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Diagnostic phylogenetics reveals a new Porcine circovirus 2 cluster

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ABSTRACT

Porcine circovirus 2 (PCV2) was prevalent in swine in the United States before PCV2-associated disease (PCVAD) appeared in 2006. Limited nucleotide sequencing of open reading frame 2 (ORF2) encoding capsid, the only structural protein, revealed the presence of two genotypes, PCV2a and PCV2b. Later, PCV2c and mutant PCV2b, or PCV2d, were also described. However, extensive PCV2 ORF2 sequence databases in veterinary diagnostic laboratories have not been analyzed systematically to determine the genetic diversity of field isolates. Here, we interrogated >1100 PCV2 ORF2 nucleotide sequences to assess population diversity and genetic variation. We detected a novel PCV2 genotype that is substantially different, primarily in ORF2, from all known PCV2. Notably, ORF2 contains a unique carboxyl terminal amino acid insertion resulting in a 238 amino acid ORF2. All other PCV2 ORF2 proteins are 233 or 234 aa in length. Phylogenetic analysis indicates that it is more ancient than other PCV2 genotypes. The findings demonstrate the value of analyzing routine diagnostic laboratory sequence databases in population genetic analyses of animal pathogens.

1. Introduction

Porcine circovirus 2 (PCV2) is a small, circular, single-stranded DNA virus of swine. PCV2 was discovered in association with outbreaks of a postweaning, multisystemic wasting syndrome (PMWS) in the 1990's in Europe and Canada (Allan et al., 1998; Ellis et al., 1998). Retrospective analysis of archived diagnostic specimens showed it was present in multiple countries in Europe, Asia and North America in previous decades (Dupont et al., 2008; Patterson and Opriessnig, 2010). A national survey of the U.S. swine herd prior to disease outbreaks showed that PCV2 infection was present in nearly all herds at a prevalence greater than 80% of pigs before diseases associated with PCV2 were observed (Haley et al., 2011; Puvanendiran et al., 2011).

Diagnostic sequencing of PCV2 open reading frame 2 (ORF2), encoding the viral capsid protein, was widely adopted in North America and other swine growing regions to assist in understanding the etiology of diseases, including PMWS, porcine dermatitis and nephropathy syndrome, porcine respiratory disease complex, and reproductive diseases, associated with the presence of PCV2 (Segales, 2012; Segales et al., 2005). In the following years, extensive phylogenetic analysis showed that PCV2 consisted of several genetic clusters, without a clear consensus relating genotypic variation to biological characteristics or to disease causation (Chae, 2015; Franzo et al., 2015; Wang et al., 2009; Xiao et al., 2015).

Numerous studies have described PCV2 field isolates and their phylogenetic relationships in swine growing regions. However, a systematic analysis of unpublished ORF2 sequences collected in veterinary diagnostic laboratories has not been conducted. We examined a repository of >1100 PCV2 ORF2 sequences dating back to 2001 in the Minnesota, USA, Veterinary Diagnostic Laboratory (MVDL). A cluster of novel sequences was identified that, by phylogenetic analysis, is ancestral to all known PCV2. Whole genome sequencing confirmed that these viruses are unique and form a new genetic group which we refer to as PCV2e.

2. Materials and methods

The MVDL PCV2 ORF2 database in August 2015 contained 1289 sequences generated by Sanger sequencing and linked by case number to date of isolation, geographic location, and descriptive reports. The files were merged and organized by geographic region and year of isolation then subjected to data quality analysis. Wild-type field origin was determined by accessing MVDL case files for

