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EXPERT REVIEWS

Best practice and future challenges for vaccination against porcine circovirus type 2

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Porcine circovirus type 2 (PCV2)-systemic disease (SD) (initially named as postweaning multisystemic wasting syndrome) was discovered as an occasional disease affecting postweaning pigs in North-America by mid-1990s. Soon afterward, it was noticed as a devastating disease worldwide. Such scenario prompted to develop vaccine prototypes that worked fairly well under experimental conditions. In spite of the multifactorial nature of the PCV2-SD, the first commercialized vaccines containing inactivated or chimeric PCV2 viruses or PCV2 Cap protein represented by far the best system to control the disease under farm conditions. Moreover, vaccination of non-clinically affected pigs demonstrated a significant improvement of average daily weight gain and, in consequence, the economic importance of the PCV2-subclinical infection. In the present review, a comprehensive overview on PCV2 vaccines and best practices on PCV2 vaccination strategies are presented and discussed.

Keywords: age of vaccination • immunity • maternally derived immunity • PCV2-subclinical infection • PCV2-systemic disease • pigs • porcine circovirus diseases • porcine circovirus type 2 • sows • vaccine

Porcine circovirus type 2, an old virus for an apparently new disease

Porcine circovirus (PCV) type 2 (PCV2) was first characterized in 1998 as an agent systematically present within the lesions of a novel disease called postweaning multisystemic wasting syndrome (PMWS) [1,2]. The condition was described sporadically during early-middle 1990s in Canada [3,4]. The name of PCV2 was used to differentiate it from an existing PCV that was considered non-pathogenic for swine (subsequently named as PCV type 1 [PCV1]) [5].

Retrospective studies have shown that PCV2 was not a novel virus and PMWS was not a new disease. The virus has been detected as early as 1962 by PCR and the disease diagnostic criteria have been fulfilled in sick pigs from 1985 onward [6]. Moreover, phylogenetic and co-phylogenetic inference studies have suggested that PCV2 has been probably circulating in pigs for more than 100 years [7].

PMWS is clinically characterized by wasting and respiratory distress, although the scope of clinical signs can vary from farm to farm due to co-existing pathogens/diseases [8]. Importantly, PMWS is considered a multifactorial disease in which PCV2 is the necessary but not sufficient infectious agent to trigger clinical signs [9]. This aspect has been widely described in the literature, and it is probably the main reason why it is almost impossible to fulfill Koch's postulates by using only PCV2 as inoculum [10–12]. As many other multifactorial diseases, PMWS perfectly fulfils Evans' postulates, which are a set of guidelines that take into account the infectious agent, the host and the spectrum of host responses in order to establish a relationship between causation and disease [13].

Porcine circovirus diseases

Besides PMWS, PCV2 has been linked to other pathological conditions, grouped together as porcine circovirus diseases (PCVDs). The most recently suggested terminology for different

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Table 1. Main clinical, pathological and diagnostic characteristics of porcine circovirus diseases.

PCVD	Main clinical signs	Main gross and histopathological findings	Diagnostic criteria
PCV2-systemic disease	Wasting, weight loss, paleness of the skin, dyspnea, diarrhea, jaundice, inguinal superficial lymphadenopathy	Lymphadenopathy (lymphocyte depletion with granulomatous inflammation in lymphoid tissues), interstitial pneumonia, interstitial nephritis and hepatitis	• Compatible clinical signs • Moderate-to-severe lymphocyte depletion with granulomatous inflammation of lymphoid tissues (plus granulomatous inflammation in a number of other tissues) • Moderate-to-high amount of PCV2 in tissues (mainly lymphoid)
PCV2-subclinical infection	Lack of clinical signs but decreased average daily weight gain	Lack of lesions or mild lymphocyte depletion with granulomatous inflammation in lymphoid tissues	• Lack of evident clinical signs • No or minimal histopathological Lymphoid lesions in tissues • Low amount of PCV2 in few (lymphoid) tissues
PCV2-reproductive disease	Return-to-estrus, late abortion, mummified fetuses, stillborns, non-viable newborns	No evidence of lesions in the sow In the fetus: heart discoloration and dilation (necrotizing and fibrosing myocarditis)	Early gestation: • Regular return-to-estrus • PCV2 seroconversion following and/or PCV2 PCR positivity around the return-to-estrus Late gestation: • Reproductive failure at late gestation • Fibrous to necrotizing myocarditis of fetuses • Moderate-to-high amount of PCV2 in heart
Porcine dermatitis and nephropathy syndrome	Red macules and papules on the skin, mainly in the hind limbs	Gross: hemorrhagic and necrotizing skin lesions and/or swollen and pale kidneys with generalized cortical petechiae Microscopic: systemic necrotizing vasculitis, and necrotizing and fibrinous glomerulonephritis	• Gross lesions • Histopathological lesions PCV2 detection is not a criteria for the diagnosis of PDNS

PCV2: Porcine circovirus type 2; PCVD: Porcine circovirus diseases: PDNS: Porcine dermatitis and nephropathy syndrome.
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PCVDs is PCV2-systemic disease (PCV2-SD, to replace PMWS), PCV2-subclinical infection (PCV2-SI), PCV2-reproductive disease (PCV2-RD) and porcine dermatitis and nephropathy syndrome (PDNS) [8]. The last one is an immune-complex disease in which PCV2 is the suspected associated antigen; however, a scientific and unequivocal demonstration of this assumption is still lacking. Also, PCV2-respiratory and PCV2-enteric diseases have been suggested as specific entities linked to the virus. Recent data suggest that those conditions are probably part of the PCV2-SD scope [14] (Baró *et al.*, UAB, Spain, Unpublished Data). Main clinical, pathological and diagnostic characteristics of PCVDs are shown in Table 1.

