How to hit the target for PCV2 protection:

An updated overview and new control opportunity

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Update on PCV2 genotype evolution, virulence and cross protection

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Introduction

Porcine circovirus type 2 (PCV2) was identified in 1998 in tissues from pigs suffering from Postweaning Multisystemic Wasting Syndrome (PMWS) (1), a syndrome initially recognized in pigs in Canadian high health herds during 1995 (2). Since this time, PCV2 has been associated with several clinical manifestations including systemic, enteric, respiratory, reproductive disease, and also porcine dermatitis and nephropathy syndrome, which are now known collectively as PCV2-associated disease or PCVAD (3). PCV2 vaccines became widely available during 2006, are considered generally to be highly effective, and are among the most widely used swine biological products (3). The objective of this short review is to summarize novel insights on PCV2 genotype evolution, characterization of isolates, virulence and vaccine cross-protection against recent PCV2 isolates.

PCV2 genotype evolution

The genetic diversity of PCV2 isolates was largely unknown until the beginning of this century. Initial reports on PCV2 indicated a single common PCV2 strain (4). When severe PCVAD outbreaks were reported in North America during 2004-2006, the genomic makeup of PCV2 isolates present in affected herds and of isolates found earlier was compared side by side. By using a technique called restriction fragment length polymorphism pattern (RFLP) a novel RFLP pattern 321 was identified in more recent PCV2 isolates compared to the previously dominant RFLP 422 viruses (5). Subsequently the novel isolates, which after sequencing and analysis formed distinct branches in dendograms, were designated as PCV2b and the older, earlier isolates were designated PCV2a (6). Since that time, many more PCV2 genotypes have been discovered and today there are nine recognized groups: PCV2a, PCV2b, PCV2c, PCV2d, PCV2e, PCV2f, PCV2g, PCV2h and PCV2i (7-9). Among these groups PCV2a, PCV2b and PCV2d are currently considered to be of greatest importance and distributed worldwide with PCV2d the current predominant genotype (9,10). The other groups including PCV2c, PCV2e, PCV2f, PCV2g and PCV2h largely represent small clusters (few isolates) of likely minor significance (9,10).

Virulence

It is recognized that PCV2 is ubiquitous and most if not all global pig herds are subclinically infected with the virus (11); yet disease is only seen in a percentage of herds and within herds in a varying percentage of pigs. The question if PCV2 isolates differ in virulence has been on people's minds essentially since the discovery of PCV2. Today we know that while PCV2 isolates themselves can differ in virulence (12), at large there is no difference among genotypes. In other words, while a given PCV2a isolate may be more virulent than another PCV2a isolate, overall PCV2a isolates are not more or less virulent than PCV2b or PCV2d isolates (12-15). Only a single study comparing all three main PCV2 genotypes side-by-side found differences (13); however, this was not repeatable by other groups. Recently, a comparison of virulence of PCV2a, PCV2b and PCV2d was done in a *Porcine reproductive and respiratory syndrome virus* (PRRSV) and PCV2 coinfection model. Interestingly, in PRRSV co-infected pigs, PCV2d appeared to be more virulent compared to PCV2a and PCV2b which was evident by an increased PCV2 load in serum and a greater severity of lymph node depletion (14). Furthermore, when the same study was repeated with *Mycoplasma hyopneumoniae* and concurrent PCV2a, PCV2b or PCV2d infection the investigators again found PCV2d to be more virulent in the context of the coinfection as measured by PCV2 load in serum and lymphoid lesion severity (15).

