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# Detection and molecular characterization of enteric viruses from poult enteritis syndrome in turkeys

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**ABSTRACT** This study was conducted to detect and characterize enteric viruses [rotavirus, turkey astrovirus-2 (TAsV-2), reovirus, and turkey coronavirus] from cases of poult enteritis syndrome (PES) in Minnesota turkeys. Of the intestinal contents collected from 43 PES cases, 25 were positive for rotavirus and 13 for small round viruses by electron microscopy (EM). Of the enteric virus-positive cases by EM (n = 27), 16 cases had rotavirus or small round viruses alone and the remaining 11 cases had both viruses. None of the cases were positive for reovirus or coronavirus by EM. However, with reverse transcription-PCR (RT-PCR), 40 cases (93%) were positive for rotavirus, 36 (84%) for TAsV-2, and 17 (40%) for reovirus. None of the cases were positive for turkey coronavirus by RT-PCR. The viruses from all cases were detected either alone or in combination of 2 or 3 by RT-PCR. Thus, 8 (19%) cases were positive for a single virus, whereas a combination

of viruses was detected in the remaining 35 (81%) cases. The rota-TAsV-2 combination was the most predominant (n = 18 cases). Fifteen cases were positive for all 3 viruses. The rotaviruses had sequence homology of 89.8 to 100% with previously published sequences of turkey rotaviruses at the nucleotide level. The TAsV-2 had sequence homology of 84.6 to 98.7% with previously published TAsV-2, whereas reoviruses had sequence homology of 91.6 to 99.3% with previously published sequences of turkey reoviruses. Phylogenetic analysis revealed that rota- and reoviruses clustered in a single group, whereas TAsV-2 clustered in 2 different groups. In conclusion, a larger number of PES cases was positive for rotavirus, TAsV-2, and reovirus by RT-PCR than with EM. The presence of more than one virus and changes at the genetic level in a virus may affect the severity of PES in turkey flocks.

**Key words:** poult enteritis syndrome, enteric virus, electron microscopy, reverse transcription-polymerase chain reaction, molecular characterization

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## INTRODUCTION

Poult enteritis syndrome (PES) is characterized by dullness, depression, and lethargy in young poults. In an earlier study, we reproduced PES experimentally by inoculating 14-d-old turkey poults orally with intestinal contents from PES-affected birds. The inoculum was positive for rotavirus, turkey astrovirus (TAsV), and *Salmonella*. The inoculated poults developed diarrhea, depression, lethargy, and an average weight loss of 31.8% at 15 to 20 d postinoculation (Jindal et al., 2009b). In another study, we observed similar clinical signs and significant growth retardation in PES-inoc-

ulated poults even at 50 d postinoculation (N. Jindal, unpublished data). These results suggest that PES may adversely affect the growth rate of young turkeys, resulting in heavy economic losses to the turkey growers.

In a retrospective study, data from the Minnesota Veterinary Diagnostic Laboratory in St. Paul were analyzed from 2002 to 2007 to detect different pathogens in PES cases. Several pathogens such as rotavirus, small round viruses (SRV), reovirus, adenovirus, *Salmonella*, *Escherichia coli*, *Enterococcus*, and *Eimeria* spp. were detected either alone or in different combinations (Jindal et al., 2009a). The viruses in these cases were detected by electron microscopy (EM), bacteria by isolation on specific bacteriological media, and protozoa by the fecal floatation technique (Jindal et al., 2009a). None of the PES-affected cases were positive for turkey coronavirus (TCV), although this virus is the primary agent identified in another syndrome known as the poult enteritis and mortality syndrome (PEMS; Guy et al., 1997; Yu et al., 2000).

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Several diarrheic syndromes such as coronaviral enteritis, malabsorption syndrome, maldigestion syndrome, PEMS, runting and stunting syndrome of turkeys, spiking mortality of turkeys, and turkey viral enteritis have been detected in turkey poults and all of them have been grouped under the poult enteritis complex (**PEC**). Enteric viruses commonly associated with these diarrheic syndromes include rotavirus, TAstV, enterovirus, reovirus, adenovirus, and TCV (Barnes et al., 2000). Electron microscopy is the most commonly used method for detecting enteric viruses and is currently favored over molecular methods because it can potentially detect all enteric viruses in a single sample preparation, whereas several polymerase or reverse transcription-PCR (**RT-PCR**) may be needed to detect all enteric viruses. The molecular techniques, on the other hand, are sensitive and specific and can detect very small quantities of viral nucleic acids. We suspect that the true role of enteric viruses in PES is underestimated because of the relative insensitivity of EM. We conducted this study to detect enteric viruses in PES cases by both EM and RT-PCR. The enteric viruses detected in these cases were characterized to determine genetic variations, if any.

## MATERIALS AND METHODS

### Source of Samples

Samples from turkeys are routinely submitted to the Minnesota Veterinary Diagnostic Laboratory for disease investigation. During 2007 and 2008, intestinal contents from 43 PES-affected cases were processed for the detection of enteric viruses (rotavirus, TAstV, reovirus, and TCV) by EM and RT-PCR. A case (flock) was diagnosed as PES on the basis of clinical findings of lethargy, depression, or diarrhea, or pathological changes of thin-walled intestines with watery contents. The intestinal contents of birds from a single case were pooled to make 1 pooled sample, which was further divided into 2 parts (parts I and II). Part I was tested for enteric viruses by EM (Goyal et al., 1987). A 10% suspension of part II of the pooled sample was made in PBS (pH 7.4). The suspension was homogenized and centrifuged at  $784 \times g$  for 20 min. The supernatant was collected and tested for enteric viruses by RT-PCR as described below.

