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List of abbreviations

BHoV	bovine hokovirus
BLAST	basic local alignment search tool
BPV	bovine parvovirus
CSFV	classical swine fever virus
ddH_2O	distilled water
DNA	deoxy ribonucleic acid
HBoV	human bocavirus
HEV	hepatitis E virus
kb	kilobase
MCV	minute canine virus
MDA	multiple displacement amplification
NP1	nucleoprotein 1
NS1	non-structural protein coding gene 1
ORF	open reading frame
PARV4	human parvovirus 4
PBo-likeV	porcine Boca-like virus
PBoV	porcine bocavirus
PCR	polymerase chain reaction
PCV2	porcine circovirus type 2
PHoV	porcine hokovirus
PMWS	post-weaning multi-systemic wasting syndrome
PPV	porcine parvovirus
PRRSV	porcine respiratory and reproductive syndrome virus
PTTV	porcine torque teno virus
qPCR	quantitative PCR
SISPA	sequence independent single primer amplification
VP1	structural viral protein 1

Summary

Thanks to the rapidly improving nucleic acid amplification technologies, novel porcine parvoviruses have been described during the last few years. One of them is the porcine hokovirus (PHoV), a newly discovered member of the family *Parvoviridae* and the proposed genus *Hokovirus*. It is considered to be phylogenetically distinct from other parvoviruses. This study reports a comprehensive spatio-temporal epidemiological and genetic characterization of porcine hokovirus infection in Transylvanian wild boars. A total of 842 wild boars from 315 hunting grounds were included in the study, sampled and grouped according to their geographical origin and the date of collection. The prevalence of PHoV differed significantly in samples from 2006/2007 (22.76%) and 2010/2011 (50.54%), respectively. Out of the 295 PHoV positive animals, 30.5% (90/295) were between 6 and 12 months of age and 69.5% (205/295) were over the age of 1 year. The same age difference was also observed when hunting seasons were compared. Sequence analysis of PHoVs from 2006/2007 showed a close relationship to PHoVs from pigs from England and wild boars from Germany, while the PHoVs from 2010/2011 were mostly similar to isolates from Hong Kong. The most variable regions were detected in the non-structural (NS1) gene and proved to be suitable for analysis of the genetic diversity of the virus. It was observed that PHoVs from older wild boar samples differed from those collected recently. The results suggested that porcine hokovirus could be a newly emerging virus of both domestic and wild pigs with yet unknown implications.

Összefoglalás

A gyorsan fejlődő nukleinsav amplifikációs technológiáknak köszönhetően az elmúlt néhány év során több új sertés parvovírust azonosítottak és írtak le. Egyikük, a sertés hokovírus (porcine hokovirus, PHoV), a *Parvoviridae* család egy újonnan felfedezett tagja, amelyet egy szintén újonnan javasolt nemzetségbe, nevezetesen a *Hokovirus* genusba soroltak, és filogenetikailag különbözik más, eddig megismert parvovírusoktól. A jelen dolgozat egy átfogó térbeli és időbeli epidemiológiai tanulmány, Erdélyben, vaddisznókból kimutatott sertés hokovírus fertőzések genetikai jellemzéséről. Összesen 842 vaddisznó, 315 vadászterületről gyűjtött mintáit elemeztük, amelyeket a begyűjtés helyének és idejének megfelelően csoportosítottunk. A kimutatott PHoV prevalencia jelentősen különbözött a 2006/2007 (22,76%) és 2010/2011 (50,54%) során vizsgált esetekben. A 295 PHoV pozitív egyedből 30,5% (90/295) volt 6 és 12 hónapos kor közötti, és 69,5% (205/295) egy éves kor feletti. Ugyanez az életkori különbözőség volt megfigyelhető az egyes vadászidényeket összehasonlítva. A 2006/2007-es PHoV szekvenciák összehasonlítása azt mutatta, hogy közeli genetikai kapcsolatban álltak az Angliában házi sertésekben és Németországban vaddisznókban kimutatott PHoV genomokkal, miközben a 2010/2011-es szekvenciák inkább a Hong Kongban azonosított PHoV szekvenciákkal mutattak közelebbi genetikai rokonságot. A legvariábilisabb szekvencia szakaszokat a nem strukturális (non-structural, NS1) génen azonosítottuk, és ezek alkalmasnak bizonyultak a vírusok genetikai diverzitásának a tanulmányozására. Megfigyeltük, hogy a régebben gyűjtött PHoV szekvenciák különböztek a frissebben szerzett mintákban azonosított vírusokéitól. Az eredmények arra utaltak, hogy a PHoV egy újonnan megjelent és terjedőben lévő vírusa a sertéseknek, nemcsak a házi sertés állományokban, de a vaddisznókban is.

