkic D, Barta JR. Use of recombinant S1 spike polypeptide to develop a TCoV-specific antibody ELISA. Vet Microbiol. 2009;138(3–4):281–8.
32. Gomaa MH, Yoo D, Ojkic D, Barta JR. Infection with a pathogenic turkey coronavirus isolate negatively affects growth performance and intestinal morphology of young turkey poults in Canada. Avian Pathol. 2009;38:279–86.
33. Gomes DE, Hirata KY, Saheki K, Rosa AC, Luvizotto MC, Cardoso TC. Pathology and tissue distribution of turkey coronavirus in experimentally infected chicks and turkey poults. J Comp Pathol. 2010;143(1):8–13.
34. Guy JS. Turkey coronavirus enteritis. In: Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, editors. Disease of poultry. 11th ed. Ames: Iowa State University Press; 2003. p. 300–7.
35. Guy JS. Turkey coronavirus enteritis. In: Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, editors. Diseases of poultry. 12th ed. Ames: Wiley-Blackwell Publishing Professional; 2008. p. 330–8.
36. Guy JS, Miles AM, Smith L, Fuller FJ, Schultz-Cherry S. Antigenic and genomic characterization of turkey enterovirus-like virus (North Carolina, 1988 isolate): identification of the virus as turkey astrovirus 2. Avian Dis. 2004;48(1):206–11.
37. Guy JS, Smith LG, Breslin JJ, Pakpinyo S. Development of a competitive enzyme-linked immunosorbent assay for detection of turkey coronavirus antibodies. Avian Dis. 2002;46(2):334–41.
38. Guy JS, Smith LG, Breslin JJ, Vaillancourt JP, Barnes HJ. High mortality and growth depression experimentally produced in young turkeys by dual infection with enteropathogenic *Escherichia coli* and turkey coronavirus. Avian Dis. 2000;44:105–13.
39. Heggen-Peay CL, Qureshi MA, Edens FW, Sherry B, Wakenell PS, O'Connell PH, Schat KA. Isolation of a reovirus from poult enteritis and mortality syndrome and its pathogenicity in turkey poults. Avian Dis. 2002;46(1):32–47.
40. Imada T, Yamaguchi S, Mase M, Tsukamoto K, Kubo M, Morooka A. Avian nephritis virus (ANV) as a new member of the family *Astroviridae* and construction of infectious ANV cDNA. J Virol. 2000;74:8487–93.
41. Ismail MM, Tang AY, Saif YM. Pathogenicity of turkey coronavirus in turkeys and chickens. Avian Dis. 2003;47:515–22.
42. Jackwood MW, Boynton TO, Hilt DA, McKinley ET, Kissinger JC, Paterson AH, Robertson J, Lemke C, McCall AW, Williams SM, Jackwood JW, Byrd LA. Emergence of a group 3 coronavirus through recombination. Virology. 2010;398:98–108.
43. Jindal N, Chander Y, Patnayak DP, Mor SK, Ziegler AF, Goyal SM. A multiplex RT-PCR for the detection of astrovirus, rotavirus, and reovirus in turkeys. Avian Dis. 2012;56:592–6.
44. Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM. Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys. Poult Sci. 2010;89:217–26.
45. Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM. Detection and molecular characterization of enteric viruses in breeder turkeys. Avian Pathol. 2010;39:53–61.
46. Jindal N, Patnayak DP, Chander Y, Ziegler AF, Goyal SM. Comparison of capsid gene sequences of turkey astrovirus-2 from poult enteritis syndrome-affected and apparently healthy turkeys. Arch Virol. 2011;156:969–77.
47. Jindal N, Patnayak DP, Ziegler A, Lago A, Goyal SM. A retrospective study on poult enteritis syndrome in Minnesota. Avian Dis. 2009;53:268–75.
48. Jindal N, Patnayak DP, Ziegler A, Lago A, Goyal SM. Experimental reproduction of poult enteritis syndrome: clinical findings, growth response and microbiology. Poult Sci. 2009;88:949–58.
49. Jindal N, Patnayak DP, Ziegler A, Lago A, Goyal SM. Duration of growth depression and pathogen shedding in experimentally reproduced poult enteritis syndrome. Avian Dis. 2009;53:517–22.
50. Johne R, Otto P, Roth B, Löhren U, Belnap D, Reetz J, Trojnar E. Sequence analysis of the VP6-encoding genome segment of avian group F and G rotaviruses. Virology. 2011;412:384–91.
