Exploring potential areas of future vaccine development for novel porcine circovirus type 3 (PCV3)

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Abstract

Porcine circoviruses have been circulating in swine herds for nearly 50 years, and from an evolutionary perspective, they may have existed before this. Most notably, pathogenic porcine circovirus type 2 (PCV2) has been a major economic concern to the swine industry. Porcine circovirus associated diseases have been linked to systemic, respiratory, enteric, and reproductive diseases, in addition to increased mortality rate in pigs. Successful vaccination programs with PCV2 vaccines have helped to control its spread and have led to a decrease in mortality rates in swine herds. Recently, porcine circovirus type 3 (PCV3), a novel porcine circovirus, has been identified and discovered to be circulating in numerous countries throughout the world. There is still more to understand about the pathogenesis and clinical presentation of PCV3, but numerous retrospective studies and some experimental infection studies have identified porcine circovirus associated disease-like syndromes in PCV3 infected pigs. Therefore, it is important to understand the potential impact this novel circovirus could have on the swine industry, including prevention strategies. Currently, there are no licensed vaccines available to aid in the prevention of disease caused by PCV3. This report aims to discuss the current state of PCV3 and explore potential areas of future vaccine development.

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Chapter 1 - Background of Porcine Circoviruses

Porcine circoviruses (PCVs) are currently classified into three distinct types with a fourth tentatively proposed. The history of porcine circoviruses dates back to their identification as a contaminant in porcine kidney cells, and these viruses have been further studied once it had been discovered as the cause a form of wasting disease. This disease has had a tremendous economic impact to the swine industry over the years. When first identified, porcine circovirus was seen as a harmless non-pathogenic viral agent. The pathogenicity remains unknown in more recently discovered porcine circoviruses, and understanding the history, genomic characterization, and clinical presentation of porcine circoviruses over the years are important points to consider for potential prevention strategies. This chapter details the background of all circoviruses including the classification and genomic characterization, history, clinical presentation, and the importance of porcine circoviruses to the swine industry.

Classification and Genomic Characterization

Circoviruses, part of the family *Circoviridae* and genus *Circovirus,* are uniquely recognized as some of the smallest viruses, at 17 nm in diameter, that are known to infect mammals. They are single stranded, non-enveloped, circular DNA viruses that tend to be very stable in the environment (Opriessnig et al., 2020). Currently, three types of PCVs are classified by the International Committee on Taxonomy of Viruses as PCV1, PCV2, and PCV3 (ICTV, 2020). However, a fourth circovirus tentatively identified as PCV4 has recently been discovered in China (Zhang et al., 2019)

The PCV2 genome contains 1767-1768 nucleotides and has a nucleotide sequence identity greater than 94% from both diseased and non-diseased pigs (Segalés et al., 2005). In

comparison, the genomic sequences of PCV1 and PCV2 have shown only an 80% overall nucleotide sequence identity (Segalés et al., 2005). Porcine circovirus type 3 was shown to be only 31-48% identical to PCV1 and PCV2 at the amino acid level (Ouyang et al., 2019). The PCV3 genome contains 2000 nucleotides and three open reading frames (ORFs). The orientation of ORF1 and ORF2 are in inverse directions, similar to PCV1 and PCV2 (Ouyang et al., 2019). Open reading frame 1 encodes the replicase (Rep) protein, involved in viral replication, which is 48% identical to PCV2, and ORF2 encodes a major structural protein, capsid (Cap), which is 26% identical to PCV2 (Ouyang et al., 2019). The Rep protein of PCV3 has shown a closer identity to the Rep protein of bat circovirus than PCV2 at around 54-55% identity (Ouyang et al., 2019). The function of ORF3 in PCV3 is currently unknown. Porcine circovirus type 3 has been shown to be genetically stable with most known mutations located in the Cap protein (Ouyang et al., 2019).

Porcine circovirus type 2 is divided into four clades, PCV2a, PCV2b, PCV2c, and PCV2d. Porcine circovirus type 2b was previously the most predominant clade, but in recent years, PCV2d has become the more dominant genotype (Iowa State University, 2016). Commercial vaccines currently available target the PCV2a subtype but may offer some cross protection to other subtypes or possibly lessen the severity of disease from coinfection of other subtypes. Porcine circovirus type 3 is divided into three clades, PCV3a, PCV3b, and PCV3c (Ouyang et al., 2019). The three clades are further divided based on mutations on the cap protein (Fu et al., 2018). Further studies are needed to identify the most predominant clade of PCV3 circulating throughout the world.

History of Porcine Circoviruses

Porcine circovirus was originally detected in Germany in 1974 as an unknown animal virus found in infected cultures of a pig kidney cell line (PK-15), and later classified as porcine circovirus (PCV) in 1982 (Tischer et al., 1982). Porcine circovirus was named due to its ability to produce antibodies in pigs and because PCV DNA was a covalently closed circular molecule as examined by electron microscopy (Tischer et al., 1982). This PK-15 contaminant is now known today by the name, porcine circovirus type 1 (PCV1).

Porcine circovirus type 2 (PCV2) was later identified and classified in the 1990s. In 1991, an otherwise healthy swine herd in Canada was experiencing symptoms of post-weaning multi-systemic wasting syndrome (PMWS), where there was an increased mortality rate of 12-15% (Ellis, 2014). Lymphoid depletion was observed in pathology samples submitted to the Western College of Veterinary Medicine from the first outbreak in Canada; however, a definitive diagnosis was not reached at that point in time (Ellis, 2014). Further studies concluded that this PCV-like virus was isolated from lesions in multiple tissues of pigs with PMWS that were experiencing both clinical signs and lesions typical of PMWS via electron microscopy, immunohistochemistry (IHC), and *in situ* hybridization (Ellis et al., 1998). Retrospective studies revealed that PCV2 may have been in existence as early as the 1960s (Ellis, 2014).

The third porcine circovirus discovered was first characterized as PCV3 in 2016 in the United States, after a herd showing clinical signs of porcine dermatitis and nephropathy syndrome (PDNS) and reproductive failure in North Carolina tested negative for PCV2 (Palinski et al., 2017). Porcine circovirus type 3 has been identified in multiple countries on multiple continents since its discovery, and it has been seen in both historical samples from retrospective studies as well as samples from live animals showing signs of clinical disease and in healthy animals (Klaumann et al., 2018). While it was first identified in 2016, historical samples have

shown it may have been circulating worldwide prior to this (Palinski et al., 2017) (further discussed in Chapter 2).

A fourth circovirus, tentatively named porcine circovirus type 4 (PCV4), was recently identified in April 2019 in China. This virus was isolated from a farm with pigs experiencing severe clinical signs of respiratory disease, diarrhea, and some skin lesions similar to PDNS lesions. This virus has a DNA length of 1,770 nucleotides and has a genomic structure typical of circoviruses. The frequency of this newly identified PCV4 in samples obtained in the same province in China was shown to be present in 12.8% of samples tested (Zhang et al., 2019). At this point, there is very little information known about this novel circovirus, and it has not yet been officially classified as PCV4 by the International Committee on Taxonomy of Viruses.

