INCIDENCE OF PORCINE CIRCOVIRUS TYPE 2 AND PORCINE PARVOVIRUSES

IN SWINE HERDS OF SOME COMMUNITIES IN

EASTERN CAPE, SOUTH AFRICA

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BY

AFOLABI KAYODE OLAYINKA

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE

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ALICE 5700

SOUTH AFRICA

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APRIL 2018

DECLARATION

I, the undersigned, declare that this thesis entitled "Incidence of porcine circovirus type 2 and porcine parvoviruses in swine herds of some communities in Eastern Cape, South Africa" submitted to the University of Fort Hare for the degree of Doctor of Philosophy in Microbiology in the Faculty of Science and Agriculture, School of Biological and Environmental Sciences, and the work contained herein is my original work with exemption to the citations and that this work has not been submitted to any other University in partial or entirely for the award of any degree.

Name: **Kayode Olayinka Afolabi**

Signature: …………………………….

Date: …..………………………...

DECLARATION ON PLAGIARISM

I, Kayode Olayinka Afolabi, student number: 201512532 hereby declare that I am fully aware of the University of Fort Hare's policy on plagiarism and I have taken every precaution to comply with the regulations.

Signature…………………………………   Date……………………………

CERTIFICATION

This thesis entitled "Incidence of porcine circovirus type 2 and porcine parvoviruses in swine herds of some communities in Eastern Cape, South Africa" meets the regulation governing the award of degree of Doctor of Philosophy of the University of Fort Hare and is approved for its contribution to scientific knowledge and literary presentation.

…………………………………..   ……………………………….

Prof. L.C. Obi   **Date**

Major Supervisor

DEDICATION

To the Immortal, Inviscible, the only Wise God; who has been my source of life, wisdom,

inspiration, knowledge and understanding,

To my darling wife, Comfort; for her unending love and unfailing support,

To my wonderful children: Goodness, Gracious, Greatness and Glorious; for their rare kind of

understanding and prayers,

To a friend like blood brother, Yinka Titilawo; for making this dream a reality,

To my lovely parents, for their perpectual songs of excellence to my hearing from my childhood

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To the memory of my dear brother, Olusola; who passed on towards the end of this programme.

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LIST OF ACRONYMS

ACT: Arctigenin

ADV: Aujeszky's disease virus

AEMREG: Applied and Environmental Microbiology Research Group

BLAST: Basic Local Alignment Search Tool

BLASTN: Basic Local Alignment Search Tool for Nucleotides

BPV2:   Bovine Parvovirus 2

CSF: Classical Swine Fever

DAFF: Department of Agriculture, Forestry and Fisheries

DNA: Deoxyribonucleic Acid

ELISA: Enzyme-Linked Immunosorbent Assay

EMCV: Encephalomyocarditis Virus

FAO: Food and Agriculture Organization

GMRDC: Govan Mbeki Research and Development Centre

HEV: Hepatitis E Virus

ICVTV: International Committee on the Taxonomy of Viruses

IHC: Immunohistochemistry

IR: Intergenic Region

ISH: *In Situ* Hybridization

LAMP: Loop-Mediated Isothermal Amplification

MDGs: Millenium Development Goals

ML: Maximum Likelihood

mPCV2b:	Mutant Porcine Circovirus Type 2b
NCBI:	National Centre for Biotechnology Information
NES:	Nuclear Export Sequence
NJ:	Neighbour Joining
ORF:	Open Reading Frame
PASC:	Pairwise Sequence Comparisons
PBo-likeV:	Porcine Boca-Like Virus
PBoVs:	Porcine Bocaviruses
PCR:	Polymerase Chain Reaction
PCR-RFLP:	polymerase chain reaction-restriction fragment length polymorphism
PCV2-IM2:	PCV2 Intermediate Clade 2
PCV2-SI:	PCV2 Subclinical Infections
PCVAD:	Porcine Circovirus-Associated Diseases
PCVs:	Porcine Circoviruses
PDNS:	Porcine Dermatitis and Nephropathy Syndrome
PMWS:	Porcine Multisystemic Wasting Syndrome
PPV-Kr:	Porcine Parvovirus Kresse
PPV-NADL2:	Porcine Parvovirus NADL-2
PPVs:	Porcine Parvoviruses
PRDC:	Porcine Respiratory Disease Complex
PRRSV:	Porcine Reproductive and Respiratory Syndrome Virus
RNA:	Ribonucleic Acid
RPA:	Recombinase Polymerase Amplification

SA:	South Africa
SAMRC:	South Africa Medical Research Council
SDG:	Sustainable Development Goals
SIV:	Swine Influenza Virus
SL:	Stem Loop
SMEDI:	Stillbirth, Mummification, Embryonic Death and Infertility
SPF:	Specific-Pathogen-Free
ssDNA:	Single-Stranded DNA
ssRNA:	Single-Stranded RNA
TTV:	Torque Teno Virus
UK:	United Kingdom
USA:	United States of America

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GENERAL ABSTRACT

GENERAL ABSTRACT

Porcine circovirus type 2 (PCV2) is one of the swine pathogens of global economic importance. Since its first detection in early 1990s as the main etiologic agent of porcine multisystemic wasting syndrome (PMWS) and many other porcine circovirus-associated diseases (PCVAD), the virus has been extensively studied and has been found to be present in virtually all the pig producing countries of the world. As a viral pathogen that brings about clinical diseases aided by co-infecting pathogens, the involvement of many other viral agents including porcine parvoviruses (PPVs) have caught the attention of stakeholders worldwide. However, no surveillance study of the viral pathogens has been carried out in South Africa as there are little or no information on their prevalence in the swine herds of the country. This present study therefore aimed at detection and molecular characterization of PCV2 and PPVs in swine herds of some selected communities in Eastern Cape Province, South Africa. A total of 375 field samples were collected from seven commercial and communal farms from three District Municipalities of Eastern Cape, South Africa between 2015 and 2016. Structured questionnaires were also administered to each farm at the time of sample collection to obtain some important information relating to health status and farm management practices in the sampled farms. With the aid of conventional PCR method, 339 samples were initially screened for the presence of PCV2; positive amplicons were sequenced and obtained partial genomes of the virus were preliminarily analyzed. In order to obtain the complete genomes of the virus, four overlapping primer pairs were used to amplify the full-genome of PCV2 from the initial positive samples; amplified genomes were sequenced using the Sanger methods, sequenced PCV2 genomes were assembled and characterized. Furthermore, the prevalences of some designated PPVs in the sampled farms were obtained using 110 samples randomly selected from the previously archived samples and

screened with 6 different primer pairs specific for the detection of 7 PPVs. All the amplified parvoviruses' genomes were sequenced; their sequenced partial genomes were subsequently base-culled and analysed. The data obtained revealed that 54/339 (15.93%) samples from the swine herds were positive for PCV2; whereas the degree of occurrence of the viral pathogen as observed at farm level ranges from approximately 5.6 to 60%. The majority 15/17 (88%) of the analyzed partial sequences were found clustering with other PCV2b strains in the phylogenetic analysis. More interestingly, two other sequences obtained were also found clustering within PCV2d genotype in the initial screening and analysis. Furthermore, 15 complete PCV2 genomes were successfully amplified, sequenced and assembled. NJ and ML phylogenetic analysis of the complete ORF2 gene and full genomes unanimously showed 11 of the assembled genomes belonging to genotype PCV2b. Another 3 of the characterized sequences formed clade with other reference mutant PCV2b and PCV2b subtype 1C (PCV2d) strains from different parts of the world. The last sequence however, clustered with other reference strains belonging to PCV2 intermediate clade 2 (PCV2-IM2) recently identified in a global PCV2 strains phylogenetic analysis. Other genetic analyses including multiple sequence alignment and p-distance analysis also confirmed the outcomes of the phylogenetic analyses of the complete capsid gene and full-genomes of the virus. On the other hand, the findings of the molecular profiling for PPVs showed that all the screened parvoviruses were present in the study area, having prevalence of 29.1% (PPV1), 21.8% (PPV2), 5.5% (PPV3), 43.6% (PPV4), 21.8% (PBo-likeV) and 44.6% for PBoV1 and PBoV2. Double infection of the screened PPVs was observed to be very rampant among the pigs as high as 20/110 (18.2%) for PPV2/PPV4 and PPV4/PBoV; followed by 19/110 (17.3%) of the samples for PPV1/PPV4 and PPV1/PBoV. Three of the viruses were found simultaneously in 19 of the screened samples representing 17.3%, whereas 8 (7.3%) samples

were positive for four of the viruses. Phylogenetic analyses of PPV1, PPV2 and PBoVs 1 and 2 were conducted with two major clades homologous for each of them. This is the first report of PCV2 in swine herds of the Province and the first detection of PCV2b, PCV2d and PCV2-IM2 strains in South African swine herds. It follows the first reported case of PCV2a in an outbreak of porcine multisystemic wasting syndrome (PMWS) in Gauteng Province, South Africa over two decades ago. Also, this is the first major epidemiologic study on PPVs in the country following the initial case study of 1975. These findings confirmed the presence of the all-important viral pathogens among pigs and also give preliminary insights into the possibility of co-infections of the pathogens in the studied area. This could however result in a serious large scale outbreak of devastating disease(s) associated with the viral pathogens, thereby ultimately resulting in huge economic losses if no appropriate measures are taken to effectively curb their spread across the country.

CHAPTER ONE

GENERAL INTRODUCTION

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1.1   Introduction and background to the study

Globally, agricultural sector provides means of livelihood for larger human population than any other industry. Livestock production, however, constitutes more than average value of the total agricultural proceeds and an appreciable amount in developing countries (Upton, 2004). Pig husbandry is one of the rapidly growing livestock sector due to some cutting-edge advantages over others (FAO, 2012). Foremost, there is an increasing demand for pork and pork products as a result of the rapid population growth and the need for cheaper and affordable source of animal protein. In addition, pig has a high fecundity with an outstanding high growth rate; i.e. a sow can farrow twice in a year with an average of 6-8 liters per time (Figure 1.1), whereas a weaned piglet of twenty-one days could attain a market size of about 280 pounds within five to six months of age (FAO, 2012; Ironkwe and Amefule, 2008).

The aforementioned benefits among others, have therefore, made pig to be one of the leading source of animal protein globally among the red-meat producing farm animals including cattle, sheep and goats; and the role played by the piggery industry in the economy of many nations remains formidable and highly significant (Ironkwe and Amefule, 2008; Khan *et al*., 2013). As a result, pig production was noted to have a great potential in achieving Millenium Development Goals (MDGs) in the area of poverty eradication and solving hunger challenges (which are still important aspect of the current United Nations' Sustainable Development Goals – SDGs) in developing regions such as Africa (FAO, 2012). South Africa is not left behind in pig production as the country has about 4,000 commercial pork producers and 19 stud breeders, producing about 1.6 million pigs commercially and boasts of R 2.7 billion average gross value of slaughtered pigs annually (DAFF, 2015). South Africa pork production industry according to DAFF (2015) provides employment for about 10,000 people. Despite the huge potentials associated with pig

rearing, the venture is faced with different challenges orchestrated by infestation of diverse kind of diseases that could hamper its economic potentials. Such diseases are those caused by porcine circovirus type 2 (PCV2) and porcine parvoviruses (PPVs).

Figure 1.1: Sow and its breastfeeding liters (*Source: FAO, 2012).*

Porcine circoviruses (PCVs) belong to the genus *Circovirus* in the family *Circoviridae*. They are group of viruses with circular single-stranded DNA genome of about 1.76 kb, packaged in a non-enveloped capsid (Tischer *et al*., 1982). Their genomes consist of two main open reading frames (ORFs) with an intergenic region that contains the origin of replication lying between them (Figure 1.2). They are the smallest known autonomous animal viruses, comprising of porcine circovirus type 1(PCV1) with genome size of about 1759 nucleotides (nt) and porcine circovirus type 2 (PCV2) with genome of 1767/1768 nt long (Hamel *et al*., 1998; Meehan *et al*., 1998;

Morozov *et al*., 1998). The detection of PCV1 occurred as a contaminant of the cell line made from kidney of pig (PK-15, ATCC CCL-33) in 1974; and further investigation into its pathogenic potential showed that it was not pathogenic (Tischer *et al*., 1974; Tischer *et al*., 1986).

However, the PCV2 which was detected about twenty years later from pigs with systemic disease called postweaning multisystemic wasting syndrome (PMWS) has now become swine pathogen of huge economic importance (Allan and Ellis, 2000). Genomic sequence comparison of PCV1and PCV2 strains generally showed about 83% and 67% sequence identity for replicase and capsid genes respectively (Allan *et al.,* 2012). Recently, another pathogenic species that has 55% and 37% identity (for replicase and capsid proteins respectively) with PCV2 has been detected in the USA (designated porcine circovirus type 3 – PCV3) from diseased pigs; having a bigger genome size of 2000 nt compared to the previous two species (Palinski *et al*., 2017) (Table 1.1).
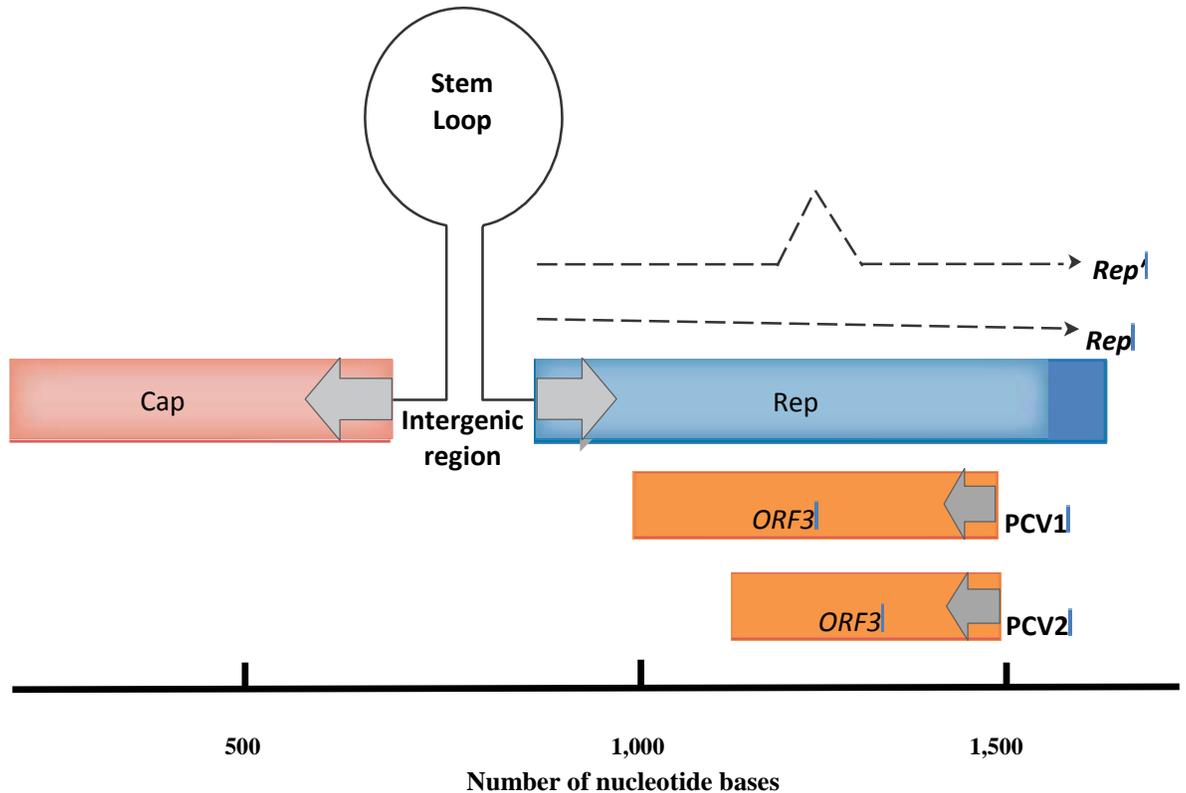
Figure 1.2: The linear format of PCV1 and PCV2 genome arrangement and their gene transcription patterns (*Adapted from Meng, 2013*).

In the diagram above, the two main ORFs for capsid and replicase genes are depicted by the two boxes consisting of inner arrows that show the direction of their translation. There is an intergenic region spanning between the two major ORFs with a stem loop structure located in between it. The ORF3 in PCV1 is a bit longer than the one in PCV2. The first replicase protein (Rep) is obtained from the translation of its whole gene, while the second one (Rep′) is formed through an alternative splicing of the first one as indicated by the upper disjointed line.

Table 1.1: Porcine circoviruses species till date

Species	Source	Place/Year of detection	References
PCV1	Pig kidney cell line (PK-15 ATCC-CCL31)	Germany / 1974	Tischer *et al*., 1974
PCV2	PMWS-affected pigs	Canada / 1991	Clark, 1996; Harding, 1996
PCV3	Sows with reproductive failure / Porcine dermatitis and nephropathy syndrome (PDNS)	North Carolina / 2015	Palinski *et al*., 2017
	Cardiac and multi-systemic inflammation	Missouri / 2015; Minnesota / 2016; South Dakota / 2016	Phan *et al*., 2016
	Sows with reproductive failure	Hubei Province, China / 2016	Fan *et al*., 2017
	Piglet with anorexia and fever	Guangdong province, China / 2016	Shen *et al*., 2018

PCV2 was initially implicated as the main causative pathogen of PMWS in weaned pigs and growers within the age range 6 to 16 weeks. Aside PMWS, the virus has been associated with arrays of swine clinical conditions generally regarded to as porcine circovirus associated diseases (PCVAD) (Table 1.2; Figure 1.3). PMWS is a globally established, multifactorial, wasting syndrome that is associated with PCV2 infection and its other co-infecting agents which include viruses such as classical porcine parvovirus (Opriessnig *et al*., 2014). The classical porcine parvovirus (PPV), also called porcine parvovirus type 1 (PPV1), is a single-stranded, negative-sense, linear DNA viral pathogen with genome length of about 5000 bases packaged in a non-enveloped icosahedral capsid (Molitor *et al*., 1983). Its genome has a characteristic hairpin terminus peculiar to it (Bergeron *et al*., 1993, Bergeron *et al*., 1996 and Tattersall, 2006).

PPV1 belongs to the genus *Protoparvovirus*, subfamily *Parvovirinae* and family *Parvoviridae.* The vertebrate's parvoviruses are classified under the subfamily *Parvovirinae,* while the subfamily *Densovirinae* is group of arthropods-infecting parvoviruses. Presently, the subfamily *Parvovirinae* is classified into eight genera in the latest proposal of International Committee on the Taxonomy of Viruses (ICTV), including four genera that contain porcine viruses namely, *Protoparvovirus, Tetraparvovirus, Copiparvovirus* and *Bocaparvovirus* (Cotmore *et al*., 2014). The advancement in molecular techniques for the detection of pathogens has led to the discovery of many other novel porcine parvoviruses which are now currently grouped into four genera of the eight presently under the subfamily earlier mentioned (Table 1.3). More recently, some other porcine parvoviruses have been detected and designated as PPV5-7, but yet to be grouped (Xiao *et al*., 2013a; Ni *et al*., 2014; Schirtzinger *et al*., 2015; Palinski *et al*., 2016; Xing *et al*., 2018).

Table 1.2: Porcine circovirus associated diseases and their clinical features

Name of disease	Type of pigs usually affected	Other implicated or co-infecting pathogens	Clinical signs
Post-weaning multisystemic wasting syndrome	Nursery, growing, and adult pigs	PPV1, porcine reproductive and respiratory syndrome virus (PRRSV), *Mycoplasma hyopneumoniae* etc.	Wasting, weight loss, pallor of the skin, ill thrift, enlarged lymph nodes, diarrhea, and respiratory distress.
Porcine dermatitis and nephropathy syndrome	Nursery, growing, and adult pigs	PRRSV and some bacteria such as *Actinobacillus pleuropneumoniae, Escherichia coli, Haemophilus parasuis*	Severe weight loss, depression, anorexia, mild pyrexia, stiff-gait, dark –red papules and macules on skin, majorly at the hind limbs and perineal region.
Porcine respiratory disease complex	Common in 8 to 26 week-old pigs	PRRSV, swine influenza virus (SIV), *A. pleuropneumoniae*, *P. multocida* and *M. hyopneumoniae*	Respiratory disorders, slow growth, Pneumonia, anorexia, dyspnea, fever, lethargy, cough and decreased feed efficiency.
Reproductive failure	Sows	PPV1, encephalomyocarditis virus (EMCV), Aujeszky's disease virus (ADV), PRRSV	Late term abortions, stillbirths, premature piglets birth, fetal mummification, mid-gestation abortion, early embryonic death and regular return-to-estrus.
Granulomatous enteritis	Common in 8 to 16 week-old pigs	*Lawsonia intracellularis*	Diarrhea, unique lesions in Peyer patches.
Exudative epidermitis	Piglets of 5-35 days old, occasional mild cases in adult pigs	*Staphylococcus hyicus*, PPV1	Skin with an odoriferous exudate of serum and sebum, resulting to a dirty, moist and greasy appearance.

(*Culled from: Chae, 2005; Segalés, 2005; Opriessnig et al., 2007*)

Figure 1.3: Typical examples of porcine circovirus associated diseases. A. PMWS–affected pigs; B. Granulomatous enteritis with inner figure (a) showing brown-stained PCV2 antigen within lymphocytes and macrophage-like cells situated in the lamina propria and Peyers patches of the ileum, the inner figure (b) showing a very thick intestinal mucosa and abnormally large mesenteric lymph node, while the inner figure (c) showed growers with mild diarrhea; C. PCV2-associated reproductive failure with lineup of foetuses at their different stages of mummification; D. A pig with clinical sign of porcine dermatitis and nephropathy syndrome (PDNS) with the entire body covered by raised coalescing red-purple lesions (*Source: Opriessnig et al., 2007*).