51. Jothikumar N, Kang G, Hill VR. Broadly reactive TaqMan assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. J Virol Methods. 2009;155:126–31.
52. Kapczynski DR, Sellers HS, Simmons V, Schultz-Cherry S. Sequence analysis of the S3 gene from a turkey reovirus. Virus Genes. 2002;25(1):95–100.
53. Ke GM, Cheng HL, Ke LY, Ji WT, Chulu JL, Liao MH, Chang TJ, Liu HJ. Development of a quantitative light cycler real-time RT-PCR for detection of avian reovirus. J Virol Methods. 2006;133(1):6–13.
54. King AMQ, Lefkowiz E, Adams M, Carstens E. Virus taxonomy: ninth report of the international committee on taxonomy of viruses. Waltham: Academic Press; 2012.
55. Koci MD, Schultz-Cherry S. Avian astroviruses. Avian Pathol. 2002;31:213–27.
56. Koci MD, Seal BS, Schultz-Cherry S. Development of an RT-PCR diagnostic test for avian astrovirus. J Virol Methods. 2000;90:79–83.
57. Koci MD, Seal BS, Schultz-Cherry S. Molecular characterization of an avian astrovirus. J Virol. 2000;74:6173–7.
58. Kool DA, Holmes IH. The avian rotavirus Ty-1 Vp7 nucleotide and deduced amino acid sequences differ significantly from those of Ch-2 rotavirus. Arch Virol. 1993;129(1–4):227–34.
59. Lin TL, Loa CC, Tsai SC, Wu CC, Bryan TA, Thacker HL, Hooper T, Schrader D. Characterization of turkey coronavirus from turkey poults with acute enteritis. Vet Microbiol. 2002;84(1–2):179–86.
60. Lin TL, Loa CC, Wu CC. Complete sequences of 3′ end coding region for structural protein genes of turkey coronavirus. Virus Res. 2004;106(1):61–70.

61. Loa CC, Wu CC, Lin TL. Comparison of 3′-end encoding regions of turkey coronavirus isolates from Indiana, North Carolina, and Minnesota with chicken infectious bronchitis coronavirus strains. Intervirology. 2006;49(4):230–8.
62. Lojkic I, Bidin M, Bidin Z, Mikec M. Viral agents associated with poult enteritis in Croatian commercial turkey flocks. Acta Vet BRNO. 2010;79:91–8.
63. Matthijnssens J, Martella V, Van Ranst M. Priority paper evaluation: genomic evolution, host-species barrier, reassortment and classification of rotaviruses. Future Virol. 2010;5: 385–90.
64. Matthijnssens J, Otto PH, Ciarlet M, Desselberger U, Van Ranst M, Johne R. VP6-sequence-based cutoff values as a criterion for rotavirus species demarcation. Arch Virol. 2012;157(6): 1177–82.
65. Maurel S, Toquin D, Briand FX, Queguiner M, Allee C, Bertin J, Ravillion L, Retaux C, Turblin V, Morvan H, Eterradossi N. First full-length sequences of the S gene of European isolates reveal further diversity among turkey coronaviruses. Avian Pathol. 2011;40:179–89.
66. McNulty MS, Allan GM, McCracken RM. Experimental infection of chickens with rotaviruses: clinical and virological findings. Avian Pathol. 1983;12:45–54.
67. Mor SK, Abin M, Costa G, Durrani A, Jindal N, Goyal SM, Patnayak DP. The role of type-2 turkey astrovirus in poult enteritis syndrome. Poult Sci. 2011;90:2747–52.
68. Mor SK, Sharafeldin TA, Abin M, Kromm M, Porter RE, Goyal SM, Patnayak DP. The occurrence of enteric viruses in light turkey syndrome. Avian Pathol. 2013;42(5):497–501.
69. Mor SK, Sharafeldin TA, Porter RE, Ziegler A, Patnayak DP, Goyal SM. Isolation and characterization of a turkey arthritis reovirus. Avian Dis. 2013;57(1):97–103.
70. Moura-Alvarez J, Chacon JV, Scanavini LS, Nunez LFN, Astolfi-Ferreira CS, Jones RC, Piantino Ferreira AJ. Enteric viruses in Brazilian turkey flocks: single and multiple virus infection frequency according to age and clinical signs of intestinal disease. Poult Sci. 2013;92:945–55.