Introduction

Parvoviruses infect a wide variety of animal species and some of them are responsible for severe clinical diseases, but the majority of these viruses cause only mild or subclinical infections. Parvoviruses are non-enveloped, small viruses containing single-stranded, linear DNA genomes of approximately 5-6 kilobases (kb). The genome consists of two main open reading frames (ORF) that encode the non-structural and capsid proteins (Soares et al., 2003; Wang et al., 2010). The family, based on host range, is currently divided into two subfamilies: the subfamily *Parvovirinae*, whose members infect vertebrates, includes five genera, namely *Dependovirus, Erythrovirus, Amdovirus, Bocavirus* and *Parvovirus*, whereas members of the subfamily *Densovirinae* are present in insects and other arthropods (Wang et al., 2010). The classical porcine parvovirus (PPV1) strains occur worldwide and cause reproductive disorders, especially in those herds where vaccination protocols are not followed correctly or where vaccine efficacy is decreased due to immunosuppressive factors.

Thanks to the rapidly improving nucleic acid amplification technologies further new porcine parvoviruses were described during the last few years. The first detection and characterization of porcine parvovirus 2 (PPV2) was in an attempt to identify the hepatitis E virus (HEV) from swine sera (Hijikata et al., 2001). Instead of HEV a new parvovirus sequence was detected by sequence analysis. The HEV specific primers amplified shorter, unspecific DNA. The 5 kilobase length genome was determined, and higher similarity was detected to Muscovy duck parvovirus and bovine parvovirus 3, but the PPV2 did not cluster with any other known parvoviruses. HEV was not detectable in the examined samples. Many years later genetically highly similar parvoviruses were identified in China (Wang et al., 2010). Recently, with the development of random amplification methods and high-throughput sequencing, new viral pathogens have been discovered from animal and human samples (Li et al., 2009; Allander et al., 2001; Finkbeiner et al., 2009). In China, faecal samples from healthy piglets (<15 days of age) were examined with a sequence independent single primer amplification (SISPA) method (Cheng et al., 2010). With this method new porcine parvoviruses were detected. According to the genome organization and sequence analysis, these sequences belong to the *Bocavirus* genus.

Members of the *Bocavirus* genus have an additional third open reading frame (ORF3) located between the original two ORFs which encode for the non-structural and caspid proteins (Blomström et al., 2009). Known members of the bocaviruses include bovine

parvovirus (BPV), minute canine virus (MCV) and human bocaviruses 1-4. MVC was first found in canine fecal samples in 1970 (Binn et al., 1970) and BPV was discovered in calves with diarrhea already in 1961 (Abinanti and Warfield, 1961). The MVC genome shares about 43% identity with that of BPV at the nucleotide level, with the non-structural (NS1) and structural (VP1 and NP1) protein coding genes, being 33.6%, 41.1% and 39% identical to those of BPV, respectively (Sun et al., 2009; Schwartz et al., 2002)**.** These studies have shown a correlation between the two viruses and also with enteric and respiratory tract diseases in younger animals (Manteufel et al., 2008; Sun et al., 2009). Human bocavirus (HBoV), an emerging human parvovirus, was first discovered in Sweden, in children with acute respiratory tract infections (Allander et al., 2005). Additionally, HBoV2, HBoV3, and HBoV4 have been identified and classified into the genus *Bocavirus* (Kapoor et al., 2009; Arthur et al., 2009). In 2009 porcine Boca-like virus (PBo-likeV) was described in Sweden, using random multiple displacement amplification (MDA) and large-scale sequencing (Blomström et al., 2009). The PBo-likeV was detected in the background of post-weaning multi-systemic wasting syndrome (PMWS), caused by porcine circovirus type 2 (PCV2). Blomström et al. (2009) suggested that PBo-likeV might also play a certain role in PMWS. In China the PBo-likeV has also been described (Zhai et al., 2010). The prevalence was 38%, based on 191 examined samples and the virus was detected mainly in weaned pigs, rather than in younger piglets or in older swine. PCV2, porcine torque teno viruses (PTTV), porcine respiratory and reproductive syndrome virus (PRRSV) and classical swine fever virus (CSFV) had higher prevalence in PBo-likeV infected pigs than in non-infected animals. Based on this finding, the authors proposed that the PBo-likeV might be an emerging virus of swine respiratory tract diseases. Among these porcine bocaviruses (PBoV), two nearly full length genomes were determined (PBoV1, PBoV2), showing 94-95% identity to each other. From the other two PBoV (labelled 6V and 7V), only 2407 and 2434 nucleotides were determined. These two sequences were not grouped with PBoV1 and PBoV2, they only shared less than 56% similarities with them. These PBoVs show less than 50% similarities to the PBo-likeV, described by Blomström et al. (2009). Based on phylognetic analysis, the PBoV-likeVs, PBoV1, 2 and 6V, 7V create three different clusters within the *Bocavirus* genus. The PBoV1 and PBoV2 were found in 50 cases from 397 stool samples, collected from healthy piglets (Cheng et al., 2010).