Table 1.3: Designated porcine parvoviruses based on ICTV latest report

Genus	Species	Virus/virus variant	Abbreviation	Reference
Protoparvovirus	*Ungulate protoparvovirus 1*	porcine parvovirus Kresse	PPV-Kr (PPV1)	Bergeron *et al*., 1996
		porcine parvovirus NADL-2	PPV-NADL2 (PPV1)	Bergeron *et al*., 1993
Tetraparvovirus	*Ungulate tetraparvovirus 3*	porcine Cn virus	CnP-PARV4 (PPV2)	Hijikata *et al*., 2001; Wang *et al*., 2010
	Ungulate tetraparvovirus 2	porcine hokovirus	PHoV, P-PARV4 (PPV3)	Lau *et al*., 2008
Copipavovirus	*Ungulate copiparvovirus 2*	porcine parvovirus 4	PPV4	Cheung *et al*., 2010
		porcine bocavirus 1	PboV1	Cheng *et al*., 2010
	Ungulate bocaparvovirus 2	porcine bocavirus 2	PboV2	Cheng *et al*., 2010
		porcine bocavirus 6	PboV6	Shan *et al*., 2011
	Ungulate bocaparvovirus 3	porcine bocavirus 5	PboV5	Zeng *et al*., 2011
Bocaparvovirus	*Ungulate bocaparvovirus 4*	porcine bocavirus 7	PboV7	Shan *et al*., 2011
		porcine bocavirus 3	PboV3	Lau *et al*., 2011
	Ungulate bocaparvovirus 5	porcine bocavirus 4-1	PboV4-1	Lau *et al*., 2011
		porcine bocavirus 4-2	PboV4-2	Lau *et al*., 2011

Parvoviruses are group of infectious viral pathogens that infect broad categories of vertebrate and invertebrate hosts with various clinical disease manifestations (Xiao *et al*., 2013b). The viral group members generally have a small, single-stranded and non-segmented linear DNA genome ranging from 4.0-6.3 kb in size (Tijssen *et al*., 2011). There are two large ORFs situated in their genome; the first one is the coding region for the non-structural proteins needed for viral replications, while the other encodes the structural capsid proteins (Figure 1.4A). One additional ORF3 has been identified in porcine parvovirus type 4 (PPV4) and bocaviruses that encodes a non-structural accessory protein (NP1) (Cheng *et al*., 2010, Cheung *et al*., 2010) (Figure 1.4B).

Among all other PPVs, PPV1 is ranking high among the pathogens that impair reproductions in pigs and accountable for the major economic losses in pig industry globally (Cartwright and Huck, 1967, Hueffer and Parrish, 2003). PPV1 is believed to be present in swine herds perpetually due to its extreme stability in the environment which exposes pigs in infected herds to repeated infections overtime (Almond *et al*., 2006; Mengeling, 2006). Previous epidemiological and diagnostic surveys have shown PPV1 as the main etiologic viral pathogen implicated in embryonic and foetal death in sows (Van-Leengoed *et al*., 1983; Mengeling *et al*., 1991). The reproductive syndrome caused by PPV1 is generally characterized by stillbirth, mummification, early embryonic and foetal mortality, as well as delayed return to estrus and infertility; with an acronym 'SMEDI' (Szelei, *et al*., 2006, Truyen and Streck, 2012) (Figure 1.5). Furthermore, PPV1 has also been implicated in clinical conditions relating to interstitial nephritis in slaughter-aged pigs, non-suppurative myocarditis in sucking piglets and swine skin lesions (Kresse *et al*., 1985; Whitaker *et al*., 1990; Lager and Mengeling, 1994; Bolt *et al*., 1997; Drolet *et al*., 2002).
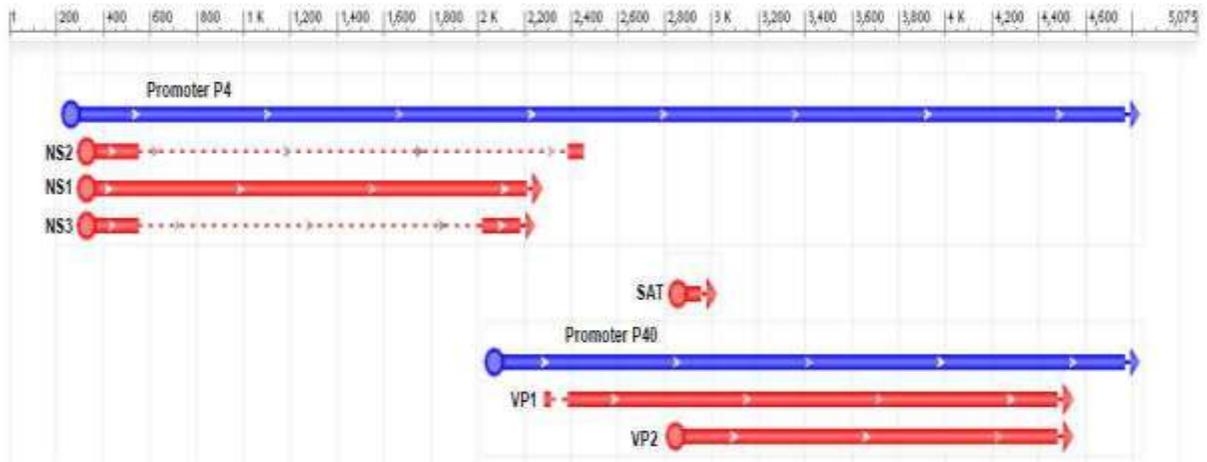
(A)
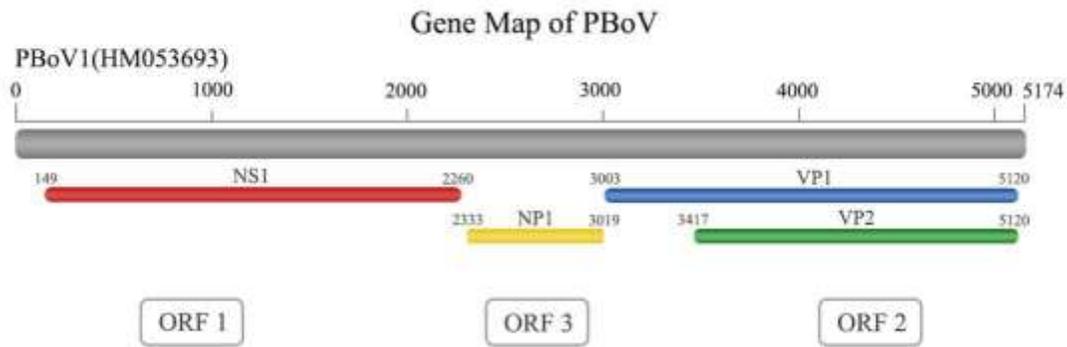
(B)

Figure 1.4: (A) Genome structure of a typical porcine parvovirus (B) Genomic map of a typical PboV (HM053693) (*Source: Streck, 2013; Zhou et al., 2014*).

In figure 1.4A, the blue arrow shows mRNA precursor while the red arrows show products of the gene; the ruler at the top represents the nucleotide numbers. The genomic structure represents that of strain NADL2 (**NC_001718)** as generated from the National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/). In figure 1.4B, the genome of PBoV contains one additional ORF3 as it could also be found in PPV4 genome. The sequences for *VP1* and *VP2* are overlapping in the genome.

Figure 1.5: Litters from a pregnant sow at the 90th gestation after the sow was inoculated with a virulent PPV isolate. It shows a typical reproductive failure associated with PPV1-infection (*Adapted from Zeeuw et al., 2007*).

As one of the PCV2 co-infecting allies, PPV1 has been confirmed to have great effects on increasing the global PCV2 infection trauma through the development of the economically significant disease called PMWS (Krakowka *et al*., 2000; Segalés *et al*., 2005). Natural co-infection of PCV2 and PPV1 has been shown in a reasonable numbers of cases of PMWS in pigs from different places (Ellis *et al*., 2000, Kim *et al*., 2002; Yue *et al*., 2009; Sun *et al*., 2015). It has equally been demonstrated in experimental cases that co-infection of both PCV2 and PPV1

aggravates the disease condition in PMWS compared to when only PCV2 is involved (Kennedy *et al*., 2000, Krakowka *et al*., 2000). However, till date, there is dearth of information on the actual pathogenicity potentials of other newly detected porcine parvoviruses (Xiao *et al*., 2013b). Nonetheless, high prevalence of the viruses has been associated with PCV2-infection and have been reported to be common in PCVAD cases; thereby showing some significant connections with the economically important, circular DNA virus (Opriessnig *et al*., 2014; Sun *et al*., 2015).

According to Alarcon *et al*. (2013), one of the PCV2 associated diseases, PMWS, is a multifactorial systemic swine disease of high economic importance in pig industry. In their investigation on the economic implication of PMWS and PCV2 subclinical infections (PCV2-SI) in some farrow-to-finish facilities in England in 2008 before the commencement of large scale vaccination, estimates of £52.6 million and £88 million per year were spent during the period of no outbreak and when there was outbreak respectively. Considering the magnitude of a typical economic loss due to PMWS in English swine industry, the need for a rigorous surveillance on PCV2 and its other co-infecting pathogens cannot be overemphasized in any pig-producing nation of the world.

Presently, there is gross neglect in determining the presence and prevalence of PCV2 and PPVs in South Africa and the entire sub-Saharan Africa region (Afolabi *et al*., 2017). Till date, only PCV2 (strain SA1) detection that was reported by Drew *et al*. (2004) from a PMWS case study in a well-managed breeding farm in Gauteng Province and PPV1 dated back as 1985 in an outbreak of swine reproductive failure are reported in the country. However, the reported previous incidence of some other related viral diseases' outbreak in the Republic and their huge

economic impact calls for proactive surveillance measures of these all-important swine viral pathogens.

According to Bührmann (2007), the outbreak of porcine reproductive and respiratory syndrome virus (PRRSV) that occurred in March 2004 at the Cape flats; which later spread to 144 farms and resulted to the killing of over fourteen thousand pigs, costs the government about 12 million rand by October 2005 to terminate. Also, in the month of July same year, there was a Classical Swine Fever (CSF) disease outbreak in a district of Western Cape; which resulted in the killing of over one thousand five hundred pigs owned by almost a hundred farmers, costing the government approximately 2 million rand as compensations for the affected farmers (Bührmann, 2007). Surprisingly, these two recorded occurrences were like a tip of an iceberg when compared to the devastating aftermath of the CSF outbreaks that eventually occurred in the Eastern Cape Province with an approximate 460,000 pigs belonging to about 120,000 farmers were culled. While compensation of about R300 million was made by the government; other costs for logistics and personnel involved in terminating the outbreak took a whooping sum of about R500 million. The overall cost implication of the outbreak amounted to R900 million (Bührmann, 2007). The funds could have been channeled for infrastructural development or any other sector deemed fit by the government. Considering the huge economic importance of PCV2 and PPVs also as reported globally, the study of their occurrence in any swine herd becomes imperative in order to employ adequate preventive and control measures; thereby forestalling large scale outbreak of their associated diseases.

1.2   Research justification

Despite the profitability of the pig farming business, diverse challenges accrued from numerous swine diseases abound to the detriment of the enterprise. Also, the possibility of growing productivity is debarred by excessive loss of animals to various diseases (Muhanguzi *et al*., 2012; Muwonge *et al*., 2012; Ndyomugyenyi and Kyasimire, 2015). This challenge is further complicated by shortage of information on the occurrence and prevalence of different diseases with their associated pathogens *vis-a-vis* their impacts on production, most especially in sub-Saharan Africa (Dione *et al*., 2014).

Till date, a vast number of PCV2 and PPVs strains from pigs of diverse health statuses have been widely documented (Mengeling *et al*., 1991, Choi *et al*., 2002, Larochelle *et al*., 2002, De boisseson *et al*., 2004, Grierson *et al*., 2004, Kim and Chae, 2004, Knell *et al*., 2005, Wen *et al*., 2005, Cheung *et al*., 2010, Cui *et al*., 2012, Ni *et al*., 2014, Jeoung *et al*., 2015, Sun *et al*., 2015). However, there is little information on the detection and characterization of the viral pathogens in circulation within swine herds of South Africa. Furthermore, the health status of pigs in Eastern Cape Province as regarding PCV2 and PPVs with their numerous associated diseases is practically unknown. Hence, this study is meant to unravel the exact picture of the prevailing disease condition orchestrated by the viruses in swine herds of the province and to give a signal to other South African provinces on the viral pathogens.

1.3   Research Hypothesis

➢ The hypothesis set for this study was that swine herds in the Eastern Cape Province, South Africa are not infected with PCV2 and porcine parvoviruses.

1.4   Broad Aim

➢ The broad aim of this study was to carry out molecular surveillance on PCV2 and porcine parvoviruses in selected swine herds from different communities in Eastern Cape Province, South Africa.

1.5   Specific objectives

The specific objectives set for this study are as follows:-

➢ To carry out molecular screening for PCV2 and PPVs genetic materials from blood, faecal and nasal swab samples obtained from swine in selected communities in Eastern Cape, South Africa

➢ To carry out partial and complete genome amplifications and sequencing of the detected viruses

➢ To carry out genetic characterization of the detected viral genomes using appropriate bioinformatics approaches

➢ To compare the sequences of PCV2 and PPVs from the Eastern Cape, South Africa with the global vaccine strains and draw logical inference by sequence similarity, effectiveness or otherwise of the vaccines on pigs in the Province.

References

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017,* 2017:6807964.

Alarcon, P., Rushton, J. and Wieland, B., 2013. Cost of post-weaning multi-systemic wasting syndrome and porcine circovirus type-2 subclinical infection in England–An economic disease model. *Preventive Veterinary Medicine*, *110*(2), pp.88-102.

Allan, G., Krakowka, S., Ellis, J. and Charreyre, C., 2012. Discovery and evolving history of two genetically related but phenotypically different viruses, porcine circoviruses 1 and 2. *Virus Research*, *164*(1), pp.4-9.

Allan, G.M. and Ellis, J.A., 2000. Porcine circoviruses: a review. *Journal of Veterinary Diagnostic Investigation*, *12*(1), pp.3-14.

Almond, G.W., Flowers, W.L., Batista, L. and D'Allaire, S., 2006. Diseases of the Reproductive system. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. 9th Eds., *Diseases of Swine*. Ames, Iowa: Blackwell Publishing, pp.113–147.

Bergeron, J., Hebert, B. and Tijssen, P., 1996. Genome organization of the Kresse strain of porcine parvovirus: identification of the allotropic determinant and comparison with those of NADL-2 and field isolates. *Journal of Virology*, *70*(4), pp.2508-2515.

Bergeron, J., Menezes, J. and Tijssen, P., 1993. Genomic organization and mapping of transcription and translation products of the NADL-2 strain of porcine parvovirus. *Virology*, *197*(1), pp.86-98.

Bolt, D.M., Häni, H., Müller, E. and Waldvogel, A.S., 1997. Non-suppurative myocarditis in piglets associated with porcine parvovirus infection. *Journal of Comparative Pathology*, *117*(2), pp.107-118.

Bührmann, G. 2007. Notifiable swine diseases in South Africa: Potential future risks, diagnosis and surveillance techniques. Web: *http://www.sapork.biz/notifiable-swine-diseases-in-south-africa-potential-future-risks-diagnosis-and-surveillance-techniques/.* Accessed on 30/04/2015.

Cartwright, S.F. and Huck, R.A., 1967. Viruses isolated in association with herd infertility absortions and stillbirths in pigs. *Veterinary Record*, *81*(8), pp.196-197.

Chae, C., 2005. A review of porcine circovirus 2-associated syndromes and diseases. *The Veterinary Journal*, *169*(3), pp.326-336.

Cheng, W.X., Li, J.S., Huang, C.P., Yao, D.P., Liu, N., Cui, S.X., Jin, Y. and Duan, Z.J., 2010. Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PloS One*, *5*(10), p.e13583.

Cheung, A.K., Wu, G., Wang, D., Bayles, D.O., Lager, K.M. and Vincent, A.L., 2010. Identification and molecular cloning of a novel porcine parvovirus. *Archives of Virology*, *155*(5), pp.801-806.

Choi, J., Stevenson, G.W., Kiupel, M., Harrach, B., Anothayanontha, L., Kanitz, C.L. and Mittal, S.K., 2002. Sequence analysis of old and new strains of porcine circovirus associated with congenital tremors in pigs and their comparison with strains involved with postweaning multisystemic wasting syndrome. *Canadian Journal of Veterinary Research*, *66*(4), pp.217-224.

Clark, E.G., 1996. Post-weaning multisystemic syndrome. *In: Proceedings of Western Canadian Association of Swine Practitioners*, pp. 22–25.

Cotmore, S.F., Agbandje-McKenna, M., Chiorini, J.A., Mukha, D.V., Pintel, D.J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D. and Davison, A.J., 2014. The family parvoviridae. *Archives of Virology*, *159*(5), pp.1239-1247.

Cui, J., Wang, X., Ren, Y., Cui, S., Li, G. and Ren, X., 2012. Genome sequence of Chinese porcine parvovirus strain PPV2010. *Journal of Virology*, *86*(4), pp.2379-2379.

DAFF, 2015. A profile of the South African goat market value chain. Web. http://www.nda.agric.za/doaDev/sideMenu/Marketing/Annual%20Publications/Commodity%20 Profiles/Livestock/Pork%20market%20value%20chain%20profile%202015.pdf. Accessed on 25/10/2017.

De Boisséson, C., Béven, V., Bigarré, L., Thiéry, R., Rose, N., Eveno, E., Madec, F. and Jestin, A., 2004. Molecular characterization of Porcine circovirus type 2 isolates from post-weaning multisystemic wasting syndrome-affected and non-affected pigs. *Journal of General Virology*, *85*(2), pp.293-304.

Dione, M.M., Ouma, E.A., Roesel, K., Kungu, J., Lule, P. and Pezo, D., 2014. Participatory assessment of animal health and husbandry practices in smallholder pig production systems in three high poverty districts in Uganda. *Preventive Veterinary Medicine*, *117*(3), pp.565-576.

Drew, T.W., Grierson, S.S., King, D.P., Hicks, D., Done, S., Banks, M., Neser, J.A., Evans, D.P.B. and Grimbeek, P., 2004. Genetic similarity between porcine circovirus type 2 isolated from the first reported case of PMWS in South Africa and North American isolates. *Veterinary Record*, *155*(5), pp.149–151.

Drolet, R., D'Allaire, S., Larochelle, R., Magar, R., Ribotta, M. and Higgins, R., 2002. Infectious agents identified in pigs with multifocal interstitial nephritis at slaughter. *Veterinary Record*, *150*(5), pp.139-142.

Ellis, J.A., Bratanich, A., Clark, E.G., Allan, G., Meehan, B., Haines, D.M., Harding, J., West, K.H., Krakowka, S., Konoby, C. and Hassard, L., 2000. Coinfection by porcine circoviruses and porcine parvovirus in pigs with naturally acquired postweaning multisystemic wasting syndrome. *Journal of Veterinary Diagnostic Investigation*, *12*(1), pp.21-27.

Fan, S., Ku, X., Chen, F., Wang, Y., Yu, X. and He, Q., 2017. Complete Genome Sequence of a Novel Porcine Circovirus Type 3 Strain, PCV3/CN/Hubei-618/2016, Isolated from China. *Genome Announcements*, *5*(15), p.e00100-17.

FAO, 2012. Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3, Rome.

Grierson, S.S., King, D.P., Wellenberg, G.J. and Banks, M., 2004. Genome sequence analysis of 10 Dutch porcine circovirus type 2 (PCV-2) isolates from a PMWS case-control study. *Research in Veterinary Science*, *77*(3), pp.265-268.

Hamel, A.L., Lin, L.L. and Nayar, G.P., 1998. Nucleotide sequence of porcine circovirus associated with postweaning multisystemic wasting syndrome in pigs. *Journal of Virology*, *72*(6), pp.5262-5267.

Harding, J.C., 1996. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. *In: Proceedings of Western Canadian Association of Swine Practitioners*, p. 21.

Hijikata, M., Abe, K., Win, K.M., Shimizu, Y.K., Keicho, N. and Yoshikura, H., 2001. Identification of new parvovirus DNA sequence in swine sera from Myanmar. *Japanese Journal of Infectious Diseases*, *54*(6), pp.244-245.

Hueffer, K. and Parrish, C.R., 2003. Parvovirus host range, cell tropism and evolution. *Current Opinion in Microbiology*, *6*(4), pp.392-398.

Ironkwe, M.O. and Amefule, K.U., 2008. Appraisal of indigenous pig procution and management practices in Rivers State, Nigeria. *Journal of Agriculture and Social Research*, *8*(1), pp.1-7.

Jeoung, H.Y., Lim, S.I., Kim, J.J., Cho, Y.Y., Kim, Y.K., Song, J.Y., Hyun, B.H. and An, D.J., 2015. Serological prevalence of viral agents that induce reproductive failure in South Korean wild boar. *BMC Veterinary Research*, *11*(1), p.78.

Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S. and Allan, G.M., 2000. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *Journal of Comparative Pathology*, *122*(1), pp.9-24.

Khan, S.U., Atanasova, K.R., Krueger, W.S., Ramirez, A. and Gray, G.C., 2013. Epidemiology, geographical distribution, and economic consequences of swine zoonoses: a narrative review. *Emerging Microbes and Infections*, *2*(12), p.e92.

Kim, J. and Chae, C., 2004. Concurrent presence of porcine circovirus type 2 and porcine parvovirus in retrospective cases of exudative epidermitis in pigs. *The Veterinary Journal*, *167*(1), pp.104-106.

Kim, J., Chung, H.K., Jung, T., Cho, W.S., Choi, C. and Chae, C., 2002. Postweaning multisystemic wasting syndrome of pigs in Korea: prevalence, microscopic lesions and coexisting microorganisms. *Journal of Veterinary Medical Science*, *64*(1), pp.57-62.

Knell, S., Willems, H., Hertrampf, B. and Reiner, G., 2005. Comparative genetic characterization of porcine circovirus type 2 samples from German wild boar populations. *Veterinary Microbiology*, *109*(3), pp.169-177.

Krakowka, S., Ellis, J.A., Meehan, B., Kennedy, S., McNeilly, F. and Allan, G., 2000. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. *Veterinary Pathology*, *37*(3), pp.254-263.

Kresse, J.I., Taylor, W.D., Stewart, W.W. and Eernisse, K.A., 1985. Parvovirus infection in pigs with necrotic and vesicle-like lesions. *Veterinary Microbiology*, *10*(6), pp.525-531.

Lager, K.M. and Mengeling, W.L., 1994. Porcine parvovirus associated with cutaneous lesions in piglets. *Journal of Veterinary Diagnostic Investigation*, *6*(3), pp.357-359.

Larochelle, R., Magar, R. and D'Allaire, S., 2002. Genetic characterization and phylogenetic analysis of porcine circovirus type 2 (PCV2) strains from cases presenting various clinical conditions. *Virus Research*, *90*(1), pp.101-112.

Lau, S.K., Woo, P.C., Tse, H., Fu, C.T., Au, W.K., Chen, X.C., Tsoi, H.W., Tsang, T.H., Chan, J.S., Tsang, D.N. and Li, K.S., 2008. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *Journal of General Virology*, *89*(8), pp.1840-1848.

Lau, S.K., Woo, P.C., Yip, C.C., Li, K.S., Fu, C.T., Huang, Y., Chan, K.H. and Yuen, K.Y., 2011. Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family Parvoviridae, and evidence for inter-and intra-host genetic diversity and recombination. *Journal of General Virology*, *92*(9), pp.2047-2059.

