Veterinary Microbiology 173 (2014) 9–16

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease

Tanja Opriessnig [a,b,*], Chao-Ting Xiao [b], Priscilla F. Gerber [a], Patrick G. Halbur [b]

[a] The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom
[b] Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, Ames, IA, USA

ARTICLE INFO

Article history:
Received 10 April 2014
Received in revised form 18 June 2014
Accepted 25 June 2014

Keywords:
Porcine parvovirus
Prevalence
Porcine circovirus type 2
PCV2
Porcine circovirus associated disease
PCVAD

ABSTRACT

The association of porcine circovirus (PCV) type 2 and porcine parvovirus (PPV) type 1 as a cause of porcine circovirus associated disease (PCVAD) is well established. The objective of this study was to investigate the prevalence rates of classical PPV1 and recently recognized PPV2-5 in serum and lung samples from pigs and farms with known PCV2 status. A total of 586 serum samples and 164 lung homogenates collected from 1996 to 2013 in the USA and Canada were utilized. All samples were tested for PPV1-5 and PCV2. PCV2 was detected in 27.7% (162/586) and PPV in 48.8% (286/586) of the serum samples, whereas 78.7% (129/164) of the lung tissues were positive for PCV2 and 56.7% (93/164) were positive for PPV. Overall, PPV2 had the highest prevalence rates in sera (35.2%) and tissues (42.7%). Concurrent infection of PCV2 and PPV occurred in 14.3% (84/586) of the serum samples and in 49.4% (81/164) of the tissue samples. Moreover, the prevalence of PPV1 or PPV2 DNA was significantly higher in tissues containing high amounts of PCV2 DNA compared to non-PCVAD cases. The frequency of concurrent PPV/PCV2 infection was higher for PCVAD herds compared to negative or subclinically infected herds. PPV2, PPV3 and PPV4 were all identified in samples collected in 1998 and PPV5 was first identified in 2006. The obtained findings indicate that similar to PCV2, PPVs are widespread in North American pigs. Nevertheless, diagnostic investigations into PCVAD cases should give more consideration to the role of PPV1 and PPV2 as contributing cofactors.

1. Introduction

Porcine parvovirus type 1 (PPV1) is a small (about 5 kb in genome size), single-stranded, linear, non-enveloped DNA virus that belongs to the family *Parvoviridae* (Molitor et al., 1984). PPV1 has been well known to pig producers for decades (Dunne et al., 1965; Mengeling and Cutlip, 1976) and is mainly associated with reproductive failure manifest as increased numbers of mummified fetuses or so called "stair-step" litters in breeding age females. To prevent PPV1-associated reproductive failure, most breeding females are routinely vaccinated. PPV1 also circulates in growing pigs but is only infrequently associated with enteric disease or dermatitis in individual pigs (Kresse et al., 1985; Dea et al., 1985; Duhamel et al., 1991) and therefore largely ignored during diagnostic investigations of disease in growing pigs.

In recent years with the wide-availability and usage of PCR-based methods and sequencing, several new members of the family *Parvoviridae* were discovered in pigs

* Corresponding author at: The Roslin Institute and The Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, United Kingdom. Tel.: +44 0131 651 9422.
E-mail address: Tanja.Opriessnig@roslin.ed.ac.uk (T. Opriessnig).

http://dx.doi.org/10.1016/j.vetmic.2014.06.024
0378-1135/

including PPV2 (Hijikata et al., 2001), PPV3 (Lau et al., 2008), PPV4 (Cheung et al., 2010) and most recently PPV5 (Xiao et al., 2013b,c). Little is known about the relevance or importance of these viruses and no information exists on pathogenic potential of any of the novel PPVs (Xiao et al., 2013d).