Another newly discovered member of porcine parvoviruses is PPV4, described in North-Carolina (USA) from samples collected during 2005 (Cheung et al., 2010). PPV4 was identified at a farm affected by PCV2, similarly to PBo-likeV in Sweden (Blomström et

al. 2009). PPV4 showed the highest similarity to bovine parvovirus 2, but the coding capacity and genome organisation was similar to that of bocaviruses, as PPV4 encodes an additional ORF3 as bocaviruses, located between ORF1 and ORF2. The PPV4 encoded putative ORF3 protein is however quite different from that of the bocaviruses (Cheung et al., 2010). After the first description, PPV4 was also detected in China (Huang et al., 2010). Although the overall PPV4 detection rate was low, the rate within each positive farm was high, between 20-50% (Huang et al., 2010). Altogether thirteen PPV4 positive samples were identified in the study, 10 samples were from adult pigs, diagnosed with reproductive failure, two from a dead piglet and one from a healthy piglet. Fever and neurologic symptoms were observed in the sick piglets. PPV4 DNA was present in the heart, serum, lymph node, lung and kidney, but the highest viral load was measured in the heart.

Furthermore, new viruses of swine and cattle were discovered in Hong Kong in 2008 (Lau et al., 2008), and the addition of a tentative genus, *Hokovirus*, to the subfamily *Parvovirinae* has been proposed (Lau et al., 2008). Comparative sequence and phylogenetic analysis showed that the genomes of the novel porcine and bovine hokoviruses (PHoV and BHoV) were most similar to those of the newly described human parvovirus types 4 and 5 (PARV4/5), forming a distinct cluster within the parvoviruses. They also differed from other parvoviruses by their relatively large predicted VP1 protein and the presence of a unique small putative protein (Lau et al., 2008). The latest taxonomic report by the International Committee on Taxonomy of Viruses (Fauquet et al., 2008) does not include these viruses or the suggested genus *Hokovirus* in the family *Parvoviridae*, nor are they listed among the proposed tentative members. Based on the observations of Lau et al. (2008) about the genomic organization of PHoV, Cheung et al. (2010) have suggested that porcine hokovirus (PHoV) should be classified as porcine parvovirus type 3 (PPV3). Considering that, until now, no official taxonomic classification of the virus is available, it will here be referred to as porcine hokovirus, as proposed by Lau et al. (2008). The worldwide occurrence of the newly discovered PHoVs is not yet known, but they have been found both in domestic (Lau et al., 2008; Szelei et al., 2010) and wild pigs (Adlhoch et al., 2010). Only 3 out of 98 investigated plasma samples that were collected from healthy pigs in England in 2001, but all 11 studied commercial clotting FVIII preparations (coagulation factor VIII) were positive for PARV4-like viruses. Molecular analysis confirmed that these viruses were related to the human PARV4 virus and closely related to the recently discovered porcine hokovirus (Lau et al., 2008; Szelei et al., 2010). Considering that the viruses initially described as porcine PARV4-

like viruses displayed high sequence similarity to the earlier discovered PHoV, it gives another reason to refer to it as such.

The prevalence of PHoV was high both in domestic 44.4% (Lau et al., 2008) and 32.7% in wild pigs, respectively (Adlhoch et al., 2010). Up to now there is a single study available about the presence and prevalence of PHoV in wild boars (Adlhoch et al., 2010). The survey performed in Germany showed that every third animal was positive for PHoV, but the prevalence showed geographical and age variations; higher positivity was detected in adults than in wild pigs less than one year of age. The presence of higher copy numbers of PHoV genomes in wild boars younger than two years old suggested that the animals were infected early in life and mostly remained persistently infected (Adlhoch et al., 2010). Phylogenetic analysis showed a close relationship of the PHoV sequences from wild boars in Germany to the isolates from Hong Kong, but the European isolates clustered together in one separate branch. The origin, epidemiology, route of transmission, clinical and pathological significance of this newly identified virus are still poorly understood, and further investigations are needed to clarify these aspects.

To contribute to the better understanding of porcine hokovirus epidemiology, a comprehensive spatio-temporal study was carried out on wild boars in Romania, and the results are reported here.

Materials and methods

Sample collection and DNA extraction

Tissue samples of shot wild boars were collected by veterinary diagnostic authorities across the Western region of Romania (Transylvania) as part of the regular classical swine fever survey, during the 2006/2007 and 2010/2011 hunting seasons. A total of 842 wild boars from 315 hunting grounds were sampled and grouped according to their geographical origin and the date of collection (Table 1). Samples from lymph nodes, lungs, liver, kidneys, spleen and tonsils were used. The samples were kept frozen at -20°C until processing.