### RNA Extraction and RT-PCR

Total RNA was extracted from the supernatant of intestinal contents using Trizol LS reagent (Invitrogen, Carlsbad CA). Total RNA was also extracted from a known rotavirus, TAstV-2, reovirus, and TCV. Extracted RNA was subjected to RT-PCR individually using primers specific to each of the 4 enteric viruses. Primers for NSP4 gene of rotavirus (Pantin-Jackwood et al., 2007), polymerase gene of TAstV (Koci et al.,

2000a), S4 gene of reovirus (Pantin-Jackwood et al., 2008a), and N gene of TCV (Sellers et al., 2004) were used. The reaction conditions and reaction mix for the amplification of rotavirus, TAstV, and TCV have been described previously (Jindal et al., 2009b). For reovirus, the reaction mix consisted of  $1 \times$  RT-PCR reaction buffer,  $320 \mu M$  of each deoxynucleotide triphosphate,  $0.6 \mu M$  of each primer,  $2 \mu L$  of enzyme blend, and  $5 \mu L$  of extracted RNA for a total volume of  $50 \mu L$ . Amplification steps consisted of reverse transcription at  $50^\circ C$  for 30 min, *Taq* activation at  $94^\circ C$  for 15 min, followed by 35 cycles of denaturation at  $94^\circ C$  for 30 s, annealing at  $53^\circ C$  for 1 min, and extension at  $72^\circ C$  for 1 min, and a step of final extension at  $72^\circ C$  for 10 min. Amplification was carried out using a OneStep RT-PCR kit (Qiagen, Valencia, CA). In positive cases, bands of 630, 802, 1,120, and 598 bp were observed on agarose gel electrophoresis for rotavirus, TAstV, reovirus, and TCV, respectively.

### Phylogenetic Analysis

To determine the sequence similarity of enteric viruses from PES cases with previously published sequences of enteric viruses, PCR products were purified using a PCR purification kit (Qiagen) followed by sequencing in both directions at the BioMedical Genomic Center, University of Minnesota, St. Paul. Using sequencer software (<http://www.msi.umn.edu>), the forward and reverse nucleotide sequences were aligned to make one sequence. Aligned nucleotide sequences were subjected to BLAST analysis (<http://www.ncbi.nlm.nih.gov>) to confirm their identity. The nucleotide sequences of each enteric virus were compared among themselves and with previously published sequences of that virus to determine differences at the nucleotide level, if any. The sequences were aligned by Clustal W method using MEGA 4.0 software (Tamura et al., 2007). The pairwise distance method using the maximum composite likelihood model was used for computing evolutionary distances. A phylogenetic tree of aligned sequences of each virus was then constructed by neighbor-joining method. The nucleotide sequences were translated to deduced amino acid sequences, which were also compared to determine differences at the amino acid level. The details of previously published sequences used for comparison are given in Table 1.

### GenBank Accession Numbers

Partial nucleotide sequences of NSP4 gene of rotavirus, polymerase gene of TAstV-2, and S4 gene of reovirus were submitted to GenBank with the following accession numbers: GQ300940 to GQ300973, GQ353331 to GQ353332, and GQ368188 (rotavirus); GQ301005 to GQ301035 and GQ353333 to GQ353334 (TAstV-2); and GQ353318 to GQ353330 (reovirus).

**Table 1.** GenBank accession numbers of previously published sequences used for comparison

Virus	Gene	Strain name	Country	GenBank accession number
Rotavirus	NSP4	Ch-1	Japan	AB065287
		Ty-3	Japan	AB065286
		Ty-1	Japan	AB065285
		CK/DE/SEP-810/05	United States	EU400305
		TK/MN/SEP-808/05	United States	EU400311
		TK/MO/SEP-814/05	United States	EU400312
		TK/NC/SEP-833/05	United States	EU400315
		TK/WI/SEP-847/05	United States	EU400327
		TK/WI/SEP-830/05	United States	EU400318
		TK/MO/SEP-822/05	United States	EU400320
		TK/NC/SEP-832/05	United States	EU400323
		AvRV-1	Korea	AY062937
		TK/MO/SEP-827/05	United States	EU400322
		TK/NC/SEP-R642/05	United States	EF204141
		TK/WI/SEP-844/05	United States	EU400326
		TK/NC/SEP-R664/05	United States	EF204137
		TK/NC/SEP-834/05	United States	EU400324
		TK/NC/SEP-R404/05	United States	EF204132
		Astrovirus	Polymerase	TAstV-2 polymerase
MN/SEP-A809/05	United States			DQ324815
NC/SEP-A41/03	United States			DQ066580
TAstV-2 complete genome	United States			AF206663
TAstV-2 complete genome	United States			NC_005790
TAstV 3910/04	Italy			DQ381356
TAstV 1619/04	Italy			DQ381372
TAstV 532/04	Italy			DQ381312
VA/SEP-A33/03	United States			DQ066598
OH-SEP-A848-86	United States			DQ324820
MO-SEP-A824-05	United States			DQ324826
TAstV-1	United States			NC_002470
Reovirus	S4			Avian orthoreovirus strain 1733
		Avian orthoreovirus strain 176	Canada	AF059724
		Avian orthoreovirus S1133	Taiwan	U95952
		CK/AR/SEP-854/06	United States	EU400297
		TK/WI/SEP-847/05	United States	EU400293
		TK/NC/SEP-832/05	United States	EU400283
		TK/MO/SEP-828/05	United States	EU400282
		TK/NC/SEP-835/05	United States	EU400285
		TK/MO/SEP-819/05	United States	EU400280
		TK/MO/SEP-827/05	United States	EU400281
		Avian orthoreovirus strain 138	Canada	AF059725