Clinical Presentation

Porcine circovirus type 2 is the main pathogenic porcine circovirus known to be associated with numerous disease syndromes in pigs. Porcine circovirus type 1, on the other hand, is considered a nonpathogenic virus. In experimental studies, the virus was recovered from pigs; however, pigs inoculated with PCV1 did not show signs of disease, and infection was apparently harmless (Segalés, 2005). Porcine circovirus type 3 will be discussed in further detail throughout this report, but it has been shown to have some similarities in clinical presentation to PCV2.

Porcine circoviruses have been linked to many different diseases with related acronyms including post-weaning multi-systemic wasting syndrome (PMWS), porcine circovirus associated diseases (PCVAD), porcine circovirus disease (PCVD), PCV2-systemic disease (PCV2-SD), PCV2 reproductive disease (PCV2-RD), PCV2 subclinical infection (PCV2-SI), PCV2 lung disease (PCV2-LD), PCV2 enteric disease (PCV2-ED), porcine dermatitis and

nephropathy syndrome (PDNS), and porcine respiratory disease complex (PRCD) (Iowa State University, 2016). Clinical findings in these disease syndromes include weight loss, wasting, diarrhea, red to purple macules and papules on the skin, late-term abortions, and stillbirths (Segalés, 2014).

With regard to PCVS-SD, high viral loads and co-infections of multiple genotypes of PCV2 as well as co-infections with porcine parvovirus, porcine reproductive and respiratory syndrome virus, *Mycoplasma hyopneumoniae*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Haemophilus parasuis, Staphylococcus* species, and *Streptococcus* species have contributed to increased clinical signs of this disease syndrome in PCV2-infected pigs (Segalés, 2014).

When PCV2 was first discovered in the 1990s, it was associated with a disease-like syndrome, unlike its predecessor PCV1. The term to describe this syndrome was coined post-weaning multi-systemic wasting syndrome (PMWS), which affected nursery pigs causing poor growth rates and wasting. The terminology for PMWS has since been replaced with PCV2-SD in recent years (Segalés, 2014).

Since PCV2 was originally discovered, additional disease syndromes have been found to affect PCV2-infected pigs, and thus, the terms porcine circovirus associated diseases (PCVAD), as it is known in North America, and porcine circovirus diseases (PCVD), as it is typically defined in Europe, have become current designations of the disease syndromes associated with PCV2. PCVD or PCVAD is the collective term used to describe systemic, respiratory, enteric, and reproductive diseases caused by porcine circovirus (Iowa State University, 2016).

Porcine circovirus type 2 is one of the numerous viral or bacterial agents that make up the porcine respiratory disease complex (PRDC). It has been found in experimental co-infection

studies that PCV2 can increase the severity of pneumonia caused by *Mycoplasma hyopneumoniae*, swine influenza virus (SIV), and porcine reproductive and respiratory syndrome virus (PRRSV) (Iowa State University, 2020).

In 2014, Ellis described PCV2 as the "necessary but not sufficient cause of PMWS"; studies showed that PCV2 could be subclinical when infecting pigs alone and that co-infection could cause more severe disease (Ellis, 2014). The model of co-infections causing more severe disease has also been observed with PRRSV and porcine parvovirus (PPV) co-infections with PCV2 (Ellis, 2014).

In 1986, studies were performed to determine if PCV1 was pathogenic, where 9-month-old pigs were infected with PCV1 but failed to show signs of clinical disease (Tischer et al., 1986). In the 1980s, it was widely accepted that PCV1 was nonpathogenic in pigs, which made it difficult for scientists to accept that viral agent PCV2 was the cause of PMWS. In the last four years, two types of porcine circoviruses have emerged. Further information will be discussed later in this report regarding the clinical presentation of PCV3. Very little is currently known about PCV4, and while this report will focus on PCV3, it is important to take note of another potentially emerging porcine circovirus that has been identified.

Economic Importance

Porcine circovirus type 2 is an important pathogen associated with the swine industry due to the economic impact it can have on swine producing countries. It has been estimated in the United States that disease associated with PCV2 can cost producers anywhere from $3 to $20 per pig (Gillespie et al., 2009). The cost of the vaccine itself is typically around $1-$2 per dose which would offset the cost of economic loss associated with disease. Mortality rates nearly doubled when PCVAD started to affect herds leading to the economic importance of

preventative measures for PCV2. The use of vaccines, developed as a preventative measure, have shown mortality rate drops in field studies from 8-10% in non-vaccinated pigs to 1-2% in vaccinated pigs (Gillespie et al., 2009).

Currently, the economic impact of PCV3 remains unknown, but it is worth noting that there have been similarities in clinical signs of PCVAD associated with PCV3 as with pathogenic PCV2, and it's important to understand the potential impact PCV3 infection has on the farm and consider the potential economic impact as more is learned about PCV3.

Chapter 2 - Origin and Prevalence of PCV3 Worldwide

An emerging circovirus, PCV3, has been identified in countries across the world over the past few years. Polymerase chain reaction (PCR) has been the main detection method to identify currently circulating swine circoviruses, and it was the method that detected PCV3 in samples dating back to the 1990s. Understanding the prevalence and geographic distribution is important with an emerging virus to help establish prevention strategies and determine if this emerging virus continues to spread. This chapter will discuss the possible origin of emerging circovirus, PCV3, and studies that have been performed to identify PCV3 in countries on four continents.

Origin

A novel circovirus, PCV3, was discovered in 2016; however, retrospective studies show it could have a longer historical past. While the exact origin is unknown, samples dating back to the 1990s demonstrate the presence of PCV3. Researchers studied the evolutionary dynamics of novel PCV3 and the impact it could have on control measures. They found that the bat clade 1 circovirus isolated in China from 2011 to 2013 was the most recent common ancestor to PCV3 that was based on GenBank sequence information in 2018 (Li et al., 2018). They also found the expected number of new infections from the original case, or reproductive number, of PCV3a and PCV3b to be somewhat similar, with values of 3.08 and 1.82, respectively. Porcine circovirus type 3a and PCV3b subtypes differ at amino acid site 24 of the ORF2 coding region. It was proposed a different epitope structure could correspond to different antigenicities of these two subtypes, but further studies would be needed. Overall, PCV3 was found to have a high substitution rate for a single stranded DNA virus, and this study concluded that this information shows the possibility of PCV3 to adapt to different biological conditions much like any RNA

virus (Li et al., 2018). Researchers concluded that the high reproductive number and high nucleotide substitution rate could be a concern for continued outbreaks, and thus, the importance of development of preventive and control measures (Li et al., 2018).

Geographic Distribution and Prevalence of PCV3

Since it was first identified in 2016, PCV3 has been isolated in countries across the world, spanning four different continents. Porcine circovirus type 3 has been found in numerous tissues from pigs of different ages and varied health conditions in Germany, United States, Mexico, Brazil, Thailand, China, Poland, Russia, Korea, Ireland, Sweden, Denmark, Italy, Spain, and Malaysia (Klaumann et al., 2018).

