PCV-2: Genetics and Immunity

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Porcine circovirus 2 (PCV-2) is a member of the family Circoviridae, genus Circovirus, featured by a remarkable evolutionary capability and genetic heterogenicity. Although an overall cross-protection among strains seems to occur, some epidemiological evidence suggests that some differences might be in place among strains, with potential consequences on vaccine efficacy also.

Keywords: PCV-2 ; Genetics ; Immunity ; Vaccines

1. Introduction

PCV-2 was initially identified from pigs suffering from postweaning multisystemic wasting syndrome (PMWS), a novel disease described in the mid-1990s [1]. PMWS is nowadays known as PCV-2-systemic disease (PCV-2-SD), and it comprises also what was initially described as PCV2-associated pneumonia and PCV-2-associated enteritis [2]. Other clinical-pathological conditions such as porcine dermatitis and nephropathy syndrome (PDNS), PCV-2-reproductive disease (PCV-2-RD) and PCV-2-subclinical infection (PCV-2-SI) have also been included into the scope of the collectively named porcine circovirus diseases (PCVD) [2]. Interestingly, the definitive causative role of PCV-2 was debated at length because of the difficulty to experimentally reproduce PCV-2-SD by viral inoculation only. Additionally, retrospective studies proved PCV-2 presence well before the emergence of PCV-2-SD [3]. Indeed, PCV-2-SD is a typical example of a multifactorial disease, where other predisposing factors, most of those featuring modern intensive farming, must be in place to elicit overt clinical signs [4]. While some farms were able to live with the infection implementing adequate management and biosecurity, the most effective control measure was represented by the development of commercial vaccines, which became available from 2004 and 2006 onwards in Europe and North-America, respectively. These products led to a remarkable decrease of economic losses attributed to PCVD including PCV-2-SI [5]. Actually, vaccine efficacy represents one of the most consistent proofs in favour of the aetiological role of PCV-2 in PCVDs. PCV-2 vaccines are the single most-sold preventive product in porcine husbandry worldwide; nowadays, the vast majority of pigs and/or sows are vaccinated against PCV-2.

Nevertheless, in the last decade, a crescent concern has risen on the protection conferred against recently emerged genetic variants of PCV-2 [6]. The purpose of this work is to summarize and critically review the current knowledge on PCV-2 genetic variability and its relationship with vaccine efficacy, based on in silico, field, and experimental evidences.

2. Genotypes of PCV-2

Similarly to other ssDNA viruses, PCV-2 is featured by a high mutation rate (i.e., 10^{-3}–10^{-4} substitution/site/year) [7][, within the range typical of RNA viruses, which has led to the emergence of a plethora of variants over time. The accumulation of molecular epidemiology studies was mirrored by the implementation of several sub-species level classification schemes and nomenclatures, often based on subjective and/or conflicting criteria. A first effective harmonization attempt was made in 2008, when two major PCV-2 groups were defined based on nucleotide diversity cut-offs for ORF2 (3.5%) and complete genome (2.0%) [8]. These criteria were adopted by a European Project on PCVDs [9] and these two groups were proposed to be named as PCV-2a and PCV-2b. Based on the same criteria, PCV-2c was then identified from archived samples in Denmark [10]. Thereafter, the progressive increase in sequence availability and the discovery of new genetically divergent clades highlighted the limitations of such stringent genetic cut-offs and a new classification was proposed based on reference sequences and/or identification of marker positions, leading to the definition of 4 genotypes [11]. Currently, the most accepted scheme allowed defining eight genotypes (PCV2a to PCV2h), based on three criteria: maximum intra-genotype p-distance of 13% (calculated on the ORF2 gene), bootstrap support at the corresponding internal node higher than 70% and at least 15 available sequences [12]. Using such classification

proposal, a new genotype PCV-2i has also been defined in the USA [13]. Therefore, the PCV-2 genetic scenario cannot be considered a static one, and new updates and changes on viral evolution are expected with a potential impact on genotype classification in the future.

