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Prevalence and complete genome characterization of turkey picobirnaviruses

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ABSTRACT

The "light turkey syndrome" (LTS), in which birds weigh less than their standard breed character at the marketing time, is believed to be a consequence of viral enteritis at an early age (3–5 weeks) from which the birds never fully recover. In a previously published study, we collected fecal pools from 2, 3, 5 and 8 week old turkey poults (80 pools from LTS farms and 40 from non-LTS farms) and examined them for the presence of astro-, rota-, reo-, and coronaviruses. To determine the presence of additional enteric viruses, we analyzed a fecal pool by Illumina sequencing and found picobirnavirus (PBV). Segments 1 and 2 of this virus shared 45.8% aa and 60.9–64.5% aa identity with genogroup I of human PBV, respectively. Primers based on RNA-dependent RNA polymerase and capsid genes were designed for detection and molecular characterization of PBVs in the 120 fecal pools described above. From LTS farms, 39 of 80 (48.8%) pools were PBV positive while 23 of 40 (57.5%) were positive from non-LTS farms. The phylogenetic analysis of 15 randomly selected strains divided them into four subgroups within genogroup I (subgroups 1A–D). Nine strains were in subgroup IA showing 69.9–76.4% nt identity with human PBV GI strainVS111 from the Netherlands. Strains in subgroup IB ($n = 2$) had 91.4–91.7% nt identity with chicken PBV GI strain AVE 42v1 from Brazil. Two strains in subgroup IC had 72.3–74.2% nt identity with chicken PBV strain AVE 71v3 from Brazil. In subgroup ID, two strains showed 72.4–81.8% nt identity with chicken PBV GI strain AVE 57v2 from Brazil. Subgroup IC and ID were the most divergent. Five of the 15 strains were typed using capsid gene primers. They showed 32.6–33.4% nt and 39.5–41.3% aa identity with VS10 human PBV strain. These results indicate co-circulation of divergent strains of PBVs among Minnesota turkeys.

1. Introduction

The family *Picobirnaviridae* contains a single genus, namely picobirnavirus (PBV). The virus is non-enveloped, 33–41 nm in diameter and contains a bi-segmented double stranded RNA genome of 4.2 kb (Silva et al., 2014). The large segment (2.2–2.7 kb), also called segment 1, contains two open reading frames encoding 224 and 552 amino acids. The first ORF encodes a protein of unknown function and the second ORF encodes the capsid protein. Small segment 2 (1.2–1.9 kb) contains a single ORF that encodes RNA-dependent RNA polymerase (RdRp) (Chandra, 1997; Wakuda et al., 2005; Smits et al., 2012).

Rosen et al. (2000) reported almost complete sequence of segment 2 and partial sequence of segment 1 by cloning and sequencing and divided human PBVs into two genogroups (genogroups I and II) based on the sequence of segment 2 that encodes for RdRp. For the specific detection and typing of PBV, they designed primer pair Pico B25 and Pico B43 for genogroup I and primer pair Pico B23 and Pico B24 for genogroup II. Viruses that do not belong to either genogroup I or II are called genogroup non-I, non-II (Ganesh et al., 2012a,b). Subsequently, Fregolente and Gatti (2009) proposed a standard nomenclature to assign a clear and unique name to each strain of PBV. According to their recommendation, the strain name starts with identification of genogroup (GI, GII, non-GI or non-GII) followed by PBV, common name of host species, three letter country code, strain name, and year of isolation.

Using primer sets designed by Rosen et al. (2000), PBVs have been detected in cases of gastroenteritis in humans, animals (including sea lions, monkeys, pigs and rats), birds (chickens, Greater rhea and turkeys) and reptiles (snakes) (Gatti et al., 1989; Wakuda et al., 2005; Wang et al., 2007; Masachessi et al., 2007; Day et al., 2010; Ganesh et al., 2012a,b; Woo et al., 2012;

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Day and Zsak, 2014; Silva et al., 2014). In animals, the role of PBV has been studied primarily in swine and most recently in chickens and turkeys. In a Venezuelan study, 27 of 244 (11.1%) samples from swine gastroenteritis cases were positive for PBV (Ludert et al., 1991). In Brazil, PBV was detected in 15.3% of pigs with diarrhea and 9.6% of pigs without diarrhea (Gatti et al., 1989).