**RESULTS**

**Occurrence of Enteric Viruses**

**Coronavirus** None of the cases were positive for coronavirus by EM. They were also not positive for coronavirus by RT-PCR.

**Rotavirus.** Of the 43 cases, rotavirus was detected in 25 (58%) cases by EM (Table 2). All 25 cases positive for rotavirus by EM were also positive by RT-PCR. In

addition, 15 more cases were positive for rotavirus by RT-PCR for a total of 40 (93%) cases being positive for rotavirus.

**TAstV-2.** Of the 43 cases, SRV were detected in 13 (30%) cases by EM (Table 2). These 13 cases were positive for TAstV-2 by RT-PCR. Twenty-three additional cases were positive for TAstV-2 by RT-PCR, bringing the total to 36 (84%) cases.

**Reovirus.** None of the cases were positive for reovirus by EM. However, when tested by RT-PCR, 17 (40%) cases were positive for this virus (Table 2).

**Table 2.** Detection of enteric viruses alone or in combination from poult enteritis syndrome (PES)-affected cases

Method	Number (%) of cases positive for indicated virus			Number (%) of cases positive for different numbers of viruses		
	Rotavirus	TAstV-2/SRV <sup>1</sup>	Reovirus	1 virus	2 viruses	3 viruses
EM <sup>2</sup>	25 (58)	13 (30)	0	16 (59)	11 (41)	0
RT-PCR <sup>3</sup>	40 (93)	36 (84)	17 (40)	8 (19)	20 (46)	15 (35)

<sup>1</sup>TAstV-2 = turkey astrovirus-2 (detected by RT-PCR); SRV = small round viruses (detected by electron microscopy).

<sup>2</sup>EM = electron microscopy; reovirus and coronavirus were not detected in any of the PES cases by EM.

<sup>3</sup>RT-PCR = reverse transcription-PCR; none of the PES cases were positive for coronavirus by RT-PCR.

**EM Versus RT-PCR.** Of the 43 cases, only 27 (63%) were positive for rotavirus or SRV by EM; of the positives, 16 (59%) were for a single virus (rotavirus in 14 and SRV in 2 cases) and 11 (41%) were for both viruses (rotavirus and SRV). However, by RT-PCR, all 43 (100%) PES cases were positive for rotavirus or TAstV-2 or reovirus (Table 2). The viruses in all cases were detected either alone or in combination of 2 or 3 by RT-PCR. Eight cases (19%) were positive for a single virus, whereas a combination of viruses was detected in the remaining 35 (81%) cases. The combination of rota-TAstV-2 viruses was the most predominant one; it was detected in 18 cases. One case each had a combination of reo-TAstV-2 and rota- and reovirus. The remaining 15 cases were positive for all 3 viruses (Table 2).

### **Phylogenetic Analysis of Rotavirus**

The PCR products from 37 rotavirus-positive cases were sequenced and compared with previously published sequences of rotaviruses. The rotaviruses had sequence homology of 93.0 to 100% among themselves at the nucleotide level, whereas the sequence homology with already published rotaviruses of turkey origin (except Ty-1) was 89.8 to 100%. The sequence homology with Ty-1 was 76.8 to 78.7%. When turkey rotaviruses were compared with previously published chicken rotaviruses, the sequence homology at the nucleotide level was only 66 to 69%. Amino acid sequences corresponding to positions 2 to 168 of NSP4 gene of Ty-3 (accession number AB065286) were analyzed to determine changes at the amino acid level and are shown in Table 3. The amino acid valine at positions 75 and 89 was observed in 18 and 24 rotaviruses, respectively; the amino acid methionine at these positions was observed in 13 and 12 rotaviruses, respectively. Similarly, the amino acids aspartic acid and asparagine at position 139 were observed in 26 and 11 rotaviruses, respectively. Phylogenetic analysis based on nucleotide sequences of NSP4 gene revealed that turkey rotaviruses were in a clade separate than that of chicken rotaviruses (Ch-1 and CK/DE/SEP-810/05; Figure 1). Of the 37 rotaviruses, 32 were phylogenetically close to each other and were also close to previously described rotaviruses from Minnesota and Wisconsin. The remaining 5 (TK/MN/D-055423/07, TK/MN/D-049317/07, TK/MN/D-53853/07, TK/MN/D-000818/08, and TK/MN/D-049007/07) were phylogenetically close to Missouri strains of rotavirus. Six strains of rotaviruses from North Carolina (TK/NC/SEP-833/05, TK/NC/SEP-R642/05, TK/NC/SEP-832/05, TK/NC/SEP-R664/05, TK/NC/SEP-834/05, TK/NC/SEP-R404/05) and 1 strain from Wisconsin (TK/WI/SEP-844/05) used for comparison were in a separate group. Similarly, 1 of the 2 strains of rotavirus (Ty-1) from Japan was also in a separate group. The other strain from Japan (Ty-3) and 1 strain from Korea (AvRV-1) were phylogenetically close to rotaviruses in this study but belonged to separate groups (Figure 1).