Meehan, B.M., McNeilly, F., Todd, D., Kennedy, S., Jewhurst, V.A., Ellis, J.A., Hassard, L.E., Clark, E.G., Haines, D.M. and Allan, G.M., 1998. Characterization of novel circovirus

DNAs associated with wasting syndromes in pigs. *Journal of General Virology*, *79*(9), pp.2171-2179.

Meng, X.J., 2013. Porcine circovirus type 2 (PCV2): pathogenesis and interaction with the immune system. *Annual. Review of Animal Biosciences*, *1*(1), pp.43-64.

Mengeling, W.L., 2006. Porcine parvovirus. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. 9th Eds., *Diseases of Swine*. Ames, Iowa, Blackwell Publishing, pp.373–385.

Mengeling, W.L., Lager, K.M., Zimmerman, J.K., Samarikermani, N. and Beran, G.W., 1991. A current assessment of the role of porcine parvovirus as a cause of fetal porcine death. *Journal of Veterinary Diagnostic Investigation*, *3*(1), pp.33-35.

Molitor, T.W., Joo, H.S. and Collett, M.S., 1983. Porcine parvovirus: virus purification and structural and antigenic properties of virion polypeptides. *Journal of Virology*, *45*(2), pp.842-854.

Morozov, I., Sirinarumitr, T., Sorden, S.D., Halbur, P.G., Morgan, M.K., Yoon, K.J. and Paul, P.S., 1998. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. *Journal of Clinical Microbiology*, *36*(9), pp.2535-2541.

Muhanguzi, D., Lutwama, V. and Mwiine, F.N., 2012. Factors that influence pig production in Central Uganda-Case study of Nangabo Sub-County, Wakiso district. *Veterinary World*, *5*(6), pp.346-51.

Muwonge, A., Johansen, T.B., Vigdis, E., Godfroid, J., Olea-Popelka, F., Biffa, D., Skjerve, E. and Djønne, B., 2012. Mycobacterium bovis infections in slaughter pigs in Mubende district, Uganda: a public health concern. *BMC Veterinary Research*, *8*(1), p.168.

Ndyomugyenyi, E.K. and Kyasimire, J., 2015. Pig production in Kichwamba Sub-county, Rubirizi district, Uganda. *Livestock Research for Rural Development*, *27*(10).

Ni, J., Qiao, C., Han, X., Han, T., Kang, W., Zi, Z., Cao, Z., Zhai, X. and Cai, X., 2014. Identification and genomic characterization of a novel porcine parvovirus (PPV6) in china. *Virology Journal*, *11*(1), p.203.

Opriessnig, T., Meng, X.J. and Halbur, P.G., 2007. Porcine circovirus type 2–associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *Journal of Veterinary Diagnostic Investigation*, *19*(6), pp.591-615.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2014. Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. *Veterinary Microbiology*, *173*(1), pp.9-16.

Palinski, R., Piñeyro, P., Shang, P., Yuan, F., Guo, R., Fang, Y., Byers, E. and Hause, B.M., 2017. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *Journal of Virology*, *91*(1), pp.e01879-16.

Palinski, R.M., Mitra, N. and Hause, B.M., 2016. Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. *Virus Genes*, *52*(4), pp.564-567.

Phan, T.G., Giannitti, F., Rossow, S., Marthaler, D., Knutson, T.P., Li, L., Deng, X., Resende, T., Vannucci, F. and Delwart, E., 2016. Detection of a novel circovirus PCV3 in pigs with cardiac and multi-systemic inflammation. *Virology Journal*, *13*(1), p.184.

Schirtzinger, E.E., Suddith, A.W., Hause, B.M. and Hesse, R.A., 2015. First identification of porcine parvovirus 6 in North America by viral metagenomic sequencing of serum from pigs infected with porcine reproductive and respiratory syndrome virus. *Virology Journal*, *12*(1), p.170.

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Segalés, J., Allan, G.M. and Domingo, M., 2005. Porcine circovirus diseases. *Animal Health Research Reviews*, *6*(2), pp.119-142.

Shan, T., Lan, D., Li, L., Wang, C., Cui, L., Zhang, W., Hua, X., Zhu, C., Zhao, W. and Delwart, E., 2011. Genomic characterization and high prevalence of bocaviruses in swine. *PLoS One*, *6*(4), p.e17292.

Shen, H., Liu, X., Zhang, P., Wang, L., Liu, Y., Zhang, L., Liang, P. and Song, C., 2018**.** Genome characterization of a porcine circovirus type 3 in South China. *Transboundary and Emerging Diseases*, *65*, pp.264–266.

Streck, A.F., 2013. Studies on genetic properties of porcine parvoviruses. *A Doctoral Dissertation*, University of Leipzig, pp.1-100.

Sun, J., Huang, L., Wei, Y., Wang, Y., Chen, D., Du, W., Wu, H. and Liu, C., 2015. Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. *Archives of Virology*, *160*(5), pp.1339-1344.

Szelei, J., Zadori, Z. and Tijssen, P., 2006. Porcine parvovirus. In: Kerr, J.R., Cotmore, S.F., Bloom, M.E., Linden, R.M. and Parrish, C.R. Eds., *Parvoviruses*, Hodder Arnold, London, UK, pp.435–445.

Tattersall, P., 2006. The evolution of parvovirus taxonomy. In: Kerr, J.R., Cotmore, S.F., Bloom, M.E., Linden, M.R. and Parrish, C.R. Eds., *Parvoviruses*, Hodder Arnold, London, United Kingdom, pp.5– 14.

Tijssen, P., Agbandje-McKenna, M., Almendral, J.M., Bergoin, M., Flegel, T.W., et al. 2011. Family Parvoviridae. In: King, A.M.Q, Adams, M.J., Carstens, E.B., Lefkowitz, E.J. eds., *Virus Taxonomy 9th report of the International Committee on Taxonomy of Viruses*. London: Academic Press. pp.405–425.

Tischer, I., Gelderblom, H., Vettermann, W. and Koch, M.A., 1982. A very small porcine virus with circular single-stranded DNA. *Nature*, *295*(5844), pp.64-66.

Tischer, I., Mields, W., Wolff, D., Vagt, M. and Griem, W., 1986. Studies on epidemiology and pathogenicity of porcine circovirus. *Archives of Virology*, *91*(3), pp.271-276.

Tischer, I., Rasch, R. and Tochtermann, G., 1974. Characterization of papovavirus and picornavirus-like particles in permanent pig kidney cell lines. *Zentralblatt fuer Bakteriologie Mikrobiologie und Hygiene series A*, *226*(2), pp.153-167.

Truyen, U. and Streck, A. F., 2012. Porcine parvovirus. In: Zimmerman, J. K., Karriker, L., Ramirez, A., Schwartz, K. and Stevenson, G. 10th Eds., *Diseases of Swine*, John Wiley and Sons, Chichester, UK, pp.447–455.

Upton, M., 2004. *The role of livestock in economic development and poverty reduction*. PPLPI Working paper No. 10. FAO, Roma, Italy.

Van Leengoed, L.A., Vos, J., Gruys, E., Rondhuis, P. and Brand, A., 1983. Clinical papers: Porcine Parvovirus infection: Review and diagnosis in a sow herd with reproductive failure. *Veterinary Quarterly*, *5*(3), pp.131-141.

Wang, F., Wei, Y., Zhu, C., Huang, X., Xu, Y., Yu, L. and Yu, X., 2010. Novel parvovirus sublineage in the family of Parvoviridae. *Virus Genes*, *41*(2), pp.305-308.

Wen, L., Guo, X. and Yang, H., 2005. Genotyping of porcine circovirus type 2 from a variety of clinical conditions in China. *Veterinary Microbiology*, *110*(1), pp.141-146.

Whitaker, H.K., Neu, S.M. and Pace, L.W., 1990. Parvovirus infection in pigs with exudative skin disease. *Journal of Veterinary Diagnostic Investigation*, *2*(3), pp.244-246.

Xiao, C.T., Gimenez-Lirola, L.G., Jiang, Y.H., Halbur, P.G. and Opriessnig, T., 2013a. Characterization of a novel porcine parvovirus tentatively designated PPV5. *PLoS One*, *8*(6), p.e65312.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2013b. Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. *Infection, Genetics and Evolution*, *16*, pp.369-376.

Xing, X., Zhou, H., Tong, L., Chen, Y., Sun, Y., Wang, H. and Zhang, G., 2018. First identification of porcine parvovirus 7 in China. *Archives of Virology*, *163*(1), pp.209-213.

Yue, F., Cui, S., Zhang, C. and Yoon, K.J., 2009. A multiplex PCR for rapid and simultaneous detection of porcine circovirus type 2, porcine parvovirus, porcine pseudorabies virus, and porcine reproductive and respiratory syndrome virus in clinical specimens. *Virus Genes*, *38*(3), pp.392-397.

Zeeuw, E.J.L., Leinecker, N., Herwig, V., Selbitz, H.J. and Truyen, U., 2007. Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. *Journal of General Virology*, *88*(2), pp.420-427.

Zeng, S., Wang, D., Fang, L., Ma, J., Song, T., Zhang, R., Chen, H. and Xiao, S., 2011. Complete coding sequences and phylogenetic analysis of porcine bocavirus. *Journal of General Virology*, *92*(4), pp.784-788.

Zhou, F., Sun, H. and Wang, Y., 2014. Porcine bocavirus: achievements in the past five years. *Viruses*, *6*(12), pp.4946-4960.

CHAPTER TWO

LITERATURE REVIEW

Global status of porcine circovirus type 2 and its associated diseases in sub-Saharan Africa

(Published in Advances in Virology)

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Abstract

Globally, porcine circovirus type 2 (PCV2) is a recognized viral pathogen of great economic value in pig farming. It is the major cause of ravaging post-weaning multi-systemic wasting syndrome (PMWS) and many other disease syndromes generally regarded as porcine circovirus associated diseases (PCVAD) in Europe. PCV2 infections, specifically PMWS, had impacted huge economic loss on swine production at different regions of the world. It has been studied and reported at different parts of the globe including: North and South America, Europe, Asia, Oceania, Middle East and the Caribbean. However, till date, this virus and its associated diseases have been grossly understudied in sub-Sahara African region and the entire continent at large. Two out of forty nine, representing just about 4% of countries that make up sub-Sahara Africa presently have limited records on reported cases and occurrence of the viral pathogen despite the ubiquitous nature of the virus. This review presents an overview of the discovery of porcine circovirus and its associated diseases in global pig herds. It also emphasizes the latest trends in PCV2 vaccines and antiviral drugs development, and the information gaps that exist on the occurrence of this important viral pathogen in swine herds of sub-Saharan Africa countries. This will serves as wake-up call for immediate and relevant actions by stakeholders in the region.

Keywords: Swine; porcine circovirus type 2 (PCV2); sub-Saharan Africa; porcine circovirus associated diseases (PCVAD).

2.1   Introduction

Pig rearing is one of the fastest growing livestock sector worldwide (FAO, 2012) as it is a valuable source of animal protein globally, and the industry contributes largely to the economy of many countries (Khan *et al*., 2013). Despite the huge economic potentials of piggery business, many farms are faced with myriads of problems orchestrated by diseases that have the capacity to decimate herds. In as much as many opportunities abound in pig rearing especially for small scale farmers, their efforts to improve on their production capacity is hampered by great loss of animals to diseases (Ndyomugyenyi and Kyasimire, 2015). Furthermore, the problem becomes more complicated by limited information on the relative frequency of occurrence of the different diseases and their detrimental effects on pig production, most especially in developing countries of Africa (Dione *et al*., 2014). Swine diseases of economic importance range from bacterial, viral, fungal to protozoan origins. According to Vidigal *et al*. (2012), swine infectious pathogens have greatly caught the attention of researchers from early 1990s when a lot of pig-producing countries experienced very huge economic losses as a result of emerging viral disease-causing agents such as porcine circovirus type 2 (PCV2).

PCV2 has been known as a universal viral pathogen because of its presence in most, if not all the swine herds (Baekbo *et al*., 2012). Its global prevalence and status has brought about its seropositivity rate of 20 - 80% in pigs coupled with very high incidence rate of 60% that is accompanied by general mortality rate of 3 - 10% and culling rate of 40% in seriously affected pig farms (Wang *et al*., 2014). PCV2 is the main etiologic entity implicated in post-weaning multi-systemic wasting syndrome (PMWS) with other remaining porcine circovirus associated diseases (PCVADs) (Chae, 2005). It has also been established to be a necessary agent in the

pathogenesis of PCVADs, but not a sufficient cause of the diseases that are known to be of great economic importance in pigs production worldwide (Allan *et al*., 1999a; Anoopraj *et al*., 2015)

2.2 Historical background, classification and genomic organization of porcine circoviruses

2.2.1 Overview of initial discovery and subsequent retrospective studies of porcine circoviruses in global swine herds

The initial discovery of porcine circovirus (PCV) in Germany occurred in a continuous pig kidney cell line (PK-15 ATCC-CCL31) as a picornavirus-like contaminant and without any cytopathic effect, with initial assumption of having an RNA genome (Tischer *et al*., 1974). However, subsequent observations led to its description as a minute, non-enveloped and icosahedral shaped virus possessing a genome with circular single-stranded DNA (Tischer *et al*., 1982). Experimentally, the PCV obtained from the PK-15 cell line did not produce any ailment in pigs (Tischer *et al*., 1986; Allan *et al*., 1995). Subsequently in 1991 at Saskatchewan, Canada, another PCV emerged in a sporadic disease called PMWS which was characterized by weight loss, breathing discomfort, jaundice and peculiar microscopic lesions in lymphoid tissues of infected pigs (Clark, 1996; Harding, 1996).

Ellis (2014) expressed the initial bias view of some investigators with "hope" of discovering a novel porcine lentivirus from the diseased pigs since they presented an AIDS-like syndrome. They hypothesized that a lentivirus caused the immunosuppression and allowed an observable, disease-causing proliferation of an endemically infectious agent proposed to be "new" circovirus (Bratanich *et al*., 1999). However, the disease condition was further investigated by its first discoverers: Dr. John Harding, a swine consultant veterinarian in a private practice and Dr. Edward Clark, a veterinary pathologist at University of Saskatchewan, Canada. Subsequently, a new DNA-virus with similar morphology to the PK-15 originated PCV was discovered not only

from PMWS-affected pigs in North America but also in European countries (Segalés *et al*., 1997; Allan *et al*., 1998a; Harding *et al*., 1998); and has virtually been described in all continents of the world. Further examination of PMWS-associated PCV showed notable variations when compared with initially defined PCV (Allan *et al*., 1998b; Meehan *et al*., 1998), and in a bid to differentiate the non-pathogenic PCV from pathogenic PMWS-associated PCV, the non-pathogenic strain was called porcine circovirus type 1 (PCV1), while the latter was named porcine circovirus type 2 (PCV2) (Allan *et al*., 1999b)

Through PCV2 serological studies, it was observed that PCV2 infection is present globally, whereas the prevalence of its associated diseases is much lower, hence, the commonest form of PCV2 manifestation is the subclinical infection (Segalés *et al*., 2005a; Segalés, 2012). However through various retrospective studies, the pathogenic PCV2 was observed to be in existence in swine herds from different regions of the world earlier before the PMWS outbreak in 1998 (Rose *et al*., 2012). Jacobsen *et al*. (2009) attempted to unravel the origin, spread and pathogenesis of PCV2 and its diseases in northern Germany, confirmed the existence of PCV2 in pigs dated back to 1962 based on archived samples taken for necropsy within the period of 1961 and 1998 with the use of *in-situ* hybridization techniques and polymerase chain reaction (PCR). Furthermore, it was discovered that the relative incidence of detectable viral nucleic acid and existence of PCV2-related lesions was significantly different by the subsequent years.

Total incidence of PCV2 infection was actually minimal within the period of 1961 to 1984, ranging from 0–11.7% but increased between 1985 and 1998 within the range of 14.3–53.3%. Also, PCV2-associated diseases were first seen in 1985 archived samples, while sequence analyses of some selected PCV2 DNA segments also showed high homology with currently

existing PCV2 strains (Jacobsen *et al*., 2009). This retrospective study by Jacobsen *et al*. (2009) is the foremost report on the detection of PCV2 in pigs worldwide. Many other retrospective studies had also confirmed the existence of the virus in swine from different countries prior to its official detection in 1998 (Table 2.1). However, associated diseases like PMWS and porcine dermatitis and nephritis syndrome (PDNS) were not diagnosed in the archived samples prior to 1985, implying that PCV2 infection solely is insufficient to bring about the onset of PCV2-associated diseases. The overall epidemiological data so far thus shows PCV2 to have probably been in existence in the swine population across the universe for more than five decades (Jacobsen *et al*., 2009; Grau-Roma *et al*., 2011; Rose *et al*., 2012)

2.2.2   Classification of Circoviruses

Historically, family *Circoviridae* comprises of two genera namely, *Gyrovirus and Circovirus* based on their morphology and genomic organisation (Pringle *et al*, 1999; McNulty *et al*., 2000) as established in the last published (ninth) report of International Committee on Taxonomy of Viruses (ICTV) of the year 2009 (Biagini *et al*., 2012); with 11 species in the genus *Circovirus* which include: *Canary circovirus, Pigeon circovirus*, *Duck circovirus, Finch circovirus, Goose circovirus, Beak and feather disease virus, Gull circovirus, Starling circovirus, Swan circovirus, Porcine circovirus type 1* and *Porcine circovirus type 2*.

Genus *Gyrovirus* consists of only *Chicken anaemia virus*, with different genome organisations compared to that of *Circovirus*. Due to the genomic organization and replication strategy of viruses listed in the genus *Circovirus,* they are known to have close relationship to the plant viruses called nanoviruses and geminiviruses, with characteristic stem loop structure situated at their origin of replication and the similarity of their replication proteins (Meehan *et al*., 1997). It

had been asserted that circoviruses are the possible genetic intermediates between nanoviruses and geminiviruses (Niagro *et al*., 1998).

Table 2.1: Retrospective studies on earlier occurrence of PCV2 in pigs from some countries of the world.

Country	Sampling period	Reference
Northern Germany	1961 - 1998	Jacobsen *et al*., 2009
Belgium	1969	Sanchez *et al*., 2000
UK	1970-1997	Grierson *et al*., 2004
Northern Ireland	1970-1971	Krakowka *et al*., 2012
Northen Ireland	1973-1999	Walker *et al*., 2000
Canada	1985	Magar *et al*., 2000
Spain	1985-1997	Rodríguez-Arrioja *et al*., 2003

However in a latest development, the taxonomy of *Circoviridae* has been revisited due to the discovery of new viruses and re-evaluation of genomic features that characterize members of the family. In a current ratification by ICTV (2016), genus *Gyrovirus* has been reassigned to the family *Anelloviridae* while genus *Circovirus* and a new genus, *Cyclovirus* were grouped together in family *Circoviridae;* consisting of twenty-seven (27) and forty-three (43) species respectively (Rosario *et al*., 2017). Cycloviruses were discovered in 2010 as a group of viruses with very high relatedness to circoviruses, having genomic features that are closely related to them; and were tentatively named cycloviruses to differentiate them from the circoviruses (Li *et al*., 2010; Delwart and Li, 2012). The establishment of the two groups in family *Circoviridae* becomes

justifiable due to phylogenetic and genomic differences that exist between them *vis-à-vis* the host range differences (Rosario *et al*., 2017).

2.2.3 Genomic organization of porcine circoviruses

Presently, PCVs are the smallest viruses found in animals. The diameter of their virions ranges from 17-21 nanometre in size (Tischer *et al*., 1982). Their genomes consist of single-stranded DNA that is circular in nature with about 1759 (PCV1) and 1767-1768 (PCV2) nucleotides sequence (Tischer *et al*., 1982; Morozov *et al*., 1998). PCV1 has only one genome map (Figure 2.1), which consists of two main open reading frames (ORFs) coding for replication initiating proteins and the structural capsid protein. The locations of the promoters of the genes (*Prep* and *Pcap)* have been determined. The capsid gene promoter is situated within the *rep* open reading frame (ORF1) precisely nucleotide position 1328 to 1252; while the replicase gene promoter is located at the intergenic area towards upstream of the replicase gene at nucleotide position 640 to 796 and forms a kind of overlapping at the origin of replication of PCV1 (Mankertz and Hillenbrand, 2002).

PCV2 genome organization is of three types: Maps 1 to 3 (Figure 2.1), corresponding to PCV2a and PCV2e; PCV2b; PCV2c and PCV2d genotypes respectively (Zhai *et al*., 2014). It also possesses three main ORFs, ORF1 (945 nucleotides at position 51 to 995) which codes for replication proteins that controls replication process of the virus (Mankertz *et al*., 1998), ORF-2 (702 or 705 nucleotide at position 1734/1735 to 1030/1033/1034) which codes for the immunogenic structural protein (Cap) that determines the virus antigenicity (Nawagitgul *et al*., 2000); and ORF3 (315 nucleotides at position 671 to 357) which was reported to encode PCV2

protein that causes apoptosis *in vitro* and also involved in the pathogenicity of the virus in mice during an *in-vivo* study (Liu *et al*., 2005; Liu *et al*., 2006).
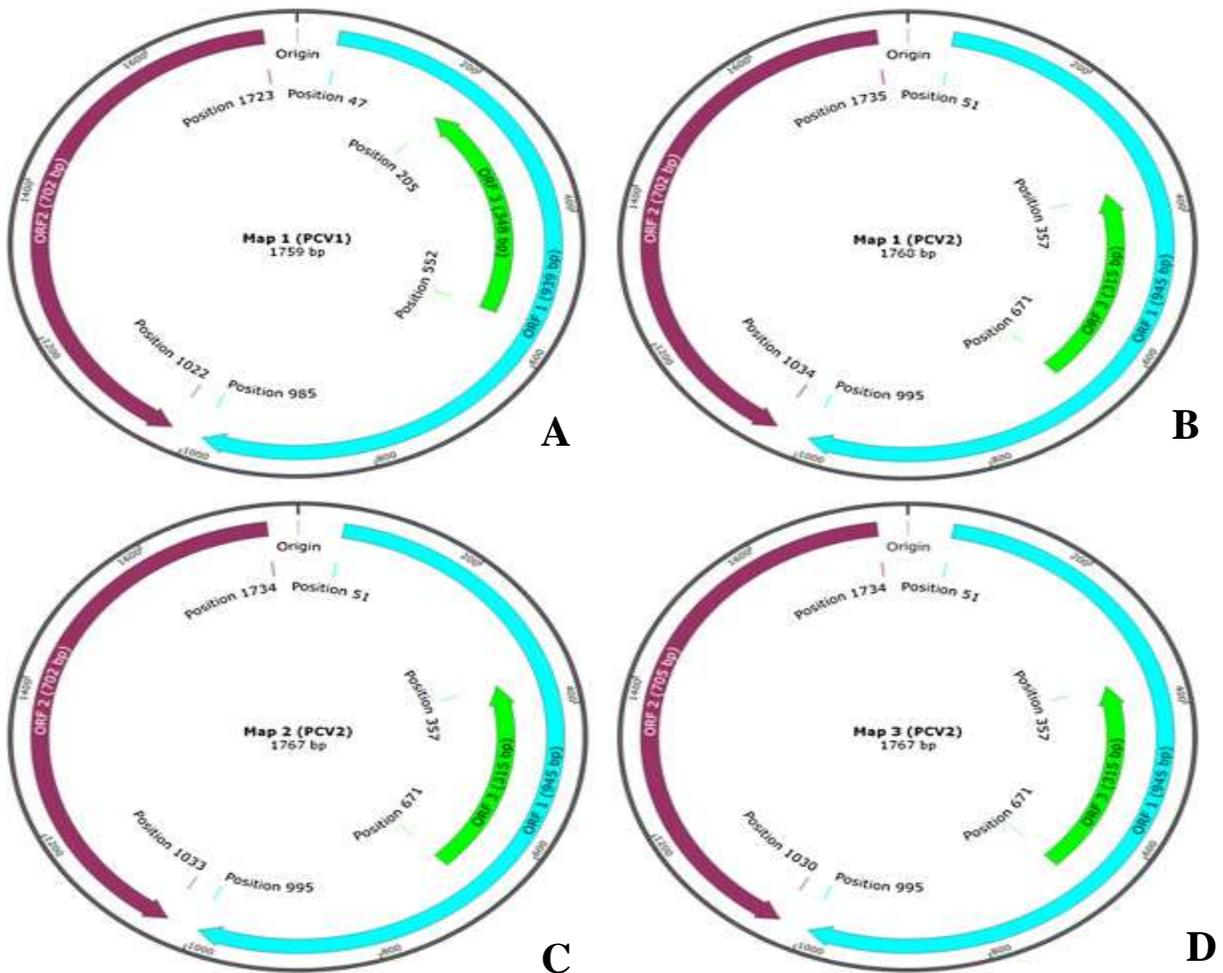
Figure 2.1: Genome organization of porcine circoviruses. (NB: Map A is for PCV1, while Map B, C and D are for PCV2a &2e; PCV2b and PCV2c & 2d respectively; adapted from Zhai *et al. (*2014)).

