Phylogenetic Analysis of Kobuvirus and Astrovirus From Korean Wild Boars: 2016-2018

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Research Article

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Abstract

The prevalence of porcine kobuvirus (PKoV) and porcine astrovirus (PAstV) in 845 Korean wild boars (KWB) during 2016-2018 were 28.0% and 10.6%, respectively, and co-infection of two viruses showed 5.1%. Phylogenetic tree analysis also revealed that 236 PKoVs from KWB were divided to diverse lineages within *Aichivirus C* group but the one strain (WKoV16CN-8627) was included the same cluster with bovine kobuvirus (*Aichivirus B*). Eighty-nine PAstVs from KWB was belonged predominantly to lineage PAstV4 and only one strain (WAsT17JN-10931) included novel to lineage PAstV2. Two viruses are epidemic more in young (≤ 12 months) than in old pigs (> 12 months).

Introduction

The genus *Kobuviruses* are small, non-enveloped viruses with single-stranded, positive-sense genomic RNA within the family *Picornaviridae*, genus *Kobuvirus*. The kobuvirus genome is approximately 8.2-8.4 kb in length, and its polyprotein includes non-structural leader (L) protein, three structural (VP0, VP3, and VP1) capsid proteins, and seven non-structural (2A-C, 3A-D) proteins [1, 2]. The genus *kobuvirus* consists of three species which were officially recognized as *Aichivirus A* (Aichi virus) [3], *Aichivirus B* (Bovine kobuvirus, BKoV) [4], and *Aichivirus C* (Porcine kobuvirus, PKoV) [5]. PKoV was first identified in farm pigs in 2008 and named as S-1-HUN strains [1]. Since then, PKoV has been detected in fecal samples of healthy and diarrhoeic pigs in diverse countries including Japan [6], China [7], Thailand [8], South Korea [9], Italy [10], and USA [11]. *Aichivirus* has been reported one of the causative pathogens of human gastroenteritis, as biological properties have been reported through cell culture system [3]. Although the pathogenicity associated with diarrhea in PKoV has not clearly determined, it is considered important that PKoV may have possibility of zoonotic transmission among other species [12].

The genus *Astroviruses* are small non-enveloped RNA viruses with single-stranded, positive-sense genomic RNA from family *Astroviridae*, genus *Mamastrovirus*. The astrovirus genome is approximately 6.1-7.9 kb in length, and its polyprotein includes three open reading frames (ORFs) with ORF1a and ORF1b encoding non-structural proteins and ORF2 encoding structural capsid proteins [13]. Porcine astroviruses (PAstV) was first detected by electron microscopy in feces of diarrheic piglets in 1980 and was isolated later in cell culture [14]. Until recently, PAstV has been detected in feces of domestic pigs worldwide, including Hungary [15], Slovakia [16], Canada [17], Thailand [18], China [19], and South Korea [20]; it was classified into five genetic variable lineages, PAstV-1 to PAstV-5.

As the population density of the wild boar (*Sus scrofa*) increases in worldwide, there are concerned that wild boar potentially causes risk of transmission of infectious pathogens which can be shared with domestic pigs and other animal species [21]. It was also reported that wild boar in Asia and Europe may act as a reservoir for economic livestock diseases such as classical swine fever virus (CSFV), african swine fever virus (ASFV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome (PRRS), hepatitis E virus (HEV), and others [22]. In this study, we determined the circulation of PKoV and PAstV infecting wild boars in the South Korea, and investigated the prevalent levels and genetic diversity.

The 729 of 845 wild boars captured by hunters for three years (2016-2018) were broadly ranged between 6 kg and 300 kg. The age of wild boars was estimated based on weight: individuals were considered as young wild boar (≤ 2 months, 3-6 months, 7-12 months) and adult wild boar (> 1 year). However, 116 of 845 wild boars without information were marked as unknown. All of captured wild boar fecal samples was no diarrhea and were collected from wild boar hunted randomly in the mountainous area in 9 provinces nationwide: Gyeonggi (n=75), Gangwon (n=112), Chungbuk (n=111), Chungnam (n=112), Jeonbuk (n=82), Jeonnam (n=88), Gyeongbuk (n=167), Gyeongnam (n=83), and Jeju (n=15).

The fecal samples were resuspended in 10 volume of phosphate-buffered saline (PBS) containing 1% gentamicin (Gibco Life Technologies, Paisley, UK), and centrifuged to obtain clarified supernatant (at 5,000 x g for 10 min). Viral RNA was extracted from the part of fecal supernatant (0.2 ml) using a RNeasy Mini kit (QIAGEN, Valencia, CA, USA) and then reverse-transcribed into viral cDNA with a random hexanucleotide primers (NanoHelix, Daejeon, Korea). To detect enteric viruses (PKoV and PAsTV) reverse transcription-polymerase chain reaction (RT-PCR) was carried out using primers conserved in 3D (RNA-dependent RNA polymerase, RdRp) of PKoV and RdRp-ORF2 region of PAsTV in previous studies, respectively [9, 20]. The amplified genes were sequenced with specific primers using an ABI Prism 3730xi DNA Sequencer (Applied Biosystems, Foster City, CA, USA) at the CosmoGenetech (CosmoGenetech co., Daejeon, Korea). Each sequence was aligned and determined with references sequences which is deposited in GenBank using Clustal X 1.83 software [23]. The phylogenetic trees for PKoV and PAsTV were constructed by Mega 6.0 software [24] using neighbor-joining method [25] and Kimura 2-parameter as genetic distance measure.

