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Epidemiological survey of enteric viruses in wild boars in the Czech Republic: First evidence of close relationship between wild boar and human rotavirus A strains

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ABSTRACT

Population of wild boar is increasing in the whole Europe, the animals migrate close to human habitats which greatly increases the possibility of natural transmission between domestic animals or humans and wild boars. The aim of the study was to estimate in population of free-living wild boar in the Czech Republic the prevalence of enteric viral pathogens, namely rotavirus groups A and C (RVA and RVC), porcine reproductive and respiratory syndrome virus (PRRSV), and members of family *Coronaviridae* (transmissible gastroenteritis virus – TGEV, porcine epidemic diarrhea virus – PEDV, porcine respiratory coronavirus – PRCV, and porcine hemagglutination encephalomyelitis virus – PHEV) and *Picornaviridae,* (teschovirus A – PTV, sapelovirus A – PSV, and enterovirus G – EV-G). In our study, stool samples from 203 wild boars culled during hunting season 2014–2015 (from October to January) were examined by RT-PCR. RVA was detected in 2.5% of tested samples. Nucleotide analysis of VP7, VP4, and VP6 genes revealed that four RVA strains belong to G4P[25]I1, G4P[6]I5, G11P[13]I5, and G5P[13]I5 genotypes and phylogenetic analysis suggested close relation to porcine and human RVAs. The prevalence of RVC in wild boar population reached 12.8%, PTV was detected in 20.2%, PSV in 8.9%, and EV-G in 2.5% of samples. During our study no PRRSV or coronaviruses were detected. Our study provides the first evidence of RVC prevalence in wild boars and indicates that wild boars might contribute to the genetic variability of RVA and also serve as an important reservoir of other enteric viruses.

1. Introduction

Numbers of wild boar (*Sus scrofa*) within Europe has been growing continuously since 1980s. Insufficient hunting together with other various causes have been identified as factors contributing to the population increase. Nowadays, wild boar is one of the most widely distributed mammals in Europe (Massei et al., 2015) and its hunting bag on many localities exceeds 1 ind/km^2. High density of wild boar increased damage on field crops and also risk of transmission of pathogens in their populations. Moreover, spreading of human habitation to suburban areas, increased use of lands for agricultural purposes, and deforestation have all enhanced chances of contact between wild boars and humans or domestic animals. Together with recreational hunting and consumption of wild boar meat, that facts form an ideal environment for the transmission of pathogens between wild boars and both humans and domestic animals (Meng et al., 2009). On the contrary, wild boars could become infected from foraged waste of human or domestic animal origin due to their typical behaviour. Wild boar is an omnivore foraging for food mainly close to the soil surface or by grubbing and digging 15 cm deep (Škrkal et al., 2015). Moreover, pigs have the ability to discriminate between food sites of different relative value and to remember their respective locations. Food foraging, reproduction, and other

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social factors may affect the aggregation of animal (Podgórski et al., 2014) presenting a determining role in epidemiology of diseases.

Wild boars have been recognized as a source of viral infectious diseases for domestic pigs. The presence of antibodies against viral pathogens or viral nucleic acids of viral pathogens representing a serious economic threat was confirmed in several epidemiological studies; it refers particularly to African swine fever virus (ASFV) (Woźniakowski et al., 2015), classical swine fever virus (CSFV) (recently reviewed by Moenning, 2015), and Aujeszky's disease virus (ADV) (Meier et al., 2015). However, not so much is known about other prevalent viral diseases of swine such as porcine circovirus type 2 (PCV-2), porcine reproductive and respiratory syndrome virus (PRRSV), influenza A virus (IAV), porcine parvovirus (PPV) (Gonzáles-Barrio et al., 2015; Sliz et al., 2015; Touloudi et al., 2015). Unexpectedly, specific antibodies against Schmallenberg virus (SBV) were detected in wild boars (Mouchantat et al., 2015). Despite of the fact that enteric viruses are significant pathogens of domestic pigs, they have been studied only sporadically in wild boar. Enteric viruses are transmitted by faecal-oral route, shed in extremely high numbers in the faeces of infected individuals, generally less susceptible to inactivation caused by environmental conditions, and can be easily transported in the environment (Fong and Lipp, 2005). Antibodies against transmissible gastroenteritis virus (TGEV) have been detected extremely rarely (Hälli et al., 2012; McGregor et al., 2015). Viral nucleic acids of porcine enteric picornaviruses, comprising teschoviruses, sapeloviruses, and enteroviruses G have been detected in stool samples (Prodělalová, 2012; Cano-Goméz et al., 2013). Moreover, wild boars represent an important source of zoonotic hepatitis E virus (Schlosser et al., 2015). The prevalence of rotaviruses in wild boars is almost unknown. The only study which detected rotavirus A (RVA) in wild boars was carried out in Japan. Okadera et al. (2013) detected RVA in four animals from 90 tested faecal samples.