Furthermore, through a latest study by Gu *et al*. (2016), a novel insight that gives deeper understanding of the biological function of PCV2 ORF3 was obtained, when, a nuclear export sequence (NES) was localized at the N-terminus of ORF3 that codes for protein which plays critical role in nuclear export activity. In another relatively recent study, a newly discovered viral protein from ORF4 (with 180 nucleotides on position 386 to 565) was reported. The open reading frame is not really essential for PCV2 replication, however according to He *et al*. (2013), it has a function of bringing down caspase activity and also helps in CD4 (+) and CD8 (+) T-lymphocytes' regulation at the time of PCV2 infection.

2.3   Porcine circovirus type 2 (PCV2)

2.3.1   Genotypic classification of porcine circovirus type 2

According to Ojok *et al*. (2013), no virulence specific DNA polymorphism has been recognized in PCV2; however, characterization of the virus is important for epidemiological purposes. Based on phylogenetic studies and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), a prototype for classifying PCV2 was given that earlier divided the strains of the virus into five genogroups namely: PCV2a to 2e (Hamel *et al*., 2000). However, through phylogenetic analysis that was based on cap gene and complete genome of PCV2 (Figure 2.2), the virus has been classified into four different genotypes which are: PCV2a with five clusters, PCV2b with three clusters, PCV2c and PCV2d (Segalés *et al*., 2008; Guo *et al*., 2010; Franzo *et al*., 2015a).

PCV2a and 2b are known to have worldwide distribution, with PCV2b being the predominant genotype detected since 2003. The third type, PCV2c was first reported in Denmark from an archived material (Dupont *et al*., 2008) and recently, it was discovered from live feral pigs in

Brazil (Franzo *et al*., 2015b) and also from various field samples obtained from sick pigs in China (Liu, *et al*., 2016). The fourth genotype, PCV2d was reported from China (Guo *et al*., 2010); more recently it has been found dominating in most cases of PCV2 infections in the United State (Xiao *et al*., 2016), South Korean pig population (Kwon *et al*., 2017) and globally (Xiao *et al*., 2015).
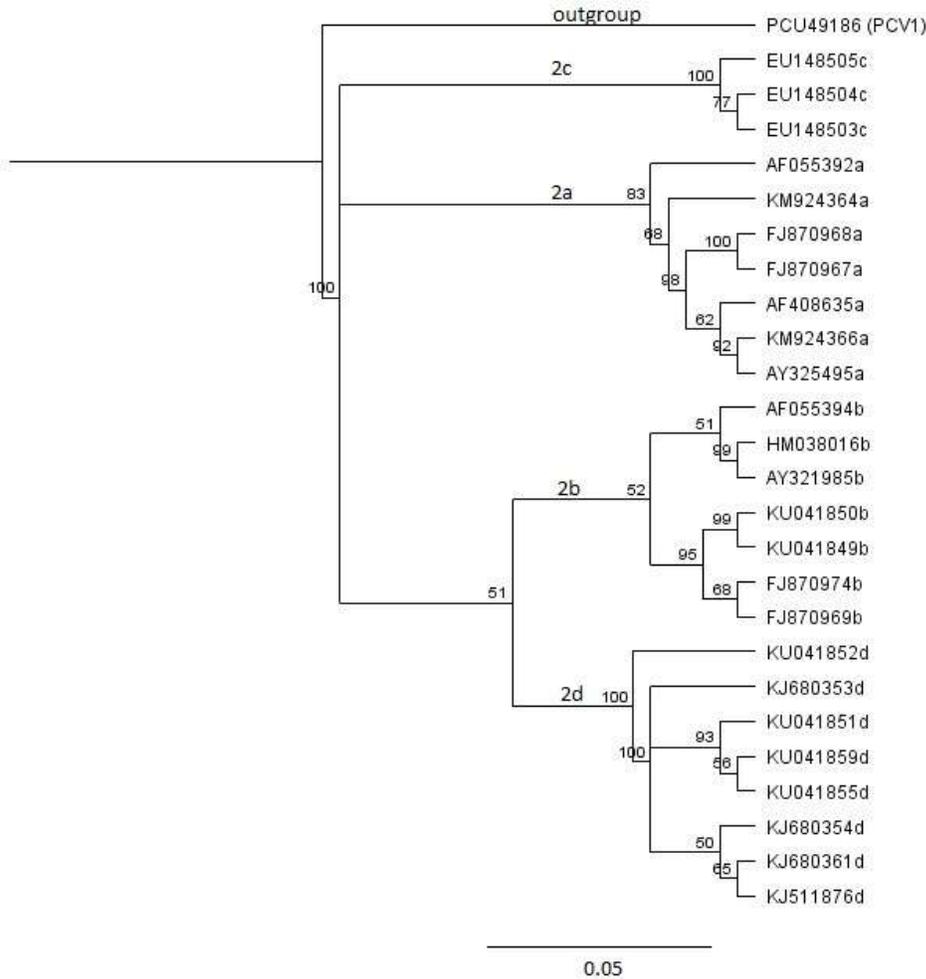
Figure 2.2: Four main genotypes of PCV2. This as shown by Phylogenetic tree that was constructed based on neighbor-joining method for complete genomes of some PCV2 sequences obtained from NCBI Genbank, genotypes of the virus were written on the main branches of the tree and PCV1 was used as outgroup. (NB: The tree was constructed using Geneious 9.1.5 - Biomatters Ltd. Boostrap values obtained from 1000 replicates are shown at the major nodes).

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Based on latest argument of Chae (2015), the classification of PCV2d is not in agreement with the standardized nomenclature rules for new PCV2 genogroups as stipulated by the European Union consortium on PCVAD (Segalés *et al*., 2008). Classification of novel PCV2 genotypes is expected to fulfill two main requirements which are (a) having cut-off value on pairwise sequence comparisons (PASC) analysis of ≥0.0351 and (b) *p*-distance cut-off value of ≥0.035 (Segalés *et al*., 2008; Cortey *et al*., 2011a). The *p*-distance that was obtained between PCV2b and PCV2d was 0.057 while the PASC value was just 0.020; hence, PCV2d according to Chae (2015) does not meet up with the criteria by which it should be classified as a different genotype. As a result of this, it was proposed that PCV2d should be renamed as mutant porcine circovirus type 2b (mPCV2b) on the account of the naming system laid down by the European Union consortium which serves to prevent any possible scientific confusion as regarding the PCV2 genotype names (Chae, 2015). However, based on the *p*-distance value of 0.055 ± 0.008 between PCV2d and PCV2b that is greater than the earlier stated PCV2 genotype definition cut-off of 0.035, Xiao *et al*. (2015) in a similar investigation, supported classification of PCV2d as an independent genotype without putting PASC cut-off value into consideration.

2.3.2   *PCV2 genotypes and their pathogenicity*

It has been confirmed that both PCV2a and PCV2b cause swine diseases with different levels of severity (Opriessnig *et al*., 2007; Beach and Meng, 2012) whereas PCV2c was discovered in Denmark from healthy pigs (Dupont *et al*., 2008). Earlier on before 2003, both PCV2a and 2b were usually prevalent in Europe and China; while in Canada and the United States, only PCV2a was common (Allan *et al*., 2007). However, an observable spontaneous shift in the prevalence of PCV2a compared to PCV2b in commercial swine populations has occurred globally since 2003 with simultaneous increment in the severity of clinical PCVAD according to many available

research findings (Gagnon *et al*., 2007; Cortey *et al*., 2011b; Constans *et al*., 2015; Huang *et al*., 2016). Irrespective of the notable shift however, there has not been any remarkable difference in pathogenicity observed between PCV2a and 2b experimentally according to Opriessnig *et al*. (2008), as their study using conventional specific-pathogen-free (SPF) pig model observed no appreciable difference in the virulence of the two viruses in pigs that were experimentally infected with them.

Also, in a similar study conducted by Lager *et al*. (2007) using germ-free experimental pigs that were infected with PCV2a or PCV2b infectious clone, it was reported that the pathological manifestations and viral antigen load observed in the two treatments were not really different, even though the pigs infected with PCV2b had quicker onset of diseases and higher overall morbidity/mortality of 100% than those infected with PCV2a which was just 25%. Similarly, in a meta-analysis experiment which focused on determining the contributory factors that culminate in development of PMWS experimentally, it was observed that inoculating the pigs with PCV2b among four other factors, favoured more successful reproduction of PMWS in the pigs (Tomás *et al*., 2008). However, the fact remains that the disease had been successfully induced in healthy pigs using either PCV2a or 2b (Opriessnig *et al*., 2008). Rather, there is possibility for the observable difference in virulence between the two main genogroups to be a function of one among many other potential factors that influence the onset of PCVADs (Cortey *et al*., 2011b).

Notwithstanding several arguments and counterarguments on virulence potentials between the two major genotypes, series of findings have shown that there's no similarity in the antigenic composition of the two genotypes (Lefebvre *et al*., 2008; Shang *et al*., 2009). The notable differences that exist between them are usually seen at the viral structural capsid gene, and these

have formed specific signature sequence motifs that are used in differentiating them (Cheung *et al*., 2007; Olvera *et al*., 2007). Furthermore, reported isolation of more virulent recombinant PCV2b strains (mPCV2b) from cases of vaccine failure from countries like China (Guo *et al*., 2010; Guo *et al*., 2012), United States of America (Xiao *et al*., 2012; Opriessnig *et al*., 2013a); and recently from Republic of South Korea (Seo *et al*., 2014), Brazil (Salgado *et al*., 2014), Uruguay (Ramos *et al*., 2015) and Germany (Eddicks *et al*., 2015) has become a serious cause for concern due to the corresponding increase in virulence and very fast spread. Series of latest findings on the escalation of genetic differences in PCV2 according to Constans *et al*. (2015) and subsequently confirmed by Reiner *et al.* (2015) suggests that current vaccines that are based on PCV2a may be the driving force behind the viral selection and evolution with resultant emergence of more virulent PCV2b strains as reported in cases from fields.

2.3.3   *Pathology, concurrent infections with other pathogens and transmission modes of PCV2 and its associated diseases*

2.3.3.1 Clinical pathology of PCV2 and its associated diseases

PCV2 and its associated diseases primarily affect pigs in their late nursery stage and growers, ranging between 7 to 16 weeks old due to presumed protective influence of maternal antibody in younger piglets (McKeown *et al*., 2005). Clinico-pathological manifestations of PCV2 infection are wasting (i.e loss of weight), unthriftiness, skin paleness, jaundice, enlarged lymph nodes and diarrhoea (Segalés *et al*., 2005a; Gillespie *et al*., 2009; Meng, 2013). PCV2 majorly has predilection for the immune system of pigs by preferentially targeting the lymphoid tissues, leading to its depletion and histiocytic replacement in them which are the observable typical histological lesions (Chae, 2012; Segalés, 2012). Pathological conditions could be exacerbated

by immunostimulation or co-infections with other pathogens which occur as a result of immunosuppression and immunity reduction in the affected pigs (Meng, 2013; Wang *et al.*, 2014).

According to Segalés (2012), the PCV2 infection's clinical scope and pathology has greatly increased over the years. Apart from the most popular and ravaging PMWS, other disease conditions and disorders which include reproductive failures which is usually characterized by abortions or stillbirths with foetuses having an observable necrotizing myocarditis, has been observed in the field cases of PCV2 infection, and has been reproduced experimentally (Park *et al.*, 2005; Salogni *et al.*, 2016). Moreover, a respiratory disease which is termed porcine respiratory disease complex (PRDC) which is known with respiratory distress has also been discovered in pigs infected with PCV2, with observable bronchiolitis with mononuclear infiltration of the lungs and interstitial pneumonia (Harms *et al.*, 2001; Kim *et al.*, 2003).

The pathogen has equally been observed to be connected with PDNS which is a disease that causes formation of skin lesions of red to purple colour in affected pigs. It is also characterized by glomerular and interstitial nephritis, and vasculitis (Rosell *et al.*, 2000; Wellenberg *et al.*, 2004), although it has not been experimentally reproduced in pigs infected by PCV2 (Meng, 2013). The list also includes enteritis and proliferative and necrotizing pneumonia (PNP) (Kim *et al.*, 2004; Segalés *et al.*, 2005a). Another disease condition in which PCV2 was initially implicated is congenital tremor type A2 (Stevenson *et al.*, 2001), which some subsequent research findings later exonerated from being the culprit etiologic agent (Kennedy *et al.*, 2003; Ha *et al.*, 2005a). Nevertheless, in a latest research work, PCV2 antigen was again found in the brain tissue of newly born piglets having congenital tremor (Tummaruk and Pearodwong, 2016).

2.3.3.2 Multifactorial status of PCVADs

Though PCV2 had been confirmed to be the primary pathogen implicated in PCVAD, under experimental conditions, it has been established that infection with PCV2 solely in most cases does not causes overt clinical disease (Opriessnig and Halbur, 2012). Based on available information, it has been shown that several infectious cofactors and non-infectious conditions such as concurrent infection with other pathogens (Hasslung *et al*., 2005; Opriessnig and Halbur, 2012), host genetic make-up (Li *et al*., 2016), and management practices (Patterson *et al*., 2015) are crucial for disease progression to PCVAD. Consequently, infections with PCV2 have been regarded as multifactorial at the instances of other underlying cofactors that exacerbate infection with the viral pathogen resulting in the clinical disease manifestations. Infectious cofactors that have been extensively studied till date consist of porcine parvovirus, PPV (Kennedy *et al*., 2000; Sun *et al*., 2015a; Lukăc *et al*., 2016), porcine reproductive and respiratory syndrome virus, PRRSV (Allan *et al*., 2000), swine influenza virus SIV (Dorr *et al*., 2007), Torque teno virus (Gagnon *et al*., 2007), swine hepatitis E virus, HEV (Yang *et al*., 2015), *M. hyopneumoniae* (Opriessnig *et al*., 2004; Dorr *et al*., 2007)*, Salmonella* spp. (Ha *et al*., 2005b), *E. coli* (Dewey *et al*., 2006) and *Metastrongylus elongatus* (Marruchella *et al*., 2012). The non-infectious co-factors include the genetic background of the pig, high stocking density and prevailing environmental conditions such as temperature fluctuation within the pen (Patterson *et al*., 2015).

2.3.3.3 Transmission modes of PCV2 infection

PCV2 is a pathogen with multi-dimensional spreading potentials. The rate at which the viral pathogen spreads brought about the analogy: "spreading like a wildfire" according to Meng (2012) in a Guest Editorial Review. The spread of PCV2 can be viewed within the scope of international transmission through trading of pigs and pig products; and also transmission among

local herds. PCV2 has been confirmed to be transmitted through trading of live pigs with subclinical infection due to the subtle nature of the disease, which invariably calls for a more critical diagnostic check on imported animals or their products (Vidigal *et al*., 2012; Franzo *et al*., 2015c). There have been reported cases of transmission through selection of boars for breeding purposes and the importation of semen for the purpose of artificial insemination (Rose *et al*., 2003; Drew *et al*., 2004; Monger *et al*., 2014).

Spreading of PCV2 among local herds has been reported in various dimensions ranging from pig to pig (Dupont *et al*., 2009; Shen *et al*., 2010), man to pig (Alarcon *et al*., 2011), rodents to pig (Lorincz *et al*., 2010), insects to pig (Blunt *et al*., 2011; Yang *et al*., 2012) and pen environment to pig (Verreault *et al*., 2010). This becomes possible due to numerous routes of shedding the virus in cases of systemic infections; as observed both in natural and experimental conditions including oro-nasal secretions, faeces, urine, colostrum, milk and semen from infected boar as earlier mentioned (Segalés *et al*., 2005b; Chiou *et al*., 2011; Patterson *et al*., 2011a; Patterson *et al*., 2011b).

2.3.4   *Diagnosis, prevention, control and treatment of PCV2 and its associated diseases*

2.3.4.1 *General diagnosis of PCV2*

As a complex disease of diverse clinical signs, accurate diagnosis of PCV2 infections is highly important in order to implement appropriate intervention strategy on affected herds (Opriessnig *et al*., 2007). Diagnosis of PCV2 infections could be done basically in two major ways. Firstly, on the basis of clinical disease manifestations, which could be regarded as tentative; and secondly, based on the confirmatory detection of PCV2 antigen in lymphoid tissues and organs such as the liver, lungs, kidney or intestine of an infected animal (Gillespie *et al*., 2009).

According to Gillespie *et al.* (2009), for a farm to be tagged as experiencing PCVAD disease, percentages of clinical signs could be seen as follows: loss of weight (98.1%), diarrhea (77.2%), lymphadenopathy (44.8%), dyspnea (75.1%), central neurologic signs (39.6%), inappetence (90.4%), jaundice (37.1%) and death (96.8%). However, in a situation whereby a farm is experiencing sub-clinical infection, true status of such herd as regards to PCV2 infection could be mistaken if based on clinical signs manifestation. This is because majority of clinically healthy pigs could be seropositive manifesting subclinical infections (Gillespie *et al.*, 2009).

PCV2 antigen or nucleic acid detection in samples from a swine herd is known as the golden standard suitable for the confirmatory diagnosis of PCVAD. This has been effectively achieved through PCR, immunohistochemistry (IHC) and *in situ* hybridization (ISH) (Shibata *et al.*, 2003; Opriessnig *et al.*, 2007). Other diagnostic tests that have been developed and used in PCV2 detection in infected pigs include enzyme linked immune-sorbent assay, immunofluorescence assay, IgM immune-peroxidase monolayer assay, serum virus neutralization assays, virus isolation, electron microscopy (Liu *et al.*, 2004; Sibila *et al*, 2004; Opriessnig *et al.*, 2007).

2.3.4.2 Prevention, control and treatment of PCV2 till date

As a multifactorial disease which has been linked to both infectious and non-infectious factors, effective control of PCVAD cannot be solely achieved by vaccine applications but also by preventing triggering factors through improved swine management, control of co-infection and change of genetic background of pigs through careful selection of boar for breeding (Beach and Meng, 2012). Though vaccination is traditionally considered as the most effective method for preventing viral diseases, it had been established that the protection period of vaccine against the disease is limited and that complete eradication of the virus has not be achieved through vaccination (Feng *et al.*, 2014).

However, the currently available commercial PCV2 vaccines (Table 2.2), all of which are either an inactivated whole virus vaccine or subunit vaccine designed base on the immunogenic Cap protein of PCV2a; have shown effectiveness against clinical disease expression and enhanced major production parameters (mortality and average daily gain) in swine herds with PCV2 occurrence. While vaccine applications did not outrightly prevent infection with PCV2 nor restrict its spread, a reasonable reduction in the systemic viral loads and shedding had been observed; which invariably help in decreasing the load of the virus in the environment (Segalés *et al*., 2009; Gerber *et al*., 2011; Fraile *et al*., 2012). Although, animals under vaccination could still be infected with the virus, however they generally do have lower viral loads compared to those not vaccinated. Thus, currently available vaccines applied most especially as a single-dose protocol do not give sterilizing immunity in swine herds (Kekarainen *et al*., 2010).

Moreover, the introduction of the PCV2a vaccines has been observed to cause a corresponding world-wide shift in the prevalence of genotypes from PCV2a to 2b with attendant severe clinical manifestations in vaccinated herds (Carman *et al*., 2008). Also, some reports on PCVAD cases have shown that a new variant named mutant PCV2b (mPCV2b) was found in diseased pigs despite their prior vaccinations (Opriessnig *et al*., 2013a). This has brought about serious concerns on possible emergence of PCV2 vaccine escape strains; however it was observed that one of the current vaccines was effective against mPCV2b infection under experimental setup (Opriessnig *et al*., 2014). Nevertheless, it has been asserted that series of recent research findings concerning the increased PCV2 genetic diversity connotes that the available vaccines which are based on PCV2a may be inducing the observable selection pressure and possibly be the driving force behind the viral evolution (Constans *et al*., 2015); being pivoted on observable high

nucleotide substitution rate earlier suggested for the continuous evolution of PCV2 and the emergence of novel PCV2 strains (Firth *et al*., 2009).

Table 2.2: Available commercial PCV2 vaccines and some of their features.