Based on morphological characterization and electropherotyping, the PBVs have been detected in chickens but are not specifically associated with enteric disease (Leite et al., 1990). Recently, distinctive sequences of PBVs were identified by metagenomic analysis in a pooled intestinal sample collected from enteritis-affected turkeys in North Carolina (Day et al., 2010). On the basis of RdRp gene sequences of turkey PBVs, only 49.5–70.0% nucleotide identity was reported with PBV strains from humans, pigs, dogs, rats and snakes. Additional characterization by RdRp gene sequences revealed that turkey PBV had 62.0% nucleotide identity with PBV of greater Rhea (*Rhea Americana*) (Day and Zsak, 2014). Although complete genome sequences of human, lapine and bovine PBVs have been reported, there is no report on complete genome characterization of PBV in birds.

Viral enteritis in domestic poultry places an economic burden on poultry producers worldwide. Recently, two new syndromes have been described in Minnesota turkeys namely, the 'poult enteritis syndrome' (PES) and 'light turkey syndrome' (LTS). The PES is an intestinal disease of young turkeys between 1 day and 7 weeks of age and is characterized by diarrhea, depression, and lethargy with pale intestines and/or excessively fluid cecal contents (Jindal et al., 2010). The LTS is characterized by lower weight of market age turkeys as compared to their standard breed character. It is believed that PES at a young age sets up conditions for the development of LTS later. Three enteric viruses (astro-, rota- and reoviruses) have been shown to cause concomitant infections in LTS and PES flocks in Minnesota (Jindal et al., 2010; Mor et al., 2013). The same three viruses have also been found in apparently healthy turkey flocks. The PES and LTS are considered a part of the poult enteritis complex (PEC). Recently, we tested a pool of fecal samples collected from LTS turkeys by NextGen Illumina sequencing and found the evidence of PBV sequences. The present study was undertaken to analyze the complete genome of turkey PBV and determine the prevalence of PBVs in Minnesota turkeys.

2. Materials and methods

2.1. Samples and RNA extraction

In 2011, we collected 80 and 40 fecal pools from LTS and non-LTS flocks, respectively. These 120 pools from 2 to 8 week old turkeys were tested for the presence of enteric viruses by a multiplex reverse transcription-polymerase chain reaction (RT-PCR; Jindal et al., 2012), the results of which have been reported previously (Mor et al., 2013). These same pools were tested in this study for the presence of PBV by RT-PCR. Briefly, the fecal pools were homogenized into a 10% suspension using sterile PBS (pH 7.4) followed by centrifugation at 1200×*g* for 20 min. The supernatants were decanted and RNA extracted using QIAamp viral RNA mini kit (Qiagen, Valencia, CA).

2.2. Illumina sequencing

RNA was extracted from a pool of fecal samples from 10 poults with LTS and submitted for Illumina HiSeq sequencing to the University of Minnesota Genomics Center (UMGC). The obtained sequence reads were analyzed by CLC Genomics Workbench 6.0 (www.clcbio.com). After trimming and sequence quality testing, contigs were prepared by *de novo* assembly. Extracted contigs were analyzed by BLAST (tBLASTx) analysis on NCBI and the ORF Finder tool (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi) was used to predict possible ORFs.

2.3. RT-PCR

Primers were designed based on RdRp and capsid gene sequences of PBV. The primer sequences used were Forward 5′ GTGGACCTACGGCTGATGAT 3′ and Reverse 5′ TCAGAT AGTTAA CTATCCACCA 3′ for RdRp gene and F1 5′ GGAAGGCGTATTCTG-GATCA 3′ and R1 5′ TGAGAGACGTTGCGTTATGC 3′ for the capsid gene. The RT-PCR was performed using Qiagen One Step RT-PCR kit (Qiagen). The reaction was done in 50 µl volume followed by 35 PCR cycles with denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and elongation at 72 °C for 1 min. The final elongation was at 72 °C for 10 min. PCR products (995 bp for RdRp and 1188 bp for capsid gene) were confirmed by analysis on ethidium bromide-stained 1.2% agarose gel followed by visualization under a UV transilluminator.