Table 1. Distribution of hunting grounds, PHoV positive wild boars across Transylvanian counties and age of animals.

Transylvanian counties	Hunting grounds (n=)	Wild boars (n=)	PHoV prevalence in wild boars during hunting seasons		Age (months)			
					6-12	12-36	6-12	12-36
			2006/2007	2010/2011	2006/2007		2010/2011	
Szatmár	26	75	8/43 (19%)	20/32 (63%)	3/8	5/8	1/20	19/20
Bihar	37	68	6/29 (21%)	23/39 (59%)	1/6	5/6	8/23	15/23
Arad	25	38	3/23 (13%)	8/15 (53%)	0/3	3/3	0/8	8/8
Temes	4	41	0/6 (0%)	21/35 (60%)	0/6	0/6	11/21	10/21
Krassó-Szörény	19	23	0/0 (0%)	8/23 (35%)	0/0	0/0	3/8	5/8
Fehér	21	41	13/29 (45%)	10/12 (83%)	4/13	9/13	4/10	6/10
Hunyad	13	45	7/33 (21%)	2/12 (17%)	5/7	2/7	1/2	1/2
Kolozs	23	59	2/28 (7%)	19/31 (61%)	0/2	2/2	6/19	13/19
Szilágy	9	20	0/3 (0%)	6/17 (35%)	0/3	0/3	1/6	5/6
Máramaros	14	22	0/11 (0%)	4/11 (36%)	0/11	0/11	0/4	4/4
Besterce-Naszód	14	38	1/8 (12%)	18/30 (60%)	0/1	1/1	4/18	14/18
Szeben	30	121	10/102 (10%)	3/19 (16%)	5/10	5/10	2/3	1/3
Brassó	10	11	6/10 (60%)	2/3 (67%)	2/6	4/6	0/2	2/2
Hargita	21	79	12/51 (23%)	8/28 (29%)	5/12	7/12	6/8	2/8
Kovászna	17	95	11/62 (18%)	19/33 (58)	5/11	6/11	2/19	17/19
Maros	32	66	28/32 (87%)	17/32 (53%)	7/28	21/28	4/17	13/17
Total	**315**	**842**	**107/470**	**188/372**	**37/107**	**70/107**	**53/188**	**135/188**
%	**100%**	**100%**	**22.76%**	**50.54%**	**34.58%**	**65.42%**	**28.2%**	**71.8%**

DNA was extracted from each tissue sample using the Viral Gene-spin™ Viral DNA/RNA Extraction Kit (Intron Biotechnology Inc., Korea), according to the protocol of the

manufacturer. The extracted DNA was used for the detection of PHoV by a polymerase chain reaction (PCR) using a diagnostic primer pair (PPV3DF-PPV3DR; Table 2; Fig. 1) designed using the Primer Designer v. 2.0 program (Scientific & Educational Software) based on conserved regions of PHoV genomes available in GenBank. Positive samples were selected based on their geographic origin and time of collection and nearly full-length genomes were amplified using the primers listed in Table 2 and Fig. 1.

Table 2. Primers used for the detection and amplification of genome sequences of porcine Hokovirus (PHoV).

Primer name	Primer sequence	Position and orientation[a]	Length[b]
PPV3DF	5'-GCAGTCTGCGCTTAACTT-3'	2587-2604 F	391
PPV3DR	5'-CTGCTTCATCCACTGGTC-3'	2961-2978 R	
PPV3F33	5'-CGGTTCCGGTTGCGTATT-3'	33-50 F	1217
PPV3R1250	5'-CGCAGCCATAAGAAGGAGC-3'	1232-1250 R	
PPV3F1040	5'-CGAGGTGGCAGTGATATTG-3'	1040-1058 F	1035
PPV3R2023	5'-TCCAGTCGTACATCCGAGA-3'	2057-2075 R	
PPV3F2011	5'-TTGGAGGTACCGGCAGA-3'	2011-2027 F	995
PPV3R3006	5'-TCATCGTACCGTTCATCG-3'	2989-3006 R	
PPV3F2859	5'-GTGAACCAGACCTTGAACG-3'	2859-2877 F	1115
PPV3R3974	5'-GAGACCATGTATCACCGGA-3'	3956-3974 R	
PPV3F3844	5'-TGGCTATCTTACTCGAGGAC-3'	3844-3863 F	1268
PPV3R5112	5'-ATGAGCTGGCCTTGTAGAC-3'	5095-5112 R	

PCR was performed in 50µl PCR reactions consisting of 10µl 5x My*Taq* Red Buffer (Bioline GmbH, Germany), 0.5µl of each primers (25 pmol), 1 U of My*Taq* DNA Polymerase (Bioline GmbH, Germany), 1µl of DNA template and ddH_2O up to 50µl. For the diagnostic primer pair, amplification of DNA was initiated by preheating for 5 min at 94°C, followed by 35 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 45 s, with a final elongation step for 7 min at 72°C. For nearly full-length genome amplification, the cycling conditions for all of the used primer pairs were as follows: 5 min at 94°C, followed by 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 3 min, and a final elongation step for 10 min at 72°C. PCR products were analyzed using 1.5% agarose gels stained with GR Safe Nucleic Acid Stain (Excellgen Inc., USA) and Dark Reader blue light transilluminator (Chemical Research, Inc., USA).