PCV2 genetic variability

PCV2 is one of the smallest DNA viruses (12–23 nm in diameter) infecting mammals, which belongs to the family Circoviridae, genus *Circovirus*. PCV2 contains a circular, covalently closed, single-stranded DNA of 1767–1768 nucleotides [1,2]. Sequence analyses of different PCV2s around the world display close phylogenetic relationship, with a nucleotide sequence identity higher than 93% [15]. Sequences with a nucleotide diversity cutoff of 3.5% have been divided into two major groups designated as PCV2a and b genotypes [16]. Moreover, a third retrospectively reported genotype from Denmark in the 1980s has been described [17] and named as PCV2c [16]. A fourth one, initially thought to be restricted in China, but nowadays detected worldwide, has been designated as PCV2d [18]. Some preliminary data suggested that a fifth genotype would exist [19]. However, a very recent update indicates the existence of 4 different genotypes [G. Franzo, Univ. Padua, Italy, Pers. Comm.]. In all cases, the most prevalent genotype in pigs worldwide is PCV2b, followed far away by PCV2a [20].

Immunity developed upon PCV2 infection

Most of the published serological techniques for PCV2 are based on the detection of total anti-PCV2 antibodies (TA), without determining their neutralizing activity. Under field conditions, seroconversion for TA occurs in both PCV2-SD-affected and PCV2-SI pigs [21–25]. Although some studies did not find differences between PCV2-SD-affected and PCV2-SI pigs regarding TA titers [22], other works have reported weaker responses in diseased pigs [25,26]. Experimentally, delayed humoral immune response or low TA titers have been related to the expression of PCV2-SD [26–30]. In consequence, it is believed that pigs suffering from the systemic disease are immunosuppressed and, therefore, their humoral immune response is

impaired or, at least, limited in comparison with PCV2-SI animals.

Importantly, field and experimental studies have shown that PCV2 can persist in tissues and blood in the presence of high TA titers [21–23,31]. Although these studies did not discriminate between neutralizing and non-neutralizing antibodies, it is very likely that complete neutralization does not occur, as it has been shown by virus neutralization tests [26,32]. Therefore, these findings would explain the long persistence of the virus in blood and tissues in spite of specific PCV2 antibody presence in serum.

Under experimental conditions, neutralizing antibodies (NA) develop between 10 and 28 days post-PCV2 inoculation (dpi) [26,32,33] and low NA titers have been related to increased PCV2 replication and development of PCV2-SD [26,32]. Noteworthy, not all pigs with low NA titers have low levels of TA [26,32]. This latter fact suggests that either some animals develop a humoral response lacking NA or that NA develop later than non-NA. Taking together, available data suggest that the sole presence of PCV2 antibodies does not fully guarantee viral clearance and points out the potential role of other immune mechanisms independent of humoral responses.

The role of adaptive cell-mediated response during PCV2 infection and disease has been less studied. However, the impaired T-cell response of PCV2-SD-affected pigs [34,35] is suggestive of its contribution to the protective immunity against PCV2 infection. In addition, it has been shown that artificially induced immunosuppression may potentiate viral replication [36–38]. The kinetics of the helper and cytotoxic T-cell responses, measured by the number of IFN-γ-secreting cells (IFN-γ-SC), are dependent on the individual animal and also on the dpi at which the cells are tested. Under experimental conditions, the increase of IFN-γ-SC production might be evidenced from 7 dpi in some animals, and is unequivocally detectable from 14 dpi onward, at least until 29 dpi [39–41]. Interestingly, virus-like particles based on the PCV2 Cap protein are also capable of stimulating cytotoxic T cells, suggesting that those PCV2-specific T cells can recognize antigen processed from virus-like particles as well as from live virus [38,39,42]. Under field conditions, PCV2-infected pigs develop a significant increase of IFN-γ-SC around 2–3 weeks post-natural exposure, and may last at least 6–7 weeks post-PCV2 exposure [43,44]. Important to note, the IFN-γ-SC response is generally very variable among pigs, suggesting the existence of some high responders and pigs with very limited response [43]. Unfortunately, such cell-mediated response studies have not been specifically performed in pigs developing PCV2-SD in comparison with PCV2-SI animals.

In summary, present knowledge on adaptive immune response against PCV2 infection suggests that cell-mediated response, measurable as IFN-γ-SC, together with a significant NA response, is mostly responsible for viral clearance in infected animals [39]. It is hypothesized that a failure in one or the other or both responses might result in PCV2-SD development. Therefore, it was anticipated that effective PCV2 vaccines should stimulate both humoral and cellular immune responses.

Experimental PCV2 vaccines

Before the advent of commercial PCV2 vaccines, a number of vaccine prototypes using a wide range of technological platforms were designed and tested in several *in vivo* models. Among them were inactivated vaccines, DNA vaccines, recombinant chimeric live and inactivated viruses and recombinant subunit vaccines expressing PCV2 viral proteins [45]. A number of them have been tested in the target species, the pig, but others have been only tested in mice. Taking into account that there are significant differences in terms of immune responses between species [46,47], it should be interesting to test how many of those prototypes that have been demonstrated to work in mice would also work in pigs. Needless to say that most, if not all, of these candidate products used in pigs were tested in the framework of PCV2 subclinical experimental models due to the lack of a systematically repeatable and reproducible disease model. In consequence, the efficacy of those prototypes in pig farms affected by severe PCV2-SD was never assessed.

