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Original Article

A survey of porcine reproductive and respiratory syndrome among wild boar populations in Korea

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No information is currently available on porcine reproductive and respiratory syndrome virus (PRRSV) infection in wild boars (*Sus scrofa*) in Korea. In this study, the status of PRRS in wild boars was investigated. Blood samples were collected from 267 wild boars from eight provinces in Korea. Four of the samples tested (1.5%) were positive for PRRSV antibodies and eight (3.0%) were positive for antigens. Of the virus-positive samples, three and five samples were typed as containing European (EU, type 1) or North American (NA, type 2) viruses, respectively. Two amplicons (one from type 1 and one from type 2) were used to analyze the PRRSV open reading frame 7 (ORF7) sequence. The nucleotide sequences of type 1 PRRSV ORF7 had identities between 96.1% and 98.4% with PRRSVs from domestic pigs in Korea. The sequences of type 2 PRRSV ORF7 had identities of 100% with the PRRSV strain VR-2332, which was prototypic North American strain. These results show that PRRSVs are present in wild boars in Korea, and effective PRRSV surveillance of the wild boar population might therefore be useful for disease control.

Keywords: ELISA, Korea, porcine reproductive and respiratory syndrome, RT-PCR, wild boar (*Sus scrofa*)

Introduction

Porcine reproductive and respiratory syndrome (PRRS) is an infectious disease characterized by reproductive disorders in sows along with respiratory signs in piglets and fatteners resulting in significant economic losses in the pig industry worldwide [21,35]. The disease is caused by the PRRS virus (PRRSV), which is classified as a member of the order *Nidovirales*, family *Arteriviridae*, and genus *Arterivirus* [6]. The genome of PRRSV is approximately 15 kb in length and consists of at least nine open reading frames (ORFs) [2,10]. ORF1a and 1b encode the enzymes responsible for replication; ORF2a and ORFs 3, 4, and 5 encode the membrane-associated glycoproteins; ORF2b and 6 encode the non-glycosylated membrane proteins, and ORF7 encodes the nucleoprotein (N) protein [34]. PRRSVs are divided into two genotypes: European (type 1) and North American (type 2) strains. The two genotypes share an approximately 67% similarity at the nucleotide level over the full genome [20,22]. The virus is primarily transmitted by contact with infected pigs but also through feces, urine, semen, and fomites. Additionally, it can be spread indirectly, presumably *via* aerosol routes and possibly by mechanical vectors [37].

The habitat of wild boar (*Sus scrofa*) has been destructed with community development and, consequently, at some area, the density and distribution of the wild boar have increased from 2010 to 2011. Without predators or competing animals, the numbers of wild boars have increased regionally [13]. Direct contact between wild boars and domestic pigs may occur rarely because all domestic pigs are reared within farming facilities in Korea. The potential role of wild boars as a reservoir for PRRSV has been reported in France, Germany, and the USA with serological evidence of infection [3,24]. Since the emergence of PRRSV in 1993, PRRS has been widespread in domestic pigs throughout Korea [6,15,17,18,35]. Furthermore, wild boars and domestic pigs have been reported to have the same susceptibility to PRRSV [1]. Monitoring PRRS in wild boars might therefore be an important factor for disease control in domestic pigs. The present study was performed to assess the prevalence of PRRSV in wild boars in Korea and provide information for developing effective PRRS surveillance programs.

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Materials and Methods

Serum sample collection and virus propagation

Blood samples collected from 267 wild boars from eight provinces of Korea (Gyeonggi, Gangwon, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, and Gyeongnam) were submitted to the Viral Disease Division of Animal, Plant and Fisheries Quarantine and Inspection Agency (Korea) during the hunting season in November 2010～February 2011. Blood samples were taken by hunters from the heart immediately after the wild boars had been shot. The collected samples were stored at 4°C and transported to the laboratory within 1～2 weeks. The sera were collected by centrifugation for 15 min at 4°C at 1,500 × g and stored at −20°C until use. The PRRSV prototype VR-2332 and Lelystad virus (LV) were propagated in MARC-145 cells, and used as positive controls for the reverse transcription-PCR assay.