PKoV-positives were 28.0% (237/845) including unknown samples for wild boar age information and the prevalence rates according to age showed 33.8% (89/263) in young pigs (≤ 12 months) and 26.2% (122/466) in old pigs (>12 months) (22.4-30.4, 95% CI; confidence interval associated with the prevalence estimate) (Table 1). PAsTV-positives were 10.6% (90/845) including unknown samples for wild boar age and the prevalence rates according to age were also 16.7% (44/263) in young pigs (≤ 12 months) and 9.2% (43/466) in old pigs (> 12 months) (6.9-12.2, 95% CI) (Table 1). Co-infection with PKoV and PAsTV detected 5.1% (43/845) from individual wild boar fecal samples including unknown age samples. The co-infection of wild boars showed 4.1% (3/74) in 3-6 months, 9.9% (18/181) in 7-12 months, and 4.3% (20/466) in > 12 months (2.8-6.6, 95% CI) (Table 1).

In terms of geographical prevalence, PKoV and PAsTV were broadly detected in most of provinces except that PAsTV had not detected in Jeju island (Fig. 1A). The prevalence of PKoV in Gyeongnam (42.2%, 35/83) was significantly higher than that in Jeju island where shows lowest PKoV-infecting rate (13.3%, 2/15) ($p < 0.05$) (Fig. 1A). The recent prevalence of PKoV-infected wild boar was increased to 35.1% in 2018, compared with last years (18.7% in 2016 and 24% in 2017) (Fig. 1B). PAsTV was detected in Gyeongbuk as highest PAsTV-occurred region (19.2%, 32/167), whereas had not detected in Jeju island (Fig. 1A). Emerging of PAsTV in Korean wild boar has been firstly reported in 2011, however its prevalent levels were only 0.7% (1/146) wild boars [11]. Recently, between 2016-2018, wild boar PAsTV tends to increase in Korea (3.2% in 2016, 8.0% in 2017, 15.9% in 2018) (Fig. 1B). These findings indicate that

enteric viruses, PKoV and PAsV are currently being transmitted in wild boar population, which consistently increase in the South Korea. In order to clarify whether the increase of virus-infected wild boars is affected epidemic circulation related to domestic pigs, additional investigation for PKoV and PAsV in farm pigs also may be required in future.

To investigate the genetic relationships among other Kobuvirus sequences, we constructed a phylogenetic tree for the 237 partial 3D gene of wild boar isolated in Korea (Fig. 2B). On the neighbor-joining tree, the 236 Korean wild boar PKoV strains were divided the diversity lineages within *Aichivirus C* (PKoV) group. Most of Korean wild boar PKoVs were belonged with domestic pig strains of many of countries: US, Japan, Thailand, China, Korea, Spain, and Hungary. Two of Korean wild boar strains (WKoV17GN-11321 and WKoV18GN-11819) were closed with WB-1-HUN2 strain isolated from Hungarian wild boar [26] (Fig. 2B).

We speculate that great spatial mobility of wild boar causes geographical circulation and genetical diverse of Korean PKoV. In comparison of the partial 3D nucleotides sequences, almost PKoV strains detected in Korean wild boars excepting one strain (WKoV16CN-8627) have high sequences identities indicating 83.5-94.5% with Korean domestic pigs and 84.6-99.7% with reference PKoV strains reported in Hungary [27], Spain, USA, China [2, 28, 29], Japan, and Thailand [30]. Interestingly, another wild boar PKoV strain, WKoV16CN-8627 showed low sequence identities ranging 50.2-54.9% with Korean domestic pigs, whereas shared relatively higher identity levels as 74.2-75.7% with those of bovine kobuvirus (BKoV) strains (EGY-1 and U-1) [4, 31]. This finding suggests that interspecies transmission between pigs and cow kobuviruses may have occurred in nature.