2.4. Sequence analysis

The PCR amplicons were purified using QIAquick PCR purification kit (Qiagen) and submitted to UMGC for sequencing. Cycle sequencing was performed separately with forward and reverse primers for the respective genes. Forward and reverse sequences were aligned together using Sequencher software version 5.1 (http://genecodes.com/). The obtained sequences were compared with the reference strains available in GenBank and aligned using CLUSTAL W in MEGA 6. Protein evolution model was selected based on lowest BIC (Bayesian Information Criterion) score using ProtTest (Abascal et al., 2005) in Phylemon 2.0 (Sanchez et al., 2011). Phylogenetic trees were constructed using the Maximum Likelihood statistical method based on the JTT model for amino acids and Kimura two-parameter model for nucleotides based tree in MEGA version 6 (Tamura et al., 2013). Numbers indicate the percentage of bootstrap support for the adjacent distal cluster. The reliability of different phylogenetic groupings was evaluated by the bootstrap method with 1000 replications. The *p*-distance method was used to calculate the evolutionary distances (percent nucleotide and amino acid identities) among the PBV strains.

Hydropathy of capsid protein sequence was evaluated with ProtScale tool (http://web.expasy.org/cgi-bin/protscale) using the method of Kyte and Doolittle (1982). The aa were classified as hydrophobic (above zero value) or hydrophilic (below zero value) on a ProtScale. Antigenic peptides were predicted in capsid protein by using the method of Kolaskar and Tongaonkar (1990) with online antigenic prediction tool (http://imed.med.ucm.es/Tools/antigenic.pl) and Bepipred Linear Epitope Prediction (http://tools.immuneepitope.org/tools/bcell/iedb_input) (Larsen et al., 2006). The comparison of predicted 2° structure as well as hydrophilic and hydrophobic aa of study strain with previously reported PBV strains was done based on complete ORF2 (capsid) aa sequence in Geneious Pro (Drummond et al., 2011).

2.5. Nucleotide sequence accession numbers

The obtained sequences have been submitted to GenBank under the following accession numbers: KJ495689 and KJ495690 for segments 1 and 2 (obtained by Illumina sequencing), KJ476119 to KJ476133 for RdRp region and KJ476134 to KJ476138 for the capsid gene.

3. Results

3.1. Complete genome characterization of PBV strain (GI/PBV/turkey/USA/MN-1/2011)

On the basis of Illumina sequencing, Contigs 157 and 151 from the NCBI BLAST analysis showed complete sequences of segments 1 and 2 of PBV. The complete genome was 4242 bp and the lengths of segments 1 and 2 were 2557 and 1685 bp, respectively. Segment 1 had two ORFs: small ORF1, 252 amino acids (aa) from nucleotide (nt) position 148 to 906 and large ORF2, 550 aa from nt position 887–2539. The ORF1 and ORF2 encode the hypothetical protein and the capsid protein, respectively. Segment 1 had 5′ UTR (untranslated region) from nucleotide 1 to 147 and 3′UTR from 2540 to 2557 (Fig. 1). Segment 2 had a single ORF (525 aa from nt 70–1647), which encodes for RdRp. Phylogenetic analysis of both segments grouped our strains in genogroup I of PBVs. Sequence analysis of segment 1 revealed 45.8% and 35.7% aa identity with that of GI human PBVs; VS10 and Hy005102 strains from the Netherlands and Thailand, respectively (Fig. 2). The study sequence had 30.5%, 32.9%, 33.1% and 35.9% aa identity with rabbit, fox, otarine, and porcine PBV sequences, respectively. Segment 2 sequences showed 60.9–64.5% aa and 59.1–63.3% nt identity with genogroup I human PBV strains (GPBV6C3 from India and VS10 from the Netherlands) (Fig. 3). The study sequence had 58.5%, 64.1%, 58.6% and 58.3% aa identity with mouse, fox, otarine, and porcine PBV sequences, respectively.