Aim of the study was to survey population of wild boar in the Czech Republic for the presence of enteric RNA-viruses including RVA, RVC, PRRSV, and members of family *Coronaviridae* and *Picornaviridae,* and to assess the role of wild boars as natural reservoirs of those important pathogens which can be transmitted to domestic pigs and in case of rotaviruses also to humans.

2. Materials and methods

2.1. Field sample collection and total RNA preparation

Faecal samples or intestinal contents from wild boar (n = 203, 91 males and 112 females) were collected from animals culled between October 2014 and January 2015 and come from four regions representing almost half of the Czech Republic area (South Moravian, Central and South Bohemian, and Vysocina Regions). The age of culled animals was determined by mandibular tooth eruption and wear and ranged between 2 and 90 months with the average age of 12.9 months and median age of 9 months. The sites of sample collection are specified on the map of the Czech Republic (Supplementary data - see the map in the online version at DOI: 10.1016/j.vetmic.2016.08.003).

In the day of collection the 10% faecal suspensions in phosphate buffered saline (PBS) were prepared and homogenized with added glass beads (diameter 2 mm) for 10 min at 2100 rpm in a vortex and then clarified in a table centrifuge for 1 min at 12,000 rpm. If not processed immediately, the supernatants were stored at −80 °C. Total RNA was extracted from 100 μl of the supernatant using 1 ml of TRI Reagent (Sigma, St. Louis, USA) according to the manufacturer's instructions. Extracted RNA was dissolved in 100 μl of RNase-free dH_2O, divided into aliquots and stored at −80 °C until further analysis.

2.2. Reverse transcription and polymerase chain reaction

Random primed reverse transcription (RT) was carried out with the use of Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany) following the manufacturer's instructions. PCR for detection of specific RNA-viruses was accomplished with Fast Start Taq DNA Polymerase (Roche, Germany) and with gene specific primers. The presence of viral RNA of following enteric pathogens was surveyed: RVA, RVC, members of family *Coronaviridae* (specifically TGEV, PEDV, porcine respiratory coronavirus – PRCV, and porcine hemagglutination encephalomyelitis virus – PHEV), PRRSV, enterovirus G (EV-G), teschovirus A (PTV), sapelovirus A (PSV). Detection of PTV and PSV was carried out with the use of one primer pair; the two virus species were distinguished according to the PCR product length (PTV – 161 bp, PSV – 180 bp) and verified with sequencing (Eurofins MWG Operon, Germany). Detection of members of *Coronaviridae* family was accomplished with the use of universal primers detecting four viral species (TGEV, PEDV, PRCV, and PHEV). The specificity of detection primers was verified on the specific pathogens deposited in the Collection of Animal Pathogenic Microorganisms (CAPM, Veterinary Research Institute, Brno) and in the case of RVC on own field isolates of porcine strains (Moutelíková et al., 2014). To increase the sensitivity of the reactions, the second round of PCR was carried out with a semi-nested pair of primers (in RVC and *Coronaviridae* detection) or with the same set of primers as in the first round. Limit of detection (LOD) of the RT-PCR assay with re-amplification was specified for RVA assay by 10-fold serial dilution of porcine RVA strain with known original concentration which was determined by transmission electron microscope virus quantitation using latex particles (Malenovska, 2013). The minimal concentration of RVA still detected was 1.2×10^3 virus particles/1 g of faeces. Used primers, sizes of the expected amplicons and references are listed in Supplementary Table S1 (see Supplementary material Table S1 in the online version at DOI: doi:10.1016/j.vetmic.2016.08.003). The PCR products were examined by electrophoreses in a 2% agarose gel, stained with Midori Green stain (Nippon Genetics Europe, Germany) and visualized by ultraviolet transillumination. Selected PCR products of used diagnostic assays were submitted to sequencing and obtained sequences were analysed with the use of BLAST on-line tool to confirm their classification into respective viral species.