Phylogenetic tree shown that 90 wild boar PAsV strains were classified into different two lineages, PAsV-2 and PAsV-4. The lineages PAsV-4 was dominantly identified in 89 of boars, followed by lineages PAsV-2 in only one of boar (strain WAsT17JN-10931) (Fig. 2A). The PAsV isolated from wild boars has previously reported in several countries including Hungary [32], Slovakia [33], and Korea [20]. Especially, PAsVs isolated in Slovakia have been typed genetic variable lineages involved in PAsV-2, PAsV-4, and AsVs derived from different species (Chicken and bat) [33]. Whereas, Korean wild boar PAsV isolated in 2013 was limited in strain typed into PAsV-4 lineage [20]. In PAsV-4 lineage, three Korean wild boar PAsV strains (WAsT18GB-11875, and WAsT18JB-11889) were included with wild boar PAsV-4 (WBAsT-V-1 strain) in Hungary [32], and other four Korean wild boar PAsV strains (WAsT17GG-10149, WAsT17GB-10880, WAsT18GB-11497, and WAsT18JB-11455) were also belonged with PAsK-31 strain detected from Korean wild boar in 2011 [20]. Other PAsV-4 strains of Korean wild boar were belonged in PAsV-4 lineage that clustered with those of domestic pigs in Hungary [26], Belgium, USA, China, and Korea [20]. Interestingly, WAsT17JN-10931 strain was identified first time in PAsV-2 lineage which had not found in Korean wild boars (Fig. 2A). Korean AsV-2 sequences previously detected in domestic pigs in South Korea (PAsK-76 and KNH14-07) [20, 34] may be closely related to wild boar PAsV-2 identified in this study.

In conclusion, PKoV and PAsTV in Korean wild boars are circulating nationwide regardless of the provinces and age. PKoV, WKoV16CN-8627 strain from Korean wild boar, showed relatively higher identity with those of bovine kobuvirus strain. It has shown the possibility of transmission between pigs and cattle kobuviruses in nature. We also suggest that PAsTV-2 infecting Korean wild boar could be involved in the interspecies transmission of domestic pig astrovirus.

Declarations

Acknowledgments

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Data availability statement

The partial 3D gene (488 bp) sequences of 135 PKoV strains (Accession number: MW297847-MW297981) and the partial RdRp-ORF2 gene (799 bp) of 48 PAsTV strains (Accession number: MW297982-MW298009, MW298011-MW298014, MW298018-MW298019, MW298023, MW298027, MW298034, MW298039-MW298041, MW298044, MW298047, MW298049-MW298053, and MW298056) detected from Korean wild boars have been deposited in GenBank.

Compliance with ethical standards

Conflict of interest The authors declare that they had no conflict of interest.

Ethical statement All animal experiments were approved by the Animal and Plant Quarantine Agency in Ministry of Agriculture Food and Rural affairs.

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Table

Table 1. Prevalence of porcine kobuvirus (PKoV) and astrovirus (PAstV) in different age groups of wild boars.

Wild boar	Age	PKoV		PAstV		Co-infection (PKoV+PAstV)	
		Positive percentage (%)	95% CI ^a	Positive percentage (%)	95% CI	Positive percentage (%)	95% CI
	≤ 2 m ^b	50.0 ^c (4/8) ^d	21.5-78.5	0 (0/8)	0-29.3	0 (0/8)	0-29.3
Young	3-6 m	33.8 (25/74)	24.0-45.2	14.9 (11/74)	8.3-24.9	4.1 (3/74)	0.9-11.7
	7-12 m	33.1 (60/181)	26.7-40.3	18.2 (33/181)	13.3-24.5	9.9 (18/181)	13.3-24.5
Adult	> 12 m	26.2 (122/466)	22.4-30.4	9.2 (43/466)	6.9-12.2	4.3 (20/466)	2.8-6.6
Unknown		22.4 (26/116)	15.7-30.9	2.6 (3/116)	0.6-7.7	1.7 (2/116)	0.1-6.5

^a, CI: Confidence interval associated with the prevalence estimate

^b, m: month

^c: positive percent

^d: (number of positive/ number of test samples).

Figures

Figure 1

Prevalence of PKoV and PAsTV in Korean wild boar. Geographic distribution of wild boar confirmed as PKoV- or PAsTV-positive in South Korea (A). Each percentage according to year indicates frequency of virus infecting wild boar with regard to PKoV and PAsTV (B). Gyeonggi (GG), Gangwon (GW), Chungbuk (CB), Chungnam (CN), Jeonbuk (JB), Jeonnam (JN), Gyeongbuk (GB), Gyeongnam (GN), and Jeju (JJ). Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

Figure 2

Phylogenetic tree analysis based on the partial RdRp-ORF2 gene (799 bp) of astrovirus (AstV) strains and the partial 3D gene (488 bp) of kobuvirus (KoV) strains. The partial RdRp-ORF2 sequences of 48 PASTV isolated from wild boar were compared with 22 global AstV strains available in GenBank database (A). The partial 3D sequences of 135 KoV isolated from wild boar were compared with 66 global KoV strains available in GenBank database (B). The trees were constructed using the neighbor-joining method (MEGA

v6.0) with bootstrap values calculated from 1,000 replicates. The PKoV (WCoV16CN-8627 strain) and PAsV (WAsV17JN-10931 strain) from Korean wild boars are marked red and blue arrows, respectively.