Currently, PCV-2a, PCV-2b, and PCV-2d display a worldwide distribution while the other genotypes have been detected sporadically and limited evidence is present on their temporal persistence [12]. Of note, PCV-2c was considered extinguished or non-detectable for a long time, before being identified again in feral pigs in the Pantanal region of Brazil [14],in domestic pig in China [15] and, more recently, in warthogs in Namibia (*manuscript in preparation*). Similarly, other genotypes could be circulating, still undetected, in unexpected ecological niches (probably other Suidae species) and may serve as source of further genetic variability in the future. Nevertheless, the most important source of variation is expected to be the domestic pig considering the abovementioned mutation rate of PCV-2 and the remarkable host population size.

Different epidemiological and phylodynamic studies revealed the occurrence of different genotype waves over time. PCV-2a was the most prevalent genotype in clinically affected pigs from 1996 to the early 2000s, after which PCV-2b predominated ("genotype shift") and was associated with the appearance of a more severe clinical disease outbreaks [16][17][18]. Thereafter, a second "genotype shift" (from PCV-2b to PCV-2d) occurred globally [7] and has sometimes been reported in cases of vaccination failure [6][19]. However, the detection of other PCV-2 genotypes in vaccinated herds is not an unusual finding and the perception of a higher PCV-2d frequency in such herds could be biased by its rising global prevalence. Simultaneously, the presence of circulating recombinant forms (CRF) displaying comparable population dynamics and spreading routes to those of major genotypes has been demonstrated, suggesting a non-negligible fitness of these variants [7].

Although different PCV-2 genotypes have been historically identified sequentially, retrospective studies and molecular-clock based analyses proved their presence and co-circulation for decades. The actual reason behind the observed epidemiological patterns is not clear. A potential higher virulence of PCV-2b and PCV-2d strains has been suggested based on epidemiological patterns and some in vivo experimental data appear to support this hypothesis [20]. However, some other studies pointed out a similar virulence among genotypes [21]. Therefore, a putative differential virulence among genotypes is still to be demonstrated, although strain-specific differences could occur [22]. Importantly, virulence markers have been not defined for PCV-2 so far.

3. PCV-2 Immunological Cross-Reactivity

The pathogenesis of PCV2-SD depends on the final balance between the virus and the host immune response [23]. Different epitopic regions have been recognized both in the Rep and Cap proteins. The latter in particular is the main target of the host immunity and can elicit antibody and lymphocyte proliferative responses to PCV-2 [24][25]. Several linear or conformational epitopes have been also identified by PEPSCAN analysis, including amino acid residues 65–87, 117–131, 157–183, and 193–207 [26]. In addition, at least three conformational neutralizing epitopes, within residues 47–63, 165–200 and 230–233, have been described using chimeric PCV-1 and PCV-2 constructs [27]. Other linear epitopes (amino acids residues 156–162, 175–192, 195–202, and 231–233) have been recognized using monoclonal antibodies [28]. Different studies done to map immunogenic epitopes in the PCV-2 Cap protein have also demonstrated that several epitopes are shared between PCV-2 genotypes [27][28].

Accordingly, an overall immune cross-protection among PCV-2 genotypes exists and polyclonal antibodies are cross-reactive and cross-neutralizing [29]. Such protection breadth has been proven also under field conditions since sera from naturally infected pigs efficiently neutralized PCV-2 strains belonging to different genotypes and collected from different part of the word. However, a differential quantitative neutralization activity was identified, being the neutralization titre higher, on average, against PCV-2a than PCV-2b, which could justify the progressive spread of the latter genotype [30]. Noteworthy, these results were obtained with non-vaccinated pigs, so the higher antibodies against PCV-2a could not be attributed to a vaccination effect.

A more detailed picture emerged from studies using monoclonal antibodies. Saha et al. (2012a) detected the presence of common epitopes between PCV-2a and PCV-2b genotypes using monoclonal antibodies. However, the existence of genotype-specific antibodies was also demonstrated and some were able to recognize specific clusters within a genotype [31]. Single amino acid mutations were thereafter proven to alter the neutralization capability of some monoclonal antibodies [32][33].