Vaccine	Antigen	Recommended usage	Manufacturer	Adjuvant
Circovac®	Inactivated PCV2a virus (whole virus)	Breeding sows (two doses at 5 weeks of age and 2 weeks ante - partum) /piglets	Merial, Lyon – Franc	Mineral oil
FosteraTM PCV	Killed chimeric PCV1-2a virus	One dose for piglets of three weeks old or above	Pfizer	SL-CD aqueous
Ingelvac CircoFLEX®	Capsid protein of PCV2a (recombinant)	One dose for piglets of three weeks of age or older	Boehringer Ingelheim Vetmedica Inc. Missouri, U.S.A.	Carbomer
Circumvent® PCV	Capsid protein of PCV2a (recombinant)	Two doses at 3 and 6 weeks of age of piglets/growers respectively	Intervet/SP (Merck)	Microsol Diluvac Forte® (MDF)
Porcilis® PCV	Capsid protein of PCV2a (recombinant)	One dose for piglets of three weeks of age or older	Schering-Plough (Merck)	Mineral oil

According to Constans *et al*. (2015), some immune cells epitopes that were known to elicit immune response in the vaccine strain were not seen in the field strains, showing that there is a silent change in the antigenic profile of the strains as many non-conserved epitopes has been predicted to have immune cells functions. Furthermore, the substitutions in the epitopes have been ascertained to affect the immune response greatly, thereby causing immune escape

(Constans *et al*., 2015). Hence, many calls and recent research findings have raised support for a rational development of PCV2 vaccines to target evolving genotypes in order to increase the current threshold of protection against PCV2 and its associated diseases (Opriessnig *et al*., 2013b; Constans *et al*., 2015; Ssemadaali *et al*., 2015).

Based on recent research findings of Peng *et al*. (2016) there is a future possibility of producing effective commercial PCV2 antibodies and vaccines that could be based on non-variable *rep* protein compared to the presently available ones that are based on variable *cap* protein of the virus. From the study, it was observed that the recombinant plasmids of *rep* gene that was constructed; shows an efficient expression in the prokaryotic system and also, the engineered proteins were immunogenic. Furthermore, the characterized polyclonal antiserum made with *rep* protein showed good reactivity and displayed considerable specificity against PCV2 in PK-15 cell line. Hence, the Rep protein seems to be having future potentials in PCV2 antibody and vaccine development (Peng *et al*., 2016).

The utility of commercially available PCV2 vaccines (both inactivated and subunit vaccines based on PCV2a genotype) has been proven over the years as they have shown effectiveness in decreasing mortality and increasing growth parameters in commercial swine herds (Beach and Meng, 2012). There are unquestionable evidences from accumulated field data that confirms the efficacy of the commercial vaccines when production parameters such as average daily weight gain and economic gains in vaccinated pigs were compared with the unvaccinated pigs. In fact, cross-protection against other co-infecting agents is an additional advantage in the use of PCV2 vaccines (Beach and Meng, 2012; Afghah *et al*., 2016). The good news is that in countries where

vaccination has been grossly employed, there has been an appreciable declination in the prevalence of PCV2 with good vaccination practices.

However, despite the huge successes recorded so far on PCV2 vaccines, it has been asserted that the protection period of a PCV2 vaccine against the disease is limited and that the virus could not be eradicated by a mass vaccination procedure when applied for a period of one year. This was because, after some months of stopping the intensive vaccination programme, there was a re-emergence of PCV2 infection (Feng *et al*., 2014). Although vaccination is recommended for healthy pigs, it is ineffective for pigs that are already infected with PCV2 (Sun *et al*., 2015b). In addition, the inherent potentials of vaccines to cause mutated viral evolution (Segalés *et al*., 2013), coupled with ineffectiveness of vaccines to prevent the multifactorial disease such as PCVAD, have necessitated urgent discovery and development of safe drugs as alternatives in a bid to eradicate or control PCV2 (Sun *et al*., 2016).

2.3.4.3 Promising efforts on Ethnobotanicals

Recently, research efforts have been focused on developing antiviral drugs from natural compounds with promising outcomes. In a recent study in which twenty natural compounds isolated from traditional Chinese plants were evaluated for their antiviral activities against PCV2 in vitro, it was observed that Matrine (an alkaloid compound purified from the dried roots of *Sophora flavescens* Ait) and Scutellarin (a flavonoid compound from *Scutellaria barbata* D. Don) showed appreciable inhibition rates of 57 and 72.69% respectively against the virus out of all the tested compounds (Sun *et al*., 2015b). Furthermore, in a more recent study which aimed at exploring the antiviral potential of Matrine against PRRSV and PCV2 concurrent infection in a porcine alveolar microphages (PAM) cell model, it was observed that the use of Matrine abated

the proliferation of PRRSV and PCV2 effectively at twelve hour post infection period. This finding further necessitates the need for immediate exploration of natural products such as Matrine as antiviral agent against PCV2 in clinical settings (Sun *et al*., 2016).

In another related study which aimed at investigating the antiviral activity of a phenylpropanoid dibenzylbutyrolactone lignan called Arctigenin (ACT), extracted from another Chinese traditional herb named *Arctium lappa* L. against PCV2 *in-vitro* and *in-vivo*, another promising result was obtained. It was observed that dosage of 15.6–62.5 µg/mL of ACT efficiently inhibit the thriving of PCV2 in PK-15 cells; while the intraperitoneal injection of 200 µg/kg of ACT into PCV2-challenged mice significantly inhibited PCV2 proliferation in the lungs, spleens and inguinal lymph nodes of the mice; showing similar effect to ribavirin, an antiviral drug that was used as positive control, thereby demonstrating the effectiveness of ACT as an antiviral agent against PCV2 both *in-vitro* and *in-vivo* (Chen *et al*., 2016).

2.4   Global status of PCV2 and its associated diseases: sub-Saharan Africa scenario

2.4.1   *PCV2 and its PMWS: Is the global disease a global concern?*

Sequel to the first known outbreak and description of Porcine multi-systemic wasting syndrome, an important PCVAD in a very healthy, farrow-to-finish swine farm situated in Northeastern Saskatchewan, Western Canada, North America in early 1990's (Clark, 1996; Harding and Clark, 1997; Ellis *et al*., 1998); PMWS and other PCVADs have been subsequently seen in all regions of the world including many European countries such as: Spain (Segalés *et al*., 1997), France (LeCann *et al*., 1997), Hungary (Dan *et al*., 2003) and United Kingdom (Allan *et al*., 1998a; Grierson *et al*., 2004); South America countries such as Brazil (De Castro *et al*., 2007), Uruguay (Ramos *et al*., 2012); Asian countries such as Korea (Choi and Chae,

2008), China (Yang *et al*., 2008); Oceania, Australia (Raye, 2004); Caribbean, Cuba (Perez *et al*., 2010); Middle East, Israel (Pozzi *et al*., 2008) and African countries such as South Africa (Drew *et al*., 2004) and, recently, Uganda (Ojok *et al*., 2013). The ubiquitous status of porcine circovirus and its numerous associated diseases has been said to be linked to the marketing of sub-clinically infected pigs *vis-á-vis* the choosing of such pigs for breeding programmes as the virus is known to be spread through semen from boars (Schmoll *et al*., 2008; Vidigal *et al*., 2012).

Vidigal *et al.* (2012) reported significant link between the routes of dispersal of PCV2 and international marketing of live pigs from obtained data, thereby showing how important the movements of livestock around the globe could be in the emergence and spread of new pathogens. Therefore, increase in the global trade of livestock and their products, increase in global livestock production as a result of the use of intensive animal rearing system have arguably contribute to the spread of infectious pathogens globally (Tilman *et al*., 2002; Vidigal *et al*., 2012; Segalés *et al*., 2013). In addition to those mentioned facts, the multiple transmission routes earlier described for PCV2 coupled with the long-lasting viral life, are also pertinent in world-wide proliferation of PCV2 resulting to the PMWS epidemics (Firth *et al*., 2009; Patterson and Opriessnig, 2010; Rose *et al*., 2012).

2.4.2   *Incidence of porcine circovirus type 2 in swine-producing countries of Sub-Saharan Africa*

2.4.2.1 First emergence of PCV2 in South African swine herds

Drew *et al.* (2004) reported what seemed to be the first cases of PCV2 associated diseases in the region, which occurred in Gauteng Province, South Africa. The cases occurred in June 2001 on a large, well-managed breeding unit which serves as supplier of breeding stock to three member

farms, separated by distances of between 100 and 500 km apart. The initial clinical manifestation observed in the pigs was PDNS which affects young pigs of two to three months old. In no distant time, the expression of clinical signs associated with PMWS became more evident, to the extent that by November 2001, the morbidity due to PMWS had increased to about 30 to 40% with mortality remaining below 10% (Drew *et al.*, 2004).

Moreover, clinical manifestations of PMWS was also seen in approximately the same percentage of pigs of the same ages bred on one of the member farms, specifically, the one situated about 100 km away from the main farm after the introduction of pigs from the main farm, on which the disease had first been noticed. However, tissues were collected from just two affected animals of about 12 to 15 weeks of age from each of the two premises and were submitted for histological, immunohistological and molecular analysis. Tissues sections from the spleen, liver, brain and heart of the four pigs did not show significant histopathology. It was observed that the lesions indicative of PMWS were concentrated in the precapsular, bronchial and gastrohepatic lymph nodes. Also in many sub-capsular areas in the lymph nodes, there were widespread areas of diffuse lymphocyte depletion with presence of multinucleate giant cells as evidence occasionally (Drew *et al.*, 2004).

Furthermore, the molecular analysis of a 501-nucleotide fragments of the viral genome amplified from tissues samples of the reported cases revealed that they were identical to a PCV2 isolate from Iowa, USA with GenBank Ascension number **AF264039** (Fenaux *et al.*, 2000); having just only two nucleotide substitutions from the USA isolate. The sequence obtained from a lymph node of a piglet from the main farm yielded the complete PCV2 genome (1768 base pairs) and was designated SAI, this is the only isolate deposited so far in the GenBank with ascension

number **AY325495** from South Africa and virtually in the entire sub-Saharan Africa. In their conclusion, Drew *et al.* (2004) recommended for further molecular epidemiological studies to investigate PCV2 at other sites in South Africa. However till this time, little or no work has been done to that effect in South African pig population.

This assertion is further confirmed by more recent research work of Mokoele *et al.* (2015) in which it was categorically stated that: "though PCV2 may be an economically important disease in South Africa, to date, no specific surveillance has been conducted to validate the current status because the disease is thought to be ubiquitous in most countries". However, seeming negligence to know the health status of swine herds of the country as regarding the ubiquitous viral pathogen could pose a serious problem for the industry in the nearest future due to a recent observation during a field trip which was part of our ongoing research work that is focused on determining the occurrence of some RNA and DNA viruses in swine herds of Eastern Cape Province South Africa; pigs with typical clinical manifestations of PMWS were seen (Figure 2.3), showing likelihood of existence of the viral pathogen in pig herds of the country.
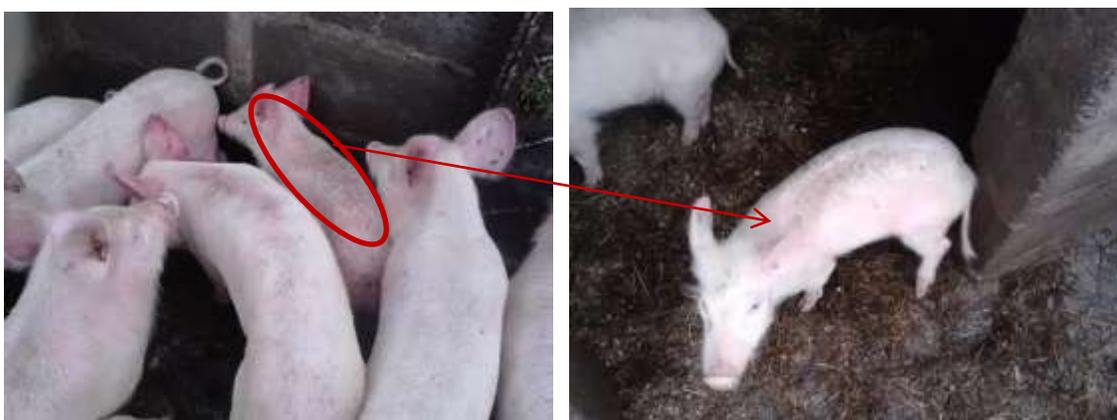
Figure 2.3: Wasting appearance of a PCV2-infected pig with a notable respiratory distress. It was within a herd of the same age at a farm in Lukhanji Local Municipality, Chris-Hani District, Eastern Cape, South Africa (*Picture taken in April, 2016 during a sampling exercise*).

In another study by An *et al*. (2007), nucleotide sequences of 197 PCV2 strains submitted to GenBank nucleotide database at the National Center for Biotechnology Information (NCBI) from all over the world and 36 PCV2 strains obtained from PMWS and PDNS cases in Korean pigs over an 8 years period; were used in grouping PCV2 into two groups (1 and 2) by phylogenetic tree analysis and multiple alignments of nucleotide sequences. In their study, it was observed that three countries namely: South Africa, United Kingdom and Thailand were having just one PCV2 complete genome sequence each on GenBank as far back as then; indicating dearth of information on PCV2 from the three countries. They recommended further studies on PCV2 from the countries so that accurate documentation of PCV2 strains circulating in the three countries could be done. However till date, nothing has been done in South Africa as regarding the clarion call.

2.4.2.2 PCV2 in Ugandan pigs

Recently, Ojok *et al*. (2013) also reported on the molecular detection and characterization of PCV2 from pigs in Uganda, however with limitation of using a relatively small sample size (n=35) like that of Drew *et al.* (2004). Only three cases of PCV2 were found in their study and they also recommended that further studies be conducted so as to fully understand the true prevalence of the virus in swine population of Uganda as well as their genetic diversity. The three PCV2 sequences in their study were observed to cluster with PCV2b genotype which was originally referred to as the European cluster or PCV2 group 1 in contrast to the South African strain which clustered with the PCV2a previously referred to as the North American strains or PCV2 group 2.

In a similar unpublished study conducted by Jonsson that aimed at investigating the disease transmission patterns in the livestock-wildlife interface in Uganda (Jonsson, 2013), being part of the Emerging Infectious Diseases (EID) surveillance programme conducted to study the prevalence of PCV2 in domestic pigs in Uganda, ninety-one domestic pigs around Murchison Falls national park were sampled and analysed. The sampled domestic pigs were all negative for PCV2a, while for PCV2b which is known to be genogroup mostly associated with PMWS, a point prevalence of 77% was reported. This is in support of the findings of Ojok *et al.* (2013) that reported the presence of PCV2b genotype in a separate study on Ugandan pigs. The point prevalence of 77% for PCV2b in the study, cannot be generalised to all of Ugandan pigs, this is because the sample selection was too small to arrive at such conclusion. As a result of this, more extensive studies were recommended by Jonsson (2013), so as to obtain more accurate data on the prevalence of the PCV2b in Ugandan pigs.

2.4.2.3 PCV2 till date in Cameroonian pigs

The study of Ndze *et al.* (2013) is another recent effort aimed at describing the occurrence and genetic diversity of selected DNA viruses belonging to different families namely: Adenoviridae, Circoviridae, Anelloviridae and Parvoviridae in Cameroonian pigs. However, only viruses belonging to the family Parvoviridae were detected, most especially those within the bocaviruses. In their remarks, they attributed their failure to detect other groups of viruses including *Circovirus* which is known to be ubiquitous to several possible factors including but not limited to short sampling period and low study sample number.

2.5   Conclusion

So far, the status of porcine circovirus type 2 is practically unknown in pigs of many swine-producing countries in sub-Saharan Africa. Moreover, countries where some research works have been done till date are having insufficient data that could enhance the detailed characterization of the viral genogroup that may likely be in circulation in swine herds of many countries within the region. There is therefore, an urgent need for large scale molecular epidemiological studies on the virus and its associated diseases in the region, including origins of observed genogroups. This will help in facilitating adequate preventive and control measures against the pathogen through the establishment of effective vaccination regime that could help in combating this globally important and emerging porcine viral pathogen with huge economic implications in the global pig industry.

References

Afghah, Z., Webb, B., Meng, X.J. and Ramamoorthy, S., 2016. Ten years of PCV2 vaccines and vaccination: is eradication a possibility?. *Veterinary Microbiology*, *206*, pp.21-28.

Alarcon, P., Velasova, M., Mastin, A., Nevel, A., Stärk, K.D. and Wieland, B., 2011. Farm level risk factors associated with severity of post-weaning multi-systemic wasting syndrome. *Preventive Veterinary Medicine*, *101*(3), pp.182-191.

Allan, G.M., Kennedy, S., McNeilly, F., Foster, J.C., Ellis, J.A., Krakowka, S.J., Meehan, B.M. and Adair, B. M., 1999a. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *Journal of Comparative Pathology*, *121*(1), pp.1–11.

Allan, G., Meehan, B., Todd, D., Kennedy, S., McNeilly, F., Ellis, J., Clark, E.G., Harding, J., Espuna, E., Botner, A. and Charreyre, C., 1998b. Novel porcine circoviruses from pigs with wasting disease syndromes. *The Veterinary Record*, *142*(17), pp.467-468.

Allan, G.M., Mc Neilly, F., Meehan, B.M., Kennedy, S., Mackie, D.P., Ellis, J.A., Clark, E.G., Espuna, E., Saubi, N., Riera, P. and Bøtner, A., 1999b. Isolation and characterisation of circoviruses from pigs with wasting syndromes in Spain, Denmark and Northern Ireland. *Veterinary Microbiology*, *66*(2), pp.115-123.

Allan, G.M., McNeilly, F., Cassidy, J.P., Reilly, G.A.C., Adair, B., Ellis, W.A. and McNulty, M.S., 1995. Pathogenesis of porcine circovirus; experimental infections of colostrum deprived piglets and examination of pig foetal material. *Veterinary Microbiology*, *44*(1), pp.49-64.

Allan, G.M., McNeilly, F., Ellis, J., Krakowka, S., Meehan, B., McNair, I., Walker, I. and Kennedy, S., 2000. Experimental infection of colostrum deprived piglets with porcine circovirus

2 (PCV2) and porcine reproductive and respiratory syndrome virus (PRRSV) potentiates PCV2 replication. *Archives of Virology*, *145*(11), pp.2421-2429.

Allan, G.M., McNeilly, F., Kennedy, S., Daft, B., Ellis, J.A., Haines, D.M., Meehan, B.M. and Adair, B.M., 1998a. Isolation of porcine circovirus-like viruses from pigs with a wasting disease in the USA and Europe. *Journal of Veterinary Diagnostic Investigation*, *10*(1), pp.3-10.

Allan, G.M., McNeilly, F., McMenamy, M., McNair, I., Krakowka, S.G., Timmusk, S., Walls, D., Donnelly, M., Minahin, D., Ellis, J. and Wallgren, P., 2007. Temporal distribution of porcine circovirus 2 genogroups recovered from postweaning multisystemic wasting syndrome-affected and-nonaffected farms in Ireland and Northern Ireland. *Journal of Veterinary Diagnostic Investigation*, *19*(6), pp.668-673.

An, D.J., Roh, I.S., Song, D.S., Park, C.K. and Park, B.K., 2007. Phylogenetic characterization of porcine circovirus type 2 in PMWS and PDNS Korean pigs between 1999 and 2006. *Virus Research*, *129*(2), pp.115-122.

Anoopraj, R., Rajkhowa, T.K., Cherian, S., Arya, R.S., Tomar, N., Gupta, A., Ray, P.K., Somvanshi, R. and Saikumar, G., 2015. Genetic characterisation and phylogenetic analysis of PCV2 isolates from India: indications for emergence of natural inter-genotypic recombinants. *Infection, Genetics and Evolution*, *31*, pp.25-32.

Baekbo, P., Kristensen, C.S. and Larsen, L.E., 2012. Porcine circovirus diseases: a review of PMWS. *Transboundary and Emerging Diseases*, *59*(s1), pp.60-67.

Beach, N.M. and Meng, X.J., 2012. Efficacy and future prospects of commercially available and experimental vaccines against porcine circovirus type 2 (PCV2). *Virus Research*, *164*(1), pp.33-42.

Biagini, P., Bendinelli, M., Hino, S., Kakkola, L., Mankertz, A., Niel, C., Okamoto, H., Raidal, S., Teo, C.G. and Todd, D., 2012. Family Circoviridae. In: King, A.M.Q., Adams, M.J., Carstens, E.B. and Lefkowitz, E.J. Eds., *Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses*, Academic Press, London, pp 343–349.

Blunt, R., McOrist, S., McKillen, J., McNair, I., Jiang, T. and Mellits, K., 2011. House fly vector for porcine circovirus 2b on commercial pig farms. *Veterinary Microbiology*, *149*(3), pp.452-455.

Bratanich, A., Lairmore, M., Heneine, W., Konoby, C., Harding, J., West, K., Vasquez, G., Allan, G. and Ellis, J., 1999. Lack of evidence of conserved lentiviral sequences in pigs with post weaning multisystemic wasting syndrome. *Canadian Journal of Veterinary Research*, *63*(3), pp.207-211.

Carman, S., Cai, H.Y., DeLay, J., Youssef, S.A., McEwen, B.J., Gagnon, C.A., Tremblay, D., Hazlett, M., Lusis, P., Fairles, J. and Alexander, H.S., 2008. The emergence of a new strain of porcine circovirus-2 in Ontario and Quebec swine and its association with severe porcine circovirus associated disease—2004–2006. *Canadian Journal of Veterinary Research*, *72*(3), pp.259-268.

Chae, C., 2005. A review of porcine circovirus 2-associated syndromes and diseases. *The Veterinary Journal*, *169*(3), pp.326-336.

Chae, C., 2012. Porcine circovirus type 2 and its associated diseases in Korea. *Virus Research*, *164*(1), pp.107-113.

Chae, C., 2015. An emerging porcine circovirus type 2b mutant (mPCV2b) originally known as PCV2d. *Veterinary Journal*, *203*(1), pp.6-9.

Chen, J., Li, W., Jin, E., He, Q., Yan, W., Yang, H., Gong, S., Guo, Y., Fu, S., Chen, X. and Ye, S., 2016. The antiviral activity of arctigenin in traditional Chinese medicine on porcine circovirus type 2. *Research in Veterinary Science*, *106*, pp.159-164.

Cheung, A.K., Lager, K.M., Kohutyuk, O.I., Vincent, A.L., Henry, S.C., Baker, R.B., Rowland, R.R. and Dunham, A.G., 2007. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. *Archives of Virology*, *152*(5), pp.1035-1044.

Chiou, M.T., Yang, C.Y., Chang, T.C., Chen, C., Lin, C.F. and Ye, L.J., 2011. Shedding pattern and serological profile of porcine circovirus type 2 infection in cesarean-derived, colostrum-deprived and farm-raised pigs. *Journal of Veterinary Medical Science*, *73*(4), pp.521-525.

Choi, K.S. and Chae, J.S., 2008. Genetic characterization of porcine circovirus type 2 in Republic of Korea. *Research in Veterinary Science*, *84*(3), pp.497-501.

Clark, E.G., 1996. Post-weaning multisystemic syndrome. *In: Proceedings of Western Canadian Association of Swine Practitioners*, pp. 22–25.

