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IDENTIFICATION OF AN ANTIGENETICALLY DIFFERENT PORCINE PARVOVIRUS (PPV) ISOLATE IN DENMARK

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Introduction
Porcine parvovirus (PPV) is a member of the family Paroviridae. PPV is widespread in swine herds and causes reproductive failure, characterized by embryonic and fetal death, mummification, stillbirths and delayed return to estrus. PPV is recognized as an economically important cause of reproductive failure and vaccines to this virus are marketed worldwide to prevent such condition.

PPV contains a single-stranded DNA genome of about 5 kb. The structural proteins VP1, VP2 and VP3 are encoded by ORF2. VP1 and VP2 are a result of alternative spliced RNAs, giving VP1 a specific N terminus of 150aa.

Recent phylogenetic analyses of German PPV sequences revealed the existence of two PPV clusters (1). In this study we have analyzed Danish PPV isolates and genetic drift similar to that found for the German isolates was found. The results support the presence of two genetic clusters for PPV.

Materials and methods
The Danish PPV isolates came from reproductive failure cases submitted to the National Veterinary Institute, Denmark for diagnostic analysis. The 5 samples (1 from 2006, 1 from 2007, and 3 from 2009) were lung and liver tissue from aborted fetuses. DNA was purified by use of QIAamp DNA Mini kit. The Danish PPV isolates were sequenced as described in Shangjin et al. (2). Phylogenetic relationships were performed with the CLC DNA Workbench software using Maximum Likelihood Phylogeny (starting tree: UPGMA, substitution model: Jukes Cantor, rate variation included). Additional representative PPV sequences were retrieved from GenBank.

Results
The sequence analyses were performed on the first 1998 nucleotides of the VP1/VP2 gene. Sequencing of the last 264 nucleotides of VP2 gene for Danish PPV isolates is in progress. Nucleotide similarities among the Danish and representative PPV sequences were 98.2-99.8% and similarities among the Danish isolates were 98.3-99.4%. When analyzing amino acid sequences the similarities among all the isolates were 96.7-100% and 96.7-99.1% between the Danish isolates.

The phylogenetic analysis of VP1/VP2 PPV sequences showed two main groups or clades. One group contained the German sequences Tornau and IDT together with 4 of the Danish PP sequences. The second main group could be separated into two branches. One of these branches contained European, American and Asian PPV sequences. The second branch of this second clade contained the German PPV-27a and one Danish PPV sequence.

The Danish PPV-2074 that group within the new German cluster show similarities of 99.8% and 100% for nucleotide and amino acid, respectively, to the PPV-27a strain. In addition, the Danish PPV-2074 contained the three amino acid substitutions (Q378E, E569Q, and S/P586T) which define this new distinct PPV cluster and all of these amino acids are exposed on the virus surface (1).

Discussion
The Danish PPV-2074 was isolated from a herd in which sows were vaccinated against PPV (Porcilis PPV, Intervet). Interestingly, based on sequence data the PPV-2074 isolate is antigenic identical to the PPV-27a strain. Previously, cross-neutralization studies of sera raised against strains included in commercial vaccines (NADL-2 and IDT) have showed low neutralization activity against PPV-27a strain, indicating incomplete protection (3). Furthermore, no protection against PPV-27a infection was found in a vaccine study using Porciparvac (IDT Biologika GmbH) (4). Unfortunately, the DNA sequence of the strain included in the Porcilis PPV vaccine is not available, however, the presence of antigenic diverse PPV strains in Danish pigs raises concern on the future protective effect of existing commercial PPV vaccines.

References