Importantly, data from these studies provided interesting insights on the immune responses generated by the viruses (inactivated or attenuated), PCV2 ORFs and specific proteins in the pig. When tested, most of these vaccine prototypes were able to develop a significant humoral immune response measured as TA; NA and cellular immune responses were sporadically investigated in these studies. However, in general terms, immunity conferred by these experimental products resembled to that generated by the natural PCV2 infection in subclinically infected pigs [39].

First attempts to find vaccine candidates included testing of conventionally inactivated vaccines. The use of such products in postweaning piglets [48,49] and sows [50,51] showed some clinical effects (measured by better growth rate of pigs) but mainly virological efficacy, with significant reduction of viral load in serum and tissues, and reduction of lesions in lymphoid lesions.

The first development of a subunit vaccine prototype indicated that the ORF2-encoded Cap protein was a major immunogen and induced protection against a subsequent challenge with PCV2; in contrast, the ORF1-encoded Rep protein was weakly immunogenic [52]. Protective immunity was attributed to the strong antibody response induced by the Cap, but no investigations on the cellular immune response were carried out. Anyway, subsequent serological studies with field samples confirmed that Rep protein is also immunogenic, generating an antibody dynamics similar to that of Cap protein [53]. Afterward, a number of subunit experimental vaccines were developed (by using recombinant viruses, bacteria, yeasts or plants expressing the Cap protein) and tested in both pig and/or mice models. These prototypes usually generated significant protective immune responses by eliciting specific humoral immune response and/or lymphocyte proliferative response to PCV2 [54–67].

DNA vaccination was also explored and compared with subunit vaccination in one of the abovementioned studies [52]. The DNA vaccine prototype was not able to reduce PCV2 replication in vaccinated pigs compared with control

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ones [52]. In contrast, other authors using a murine model [68] found that vaccination with an ORF2 plasmid vaccine resulted in a higher efficacy upon challenge with PCV2 compared with the Cap protein. So far, the DNA immunization model has been used in many experiments demonstrating generation of (sometimes protective) immunity [56,69–72].

A chimeric infectious DNA clone containing the immunogenic ORF2 capsid gene of a PCV2a cloned into the non-pathogenic PCV1 genetic backbone (PCV1-2a) induced antibody response to PCV2 capsid when inoculated in pigs and was shown to be attenuated compared with the wild-type PCV2 [73]. When the PCV1-2a DNA clone was used as a candidate DNA vaccine, it induced protective immunity against PCV2 infection [74]. Moreover, the resulting virus propagated from the PCV1-2a DNA clone was also used as an attenuated chimeric virus, demonstrating also protective capabilities. Subsequently, a chimeric attenuated PCV1-2b has been also developed and demonstrated to induce cross-protective immunity against PCV2b and PCV2a in pigs [75]. Finally, an experimental live-attenuated chimeric PCV1-2 vaccine (presumably a PCV1-2a vaccine, although not clarified in the article) was tested in sows using a PCV2-spiked semen model [76]. Such vaccine prototype was effective at inducing a humoral immune response and reducing PCV2 viremia in the sow, although vertical transmission to the fetuses was not prevented [76]. At least another chimeric virus PCV1-2 has been developed experimentally [77].

PCV2 vaccination & protection

Four commercial vaccines for use in piglets and/or breeding animals are currently available in all major swine-producing countries worldwide [45]. The number of commercial PCV2 vaccines is even higher in some areas of the world like Southeast Asia, where at least 6 more products are available in South Korea [78] and 16 in China [79]. Curiously, in this latter country, only one of the four major PCV2 vaccines commercialized worldwide is available [79]. A summary of characteristics of most currently used vaccines worldwide is given in Table 2.

The first vaccine on the market was Circovac® (Merial), an inactivated PCV2, oil-adjuvanted (o/w) vaccine for use in sows and gilts. Circovac was first used in Europe under temporary license in 2004 in France and Germany, and by 2006–07 was already available in most European countries and Canada. By 2010, this product got the license for its use in piglets. The other three most used commercial products are subunit and recombinant vaccines designed for use in growing pigs, with application around 2–4 weeks of age. Their names might vary depending on the part of the world where they are being commercialized, but their basic design is equivalent among products from the same pharmaceutical company. Suvaxyn® PCV2, Fostera™ PCV and Relsure™ PCV (Zoetis) are recombinant vaccines based on an inactivated chimeric virus containing the immunogenic ORF2 Cap gene of PCV2 into the non-pathogenic genomic backbone of PCV1 [73]. Ingelvac CircoFLEX® (Boehringer Ingelheim) and the vaccines from Merck/MSD Animal Health (Porcilis® PCV, Circumvent™ PCV and Circumvent G2 PCV) are subunit vaccines based on the product of the ORF2 gene expressed on baculovirus systems. All these vaccine products are based on PCV2a strains.

Vaccination of breeding stock

The rationale of stimulating active immunity in gilts and sows is to provide passive immunity to piglets through colostrum intake. Experimental and field studies demonstrated that sow and gilt vaccination allowed preventing PCV2-SD development in the offspring [80], decreasing lesion severity in lymphoid tissues, viremia and viral load in pig tissues [81] and PCV2 prevalence in the offspring [82], and improving the average daily weight gain (ADWG) in PCV2-SI pigs [83]. However, sow vaccination does not prevent early PCV2 infection and viremia in piglets from the first day of life, peri-partum maternal viremia and virus shedding into the lacteal secretions [84,85]. Although only Circovac has been specifically licensed for its use in sows and gilts, other vaccine trademarks have also been occasionally used off-label in these collectives, showing reduction of viremia and increased ADWG in the offspring [86]. As a curiosity, one field study concluded that sow vaccination in a farm decreased significantly the prevalence of ear necrosis syndrome in nursery pigs [87].