3.2. Prevalence of PBVs in LTS and non-LTS flocks

Of the 80 LTS and 40 non-LTS sample pools, 39 (48.8%) and 23 (57.5%) were positive for PBV, respectively. When the samples were stratified by age (2, 3, 5 and 8 weeks), the maximum number of samples were positive in LTS flocks at 3 weeks of age (20 of 20) followed by 2 weeks of age (15 of 20). Subsequently, there was a decrease in the number of positive samples (2/20 were positive at both 5 and 8 weeks of age). However, in non-LTS flocks 3/10 (30%), 5/10 (50%), 10/10 (100%) and 5/10 (50%) were found positive at 2, 3, 5 and 8 weeks of age (Table 1).

3.3. Co-infections with other enteric viruses

Six of 80 (7.5%) and five of 40 (12.5%) pools from LTS and non-LTS flocks, respectively, were positive for PBV only. The remaining samples were positive for PBV in addition to having astro-, rota-, or orthoreovirus. Dual infection of PBV with astrovirus was the highest; 32.5% and 15.0% in LTS and non-LTS flocks, respectively. Triple infection with PBV, astrovirus, and rotavirus was observed in 7.5% and 27.5% of LTS and non-LTS flocks, respectively (Table 2).

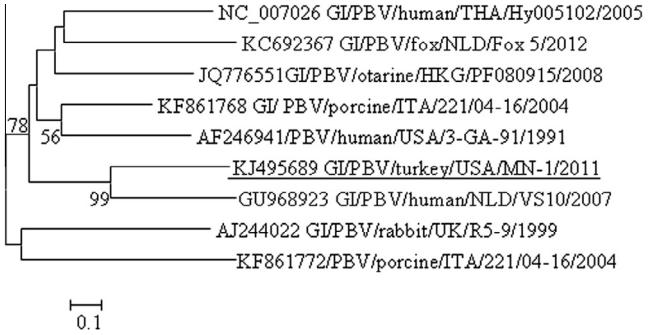

Fig. 2. Phylogenetic tree based on complete ORF 2 amino acid sequence (550 aa) of segment 1 of KJ495689 GI/PBV/turkey/USA/MN-1/2011 strain from Minnesota turkeys. Phylogenetic tree was constructed using the Maximum Likelihood statistical method based on the JTT model with 1000 bootstraps.

3.4. Sequence analysis of PBVs from LTS and non-LTS flocks

Fifteen randomly selected samples representative of different age groups of LTS and non-LTS flocks (nine from LTS and 6 from non-LTS flocks) were sequenced using RdRp primers. The study sequences were ~1000 bp in size but all chicken PBVs and most of PBV sequences from different host species available in GenBank were of ~200 bp. Hence, for better comparison with previously reported PBVs, the phylogenetic analysis was constructed based on 200 bp of the RdRp gene. The sequence comparison and phylogenetic analysis divided these 15 strains into four subgroups (IA, IB, IC, ID) within genogroup I (Fig. 4). Subgroup IA included three non-LTS strains (non-LTS 22, non-LTS 31 and non-LTS 35), and six LTS strains (LTS 101, LTS 116, LTS 206, LTS 215, LTS 303, LTS 307), which showed 69.9–76.4% nt identity with the human PBV GI strainVS111 from the Netherlands. The GI/PBV/turkey/USA/MN-1/2011 strain also belonged to this subgroup. Subgroup IB included two LTS strains (LTS 108, LTS 115) and grouped together with chicken PBV GI, AVE 42v1 strain from Brazil (KC865798) with 91.4–91.7% nt identity. Subgroup IC included one LTS (LTS 302) and one non-LTS (non-LTS 11) strain and had 72.3–74.2% nt identity with chicken PBV, AVE 71v3 strain from Brazil. Subgroup ID included two non-LTS strains (non-LTS 25, non-LTS 27), which showed 72.4–81.8% nt identity with chicken PBV GI, AVE 57v2 strain from Brazil.