Cross protection

Most of our knowledge on cross-protection among PCV2 genotypes results from using available vaccines (commonly commercial PCV2a vaccines) in commercial herds with ongoing field PCV2 infections. Based on this we know that there is good cross protection as many field isolates are PCV2b or PCV2d. Severe PCVAD problems in large percentages of pigs in a given herd are rare in vaccinated pig populations today. However, there is evidence that certain PCV2 genotypes such as PCV2d manage to replicate and increase in prevalence in vaccinated herds and can be associated with clinical PCVAD in smaller numbers of pigs (16,17). Numerous experimental studies have been conducted to show cross-protection with the majority of the studies using PCV2a vaccines (commercial vaccines are commonly tested) using a PCV2a (18-19), a PCV2b (20,21) or a PCV2d challenge (22, 23). The outcomes overall were essentially always positive; vaccinated pigs have a clear advantage over non-vaccinated pigs regardless of PCV2 type present in the vaccine. However, one study investigating chimeric live PCV1-2a and PCV1-2b found better protection against PCV2b challenge by the homologous vaccine (24) possibly suggesting that homologous protection may be better compared to heterologous protection. In support of this, a field investigation was conducted in 2012 in the USA after PCV2a vaccination had been used for approximately 5 years in almost all growing pigs (26). Interestingly, PCV2b (9.9%) and not PCV2a was found in serum from healthy vaccinated pigs whereas both PCV2a and PCV2b were found in serum from non-vaccinated pigs (26). In a recent field study, a PCV2d vaccine had some advantages over a PCV2a vaccine in protecting pigs from concurrent PCV2d/PRRSV 1-7-4 challenge and endemic IAV and bacterial coinfections. In non-vaccinated pigs severe PCVAD was reproduced (27).

Conclusions

Almost 25 years since its discovery in 1998, PCV2 remains an important pig pathogen on a global scale and needs to be managed properly. PCV2 likely will continue to evolve. As is clear from previous PCVAD outbreaks and investigations, monitoring for emergence of potential novel PCV2 genotypes or groups and subsequent identification of genetic shifts is important and routine surveillance protocols for emerging PCV2 genotypes should be in place. Vaccination has been found the best tool to deal with this virus in pigs. Increasing numbers of vaccines based on different genotypes offer pig farm owners and pig veterinarians more choices for customized protection than ever before.

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PCV2 SITUATION IN BRAZIL: GENOTYPES EVOLUTION, VACCINATION EFFICACY.

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Presentation of the disease and the agent in Brazil

Porcine circovirus type 2 or PCV2 is an important viral agent of PCV2 infectious disease, which is considered one of the main diseases of pig farming (Opriessnig, Karuppannan *et al*. 2020). PCV2-associated disease (PCVAD or PCVD) was first described in 1997(Allan and Ellis 2000). The disease, characterized by wasting and severe lesions in lymphoid tissue, was diagnosed in Brazil in 1999(Ciacci-Zanella and Mores 2003). Rapidly, it was observed in all areas of intensive pig production and became endemic in technical pig farming. Porcine multisystemic wasting syndrome (PMWS) is one of the most prevalent and severe clinical manifestations of PCV2 infection. Its control is performed by correcting risk factors and by using vaccines (Zanella, Morés *et al*. 2016). PCV2 is one of the most important pathogens in pigs, causing economic losses due to high mortality, delay in production or the manifestation of secondary infections associated with the virus. PCV2 is part of the Porcine Respiratory Disease Complex (PRDC), aggravating pneumonia and other respiratory signs (Rech, Gava *et al*. 2018). Nowadays, PCV2 infection is characterized by Systemic or Subclinical Disease.

Circoviridae family includes other porcine viruses, such as PCV1, which was first isolated from cell culture in 1982 and has unknown pathogenesis. In addition, together with PCV2, newly identified PCV3 and PCV4 are associated with potentially fatal diseases. PCV3 has also been identified in Brazilian pigs and PCV3a genotype characterized (de Souza, Gava *et al*. 2021). PCV2 can be classified into eight different genotypes, including PCV2a, PCV2b, PCV2c, PCV2d, PCV2e, PCV2f, PCV2g and PCV2h of which PCV2a is the oldest (Franzo and Segalés 2020). PCV2c has only been identified in tissues of pigs archived from Denmark and in feral pigs from Brazil and is considered of lesser importance. Around 2003, there was a major change in genotype from PCV2a to PCV2b. Severe PCV2 epidemics linked to the introduction of PCV2b occurred in North America during 2005/2006 and subsequently led to the introduction and large-scale use of PCV2 vaccines in pigs (Karuppannan and Opriessnig 2017). However, PCV2b emerged globally, being associated with more severe outbreaks of circovirus, becoming the prevalent genotype in pigs worldwide. In Brazil, mostly of the commercial vaccines are based on the PCV2a genotype, used in both, sows and piglets at weaning and have been effective in controlling the genotypes PCV2a and PCV2b (Zanella 2017).