Generally speaking, PCV2-RD is seldom occurring, probably due to the widespread positive serological status of gilts and sows [8]. Therefore, this is the main reason why there are few reports on the use of PCV2 vaccination to control PCV2-RD or to potentially improve reproductive parameters. However, the repeated use of sow vaccination has also demonstrated significant effects on the reproductive side, although these effects have not been sufficiently documented in the peer-reviewed literature. In one particular study, long-term vaccination with Circovac in sows influenced positively the insemination rate, number of piglets born alive and weaned per litter as well as birth body weight and percentage of piglets weighing <1 kg [88]. In other non-peer reviewed reports, prevention of abortions, increased fertility and reduction in returns to service of sows and gilts following the use of PCV2 sow vaccine have been described, as well as increase in the number of live born pigs and the number of pigs per sow per year, and to reduce the number of mummies per sow [89,90]. It must be emphasized as well that the use of PCV2 vaccines in gilts/sows does not necessarily implies a significant improvement of reproductive parameters based on some observations from field veterinarians. As previously indicated, the major driving force to vaccinate sows relies today on protection of the offspring.

Dam vaccination to prevent PCV2 fetal/newborn infections and reproductive failure has also been investigated under experimental conditions. Vaccinated sows at 28 days of gestation with CircoFLEX and inoculated with PCV2b at 56 days of gestation displayed no viremia and lack of microscopic lesions and detection of PCV2 antigen in their fetuses and live born piglets; however, PCV2 DNA was detected in colostrum samples, fetuses and live born pigs [91]. In another study, Fostera

Table 2. Major porcine circovirus type 2 vaccines available on the worldwide market, with their main characteristics.

Manufacturing company	Vaccine name	Antigen	Licensed for	Administration dosage	Vaccination schedule	Geographical availability
Merial	Circovac®	Inactivated PCV2a	Sows/Gilts	2 ml, im.	Basic vaccination: • Gilts: One injection, followed 3–4 weeks later by a second injection, at least 2 weeks before mating. One further injection must be given, at least 2 weeks before farrowing • Sows: One injection, followed 3–4 weeks later by a second injection, at least 2 weeks before farrowing Re-vaccination: • One injection at each gestation, at least 2–4 weeks before farrowing	Worldwide; not licensed in the USA
			Pigs	0.5 ml, im.	Single dose, from 3 weeks of age onward	Worldwide
Boehringer Ingelheim	Ingelvac CircoFLEX®	PCV2a Cap protein	Pigs	1 ml, im.	Single dose, from 2 weeks of age onward	Worldwide
MSD/Merck Animal Health	Porcilis® PCV	PCV2a Cap protein	Pigs	2 ml, im.	Single dose, from 3 weeks of age onward[†]	Europe, several South- and Central-American and Asian countries
	Circumvent™ PCV	PCV2a Cap protein	Pigs	2 ml, im.	Double dose, from 3 weeks of age onward (suggested at 3 and 6 weeks of age)	North-America, several South- and Central-American and Asian countries
	Circumvent G2 PCV	PCV2a Cap protein	Pigs	1 (×2) or 2 ml, im.	One-dose option: One 2-ml dose from 3 weeks of age onward Two-dose option: One 1-ml dose as early as 3 days of age with the second 1-ml dose 3 weeks later	USA and Canada
Zoetis	Suvaxyn® PCV	Inactivated recombinant PCV1 expressing the PCV2a Cap protein	Pigs	2 ml, im.	Single dose, from 3 weeks of age onward	Europe, several Asian countries
	Fostera™ PCV[‡]	Inactivated recombinant PCV1 expressing the PCV2a Cap protein	Pigs	1 (×2) or 2 ml, im.	Single dose: 2 ml from 3 weeks of age onward Flexible dose: 1 ml dose at 3 weeks of age followed by a second 1 ml dose 2–3 weeks later	North-America, South Africa, several Asian countries

[†]The summary of product characteristics indicates the following recommendations in relation to maternally derived antibodies (MDA): 'In the case of low-to-medium levels of DA against PCV2a single vaccination (2 ml) to pigs from an age of 3 weeks onward is advised. When it is expected that higher levels of MDA against PCV2 are present, the following schedule of two vaccinations is advised: the first injection (2 ml) can be given from an age of 3–5 days, the second injection (2 ml) 2–3 weeks later. High levels of MDA may be expected when sows/gilts are vaccinated against PCV2 virus or when sows/gilts have recently been exposed to high levels of PCV2 virus. In such cases, it is advised to perform PCV2 serology, using suitable diagnostics, to select the most appropriate vaccination schedule. In case of doubt, apply the two shot vaccination schedule'.
[‡]Named as Relsure™ PCV in some South- and Central-American countries.
im.: Intramuscular; PCV2: Porcine circovirus type 2; PCVD: Porcine circovirus diseases: PDNS: Porcine dermatitis and nephropathy syndrome.

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PCV applied before artificial insemination was shown to be effective at inducing humoral immune response and reducing PCV2 viremia and intrauterine infection in sows [76]. Results from both studies suggest that PCV2 vaccination may control the detrimental effects of this virus, thereby preserving and maintaining gestation upon inoculation; however, it is not able to prevent vertical transmission.