The nt identity within strains of subgroups IA, IB, IC and ID was 97.5–100%, 100% and 100%, respectively. The nt identity of subgroup IA sequences was 67.5–74.3%, 64.5–71.3% and 63.2–70.5% with subgroup IB, IC and ID strains, respectively. Subgroup IB sequences had 74.3% and 67.7–71.4% nt identity with subgroup IC and ID strains. Subgroup IC strains showed 76.3–86.8% nt identity with subgroup ID strains. Subgroup IC and ID were the most divergent subgroups within genogroup I (Fig. 4). The previously reported turkey PBV strains formed two separate subgroups: 1E (USA-1512, JX680468 and USA-1507, JX680467) and 1F (MD-2010, HM803965). Subgroup 1E strains had 65.3–74.4%,

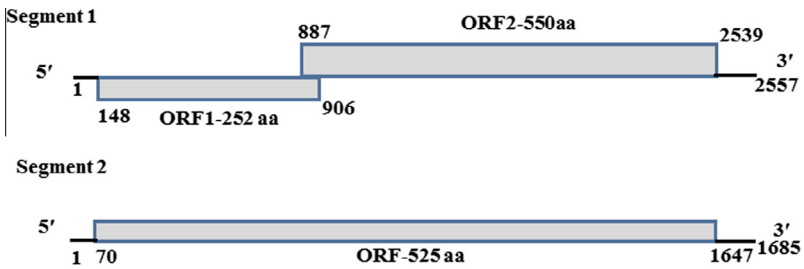

Fig. 1. Genome organization of a PBV strain from Minnesota turkeys (GI PBV/turkey/USA/MN-1/2011) based on Illumina sequencing.

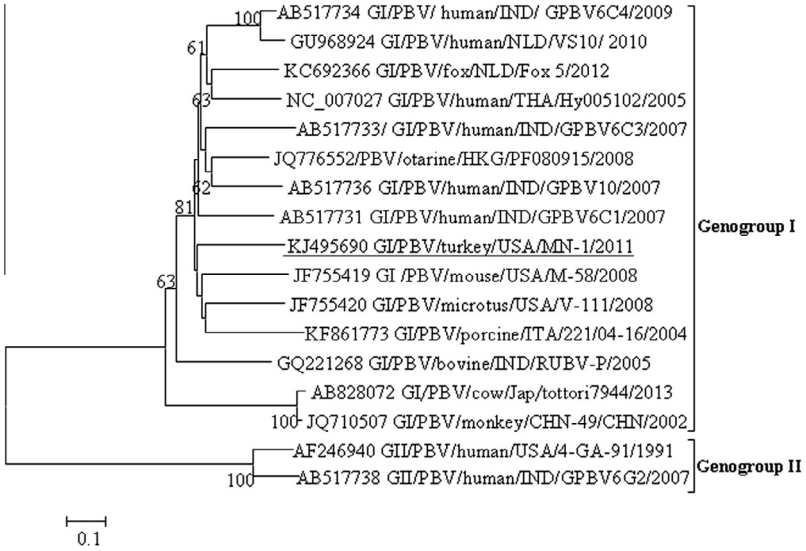

Fig. 3. Phylogenetic tree based on complete amino acid sequence (525 aa) of segment 2 of KJ495690 GI/PBV/turkey/USA/MN-1/2011 strain from Minnesota turkeys. Phylogenetic tree was constructed using the Maximum Likelihood statistical method based on the JTT model with 1000 bootstraps.

Table 1
Prevalence of PBV in LTS and Non-LTS flocks.

Age	LTS (% positive)	Non-LTS (% positive)
2 week	15/20 (75)	3/10 (30)
3 week	20/20 (100)	5/10 (50)
5 week	2/20 (10)	10/10 (100)
8 week	2/20 (10)	5/10 (50)

Table 2
Prevalence of PBV and other enteric viruses in LTS and Non-LTS flocks.[a]

Enteric viruses[b]	Number (%) of LTS fecal pools positive	Number (%) of non-LTS fecal pools positive
PBV	6 (7.5)	5 (12.5)
PBV + AstV	26 (32.5)	6 (15.0)
PBV + RV	0 (0.0)	1 (2.5)
PBV + AstV + RV	6 (7.5)	11 (27.5)
PBV + AstV + RV + ReoV	1 (1.3)	0 (0.0)

[a] LTS flocks = 80 pools; Non-LTS flocks = 40 pools.
[b] PBV = picobirnavirus; RV = rotavirus; AstV = astrovirus; ReoV = reovirus.