Porcine circovirus type 3 has been detected in parts of North America and South America. It was first identified in the United States in 2015 on a commercial sow farm in North Carolina after an outbreak of PDNS led to a mortality rate of more than 10% (Palinski et al., 2017). Since this discovery, swine herds in multiple states throughout the US have experienced cases of reproductive failure possibly linked to PCV3. Between January and November of 2018, cases of reproductive failure were identified and positive for PCV3 at 20 different sites based on quantitative RT-PCR (qPCR) results. Effected sites included swine herds in Iowa, Indiana, Nebraska, Kansas, South Dakota, Michigan, Minnesota, North Carolina, and Ohio (Arruda et al., 2019). In South America, PCV3 has been isolated from five different states in Brazil, including Rio Grande do Sul-RS, Santa Catarina-SC, Parana-PR. Mato Grosso do Sul-MS, and Goias State-GO, and was detected in cases of reproductive failure (Dal Santo et al., 2020).

Porcine circovirus type 3 has been detected in multiple countries throughout Europe. It was first detected in Europe in samples collected from five different provinces in Poland from 2014 to 2017 made up of fourteen farms with sow herds composed of pigs of different genetic

backgrounds (Stadejek et al., 2017). Seven of the fourteen farms had pigs that exhibited signs of PCVD or skin lesions consistent with PDNS and all but one farm vaccinated against PCV2 (Stadejek et al., 2017). Out of the fourteen farms, twelve tested positive for PCV3 by qPCR that target the ORF2 region of PCV3. Serum pools were collected from pigs of various ages noting a lower rate of PCV3 in the youngest age group. Serum pools from sows were positive for PCV3 in 9 out of 31 (29%) of pools. Serum pools from fatteners aged 9 weeks or older were positive for PCV3 in 33 out of 118 (28%) of pools. Serum pools from weaned pigs aged 5-8 weeks were positive for PCV3 in 12 out of 46 (26.1%) of pools, and serum pools from suckling pigs aged 3-4 weeks were positive for PCV3 in 1 out of 20 (5%) of pools (Stadejek et al., 2017).

In an effort to better understand PCV3 in Europe after its detection in Poland, samples that were collected for routine diagnostic purposes in 2016 and 2017. These samples were tested for PCV3 by qPCR in countries throughout Europe, including samples collected from previous studies performed on herds in Denmark, Italy, and Spain. Results showed the PCV3 genome sequences were similar to strains from South Korea, Brazil, and China (Franzo et al., 2018). In Denmark, 36 of 38 lymph node samples, 6 of 20 serum samples, and 2 of 20 lung samples tested positive for PCV3. In Italy, 10 of 29 lung samples, 20 of 29 organ pools, 6 of 33 serum samples, and 1 of 8 nasal swab samples tested positive for PCV3. In Spain, half of the farms tested were positive for PCV3, and of the positive farms, 14 of 94 serum samples tested positive for PCV3 (Franzo et al., 2018).

A high prevalence of PCV3 has also been found in wild boars in Eastern and Western Germany (Prinz et al., 2019). Samples collected from 2011 to 2015 were evaluated for PCV3 via qPCR. Wild boars in the Western region showed positive PCV3 results in 10 of 20 wild boars, and the Eastern region showed PCV3 positive results in 16 of 69 wild boars (Prinz et al., 2019).

It is also worth noting that in addition to Germany, PCV3 was detected in wild boars in Italy and Spain (Prinz et al., 2019).

Data on PCV3 in pigs in Sweden was first published in 2018 (Ye et al., 2018). The aims of the study were to detect PCV3 for the first time in Sweden and genetically characterize it from samples that had been collected over time. One sample that tested positive for PCV3 by PCR was collected from a pig in a post-weaning multi-systemic wasting syndrome positive herd, and the other 9 samples that were identified to be PCV3 positive came from healthy pigs (Ye et al., 2018). It is also interesting to note that the samples positive for PCV3 were collected years prior to the first discovery of PCV3, between 1993 and 2007 (Ye et al., 2018).

Porcine circovirus type 3 has also been detected in multiple countries throughout Asia. It was first detected in Russia from samples taken on two commercial farms from different geographic locations, including the Tyumen and Smolensk regions from herds displaying reproductive failure and PDNS (Yuzhakov et al., 2018). In 2017, a total of 77 tissue and serum samples were tested for PCV3 from pigs of different ages, including sows, weaned pigs, and fattening pigs (Yuzhakov et al., 2018). Fluid samples from the thoracic cavity were 100% positive for PCV3, and other tissue samples showed varying rates of positivity for PCV3 (Yuzhakov et al., 2018). Strains sequenced were shown to be 99.3% identical between the two regions in Russia and 97.0-99.6% identical to strains from other countries (Yuzhakov et al., 2018).

In Thailand, PCV3 was identified in samples dating back to 2006, collected from both healthy pigs and those showing clinical signs of PRDC (Sukmak et al., 2018). Serum and tissue samples from 26 farms, previously collected between 2006 and 2017, were tested by PCR for PRRS, PCV2, and PCV3. Porcine circovirus type 3 was detected in 14 of the 26 pig farms and in

29 of the 79 samples. Positive PCV3 samples originated from pigs ranging in age from 1 day to 24 weeks old as well as gilts and multiparous sows. There was a high rate of co-infection, with samples showing a positive rate of 61.54% for PCV3 and PCV2, 61.54% for PCV3 and PRRS, and 30.76% for PCV3, PCV2, and PRRS (Sukmak et al., 2018).

Porcine circovirus type 3 was the only viral pathogen detected on three farms from two provinces in China that were experiencing an outbreak of reproductive failure and loss of neonatal piglets from 356 sows (Ku et al., 2017). Further studies were conducted on 35 farms across one province in China, which found positive PCV3 pigs on 24 of the 35 sampled farms and a PCV3 and PCV2 co-infection rate of 15.8% in all samples collected (Ku et al., 2017). PCV3 was seen in multiple tissue types, including brain, lung, lymph nodes, tonsils, semen, serum as well as the brains and lungs of stillbirth samples (Ku et al., 2017).

In Korea in 2016, samples were collected from pigs on 73 different farms to determine the prevalence of PCV3. Porcine circovirus type 3 was detected by PCR in 53 of the 73 farms tested and distributed throughout all provinces of Korea (Kwon et al., 2017). The study broke the samples up into four groups composed of weaned, growing, finisher, and sick pigs. The sick group had the highest rate of PCV3 at 51.5%, followed by the weaned group at 49.3%, the growing group at 42.6%, and the finisher group at 41.1% (Kwon et al., 2017).

More recently, a total of 141 pigs were sampled from commercial swine farm abattoirs and retail shops from northern, central, and southern regions of Malaysia, specifically to test for PCV3 (Tan et al., 2020). Animals were divided into multiple age groups, including fetuses, piglets, weaned pigs, growers (also called finishers), and sows (Tan et al., 2020). Porcine circovirus type 3 was detected by PCR on 10 of 24 farms tested, and a total of 24 out of 141 samples were positive for PCV3 (Tan et al., 2020). Porcine circovirus type 3 was detected in all

nine organs tested with the highest positive rate found in inguinal lymph node samples at a rate of 81.82% (Tan et al., 2020). Archived lung samples, collected in 2016 to 2017 from wild boars, were also tested in this study for PCV3, and all samples were determined negative (Tan et al., 2020).