2.3. Phylogenetic analysis of RVA-positive samples

To further characterize RVA-positive samples we attempted to determine the genotypes of the VP7, VP4, and VP6 segments. The primers used for the whole (VP7, VP6) or partial (VP4) ORF preparation are described in the Supplementary Table S2 (see Supplementary material Table S2 in the online version at DOI: http://dx.doi.org/10.1016/j.vetmic.2016.08.003). The obtained sequences were phylogenetically analysed with the MEGA version 6 (Tamura et al., 2013). The dendrograms were prepared with the neighbor-joining method and the evolutionary distances were calculated with the use of Kimura 2-parameter model (Kimura, 1980). To assess the reliability of constructed phylogenetic trees, the bootstrap test with 1000 of replicates was used. The bootstrap value >75% indicates satisfactory topology of phylogenetic tree branches; the bootstrap value of 95–100% is very good. To classify the RVA sequence into the corresponding genotype, the percentage of nucleotide sequence similarity between the Czech RVA strains and RVA sequences deposited in the GenBank was calculated using the *p*-distances method. The VP7, VP4, and VP6 genotypes of RVA strains were determined with the use of previously published cut-off values (Matthijnssens et al., 2008). The assigned genotypes were verified with RotaC online

Table 1
The distribution of enteric viruses in single or mixed infections in faecal samples from wild boars of different age categories.

Detected viral pathogens	Age categories		
	Number of positive samples (%)		
	≤6 months of age (n = 64)	>6 months of age (n = 139)	Total (n = 203)
RVA	2 (3.1)	0	2 (1.0)
RVC	8 (12.5)	10 (7.2)	18 (8.9)
PTV	7 (10.9)	22 (15.8)	29(14.3)
PSV	2 (3.1)	7 (5.0)	9 (4.4)
EV-G	2 (3.1)	0	2 (1.0)
RVA + RVC	2 (3.1)	0	2 (1.0)
RVA + PSV + PTV	1 (1.6)	0	1 (0.5)
RVC + PTV	0	3 (2.2)	3 (1.5)
RVC + PSV	0	2 (1.4)	2 (1.0)
RVC + PTV + PSV	0	1 (0.7)	1 (0.5)
PTV + PSV	1 (1.6)	4 (2.9)	5 (2.5)
PTV + EV-G	1 (1.6)	1 (0.7)	2 (1.0)
Coronaviridae	0	0	0
PRRSV	0	0	0
Total positive samples	27 (42.2)	51 (36.7)	78(38.4)

tool (Maes et al., 2009) in accord with the Rotavirus Classification Working Group (RCWG) guidelines.

3. Results

3.1. Detection of viral pathogens in faeces of wild boars

In total, 203 faeces samples from wild boars were surveyed for RNA-viruses. The screening revealed presence of RVA, RVC, and members of family *Picornaviridae*. Single infections with enteral viruses as well as mixed infections with two or three viruses were also evaluated. No members of family *Coronaviridae* (TGEV, PEDV, PRCV, and PHEV) or PRRSV were found. The results are summarized in Table 1. The distribution of detected viruses on different sites of collection may be seen on the map of the Czech Republic (Supplementary data).

3.2. Detection and characterization of RVAs in wild boars

RVA was detected in 5 samples, e.g. in 2.5% of all examined samples. All RVA-positive samples (P70/2015, P140/2015, P211/2014, P218/2014, P245/2014) were detected in 5- or 6-month-old animals. Their body weights were approximately 33 kg, 27 kg, 27 kg, 23 kg, and 53 kg, respectively. Two RVA-positive sample contained RVA as a single detected pathogen, other three samples contained either another viral pathogen (RVC) or two viral pathogens in coinfection (PSV + PTV).

Out of the five RVA-positive samples, four contained enough quality RNA for the sequencing reaction. The sequences of complete CDS of *VP6* gene were obtained in four samples (P70/2015, P211/2014, P218/2014, and P245/2014) and in three samples (P211/2014, P218/2014, and P245/2014) were obtained complete CDS of *VP7* gene. The partial CDS of *VP7* gene (654 nt) was sequenced in the sample P70/2015 and the partial CDS of *VP4* gene (VP8* region) was obtained in all four sequenced RVA-positive samples. All obtained sequences were deposited in the GenBank database and received accession numbers KU887645-KU887656 which are listed in the Supplementary Table 2.