Phylogenetic analysis

Twenty amplicons of partial VP1 gene sequences were selected and verified by sequencing (Macrogen, Korea). The nucleotide sequences of PHoV were adjusted and aligned using the BioEdit program (Hall, 1999). Seven close to full-length genomes, a large fragment of NS1 and VP1/2 for three and partial NS1 for 10 additional isolates were sequenced and used for phylogenetic analysis. The created sequences were used in a Blast search of the GenBank database (Altschul et al., 1997). Phylogenetic tree was determined with the neighbor-joining (NJ) method with a bootstrap value of 1000 using the MEGA5 software (Tamura et al., 2011).

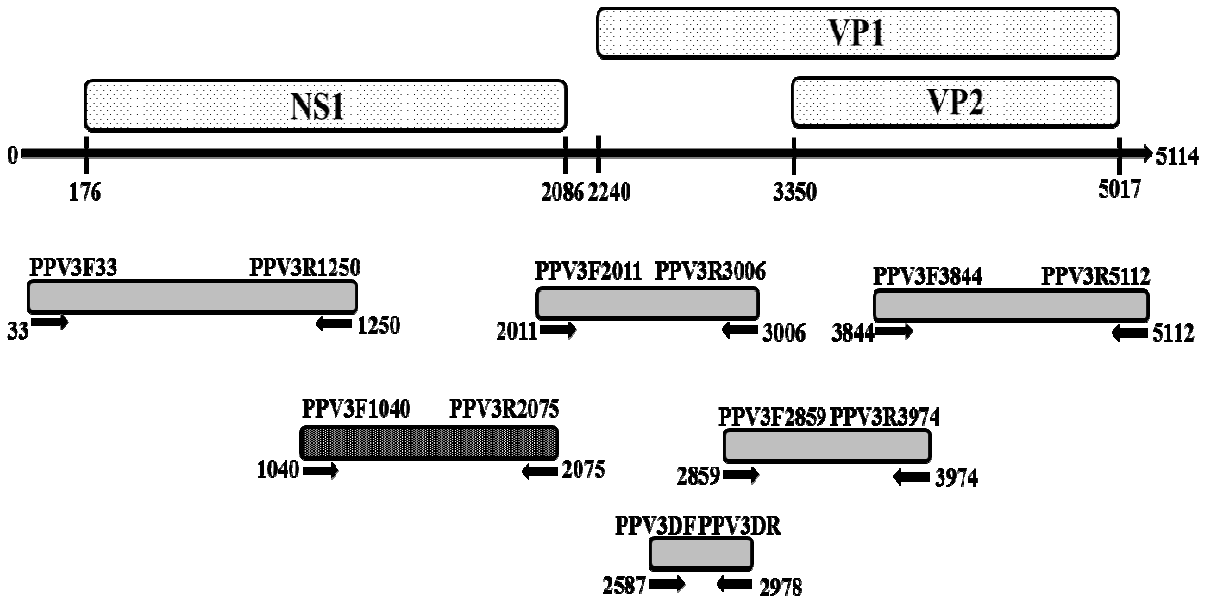

Figure 1. Genome organization of PHoV and the relative position of the amplified sequences. Numbers indicate nucleotide positions corresponding to the PHoV HK7 genome (GenBank accession no. EU200677). Boxes above the arrow represent the three major open reading frames (NS1, VP1 and VP2). Grey boxes indicate the relative position of the primers and the amplified fragments. The dark box represents the partial NS1 gene sequence used for the phylogenetic analysis shown in Figure 3.