Prevalence studies may not identify a causal role in the pathogenicity of PCV3; however, they provide information with regard to the presence of circulating PCV3 in different geographic regions as well as the prevalence of infection among pigs of different ages. While it was first detected in the United States in 2015, retrospective studies have found that PCV3 has actually been circulating among swine herds earlier than this based on the detection of PCV3 in archived samples dating back to the early 1990s. As more information is revealed about the pathogenesis of PCV3, understanding the geographic distribution of this virus may be especially beneficial in determining how to effectively prevent and control the spread of PCV3.

Chapter 3 – Clinical Presentation of PCV3

Countries across the world have identified PCV3 in pigs displaying different clinical presentations as well as in healthy appearing pigs. Retrospective studies of PCV3 have shed some light on the types of clinical presentations in infected swine; however, it is not clear how PCV3 contributes to the pathogenesis of disease. A porcine dermatitis and nephropathy syndrome (PDNS)-like presentation is what originally alerted researchers to suspect a novel virus after samples from swine cases tested negative for PCV2. Reproductive failure has also been found in PCV3 infected herds, and it has been hypothesized that PCV3 is the cause. Less reported, clinical cases of respiratory and neurologic disease have been linked to PCV3 in swine herds. This chapter will discuss the different disease syndromes thought to be linked to PCV3.

Porcine Dermatitis and Nephropathy Syndrome

Porcine dermatitis and nephropathy syndrome was linked to a novel circovirus, and then, later characterized as PCV3 when it was first discovered on a sow farm in North Carolina. Clinical presentation of these sows included anorexia, multifocal papules, macules, and superficial dermatitis. Histological analysis of skin lesions observed acute necrotic dermatitis and epidermitis with lymphoplasmacytic perivascular cuffs (Palinski et al., 2017). These tissues tested negative for PCV2 by immunohistochemistry (IHC) and quantitative RT-PCR (qPCR). A retrospective study looking at samples from 48 cases with histological lesions typical of PDNS were negative for PCV2, and out of these cases, 45 were positive for PCV3 by qPCR analysis with viral titers of 1.60×10^4 to 3.47×10^4 genomic copies (gc)/mL (Palinski et al., 2017).

The first and only study to date to assess clinical signs of PDNS caused by PCV3 in experimentally inoculated piglets was conducted recently. The aim of the study was to evaluate

pathogenesis and reproduce clinical signs of disease. Specific-pathogen-free (SPF) piglets of the breed Duroc crossed with Large White were experimentally inoculated with PCV3 with the aim of reproducing PDNS-like clinical disease and establishing a better understanding of pathogenesis (Jiang et al., 2019). Prior to the experiment, all SPF pigs were negative for PCV1, PCV2, PCV3, PPV, torque teno virus (TTV), pseudorabies virus, swine influenza virus, classical swine fever virus, porcine epidemic diarrhea virus, Japanese encephalitis virus, and PRRSV antigens, and they were observed for clinical signs of disease. Fifteen 4-week-old piglets were divided into three groups of five piglets each. Group one of this experiment was given a sham inoculation and served as the negative control. Group two of piglets was inoculated intranasally with a PCV3 infectious DNA clone. Group three was given the same inoculation of a PCV3 infectious DNA clone in addition to keyhole limpet hemocyanin in incomplete Freund's adjuvant to serve as an immunostimulant and assess the effect of immunostimulation. This had previously been shown to be an important component of pathogenesis in PCV2-associated PMWS. These treatments were carried out in both 4-week-old and 8-week-old SPF piglets; however, the 8-week-old group did not include the immunostimulant treatment group (Jiang et al., 2019). Serum samples were tested for PCV3 by quantitative real-time PCR at days 0,7, 14,21, and 28 and observations of clinical disease were made throughout the study. Sham inoculated piglets remained PCV3 negative throughout the study, while inoculated groups showed an increase in PCV3 viral load. The infectious clone was able to reproduce a PDNS-like disease in both treatment groups of 4-week-old and 8-week-old piglets. Both inoculated groups also showed a reduction in body weight gain compared to the negative control and developed multifocal papules, macules, and superficial dermatitis typical of PDNS (Jiang et al., 2019). In addition to clinical signs of PDNS, piglets inoculated with PCV3 experienced anorexia, coughing, sneezing,

diarrhea, increased respiratory rates, lethargy, shivering, and hyperspasmia (Jiang et al., 2019). No deaths occurred in the negative control group of 4-week-old and 8-week-old piglets and no deaths occurred in the PCV3 inoculated pigs from the 8-week-old group. The 4-week-old piglets had a total of 4 deaths associated with PCV3, 2 from group 2, the PCV3 inoculated group and 2 from group 3, the PCV3 inoculated group plus immunostimulant group. In the study, which controlled environmental factors and other swine pathogens, piglets showed signs of clinical disease, with some disease symptoms presenting as severe with a PDNS-like presentation. Currently, this is the only study to reproduce signs of clinical disease by PCV3 and shows the importance of being able to reproduce these clinical signs as a possible challenge model for vaccine development.

Reproductive Failure Cases

Reproductive failure that was likely caused by PCV3 was first described in a swine herd in North Carolina in June of 2015. Higher mortality rates in sows and lower conception rates at 10.2% and 0.6%, respectively, were reported along with clinical signs of PDNS. Fetuses were also affected during the same outbreak, showing an increase of 1.19 times the number of aborted mummified fetuses per litter when compared to the average historical abortion rate on the same farm (Palinski et al., 2017). Fetal tissues were sent to the Iowa State University Veterinary Diagnostic Laboratory for diagnostic testing to determine the causative agent, which eventually ruled out PCV2, PRRSV, and PPV after testing negative via qPCR. Metagenomic sequencing was also performed on a pool of fetal tissues. Positive samples were found to be most closely related to the viral genome, PorkNW2/USA/2009, discovered in commercial ground pork. The novel genome isolate from these fetal tissues were proposed to be a new type of porcine circovirus, called PCV3 (Palkinsi et al., 2017).

Another study conducted in the United States detected PCV3 in fetal tissues (Arruda et al., 2019). Routine diagnostic cases submitted to the Iowa State University Diagnostic Laboratory for diagnostic testing over a 10-month period in 2018 were evaluated based on cases specifically involving reproductive failure. Fetal heart and lung tissue were evaluated from samples that contained PCV3 cycle quantification (cq) values of less than or equal to 30 by qPCR from these reproductive cases. Three farms experienced early embryonic death, increased mummified fetal rates, and decreased farrowing rates ranging from 77-83%, which was approximately 10% lower than the historical average of these farms. It is important to note, however, that the gilts from each of these farms were all sourced from the same location (Arruda et al., 2019). Overall, the percentage of positive PCV3 cases involved in reproductive failure was 13% over a 10-month period in 2018. All PCV3 positive cases evaluated were negative for PCV2, and most, but not all, samples tested for PRRSV and PPV were negative for these viral agents (Arruda et al., 2019).