In 2012, a new variant virus of the genotype PCV2b mutant or mPCV2b – later called PCV2d – was isolated in vaccinated pig herds from the United States (Opriessnig, Xiao *et al*. 2013), and this viral strain was almost identical to a virus initially identified in China between 2004 and 2008 (Guo *et al*. 2010). Consequently, the presence of the new virus was investigated in cases of apparent vaccine failure and experiments were carried out to evaluate the efficacy of vaccines against the new discovered virus (Opriessnig *et al*. 2014, Gerber *et al*. 2013). In Brazil it was no different. As of 2012, a new variant or strain of PCV2 (mPCV2) has also been detected in pigs and has been associated with cases of vaccine failures (Ciacci-Zanella 2015). Pigs vaccinated against PCV2 showed typical clinical signs of PMWS, and the laboratory diagnosis confirmed PCV2 infection. Thus, the virus identified in these cases as genotype b. Genomic analyses indicated additions or substitutions of nucleotides generating modifications in the genome of the virus, called mPCV2 (later classified as PCV2d), and which in turn led to changes in amino acids located in epitopes responsible for the activation of the immune system. In fact, this variant has 3 extra amino acids at the end of ORF2 (viral capsid protein). The evolutionary rates of PCV2 are higher than expected for a DNA virus, which has historically been proven by the emergence of different genotypes (PCV2a, PCV2b, etc.). PCV2 genotype studies in 2019 in vaccinated pig herds characterized PCV2b (11/26) and PCV2d (13/26) in 26 PCV2 positive samples (Nascimento HIJ 2021). Moreover, different populations of PCV2 could circulate in the same farm and in the same animal, and can infect both vaccinated animals and unvaccinated animals. In fact, the possibility of the future may generate mutant viruses due to vaccination pressure (Dvorak, Yang *et al*. 2016) has been speculated. Therefore, adequate genotypic monitoring is key to assess vaccine efficiency. There is also discussion about the role of vaccination in modulating the genetic variability of PCV2 and the need to update current vaccines.

Even though studies on the efficacy of commercial vaccines based on PCV2a indicate failure of vaccines to protect infection for mPCV2, currently called PCV2d, they prevent the clinical manifestation of the disease. For the control of circovirus, it is important to implement biosecurity measures in pig farms, maintenance of the immune balance of the herds, low microbial load (viral), as well as the good practices of conservation and administration of vaccines, avoiding the occurrence of vaccine failures and consequently greater severity of the disease (Opriessnig, Karuppannan *et al*. 2020).

PCV2 infects domestic and wild boars and may be present in herds with different health standards and production systems. Since the initial description of PMWS, other infections or syndromes have been identified and associated with PCV2 infection. Among these, porcine dermatitis and nephropathy syndrome (PNDS) and enteric, respiratory and reproductive diseases associated to PCV2 (Segales 2012) have been described. PMWS predominantly affects piglets between five and 12 weeks of age, although the disease has already been described in piglets at four to 24 weeks of age. Morbidity and mortality range from 70-80% and 4-30%, respectively, according to the farm, phase in which the outbreak appears and according to the type of management used in the rearing. Mortality rates in daycare and growth-termination triple in relation to normal farm averages, and in several herds these rates can normalize in a few months.

