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Genetic diversity of porcine circovirus type 2 and implications for detection and control

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ABSTRACT

Porcine circovirus type 2 (PCV2) is the etiological agent of post- weaning multi-systemic wasting syndrome (PMWS), an economically important disease of swine. Severe wasting and lymphadenopathy are typical signs of PMWS. Effective vaccines against PCV2 and reliable diagnostic tests are available. Since PCV2's discovery in the mid-90s and the introduction of commercial vaccines, several new recombinant strains and variants with genetic mutations have emerged. Two noteworthy changes include; a major type switching event that resulted in the previously predominant PCV2a genotype being replaced by PCV2b, and the recent emergence of a mutant PCV2b with a capsid protein containing an additional lysine. The mutant PCV2b exhibits increased virulence and is spreading rapidly in various regions of the world. This article provides an overview of the recent molecular epidemiology in the context of the current methods for the detection and prevention of PCV2, emphasizing the need for updated PCV2 vaccines.

Contents

1. Introduction

Porcine circovirus type 1 (PCV1), which is non-pathogenic, was discovered as a contaminant of porcine kidney cell lines (Tischer et al., 1974). Porcine circovirus type 2 (PCV2) was identified in the mid-1990s, as the primary etiological agent of post weaning multi-systemic wasting syndrome (PMWS) in weaning piglets (Harding, 2004). PCV2 causes anemia, weight loss, diarrhea, jaundice and lymph node enlargement in 10–15 week old piglets, resulting in low productivity and economic devastation (Ramamoorthy and Meng, 2009; Segales et al., 2013). Although PMWS was the first clinical manifestation associated with PCV2, several other syndromes such as porcine dermatitis and nephropathy syndrome (PDNS), PCV2-associated pneumonia, reproductive failure, and enteritis were subsequently recognized as sequelae of PCV2 infections and reviewed extensively elsewhere (Opriessnig and Langohr, 2013; Segales, 2012). Currently, the term porcine circovirus associated diseases (PCVAD) collectively represents the many clinical manifestations of PCV2 infections.

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The genomes of the non-pathogenic PCV1 and the pathogenic PCV2 are similarly organized. The complete PCV2 genome is about 1.7 Kb in length with two main open reading frames (ORFs); ORF1 and ORF2. They are oriented in opposite directions, make up about 93% of the PCV2 genome and encode the replicase and capsid proteins, respectively. The replicase gene, encoding the Rep and Rep′ proteins, is highly conserved in PCV2 isolates, and also between PCV1 and PCV2 (Mankertz et al., 1998). The capsid protein is encoded by ORF2. It is the most important target for vaccine and diagnostic test development due to its immunogenicity, and as it exhibits the greatest sequence variation between genotypes. Two other genes, the ORF3 and 4 play a role in PCV2 induced apoptosis and suppression of caspase activity, respectively (He et al., 2012; Liu et al., 2005). However, the exact genetic basis of PCV2 virulence is not clearly defined as yet.

Several commercial vaccines against PCV2 are available and were introduced in the U.S. market in 2006. All current commercial vaccines target the PCV2a genotype. PCV2 vaccination is considered a success-story in veterinary vaccinology, as PCV2 vaccines effectively prevent clinical signs and economic losses due to PCVAD. Between 2004 and 2008, a major type switching event resulted in PCV2b replacing PCV2a as the predominantly circulating strain in the U.S. and other parts of the world. In addition, several recent reports document the emergence of new viral variants which are recombinants composed of genomic segments derived from both the major genotypes, PCV2a and PCV2b (Li et al., 2012; Opriessnig et al., 2013b; Ramos et al., 2013), as well as those generated by mutation.

Laboratory diagnosis of PCV2 infections is of specific importance in designing intervention strategies. Current laboratory tests support the reliable diagnosis of PCV2 for a majority of the newly evolved strains. However, monitoring for the emergence of new strains is important and may necessitate the development of new tests. Similarly, current vaccines induce cross-protection against the newly evolved strains at the level of preventing clinical signs. However, they appear to induce selection pressure and promote genetic diversity. Therefore, updating vaccines to include contemporary strains is warranted and will likely increase the threshold of immunity to sterilizing or near-sterilizing immunity to reduce the rate of PCV2 evolution. This article discusses the implications of genetic variation on the diagnosis and prevention of PCV2 while summarizing the current findings in this area.