Cases of reproductive failure have been found to be PCV3 positive in countries outside of the United States, and one study questioned the association between PCV3 and reproductive failure. More specifically, a study conducted in Brazil took a closer look at the association between PCV3 stillbirths from six piglet-producing farms (Tochetto et al., 2020). Serum samples were collected from sows just after farrowing. Samples were chosen from sows that experienced at least one stillbirth as well as a second group of samples from sows with no stillbirths. Positive PCV3 samples were identified by qPCR. Of the two groups, there was a 67.4% frequency of PCV3-positive animals in sows with stillbirths and 60.5% in sows without stillbirths. While the frequency was higher in the stillbirth group, there was no statistically significant difference by

chi-square analysis between the two groups (Tochetto et al., 2020). Contrary to other studies, these findings did not support the association between PCV3 positive infections and stillbirths.

Further cases have been identified in stillborn tissues, serum, and in sow colostrum, offering more information on the possibility of vertical transmission of PCV3. A study in China identified PCV3 positive cases across 35 farms in different geographical locations (Ku et al., 2017). A total of 222 samples were collected. Of these samples, 8 out of 14 samples identified PCV3 in the brain of stillbirth samples, and 4 out of 47 semen samples were positive for PCV3. Other than PCV2, it was not noted if other viral agents were tested in these samples. They did note the co-infection rate of both PCV3 and PCV2 across all 222 samples was 15.8% (Ku et al., 2017). This association demonstrates the potential for vertical transmission of PCV3.

Vertical transmission cases have also been studied in Thailand, where PCV3 has been shown to be present in sow colostrum. A study analyzing serum and colostrum samples in 38 sows infected with PCV3 from a farm in Thailand was the first known study to report on PCV3 shedding in colostrum (Kedkovid et al., 2018b). PCR and sequencing were used to identify PCV3 in colostrum samples and serum samples. Out of the 38 sows, 18 were PCV3 positive in serum samples, 17 were PCV3 positive in colostrum samples, and 25 were positive in either serum or colostrum samples. Serum titers were assessed, and results were categorized into groups of high-viremic sows, low-viremic sows, and non-viremic sows based on PCV gc/mL.

Results showed that the high-viremic group of sows with higher PCV3 levels also had high titers in the colostrum samples, which were significantly higher than low-viremic and non-viremic serum groups. Seven out of 17 pigs with PCV3 in their colostrum were categorized in the non-viremic serum group; a similar occurrence has been seen in PCV2 infected pigs (Kedkovid et al., 2018b). Information was gathered on parity of the sows involved in this study,

showing that PCV3 was more prevalent in primiparous sows compared to multiparous sows; a similar occurrence has been shown in sows co-infected with PCV2 and PRRSV (Kedkovid et al., 2018b). The study did not look at the clinical outcome, and to date, this seems to be an area that still needs to be investigated along with the mechanism of colostrum shedding.

Information on the frequency of PCV3 in healthy primiparous and multiparous sows with mummified fetuses and stillborns of typical reproductive parameters in Spain has recently been assessed (Sapotiri et al., 2020). Three farms in Spain found PCV3 positive rates in 77.8% of mummified fetuses and 67.8% of stillborns. Primiparous sows were found to have a higher number of PCV3-infected fetuses as compared to fetuses from multiparous sows. Due to the high level of PCV3 infected fetuses from primiparous sows compared to multiparous sows, the researchers suggest the possibility of immunity in multiparous sows that was able to prevent PCV3 infection (Sapotiri et al., 2020).

A high rate of mummified fetuses with PCV3 infections was also seen in Brazil. A total of 276 mummified fetuses were evaluated, and 270 were shown to be PCV3 positive, a rate of 97% (Dal Santo et al., 2020). The study also demonstrated a co-infection rate of 93.1% of positive infections with at least one of the following agents: PPV, PCV2 and Leptospira (Dal Santo et al., 2020).

Multiple studies have identified PCV3 in tissue from reproductive failure, suggesting vertical transmission is possible in PCV3 infections. Porcine circovirus type 3 has also been identified in colostrum and in fetal tissue from stillborns. While PCV3 has been detected, further studies are warranted to determine if PCV3 is the causal agent of disease and reproductive loss. Few studies have investigated the transmission of PCV3 in colostrum, but it is important based on the data available to continue to investigate mechanisms of colostrum shedding. There have

been numerous studies looking at the association of PCV3 in reproductive failure, and other investigations have focused on determining if infection from vertical transmission can cause disease and whether or not there is a potential for co-infections to cause reproductive failure in PCV3-infected sows.

Recently, researchers were able to successfully isolate PCV3 from perinatal and reproductive cases of PCV3-associated disease and show successful infection and replication *in vivo* by experimentally inoculating PCV3 isolates in cesarean-derived, colostrum-deprived (CD/CD) pigs. Viral isolates originated from cases involved in reproductive failure. Samples were derived from brain, heart, liver, kidney, and lung tissues in cases that had tested negative for PCV2, porcine reproductive and respiratory syndrome virus (PRRSV), porcine parvovirus, and atypical porcine pestivirus, which are all known to be associated with reproductive failure (Diaz et al., 2020). Isolates were cultured in PK-15 cells free of both PCV1 and PCV2. One of the PCV3 isolates, specifically PCV3/USA/MO/ISU27734/2018, was found to be stable after 9 passages in cell culture. This PCV3 isolate, ISU27734, was used to experimentally inoculate the CD/CD pigs, of which 8 out of 8 showed viremia and 4 out of 8 had an IgM antibody response. While the aim of the study was not to determine the clinical presentation of PCV3 in experimentally inoculated pigs, the authors did note that experimentally infected pigs did not show any clinical signs of disease (Mora-Díaz et al., 2020).

Respiratory Cases

A farm in Thailand experiencing an outbreak of PRDC-related clinical signs was investigated from the presence of PCV3 (Kedkovid et al., 2018a). Porcine circovirus type 3 was detected in 60% (15 out of 25) pigs experiencing signs of PRDC compared to only 28% (7 out of 25) in clinically healthy pigs. Porcine circovirus type 3 titers were also higher in the PRDC pigs

at 3.24 ± 2.80 log gc/mL compared to the titers from the clinically healthy pigs at 1.61 ± 2.67 log gc/mL (Kedkovid et al., 2018a). Porcine reproductive and respiratory syndrome virus was also seen in some samples and known to be endemic on this particular farm. Porcine circovirus type 3 infection was seen in pigs at 12 weeks of age, followed by PRRSV infection at 16 weeks of age, and a high prevalence of PRDC was seen at 18 weeks of age (Kedkovid et al., 2018a). While this study did not lead to a causative role for PCV3 in PRDC, it is worth noting the higher titers in PRDC cases and higher frequency of pigs experiencing PRDC that were positive for PCV3. Further studies into the causal role of PCV3 in PRDC are needed and the possible role of co-infections.