Constans, M., Ssemadaali, M., Kolyvushko, O. and Ramamoorthy, S., 2015. Antigenic determinants of possible vaccine escape by porcine circovirus subtype 2b viruses. *Bioinformatics and Biology Insights*, *9*, pp.1-12

Cortey, M., Olvera, A., Grau-Roma, L. and Segalés, J., 2011a. Further comments on porcine circovirus type 2 (PCV2) genotype definition and nomenclature. *Veterinary Microbiology*, *149*(3), pp.522-523.

Cortey, M., Pileri, E., Sibila, M., Pujols, J., Balasch, M., Plana, J. and Segalés, J., 2011b. Genotypic shift of porcine circovirus type 2 from PCV-2a to PCV-2b in Spain from 1985 to 2008. *The Veterinary Journal*, *187*(3), pp.363-368.

Dán, Á., Molnár, T., Biksi, I., Glávits, R., Shaheim, M. and Harrach, B., 2003. Characterisation of Hungarian porcine circovirus 2 genomes associated with PMWS and PDNS cases. *Acta Veterinaria Hungarica*, *51*(4), pp.551-562.

De Castro, A.M.G., Cortez, A., Heinemann, M.B., Brandão, P.E. and Richtzenhain, L.J., 2007. Genetic diversity of Brazilian strains of porcine circovirus type 2 (PCV-2) revealed by analysis of the cap gene (ORF-2). *Archives of Virology*, *152*(8), pp.1435-1445.

Delwart, E. and Li, L., 2012. Rapidly expanding genetic diversity and host range of the Circoviridae viral family and other Rep encoding small circular ssDNA genomes. *Virus Research*, *164*(1), pp.114-121.

Dewey, C.E., Johnston, W.T., Gould, L. and Whiting, T.L., 2006. Postweaning mortality in Manitoba swine. *Canadian Journal of Veterinary Research*, *70*(3), pp.161-167.

Dione, M.M., Ouma, E.A., Roesel, K., Kungu, J., Lule, P. and Pezo, D., 2014. Participatory assessment of animal health and husbandry practices in smallholder pig production systems in three high poverty districts in Uganda. *Preventive Veterinary Medicine*, *117*(3), pp.565-576.

Dorr, P.M., Baker, R.B., Almond, G.W., Wayne, S.R. and Gebreyes, W.A., 2007. Epidemiologic assessment of porcine circovirus type 2 coinfection with other pathogens in swine. *Journal of the American Veterinary Medical Association*, *230*(2), pp.244-250.

Drew, T.W., Grierson, S.S., King, D.P., Hicks, D., Done, S., Banks, M., Neser, J.A., Evans, D.P.B. and Grimbeek, P., 2004. Genetic similarity between porcine circovirus type 2 isolated

from the first reported case of PMWS in South Africa and North American isolates. *Veterinary Record*, *155*(5), pp.149–151.

Dupont, K., Hjulsager, C.K., Kristensen, C.S., Baekbo, P. and Larsen, L.E., 2009. Transmission of different variants of PCV2 and viral dynamics in a research facility with pigs mingled from PMWS-affected herds and non-affected herds. *Veterinary Microbiology*, *139*(3), pp.219-226.

Dupont, K., Nielsen, E.O., Baekbo, P. and Larsen, L.E., 2008. Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case–control study supports a shift in genotypes with time. *Veterinary Microbiology*, *128*(1), pp.56-64.

Eddicks, M., Fux, R., Szikora, F., Eddicks, L., Majzoub-Altweck, M., Hermanns, W., Sutter, G., Palzer, A., Banholzer, E. and Ritzmann, M., 2015. Detection of a new cluster of porcine circovirus type 2b strains in domestic pigs in Germany. *Veterinary Microbiology*, *176*(3), pp.337-343.

Ellis, J., 2014. Porcine circovirus: a historical perspective. *Veterinary Pathology*, *51*(2), pp.315-327.

Ellis, J., Hassard, L., Clark, E., Harding, J., Allan, G., Willson, P., Strokappe, J., Martin, K., McNeilly, F., Meehan, B. and Todd, D., 1998. Isolation of circovirus from lesions of pigs with postweaning multisystemic wasting syndrome. *The Canadian Veterinary Journal*, *39*(1), pp.44-51.

FAO, 2012. Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3, Rome.

Fenaux, M., Halbur, P.G., Gill, M., Toth, T.E. and Meng, X.J., 2000. Genetic characterization of type 2 porcine circovirus (PCV-2) from pigs with postweaning multisystemic wasting syndrome in different geographic regions of North America and development of a differential PCR-restriction fragment length polymorphism assay to detect and differentiate between infections with PCV-1 and PCV-2. *Journal of Clinical Microbiology*, *38*(7), pp.2494-2503.

Feng, H., Blanco, G., Segalés, J. and Sibila, M., 2014. Can Porcine circovirus type 2 (PCV2) infection be eradicated by mass vaccination?. *Veterinary Microbiology*, *172*(1), pp.92-99.

Firth, C., Charleston, M.A., Duffy, S., Shapiro, B. and Holmes, E.C., 2009. Insights into the evolutionary history of an emerging livestock pathogen: porcine circovirus 2. *Journal of Virology*, *83*(24), pp.12813-12821.

Fraile, L., Sibila, M., Nofrarías, M., López-Jimenez, R., Huerta, E., Llorens, A., López-Soria, S., Pérez, D. and Segalés, J., 2012. Effect of sow and piglet porcine circovirus type 2 (PCV2) vaccination on piglet mortality, viraemia, antibody titre and production parameters. *Veterinary Microbiology*, *161*(1), pp.229-234.

Franzo, G., Cortey, M., de Castro, A.M.M.G., Piovezan, U., Szabo, M.P.J., Drigo, M., Segalés, J. and Richtzenhain, L.J., 2015b. Genetic characterisation of Porcine circovirus type 2 (PCV2) strains from feral pigs in the Brazilian Pantanal: an opportunity to reconstruct the history of PCV2 evolution. *Veterinary Microbiology*, *178*(1), pp.158-162.

Franzo, G., Cortey, M., Olvera, A., Novosel, D., De Castro, A.M.M.G., Biagini, P., Segalés, J. and Drigo, M., 2015a. Revisiting the taxonomical classification of Porcine Circovirus type 2 (PCV2): still a real challenge. *Virology Journal*, *12*(1), p.131.

Franzo, G., Tucciarone, C.M., Dotto, G., Gigli, A., Ceglie, L. and Drigo, M., 2015c. International trades, local spread and viral evolution: The case of porcine circovirus type 2 (PCV2) strains heterogeneity in Italy. *Infection, Genetics and Evolution*, *32*, pp.409-415.

Gagnon, C.A., Tremblay, D., Tijssen, P., Venne, M.H., Houde, A. and Elahi, S.M., 2007. The emergence of porcine circovirus 2b genotype (PCV-2b) in swine in Canada. *The Canadian Veterinary Journal*, *48*(8), pp.811-819.

Gerber, P.F., Garrocho, F.M., Lana, Â.M. and Lobato, Z.I., 2011. Serum antibodies and shedding of infectious porcine circovirus 2 into colostrum and milk of vaccinated and unvaccinated naturally infected sows. *The Veterinary Journal*, *188*(2), pp.240-242.

Gillespie, J., Opriessnig, T., Meng, X.J., Pelzer, K. and Buechner-Maxwell, V., 2009. Porcine circovirus type 2 and porcine circovirus-associated disease. *Journal of Veterinary Internal Medicine*, *23*(6), pp.1151-1163.

Grau-Roma, L., Fraile, L. and Segalés, J., 2011. Recent advances in the epidemiology, diagnosis and control of diseases caused by porcine circovirus type 2. *The Veterinary Journal*, *187*(1), pp.23-32.

Grierson, S.S., King, D.P., Sandvik, T., Hicks, D., Spencer, Y., Drew, T.W. and Banks, M., 2004. Detection and genetic typing of type 2 porcine circoviruses in archived pig tissues from the UK. *Archives of Virology*, *149*(6), pp.1171-1183.

Gu, J., Wang, L., Jin, Y., Lin, C., Wang, H., Zhou, N., Xing, G., Liao, M. and Zhou, J., 2016. Characterization of specific antigenic epitopes and the nuclear export signal of the Porcine circovirus 2 ORF3 protein. *Veterinary Microbiology*, *184*, pp.40-50.

Guo, L., Fu, Y., Wang, Y., Lu, Y., Wei, Y., Tang, Q., Fan, P., Liu, J., Zhang, L., Zhang, F. and Huang, L., 2012. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. *PLoS One*, *7*(7), p.e41463.

Guo, L.J., Lu, Y.H., Wei, Y.W., Huang, L.P. and Liu, C.M., 2010. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virology Journal*, *7*(1), p.273.

Ha, Y., Jung, K. and Chae, C., 2005a. Lack of evidence of porcine circovirus type 1 and type 2 infection in piglets with congenital tremors in Korea. *The Veterinary Record*, *156*(12), pp.383-384.

Ha, Y., Jung, K., Kim, J., Choi, C. and Chae, C., 2005b. Outbreak of salmonellosis in pigs with postweaning multisystemic wasting syndrome. *Veterinary Record*, *156*(18), pp.583-583.

Hamel, A.L., Lin, L.L., Sachvie, C., Grudeski, E. and Nayar, G.P., 2000. PCR detection and characterization of type-2 porcine circovirus. *Canadian Journal of Veterinary Research*, *64*(1), pp.44-52.

Harding, J.C., 1996. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. *In: Proceedings of Western Canadian Association of Swine Practitioners*, p. 21.

Harding, J.C. and Clark, E.G., 1997. Recognizing and diagnosing postweaning multisystemic wasting syndrome (PMWS). *Journal of Swine Health and Production*, *5*(5), pp.201-203.

Harding, J.C., Clark, E.G., Strokappe, J.H., Wilson, P.I. and Ellis, J. A., 1998. Post-weaning multisystemic wasting syndrome (PMWS): epidemiology and clinical presentation. *Swine Health and Production, 6*(6), pp.249-254.

Harms, P.A., Sorden, S.D., Halbur, P.G., Bolin, S.R., Lager, K.M., Morozov, I. and Paul, P.S., 2001. Experimental reproduction of severe disease in CD/CD pigs concurrently infected with type 2 porcine circovirus and porcine reproductive and respiratory syndrome virus. *Veterinary Pathology*, *38*(5), pp.528-539.

Hasslung, F., Wallgren, P., Ladekjaer-Hansen, A.S., Bøtner, A., Nielsen, J., Wattrang, E., Allan, G.M., McNeilly, F., Ellis, J., Timmusk, S. and Belak, K., 2005. Experimental reproduction of postweaning multisystemic wasting syndrome (PMWS) in pigs in Sweden and Denmark with a Swedish isolate of porcine circovirus type 2. *Veterinary Microbiology*, *106*(1), pp.49-60.

He, J., Cao, J., Zhou, N., Jin, Y., Wu, J. and Zhou, J., 2013. Identification and functional analysis of the novel ORF4 protein encoded by porcine circovirus type 2. *Journal of Virology*, *87*(3), pp.1420-1429.

Huang, L., Wang, Y., Wei, Y., Chen, D., Liu, D., Du, W., Xia, D., Wu, H., Feng, L. and Liu, C., 2016. Capsid proteins from PCV2a genotype confer greater protection against a PCV2b strain than those from PCV2b genotype in pigs: evidence for PCV2b strains becoming more predominant than PCV2a strains from 2000 to 2010s. *Applied Microbiology and Biotechnology*, *100*(13), pp.5933-5943.

Jacobsen, B., Krueger, L., Seeliger, F., Bruegmann, M., Segalés, J. and Baumgaertner, W., 2009. Retrospective study on the occurrence of porcine circovirus 2 infection and associated entities in Northern Germany. *Veterinary Microbiology*, *138*(1), pp.27-33.

Jonsson, L., 2013. *Emerging infectious diseases: using PCV2 as a model of disease transmission dynamics at the livestock-wildlife interface in Uganda* (Doctoral dissertation, Swedish University of Agricultural Sciences).

Kekarainen, T., McCullough, K., Fort, M., Fossum, C., Segalés, J. and Allan, G.M., 2010. Immune responses and vaccine-induced immunity against Porcine circovirus type 2. *Veterinary Immunology and Immunopathology*, *136*(3), pp.185-193.

Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S. and Allan, G.M., 2000. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *Journal of Comparative Pathology*, *122*(1), pp.9-24.

Kennedy, S., Segalés, J., Rovira, A., Scholes, S., Domingo, M., Moffett, D., Meehan, B., O'Neill, R., McNeilly, F. and Allan, G., 2003. Absence of evidence of porcine circovirus infection in piglets with congenital tremors. *Journal of Veterinary Diagnostic Investigation*, *15*(2), pp.151-156.

Khan, S.U., Atanasova, K.R., Krueger, W.S., Ramirez, A. and Gray, G.C., 2013. Epidemiology, geographical distribution, and economic consequences of swine zoonoses: a narrative review. *Emerging Microbes and Infections*, *2*(12), p.e92.

Kim, J., Chung, H.K. and Chae, C., 2003. Association of porcine circovirus 2 with porcine respiratory disease complex. *The Veterinary Journal*, *166*(3), pp.251-256.

Kim, J., Ha, Y., Jung, K., Choi, C. and Chae, C., 2004. Enteritis associated with porcine circovirus 2 in pigs. *Canadian Journal of Veterinary Research*, *68*(3), pp.218.

Krakowka, S., Allan, G., Ellis, J., Hamberg, A., Charreyre, C., Kaufmann, E., Brooks, C. and Meehan, B., 2012. A nine-base nucleotide sequence in the porcine circovirus type 2 (PCV2) nucleocapsid gene determines viral replication and virulence. *Virus Research*, *164*(1), pp.90-99.

Kwon, T., Lee, D.U., Yoo, S.J., Sang, H.J., Shin, J.Y. and Lyoo, Y.S., 2017. Genotypic diversity of porcine circovirus type 2 (PCV2) and genotype shift to PCV2d in Korean pig population. *Virus Research*, *228*, pp.24-29.

Lager, K.M., Gauger, P.C., Vincent, A.L., Opriessnig, T., Kehrli Jr, M.E. and Cheung, A.K., 2007. Mortality in pigs given porcine circovirus type 2 subgroup 1 and 2 viruses derived from DNA clones. *Veterinary Record*, *161*(12), pp.428-428.

LeCann, P., Albina, E., Madec, F., Cariolet, R. and Jestin, A., 1997. Piglet wasting disease. *The Veterinary Record*, *141*(25), p.660.

Lefebvre, D.J., Costers, S., Van Doorsselaere, J., Misinzo, G., Delputte, P.L. and Nauwynck, H.J., 2008. Antigenic differences among porcine circovirus type 2 strains, as demonstrated by the use of monoclonal antibodies. *Journal of General Virology*, *89*(1), pp.177-187.

Li, L., Kapoor, A., Slikas, B., Bamidele, O.S., Wang, C., Shaukat, S., Masroor, M.A., Wilson, M.L., Ndjango, J.B.N., Peeters, M. and Gross-Camp, N.D., 2010. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *Journal of Virology*, *84*(4), pp.1674-1682.

Li, Y., Liu, H., Wang, P., Wang, L., Sun, Y., Liu, G., Zhang, P., Kang, L., Jiang, S. and Jiang, Y., 2016. RNA-seq analysis reveals genes underlying different disease responses to porcine circovirus type 2 in pigs. *PloS One*, *11*(5), p.e0155502.

Liu, C., Ihara, T., Nunoya, T. and Ueda, S., 2004. Development of an ELISA based on the baculovirus-expressed capsid protein of porcine circovirus type 2 as antigen. *Journal of Veterinary Medical Science*, *66*(3), pp.237-242.

Liu, J., Chen, I. and Kwang, J., 2005. Characterization of a previously unidentified viral protein in porcine circovirus type 2-infected cells and its role in virus-induced apoptosis. *Journal of Virology*, *79*(13), pp.8262-8274.

Liu, J., Chen, I., Du, Q., Chua, H. and Kwang, J., 2006. The ORF3 protein of porcine circovirus type 2 is involved in viral pathogenesis in vivo. *Journal of Virology*, *80*(10), pp.5065-5073.

Liu, X., Wang, F.X., Zhu, H.W., Sun, N. and Wu, H., 2016. Phylogenetic analysis of porcine circovirus type 2 (PCV2) isolates from China with high homology to PCV2c. *Archives of Virology*, *161*(6), pp.1591-1599.

Lőrincz, M., Cságola, A., Biksi, I., Szeredi, L., Dán, Á. and Tuboly, T., 2010. Detection of porcine circovirus in rodents. *Acta Veterinaria Hungarica*, *58*(2), pp.265-268.

Lukač, B., Knežević, A., Milić, N., Krnjaić, D., Veljović, L., Milićević, V., Zorić, A., Đurić, S., Stanojević, M. and Nišavić, J., 2016. Molecular detection of PCV2 and PPV in pigs in Republic of Srpska, Bosnia and Herzegovina. *Acta Veterinaria*, *66*(1), pp.51-60.

Magar, R., Müller, P. and Larochelle, R., 2000. Retrospective serological survey of antibodies to porcine circovirus type 1 and type 2. *Canadian Journal of Veterinary Research*, *64*(3), pp.184-186.

Mankertz, A. and Hillenbrand, B., 2002. Analysis of transcription of Porcine circovirus type 1. *Journal of General Virology*, *83*(11), pp.2743-2751.

Mankertz, A., Mankertz, J., Wolf, K. and Buhk, H.J., 1998. Identification of a protein essential for replication of porcine circovirus. *Journal of General Virology*, *79*(2), pp.381-384.

Marruchella, G., Paoletti, B., Speranza, R. and Di Guardo, G., 2012. Fatal bronchopneumonia in a Metastrongylus elongatus and Porcine circovirus type 2 co-infected pig. *Research in Veterinary Science*, *93*(1), pp.310-312.

McKeown, N.E., Opriessnig, T., Thomas, P., Guenette, D.K., Elvinger, F., Fenaux, M., Halbur, P.G. and Meng, X.J., 2005. Effects of porcine circovirus type 2 (PCV2) maternal antibodies on experimental infection of piglets with PCV2. *Clinical and Diagnostic Laboratory Immunology*, *12*(11), pp.1347-1351.

McNulty, M., Dale, J., Lukert, P., Mankertz, A., Randles, J. and Todd, D., 2000. Circoviridae. In*:* van Regenmortel, M.H.V., Fauquet, C.M., Bishop, D.H.L., Carstens, E.B., Estes, M.K., Lemon, S.M., Maniloff, J., Mayo, M.A., McGeoch, D.J., Pringle, C.R. and Wickner, R.B. Eds., *Seventh Report of the International Committee on Taxonomy of Viruses*, Academic Press, San Diego, pp. *299*-303.

Meehan, B.M., Creelan, J.L., McNulty, M.S. and Todd, D., 1997. Sequence of porcine circovirus DNA: affinities with plant circoviruses. *Journal of General Virology*, *78*(1), pp.221-227.

Meehan, B.M., McNeilly, F., Todd, D., Kennedy, S., Jewhurst, V.A., Ellis, J.A., Hassard, L.E., Clark, E.G., Haines, D.M. and Allan, G.M., 1998. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. *Journal of General Virology*, *79*(9), pp.2171-2179.

Meng, X.J., 2012. Spread like a wildfire—the omnipresence of porcine circovirus type 2 (PCV2) and its ever-expanding association with diseases in pigs. *Virus Research*, *164*(1-2), pp.1–3.

Meng, X.J., 2013. Porcine circovirus type 2 (PCV2): pathogenesis and interaction with the immune system. *Annual Review of Animal Biosciences*, *1*(1), pp.43-64.

Mokoele, J.M., van Rensburg, L.J., Van Lochem, S., Bodenstein, H., Du Plessis, J., Carrington, C.A., Spencer, B.T. and Fasina, F.O., 2015. Overview of the perceived risk of transboundary pig diseases in South Africa. *Journal of the South African Veterinary Association*, *86*(1), pp.1-9.

Monger, V.R., Stegeman, J.A., Koop, G., Dukpa, K., Tenzin, T. and Loeffen, W.L.A., 2014. Seroprevalence and associated risk factors of important pig viral diseases in Bhutan. *Preventive Veterinary Medicine*, *117*(1), pp.222-232.

Morozov, I., Sirinarumitr, T., Sorden, S.D., Halbur, P.G., Morgan, M.K., Yoon, K.J. and Paul, P.S., 1998. Detection of a novel strain of porcine circovirus in pigs with postweaning multisystemic wasting syndrome. *Journal of Clinical Microbiology*, *36*(9), pp.2535-2541.

Nawagitgul, P., Morozov, I., Bolin, S.R., Harms, P.A., Sorden, S.D. and Paul, P.S., 2000. Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein. *Journal of General Virology*, *81*(9), pp.2281-2287.

Ndyomugyenyi, E.K. and Kyasimire, J., 2015. Pig production in Kichwamba Sub-county, Rubirizi district, Uganda. *Livestock Research for Rural Development*, *27*(10).

Ndze, V.N., Cadar, D., Cságola, A., Kisfali, P., Kovács, E., Farkas, S., Ngu, A.F., Esona, M.D., Dán, Á., Tuboly, T. and Bányai, K., 2013. Detection of novel porcine bocaviruses in

fecal samples of asymptomatic pigs in Cameroon. *Infection, Genetics and Evolution*, *17*, pp.277-282.

Niagro, F.D., Forsthoefel, A.N., Lawther, R.P., Kamalanathan, L., Ritchie, B.W., Latimer, K.S. and Lukert, P.D., 1998. Beak and feather disease virus and porcine circovirus genomes: intermediates between the geminiviruses and plant circoviruses. *Archives of Virology*, *143*(9), pp.1723-1744.

Ojok, L., Okuni, J.B., Hohloch, C., Hecht, W. and Reinacher, M., 2013. Detection and characterisation of porcine circovirus 2 from Ugandan pigs. *Indian Journal of Veterinary Pathology*, *37*(1), pp.77-80.

Olvera, A., Cortey, M. and Segalés, J., 2007. Molecular evolution of porcine circovirus type 2 genomes: phylogeny and clonality. *Virology*, *357*(2), pp.175-185.

Opriessnig, T. and Halbur, P.G., 2012. Concurrent infections are important for expression of porcine circovirus associated disease. *Virus Research*, *164*(1), pp.20-32.

Opriessnig, T., Meng, X.J. and Halbur, P.G., 2007. Porcine circovirus type 2–associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *Journal of Veterinary Diagnostic Investigation*, *19*(6), pp.591-615.