1.1. Coinfections and viral evolution

Nearly 98% of swine herds in the U.S. are PCV2 positive. Similar rates of prevalence have been reported on every continent in the world (Shen et al., 2012). A major reason for the high rates of prevalence is that PCV2 infections are persistent. Transmission of PCV2 occurs both through direct contact by susceptible animals with contaminated milk, oral, fecal and nasal discharges (Shibata et al., 2003) as well as artificial insemination with semen from infected boars and vertical transmission of the virus (Madson et al., 2009; Madson et al., 2008). In a recent study, Cortey et al. (2011)determined that 100% of the tissues selected from cases showing clinical signs of PCVAD were infected by both PCV2a and b genotypes, even at the cellular level. High rates of coinfection with multiple PCV2 strains were also recorded in herds in China (Zhai et al., 2011). While both studies were designed to explore the correlation between co-infection and disease severity, the finding that high rates of PCV2a/b coinfections are common has important implications for viral evolution, as they facilitate genetic recombinations [Table 1]. Information about the current rates of coinfection of PCV1 and 2 is an unavailable but important piece in the puzzle of predicting PCV2's evolution. Antigenic variation which can occur as a consequence of genetic changes, has negative implications for both the prevention and detection of PCV2.

The role of co-infecting pathogens, such as the porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae* and swine influenza virus (SIV), in exacerbating PCV2 viral pathogenesis is well established (Ramamoorthy et al., 2009; Ramamoorthy et al., 2011; Rammohan et al., 2012; Sinha et al., 2011; Ticó et al., 2013). For example, PCV2 and PRRSV can be co-isolated in approximately 40% of PCVAD cases (Ramamoorthy et al., 2009) and pigs co-infected with PCV2 and PRRSV mount significantly reduced IgG responses to both pathogens (Park et al., 2014). It is very likely that the natural selection pressure exerted by the altered host immunity in viral coinfections has a significant effect on PCV2's evolution. Indeed, the mutation rate of PRRSV genes is increased during coinfection with PCV2 when compared to singular infections with PRRSV alone (Yin et al., 2013). Similar data for PCV2 is as yet unavailable; nor have detailed studies been conducted on the viral genetic elements which modulate host protective immunity in singular or coinfections with PCV2a/b or with other agents. The availability of such data is critical for evaluating and predicting how genetic changes in PCV2 can influence diagnostic, vaccine-related or disease outcomes. Therefore, the influence of coinfections in driving PCV2 evolution is an important but under-studied area.

1.2. Recombination and PCV2 evolution

While PCV2a and PCV2b show a high level of nucleotide identity with approximately 97–100% of the rep gene and 91–96% of the capsid gene being similar (Larochelle et al., 2002), they are believed to have evolved independently from a common ancestor about 100 years ago (Firth et al., 2009). Three major genotypes of PCV2, each containing several subtypes, have been described to date (Cortey et al., 2011; Olvera et al., 2007). PCV2a and PCV2b are clearly identified as cause of PWMS, while the third genotype (PCV2c) has only been reported in Denmark among healthy swine (Dupont et al., 2008). Attempts to determine whether there is a difference in the virulence properties of these subtypes have not provided clear cut information (de Boisseson et al., 2004; Larochelle et al., 2002; Reiner and Willems, 2008).

Both mutation and genetic recombination are common and important mechanisms in the evolution of PCV2. The ORF1 gene is highly conserved in PCV2 isolates, and also between PCV1 and PCV2 (Mankertz et al., 1998). Initially, the PCV2 ORF1 gene was identified as harboring the most likely sites for recombination at the first exon of the rep' transcript (Olvera et al., 2007) and the PCV2a cluster 1B was one of the earliest identified recombinants between PCV2a and b. Due to its highly conserved nature, exchange of segments within the ORF1 gene is unlikely to alter the phenotypic or virulence properties of the recombinants. Indeed, studies describing recombinations within the ORF1 or origin of replication do not call attention to alteration in virulence properties (Ma et al., 2007). Recombinations within the ORF2 gene are more likely to influence PCV2's phenotypic and antigenic diversity as the capsid protein is the major immunogenic protein and the primary component of all PCV2 vaccines. Both inter and intra genotype recombinations within the ORF2 with break points in the antigenic epitope regions have been reported (Fraile et al., 2012; Cheung et al., 2007; Saha et al., 2012a; Guo et al., 2011) [Table 2]. However, possible differences in antigenicity or virulence for these isolates were either not observed or characterized. Recently, several truncated PCV2 genomes of 600–800 bp size, sometimes including extraneous coding or non-coding nucleotides were discovered. The significance of these truncated PCV2 variants is as yet unknown (Stephenson et al., 2015; Luo et al., 2013) [Table 1] but require further exploration to complete our understanding of PCV2 replication and evolution.

Similar to PRRSV (Franzo et al., 2014), while recombination appears to be an important mechanism by which genetic diversity is generated, as described below, a combination of recombination and mutation appears to be most likely to result in altered fitness or phenotypic properties for PCV2.

1.3. Mutation and PCV2 evolution

When compared to DNA viruses, genetic instability is more common in RNA viruses due to the poor proof reading abilities of RNA