Serological test

Anti-PRRSV antibody titers were determined using a commercially available HerdChek PRRS 2XR Virus Antibody Test Kit (Idexx Laboratories, USA) according to the manufacturer's instructions. Samples were considered to be positive for PRRSV antibodies if the ratio of sample absorbance to positive control absorbance (S/P) was greater than 0.4.

RT-PCR for the detection and differentiation of PRRSV

Total RNA was extracted from 100 μL of each serum sample using an RNeasy mini kit (Qiagen, Germany) according to the manufacturer's instructions. RT-PCR was carried out using a OneStep RT-PCR kit (Qiagen, Germany) and a PRRSV common primer set (Table 1) derived from the sequences of ORF7 and the 3′ non-coding region of the VR-2332 and LV strains. The primer set was designed to detect and differentiate between PRRSV types 1 and 2. The PCR reaction contained 5 μL 5× RT-PCR buffer (2.5 mM $MgCl_2$), 0.4 mM dNTPs, 0.5 μM of each of the four primers shown in Table 1, 1 μL enzyme mix, and 5 μL RNA in a final volume of 25 μL. RT-PCR amplification was carried out as follows: a reverse transcription step at 50°C for 30 min; RTase inactivation and initial PCR activation at 95°C for 15 min followed by 35 cycles of denaturation at 94°C for 20 sec, annealing at 55°C for 20 sec, and extension at 72°C for 30 sec, and a final elongation step at 72°C for 10 min. The amplicons were separated by electrophoresis in a 1.5% agarose gel and stained with ethidium bromide.

Sequencing and phylogenetic analysis of ORF7

For the complete ORF7 sequencing, PCR was performed using SuperScript One-Step RT-PCR with Platinum *Taq* (Invitrogen, USA) according to the manufacturer's

Table 1. Primers for the detection and differentiation of porcine reproductive and respiratory syndrome virus (PRRSV)

Genotype	Strain	GenBank accession No.	Primer			Product size
				Position*	Sequence	
Type 1	LV	M9662	Forward	14653-14671 (ORF7)	5′ ATGGCCAGCCAGTCAATCA 3′;	398 bp
			Reverse	15030-15050 (3′NCR)	5′ TCGCCCTAATTGAATAGGTGA 3′	
Type 2	VR-2332	AY150564	Forward	14933-14951 (ORF7)	5′ ATGGCCAGCCAGTCAATCA 3′;	433 bp
			Reverse	15346-15365 (3′NCR)	5′ TCGCCCTAATTGAATAGGTGA 3′	

*Primer position: forward primers are from ORF7 and reverse primers are from the 3′NCR region. ORF: open reading frame, NCR: non-coding region.

Table 2. Primers for propagating the complete ORF7 region of PRRSV

Genotype	Strain	GenBank accession No.	Primer			Product size
				Position*	Sequence	
Type 1	LV	M9662	Forward	14413-14429 (ORF6)	5′ GGCCCCTGCCCACCACG 3′	638 bp
			Reverse	15030-15050 (3′NCR)	5′ TCGCCCTAATTGAATAGGTGA 3′	
Type 2	VR-2332	AY150564	Forward	14705-14721 (ORF6)	5′ GGCCCCTGCCCACCACG 3′	661 bp
			Reverse	15346-15365 (3′NCR)	5′ TCGCCCTAATTGAATAGGTGA 3′	

*Primer position: forward primers are from ORF6 and reverse primers are from 3′NCR region.

instructions with primers (Table 2) designed to include the full ORF7 area. The PCR amplicons were purified using a MiniElute gel extraction kit (Qiagen, Germany) and cloned using a pGEMT easy vector system (Promega, USA). Sequencing was then performed using a GenomeLab DTCS-Quick Start Kit (Beckman Coulter, USA) and CEQ8000 automated sequencer (Beckman Coulter, USA). Multiple sequence alignment of the individual sequences was performed using CLUSTALX 1.81, and nucleotide sequence identities among the Korean PRRSV isolates were calculated using BioEdit software (Ibis Biosciences, USA).