77.1–72.9%, 67.7–71.3% and 69.7–77.3% nt identity with subgroup IA, IB, IC and ID strains, respectively (Fig. 4).

We observed insertions and deletions in RdRp region most of which were at the 5′ end (from 549 to 576). Compared to previously reported turkey PBV strain USA-1507 from North Carolina, there was deletion of three nucleotides at position 550–552 in one strain (Non-LTS 22) and two deletions each in two strains (LTS 206 and LTS 307). Another two strains (Non-LTS 25 and Non-LTS 27) showed insertion of two nucleotides at position 1009–1010 and 1225–1226, respectively.

Five of the fifteen strains (Non-LTS 22, LTS 101, LTS 206, LTS 215 and LTS 303) were further typed using capsid gene primers. Since sequences of capsid genes of turkey and chicken PBV strains are not available in GenBank, we compared our sequences with those of other host species. Based on phylogenetic analysis all five strains grouped together with 97.4–98.0% nt and 97.8–98.9% aa identity. They showed 32.6–33.4% nt and 39.5–41.3% aa identity with VS10 human PBV strain (Fig. 5).

Further analysis indicated that the first 65 aa from 5′ end were hydrophilic and were divergent from PBVs of different host species. Hence they differed in their predicted 2° structure (Figs. S1 and S2). The average antigenic propensity for complete capsid protein of segment 1 was 1.0334 and 23 antigenic determinants were found in capsid protein of KJ495689 GI/PBV/turkey/USA/MN-1/2011 (Fig.S3, Tables S1 and S2). The largest antigenic peptide (144–188) was found to be more conserved among PBVs of different host species (Fig. S2).

4. Discussion

The PBVs are newly discovered enteric viruses of chickens and turkeys (Day and Zsak, 2014; Silva et al., 2014). We designed this study to test the prevalence of PBVs in LTS and non-LTS flocks in Minnesota turkeys. The PBV was found in both LTS and non-LTS flocks with maximum prevalence at 3 and 5 weeks of age in LTS and non-LTS flocks, respectively. In a recent study in turkeys, Day and Zsak (2014) tested five turkey flocks from North Carolina with historically high incidence of enteric diseases. Samples were collected and tested by RT-PCR from week 1 to 5 after placement. The maximum number of flocks was positive after 4 and 5 weeks of placement. However, the maximum numbers of samples in our study were PBV-positive at 2 and 3 weeks of age in LTS flocks, which is in contrast to Day and Zsak (2014) who reported that PBV infection establishes in PEC flocks after fourth week of placement.

In non-LTS flocks, the maximum number of samples was positive at 5 weeks of age, the significance of which is not known at this point. In this study we tested LTS and non-LTS flocks for up to 8 weeks of age and observed a decrease in prevalence at 8 weeks in both LTS and non-LTS flocks. This may signify that 2–5 weeks of age is critical for PBV infection in turkey flocks. This trend of PBV prevalence is similar to other enteric viruses such as astrovirus, rotavirus and reovirus in LTS and non-LTS flocks (Mor et al., 2013).

In a previous study, Mor et al. (2013) reported that enteric virus infection at young age (2–3 weeks of age) may lead to PES in turkey poults. The PES-affected birds continue to be uneven in size and may develop LTS at marketing age. In this study, we found high prevalence of PBVs at 2–3 weeks of age in LTS flocks (as compared to non-LTS flocks), which indicates possible involvement of PBVs in LTS. This is well known that as the age advances, poults become more resistant to enteric virus infections; hence, high incidence of PBV at 5 weeks of age in non-LTS flocks may not affect the growth of the birds. Future experimental studies with PBVs are indicated to determine their impact on LTS in poults.

