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Genetic characterization of porcine circovirus type 2 in the Korean wild boar population

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ABSTRACT

The porcine circovirus type 2 (PCV2) has reached very high levels in the pig population in South Korea and throughout the world since it was first described in the late 1990s. In this study, we found that the prevalence of PCV2 in the Korean wild boar population was 4.98% (91/1825). Interestingly, 19 PCV2 ORF2 sequences that could be completely sequenced showed that they belonged only to genotype PCV2b, subgroup 1A/B ($n = 16$) and 1 C ($n = 3$). We suggest that sites potentially under positive selection are responsible for the antigenicity changes and phenotypic switch patterns in the capsid gene of 55 PCV2s from Korean domestic pigs, but the sites potentially under positive selection in the 19 PCV2 ORF2 genes from Korean wild boar are not responsible for antigenicity.

1. Introduction

Porcine circovirus type 2 (PCV2) is associated with various pig disease manifestations including post-weaning multisystemic wasting syndrome (PMWS) and porcine dermatitis and nephropathy syndrome (PDNS) (Opriessnig et al., 2007). Porcine circovirus (PCV), including PCV type 1 (PCV1) and PCV type 2 (PCV2), is a circular single-strand DNA virus of the family *Circoviridae* (Tischer et al., 1982). A unified nomenclature for PCV2 genotypes (PCV2a, PCV2b, and PCV2c) has been proposed (Segales et al., 2008). PCV2a is subdivided into five clusters (2A-2E), whereas PCV2b is subdivided into three clusters (1A–1C) (Olvera et al., 2007). PCV2c was identified in pigs from Denmark (Dupont et al., 2008). Recent epidemiological studies have strongly suggested an association between the genotype shift from PCV2a to PCV2b with the occurrence of PMWS. A genotype shift was reported in Switzerland and Denmark in 2003 (Dupont et al., 2008; Wiederkehr et al., 2009), Canada in 2005 (Ellis et al., 2006), and the USA in 2005 (Cheung et al., 2007). In South Korea, the genotype shift from PCV2a to PCV2b presumably occurred in 2002 or even earlier (Kim et al., 2011a,b). To date, extensive studies of PCV2 circulation and genetic diversity in both domestic and wild boar populations (Knell et al., 2005; Grierson et al., 2004; Wen et al., 2005; Sofia et al., 2008; Csagola et al., 2006) have shown a high degree of heterogeneity among isolates within a specific geographic region. Recently, PCV2 isolated from the Romanian wild boar population was found to belong to the previously described PCV2a and PCV2b genotypes and to possess a high degree of sequence heterogeneity (Turcitu et al., 2011). Furthermore, an antigen shift from PCV2a to PCV2b was found to have occurred in the Romanian wild boar. This study investigates the antigen prevalence and genetic diversity of PCV2. Also, the 19 PCV2 ORF2 genes that could be completely sequenced from Korean wild boar were analyzed by comparing them with 118 PCV2 ORF2 genes in worldwide boar populations, including PCV2 from wild boar, and the sites that were potentially under positive selection were investigated.

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2. Material and methods

2.1. Wild boar sample collection

All wild boar samples were collected between 2010 and 2012 during the classical swine fever virus (CSFV) surveillance campaign. The capture of wild boars in South Korea is permitted mainly during the winter season, usually between November and February. The total number of wild boars hunted during the three year surveillance period was 1,825 heads from nine provinces: Gyeonggi ($n = 354$), Gangwon ($n = 397$), Chungnam ($n = 144$), Chungbuk ($n = 176$), Jenbuk ($n = 62$), Jennam ($n = 152$), Gyungnam ($n = 288$), and Gyungbuk ($n = 252$). The collected samples were meat, blood, and feces of wild boars with ages of 1–10 years.