The acquired sequences were compared with RVA sequences available in the GenBank and the phylogenetic trees based on the nucleotide sequences of *VP7*, *VP4* (VP8* region), and *VP6* genes were constructed (Figs. 1–3 respectively). The genotypes of the detected RVA strains were determined as follows: G4P[25]I1 for P70/2015 strain, G4P[6]I5 for P211/2014 strain, G11P[13]I5 for P218/2014 strain, and G5P[13]I5 for P245/2014 strain. The P70/2015 showed the highest similarities in all studied nucleotide sequences to human RVA strains (91.6% to RVA/Human-wt/CHN/E931/2008 in *VP7* gene, 85.6% to RVA/Human-wt/NPL/KTM368/2004 in VP8* region, and 98.1% to RVA/Human-wt/HUN/BP1231/2002 in *VP6* gene). The other two Czech RVA strains from wild boars P211/2014 and P218/2014 displayed the highest similarities in some segments to the human strains (VP7 and VP4 in P211/2014 and VP6 in P218/2014) while in the rest of segments to the porcine strains. The strain P245/2014 showed the highest similarities to RVA strains isolated from domestic pigs in all studied segments (Table 2). Although some of the Czech wild boar RVA strains shared the same genotypes (e.g. P211/2014, P218/2014, and P245/2014 were I5 genotype the in the segment coding VP6, or P70/2015 and P211/2014 were G4 genotype in the segment coding VP7), the cross similarities of their sequences on nucleotide level were quite low for most of the analysed gene segments (68.1–81.6% for VP7, 57.5–83.3% for VP4, and 83.4–93.5% for VP6).

3.3. Detection of RVCs in wild boars

RVC was detected in 12.8% (n = 26) of the faecal samples. Two thirds of RVC-positive samples (n = 18) contained RVC as a single detected pathogen. In the rest of RVC-positive samples one or more other pathogens were detected (RVA, PSV, PTV). The percentage of RVC-positive samples was higher in the group of animals younger than 6 months of age (15.6%) than among older animals (11.5%) but the difference was not statistically significant ($p < 0.05$, Fisher's exact test, GraphPad Prism 5). Sixteen randomly selected RVC-positive PCR products (326 bp) were submitted to sequencing. The similarity of analysed sequences detected in the Czech wild boars and sequences of VP6 segment of porcine RVC strains deposited in the GenBank ranged between 87 and 98% on nucleotide level (data on request).

3.4. Detection of members of family Picornaviridae in wild boars

The most prevalent virus detected during our study in wild boars was PTV which was detected in 20.2% (n = 41) of all tested samples including coinfections with other viruses. PSV was less prevalent with 8.9% (n = 18) of positive samples. Infections with PTV were found evenly in both age groups of animals while PSV was more often detected in the group of animals above 6 months of age (10.1% of samples were PSV-positive) than in the group of younger animals (6.3% of samples PSV-positive) but the difference was not found statistically significant ($p < 0.05$, Fisher's exact test,