Little is known about the effect of PCV2 vaccines in boars and viral excretion in semen. It is well known that the virus can be shed by semen at least until 60 dpi [92,93]. Moreover, one study demonstrated that PCV2 DNA-positive semen did not infect artificially inseminated gilts, although it was demonstrated to be infectious in a swine bioassay model [94]. Based on these results, the likelihood of having PCVD problems propitiated by viral shedding by semen is very low; anyway, some vaccination studies in boars have been performed. A first study was carried out with a commercial inactivated tissue homogenate PCV2 vaccine based on PCV2b (CircoPrime, Komipharm), a product mainly available in some South Asian countries. After two doses applied 3 weeks apart, boars were challenged with a PCV2b strain 3 weeks after last injection. Vaccinated boars had significantly lower amount of PCV2b shedding in semen compared with non-vaccinated ones [93]. Subsequently, same authors compared the efficacy of three different PCV2 vaccines in boars. The vaccination protocol reduced the amount of PCV2 shed in the semen with all products, but there was a significantly different amount of PCV2 shed in semen among the three vaccinated and challenged boar groups [95]. PCV2 vaccination has also been demonstrated to reduce disease and PCV2 shedding in semen of boars concurrently infected with PCV2 and *Mycoplasma hyopneumoniae* [96]. Importantly, PCV2 vaccines do not apparently have a major impact on sperm quality or quantity; in one study, however, one boar treated with an oil-based vaccine showed a temporarily impaired semen quality after elevated body temperature post-vaccination [97]. Generally speaking, the use of PCV2 vaccines in boars is very limited, probably because existing data indicate that semen is not a significant transmission route of the virus. Moreover, there is discussion about whether the virus found in semen is truly coming from an infected boar or from potentially environmental contamination at collection.

Vaccination of piglets

The rationale of stimulating active immunity in piglets is to provide protection against PCV2 infection in the group of animals that specifically suffers from PCV2-SD and PCV2-SI. Both experimental and field studies have demonstrated that PCV2 vaccination using commercial products in piglets is very efficient.

Experimental works have hardly reproduced clinical disease, but have been shown to reduce viremia, viral load in tissues, microscopic lymphoid lesions and, occasionally, to increase ADWG in the group of vaccinated pigs compared with non-vaccinated ones [40,41,98–105]. Moreover, these effects were observed in both PCV2 seropositive and seronegative piglets at the time of vaccination. In addition, one study demonstrated that vaccination was also effective at reducing viremia and lymphoid PCV2 load in PCV2-viremic pigs with passively acquired maternally derived antibodies (MDA) at the time of vaccination [106].

Data coming from field studies have demonstrated that all vaccine products on the market show remarkable efficacy, showing a drastic reduction in PCV2-associated productive losses at the growing-finishing stage. Listing all effects together, these studies have shown significant reduction of PCV2-SD prevalence and clinical severity, lesser mortality and cull rates, improvement of ADWG and feed conversion rate, a decrease in the frequency of co-infections and reduction of viral-induced microscopic PCV2-SD-like lymphoid lesions and viral load in tissues and blood [43,80,107–113]. In addition, a comparative field study using three PCV2 vaccines indicated no significant differences in efficacy among them [114]. Indeed, a meta-analysis study of 66 published field trials performed in clinically affected farms found a positive effect of all PCV2 vaccines on productivity measured as increase of ADWG (10–40 g/day in vaccinated pigs compared with non-vaccinated controls) and reduction in mortality rates [115]. In this study, no statistically significant differences in efficacy among tested commercial PCV2 vaccines were observed, although the number of trials analyzed was not the same for each PCV2 vaccine product.

Importantly, some studies have shown beneficial effects of PCV2 vaccination in farms not clinically affected by overt PCVDs. As observed with sow vaccination [83], piglet vaccination in PCV2-SI scenarios has resulted in significant improvement of ADWG, and reduction of prevalence of infection and PCV2 viral load in serum and feces [116–118]. Taking into account the ubiquitous nature of the virus, such positive effects on subclinical situations are probably the main cause of the extraordinary widespread use of PCV2 vaccines worldwide.

In 2008, the inactivated chimeric PCV1-2a vaccine product was temporarily removed from the markets because a chimeric PCV1-2a virus was incidentally detected in the field due to incomplete inactivation of the vaccine [119]. In 2011, a reformulated version of the chimeric PCV1-2a vaccine re-entered the market and tested efficacious and safe for piglets [120].

Mechanisms underlying vaccine-induced protection

Protective immunity against PCV2 infection relies on humoral (including NA) and cellular immune responses [26,32,39,42]. Although commercial vaccines reached the market before studying in depth their immune response, multiple studies have shown the importance of both NA and cellular immune vaccine-induced responses over the last 5 years.

At the starting of vaccine use, it was assumed that the main basis of efficacy relied on the protective effect of PCV2 antibodies, either passively acquired (sow/gilt vaccination) or actively induced (piglet vaccination). Subsequent studies demonstrated that all licensed vaccines were able to elicit a detectable humoral immune response of TA and, although less

studied, also NA. Moreover, such antibody generation has been observed in studies using PCV2 seropositive and seronegative piglets at the time of vaccination, including PCV2-viremic and seropositive pigs [40,41,43,86,98–100,105,106,113,117,120,121]. It is noteworthy that the amount of antibodies elicited is vaccine-dependent. In other words, not all vaccines on the market are able to produce a similarly detectable humoral response. As expectable, the type of adjuvant may play a major role in antibody development, together with the amount of antigen formulated into the vaccine [122]. In spite of the fact that some vaccines may not elicit a detectable PCV2 seroconversion after vaccination, the longitudinal monitoring of vaccinated pigs show that there is a certain production of TA when compared with non-vaccinated animals [41]. Therefore, these vaccines do really elicit an antibody response, but this weak response might not be enough to cause seroconversion upon vaccination.