Results

Out of the 842 wild boars sampled all over Transylvania, 22.76% (107/470) from the 2006/2007 and 50.54% (188/372) of the 2010/2011 huntig season were found positive for PHoV. The prevalence of the virus also differed among sampling regions: PHoV could not be detected in 3 out of 16 Transylvanian counties in 2006/2007 (Timisoara, Salaj, Maramures), but it was already present in the entire region of Transylvania by the 2010/2011 hunting season (Table 1; Fig. 2). The prevalence of PHoV infection varied significantly with age (Table 1). Out of the 295 PHoV positive animals, 30.5% (90/295) were between 6 and 12 months of age and 69.5% (205/295) were over the age of 1 year. The same age difference was also observed when hunting seasons were compared (Table 1). Twenty PHoV positive samples were selected based on geographic origin and time of collection and sequenced for phylogenetic analyses. According to the nucleotide sequence, the major differences were present in the NS1 gene sequences. To verify if the NS1 sequences were the most variable regions and to establish the phylogenetic relationship between Transylvanian PHoVs and those available in GenBank, seven nearly full-length genome sequences (JF738351; JF738357; JF738362; JF738364; JF738366-JF738368), a large fragment of NS1 and VP1/2 from three (JF738350; JF738352; JF738354) and partial NS1 sequences from 10 PHoV strains (JF738349; JF738353; JF738355; JF738356; JF738358-JF738361; JF738363; F738365) were generated and deposited in GenBank.

Comparing our PHoVs with those detected in wild boars in Germany (Adlhoch et al., 2010), domestic pigs in Hong Kong (Lau et al., 2008) and England (Szelei et al., 2010), 98-99% similarity between the 2006/2007 samples with PHoV from England and Germany and only 96-97% with Hong Kong isolates were observed, while the 2010/2011 samples showed 96-97% and 98-100% similarity with PHoV from Europe and Hong Kong, respectively. Phylogenetic analysis based on partial NS1 sequences revealed that PHoVs from the 2006/2007 wild boars, except one, were closely related to PHoV sequences from England and Germany, and formed a separate cluster in the phylogenetic tree (Fig. 3). The PHoVs collected during 2010/2011 were present in both clusters, one of them including the PHoVs from England and Germany and the other one the Hong Kong isolates (Fig. 3). Phylogenetic analysis showed that PHoVs varied depending on the sampling region. Thus, some samples from both studied times and the same county, were very similar to each other and differed from sequences from other regions (Fig. 3).