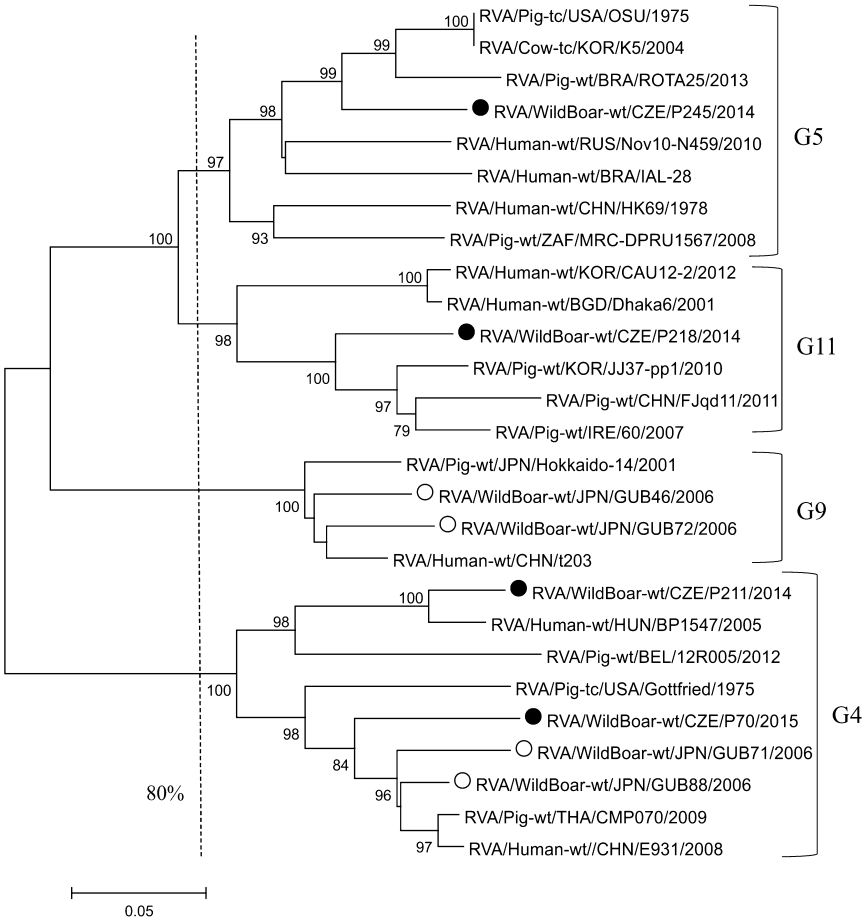

Fig. 1. Phylogenetic tree based on the complete CDS (981 bp) of the *VP7* gene (G-genotypes). The Czech RVA strains described in this study are marked with a black dot (●). All other available RVA strains obtained from wild boars are marked with a black circle (○). The tree was generated by the neighbor-joining method using MEGA version 6. Bootstrap values (1000 replicates) below 70% were hidden. The cut-off value of 80% is indicated with a dashed line.

GraphPad Prism 5). EV-G was detected only in 2.5% (n = 5) of all tested samples (including mixed infections). All PCR products of PTV-, PSV-, and EV-G-positive samples were sequenced and nucleotide sequences compared to sequences available in the GenBank. The similarities of picornavirus sequences detected in the Czech wild boars and the sequences in the GenBank were 97–100% for PTV and EV-G, and 97–99% for PSV (data on request).

4. Discussion

This study investigated the prevalence of eleven enteric viral infections in 203 wild boars culled during the 2014/2015 hunting season in the Czech Republic. Previously described RT-PCR primers were used to detect RVA, RVC, PRRSV and members of families *Coronaviridae* (TGEV, PEDV, PRCV, and PHEV) and *Picornaviridae* (PTV, PSV, and EV-G).

Porcine rotaviruses (both RVA and RVC) are considered to be major pathogens causing diarrhoea in pigs. Transmission of RVC from animal reservoir to human host was reported only once in Brazil (Gabbay et al., 2008) and owing to high degree of conservation (>93% of nucleotide sequence identity) of all segments of human RVC isolates (except for the VP3 gene) (Ghosh and Kobayashi, 2011) it may be presumed that zoonotic transmission of RVC strains is a rare event. However, zoonotic potential of RVA strains has been proven repeatedly (Zeller et al., 2012; Papp et al., 2013). So far, there is no available information concerning RVC prevalence in wild boars and only very limited knowledge about RVA detected in wild boars in Japan (Okadera et al., 2013). The detected RVC prevalence in our study was 12.8% (26/203) including coinfections with other enteral viruses. Similar prevalence of RVC was detected in domestic pigs in the Czech Republic (25.6%) (Moutelíková et al., 2014), in South Korea (26.2%) (Jeong et al., 2009) or in Italy (31.3%) (Martella et al., 2007). Although the RVC infection can cause severe gastroenteritis in pigs of all ages, it may also have subclinical course (Amimo et al., 2013). The samples collected during our study came from animals without any apparent clinical signs of a disease and the faeces processed were of normal consistence. RVA was in the Czech wild boars detected in 2.5% (5/203) of tested samples which is in agreement with results of Okadera et al. (2013). They found RVA in 4.4% of tested faecal samples from wild boars in Japan. The possibility of RVA transmission between wild boars and domestic pigs in Japan was supported by very close phylogenetic relationship between their VP7 and VP4 nucleotide sequences. The RVA-positive strain P70/2015 detected during our survey showed the highest similarities of all studied genomic segments to human RVA strains which were described elsewhere as porcine-human reassortments (Mullick et al., 2013; Zhou et al., 2015). It is of great interest that the P[25] genotype of VP4 segment was found so far only very rarely in several RVA strains isolated from humans. This genotype is thought to be of porcine origin but so far no porcine (or other animal) sample was described to bear this scarce VP4 genotype (Matthijnssens et al., 2010). This is the first time the possible animal progenitor strain reservoir of P[25] genotype was