2.2. Isolation of viral DNA and detection of PCV2 ORF2

Total viral DNA was extracted directly from blood samples using a DNeasy tissue kit (Qiagen Inc., Valecia, CA, USA) according to manufacturer's instructions. PCR for PCV2 genomic DNA was performed as described previously (An et al., 2007). Out of the 1825 samples, 91 tested positive for PCV2, and 19 of these were completely sequenced for ORF2 genes. Products of the expected size were cloned by using the pGEM-T Vector System II™ (Promega, Cat. No. A3610, USA). The cloned genes were sequenced with T7 and SP6 sequencing primers using the ABI Prism® 3730xl DNA Sequencer at the Macrogen Institute (Macrogen Co., Ltd.). The sequences of 19 PCV2 ORF2 were submitted to the GenBank under accession numbers KC620502-KC620520.

2.3. Phylogenetic analysis

All porcine circovirus sequences were aligned initially by using the CLUSTAL X alignment program (Thompson et al., 1997). The nucleotide sequences were translated and nucleotide and amino acid sequence identities among the porcine circovirus strains were calculated by using BIOEDIT 7.053 (Hall, 1999). The transition/transversion (T_s/T_v) rate was estimated from the data set using TreePuzzle (Version 5.2) (Strimmer and von Haeseler, 1997) and found to be 0.73. The bootstrap values were calculated with the modules SEQBOOT, DNADIST, NEIGHBOUR and CONSENSE of PHYLIP (Ver. 3.59 package) (Felsenstein, 1995). The phylogenetic tree was calculated using the Neighbor-joining method and was computed by using the DNADIST and NEIGHBOR modules with the same parameters. Another calculation method employing the Neighbor-joining method of the MEGA 4.1 program (Tamura et al., 2007) was also used. Trees were visualized using the TREEVIEW (ver. 1.6.6) program (Page, 1996).

2.4. Positive Darwinian selection analysis

A total of 74 ORF2 sequences isolated from Korean wild boars and domestic pigs from 1999 to 2010 were examined for evidence of positive selection. Several models available in the CODEML module of the PAML 4.3 software package (Yang, 2007) were used to detect positive selection acting on a particular lineage as well as on sites within the complete ORF2 gene. Different values for the non-synonymous/synonymous dN/dS rate ratio (ω parameter) were calculated according to the user guide manual (Yang, 2007). The models used to detect sites under positive pressure were contrasted with models used to detect neutral selection (Anisimova and Yang, 2007). The Bayes Empirical Bayes (BEB) calculation of posterior probabilities for site classes was used to calculate the probabilities of sites under positive selection (Yang et al., 2005).

3. Results

3.1. Monthly PCV2 antigen prevalence by region

Antigen prevalence analysis of 1,825 hunted wild boars revealed that 91 samples were positive for PCV2 by PCR sequencing. Detection of positive antigens was done at

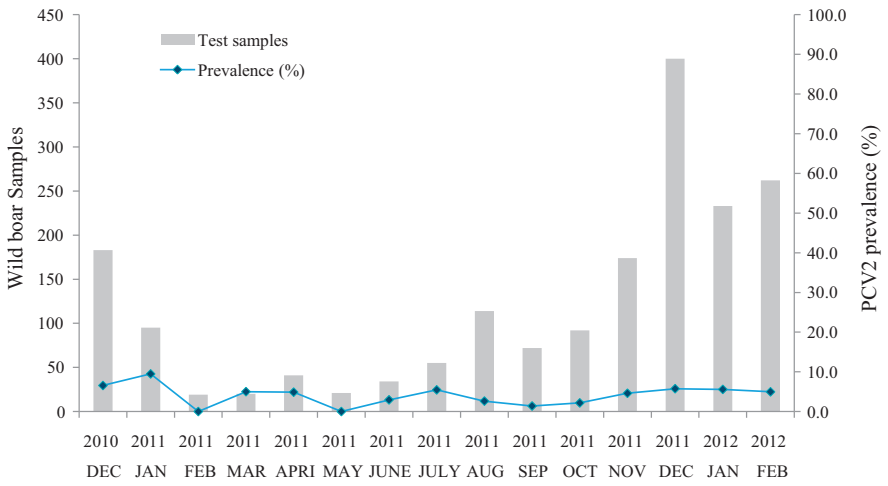

Fig. 1. Monthly prevalence rates of PCV2 in Korean wild boars.
The grey bar indicates the number of samples analyzed and the blue diamond represents the antigen prevalence rates for PCV2 by month.