71. Otto PH, Ahmed MU, Hotzel H, Machnowska P, Reetz J, Roth B, Trojnar E, Johne R. Detection of avian rotaviruses of groups A, D, F and G in diseased chickens and turkeys from Europe and Bangladesh. Vet Microbiol. 2012;156(1–2):8–15.
72. Otto P, Liebler-Tenorio EM, Elschner M, Reetz J, Lohren U, Diller R. Detection of rotaviruses and intestinal lesions in broiler chicks from flocks with runting and stunting syndrome (RSS). Avian Dis. 2006;50:411–8.
73. Pakpinyo S, Ley DH, Barnes HJ, Vaillancourt JP, Guy JS. Prevalence of enteropathogenic *Escherichia coli* in naturally occurring cases of poult enteritis-mortality syndrome. Avian Dis. 2002;46(2):360–9.
74. Palade EA, Demeter Z, Hornyak A, Nemes C, Kisary J, Rusvai M. High prevalence of turkey parvovirus in turkey flocks from Hungary experiencing enteric disease syndromes. Avian Dis. 2011;55(3):468–75.
75. Pantin-Jackwood MJ, Day JM, Jackwood MW, Spackman E. Enteric viruses detected by molecular methods in commercial chicken and turkey flocks in the United States between 2005 and 2006. Avian Dis. 2008;52:235–44.
76. Pantin-Jackwood MJ, Spackman E, Day JM. Pathogenesis of type 2 turkey astroviruses with variant capsid genes in 2-day-old specific pathogen free poults. Avian Pathol. 2008;37:193–201.
77. Pantin-Jackwood MJ, Spackman E, Day JM, Rives D. Periodic monitoring of commercial turkeys for enteric viruses indicates continuous presence of astrovirus and rotavirus on the farms. Avian Dis. 2007;51:674–80.
78. Pantin-Jackwood MJ, Spackman E, Woolcock PR. Molecular characterization and typing of chicken and turkey astroviruses circulating in the United States: implications for diagnostics. Avian Dis. 2006;50:397–404.
79. Pantin-Jackwood MJ, Strother KO, Mundt E, Zsak L, Day JM, Spackman E. Molecular characterization of avian astroviruses. Arch Virol. 2011;156:235–44.
80. Qureshi MA, Saif YM, Heggen-Peay CL, Edens FW, Havenstein GB. Induction of functional defects in macrophages by a poult enteritis and mortality syndrome-associated turkey astrovirus. Avian Dis. 2001;45(4):853–61.
81. Ramig RF, Ciarlet M, Mertens PPC, Dermody TS. Rotavirus. In: Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA, editors. Virus taxonomy: eighth report of the international committee on taxonomy of viruses. Amsterdam: Academic Press; 2005. p. 484–96.
82. Reynolds DL, Saif YM. Astrovirus: a cause of an enteric disease in turkey poults. Avian Dis. 1986;30:728–35.
83. Robbins KM. Turkey coronavirus epidemiology and diagnostics. Paper presented in annual meeting of American Association of Avian Pathologist held at Chicago, Illinois. 2013.
84. Rodriguez-Diaz J, Rubilar-Abreu E, Spitzner M, Hedlund KO, Liprandi F, Svensson L. Design of a multiplex nested PCR for genotyping of the NSP4 from group A rotavirus. J Virol Methods. 2008;149:240–5.
85. Ruff MD, Rosenberger JK. Concurrent infections with reoviruses and coccidia in broilers. Avian Dis. 1985;29(2):465–78.
86. Schultz-Cherry S, Kapczynski DR, Simmons VM, Koci MD, Brown C, Barnes HJ. Identifying agent(s) associated with poult enteritis mortality syndrome: importance of the thymus. Avian Dis. 2000;44:256–65.
87. Schumann T, Hotzel H, Otto P, Johne R. Evidence of interspecies transmission and reassortment among avian group A rotaviruses. Virology. 2009;386(2):334–43.
88. Sellers HS, Linnemann EG, Pereira L, Kapczynski DR. Phylogenetic analysis of the sigma 2 protein gene of turkey reoviruses. Avian Dis. 2004;48(3):651–7.
89. Sellers HS, Koci MD, Linnemann E, Kelley LA, Schultz-Cherry S. Development of a multiplex reverse transcription-polymerase chain reaction diagnostic test specific for turkey astrovirus and coronavirus. Avian Dis. 2004;48:531–9.