Phylogenetic reconstructions were generated with PHYLIP (ver. 3.572c) using the neighbor-joining method based on the Kimura two-parameter model [11,16]. Robustness of the phylogenetic analysis was measured by bootstrap analysis with 1,000 replications. Graphic output was produced by TreeView (ver. 1.6.1) [35]. Evolutionary history was inferred using the neighbor-joining method [36]. An optimal tree in which the sum of branch length was 1.90,543,171 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches [12]. The tree was drawn to scale with branch lengths in the same units as those of the evolutionary distances used to establish the phylogenetic tree. Evolutionary distances were calculated using the Kimura two-parameter method [11] and are expressed as units of the number of base substitutions per site. The analysis included 40 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 363 positions in the final dataset. Evolutionary analyses were conducted with Molecular Evolutionary Genetics Analysis version 5 (MEGA 5) [29].

Results

Out of the 267 sera tested, four (1.5%) were positive for PRRSV antibodies (Table 3). The ELISA S/P ratios for the positive samples were 0.46, 0.74, 0.77, and 0.91. Eight sera samples (3.0%) were positive for PRRSV antigens (Fig. 1). All PRRSV-positive wild boars were infected with only one genotype. Type 1 virus was detected in three wild boars from two provinces (Gyeonggi and Chungbuk), and type 2 virus was detected in five animals from three provinces (Gyeonggi, Jeonnam, and Gyeongbuk).

Two amplicons (sample No. 49 from Gyeongbuk and sample No. 129 from Chungbuk) from the positive samples were subjected to ORF7 sequencing (Fig. 2). Homology of the deduced amino acid (aa) sequences between the wild boar type 1 virus (sample No. 129) and LV strain was 92.2% (Fig. 2A). Homology between the wild boar type 2 virus (No. 49) and VR-2332 strain was 100% (Fig. 2B). The wild boar type 1 virus had amino acid sequence identities between 96.0% and 98.4% with PRRSVs from domestic pigs in Korea (Table 4). Phylogenetic analysis revealed that the wild boar type 1

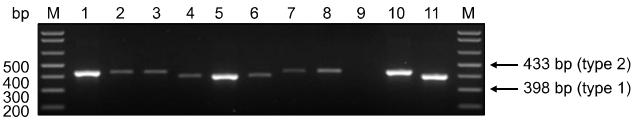


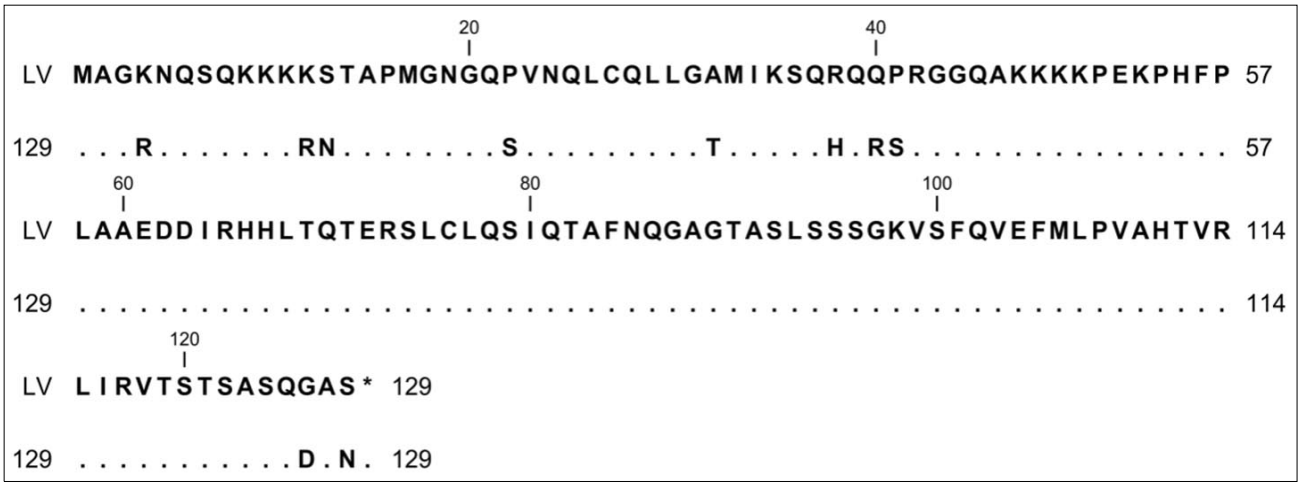
Fig. 1. RT-PCR results for the detection and differentiation of PRRSV in wild boar serum samples. Lane M: 100-bp DNA ladder, Lanes 1, 2, 3, 7, and 8: sample Nos. 49, 77, 167, 193, and 227, respectively (433-bp band), Lanes 4, 5, and 6: sample Nos. 110, 129, and 258, respectively (398-bp band), Lane 9: normal wild boar serum used as a negative control, Lane 10: VR-2332 strain used as a positive control for type 2, Lane 11: Lelystad virus (LV) used as a positive control for type 1.