A prevalence study was conducted recently in Spain to look at the frequency of PCV3 in swine serum samples from post-weaning respiratory or digestive disorders and from healthy pigs (Saporiti et al., 2019). Swine serum samples that had previously been collected between 1997 and 2018 were tested for PCV3 by PCR analysis. The samples were from pigs that were 1-4 months of age, and analyzed as three separate groups: 129 samples from pigs that had been clinically diagnosed with respiratory disease, 126 samples from pigs that had been clinically diagnosed with enteric disease, and 60 samples from clinically healthy pigs. All three groups showed a similar frequency of positive PCV3 cases. Porcine circovirus type 3 was detected in 6.2% (8 of 129) of samples from the respiratory disease group, in 5.65% (7 of 126) samples from the enteric disease group, and in 6.7% (4 of 60) samples from the healthy group. This study concluded that the results did not support an association of PCV3 with respiratory disease or enteric disease (Saporiti et al., 2019). Overall, further studies need to be conducted to determine if PCV3 is associated with respiratory disease. Current information does not show strong evidence that PCV3 is associated with the porcine respiratory disease complex (PRDC).

Neurologic Cases

One study in China identified PCV3 in neonatal pigs experiencing signs of congenital tremors (Chen et al., 2017). The clinical signs affecting these pigs included severe shaking of both the head and limbs of neonatal pigs, which led to a high mortality rate and economic loss on the farms experiencing these congenital tremors. Polymerase chain reaction was performed on tissue samples of the brains, hearts, kidneys, livers, lungs and spleens of the piglets. In severely affected herds, PCV3 was found in the highest concentration in both the heart and brain. All samples were tested for PCV3 along with other viral pathogens, including atypical porcine pestivirus (APPV), bovine viral diarrhea virus (BVDV), classical swine fever virus (CSFV), foot-and-mouth disease virus (FMDV), hepatitis E virus (HEV), swine Japanese encephalitis virus (JEV), porcine bocavirus (PBoV), porcine circovirus 2 (PCV2), porcine delta coronavirus (PDCoV), porcine epidemic diarrhea virus (PEDV), porcine enterovirus (PEV), porcine kobuvirus (PKV), porcine parvovirus (PPV), porcine reproductive and respiratory syndrome virus (PRRSV), porcine pseudorabies virus (PRV), porcine sapelovirus (PSV), swine influenza virus (SIV), Senecavirus A (SVA), and swine vesicular disease (SVDV). In the samples that were PCV3 positive, several other viral pathogens were also present, including APPV, PDCoV, PEDV, PKV, PRRSV, PRV, and/or PSV.

Atypical porcine pestivirus has been associated with causing congenital tremors and was present in 5 out of 7 samples positive for PCV3. There were 2 out of 7 samples positive for PCV3 that were negative for other pathogenic viruses that caused congenital tremors. While this study did not definitively determine that PCV3 was the cause congenital tremors, it did detect a tissue tropism for the brain in PCV3-infected piglets, and further analysis of the relationship between PCV3 and neurologic issues should be further investigated. In addition to that study,

there has since been one neurologic case sample in the United States, which also found PCV3 in the brain of a PCV3-infected piglet via PCR detection. The case was a one-day-old piglet that was experiencing congenital tremors (Diaz et al., 2020).

Subclinical and Co-infections

A study in the Shandong Province of China identified PCV3 in samples from seven large pig farms that showed no clinical signs in multiparous sows nor in live-born piglets (Zheng et al., 2017). A total of 222 tissue samples, made up of heart, liver, lung, kidney, spleen and umbilical cord from 37 stillborn fetuses were tested for PCV3 on farms with sows and live-born piglets that did not show any clinical signs of disease. The PCV3 positivity rate was 59.5% (132 out of 222) in the tissue samples from stillborn fetuses. Porcine circovirus type 2 was also found in 52 out of the 132 samples (39.4%) that were tested for PCV2 (Zheng et al., 2017). While the cause of the stillborn fetuses could not be fully attributed to an infectious agent in this study, the researchers observed no clinical signs of neither PMWS nor PCV-associated disease in the sows and live-born piglets.

Co-infections have been suggested as a possibility with PCV3 and other viruses that impact the swine industry. While there is still more to understand about the role of co-infections with PCV3, there have been a few studies aimed at evaluating the status of co-infections of swine viruses that cause enteric, reproductive, and respiratory disease. One study did look at the status of co-infection with PCV2, PCV3, and PEDV in piglets with severe diarrhea and found a high rate of co-infection (Guo et al., 2020). In this study, out of 22 pig farms distributed throughout China, 76 samples were collected and showed a low rate of single viral agent infections of PCV2 (2 of 76), PCV3 (3 of 76), and PEDV (5 of 76), but a high rate of infection

with all three viral agents (37 of 76) (Guo et al., 2020). Further information is still needed to understand whether or not co-infection has an effect on the severity of clinical disease outcomes.

Chapter 4 - Vaccines and Prevention Strategies

There have been numerous retrospective studies that have looked at PCV3 and the role it could play in causing porcine circovirus-associated diseases. Fewer studies have been performed to assess whether or not PCV3 is pathogenic on its own. Further knowledge in understanding the pathogenicity and the clinical presentation of PCV3 will help determine how critical of a role PCV3 may play in swine herds and what preventive strategies are necessary. Veterinary vaccines may play an important role in prevention strategies for PCV3 as they have for PCV2, and considerations of the regulatory requirements and gaps in knowledge of PCV3-associated disease are needed to understand possible areas of vaccine development for this novel virus. This chapter will discuss veterinary vaccines and USDA regulations to consider for possible prevention strategies of an emerging virus, the current state of porcine circoviruses for all porcine circovirus types, and considerations for potential development of PCV3 vaccines.

Veterinary Vaccines

The United States Department of Agriculture (USDA) – Animal and Plant Inspection Service (APHIS) is the regulating authority over veterinary biologics, including veterinary vaccines in the United States. The USDA provides guidelines and regulations for veterinary vaccine licensure in the form of Veterinary Services Memoranda (VSM) and Title 9 of the Code of Federal Regulations (CFR), respectively. Vaccines must be licensed and produced in a USDA-licensed production facility to prepare biological products in accordance with 9 CFR Part 102 – Licenses For Biological Products. Overall, based on the Virus-Serum-Toxin Act (VSTA) of 1913, the core principles of all veterinary vaccines are that they are required to be pure, safe, potent, and efficacious. VSM 800.202 provides licensing considerations involving efficacy

studies for prophylactic veterinary biological products including vaccines. The intent of these products is to prevent or control a disease and are efficacious, as defined in VSM 800.202, where they have the capacity to produce a desired or intended result. Efficacy studies are performed in support of the label claims prior to the approval and issuance of a license by the USDA.

Both efficacy studies and field safety studies are the main studies performed in target animals during vaccine development and prior to licensure. An understanding of how emerging viruses, such as PCV3, are defined in their clinical presentation is one of the most important aspects that contribute to the overall success of efficacy and safety studies for the licensure of a vaccine

Veterinary Services Memorandum (VSM) 800.204 provides guidance on target animal safety studies that support product licensure. The overall aim of a target animal study is to assess the safety of the vaccine according to its labeled use. Considerations should be taken into account based on the current knowledge of PCV3 and the regulatory requirements for licensing a vaccine.