Opriessnig, T., O'Neill, K., Gerber, P.F., de Castro, A.M., Gimenéz-Lirola, L.G., Beach, N.M., Zhou, L., Meng, X.J., Wang, C. and Halbur, P.G., 2013b. A PCV2 vaccine based on genotype 2b is more effective than a 2a-based vaccine to protect against PCV2b or combined PCV2a/2b viremia in pigs with concurrent PCV2, PRRSV and PPV infection. *Vaccine*, *31*(3), pp.487-494.

Opriessnig, T., Ramamoorthy, S., Madson, D.M., Patterson, A.R., Pal, N., Carman, S., Meng, X.J. and Halbur, P.G., 2008. Differences in virulence among porcine circovirus type 2 isolates are unrelated to cluster type 2a or 2b and prior infection provides heterologous protection. *Journal of General Virology*, *89*(10), pp.2482-2491.

Opriessnig, T., Thacker, E.L., Yu, S., Fenaux, M., Meng, X.J. and Halbur, P.G., 2004. Experimental reproduction of postweaning multisystemic wasting syndrome in pigs by dual infection with Mycoplasma hyopneumoniae and porcine circovirus type 2. *Veterinary Pathology*, *41*(6), pp.624-640.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2013a. Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the US concurrently infected with PPV2. *Veterinary Microbiology*, *163*(1), pp.177-183.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2014. Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. *Veterinary Microbiology*, *173*(1), pp.9-16.

Park, J.S., Kim, J., Ha, Y., Jung, K., Choi, C., Lim, J.K., Kim, S.H. and Chae, C., 2005. Birth abnormalities in pregnant sows infected intranasally with porcine circovirus 2. *Journal of Comparative Pathology*, *132*(2), pp.139-144.

Patterson, A.R. and Opriessnig, T., 2010. Epidemiology and horizontal transmission of porcine circovirus type 2 (PCV2). *Animal Health Research Reviews*, *11*(2), pp.217-234.

Patterson, A.R., Madson, D.M., Halbur, P.G. and Opriessnig, T., 2011a. Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after natural exposure. *Veterinary Microbiology*, *149*(1), pp.225-229.

Patterson, A.R., Ramamoorthy, S., Madson, D.M., Meng, X.J., Halbur, P.G. and Opriessnig, T., 2011b. Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after experimental infection. *Veterinary Microbiology*, *149*(1), pp.91-98.

Patterson, R., Nevel, A., Diaz, A.V., Martineau, H.M., Demmers, T., Browne, C., Mavrommatis, B. and Werling, D., 2015. Exposure to environmental stressors result in increased viral load and further reduction of production parameters in pigs experimentally infected with PCV2b. *Veterinary Microbiology*, *177*(3), pp.261-269.

Peng, Z., Ma, T., Pang, D., Su, D., Chen, F., Chen, X., Guo, N., Ouyang, T., Ouyang, H. and Ren, L., 2016. Expression, purification and antibody preparation of PCV2 Rep and ORF3 proteins. *International Journal of Biological Macromolecules*, *86*, pp.277-281.

Pérez, L.J., de Arce, H.D., Percedo, M.I., Domínguez, P. and Frías, M.T., 2010. First report of porcine circovirus type 2 infections in Cuba. *Research in Veterinary Science*, *88*(3), pp.528-530.

Pozzi, S.P., Yadin, H., Lavi, J., Pacciarini, M. and Alborali, L., 2008. Porcine circovirus type 2 (pcv2) infection of pigs in Israel: Clinical presentation, diagnosis and virus identification. *Israel Journal of Veterinary Medicine*, *63*(4), pp.122-125.

Pringle, C.R., 1999. Virus taxonomy at the XIth international congress of virology, Sydney, Australia, 1999. *Archives of Virology*, *144*(10), pp.2065-2070.

Ramos, N., Mirazo, S., Castro, G. and Arbiza, J., 2012. Detection and molecular characterization of porcine circovirus type 2 (PCV2) from piglets with exudative epidermitis in Uruguay. *Research in Veterinary Science*, *93*(2), pp.1042-1045.

Ramos, N., Mirazo, S., Castro, G. and Arbiza, J., 2015. First identification of Porcine Circovirus Type 2b mutant in pigs from Uruguay. *Infection, Genetics and Evolution*, *33*, pp.320-323.

Raye, W., 2004. *An investigation into the status of porcine circovirus in Australia* (Doctoral dissertation, Murdoch University).

Reiner, G., Hofmeister, R. and Willems, H., 2015. Genetic variability of porcine circovirus 2 (PCV2) field isolates from vaccinated and non-vaccinated pig herds in Germany. *Veterinary Microbiology*, *180*(1), pp.41-48.

Rodríguez-Arrioja, G.M., Segalés, J., Rosell, C., Rovira, A., Pujols, J., Plana-Durán, J. and Domingo, M., 2003. Retrospective study on porcine circovirus type 2 infection in pigs from 1985 to 1997 in Spain. *Zoonoses and Public Health*, *50*(2), pp.99-101.

Rosario, K., Breitbart, M., Harrach, B., Segalés, J., Delwart, E., Biagini, P. and Varsani, A., 2017. Revisiting the taxonomy of the family Circoviridae: establishment of the genus Cyclovirus and removal of the genus Gyrovirus. *Archives of Virology*, *162*(5), pp.1447-1463.

Rose, N., Larour, G., Le Diguerher, G., Eveno, E., Jolly, J.P., Blanchard, P., Oger, A., Le Dimna, M., Jestin, A. and Madec, F., 2003. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. *Preventive Veterinary Medicine*, *61*(3), pp.209-225.

Rose, N., Opriessnig, T., Grasland, B. and Jestin, A., 2012. Epidemiology and transmission of porcine circovirus type 2 (PCV2). *Virus Research*, *164*(1), pp.78-89.

Rosell, C., Segalés, J., Ramos-Vara, J.A., Folch, J.M., Rodriguez-Arrioja, G.M., Duran, C.O., Balasch, M., Plana-Duran, J. and Domingo, M., 2000. Identification of porcine

circovirus in tissues of pigs with porcine dermatitis and nephropathy syndrome. *The Veterinary Record*, *146*(2), pp.40-43.

Salgado, R.L., Vidigal, P.M., de Souza, L.F., Onofre, T.S., Gonzaga, N.F., Eller, M.R., Bressan, G.C., Fietto, J.L., Almeida, M.R. and Júnior, A.S., 2014. Identification of an emergent porcine circovirus-2 in vaccinated pigs from a Brazilian farm during a postweaning multisystemic wasting syndrome outbreak. *Genome Announcements*, *2*(2), pp.e00163-14.

Salogni, C., Lazzaro, M., Giacomini, E., Giovannini, S., Zanoni, M., Giuliani, M., Ruggeri, J., Pozzi, P., Pasquali, P., Boniotti, M.B. and Alborali, G.L., 2016. Infectious agents identified in aborted swine fetuses in a high-density breeding area: A Three-Year Study. *Journal of Veterinary Diagnostic Investigation*, *28*(5), pp.550-554.

Sanchez, R., Nauwynck, H. and Pensaert, M., 2001. Serological survey of porcine circovirus 2 antibodies in domestic and feral pig populations in Belgium. *Proc. ssDNA viruses of plants, birds, pigs and primates. St. Malo (France)*, p.122.

Schmoll, F., Lang, C., Steinrigl, A.S., Schulze, K. and Kauffold, J., 2008. Prevalence of PCV2 in Austrian and German boars and semen used for artificial insemination. *Theriogenology*, *69*(7), pp.814-821.

Segalés, J., 2012. Porcine circovirus type 2 (PCV2) infections: clinical signs, pathology and laboratory diagnosis. *Virus Research*, *164*(1), pp.10-19.

Segalés, J., Allan, G.M. and Domingo, M., 2005a. Porcine circovirus diseases. *Animal Health Research Reviews*, *6*(2), pp.119-142.

Segalés, J., Calsamiglia, M., Olvera, A., Sibila, M., Badiella, L. and Domingo, M., 2005b. Quantification of porcine circovirus type 2 (PCV2) DNA in serum and tonsillar, nasal, tracheo-

bronchial, urinary and faecal swabs of pigs with and without postweaning multisystemic wasting syndrome (PMWS). *Veterinary Microbiology*, *111*(3), pp.223-229.

Segalés, J., Kekarainen, T. and Cortey, M., 2013. The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?. *Veterinary Microbiology*, *165*(1), pp.13-20.

Segalés, J., Olvera, A., Grau-Roma, L., Charreyre, C., Nauwynck, H., Larsen, L., Dupont, K., McCullough, K., Ellis, J., Krakowka, S. and Mankertz, A., 2008. PCV-2 genotype definition and nomenclature. *Veterinary Record*, *162*(26), pp.867-868.

Segalés, J., Sitjar, M., Domingo, M., Dee, S., Pozo, M.D., Noval, R., Sacristan, C., Heras, A., Ferro, A. and Latimer, K.S., 1997. First report of post-weaning multisystemic wasting syndrome in pigs in Spain. *Veterinary Record*, *141*(23), pp.600-601.

Segalés, J., Urniza, A., Alegre, A., Bru, T., Crisci, E., Nofrarías, M., López-Soria, S., Balasch, M., Sibila, M., Xu, Z. and Chu, H.J., 2009. A genetically engineered chimeric vaccine against porcine circovirus type 2 (PCV2) improves clinical, pathological and virological outcomes in postweaning multisystemic wasting syndrome affected farms. *Vaccine*, *27*(52), pp.7313-7321.

Seo, H.W., Lee, J., Han, K., Park, C. and Chae, C., 2014. Comparative analyses of humoral and cell-mediated immune responses upon vaccination with different commercially available single-dose porcine circovirus type 2 vaccines. *Research in Veterinary Science*, *97*(1), pp.38-42.

Shang, S.B., Jin, Y.L., Jiang, X.T., Zhou, J.Y., Zhang, X., Xing, G., He, J. and Yan, Y., 2009. Fine mapping of antigenic epitopes on capsid proteins of porcine circovirus, and antigenic phenotype of porcine circovirus type 2. *Molecular Immunology*, *46*(3), pp.327-334.

Shen, H., Wang, C., Madson, D.M. and Opriessnig, T., 2010. High prevalence of porcine circovirus viremia in newborn piglets in five clinically normal swine breeding herds in North America. *Preventive Veterinary Medicine*, *97*(3), pp.228-236.

Shibata, I., Okuda, Y., Yazawa, S., Ono, M., Sasaki, T., Itagaki, M., Nakajima, N., Okabe, Y. and Hidejima, I., 2003. PCR detection of porcine circovirus type 2 DNA in whole blood, serum, oropharyngeal swab, nasal swab, and feces from experimentally infected pigs and field cases. *Journal of Veterinary Medical Science*, *65*(3), pp.405-408.

Sibila, M., Calsamiglia, M., Segalés, J., Blanchard, P., Badiella, L., Le Dimna, M., Jestin, A. and Domingo, M., 2004. Use of a polymerase chain reaction assay and an ELISA to monitor porcine circovirus type 2 infection in pigs from farms with and without postweaning multisystemic wasting syndrome. *American Journal of Veterinary Research*, *65*(1), pp.88-92.

Ssemadaali, M.A., Ilha, M. and Ramamoorthy, S., 2015. Genetic diversity of porcine circovirus type 2 and implications for detection and control. *Research in Veterinary Science*, *103*, pp.179-186.

Stevenson, G.W., Kiupel, M., Mittal, S.K., Choi, J., Latimer, K.S. and Kanitz, C.L., 2001. Tissue distribution and genetic typing of porcine circoviruses in pigs with naturally occurring congenital tremors. *Journal of Veterinary Diagnostic Investigation*, *13*(1), pp.57-62.

Sun, J., Huang, L., Wei, Y., Wang, Y., Chen, D., Du, W., Wu, H. and Liu, C., 2015a. Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. *Archives of Virology*, *160*(5), pp.1339-1344.

Sun, N., Sun, P., Lv, H., Sun, Y., Guo, J., Wang, Z., Luo, T., Wang, S. and Li, H., 2016. Matrine displayed antiviral activity in porcine alveolar macrophages co-infected by porcine

reproductive and respiratory syndrome virus and porcine circovirus type 2. *Scientific Reports*, *6*, Article ID 24401.

Sun, N., Yu, T., Zhao, J.X., Sun, Y.G., Jiang, J.B., Duan, Z.B., Wang, W.K., Hu, Y.L., Lei, H.M. and Li, H.Q., 2015b. Antiviral activities of natural compounds derived from traditional chinese medicines against porcine circovirus type 2 (PCV2). *Biotechnology and Bioprocess Engineering*, *20*(1), p.180.

Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R. and Polasky, S., 2002. Agricultural sustainability and intensive production practices. *Nature*, *418*(6898)*,* pp.671-677.

Tischer, I., Gelderblom, H., Vettermann, W. and Koch, M.A., 1982. A very small porcine virus with circular single-stranded DNA. *Nature*, *295*(5844), pp.64-66.

Tischer, I., Mields, W., Wolff, D., Vagt, M. and Griem, W., 1986. Studies on epidemiology and pathogenicity of porcine circovirus. *Archives of Virology*, *91*(3), pp.271-276.

Tischer, I., Rasch, R. and Tochtermann, G., 1974. Characterization of papovavirus and picornavirus-like particles in permanent pig kidney cell lines. *Zentralblatt fuer Bakteriologie Mikrobiologie und Hygiene series A*, *226*(2), pp.153-167.

Tomás, A., Fernandes, L.T., Valero, O. and Segalés, J., 2008. A meta-analysis on experimental infections with porcine circovirus type 2 (PCV2). *Veterinary Microbiology*, *132*(3), pp.260-273.

Tummaruk, P. and Pearodwong, P., 2016. Porcine circovirus type 2 expression in the brain of neonatal piglets with congenital tremor. *Comparative Clinical Pathology*, *25*(4), pp.727-732.

Verreault, D., Létourneau, V., Gendron, L., Massé, D., Gagnon, C.A. and Duchaine, C., 2010. Airborne porcine circovirus in Canadian swine confinement buildings. *Veterinary Microbiology*, *141*(3), pp.224-230.

Vidigal, P.M., Mafra, C.L., Silva, F.M., Fietto, J.L., Júnior, A.S. and Almeida, M.R., 2012. Tripping over emerging pathogens around the world: a phylogeographical approach for determining the epidemiology of Porcine circovirus-2 (PCV-2), considering global trading. *Virus Research*, *163*(1), pp.320-327.

Walker, I.W., Konoby, C.A., Jewhurst, V.A., McNair, I., McNeilly, F., Meehan, B.M., Cottrell, T.S., Ellis, J.A. and Allan, G.M., 2000. Development and application of a competitive enzyme-linked immunosorbent assay for the detection of serum antibodies to porcine circovirus type 2. *Journal of Veterinary Diagnostic Investigation*, *12*(5), pp.400-405.

Wang, X., He, K., Wang, W., Zhou, Z., Yang, G., Mao, A., Yu, Z. and Ni, Y., 2014. Genetic variation of porcine circovirus type 2 isolate 201105ZJ. *Agricultural Science and Technology*, *15*(11), p.1860-1864.

Wellenberg, G.J., Stockhofe-Zurwieden, N., De Jong, M.F., Boersma, W.J.A. and Elbers, A.R.W., 2004. Excessive porcine circovirus type 2 antibody titres may trigger the development of porcine dermatitis and nephropathy syndrome: a case-control study. *Veterinary Microbiology*, *99*(3), pp.203-214.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2012. Complete genome sequence of a novel porcine circovirus type 2b variant present in cases of vaccine failures in the United States. *Journal of Virology*, *86*(22), pp.12469-12469.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2015. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. *Journal of General Virology*, *96*(7), pp.1830-1841.

Xiao, C.T., Harmon, K.M., Halbur, P.G. and Opriessnig, T., 2016. PCV2d-2 is the predominant type of PCV2 DNA in pig samples collected in the US during 2014–2016. *Veterinary Microbiology*, *197*, pp.72-77.

Yang, X., Hou, L., Ye, J., He, Q. and Cao, S., 2012. Detection of porcine circovirus type 2 (PCV2) in mosquitoes from pig farms by PCR. *Pakistan Veterinary Joutnal*, *32*, pp.134-135.

Yang, Y., Shi, R., She, R., Mao, J., Zhao, Y., Du, F., Liu, C., Liu, J., Cheng, M., Zhu, R. and Li, W., 2015. Fatal disease associated with Swine Hepatitis E virus and Porcine circovirus 2 co-infection in four weaned pigs in China. *BMC Veterinary Research*, *11*(1), p.77.

Yang, Z.Z., Shuai, J.B., Dai, X.J. and Fang, W.H., 2008. A survey on porcine circovirus type 2 infection and phylogenetic analysis of its ORF2 gene in Hangzhou, Zhejiang Province, China. *Journal of Zhejiang University-Science B*, *9*(2), pp.148-153.

Zhai, S.L., Chen, S.N., Xu, Z.H., Tang, M.H., Wang, F.G., Li, X.J., Sun, B.B., Deng, S.F., Hu, J., Lv, D.H. and Wen, X.H., 2014. Porcine circovirus type 2 in China: an update on and insights to its prevalence and control. *Virology Journal*, *11*(1), p.88.

CHAPTER THREE

LITERATURE REVIEW

Epidemiology of Porcine parvoviruses in African swine herds

(*To be submitted for publication in Animal Health Research Reviews*)

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Abstract

Detection of infectious viral agents has been on the increase globally with the advent and usage of more sensitive and selective novel molecular techniques in the epidemiological study of viral diseases of economic importance to the swine industry. The story is not different for the pig-infecting member of the subfamily *Parvovirinae* in the family *Parvoviridae* as the application of novel molecular methods like metagenomics has led to the detection of many other novel members of the group. Surprisingly, the list kept increasing day by day with some of them possessing zoonotic potentials. In the last one decade, not less than 10 novel swine-infecting viruses have been added to the subfamily; and ceaseless efforts have been in top gear in determining the occurrence and prevalence of the old and new porcine parvoviruses in herds of pig-producing countries worldwide. The story, however, is on the contrary on the African continent as there is presently a dearth of information on surveillance initiatives of the viruses among swine herds of pig-producing countries in the region. Timely detection and characterization of the viral pathogens is highly imperative for the implementation of effective control and prevention of its spread. This current review therefore presents a concise overview on the epidemiology of novel porcine parvoviruses globally; and also provides up-to-date highlights on the reported cases of the viral agents in the African sub-region.

Keywords: Porcine parvoviruses, Epidemiology, Africa, Swine

3.1   Introduction

3.2   Diversity and classifications of parvoviruses

The prefix "parvo" in parvoviruses emanated from the Latin word "parvum" and means "small". Parvoviruses are therefore, a group of relatively small viruses with linear, single stranded DNA (ssDNA) genome ranging from 4-6.3 kb in size, packaged in a non-enveloped, icosahedral capsid (Tijssen *et al*., 2011). They are ubiquitous in nature and common infectious agents of numerous hosts ranging from non-vertebrate arthropods to vertebrates and higher mammals including human beings. Their ancestors, according to Kailasan *et al*. (2015) seem to have emerged several millions of years past and have been widely distributed ever since. Parvoviruses, though appear to evolve from the same ancestor and have similar genomic features, they usually exhibit a very low relatedness at the nucleotide or protein level thereby depicting their highly extensive diversity. Their diversity seems to also influence their clinical effects on their infected hosts which could range from non-pathogenic infections to severely lethal diseases' manifestations (Kailasan *et al*., 2015).

Parvoviruses belong to the family *Parvoviridae* that comprises two subfamilies namely: *Densovirinae* and *Parvovirinae.* Members of the family *Parvoviridae* were assigned into the two subfamilies based on the kind of hosts they infect. Groups of parvoviruses that infect invertebrate hosts (arthropods and crustaceans) belong to the subfamily *Densovirinae*, whereas those that infects vertebrate hosts belong to the subfamily *Parvovirinae*. In the ninth edition of the taxonomical grouping that was made by the International Committee on Taxonomy of Viruses (ICTV), the subfamily *Densovirinae* consists of four genera namely: *Densovirus, Brevidensovirus, Iteravirus* and *Pefudensovirus*; whereas the subfamily *Parvovirinae* comprises five genera including *Parvovirus, Erythrovirus, Dependovirus, Amdovirus* and *Bocavirus* (Tijssen *et al*., 2011). However, in the latest ICTV report, series of systematic changes were

made using a modified definition for classification that requires complete or nearly complete genome of the viruses to arrive at an improved taxonomic clarity of parvoviruses; and this gave rise to the introduction of new species and genera into the two subfamilies of the family *Parvoviridae*.

The sub-family *Densovirinae* in the latest report now consists of five genera namely: *Ambidensovirus* (*Densovirus*), *Brevidensovirus, Hepandensovirus, Iteradensovirus* (*Iteravirus*) and *Penstyldensovirus*. Members of the subfamily *Parvovirinae* are now categorized into two major groups namely: dependoparvoviruses and autonomous parvoviruses based on their replication requirement. While the dependoparvoviruses require helper virus for successful replication within cells, the autonomous types do not (Kailasan *et al*., 2015). Altogether, the subfamily *Parvovirinae* consists of eight genera including: *Amdoparvovirus* (*Amdovirus*), *Aveparvovirus, Dependoparvovirus* (*Dependovirus),* *Erythroparvovirus* (*Erythrovirus*), *Copiparvovirus, Bocaparvovirus* (*Bocavirus*), *Protoparvovirus* (*Parvovirus*) and *Tetraparvovirus*. The last four genera contain the classical porcine parvovirus and other new porcine parvoviruses on which this review focuses (Table 3.1) (Cotmore *et al*., 2014).