### **Phylogenetic Analysis of TAstV**

The PCR products from 33 TAstV-2-positive cases were sequenced and compared with previously published sequences. These viruses had a sequence homology of 85.9 to 100% at the nucleotide level among themselves and 84.6 to 98.7% with previously published TAstV-2. Amino acid sequence analysis corresponding to positions 253 to 491 (based on TAstV-2 GenBank accession number NC\_005790) revealed changes at the amino acid level (Table 3). Changes such as cysteine to tyrosine at position 265, asparagine to serine at position 414, serine to asparagine or threonine at position 423, serine to asparagine or lysine at position 445, and threonine to alanine at position 446 were observed in 10, 15, 13, 13, and 20 TAstV-2, respectively (Table 3). Astroviruses that revealed the change at position 265 also revealed the changes at other positions such as 414, 423, 445, and 446. Phylogenetic analysis revealed that all TAstV-2 were in a group separate from TAstV-1 (Figure 2). The TAstV-2 viruses assorted in 2 groups (I and II); 30 were in group I and 3 in group II. Of the 10 previously published TAstV-2 used for comparison, 5 each were assorted with group I or group II TAstV-2, respectively (Figure 2).

### **Phylogenetic Analysis of Reovirus**

The PCR products from 13 reovirus-positive cases were sequenced and compared with previously published sequences of reovirus. Sequence homology among reoviruses of this study ranged from 91.6 to 99.8% and was 91.6 to 99.3% when compared with previously published sequences of turkey reoviruses. The sequence homology was low (80.6 to 83.1%) with reoviruses of chicken origin (CK/AR/SEP-854/06, strain 1733, strain 138, strain 176, and strain 1133). Amino acid sequences corresponding to positions 26 to 344 of avian orthoreovirus strain 176 (GenBank accession number AF059724) revealed changes at the amino acid level (Table 3). Changes at position 138 (from isoleucine to valine) and at position 183 (from methionine to leucine) were observed in 3 of the 13 reoviruses, and both of these changes were noticed in reoviruses from the same cases (Table 3). Phylogenetic analysis based on nucleotide sequences of S4 gene revealed that all reoviruses of this study clustered in a single group (Figure 3). Within this group, 9 of the 13 reoviruses were phylogenetically close to a previously published reovirus strain from Missouri (TK/MO/SEP-828/05). The remaining 4 reoviruses of this study were close to a strain from Wisconsin (TK/WI/SEP-847/05). The other 2 strains from Missouri (TK/MO/SEP-819/05 and TK/MO/SEP-827/05) and 2 strains from North Carolina (TK/NC/SEP-832/05 and TK/NC/SEP-835/05) were in a separate clade. All other previously published reoviruses of chicken origin (CK/AR/SEP-854/06, strain

1733, strain 138, strain 176, and strain 1133) were distantly placed from reoviruses of this study (Figure 3).

### DISCUSSION

We tested intestinal contents from PES-affected cases for 4 viruses by EM and RT-PCR. None of the cases positive for a virus by EM were negative for that virus by RT-PCR. The number of cases positive for enteric viruses was higher by RT-PCR than EM. In addition, reovirus was detected in 40% of cases by RT-PCR and not by EM. It is possible that some of the PES cases had lower virus concentrations that went undetected by EM thereby leading to classification of such cases as negative. It has been reported that SRV (TAsTV, enterovirus, picorna like-virus) may not be clearly differentiated by EM because of similarity of their sizes (van Regenmortel et al., 2000) albeit with distinct morphologies (Caul and Appleton, 1982). This may be true in this study, too, because TAsTV was not detected even from a single case by EM and the viruses resembling TAsTV were classified as SRV by EM. However, when the samples were processed by RT-PCR, we could detect TAsTV in 84% of cases. These results indicate the

importance of using molecular techniques for detection of enteric viruses.

None of the PES cases were positive for TCV either by EM or RT-PCR. Turkey coronavirus is the cause of an acute contagious enteric disease of turkeys referred to as bluecomb disease. The disease was first described in turkeys in 1951 and a coronavirus was identified as the cause of the disease in 1973 (Pomeroy and Nagaraja, 1991). Turkey coronavirus has also been identified in PEMS cases in the United States (Guy et al., 1997; Yu et al., 2000) and United Kingdom (Culver et al., 2006) and has also been detected in turkey poult affected with PEC in Brazil (Teixeira et al., 2007). Pantin-Jackwood et al. (2008a) reported that coronavirus is strongly associated with enteric disease and is rarely observed in healthy poults. The absence of this virus in our study indicates a basic difference between PES and PEMS with respect to coronavirus.