Table 3. Results of PRRSV detection in wild boars from different provinces of Korea

Province	Number of samples tested	Antibody		Antigen	
		Number of positive samples	S/P	Number of positive samples	Genotype (numberof positive)
Gyeonggi	108	0	−	5	Type 1 (2), Type 2 (3)
Gangwon	30	0	−	0	−
Chungbuk	32	2	0.77, 0.91	1	Type 1 (1)
Chungnam	20	0	−	0	−
Jeonbuk	7	0	−	0	−
Jeonnam	31	1	0.74	1	Type 2 (1)
Gyeongbuk	32	0	−	1	Type 2 (1)
Gyeongnam	7	1	0.46	0	−
Total	267	4 (1.5%)	−	8 (3%)	−

S/P: the ratio of sample absorbance to positive control absorbance.

A



B

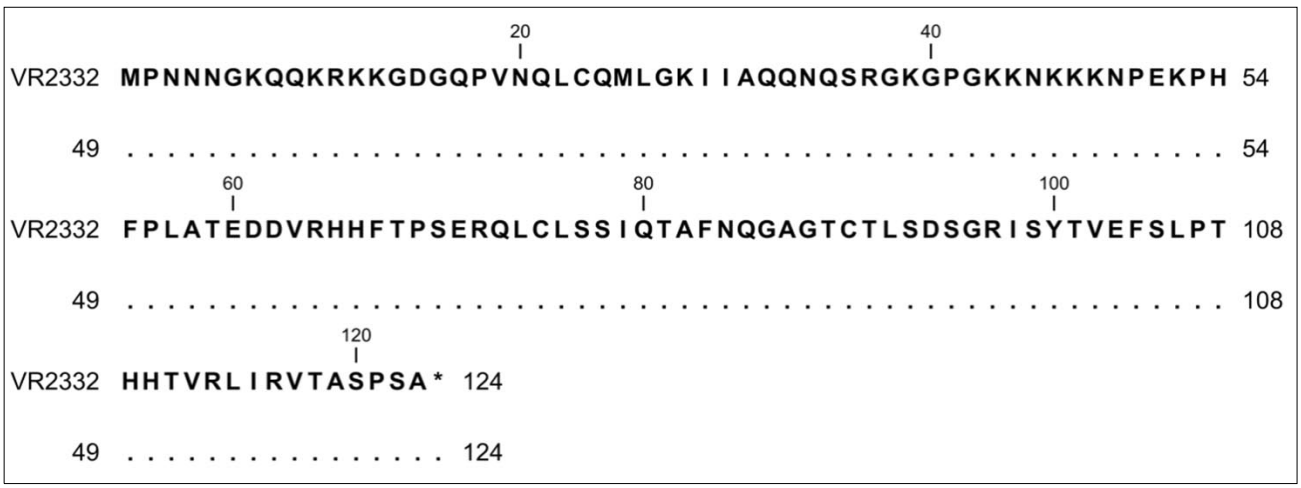


Fig. 2. Alignments of the putative amino acid sequences based on ORF7 of wild boar-derived PRRSVs and prototype virus strains. (A) No. 129 and Lelystad virus (LV). (B) No. 49 and VR-2332 strain. Dots indicate identical amino acids.