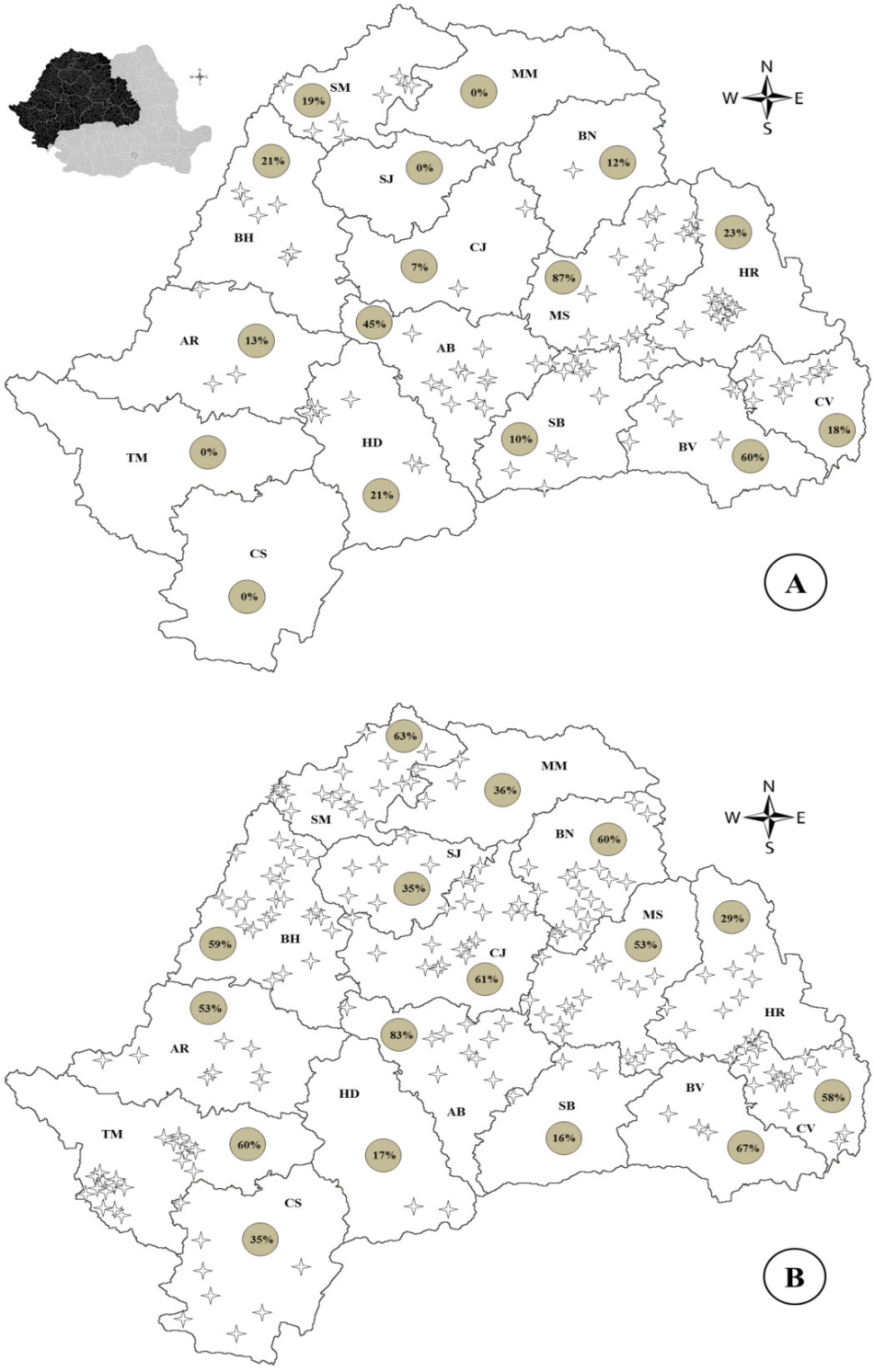

Figure 2. Spatio-temporal distribution of PHoV infected wild boars in the Western part of Romania (Transylvania) during the 2006/2007 (A) and the 2010/2011 (B) hunting seasons. Stars indicate the spread of PHoV positive animals and hunting grounds; the numbers show PHoV prevalences within counties. Bold letters (counties): SM, Satu Mare; BH, Bihor; AR, Arad; TM, Timisoara; CS, Caras Severin; AB, Alba; HD, Hunedoara; SB, Sibiu; BV, Brasov; CV, Covasna; HR, Harghita; MS, Mures; BN, Bistrita Nasaud; CJ, Cluj; SJ, Salaj; MM, Maramures. The area of the small map marked black in the upper left corner represents the Western part of Romania (Transylvania).