The criteria for PCV2 infection diagnosis should include clinical disease, macro / microscopic lesions and the association with the presence of PCV2 nucleic acid or antigen. Among the various clinical signs observed in piglets of herds infected with PCV2, rapid and progressive weight loss and signs of pneumonia, are the most typical signs (Grau-Roma, Hjulsager *et al*. 2009). However, the subclinical form can occur in many affected herds, where in these piglets there is only insufficient performance and higher occurrence of other health problems, especially diarrhea and pneumonia (Segales 2011). In the reproductive area, PCV2 has been associated with the occurrence of fetal mummification, stillbirths, birth of weak piglets and abortions, both in experimental and field-level conditions (Pensaert, Sanchez *et al*. 2004, Brunborg, Jonassen *et al*. 2007). In a study carried out in Brazil investigating these reproductive failures, among the infectious agents that cause reproductive problems, PCV2 was the most frequent agent associated with the pathological conditions of piglets (Ritterbusch, Sa Rocha *et al*. 2011). In Brazil there are few reports, but PCV2 was also implicated as a cause of enteric disease, and the diagnosis was characterized by diarrhea, granulomatous enteritis injury and lymphoid tissue depletion (Peyer plaques) and detection of the agent in the intestine. However, because it is an immunosuppressive agent, it is currently considered that enteric problems are part of the systemic picture of circovirus. Thus, enteric disorders occur due to immunosuppression and consequent manifestation of "secondary" enteric pathogens, or granulomatous enteritis that could alter the permeability of the intestinal wall and lead to diarrhea (Baró, Segalés *et al*. 2015). With the introduction of vaccination, in which only subclinical disease is observed most often, enteric problems are possibly caused by other concomitant pathogens such as E. coli, Salmonella spp, among others (Baró, Segalés *et al*. 2015). Similarly, clinical presentation of circovirus can vary greatly among herds due to other diseases that may occur simultaneously. These include: Aujeszky's disease (AD), Porcine Reproductive and Respiratory Syndrome (PRRS), Glässer's disease, swine parvovirus, Meningitis by Streptococcus suis, salmonellosis, colibacillosis, colitis, dietary hepatosis and purulent bronchopneumonia. Among these, PRRS seems to be the most important, since PMWS is more frequent and severe in PRRSV infected herds (Segales 2011). Since PRRSV has never been identified in Brazil, this is not an important finding.

Macroscopic lesions include lymph node hypertrophy, thymus atrophy and non-glued lung (pulmonary edema), sometimes with small disseminated areas of hepatization (Segales 2011). In addition, in Brazil, polyserosites and colitis are the most frequent concomitant lesions (Correa *et al*., 2006). The most consistent changes in histopathological examination are found in lymphoid tissues (lymph nodes, spleen, thymus, tonsils and Peyer plaques), lung and kidneys. In the lung there is thickening of the alveolar wall by proliferation of septal cells and infiltration of lymphocytes and histiocytic cells that also appear around vessels, bronchi and bronchioles (interstitial pneumonia) (Correa *et al*. 2006). In lymphoid tissue there is loss of the architectural structure of the tissue, lymphoid depletion in different degrees of severity, necrosis of lymphoid cells, especially in follicular centers, infiltration of histiocytic cells, sometimes with formation of granulomas and presence of multinucleated cells (Guo, Lu *et al*. 2010, Gerber, Johnson *et al*. 2013, Opriessnig, Xiao *et al*. 2014). The kidneys have focal areas of nephritis/glomerulonephritis, especially by lymph histiocytic infiltration (Segales 2011).

Control, vaccines, failures and comparison of the importance of swine circovirus today and 20 years ago

Control of PCV2 can be divided into before and after pig's vaccination with commercial vaccines for PCV2. Before vaccines were available, control strategies focused on minimizing pig infection by secondary agents (or other diseases) and correcting/eliminating risk factors associated with the occurrence of the disease (Lopez-Soria, Segales *et al*. 2005). The use of spray dried porcine plasma in nursery and grower feed showed reduction and the severity of PCVD.

Piglet vaccination should combine low levels of maternal antibodies with the development of protective immunity against PCV2 through the vaccine before the onset of natural infection. Each farm must develop its own vaccination program according to the challenges, immunity of sows and dynamics of infection (Oliver-Ferrando, Segalés *et al*. 2016). Vaccines indicated for use in piglets should be applied before the phase of increased exposure to the agent, and some preparations recommend two applications and other vaccines are recommended for single-dose application. The rate of pig herds vaccinated for PCV2 in Brazil varies between 80-98% and several field studies have shown that the vaccines used are efficient in controlling circovirus.