Table 4. Nucleocapsid amino acid sequence pair distances between Korean PRRSV isolates and comparison of the identity and divergence percentages

		Percent identity (%)							
		129	D82-1	D163-	G210	G2448	KNU-07	V0773	V1294
Percent divergence (%)	129		96.0	98.4	96.8	96.8	98.4	96.0	96.0
	D82-1	4.0		97.6	96.0	96.0	97.6	95.3	95.3
	D163-	1.6	2.4		98.4	98.4	100.0	97.6	97.6
	G210	3.2	4.0	1.6		96.8	98.4	96.0	96.0
	G2448	3.2	4.0	1.6	3.2		98.4	96.0	96.0
	KNU-0	3.6	2.4	0.0	1.6	1.6		97.6	97.6
	V0773	4.0	4.7	2.4	4.0	4.0	2.4		95.3
	V1294	4.0	4.7	2.4	4.0	4.0	2.4	4.7	

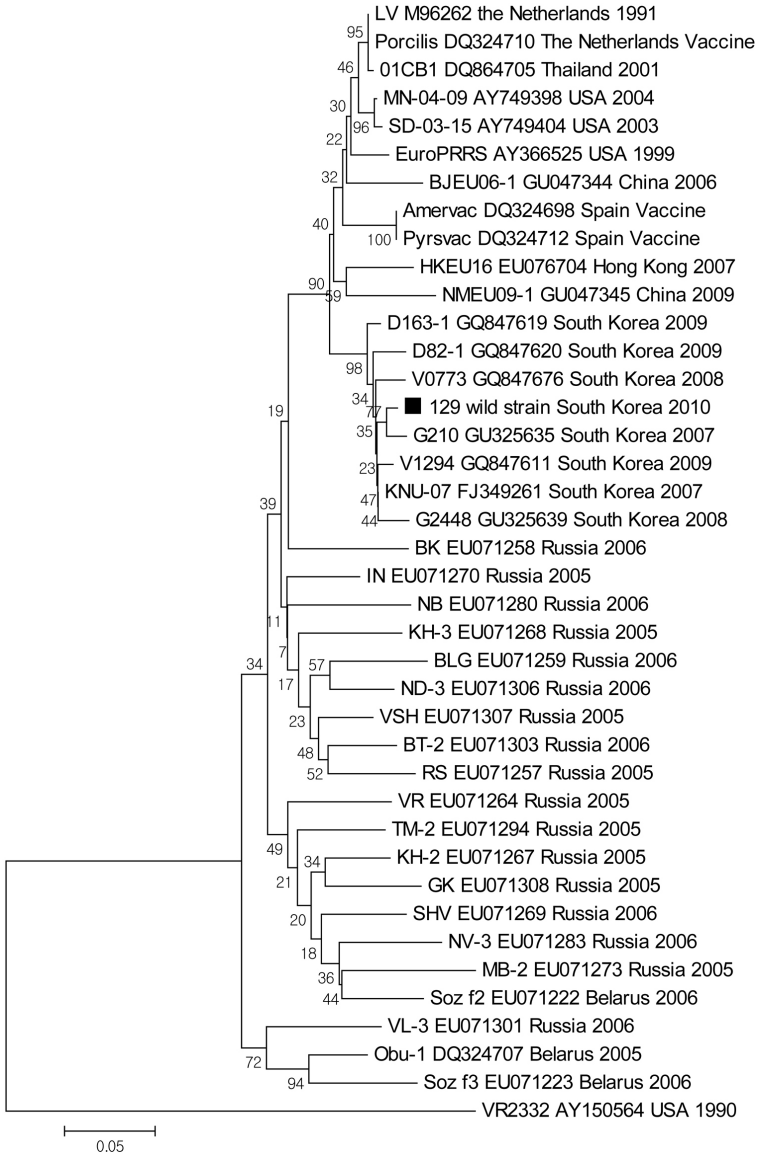


Fig. 3. Phylogenetic analysis of ORF7 nucleotide sequences of a wild boar-derived and other various PRRSVs. The phylogenetic tree was constructed with wild boar type 1 (No. 129) PRRSV and PRRSV strains isolated worldwide. PRRSV strains are denoted as follows: PRRSV strain/GenBank accession No./country/ collection time, publication year, or vaccine.

virus (No. 129) is closely related to existing PRRSVs recovered from domestic pigs based on ORF7 sequences (Fig. 3). The percentages of identity between ORF7 sequences in wild boar type 1 virus and seven Korean type 1 PRRSVs analyzed in 2007～2009 (D163-1, D82-1, V0773, G210, V1294, KNU-07, and G2448) were found to be 97.2～99.0%.