Porcine circovirus (PCV) type 2 is another small single-stranded DNA virus but, unlike PPV1, is circularly arranged (Tischer et al., 1974, 1982). PCV2 is associated with many disease manifestations in pigs of all ages collectively summarized as PCV associated disease (PCVAD) (Opriessnig et al., 2007). In 1999, due to unintended contamination of PCV2 inoculum with PPV1, concurrent PCV2–PPV1 infection resulted in reproduction of characteristic PCV2 lesions in lymphoid tissues including severe lymphoid depletion and granulomatous lymphadenitis in experimentally infected pigs (Ellis et al., 1999). Subsequent studies confirmed the initial results and demonstrated enhanced PCVAD and PCV2-associated lesions in pigs coinfected with PPV1 and PCV2 compared to singularly PCV2 infected pigs (Allan et al., 1999; Hasslung et al., 2005; Opriessnig et al., 2004; Ostanello et al., 2005). A field investigation examining the prevalence of PPV1 and PCV2 in Canadian pigs demonstrated concurrent PPV1–PCV2 infection in 17.4% of pigs (12/69) with PCVAD (Ellis et al., 2000).

The two main PCV2 genotypes are PCV2a and PCV2b. During 2012, a mutant PCV2 strain (PCV2d) was identified in the U.S. pig population which can be differentiated from classical PCV2a and PCV2b strains (Xiao et al., 2012b). A high prevalence of PPV2 was identified in cases where PCV2d was detected (Opriessnig et al., 2013) raising concerns that PPV2, similar to PPV1, may be able to enhance PCV2 replication to levels that may interfere with successful vaccine protection. This was further supported by findings in China were PPV2 was also identified in cases of PCVAD (Wang et al., 2010).

The objective of this study was to investigate the prevalence rates of the different PCV2 sub-genotypes together with classical PPV1 and emerging PPV2-5 in archived and recent serum and lung tissue samples collected in the USA and Canada from 1996 to 2013.

2. Materials and methods

2.1. Ethics statement

All samples utilized were arbitrarily selected and originated from pig case submissions to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) for diagnostic work-up. The sample collection and submission was unrelated to and not part of this study. The protocol for this study was approved by the Iowa State University Institutional Biosafety Committee.

2.2. Samples

2.2.1. Serum samples

A total of 586 serum samples collected from 2006 to 2013 were utilized (Table 1). A total of 13 farms were sampled and the sample size ranged from 2 to 80 samples with a mean sample size of 30 samples (25% quartile 21, 75% quartile 60). The pigs included nursery pigs between 3 and 9 weeks of age ($n = 242$), grow-finish pigs from 9 to 25 weeks of age ($n = 338$) and adult pigs older than 25 weeks of age ($n = 6$).

2.2.2. Lung homogenates

A total of 164 lung homogenates collected from 1996 to 2013 were included (Table 1). The tissue samples were obtained from Canada ($n = 3$) and U.S. including Iowa ($n = 126$), Illinois ($n = 2$), Maryland ($n = 1$), Minnesota ($n = 2$), Missouri ($n = 7$), Nebraska ($n = 1$), New Hampshire ($n = 1$), North Carolina ($n = 14$), North Dakota ($n = 1$), Texas ($n = 3$) and Wisconsin ($n = 3$). The age groups of the pigs that were sampled ranged from nursery pigs ($n = 44$), grow-finish pigs ($n = 116$) to adult pigs ($n = 4$). The number of individual pigs sampled from a farm ranged from one to three.

2.3. DNA extraction

Lung samples of approximately 1 g were minced and diluted 1:10 in Hank's balanced salt solution (HBSS), homogenized by using a Stomacher® 80 (Seward Laboratory Systems, Inc., Bohemia, NY, USA), and centrifuged at $1500 \times g$ for 10 min to obtain supernatant. Total nucleic acids were extracted from serum samples or lung homogenates using the MagMax™ Pathogen RNA/DNA Kit (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) and an automated DNA/RNA extraction system (Thermo Scientific Kingfisher® Flex, Thermo Fisher Scientific, Pittsburgh, PA, USA) according to the instructions of the manufacturer.

2.4. Detection and quantification of viral nucleic acids

For PPVs, a PCR panel composed of a duplex real-time PCR for PPV1 and PPV2 and a triplex real-time PCR for

Table 1
Information on collection year of the samples utilized in this study.

Collection year	Serum samples (number of farms)	Tissues
1996	–	2
1997	–	–
1998	–	7
1999	–	2
2000	–	–
2001	–	80
2002	–	2
2003	–	–
2004	–	1
2005	–	6
2006	118 (2)	3
2007	–	–
2008	12 (1)	6
2009	–	16
2010	–	7
2011	–	19
2012	427 (9)	3
2013	29 (1)	10
Total	586 (13)	164