The detection of multiple enteric viruses in PES cases has the support of Saif et al. (1985), who reported co-infection of turkeys with both rotavirus and astrovirus. Pantin-Jackwood et al. (2008a) also detected combinations of astrovirus, rotavirus, and reovirus in chicken and turkey flocks. Woolcock and Shivaprasad (2008)

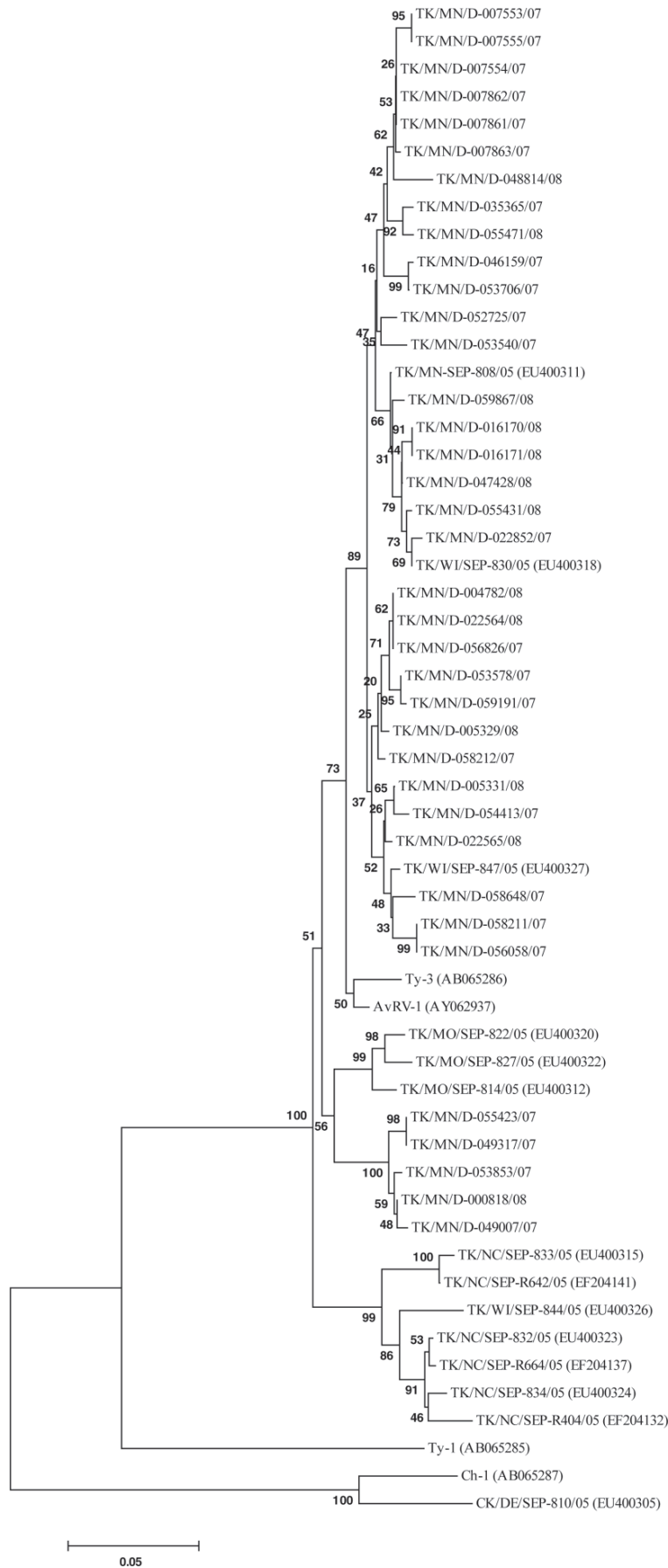
**Table 3.** Amino acid changes in rotavirus, turkey astrovirus-2, and reovirus detected in poult enteritis syndrome (PES)-affected poults

Gene (virus)	Position	Amino acid (number of cases)	
NSP4 gene <sup>1</sup> (avian rotavirus)	31	Lysine (33), arginine (4)	
	43	Alanine (31), valine (6)	
	75	Valine (18), methionine (13), alanine (6)	
	89	Valine (24), methionine (12), isoleucine (1)	
	104	Alanine (28), threonine (9)	
	139	Aspartic acid (26), asparagine (11)	
	140	Glutamic acid (31), aspartic acid (5), glycine (1)	
	141	Valine (34), isoleucine (3)	
	Polymerase gene <sup>2</sup> (turkey astrovirus-2)	265	Cysteine (23), tyrosine (10)
		287	Serine (30), proline (3)
		306	Threonine (30), serine (3)
		309	Methionine (31), isoleucine (2)
		324	Isoleucine (31), valine (2)
359		Threonine (30), alanine (3)	
362		Valine (30), isoleucine (3)	
370		Arginine (30), phenylalanine (1), histidine (2)	
406		Aspartic acid (30), glutamic acid (3)	
414		Asparagine (18), serine (15)	
422		Arginine (30), lysine (3)	
423		Serine (20), asparagine (10), threonine (3)	
445		Serine (20), asparagine (10), lysine (3)	
446		Alanine (20), threonine (13)	
448		Alanine (30), threonine (3)	
S4 gene <sup>3</sup> (avian reovirus)	82	Leucine (11), methionine (2)	
	89	Isoleucine (10), valine (3)	
	131	Leucine (9), alanine (4)	
	138	Isoleucine (10), valine (3)	
	152	Arginine (9), cysteine (3), histidine (1)	
	160	Asparagine (9), serine (4)	
	183	Methionine (10), leucine (3)	
	202	Alanine (9), threonine (4)	
	205	Glycine (9), arginine (4)	
	321	Valine (11), isoleucine (2)	