Discussion

The significance of wild boars as potential vectors or reservoirs for PRRSV and other viral diseases in France, the USA, Italy, and other countries has been evaluated [1,4,8,9,19,24,25,30-32,38]. The wild boar population density in Korea has increased between 1982 and 1997 and it showed an average of 3.7 wild boars per 100 hectares in 2005 [33]. Wild boar blood samples were collected to monitor foot and mouth disease (FMD) and classical swine fever (CSF) in Korea in 2010 and 2011 by Animal, Plant and Fisheries Qurantine and Inspection Agency as a part of National Animal Disease Monitoring Program. In addition to FMD and CSF, the samples were also tested for many other porcine viral diseases. These samples were screened for PRRSVs antibodies and antigens to evaluate PRRS in Korean wild boars. In the present study, prevalence of PRRS in wild boars was not high (less than 3%).

Spread of the virus from domestic pigs to wild boar populations cannot be excluded because of the high prevalence of PRRS in domestic pigs in Korea. Recent data for serological prevalence of PRRS have not been published, but it was estimated to be approximately 70% in the 1990s [7]. Herd prevalence of a newly emerging Korean type 1 PRRSV was 29.4% during 2007～2008 [18].

The N protein encoded by ORF7 is the most abundant, immunogenic, and conserved of all PRRSV proteins. ORF6 and ORF7 are the most conserved nucleotide sequences among the different strains of PRRSV [14,37]. Thus, ORF7 was selected to detect PRRSV in our PCR-based assay. The ORF7 sequence of the wild boar-derived type 2 virus analyzed in the current study was identical to that of the VR-2332 strain, a prototype vaccine virus used worldwide. It is possible that vaccine viruses have spread from domestic pigs to wild boars because vaccination of domestic pigs with an attenuated type 2 VR-2332 is associated with type 2-positive PCR results in Europe [5,23,28].

The wild boar-derived type 1 virus had 92.2% homology with the LV strain, which was prototypic European strain, and very similar identities (96.0% to 98.4%) with PRRSVs from domestic pigs in Korea. ORF7-based phylogenetic analysis showed that the wild boar type 1 virus (No. 129) and other Korean type 1 isolates appeared to be in a cluster of the pan-European subtype 1 proposed by Stadejek *et al*. [27] and Lee *et al*. [18]. However, further studies are needed to confirm this finding.

In Korea, some farmers raise their pigs on the edge of mountains with rough fence around the farmland. Sometimes the housed pigs escaped from their pen. Recently, there were increased numbers of reports about damages by wild boars roaming around residential areas including the outskirts of cities. The boars had started to invade residential areas to search for food. Compared to other less densely human and animal populated countries such as the USA and Canada, the environment in Korea provides a better chance for domestic animals to encounter wild animals. It is highly possible for domestic pigs and wild boars to come into contact and exchange pathogens. Recent evidence of this was provided by detection of the classical swine fever virus genome and antibodies in wild boar samples collected in 2011 [26]. Surveys of animal disease in wildlife would be important for national animal disease monitoring programs to determine the status of diseases in specific territories.

In conclusion, the present study showed that PRRSVs are present in Korean wild boar populations. Low prevalence rates of the virus among these animals suggest that PRRS is not endemic in wild boar populations in Korea. Our study explained the need to perform continual investigation of PRRS in wild boar population for efficient national PRRS monitoring.