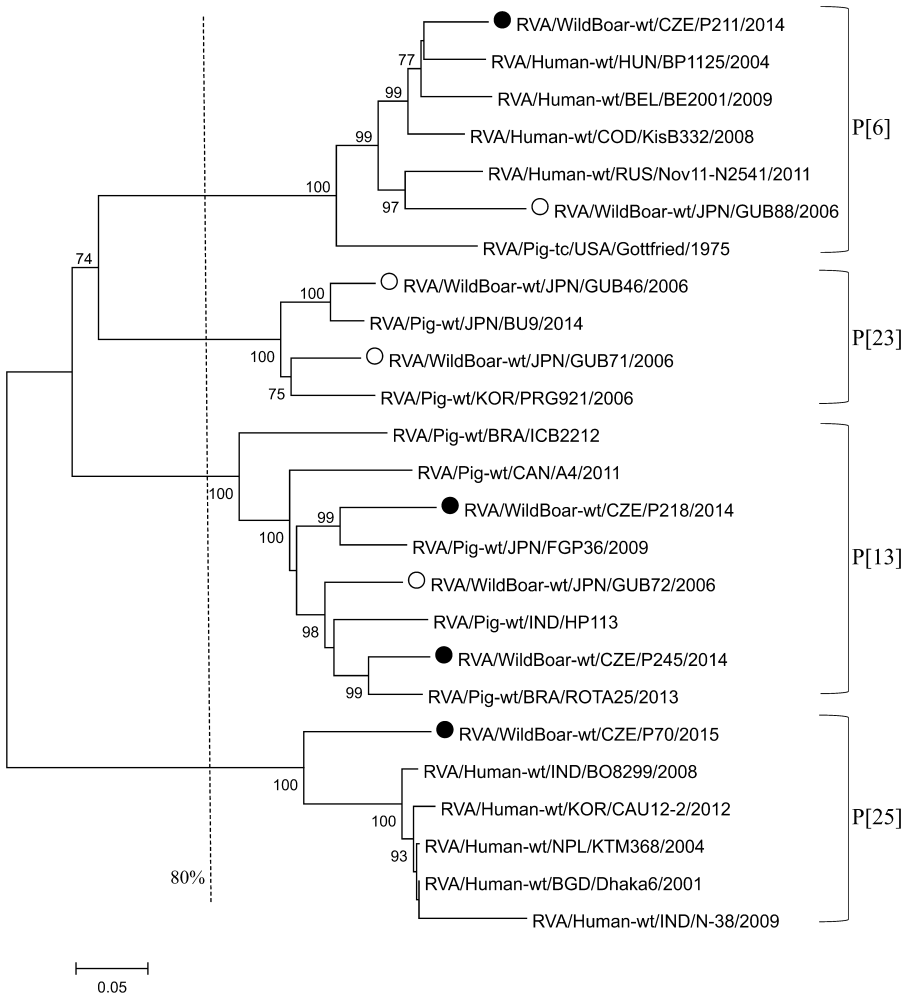

Fig. 2. Phylogenetic tree based on the partial CDS (839 bp) of the *VP4* (VP8* region, nucleotide 16-854 of the Gottfried strain KR052749) gene (P-genotypes). The Czech RVA strains described in this study are marked with a black dot (●). All other available RVA strains obtained from wild boars are marked with a black circle (○). The tree was generated by the neighbor-joining method using MEGA version 6. Bootstrap values (1000 replicates) below 70% were hidden. The cut-off value of 80% is indicated with a dashed line.

described and surprisingly it might be the wild boar population. Up to date, only eight RVA strains isolated from wild boars were genetically described so we cannot reason out how frequent separate RVA genotypes might be among wild boars and whether the proportional representation of genotypes is similar as in domestic pigs. Phylogenetic analysis of VP7 and VP4 genome segments of another RVA strain from our study (P211/2014) also clustered this wild boar RVA strain together with human RVA strains isolated in Hungary and Belgium (Zeller et al., 2012; Papp et al., 2013). The whole genomic sequences of those human RVA strains were described and it was found that those human strains originated from porcine or porcine-human reassortment RVA strains. It is of interest, that all analysed wild boar RVA strains were found in the region of South Moravia, namely near the town of Brno (RVA strain P245/2014) and in the vicinity of the confluence of Morava and Thaya Rivers very close to the Czech and Slovakian borders (RVA strains P70/2015, P211/2014, P218/2015). This area of the confluence is regularly flooded which forces increased migration of wildlife animals and it can also contribute to spreading of enteric viruses through contaminated water. The Morava River later joins the Danube River near Slovakian and Hungarian borders. At present, it is not possible to answer if the wild boar RVA strains which show close relationship to Hungarian human strains (P70/2015, P211/2014) are also circulating among wild boars in Hungary, as there is no relevant data available. Considering very low nucleotide sequences similarities of the Czech wild boar RVA strains we may presume that a large numbers of different RVA strains is currently circulating among wild boars.