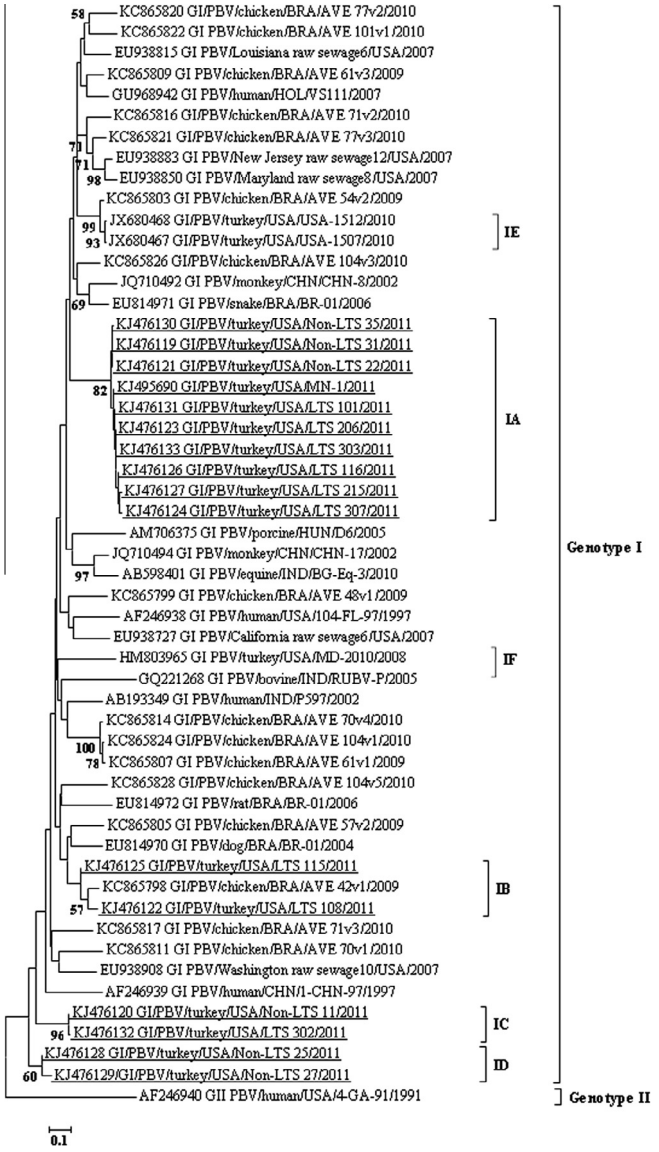

Fig. 4. Phylogenetic tree of 16 Minnesota turkey PBVs (underlined) based on partial nucleotide sequences (182 bp) from RdRp region (segment 2). Bar indicates 0.1 substitutions per nucleotide. Phylogenetic tree was constructed using the Maximum Likelihood statistical method based on the Kimura two-parameter model with 1000 bootstraps.

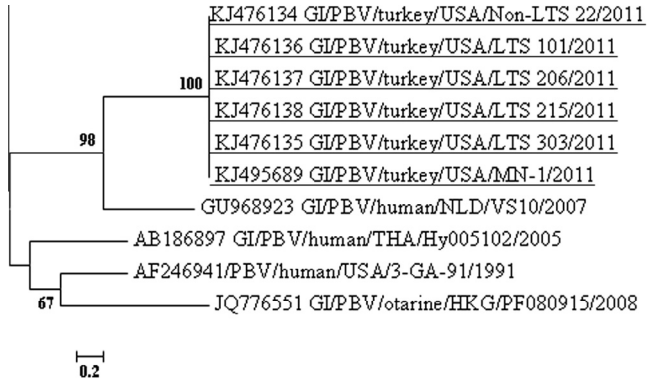

Fig. 5. Phylogenetic tree of 6 Minnesota turkey PBVs (underlined) based on 350 amino acids from the capsid gene (segment 1). Phylogenetic tree was constructed using the Maximum Likelihood statistical method based on the JTT model with 1000 bootstraps.