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References

1. **Albina E, Mesplède A, Chenut G, Le Potier MF, Bourbao G, Le Gal S, Leforban Y.** A serological survey on classical swine fever (CSF), Aujeszky's disease (AD) and porcine reproductive and respiratory syndrome (PRRS) virus infections in French wild boars from 1991 to 1998. Vet Microbiol 2000, **77**, 43-57.
2. **Allende R, Lewis TL, Lu Z, Rock DL, Kutish GF, Ali A, Doster AR, Osorio FA.** North American and European porcine reproductive and respiratory syndrome viruses differ in non-structural protein coding regions. J Gen Virol 1999, **80**, 307-315.
3. **Baker SR, O'Neil KM, Gramer MR, Dee SA.** Estimates of the seroprevalence of production-limiting diseases in wild pigs. Vet Rec 2011, **168**, 564.
4. **Bartak P, Greiser-Wilke I.** Genetic typing of classical swine fever virus isolates from the territory of the Czech Republic. Vet Microbiol 2000, **77**, 59-70.
5. **Bøtner A, Strandbygaard B, Sørensen KJ, Have P, Madsen KG, Madsen ES, Alexandersen S.** Appearance of acute PRRS-like symptoms in sow herds after vaccination with a modified live PRRS vaccine. Vet Rec 1997, **141**, 497-499.
6. **Cha SH, Choi EJ, Park JH, Yoon SR, Song JY, Kwon JH, Song HJ, Yoon KJ.** Molecular characterization of recent Korean porcine reproductive and respiratory syndrome (PRRS) viruses and comparison to other Asian PRRS viruses. Vet Microbiol 2006, **117**, 248-257.
7. **Cheon DS, Chae C, Lee YS.** Seroprevalence of antibody to porcine reproductive and respiratory syndrome virus using enzyme-linked immunosorbent assay in selected herds in Korea. J Vet Diagn Invest 1997, **9**, 434-436.
8. **Cságola A, Kecskeméti S, Kardos G, Kiss I, Tuboly T.** Genetic characterization of type 2 porcine circoviruses detected in Hungarian wild boars. Arch Virol 2006, **151**, 495-507.
9. **de Deus N, Casas M, Peralta B, Nofrarías M, Pina S, Martín M, Segalés J.** Hepatitis E virus infection dynamics and organic distribution in naturally infected pigs in a farrow-to-finish farm. Vet Microbiol 2008, **132**, 19-28.
10. **Dea S, Gagnon CA, Mardassi H, Pirzadeh B, Rogan D.** Current knowledge on the structural proteins of porcine reproductive and respiratory syndrome (PRRS) virus: comparison of the North American and European isolates. Arch Virol 2000, **145**, 659-688.
11. **Felsenstein J.** An alternating least squares approach to inferring phylogenies from pairwise distances. Syst Biol 1997, **46**, 101-111.
12. **Felsenstein J.** Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985, **39**, 783-791.