Low antibody responses or lacking antibody development after experimental vaccination apparently do not always rule out protection [60,74]. These authors already suggested a potential role of cell-mediated immunity in vaccine-induced protection. Development of PCV2-specific IFN-γ-SC has been demonstrated by means of vaccination under both experimental and natural conditions for all commercially available vaccines [40,41,43,44,123]. Moreover, it has been shown that PCV2 vaccination induces a long-lasting immunity sustained by memory T cells and IFN-γ-SC that potentially play a role in preventing the onset of natural infection [44]. Recently, a side-by-side immune response comparison study among the four major vaccines available on the market has been published. As it occurred with TA and NA, all vaccines elicited a cellular immune response measured by PCV2-specific IFN-γ-SC, but with different amounts [122].

Regarding sow vaccination, it is also believed that both humoral and cellular immune responses play a role. Vaccination elicits antibodies in sows and their transfer to piglets has been shown in several studies [76,83,86,121,124]. An early report indicated that colostrum of vaccinated SPF sows contained PCV2-specific IFN-γ-SC [125]. The transfer of those cells to offspring was proved, but their protective effect (at ages where PCV2-SD or PCV2-SI occurs) could not be elucidated since IFN-γ-SC were only detected in newborn piglets within a very short period of time. A more recent study has demonstrated not only the passive transfer of functional PCV2-specific lymphocytes to newborn piglets, but also their protective role upon viral challenge in piglets [126].

Practical tips on PCV2 vaccination

Vaccine summary of product characteristics states the indications, contraindications, adverse reactions, target species, dosage for each species and route/s and method of administration (Table 2). Therefore, practicalities on how, to whom and when to vaccinate against PCV2 are already given for each particular product.

To decide the corresponding vaccine schedule to apply in a particular farm, a number of aspects must be considered initially. A first level of decision is the convenience of vaccinating piglets or breeding stock, or both. Such decision level should also include the selection of the particular product to be applied, since there are a number of vaccines licensed for pigs, but only one specifically licensed for breeding stock. The following step depends on the first one, and includes the timing of vaccination. This latter issue is especially important when vaccinating piglets, since interference of maternally derived immunity (MDI) might exist with vaccine efficacy.

If the first decision level is to vaccinate sows, schedule might be primarily directed to prevent PCVDs of the offspring or, alternatively, to protect against PCV2-RD. In the first case, vaccination should take place at the end of gestation, as it is recommended by the single manufacturer of the PCV2 vaccine intended for sows (Table 2). If the objective it to prevent PCV2-RD, vaccination might be applied before mating, being at the lactating period or at weaning for first parity or older sows, or during the acclimatization in gilts. Based on vaccine indications, this latter applicability should be considered off-label, since licensing of these products were intended to control PCVDs in the growing-finishing pigs. In any case, the use of sow vaccination at the end of the gestation period in a repeated fashion in the farm also provides protection against PCV2-RD [88].

Alternatively, one may select piglet vaccination as the way to control PCVDs in the farm. It is nowadays known that control of PCV2-SD in affected farms is quicker if piglet instead of sow vaccination is used. The main reason is that the vaccine applied in pigs is able to elicit protective immune responses in the animal that subsequently suffer from the disease, therefore, having a positive effect in the very first vaccinated batch. Non-published field data indicate that sow vaccination helps controlling clinical disease in growing-finishing pigs, but needs 6 months to 1 year of continuous use of vaccine in sows to generate comparable effects to piglet vaccination in a single batch.

Finally, the third option is to vaccinate both sows and piglets. There are already reports on the benefits of this schedule at productive and virological levels [86,121]. In this scenario, it is important to evaluate the putative interference of MDI upon PCV2 vaccine efficacy in piglets, since colostrum intake provides higher amounts of PCV2 antibodies. Within this scenario, an interesting point would be to analyze the benefit of leaving only one of the vaccine applications (sows or piglets) once the disease situation is under control. However, this strategy and its results have not been described in the literature yet.

The effect of MDI (measured as MDA) is significant, and may protect against PCV2 challenge [40] and influence the humoral response developed after vaccination [40,86,117,121]. In these later reports, a significant negative correlation between MDA at the day of vaccination and the increment of antibody titers to PCV2 4 weeks post-vaccination was confirmed. In other words, the higher the antibody titer at vaccination (generally at weaning), the lower the degree of seroconversion 4 weeks later. Moreover, one of these studies compared a protocol of both sow and piglet double vaccination, with application of the

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product in pigs at 3 and 7 weeks of age [121]. Evident MDA interference with vaccine seroconversion was only observed in the 3-week-old piglet vaccination schedule. Based on these observations, optimal vaccination strategies must balance the advantage of delayed vaccination in case of high antibody titers at weaning age with the need to induce immunity prior to exposure to pathogens under field conditions. In consequence, a 'vaccination window' has been proposed, defined as the range of antibody titers at which piglets should be vaccinated to minimize interference with MDA and, at the same time, ensure the development of protective immunity before PCV2 exposure [40].