Although PTVs, PSVs, and EV-Gs are generally considered to be nonpathogenic (causing asymptomatic infections in most cases), PTV-1 strains are causative agents of acute disease of swine called Teschovirus encephalomyelitis, which is characterised by central nervous system disorders. Some PTV serotypes were detected rarely in association with reproductive failure, diarrhoea, pneumonia, pericarditis, and myocarditis (Knowles, 2006). PSVs were connected with clinical signs of diarrhoea, pneumonia, and reproductive disorders. EV-G was originally isolated from atypical skin lesions and the role of the virus as enteric pathogen causing diarrhoea was discussed (Knowles, 2006). Infected pigs are an important source of PTV, PSV, or EV-G infection and contamination of water and environment since these viruses are easily transferred by faecal-oral route. The zoonotic potential of porcine enteric picornaviruses was not described so far but the transmission between domestic pigs and wild boars is highly probable (Prodělalová, 2012). One or more members of family *Picornaviridae* (PTV, PSV, and EV-G) were detected in 26.6% of wild boars tested in our study. PTV was the most prevalent of all detected viruses and was found in 20.2% of all samples. PSV was found less often (8.9% of

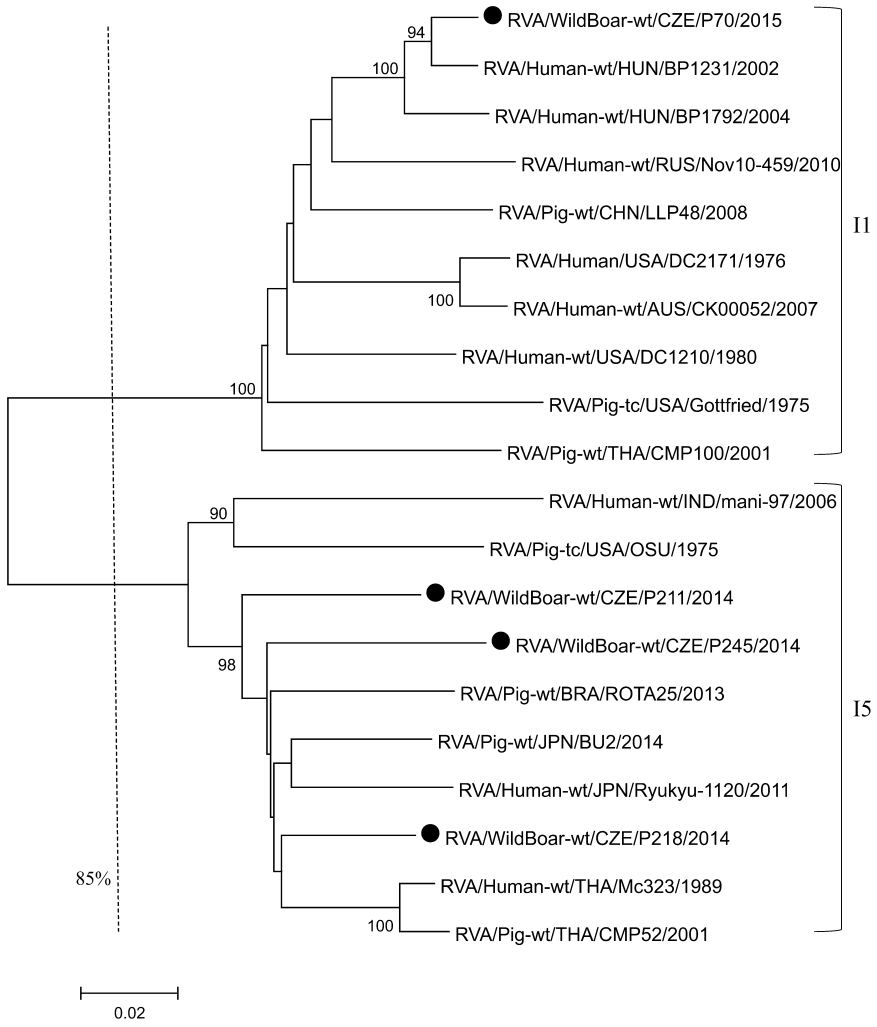

Fig. 3. Phylogenetic tree based on the complete CDS (1194 bp) of the *VP6* gene (I-genotypes). The Czech RVA strains described in this study are marked with a black dot (●). The tree was generated by the neighbor-joining method using MEGA version 6. Bootstrap values (1000 replicates) below 70% were hidden. The cut-off value of 85% is indicated with a dashed line.