Viral veterinary vaccines can be classified into two broad categories of conventional and genetically modified vaccines. Conventional vaccines are made up of killed or inactivated viruses and modified live (MLV) viruses. Different vaccine types have certain advantages and disadvantages that should be considered during vaccine development. Overall cost is an advantage of killed vaccines as these tend to be less expensive to produce. They also will not revert to a virulent form, like a modified live may, which could be a potential safety issue. Modified live vaccines are not suitable for pregnant animals as this would also pose a potential safety concern which should be considered during vaccine development if this is a vaccine that may be used on pregnant animals. Modified live vaccines do tend to mount a better immune

response over killed vaccines, which is quite an advantage. Currently, there are no modified live veterinary vaccines for porcine circoviruses. Recombinant vaccines are a type of genetically modified vaccines and offer safety advantages as compared to conventional vaccines. (Nascimento et al., 2012). All current circovirus vaccines are recombinant, as defined by the "R" in each licensed product code as seen in table 4.1.

Current State of Circovirus Vaccines

While there are multiple licensed veterinary vaccines for PCV2, there are no licensed veterinary vaccines for PCV3. Currently, porcine circovirus vaccines are licensed under the true name, Porcine Circovirus Type 1 and Type 2 Chimera Killed Virus and Type 2 Killed Baculovirus vector (USDA, 2020). All licensed vaccines that include PCV1 are known as true name, Type 1-Type 2 Chimera, Killed Virus. The Chimeric PCV1-2 vaccine is a genetically engineered vaccine that uses the genetic backbone of nonpathogenic PCV1 with the immunogenic capsid protein of PCV2. This was the first USDA fully licensed PCV2 vaccine (Gillespie et al., 2009). Monovalent vaccines are available that protect against PCV2, known by true name, Type 2, Killed Baculovirus Vector (USDA, 2020). The baculovirus vector vaccines are recombinant, subunit vaccines that contain a baculovirus expressed PCV2 capsid protein (Afghah et al., 2017). Porcine circovirus vaccines are also found in combination products, specifically multivalent vaccines with other viral agents and bacterins, described by true names, Porcine Circovirus Vaccine-Mycoplasma Hyopneumoniae Bacterin and Porcine Reproductive and Respiratory Syndrome-Circovirus Vaccine-Mycoplasma Hyopneumoniae Bacterin (USDA). In the most recent publication of Licensed Veterinary Biological Product Information, prepared by the USDA on October 8, 2020, there were no active full licenses or conditional licenses for PCV Type 3 (USDA, 2020). Table 4.1 shown below lists all current USDA licensed porcine

circovirus vaccines from the USDA Current Veterinary Biologics Product Catalog, published on October 8, 2020 (USDA, 2020). The USDA product code is the name given to each licensed vaccines. The product and form describe the type of vaccine product, and the license number of the producer is the number given to each facility that is licensed to develop vaccines under USDA regulation.

Table 4.1. Current PCV Vaccine Licenses

USDA Product Code	Product and Form	License Number of Producer
19K5.R1	Porcine Circovirus Vaccine, Type 1 – Type 2 Chimera, Killed Virus	190
19K5.R3	Porcine Circovirus Vaccine, Type 1 – Type 2 Chimera, Killed Virus	190
19K5.R5	Porcine Circovirus Vaccine, Type 1 – Type 2 Chimera, Killed Virus	190
19K5.R0	Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector	124, 165A, 329
19K5.R2	Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector	124
19K5.R4	Porcine Circovirus Vaccine, Type 2, Killed Baculovirus Vector	124 165A
49K5.R1	Porcine Circovirus Vaccine- Mycoplasma Hyopneumoniae Bacterin, Type 2, Killed Baculovirus Vector	124 165A 329
49K5.R6	Porcine Circovirus Vaccine- Mycoplasma Hyopneumoniae Bacterin, Type 2, Killed Baculovirus Vector	165A
49K5.R5	Porcine Circovirus Vaccine-Mycoplasma Hyopneumoniae Bacterin, Type 1 -Type 2 Chimera, Killed Virus	190
49K5.R7	Porcine Circovirus Vaccine-Mycoplasma Hyopneumoniae Bacterin, Type 1 -Type 2 Chimera, Killed Virus	190
49K9.R0	Porcine Reproductive & Respiratory Syndrome-Circovirus Vaccine-Mycoplasma Hyopneumoniae Bacterin, Reproductive & Respiratory Form, Type 2, Modified Live Virus, Killed Baculovirus Vector	124
A9K5.R2	Porcine Circovirus, Type 1 – Type 2 Chimera, Killed Virus (For further manufacture)	190
A9K5.R0	Porcine Circovirus, Type 2, Killed Baculovirus Vector (For further manufacture)	329

D9K5.R0	Porcine Circovirus Vaccine- Mycoplasma Hyopneumoniae Bacterin, Type 2, Killed Baculovirus Vector (For further manufacture)	329

Vaccine Development Considerations

Overall current porcine circovirus vaccines for PCV2 have been effective in reducing clinical signs and have had a positive impact on the swine industry. The first PCV2 vaccine became commercially available in the US in 2006, at that time PCV2a was the predominant subtype circulating in the US and the target for the first PCV2 vaccine (Afghah et al., 2017). Currently PCV2d is emerging as the dominant strain. (Afghah et al., 2017). The PCV2 vaccines have demonstrated improvements in average daily weight gain and economic benefits in vaccinated pigs compared to unvaccinated pigs (Afghah et al., 2017).

Today, there are two main categories of PCV2 vaccines (as discussed above), an inactivated PCV1-2 chimeric virus vaccine for use in vaccinating three-week-old piglets and a subunit vaccine containing baculovirus expressed PCV2 capsid protein for use in vaccinating three-week-old piglets or older. When considering vaccine technologies for PCV3, a killed option similar to the PCV2 vaccines would be beneficial over modified live vaccines due to the potential association of PCV3 and reproductive failure. Both vaccine technology options that are currently used for PCV2 would be safe options for PCV3 and could be considered as potential technologies for future vaccine development.

Once an initial experimental vaccine technology has been developed, efficacy studies and target animal studies are a large component in the vaccine development process of licensure for veterinary vaccines under USDA regulations. Detailed information in understanding the disease in the target animal is essential to initiate proper animal studies to support the label claim and gain approval from the USDA to move forward with licensure. Prior to initiating studies to

support licensure of vaccines, considerations including detailed target animal information need to be assessed based on the intended use of the vaccine.

While thinking about the efficacy of a potential vaccine, the following regulatory considerations should be taken into account. The final label indication explains what type of animal the vaccine will protect and what it is going to protect against. This is important to consider this early in the development process. The label indication statement per 9CFR112.2(a)(12) states "This product has been shown to be effective for the vaccination of healthy (insert name of species) ___ weeks of age or older against ____.". General indications of target species, minimum age of administration, route of administration, vaccination schedule and any restrictions will be required on the container label per 9CFR112.2(a)(5). The target species, swine in this case, is known but it is important to keep other indications in mind for developing an effective efficacy study for the purpose of gaining licensure. Overall, efficacy studies are designed to support the label claim.