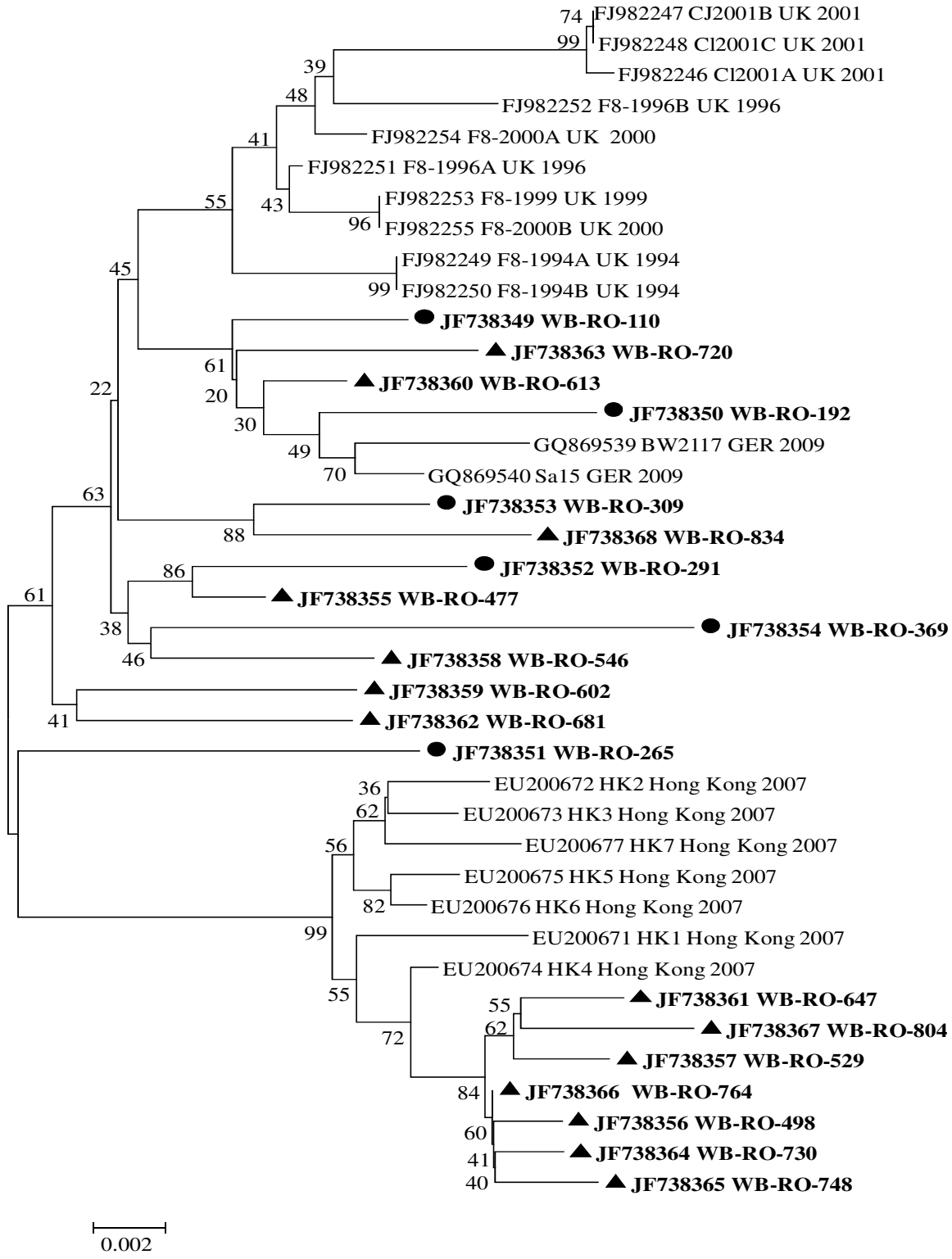

Figure 3. Construction of phylogenetic tree of twenty Transylvanian PHoV partial NS1 gene sequences and comparison with previously identified porcine hokoviruses available in the GenBank. The Romanian PHoVs are bold and indicated with "●" (2006/2007) and "▲" (2010/2011). Sequences of other known porcine hokoviruses are indicated by the accession number, year of isolation and their origin. The phylogenetic tree was constructed by the neighbor-joining method with 1000 bootstrap replicates using the MEGA5 software.

Discussion

Wild boars (*Sus scrofa*) are widespread around the world and play a reservoir role for many important infectious agents with economic or zoonotic significance. The natural habitat of the species is shrinking gradually and the chances of contact among wild boars, domestic pigs and humans are increasing, creating a new environment for the transmission of pathogens. This event is mainly important for disease-free populations of animals that were not exposed previously to the agents from wild boars. Thanks to the rapidly improving nucleic acid amplification technologies new porcine parvoviruses have been described during the last years.

The present study shows that the newly discovered PHoV is present in Romanian free living wild boar populations. Variation in the prevalence was detected when samples from different age groups, years and areas were studied. When looking at samples from 2006/2007, practically every fourth, in 2010/2011 every second animal was PHoV positive. Geographic differences were also observed, especially for the older samples, when at the time of collection some counties were still free of PHoV or with very low prevalence. By today the virus had become widespread in these areas as well. Limited data are available about the presence of PHoV in commercial pigs and wild boars, prevalences of 44.4% in pigs from Hong Kong and 32.7% in wild boars were observed (Adlhoch et al., 2010; Lau et al., 2008), suggesting that in some geographic areas PHoV infection can be endemic both in domestic and wild pigs. The increased prevalence of PHoV infection in wild boars older than one year as observed in our study, is similar to the results obtained by Adlhoch et al. (2010) for wild boars of Germany. It is difficult to estimate the time of infection, but qPCR performed in wild boars in Germany suggested that animals were infected early in their lives and the infection persisted through older ages. The persistence of the infection might be a common feature for this newly proposed group of parvoviruses (Adlhoch et al., 2010). However, to clarify these aspects further investigations are required.

Comparison of the genomic sequences showed that the most variable regions of PHoVs were located in the NS1 gene, and this area was suitable for analysis of the genetic diversity of PHoV. Phylogenetic analysis of the Transylvanian PHoVs showed that the older samples and some of the recent ones were closely related to PHoVs isolated from pigs from England and wild boars in Germany, while the rest of the recent PHoVs showed similarity

with the Hong Kong isolates, forming two separate clusters. The older PHoVs (PARV4-like viruses) from domestic pigs shared higher identity with BHoV and human PARV4-g2 (PARV4-genotype 2), but the major differences in the genome sequences of these viruses from different species (human, bovine, pig) suggested that they had diverged a long time ago (Szelei et al., 2010). Detection of PARV4-like viruses from older samples and recent ones suggested that the porcine PARV4-like and human PARV4 viruses may have evolved similarly (Simmonds et al., 2008; Szelei et al., 2010).

The phylogenetic study showed that the PHoVs, especially older ones isolated from European domestic and wild pigs formed a separate cluster within the phylogenetic tree from the recent ones and the Hong Kong strains. It seems that they represent a cluster of viruses circulating both in domestic and in wild pig populations.

Since PHoV has been detected in a variety of porcine tissues with high virus loads (Adlhoch et al., 2010; Lau et al., 2008) the risk of transmission to humans must be considered. However, until now no mutation or recombination was observed in animal hokoviruses, so the risk to human health could be considered minimal (Szelei et al., 2010). The possible zoonotic role or impacts on domestic and wild pigs are unknown, and also further studies are required. Up to now, no evidence of disease or implications of PHoV in other swine diseases in domestic or wild pigs were reported. Nevertheless, based on our results indicating an increasing prevalence of PHoV in wild boars we proposed PHoV an emerging infection of both domestic and wild pigs.

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