In LTS flocks, 32.5% samples showed dual infection with astrovirus and PBV. Mor et al. (2011) have reported that type-2 astrovirus from PES cases caused up to 16% reduction in weight gain as compared to type-2 astrovirus from apparently healthy cases after oral inoculation in 2-week-old turkey poults. It is possible that dual infection with astrovirus and PBV at 2–3 weeks of age may lead to PES in young birds followed by LTS in market age birds.

The sequence analysis of segment 1 in this study confirmed the presence of two ORFs in turkey PBVs as has been reported for human, porcine, bovine and otarine PBVs. This is different from rabbit PBV which contains three ORFs (one large ORF and two smaller ORFs). Segment 1 of PBVs from humans, fox, otarine, porcine and rabbits is well characterized but this information is lacking for turkey PBVs (Gatti et al., 1989; Ludert and Liprandi, 1993; Green et al., 1999; Wakuda et al., 2005; Van Leeuwen et al., 2010; Ganesh et al., 2012a,b). In this study, we described sequences of segment 1 of five turkey PBV strains on the basis of 1050 bp/350 aa for the first time. The 23 predicted antigenic peptides were determined in capsid protein of study strains, which will be helpful in future for conducting experimental studies.

We found that segment 2 had one ORF as has been previously reported for other PBVs (Green et al., 1999; Wakuda et al., 2005; Ghosh et al., 2009; Woo et al., 2012; King et al., 2012). Three conserved motifs (WMFP, SGSG and GDDG) were observed in *GI/PBV/turkey/USA/MN-1/2011* strain from Minnesota turkeys which is a characteristic of the RdRp gene of dsRNA viruses (Bruenn, 1993).

Phylogenetic analysis based on segment 1 and segment 2 sequences showed that our strains grouped with genogroup I, which is consistent with previous studies on chicken and turkey PBVs (Day and Zsak, 2014; Silva et al., 2014). Phylogenetic analysis of 15 sequences based on RdRp gene divided them into four subgroups (IA to ID) under genogroup I. An interesting finding was that the previously reported turkey PBVs from cases of PEC in North Carolina formed different subgroups (IE and IF) indicating that there may be geographical differences in turkey PBV strains in the USA. The divergence observed in our sequences is in correlation with previous findings in chicken and turkey PBVs (Day and Zsak, 2014; Silva et al., 2014). Day and Zsak (2014) reported that turkey PBVs were distinctly related to genogroup I. However, in this study we observed that only subgroups IC and ID were distinctly related to genogroup I and all other chicken and turkey PBVs are related to PBVs from different species. This indicates that sequencing of additional chicken and turkey PBV sequences is necessary to obtain a better comparison of PBVs from chickens and turkeys.

Similar to this study, Silva et al. (2014) also reported different subgrouping of PBVs variants in Brazilian broiler chickens. They reported mixed infection of different chicken PBV variants in the same sample. In this study, we did see some sequences with dual peaks but did not pursue this work further to determine the types of variants present in a single sample. Similar to Day and Zsak (2014), who developed an RT-PCR of amplicon size ~1100 bp, we developed RdRp primers with a product size of 995 bp. Due to high variations among PBVs, the large sequence size will be helpful to make better comparison among PBV variants. We detected insertions and deletions along with nucleotide substitutions in RdRp region of the virus; however, significance of these findings is not yet clear. This may possibly be the reason for low typing rate using capsid gene primers in the present study.

Phylogenetic analysis indicates that genotype I PBVs are distributed worldwide and can infect different hosts including reptiles, animals, birds, and humans. The grouping of genogroup 1 PBVs from different host species indicates that these are not host specific and could transmit from one host species to another. This study documented the prevalence, age related distribution, and molecular characterization of PBV strains associated with LTS and non-LTS turkeys. The high prevalence of PBV at 2–3 weeks of age in LTS

flocks indicates possible involvement of PBVs (alone or in association with astrovirus) in PES and LTS. Also, a considerable proportion of PBV infection was observed in non-LTS cases, which emphasizes the need for further studies on transmission pattern and pathogenesis of this virus to determine its etiological role as a pathogen in turkeys.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.meegid.2014.12.014.

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