When developing an efficacy study, materials for performing the study will be documented in the protocol and must include detailed formulations of the vaccine experimental product, essential materials or reagents, and information about the placebo as per VSM 800.200. Challenge material must be defined and include the pathogenic new viral agent, PCV3. Previous studies have successfully isolated and cultured PCV3 in PK-15 cells. This information could be beneficial for determining the challenge material; however, further information regarding the appropriate subtype of PCV3a, PCV3b, or PCV3c may need further studies. It is currently unknown which of these subtypes are more virulent than the others, further research into the virulence as well as prevalence and geographic distribution of these three, would be important for development of a vaccine against the emerging subtype. It is important to consider the

subtype upfront to avoid a situation of creating a vaccine to one subtype that does not offer cross-protection to another subtype that might be the more dominant subtype in the field. This has been the case with PCV2 vaccines. When PCV2 vaccines were first created, PCV2a was the dominant subtype in the United States and vaccines targeting PCV2a were created and continue to be used today (Afghah et al., 2017). Recently, however, PCV2d is emerging as the dominant strain, and studies have found that the current vaccine may not show complete reduction in viremia (Afghah et al., 2017).

The pigs used in efficacy studies would need to be defined by the age, breed, and sex. Retrospective studies have found PCV3 detection in pigs of all ages and further experimental studies have shown signs of clinical disease in young piglets. A gap in current knowledge that is important for methods and criteria of efficacy studies is breed type. Very few published articles on PCV3 specified breed type. One article that was able to reproduce clinical signs of disease reported using Duroc crossed with Large White pigs (Jiang et al., 2019). Statistical information must also be considered, including experimental unit, number of experimental units, criteria for inclusion or exclusion, and randomizations plans per VSM 800.200. Careful consideration should include how animals will be identified, information on co-mingling, and detailed housing arrangements of the animals as per VSM 800.200.

Once the focus of the study is determined, information on expected observations and outcomes should be considered. Timing and frequency of observations, the expected outcome for clinical trials, and criteria for interpreting results per VSM 800.200 to finalize efficacy studies must be considered based on current knowledge of PCV3. It is important to note that efficacy studies need to define a measurable effect. Statistics can play a large role in this especially in the

case that there may be a small number of animals that develop clinical signs of disease in order to detect a statistical difference in efficacy.

As more controlled studies are performed using a PCV3 challenge model to help determine signs of clinical presentation. These signs are essential in determining efficacy study observations and outcomes to ensure a measurable effect during challenge. Current knowledge has shown clinical signs of PDNS in an experimental infection study (Jiang et al., 2019), and it is possible this information could be used as a clinical measurement for challenge studies. Although numerous retrospective studies have linked PCV3 to cases of reproductive failure, it is important to note that it has not been determined that PCV3 is the primary cause reproductive failure. However, when considering vaccine development, if a reproductive claim is considered, then efficacy studies will need to support a reproductive claim. Numerous retrospective articles on reproductive failure with the detection of PCV3 have shown that vertical transmission is possible (Ku et al., 2017). This is also a key element to efficacy and safety studies if this label claim is intended. Identifying an experimental model of disease is a key component of having a successful challenge study.

Veterinary Services Memorandum (VSM) 800.204 provides guidance on target animal safety studies that support product licensure. Field safety studies require testing in three distinct regions in the United States, with the possibility of data from countries outside of the U.S. Since it is known that PCV3 has been identified in North Carolina and states throughout the Midwest, these could be potential regions chosen for field safety studies. Similar to efficacy studies, the number and age of animals must be considered for field safety studies. Further studies into what age groups are most affected by clinical signs and symptoms of disease still need to be investigated, but PCV3 has been detected in young piglets and should be considered for the

minimum age testing as required in VSM 800.204. Pigs of breeding age would also need to be considered with the known information on vertical transmission of PCV3 (Ku et al., 2017). Further information on passive immunity would need to be studied, but as more evidence points to the possibility of reproductive failure associated with PCV3, safety testing in both adults and neonates would be required to support possible label claims for passive immunity.

Full license efficacy considerations are listed above; however, there are alternate licenses that could be considered for an emerging virus like PCV3. Conditional licenses could be considered as they have less efficacy requirements than a full license. The efficacy requirements, as defined in 9CFR102.6, state these vaccines must show a reasonable expectation of efficacy for a conditional license. These vaccine licenses are typically used for emerging diseases when a full license is not available. In addition to types of licensure, while a monovalent vaccine may be appropriate for an emerging virus, multivalent vaccines should also be considered as a potential option for licensure. Porcine circovirus type 3 has been detected in retrospective studies with other viral and bacterial agents and thought to possibly play a role in co-infections. Pending more information on the disease-causing status of PCV3, the potential of a multivalent vaccine for PDNS or multivalent vaccine for reproductive failure should also be considered.

Export Considerations

Porcine circovirus type 3 has been identified in multiple countries across the world and country specific regulations should be considered during vaccine development. Currently, PCV3 has been identified in North America, South America, Europe, and Asia. While the USDA regulates US Veterinary Biologics, the following regulatory agencies will need to be considered in regard to potential vaccine exports to countries with positive PCV3 cases: SAGARPA, European Medicines Agency (EMA), and China Institute of Veterinary Drug Control. Different

regulatory agencies have different requirements in the vaccine development pathway. As more testing is performed and further information about the virus is understood, PCV3 may be identified in more countries, thus expanding the need for additional country/region specific regulatory requirements.

Chapter 5 – Conclusion

Porcine circovirus type 3 (PCV3) has been detected in swine on four continents spanning numerous countries across the world. Porcine circovirus type 3 has been detected in healthy animals as well as diseased herds showing signs of reproductive failure, porcine dermatitis and nephropathy syndrome (PDNS), respiratory and neurologic issues as well as subclinical infections and co-infections with other viral and bacterial agents. While PCV3 pathogenesis remains unclear, retrospective studies have recently uncovered more information about the frequency of PCV3 in reproductive cases as well as more information into the possibility of vertical transmission. Experimental studies have also linked clinical signs of PDNS to PCV3 infection, which is important information to develop successful challenge models for vaccine efficacy studies.

As with any novel virus, it is important to consider prevention strategies early in discovery. Veterinary vaccines have been essential in controlling and preventing disease caused by pathogenic PCV2, and currently, PCV2 is the only porcine circovirus with licensed vaccines. It is too early to tell if the novel circovirus PCV3 will overtake the impact of PCV2, but determining gaps in information that may help determine specific vaccine claims will be beneficial to consider as more is understood about the virus.

Although PCV3 was first discovered in the US, it has worldwide implications for the swine industry. Regulatory requirements to successfully license a PCV3 vaccine in the US and elsewhere would be important to consider. In the US, this would fall under the authority of the USDA, but it is also important to consider regulatory requirements for vaccine development in other countries where PCV3 has been associated with disease. Efficacy studies and field safety

studies play an important role in part of the vaccine licensure process. Understanding certain aspects of the known clinical presentation of PCV3 and detailed information about animals affected by PCV3 would be essential. In addition, any gaps in information, including subtype virulence and frequency, along with co-infection status, and proper challenge models should be considered to develop successful animal studies for vaccine licensure. Overall, it appears that PCV3 could have the potential of causing a widespread negative impact on the swine industry and considering future areas of vaccine development early on could assist in controlling and preventing possible disease outbreaks and transmission.

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