After registration and wide use of commercial vaccines for PCV2 in Brazil, when well used, infection and disease were controlled and the productive rates improved (Zanella, Morés *et al*. 2016). Thus, the wide and continuous use of these vaccines gave rise, in most farms, to a scenario of subclinical infection by PCV2, finally achieving seronegative pig herds at slaughter. However, practices inherent to the application of the vaccine (half dose, change of manufacturers' marks, packaging temperature, application in part of the group) possibly cause the virus (of all genotypes) to continue circulating and causing disease. In necropsies of varied cases, an increase in lymph nodes is already noticed again and in some cases there are typical lesions of PCV2 infection. It is also observed the worsening of cases of salmonellosis in nurseries and late presentation in termination by insufficient immunity. However, the picture seen today does not compared with that before the advent of vaccines. In all cases, vaccination against PCV2 is, in most scenarios, economically profitable. The main changes in clinical presentation in recent years are for failure or even incomplete vaccination of all piglets, but vaccines (when well used) are efficient.

In summary, since the introduction of vaccines, the presence and viral load has decreased greatly in the swine population. Currently pigs have a lower viral load and no PCR viruses are detected in most farms, suggesting that continuous vaccination has managed to get some farms to negative towards PCV2 over time. Although the mechanism by which vaccination controls the infection is not entirely clear, its continuous use has strongly reduced, below detectable levels, the prevalence of PCV2 in pigs. At the same time, it was concluded that most pigs remain infected with PCV2 at levels below the sensitivity limit of the qPCR tests, since more than 70% of farms have animals with anti-replication viral protein antibodies induced by natural infection. The infection would also explain the cases of circovirus associated with vaccination interruptions. Therefore, vaccination remains important to protect animals against circovirus.

Compared to 20 years ago, circovirus today is a controlled disease, including subclinical infection. However, as in other pig-producing countries, in Brazil, even with almost massive vaccination, PCV2 infection is still diagnosed and there is a risk of re-emergence. Among the critical points for worsening infection are the factors of ambience, mix of lots, overcrowding, failures in the sanitary measures and high pressure of infection. Although it is not the main disease of Brazilian pig farming today, circovirus remains a multifactorial disease and not all factors that play a role in the development of the disease are completely known. For a long time we searched for an unknown agent or "Agent X" that could be a virus (torquetenovirus, parvovirus, or other circular DNA simple-type virus) or other microorganism. Thus, a constant monitoring is needed that includes: management measures with correction of risk factors, adequate vaccination protocols, control of concomitant infections, research on PCV2 genotype evolution (or PCV3, PCV4 or others), dynamic of infection and pathogen-host interactions.

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Safety and efficacy of a new PCV2 vaccine (Suigen® PCV2, Virbac)

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Introduction

Porcine circovirus 2 (PCV2) is a single-stranded DNA virus infecting most of pigs worldwide and responsible for important economic losses due to mortality and impaired animals growth. PCV2 vaccination has become a major success to improve productive parameters, particularly in subclinical infection situations, thus providing a definite return on investment (1). Mutation rate of PCV2 is the highest recorded for a single-stranded DNA virus, close to single-stranded RNA viruses, allowing genotype dominance shifts over time (2-3). PCV2 was subjected to a drastic global genotype shift from PCV2a to PCV2b around 2003 (2). This shift was often associated with apparent increased severity of clinical disease and high morbidity and mortality in regions where PCV2b was introduced (4). A recent phylodynamic analysis of around 900 PCV2 genome sequences from 32 countries evidences the current occurrence of a second major genotype shift characterized by the emergence and spread of PCV2d, which appears to be replacing PCV2b (5). A retrospective phylogenetic analysis of 5120 PCV2-ORF2 sequences confirmed the current predominance of PCV2d worldwide (6).

PCV2 genotype evolution is linked with its high mutation rate, lack of eradication and possibly vaccination pressure (3). Though cross-protection of the current PCV2a based vaccines has been proven, emergence of immune escape mutants is feasible (1). Moreover homologous genotype vaccination has been reported to better reduce viremia in some challenge tests (7). At least three publications from USA, Korea and Germany reported clinical cases of PCV2 disease in herds routinely vaccinating against PCV2, linked to isolation of PCV2b variant strains recognized later as PCV2d (8-9-10). However definitive conclusion on failure due to vaccination procedure or vaccine *per se* is lacking (3). Thus vaccines development should consider the viral evolution.

Development of a new PCV2 vaccine

A whole inactivated virus vaccine (Suigen® PCV2, Virbac) has been recently developed from a PCV2d strain formulated in a ready to use double emulsion (water in oil in water) designed to vaccinate piglets from 3 weeks of age by a one shot intramuscular dose of 1 ml. This vaccine has been tested to assess its safety and efficacy in induction of immunity, control of PCV2 infection and improvement of herds performances.