This latter point is the one that should define the age at piglet vaccination. Product label indicates vaccination from 2 to 3 weeks of age onward, although one vaccine is licensed for an earlier age if a double dosage is used (Table 2). In fact, and taking into account that MDA interference with vaccine seroconversion has been demonstrated, the important point is to ascertain if this is paralleled with interference with vaccine efficacy. A first study indicated that efficacy is not jeopardized by MDA, with independence of antibody titers at vaccination [108]. However, if such interference does exist, it is very likely that most of the pigs in a batch overcome MDI on a practical basis. In the worst case scenario, such putative interference would happen in the proportion of pigs with relatively high or very high antibody titers. Such proportion might be different from batch to batch. A very recent study concluded that PCV2 vaccination in the presence of high MDA levels is efficacious when used in 3-week-old but not in 1-week-old pigs [127]. This paper indicated that PCV2 vaccine efficacy was independent of the level of MDA, since MDA titers of pigs vaccinated at both 1 and 3 weeks of age were apparently comparable. Anyway, when analyzing the data of this particular work, the pig group that performed better was the one vaccinated at 3 weeks of age in one of the studied farms, being the batch with the lower antibody titers [127]. Therefore, an alternative interpretation of these results would suggest interference of vaccine efficacy by means of high titers of MDA.

Expert commentary

PCV2 vaccination probably represents one of the most revolutionary successes of the pig industry in the last 30 years. PCV2-SD and PCV2-SI are the most detrimental PCVDs in terms of economic losses worldwide. The first one was the major reason that prompted scientists and vaccine-manufacturing companies to develop vaccines. Looking closely to PCV2-SD, some early reports considered that losses attributable to this disease in Europe were around €900 million per year (data from 2004) [128]. However, more accurate calculations from the UK have shown that if weaner/grower and finisher mortality is calculated during the whole epidemic period of PCV2-SD, economic losses could be as high as €5.76 billion for the 27 countries of the EU [129]. In contrast, the PCV2-SI was discovered once the commercial vaccines were used under field conditions worldwide and veterinarians realized that their use improved significantly the ADWG, even in pigs without overt clinical signs. In fact, a recent report indicated that the greatest proportion of negative economic impact at farm level was attributed to the subclinical infection, and not PCV2-SD [118]. Therefore, PCVDs have represented and still represent one of the major economic threats for the swine industry worldwide, but the use of vaccine products has allowed switching from a devastating disease to a practically negligible disease if pigs are properly vaccinated [20].

Sporadic reports have appeared regarding potential vaccine failure or, alternatively, lack of expected effect of the vaccine product. In 2009, pigs from PCV2-vaccinated herds in the USA experienced a peracute syndrome, referred as acute pulmonary edema, which mainly affected nursery and younger finisher pigs [130]. The only consistent infectious agent detected in a number of examined tissues was PCV2. It was speculated that acute pulmonary edema-susceptible pigs would lack sufficient levels of pre-existing antibodies against PCV2 and would become infected prior to vaccination. Therefore, it was suggested that vaccination was applied too late in these particular animals and an earlier administration of the vaccine may prevent the condition [130]. However, this potential new condition linked to PCV2 has only been described once and it might simply reflect a case of too late vaccination of pigs once the infection has already been established.

In 2010, Japanese researchers suggested that the mortality rate reduction by means of PCV2 vaccination might depend on the genotypes and subtypes (clades) of PCV2 [131]. In other words, their results pointed out to the possibility that existing vaccines based on PCV2a (and a specific clade within this genotype [132]) would not be equally effective in front of wild viruses of different genotypes or clades. Such idea, although indirectly, could be considered supported by a recent study that found better effectiveness of a vaccine based on PCV2b compared with one based on PCV2a in order to protect against PCV2b or combined PCV2a/2b challenges in pigs [133].

The most concerning issue regarding PCV2 vaccination is the possibility that new genotypes or variants of the virus may eventually escape vaccine-induced immunity, totally or partially. This concern reached its highest peak in 2012–2013, when PCV2-SD was diagnosed in two vaccinated US farms. The problem appeared in growing pigs from two wean-to-finish farms within the same production system. These animals were vaccinated with one single dose of a commercial vaccine at 3 weeks of age [134]. Further sequencing studies indicated that the strain linked to those PCV2-SD cases was a mutant PCV2b (mPCV2b) [135], which was previously described in China and apparently linked to increased virulence [136]. Surprisingly, when the producer changed to a double vaccination program (applied at 3 and 6 weeks of age), the problem was solved and the mPCV2b was no longer detected [134]. Such mPCV2b has been further detected in other countries, also linked with perceived vaccine failure, such as Brazil [137] and Korea [138]. The same variant has also been found in Europe, but not specifically related to vaccine failure cases (Kekarainen, CReSA, Spain, Unpublished Data). Importantly, a

recent study indicated that current commercial vaccines based on PCV2a strains are able to protect against the mPCV2b in an experimental model [139]. Furthermore, in a caesarian-derived, colostrum-deprived piglet model, virulence of this variant has not been found different from that of classical PCV2a and PCV2b genotypes [140].

Since viral evolution under vaccination pressure may occur due to the non-sterilizing immunity elicited by current vaccines [141], the potential generation of immune escape mutants in PCV2 is perfectly feasible. However, so far existing data are relatively contradictory and it is still to be demonstrated that these perceived vaccine failures are true ones. It must be emphasized that PCV2-SD is a multifactorial disease and the counteraction of infectious and non-infectious triggering factors is still paramount to control disease outcome. Anyway, permanent surveillance on emerging new variants potentially escaping vaccine-induced immunity is guaranteed.

Five-year view

PCV2 vaccines are currently the most sold preventive products in swine worldwide. Some countries are vaccinating almost if not all pigs that reach slaughter, and this trend is increasing in other parts of the world. This situation is understandable, since vaccines are able to improve productive parameters (ADWG, percentage of runts, body condition and carcass weight) in PCV2-SI situations. The ubiquity of PCV2 may prompt, therefore, to further intensify vaccination in these countries in which the vaccination rate is still medium to low. At the very end, the return of inversion with this vaccine is one of the highest among available biological products in swine.