all samples). Even higher prevalence of PTV was described in wild boars in Spain where 50.8% of tested samples were PTV-positive, while only 6.4% of samples contained PSV (Cano-Goméz et al., 2013). Similarly high prevalence of porcine enteric picornaviruses was earlier shown in wild boars in the Czech Republic (Prodělalová, 2012), where 44.4% of tested samples were positive for PTV and 27.8% for PSV. EV-G was in the Czech Republic detected in 69.4% which is more than ten times higher prevalence than the number of EV-G-positive samples detected in this study. The reason might be that wild boars tested previously in the Czech Republic were animals kept in enclosure in four wild boar farms where there is a higher probability of virus transmission between animals. In the present study the animals tested were free-ranging wild boars culled during hunting season in at least 16 places located in four regions covering over 35 000 km^2.

Table 2
The highest nucleotide (nt) identities of VP7, VP4 (VP8* region), and VP6 genes of the Czech RVA strains detected in wild boars and other available RVA sequences specified with the use of the *p*-distances method of MEGA version 6.

Strain	Gene segment	Geno-type	Strain with the highest identity	
			Strain/Host	nt identity (%)
P70/2015	VP7	G4	E931/Human	91.6
	VP4	P[25]	KTM368/Human	85.6
	VP6	I1	BP1231/Human	98.1
P211/2014	VP7	G4	BP1547/Human	95.3
	VP4	P[6]	PE2001/Human	92.3
	VP6	I5	DPRU1487/Pig	93.5
P218/2014	VP7	G11	IRE/60/Pig	91.0
	VP4	P[13]	FGP36/Pig	89.7
	VP6	I5	BP1547/Human	96.1
P245/2014	VP7	G5	OSU/Pig	91.7
	VP4	P[13]	ROTA25/Pig	92.5
	VP6	I5	BU2/Pig	93.0G

No members of family *Coronaviridae* were detected during our study. It is in agreement with serological surveys in wild boar population which were carried out in Slovenia, Finland, or in Canada and did not detect antibodies against TGEV (Vengust et al., 2006; Hälli et al., 2012; McGregor et al., 2015). In Slovenian wild boars 3% of animals tested positive for anti-PRCV antibodies (Vengust et al., 2006). The viral loads of PRCV in faeces would be very low (if present at all), so no PRCV was detected during our survey.

Antibodies against PRRSV were detected in wild boars in 12.8% of tested animals in Greece (Touloudi et al., 2015) or in 1.9% of tested animals in Spain (Cano-Manuel et al., 2014), other

studies carried out in Slovenia, Finland, and Canada did not find any anti-PRRSV antibodies (Vengust et al., 2006; Hälli et al., 2012; McGregor et al., 2015). So far, the evidence of PRRS virus presence in wild boar was confirmed in Germany (Reiner et al., 2009) or recently in Slovakia (Vilcek et al., 2015). In those studies, tissues of lungs, tonsils or spleen were used for PRRSV detection, which are the places of virus replication. Our study did not confirm presence of PRRSV nucleic acid in wild boar faeces although the shedding of virus in faeces of domestic pigs was previously documented (Ramírez et al., 2008).

5. Conclusion

In summary, our survey shows that RVA and RVC are circulating in the population of wild boar in the Czech Republic. To the best of our knowledge, this is the first time the prevalence of RVC was assessed in wild boars. RVA strains isolated from wild boars were genetically described and close relationship with human and domestic pig RVA strains was found so the transmission of RVA between wild boars and humans or between domestic pigs and humans and subsequently between domestic pigs and wild boars is highly probable. Furthermore, high prevalence of PTV in Czech wild boars was confirmed, PSV and EV-G were found less frequently. During our study no PRRSV or members of family *Coronaviridae* were detected. Our findings indicate that wild boars might contribute to the genetic variability of RVA and also serve as an important reservoir of other enteric viruses including RVC, PTV, PSV, and EV-G.

Conflict of interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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