90. Shivaprasad HL, Franca M, Woolcock PR, Nordhausen R, Day JM, Pantin-Jackwood M. Myocarditis associated with reovirus in turkey poults. Avian Dis. 2009;53:523–32.
91. Spackman E, Day JM, Pantin-Jackwood MJ. Astrovirus, reovirus, and rotavirus concomitant infection causes decreased weight gain in broad-breasted white poults. Avian Dis. 2010;54:16–21.
92. Spackman E, Kapczynski D, Sellers H. Multiplex real-time reverse transcription-polymerase chain reaction for the detection of three viruses associated with poult enteritis complex: turkey astrovirus, turkey coronavirus, and turkey reovirus. Avian Dis. 2005;49:86–91.
93. Spackman E, Pantin-Jackwood M, Day JM, Sellers H. The pathogenesis of turkey origin reoviruses in turkeys and chickens. Avian Pathol. 2005;34:291–6.
94. Strain E, Kelley LA, Schultz-Cherry S, Muse SV, Koci MD. Genomic analysis of closely related astroviruses. J Virol. 2008;82:5099–103.
95. Sugiyama MK, Goto K, Uemukai H, Mori Y, Ito N, Minamoto N. Attachment and infection to MA104 cells of avian rotaviruses require the presence of sialic acid on the cell surface. J Vet Med Sci. 2004;66:461–3.
96. Tang Y, Ismail MM, Saif YM. Development of antigen-capture enzyme-linked immunosorbent assay and RT-PCR for detection of turkey astroviruses. Avian Dis. 2005;49(2):182–8.
97. Tang Y, Murgia AM, Saif YM. Molecular characterization of the capsid gene of two serotypes of turkey astroviruses. Avian Dis. 2005;49(4):514–9.

98. Teixeira MC, Luvizotto MC, Ferrari HF, Mendes AR, da Silva SE, Cardoso TC. Detection of turkey coronavirus in commercial turkey poults in Brazil. Avian Pathol. 2007;36(1):29–33.
99. Theil KW, Saif YM. Age-related infections with rotavirus, rotaviruslike virus, and atypical rotavirus in turkey flocks. J Clin Microbiol. 1987;25(2):333–7.
100. Tilley BJ, Gonder E, Mason S, Weeks J. Turkey coronavirus outbreak in North Carolina. Paper presented in annual meeting of American Association of Avian Pathologist held at Chicago, Illinois. 2013.
101. Toffan A, Catania S, Salviato A, De Battisti C, Vascellari M, Toson M, Capua I, Cattoli G. Experimental infection of poults and guinea fowl with genetically distinct avian astroviruses. Avian Pathol. 2012;41:429–35.
102. Trojnar E, Otto P, Roth B, Reetz J, Johne R. The genome segments of a group D rotavirus possess group A-like conserved termini but encode group-specific proteins. J Virol. 2010;84:10254–65.
103. Trojnar E, Sachsenroder J, Twardziok S, Reetz J, Otto PH, Johne R. Identification of an avian group A rotavirus containing a novel VP4 gene with a close relationship to those of mammalian rotaviruses. J Gen Virol. 2013;94:136–42.
104. Velayudhan BT, Shin HJ, Lopes VC, Hooper T, Halvorson DA, Nagaraja KV. A reverse transcriptase-polymerase chain reaction assay for the diagnosis of turkey coronavirus infection. J Vet Diagn Invest. 2003;15(6):592–6.
105. Woolcock PR, Shivaprasad HL. Electron microscopic identification of viruses associated with poult enteritis in turkeys grown in California 1993–2003. Avian Dis. 2008;52:209–13.
106. Wooming B. A coronavirus outbreak in Northwest Arkansas. Paper presented in annual meeting of American Association of Avian Pathologist held at Chicago, Illinois. 2013.
107. Yu M, Ismail MM, Qureshi MA, Dearth RN, Barnes HJ, Saif YM. Viral agents associated with poult enteritis and mortality syndrome: the role of a small round virus and a turkey coronavirus. Avian Dis. 2000;44(2):297–304.
108. Yu M, Tang Y, Guo M, Zhang Q, Saif YM. Characterization of a small round virus associated with the poult enteritis and mortality syndrome. Avian Dis. 2000;44(3):600–10.
109. Zsak L, Strother KO, Kisary J. Partial genome sequence analysis of parvoviruses associated with enteric disease in poultry. Avian Pathol. 2008;37(4):435–41.