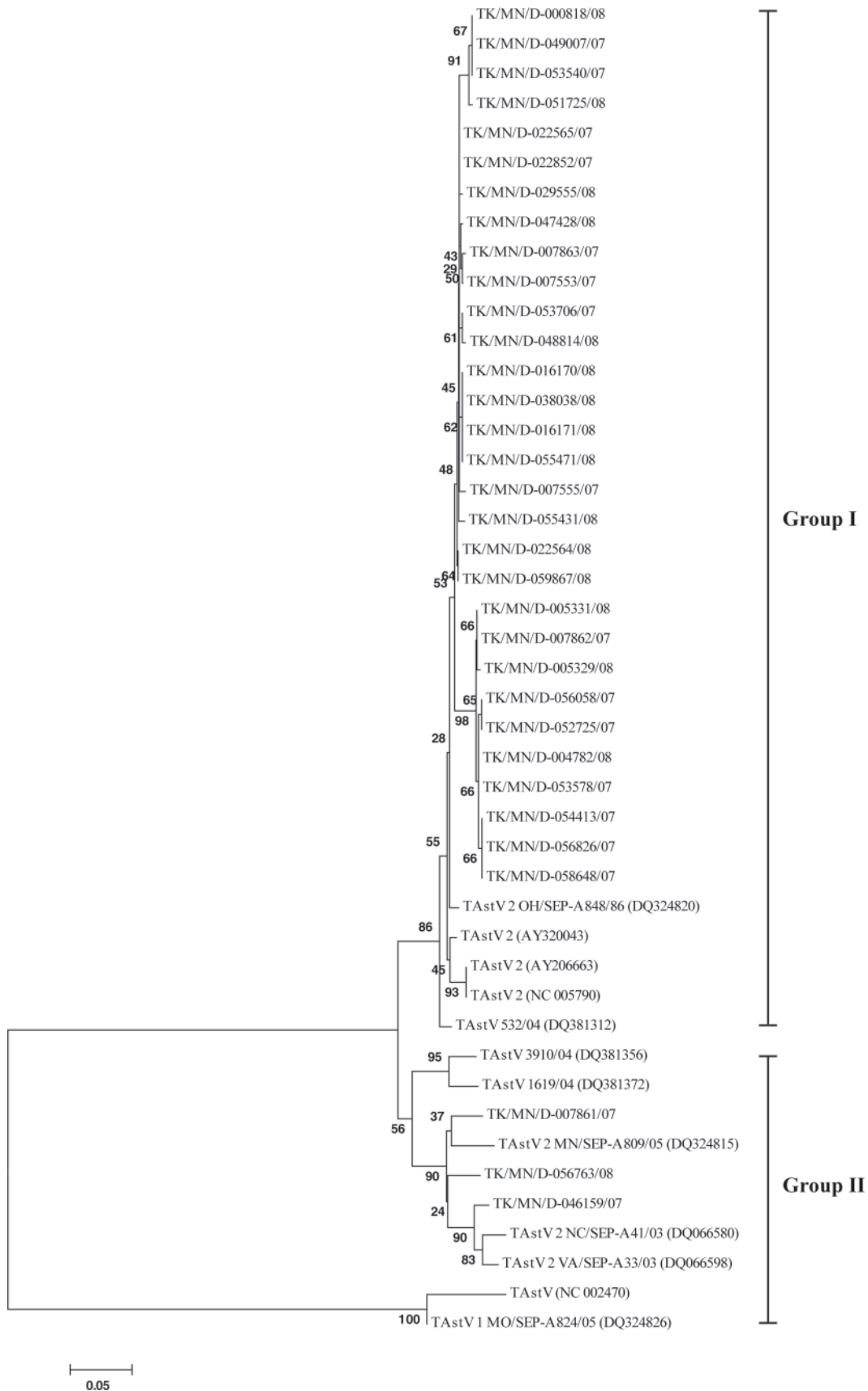
<sup>1</sup>Amino acid positions correspond to positions 2 to 168 of NSP4 gene of Ty-3 (accession number AB065286).

<sup>2</sup>Amino acid positions correspond to positions 253 to 491 of polymerase gene of turkey astrovirus-2 (TAsTV-2; accession number NC\_005790).

<sup>3</sup>Amino acid positions correspond to positions 26 to 344 of S4 gene of avian orthoreovirus strain 176 (accession number AF059724).