13. **Han SH.** Survey and Resource Management of Wildlife. p.14, National Institute of Biological Resources, Seoul, 2011.
14. **Inoue R, Tsukahara T, Sunaba C, Itoh M, Ushida K.** Simple and rapid detection of the porcine reproductive and respiratory syndrome virus from pig whole blood using filter paper. J Virol Methods 2007, **141**, 102-106.
15. **Kim SH, Roh IS, Choi EJ, Lee C, Lee CH, Lee KH, Lee KK, Song YK, Lee OS, Park CK.** A molecular analysis of European porcine reproductive and respiratory syndrome virus isolated in South Korea. Vet Microbiol 2010, **143**, 394-400.
16. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 1980, **16**, 111-120.
17. **Kweon CH, Kwon BJ, Lee HJ, Cho JJ, Hwang EK, Shin JH, Yoon YD, Kang YB, An SH, Kim YH, Huh W, Jun MH, Wensvoort G.** Isolation of porcine reproductive and respiratory syndrome virus (PRRSV) in Korea. Korean J Vet Res 1994, **34**, 77-83.
18. **Lee C, Kim H, Kang B, Yeom M, Han S, Moon H, Park S, Kim H, Song D, Park B.** Prevalence and phylogenetic analysis of the isolated type I porcine reproductive and respiratory syndrome virus from 2007 to 2008 in Korea. Virus Genes 2010, **40**, 225-230.
19. **Leifer I, Hoffmann B, Höper D, Rasmussen TB, Blome S, Strebelow G, Höreth-Böntgen D, Staubach C, Beer M.** Molecular epidemiology of current classical swine fever virus isolates of wild boar in Germany. J Gen Virol 2010, **91**(Pt 11), 2687-2697.
20. **Mardassi H, Wilson L, Mounir S, Dea S.** Detection of porcine reproductive and respiratory syndrome virus and efficient differentiation between Canadian and European strains by reverse transcription and PCR amplification. J Clin Microbiol 1994, **32**, 2197-2203.
21. **Mateu E, Diaz I.** The challenge of PRRS immunology. Vet J 2008, **177**, 345-351.
22. **Meng XJ, Paul PS, Halbur PG, Lum MA.** Phylogenetic analyses of the putative M (ORF 6) and N (ORF 7) genes of porcine reproductive and respiratory syndrome virus (PRRSV): implication for the existence of two genotypes of PRRSV in the U.S.A. and Europe. Arch Virol 1995, **140**, 745-755.
23. **Nielsen J, Bøtner A, Bille-Hansen V, Oleksiewicz MB, Storgaard T.** Experimental inoculation of late term pregnant sows with a field isolate of porcine reproductive and respiratory syndrome vaccine-derived virus. Vet Microbiol 2002, **84**, 1-13.
24. **Reiner G, Fresen C, Bronnert S, Willems H.** Porcine reproductive and respiratory syndrome virus (PRRSV) infection in wild boars. Vet Microbiol 2009, **136**, 250-258.
25. **Ruiz-Fons F, Segalés J, Gortázar C.** A review of viral diseases of the European wild boar: effects of population dynamics and reservoir rôle. Vet J 2008, **176**, 158-169.
26. **Seo SW, Sunwoo SY, Hyun BH, Lyoo YS.** Detection of antibodies against classical swine fever virus in fecal samples from wild boar. Vet Microbiol 2012, **161**, 218-221.
27. **Stadejek T, Oleksiewicz MB, Scherbakov AV, Timina AM, Krabbe JS, Chabros K, Potapchuk D.** Definition of subtypes in the European genotype of porcine reproductive and respiratory syndrome virus: nucleocapsid characteristics and geographical distribution in Europe. Arch Virol 2008, **153**, 1479-1488.
28. **Storgaard T, Oleksiewicz M, Bøtner A.** Examination of the selective pressures on a live PRRS vaccine virus. Arch Virol 1999, **144**, 2389-2401.
29. **Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S.** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 2011, **28**, 2731-2739.
30. **Vengust G, Grom J, Bidovec A, Kramer M.** Monitoring of classical swine fever in wild boar (*Sus scrofa*) in Slovenia. J Vet Med B Infect Dis Vet Public Health 2006, **53**, 247-249.
31. **Vengust G, Valencak Z, Bidovec A.** A serological survey of selected pathogens in wild boar in Slovenia. J Vet Med B Infect Dis Vet Public Health 2006, **53**, 24-27.
32. **Vicente J, Ruiz-Fons F, Vidal D, Höfle U, Acevedo P, Villanúa D, Fernández-de-Mera IG, Martín MP, Gortázar C.** Serosurvey of Aujeszky's disease virus infection in European wild boar in Spain. Vet Rec 2005, **156**, 408-412.
33. **Won CM, Yoo BH, Yang BG, Kim WM, Moon JS, Oh H, Lee H.** Wildlife Survey. p.18, National Institute of Environmental Research, Incheon, 2005.
34. **Wu WH, Fang Y, Farwell R, Steffen-Bien M, Rowland RRR, Christopher-Hennings J, Nelson EA.** A 10-kDa structural protein of porcine reproductive and respiratory syndrome virus encoded by ORF2b. Virology 2001, **287**, 183-191.
35. **Yoon SH, Song JY, Lee CH, Choi EJ, Cho IS, Kim B.** Genetic characterization of the Korean porcine reproductive and respiratory syndrome viruses based on the nucleocapsid protein gene (ORF7) sequences. Arch Virol 2008, **153**, 627-635.
36. **Zhang W, Sun Z.** Random local neighbor joining: a new method for reconstructing phylogenetic trees. Mol Phylogenet Evol 2008, **47**, 117-128.
37. **Zimmerman JJ, Benfield DA, Murtaugh MP, Osorio F, Stevenson GW, Torremorell M.** Porcine reproductive and respiratory syndrome virus (porcine arterivirus). In: Straw BE, Zimmerman JJ, D'Allaire S, Taylor DJ (eds.). Diseases of Swine. 9th ed. pp. 387-417, Blackwell Publishing, Ames, 2006.
38. **Župančić Ž, Jukić B, Lojkić M, Čač Ž, Jemeršić L, Starešina V.** Prevalence of antibodies to classical swine fever, Aujeszky's disease, porcine reproductive and respiratory syndrome, and bovine viral diarrhoea viruses in wild boars in Croatia. J Vet Med B Infect Dis Vet Public Health 2002, **49**, 253-256.