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0168-1702/

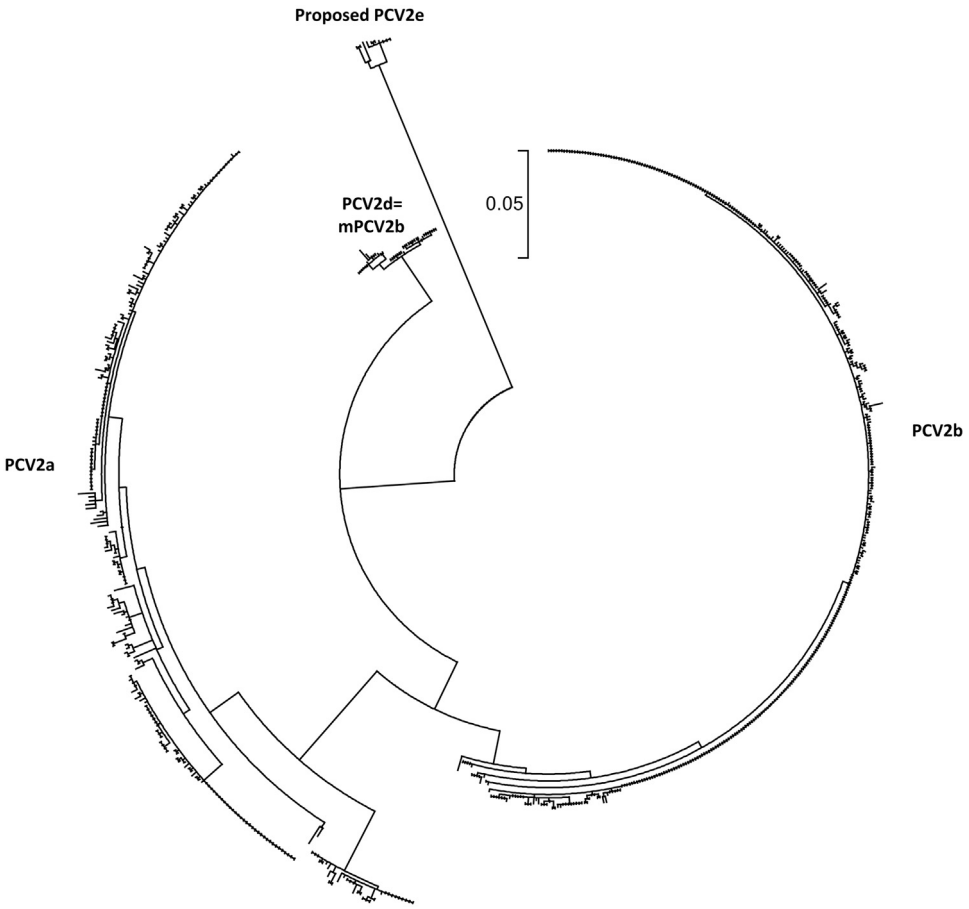

Fig. 1. Maximum likelihood (ML) phylogenetic analysis of 729 PCV2 ORF2 sequences in the Minnesota Veterinary Diagnostic Laboratory database. Substitution pattern and rates were estimated under the Tamura-Nei model (+G) (Tamura and Nei, 1993), and a discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories [+G]). For estimating ML values, a tree topology was automatically computed. Evolutionary analyses were conducted in MEGA6 (Tamura et al., 2013).

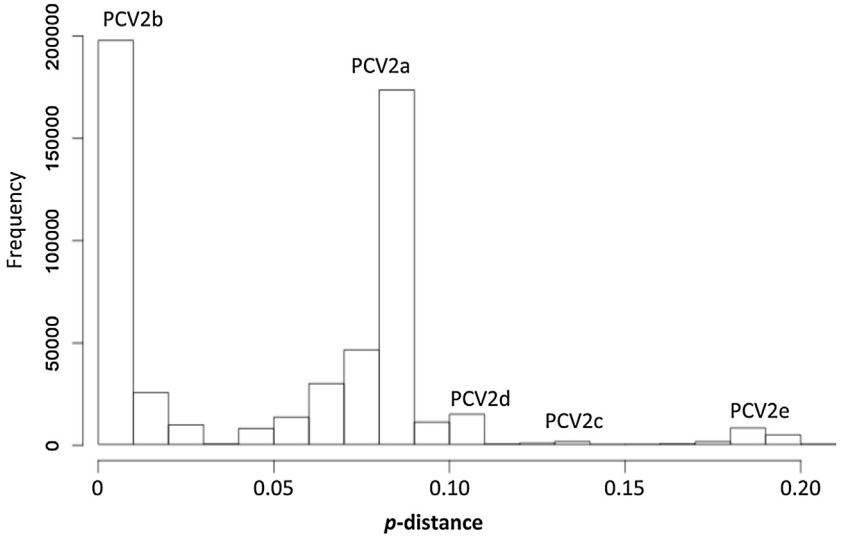

Fig. 2. Pairwise similarity analysis of 729 Minnesota Veterinary Diagnostic Laboratory PCV2 ORF2 sequences. Histogram bins are in 1% increments. Genotypes are shown relative to PCV2b. Analysis was performed in MEGA6 (Tamura et al., 2004, 2013). The total number of comparisons was 531,448.

missing or suspect information and to remove sequences generated in research studies. Sequences that could not be verified as field isolates were discarded. A fasta file was generated with the resulting list of 905 sequences, renamed to show virus name (PCV2), geographic location (country, state or province), a unique identifier, and year of isolation.

The fasta file was imported into Geneious v6 (Biomatters Limited, Auckland NZ) and the "Find ORFs" tool was used to search for intact ORFs over 650 nucleotides in length since PCV2 ORF2