Although less characterized, cell immunity plays a relevant role in protection against PCV-2 and the number of PCV-2 specific INFγ secreting cells (INFγ-SC) is inversely correlated to viral load and lesions [34][35]. Both Cap and Rep proteins are targeted by INFγ-SC, although a significant reactivity against Rep was reported in subjects with high viral titres and

typical lesions, suggesting that high viral replication levels are necessary to elicit a significant response against non-structural proteins; such immunity could be related in preventing the progression towards PCV-2-SD [36]. Also in this case, experimental data showed that the cellular immunity induced by PCV-2a proteins is protective against PCV-2b challenge [36]. However, in silico epitope prediction revealed the presence of several potential cellular epitopes located both on Cap and Rep, some of those slightly differing among circulating genotypes [37].

Therefore, based on the observed evidence, while an overall cross-reactivity can safely be stated, some qualitative differences in the breadth and efficacy of immunity can be expected and involved in the PCV-2 epidemiologic patterns observed over time. The lower protection conferred by the immunity arisen against the prevalent genotypes and/or administered vaccines, based on PCV-2a, could have resulted in a fitness advantage of other genetic groups and, thus, their emergence in the world limelight. However, the generalized use of PCVC-2a vaccines all over the world also coincided with a “genotype shift” from PCV-2b to PCV-2d. Whether these vaccines are more effective on PCV-2b or a fast-evolving virus such as PCV-2 simply produced a novel genotype (PCV-2d) with better biological fitness is currently unknown.

Taken as a whole, current evidence suggests that PCV-2 products are “leaky vaccines”, which can elicit adequate protection against clinical disease and reduce viral replication even when heterologous strains are involved. The presence of different epitopes, including neutralizing ones, is apparently balanced out by the efficient protective activity against shared ones. Nevertheless, viral infection and replication are not prevented [38] and under less optimal conditions (e.g. inaccurate vaccine administration, immunosuppression, declining maternally derived immunity, concomitant infections, etc.) the generated immunity could be less effective. Vaccines have traditionally been considered much more resistant to pathogen evolution than antimicrobials [39]. Nevertheless, when immunity is not sterilizing, wild strains can circulate in a new “challenging” environment, made of less susceptible-immune hosts, adapting to it. Such scenario seems to occur for PCV-2 also. Analysis of selective pressures strength acting on PCV-2a highlighted a higher diversification tendency after vaccination introduction. Similarly, the viral population circulating in unvaccinated wild boar populations appears under lower selective pressures compared to domestic pigs [40].

Interestingly, PCV-2d was first detected retrospectively in Switzerland already in 1998, but an increase in detection frequency of a sub-clade of PCV-2d has been reported in the years following vaccination introduction [8], which is indicative of a putative vaccine-induced replacement of a subset of genetic variants. Accordingly to this hypothesis, a statistically significant tendency of PCV-2a strains to mutate towards amino acids different from those of one commercial vaccine based on an inactivated PCV-2a virus, and identical to the amino acid profile of PCV-2d, was detected in at least 3 Cap sites after vaccination introduction, suggesting the appearance of vaccine-induced immuno-escaping evolutive trajectories [40]. Most interestingly, changes in each of these three amino acids (59-206-210) were experimentally demonstrated to impair the binding of monoclonal antibodies [27][32]. These data propose that the ancient PCV-2d strains had phenotypic features favouring them on a global scale in presence of vaccine immunity.

Although challenging to be consistently proven, the congruent pieces of evidence support the action of genotype-specific vaccine-induced immunity in progressively driving PCV-2 evolution, with a theoretical putative detrimental effect on vaccine efficacy in the long term. If this path would ultimately lead to actual PCV-2a-based vaccine failure or certain loss of overall efficacy, is still a matter of discussion and definitively not yet proven.

In conclusion, PCV-2 is a fast-evolving virus that prompted the definition of a plethora of variants named genotypes. Although genotype variability is likely to increase in the future, current data indicates that cross-immunity is present among major genotypes (PCV-2a, PCV-2b and PCV-2d) which, to date, guarantees vaccine induced protection by those products based on PCV-2a. Therefore, the different genotypes of PCV-2 still represent one single viral serotype; in other words, PCV-2 genotypes conform a unique immunological unit with common antigenic properties so far covered by existing commercial vaccines.

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