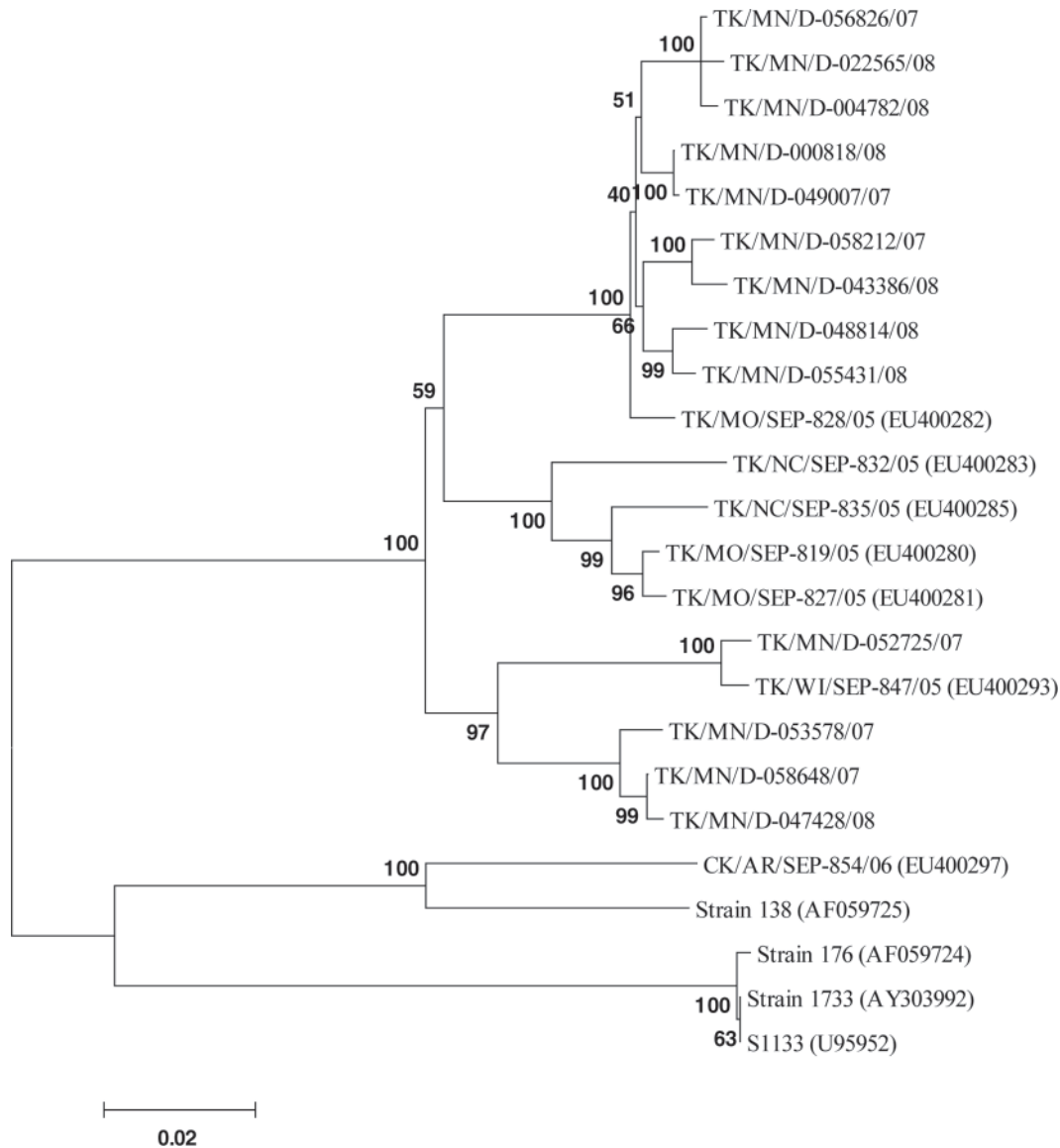


**Figure 1.** Phylogenetic tree of nucleotide sequences of NSP4 gene of rotavirus from poult enteritis syndrome-affected cases. The sequence names starting with TK/MN and without GenBank accession numbers are from the present study and the strain names (GenBank accession numbers) are previously published sequences. Bootstrap values are shown on the tree.



**Figure 2.** Phylogenetic tree of nucleotide sequences of polymerase gene of turkey astrovirus from poult enteritis syndrome-affected cases. The sequence names starting with TK/MN and without GenBank accession numbers are from the present study and the strain names (GenBank accession numbers) are previously published sequences. Bootstrap values are shown on the tree.





**Figure 3.** Phylogenetic tree of nucleotide sequences of S4 gene of reovirus from poult enteritis syndrome-affected cases. The sequence names starting with TK/MN and without GenBank accession numbers are from the present study and the strain names (GenBank accession numbers) are previously published sequences. Bootstrap values are shown on the tree.

reported the presence of multiple enteric viruses in cases of poult enteritis in turkeys. In an earlier study, we detected the combinations of 2 or 3 viruses from apparently healthy breeder turkeys (N. Jindal, unpublished data) with a notable difference that 81.4% cases were positive for 2 or 3 viruses in the current study, whereas only 40.6% of the enteric virus-positive samples from breeder turkeys were positive for combinations of viruses. The increased frequency of multiple viruses in PES cases as compared with those in apparently healthy flocks is not surprising. All 3 viruses (rotavirus, astrovirus, and reovirus) individually have been reported to cause diarrhea, depression, or stunted growth in turkeys and chickens (Hieronymus et al., 1983; Nersessian et al., 1986; Yason and Schat, 1986, 1987; Heggen-Peay et al., 2002; Otto et al., 2006; Pantin-Jackwood et al., 2008b). It is possible that these viruses in combination may cause more increased adverse effects in poults than

an individual virus. Such a possibility under field conditions cannot be ruled out based on our observations of detecting these viruses in combination from PES cases. In experimental studies, we have reported that oral inoculation of poults with PES material (positive for rotavirus, TAsV-2, and *Salmonella*) led to significant growth retardation for up to 50 d postinoculation. At no point postinoculation did any of the challenged birds' weight converge with that of controls (N. Jindal, unpublished data). This extent of growth depression due to PES may lead to considerable economic losses to turkey growers.