Early reports of PCV2-SD already indicated an increased co-infection rate in affected pigs [9], probably due to the immunosuppressive nature of the disease [142]. Concomitant infections included not only viruses, but bacteria and, to a lesser extent, parasites [143]. Therefore, it is not surprising that, in Denmark, herds experiencing PCV2-SD had a usage of antimicrobials 37 and 19% higher before the outbreak in nursery pigs and finishers, respectively, compared with herds not experiencing the disease [144]. Subsequently, it has been observed that vaccination against PCV2 does not only imply direct beneficial effects on pig productivity, but also contributes to reduction of antimicrobial use [145]. This issue is and will be of special importance taking into account the antimicrobial use policies being implemented worldwide.

Although the initial tendency was mainly to vaccinate piglets, the number of vaccinated sows and gilts is increasing over time. Taking into account that combined vaccination of piglets and sows offer the best performance of animals [80,86], it is likely that a 'continuous protection fashion' will gain ground in the future. Initial assessments point out that besides getting the best ADWG of pigs, vaccination of both pigs and sows is overall cost-effective.

Continuous surveillance for new PCV2 variants is paramount for a DNA virus that has evolutionary rates resembling those of RNA viruses [7]. So far it seems that current vaccines based on PCV2a strains are able to cope with major circulating strains worldwide (PCV2a, PCV2b), including with the mPCV2b [139], due to cross-reactivity among genotypes [98,146]. However, limited existing data suggest that PCV2b vaccines might be even more efficient to counteract currently circulating viruses [133]. Therefore, vaccine manufacturers should consider licensing products based on PCV2b (some countries already have them [78,79]) or, alternatively, to develop polyvalent vaccines including two or more genotypes.

Elimination of PCV2 from a herd or a batch is feasible without vaccination [147,148]. On the other hand, since viremia control is very efficient by means of PCV2 vaccination [45] and that a double dose can prevent viremia in experimentally challenged pigs [98], it was hypothesized that a continuous, high vaccination pressure of piglets and sows might be able to control, and eventually eradicate, PCV2 infection in a farm. This hypothesis was recently tested and after 1 year of such mass vaccination program, PCV2 was undetectable by conventional PCR techniques and piglets reached slaughter seronegative [149]. Discontinuity of this vaccination program resulted in re-detection of the virus after 4 months. Evidence of infection after stopping the vaccination program might indicate either re-infection or that the virus was never cleared out from the farm, the second hypothesis being the most probable reason. Although this was just a punctual study, it would be interesting to assess if the application of such control measure extended over an undetermined period of time in a local, regional or country basis would result in the eradication of PCV2 infection.

A still fairly naïve field of research is the PCV2-RD. Although there are consistent criteria to diagnose the late-term reproductive disease by means of fetal analyses, there are still a number of gaps on the real effects of PCV2 on reproductive parameters as well as regarding the subclinical infection of the sow and fetuses. Vaccines allow preventing reproductive failure caused by PCV2, but the extent of their effects on general reproductive performance is still poorly known.

Novel research on PDNS is not really expected. The occasional occurrence of this disease and the complete lack of an experimental model make difficult to progress in the knowledge of its pathogenesis. The involvement of PCV2 antigen in this immune-complex disease has never been scientifically demonstrated, but field data indicate that farms that experienced PDNS together with PCV2-SD (they usually went together), the PDNS picture virtually disappeared after vaccination. If such effect is due to the control of PCV2 by itself or because of the general health improvement of the farm is not known.

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Key issues

- Porcine circovirus type 2 (PCV2)-systemic disease (SD) resulted in a devastating disease for the swine sector; subsequently, PCV2-subclinical infection (SI) has been identified as the porcine circovirus diseases representing the greatest proportion of negative economic impact.
- Several PCV2 vaccines are commercialized in the worldwide market based on inactivated wild or chimeric viruses, or Cap protein expressed in baculovirus systems.
- PCV2 vaccination is able to induce full clinical protection and to develop both humoral (total anti-PCV2 antibodies and neutralizing antibodies) and cellular (IFN-γ-SC) immunity.
- The rationale of stimulating active immunity in gilts and sows is to provide passive immunity to piglets, through colostrum intake, allowing prevention of PCV2-SD development in the offspring. However, the repeated use of sow vaccination seem to prevent effects of PCV2 on the reproductive parameters.
- The rationale of stimulating active immunity in piglets is to provide protection against PCV2 infection in the group of animals that are specifically suffering from PCV2-SD and PCV2-SI.
- The most efficient way to prevent porcine circovirus diseases in the shorter term is by means of piglet vaccination.
- A 'vaccination window' has been defined as the range of antibody titers at which piglets should be vaccinated to minimize interference with maternally derived antibodies and, at the same time, ensure the development of protective immunity before PCV2 infection. Vaccination of piglets around weaning in absence of sow vaccination is usually sufficient to overcome maternally derived immunity.
- Perceived vaccine failures have been described over the world; however, no definitive clue is still available in regards the existence of true vaccine escape mutants for PCV2. Continuous surveillance for new PCV2 variants will be paramount in the future.
- Future insights point out to increase the use of PCV2b-based vaccines or the putative development of polyvalent vaccines including two or more genotypes.
- PCV2 eradication or elimination at a local, regional or country basis is still far from the mind of veterinarians and producers, but apparently feasible by means of mass vaccination programs used in a continued form.

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