Induction of immunity and control of PCV2 infection

Humoral immunity through neutralizing antibodies (NA) and cell mediated immunity by interferon gamma secreting cells (IFN-γ-SC) are the 2 pillars of immune protection against PCV2 (1-11). Two challenge tests showed significantly higher NA and IFN-γ-SC, and lower viremia and viral tissue burdens in Suigen® PCV2 vaccinated pigs than in non vaccinated pigs. Both immunity criteria and viral burdens were similar between Suigen® PCV2 and reference subunit vaccine groups when compared in one of these challenges (Table 1). A multicenter field study confirmed significant reduction of PCV2 viremia and tissue burdens till finishing in Suigen® PCV2 vaccinated pigs compared to non vaccinated pigs (Table 2).

Table 1: Mean ± SD of peak PCV2 immunity criteria, viremia and inguinal lymph node viral burden after two different challenge models

Challenge	PCV2b (12)		PCV2d (13)		
Group	Suigen® PCV2 (n=4)	Saline (n=4)	Suigen® PCV2 (n=20)	Subunit vaccine (n=20)	Saline (n=20)
Log_2 NA titers	6.8 ± 0.7[a]	2.5 ± 0.5[b]	7.0 ± 1.5[a]	7.3 ± 1.2[a]	2.3 ± 1.0[b]
IFN-γ-SC/10^6 PBMC	NA	NA	76 ± 27[a]	87 ± 30[a]	36 ± 28[b]
PCR serum Log_{10} copies/ml	3.3 ± 0.5[a]	5.6 ± 0.7[b]	3.4 ± 1.8[a]	3.1 ± 1.9[a]	6.2 ± 0.8[b]
IHC scores	9.7 ± 1.6[a]	52.5 ± 20.1[b]	3.7 ± 1.4[a]	2.8 ± 1.1[a]	18.3 ± 4.0[b]

PBMC : Peripheral blood mononuclear cells IHC : Immunohistochemistry

NA : not available [a,b] : Different superscripts indicate significant differences

Table 2: Rate of PCV2 PCR positive pigs at finishing in a multicenter field trial (12)

Tissue	Age (weeks)	Suigen® PCV2	Non vaccinated
Serum	19	5.2% [a] (5/97)	50% [b] (46/92)
Inguinal lymph node	25	10% [a] (3/30)	100% [b] (30/30)
Lung		6.7% [a](2/30)	100% [b] (30/30)
Tonsil		20% [a] (3/15)	100% [b] (15/15)

[a,b] : Different superscripts indicate significant differences

Safety

Safety of Suigen® PCV2 was assessed from 15 experimental or field sites in 6 countries from Latin America and South East Asia representing more than 2500 piglets and including two overdose studies (2 ml/piglet). Very rare and reversible post vaccinal reactions were observed, local reactions being significantly lower than with a reference subunit vaccine in one study (14).

Field performance data

Seven controlled field trials have been performed during or after the development of Suigen® PCV2 in 4 countries from Latin America and South East Asia representing more than 4500 piglets (12-15-16-17). These studies generally compared this PCV2 vaccine to a reference one following the piglets vaccination at weaning. In 2 trials, Suigen® PCV2 could be compared to a non vaccinated group. The main criteria of evaluation were the wean to finish mortality or culling rates and finishing weights or wean to finish ADGs.

When compared to an unvaccinated group, a significant reduction of wean to finish mortality rate (7.6% vs 12.4%) and increase of growth (78 kg vs 73 kg for mean pig life weight at 19 weeks of age) was recorded in the Suigen® PCV2 vaccinated group (12).

When compared to another commercial vaccine (whole inactivated virus, subunit or recombinant vaccine), the mortality/culling rates and growth indicators from weaning to finishing were generally similar between vaccines. Nevertheless the performances outcome may depend on the field situation (PCV2 circulating field strains genotype and virulence, coinfections…).

Conclusion

Performed studies confirmed safety and efficacy of Suigen® PCV2 (based on the current dominant PCV2d genotype) in induction of specific immunity, reduction of viral burden and improvement of field performances in various field conditions.

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