A higher number of PES cases was positive for rotavirus than astrovirus or reovirus, indicating that rotaviruses may be associated with PES. In a retrospective study, we observed that the proportion of rotavirus and *Salmonella* was significantly higher than the other pathogens (SRV, reovirus, adenovirus, *E.*

*coli*, *Enterococcus*, *Eimeria* sp.) in PES cases (Jindal et al., 2009a). Close association of rotaviruses with previously published rotaviruses from the Upper Midwest indicated that turkey rotaviruses may assort based on geographic locations. Assortment of turkey rotaviruses based on different geographical locations in the United States has earlier been reported (Pantin-Jackwood et al., 2008a). Different groups of rotaviruses (groups A, D, F, and G) have been detected in poultry (McNulty et al., 1984), with group D rotaviruses being the most common (McNulty, 2003). We do not know to which group(s) the rotaviruses of this study belong. We also do not know whether the changes observed at amino acid level in rotaviruses of this study are group-related or not. Further studies are required to determine the groups of rotaviruses involved in PES cases. Assortment of turkey rotaviruses in a separate group from that of chicken rotaviruses based on NSP4 gene indicates that interspecies transmission of rotaviruses may not be a common phenomenon. However, Schumann et al. (2009) did report on the possibility of interspecies transmission and reassortment among group A avian rotaviruses.

The TAsV have been classified into 2 types: TAsV-1 and TAsV-2. The TAsV have been detected from poult in the United States (Reynolds and Saif, 1986; Pantin-Jackwood et al., 2008a). The TAsV-2 has also been detected from PEMS cases (Koci et al., 2000b; Schultz-Cherry et al., 2000; Yu et al., 2000). In this study, we tested the intestinal contents from PES cases for the presence of TAsV-2 only. Hence, the presence of TAsV-1 in PES cases cannot be ruled out and needs further studies. Substantial changes were observed in polymerase gene of TAsV-2 both at nucleotide and amino acid levels. The effect of these changes on antigenicity or pathogenicity of TAsV is not known. The TAsV-2 assorted into 2 groups. In a previous study, the TAsV-2 also assorted into 2 groups based on polymerase phylogeny (N. Jindal, unpublished data). These findings have the support of Pantin-Jackwood et al. (2006), who also reported the assortment of TAsV from different regions of the United States into 2 groups on the basis of polymerase gene. It is not known whether these 2 groups of TAsV-2 belong to different serotypes or not, although it is possible that different serotypes of TAsV may exist (Strain et al., 2008). In this study, we used primers that could amplify a part of the polymerase gene of TAsV-2. To obtain even better understanding of TAsV, it would be interesting to study polymerase gene in full length from PES cases. Such a study would provide more information about the changes at a genetic level in this gene of TAsV.

Avian reoviruses have been associated with a wide range of disease presentations in avian species (Nibert and Schiff, 2001). This virus has also been isolated from PEMS and PEC (Heggen-Peay et al., 2002; Kapczynski et al., 2002; Spackman et al., 2005). This study revealed the clustering of reoviruses on the basis of species. A similar assortment of reoviruses based on species

has been reported by Pantin-Jackwood et al. (2008a). Due to a lower number of S4 gene sequences of avian reoviruses of turkey origin available in GenBank from different geographic locations, a few were used for comparison with reoviruses of this study. This study did not reveal a specific pattern of assortment based on geographical locations. A better picture may emerge when more sequences of this gene from different geographic locations are available in GenBank.

The aim of this study was to test intestinal contents from PES cases for rotavirus, astrovirus, reovirus, and coronavirus and to determine the variations in these viruses at nucleotide and amino acid levels. We did not determine the correlation of enteric viruses with PES. We also did not test the intestinal contents for the presence of other enteric pathogens; their role in causing or increasing the severity of PES cannot be ruled out. Further studies are needed to determine the presence of other pathogens in PES cases and their possible role in the disease process. Based on our results, we cannot say whether rotavirus or astrovirus initiates the disease process; however, their prevalence in PES cases indicates that both viruses may do so on their own. It is also possible that these viruses may assume a more virulent role when birds are under stress due to concurrent infection(s) or climate changes. In this study, we did not analyze our data with age of birds involved; however, our previous study (Jindal et al., 2009a) suggests that young birds up to 3 wk of age are more susceptible to PES. The disease has been seen in poult as young as 2 d old. It remains to be seen how young poult of 1 to 2 d of age get exposed to these enteric viruses, even though fecal-oral transmission has been well established for these viruses.

In conclusion, this study indicates that testing of intestinal contents from PES cases by RT-PCR may provide a better picture of the presence of enteric viruses in these cases than EM. Presence of more than one virus may affect the severity of PES in turkey flocks. The changes observed at nucleotide and amino acid levels in each virus may affect the pathogenicity of the virus and needs further studies. Considering the high prevalence of PES in Minnesota turkeys and its adverse effects, particularly the enteritis and growth retardation, the potential economic ramifications are apparent.

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