Table 3.1: Newly proposed taxonomy for the subfamily *Parvovirinae*

Genus	Species	Virus or virus variants	Abbreviation	Accession #
Amdoparvovirus	*Carnivore amdoparvovirus 1*	Aleutian mink disease virus	AMDV	JN040434
	Carnivore amdoparvovirus 2	gray fox amdovirus	GFAV	JN202450
Aveparvovirus	*Galliform aveparvovirus 1*	chicken parvovirus	ChPV	GU214704
		turkey parvovirus	TuPV	GU214706
Bocaparvovirus	*Carnivore bocaparvovirus 1*	canine minute virus	CnMV	FJ214110
	Carnivore bocaparvovirus 2	canine bocavirus 1	CBoV	JN648103
	Carnivore bocaparvovirus 3	feline bocavirus	FBoV	JQ692585
	Pinniped bocaparvovirus 1	California sea lion bocavirus 1	CslBoV1	JN420361
		California sea lion bocavirus 2	CslBoV2	JN420366
	Pinniped bocaparvovirus 2	California sea lion bocavirus 3	CslBoV3	JN420365
	Primate bocaparvovirus 1	human bocavirus 1	HBoV1	JQ923422
		human bocavirus 3	HBoV3	EU918736
		gorilla bocavirus	GBoV	HM145750
	Primate bocaparvovirus 2	human bocavirus 2a	HBoV2a	FJ973558
		human bocavirus 2b	HBoV2b	FJ973560
		human bocavirus 2c	HBoV2c	FJ170278
		human bocavirus 4	HBoV4	FJ973561
	Ungulate bocaparvovirus 1	bovine parvovirus	BPV	DQ335247
	Ungulate bocaparvovirus 2	porcine bocavirus 1*	PBoV1	HM053693
		porcine bocavirus 2*	PBoV2	HM053694
		porcine bocavirus 6*	PBoV6	HQ291309
	Ungulate bocaparvovirus 3	porcine bocavirus 5*	PBoV5	HQ223038
	Ungulate bocaparvovirus 4	porcine bocavirus 7*	PBoV7	HQ291308
	Ungulate bocaparvovirus 5	porcine bocavirus 3*	PBoV3	JF429834
		porcine bocavirus 4-1*	PBoV4-1	JF429835
		porcine bocavirus 4-2*	PBoV4-2	JF429836
Copiparvovirus	*Ungulate copiparvovirus 1*	bovine parvovirus 2	BPV2	AF406966
	Ungulate copiparvovirus 2	porcine parvovirus 4*	PPV4	GQ387499
Dependoparvovirus	*Adeno-associated dependoparvovirus A*	adeno-associated virus-1	AAV1	AF063497
		adeno-associated virus-2	AAV2	AF043303
		adeno-associated virus-3	AAV3	AF028705
		adeno-associated virus-4	AAV4	U89790
		adeno-associated virus-6	AAV6	AF028704
		adeno-associated virus-7	AAV7	AF513851
		adeno-associated virus-8	AAV8	AF513852
		adeno-associated virus-9	AAV9	AX753250
		adeno-associated virus-10	AAV10	AY631965
		adeno-associated virus-11	AAV11	AY631966

Table 3.1 (contd.): Newly proposed taxonomy for the subfamily *Parvovirinae*

Genus	Species	Virus or virus variants	Abbreviation	Accession #
		adeno-associated virus-12	AAV12	DQ813647
		adeno-associated virus-13	AAV13	EU285562
		adeno-associated virus-S17	AAVS17	AY695376
	Adeno-associated dependoparvovirus B	adeno-associated virus-5	AAV5	AF085716
		bovine adeno-associated virus	BAAV	AY388617
		caprine adeno-associated virus	CapAAV	DQ335246
	Anseriform dependoparvovirus 1	duck parvovirus	DPV	U22967
		goose parvovirus-PT	GPV2	JF926695
		goose parvovirus	GPV	U25749
	Avian dependoparvovirus 1	avian adeno-associated virus	AAAV	AY186198
	Chiropteran dependoparvovirus 1	bat adeno-associated virus	BtAAV	GU226971
	Pinniped dependoparvovirus 1	California sea lion adeno-associated virus	CslAAV	JN420372
	Squamate dependoparvovirus 1	snake adeno-associated virus	SAAV	AY349010
Erythroparvovirus	*Primate erythroparvovirus 1*	human parvovirus B19-Au	B19V-Au	M13178
		human parvovirus B19-J35	B19V-J35	AY386330
		human parvovirus B19-Wi	B19V-Wi	M24682
		human parvovirus B19-A6	B19V-A6	AY064475
		human parvovirus B19-Lali	B19V-Lali	AY044266
		human parvovirus B19-V9	B19V-V9	AJ249437
		human parvovirus B19-D91	B19-D91	AY083234
	Primate erythroparvovirus 2	simian parvovirus	SPV	U26342
	Primate erythroparvovirus 3	rhesus macaque parvovirus	RhMPV	AF221122
	Primate erythroparvovirus 4	pig-tailed macaque parvovirus	PtMPV	AF221123
	Rodent erythroparvovirus 1	chipmunk parvovirus	ChpPV	GQ200736
	Ungulate erythroparvovirus 1	bovine parvovirus 3	BPV3	AF406967
Protoparvovirus	*Carnivore protoparvovirus 1*	feline parvovirus	FPV	EU659111
		canine parvovirus	CPV	M19296
		mink enteritis virus	MEV	D00765
		racoon parvovirus	RaPV	JN867610
	Primate protoparvovirus 1	bufavirus 1a	BuPV1a	JX027296
		bufavirus 1b	BuPV1b	JX027295
		bufavirus 2	BuPV2	JX027297
	Rodent protoparvovirus 1	H-1 parvovirus	H1	X01457
		Kilham rat virus	KRV	AF321230
		LuIII virus	LuIII	M81888
		minute virus of mice (prototype)	MVMp	J02275
		minute virus of mice (immunosuppressive)	MVMi	M12032
		minute virus of mice (Missouri)	MVMm	DQ196317
		minute virus of mice (Cutter)	MVMc	U34256
		mouse parvovirus 1	MPV1	U12469

Table 3.1 (contd.): Newly proposed taxonomy for the subfamily *Parvovirinae*

Genus	Species	Virus or virus variants	Abbreviation	Accession #
		mouse parvovirus 2	MPV2	DQ196319
		mouse parvovirus 3	MPV3	DQ199631
		mouse parvovirus 4	MPV4	FJ440683
		mouse parvovirus 5	MPV5	FJ441297
		hamster parvovirus	HaPV	U34255
		tumor virus X	TVX	In preparation
		rat minute virus 1	RMV1	AF332882
	Rodent protoparvovirus 2	rat parvovirus 1	RPV1	AF036710
	Ungulate protoparvovirus 1	porcine parvovirus Kresse*	PPV-Kr	U44978
		porcine parvovirus NADL-2*	PPV- NADL2	L23427
Tetraparvovirus	*Chiropteran tetraparvovirus 1*	Eidolon helvum (bat) parvovirus	Ba-PARV4	JQ037753
	Primate tetraparvovirus 1	human parvovirus 4 G1	PARV4G1	AY622943
		human parv4 G2	PARV4G2	DQ873391
		human parv4 G3	PARV4G3	EU874248
		chimpanzee parv4	Ch-PARV4	HQ113143
	Ungulate tetraparvovirus 1	bovine hokovirus 1	B-PARV4-1	EU200669
		bovine hokovirus 2	B-PARV4-2	JF504697
	Ungulate tetraparvovirus 2	porcine hokovirus*	P-PARV4	EU200677
	Ungulate tetraparvovirus 3	porcine Cn virus*	CnP-PARV4	GU938300
	Ungulate tetraparvovirus 4	ovine hokovirus	O-PARV4	JF504699

Notes: The genera and type species into which pigs' parvoviruses belong are indicated in bold type, whereas porcine viruses or virus variants are indicated by the asterisks (*Source: Cotmore et al., 2014*).

3.3 The ever-increasing ungulate porcine parvoviruses species of the subfamily *Parvovirinae*

The sporadic revolutions in molecular technology which brought about the use of nucleic acid amplification techniques in pathogen detection and the use of more recent; novel molecular tools such as high-throughput sequencing, has led to the detection of some novel porcine parvoviruses. The new viruses have been characterized in various research proposals with most of the classifications being considered in the latest ICTV report that is awaiting ratification. As earlier mentioned, four genera now consist the designated eight ungulate porcine parvoviruses species

containing about twelve pig-infecting viruses and their variants (Cotmore *et al*., 2014) (Table 3.1). More recent ones are yet to be assigned into species and genus under the subfamily. These include: PPVs 5, 6 and 7 (Xiao *et al*., 2013a; Ni *et al*., 2014; Schirtzinger *et al*., 2015; Palinski *et al*., 2016; Xing *et al*., 2018).

However, in another recent classification proposal based on molecular evolutionary genetics of the parvoviruses' genera that infect pigs, PPVs 5 and 6 have been assigned into a novel clade 2 (tagged unassigned) with PPV4 that is currently grouped under genus *Copiparvovirus* together with bovine parvovirus 2 (BPV2) in the ICTV latest classification. While PPV1 remains the only pig-infecting virus of genus *Parvovirus* (i.e. *Protoparvovirus*) in the classification; PPVs 2 and 3 were categorized together with human parvovirus 4 (*PARV4*) due to their relatively close genomic homologies, under a non-designated genus provisionally named as *PARV4-like* (Xiao *et al*., 2013b). Also, seven bocaviruses consisting of porcine bocaviruses (PBoVs) 1, 2, 3A, 3B, 3C, 3D and 3E were assigned into the genus *Bocavirus* in the proposed classification (Xiao *et al*., 2013b).

Presently, majority of the novel porcine parvoviruses are yet to be fully studied while very limited information is available on the importance and pathogenicity potential of these viruses to global swine population (Xiao *et al*., 2013b). Hence, for the purpose of this current review, the classical porcine parvovirus (PPV) or porcine parvovirus type 1 (PPV1) will be briefly reviewed as a representative of other porcine parvoviruses, while an overview on the detection and prevalence of other viruses will be duly stressed. Also, the later part of this review will bring to the fore, the epidemiology of the porcine parvoviruses in swine herds of African countries taking cognizance of the past, present and future.

3.4   Classical porcine parvovirus: a known pathogen of swine reproductive disorder

3.4.1   History of porcine parvovirus type 1 (PPV1)

Early in the 1960s, cases of reproductive failure were rampant in commercial swine farms due to unknown causes which experts thought could be attributed to nutritional and environmental factors among many others (Lawson, 1961). PPV1 was subsequently isolated in Germany as a contaminant of pig cell cultures used for the cultivation of classical swine fever virus in mid 1960s (Mayr and Mahnel, 1964) and was later confirmed to be associated with reproductive losses in swine (Dunne *et al.*, 1965; Cartwright and Huck, 1967). In the subsequent years, the clinical manifestations of PPV1 reproductive disease were stated as reoccurring of oestrus in sows, abortion and farrowing of mummified or stillborn foetuses; generally regarded as SMEDI meaning: stillbirth, mummification, embryonic death and infertility (Thompson and Prozesky, 1994; Mengeling *et al.*, 2000).   The virus is considered to be extremely stable in the environment, endemic in many parts of the world and capable of infecting pig herd of all categories (Almond *et al.*, 2006; Truyen and Streck, 2012).

3.4.2   Genomic structure and organization in PPV1

PPV1 has a small, single-stranded, negative-sense DNA genome of approximately 5 kb which is packaged in a non-enveloped viral capsid (Molitor *et al.*, 1984). The genome has a unique feature of distinct palindromic hairpin termini and contains two major open reading frames (ORFs) (Bergeron *et al.*, 1993, Bergeron *et al.*, 1996). The ORF1 found at the G- terminus end of the viral genome codes for non-structural proteins (NS1) which could be spliced alternatively to obtain two additional non-structural proteins (NS2 and NS3). The non-structural proteins have some enzymatic functions that are important in the viral replication and packaging (Bergeron *et al.*, 1996). The ORF2 which codes for capsid proteins (VP1, 2 and 3) is located at the C-terminus

end of the viral genome. The VP1 and 2 proteins are formed from differently spliced mRNAs, while VP3 is formed by proteolytic cutting of VP2 (Bergeron *et al*., 1996). About sixty copies of the capsid proteins are used in the assembly of the virus' icosahedral capsid, as previously reviewed elsewhere (Streck *et al*., 2015a).

3.4.3   Epidemiology of PPV1

PPV1 is the earliest known swine parvovirus of veterinary importance (Dunne *et al*., 1965). The virus is highly ubiquitous and has been found in swine herds of different categories. Notable types include domestic and wild pigs, breeding age females and boars, young piglets and fattening pigs (Dea *et al*., 1985; Duhamel *et al*., 1991; Lager and Mengeling, 1994; Drolet *et al*., 2002; Cadar *et al*., 2012; Truyen and Streck, 2012). Also, it has been discovered amongs pigs of different health status including vaccinated and non-vaccinated, healthy and sick pigs (Jóźwik *et al*. 2009). The exceptional stableness of the virus in the surroundings influences its infectivity and its spread. It is highly thermo-stable, having the ability of surviving dry heat to an extent of 90 °C (Eterpi *et al*., 2009).

Furthermore, the virus could resist 70% ethanol and disinfectants like sodium hypochlorite at its low concentration of 2500 ppm; hence, the viral pathogens can remain infectious in a contaminated pen, farm tools and wears for months thereby enhancing its transmission from one farm to another (Truyen and Streck, 2012). Farm to farm transmission could also be enhanced through gilt replacement when asymptomatic gilts are acquired. This is because infected pigs may not necessarily show any clinical manifestation if well vaccinated (Truyen and Streck, 2012; Foerster *et al*., 2016). Also, the virus can be introduced into a vulnerable farm when an infected boar or sperm is used for breeding purposes; a seronegative boar can also be infected during

mating from vaginal mucus of an infected sow (Szelei *et al*., 2006). Furthermore, the viral pathogen could spread within a herd through faecal droppings, nasal and oral secretions from infected pigs (Truyen and Streck, 2012).

3.4.4 *Viral pathogenesis and clinical manifestations*

Porcine parvovirus type 1, which is currently referred to as u*ngulate protoparvovirus 1* species under genus *Protoparvovirus* in the latest ICTV classification, consists of two variants namely porcine parvovirus Kresse (PPV-Kresse) and NADL-2 (PPV-NADL2). The two strains could be differentiated by the degree of their pathogenicity in infected pigs (Tijssen *et al*., 1995). The disparity in their pathogenic potential is believed to be as a result of some residues substitution in their VP2 structural protein. While the former is known to be virulent variant, the later is non-pathogenic and generally employed in producing the viral inactivated and modified live vaccines (Paul and Mengeling, 1984; Mengeling *et al*., 1984; Bergeron *et al.*, 1996; Simpson *et al.*, 2002). Although the avirulent PPV-NADL2 had history of causing limited viraemia without crossing the placenta barrier (Mengeling *et al*., 1984); however, PPV-Kresse is exceptionally pathogenic, having strange capacity to cause mortality not only in susceptible foetuses, but also in immuno-competent ones in cases of maternal infections (Zeeuw *et al*., 2007); and could also induce dermatitis in immuno-competent foetuses and young pigs (Choi *et al*., 1987; Whitaker *et al*., 1990; Lager and Mengeling, 1994).

The main clinical manifestations of PPV1 infections in swine are the reproductive disorders in breeding sows. When a seronegative pregnant sow is infected at the early stage of gestation, its conceptus is usually not affected due to an initial protection received (Mengeling *et al*., 2000). However, during the embryonic stage, occurrence of PPV1 infection normally results to transplacental infections of the embryo leading to embryonic death and resorption. At gestation

period of 35 days and above when bone formation begins (Figure 3.1), the viral infection gives rise to foetal death and mummification. Finally, if a pregnant sow is infected at the advance stage of gestation of 70 days and above, its foetus has already become immuno-competent at that point; hence, the foetus can independently resist the viral infection and becomes seropositive at birth with likelihood of having a subclinical infection status (Lenghaus *et al*. 1978; Mengeling *et al*., 2000).
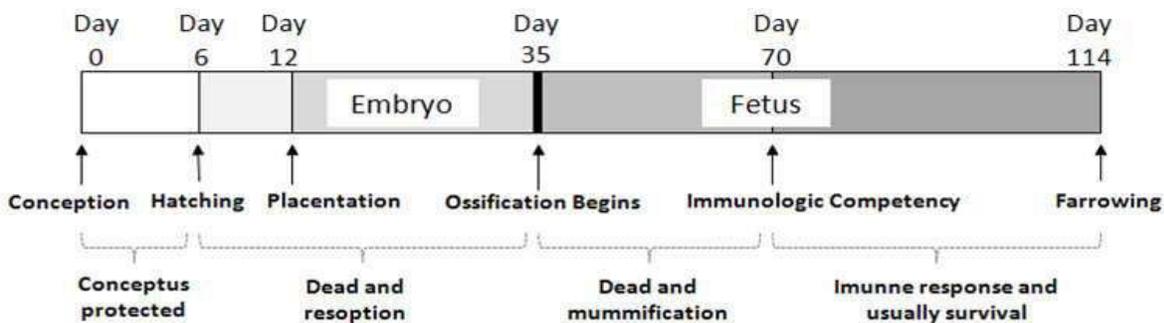
Figure 3.1: Clinical implications of PPV1 infection in pregnant sows as observed at various periods of gestation (*Source: Truyen and Streck, 2012*).

Although, the viral pathogen has been linked to many other disease conditions including non-suppurative myocarditis in suckling piglets, diarrhoea, interstitial nephritis in slaughter-aged pigs and skin lesions, (Kresse *et al*., 1985; Whitaker *et al*., 1990; Lager and Mengeling, 1994; Bolt *et al*., 1997; Drolet *et al*., 2002); yet, its etiological role in the disease manifestations is not fully elucidated. Also, PPV1 is a confirmed co-infecting pathogen that aggravates the disease conditions in PCV2 infection by facilitating the onset of postweaning multisystemic wasting syndrome (PMWS) which is presently one of the viral swine diseases of huge economic importance globally (Allan *et al*., 1999; Krakowka *et al*., 2000; Segalés *et al*., 2005).

3.4.5 Clinical diagnosis of PPV1 infection

As the major clinical effect of PPV1 infection in susceptible pigs, precisely in adult reproducing sows, is the expression of reproductive failure, proper diagnosis of the virus in case of swine reproductive disorders becomes imperative as there are other viral and bacterial pathogens that express related symptoms in pigs. However, in cases of embryonic and foetal death, preterm birth or abortion, stillbirth, farrowing mummified foetuses and reoccurrence of oestrus, PPV1 infection should be suspected (Truyen and Streck, 2012). On the basis of its ability to clump together the erythrocytes from some specific animals including mouse, chicken, rat, human being and so on; haemagglutination method could be conducted for the detection of the virus in the mummified foetal samples. Alternatively, serum samples collected from sows after exhibiting typical PPV1 reproductive disorders can be used in heamagglutination inhibition assay to detect and quantify specific antibodies to the virus (Cartwright *et al*., 1971; Joo *et al*., 1976).

Also, serological assays including serum neutralization assay and enzyme-linked immunosorbent assay (ELISA) could be used to detect specific antibodies of PPV1 in the serum of infected pigs. A differential ELISA that distinguishes developed antibodies due to vaccination from antibodies as a result of natural infection could also be used in PPV1 diagnosis (Hohdatsu *et al*., 1988; Westenbrink *et al*., 1989; Qing *et al*., 2006). Moreover, with the advent of molecular techniques, viral DNA could be detected with more precision and accuracy from various samples including faecal and nasal swab samples, tissues and various organ samples. Several molecular techniques including the conventional polymerase chain reaction (PCR), quantitative PCR, recombinase polymerase amplification (RPA) assay, loop-mediated isothermal amplification (LAMP) assay and nanoPCR have been used in porcine parvovirus' detection (Chen *et al*., 2009; Csagola *et al*., 2012; Cui *et al*., 2014; Streck *et al*., 2015b; Yang *et al*., 2016). Recently, metagenomics has

become a novel tool for the discovery of novel viruses from clinical samples (Belák *et al*., 2013; Blomström *et al*., 2016).

3.4.6   *Prevention and control of PPV1 infection*

Control of PPV1 infection has been a difficult task due to its stable nature, which normally enhances the persistence of the pathogen in swine herds (Truyen and Streck, 2012). However, reproductive failure due to PPV1 infection is usually prevented through vaccination of the gilts prior to insemination and this has proven effective and economical over the years for a successful farm operation (Wrathall *et al*.1984; Pye *et al*., 1990; Mengeling *et al*., 1991; Parke and Burgess, 1993). The recorded success, however, has been a function of effective vaccination regime in terms of timing and consistency (Truyen and Streck, 2012). Firstly, it has been shown that a trans-placental passage of maternal antibodies does not occur in pigs (Salmon *et al*., 2009); hence, a surviving piglet is expected to obtain antibodies from its mother against PPV1 through colostrum intake at its early stage of life (Devillers *et al*., 2011). However, the depletion of the maternally acquired antibodies is a normal phenomenon that determines when vaccination of pigs could be carried out in order for it to be effective as vaccination at high level of maternal antibodies brings about negative interference (Truyen and Streck, 2012). An appropriate timing has been recently determined to be after 3 months of age for a gilt to be inseminated prior to 5 or 6 months old (Gava *et al*., 2017).

Commercially available PPV1 vaccines are made through whole-virus inactivation of the avirulent PPV-NADL2 strain and have been effective in preventing reproductive disorders in sows (Mengeling *et al*., 1980; Paul *et al*., 1980; Mengeling *et al*., 1984). However, it has been proven experimentally that both homologous and heterologous inactivated PPV1 vaccines could not prevent viral infection and shedding, thereby making total immunization and subsequent

eradication of the virus impossible. This, therefore, necessitates the need for a consistent follow-up vaccination of all breeders appropriately (Jóźwik *et al*., 2009; Foerster *et al*., 2016). This limitation, coupled with seemingly emergence of new strains of PPV1 that show lower homology with the vaccine strains, have therefore necessitated future need in developing more efficient vaccines (Jóźwik *et al*., 2009; Ren *et al*., 2013; Streck *et al*., 2015a).

3.5   General epidemiology of other known porcine parvoviruses

Since about five decades ago that PPV1 was first detected in Germany (Mayr *et al*., 1968), many more porcine parvoviruses other than those in bocaviruses' group have been detected in swine population with the recent advancement in the use of molecular techniques in pathogen discovery and epidemiology (Table 3.2) (Ni *et al*., 2014). Generally, the new development has brought about continuing increase in the number of new parvoviruses grouped under the subfamily *Parvovirinae* including those that have been found in other animal species such as Ovine hokovirus (O-PARV4)*;* and also, group of novel porcine bocaviruses (Lau *et al*., 2011; Tse *et al*., 2011; Huang *et al*., 2014). Human parvovirus 4 (PARV4) is also a good example of humans' parvoviruses that has recently been detected with a close relationship with O-PARV4 and porcine hokovirus (PPV3) and as such they have been currently grouped in the same genus *Tetraparvovirus* (Cotmore *et al*., 2014). In this review, porcine parvoviruses other than PPV1 will be concisely discussed including the very latest PPV7.

Table 3.2: Detected porcine parvoviruses till date

Virus	Abbreviation	Year first detected	Place	Source	Reference
Porcine parvovirus 1	PPV, PPV1	1965	Germany	Porcine primary cell culture used for the propagation of classical swine fever (CSF) virus	Cartwright and Huck, 1967
Porcine parvovirus 2	PPV2, CnP-PARV4	2001	Myanmar	Serum samples from pigs obtained for HEV screening	Hijikata *et al.*, 2001
Porcine parvovirus 3	PPV3, P-PARV4, porcine hokovirus	2008	Hong Kong	Multiple samples from healthy, sick and dead pigs	Lau *et al.*, 2008
Porcine parvovirus 4	PPV4	2010	USA	Lung lavage of a diseased pig co-infected with PCV2	Cheung *et al.*, 2010
Porcine parvovirus 5	PPV5	2013	USA	Lung tissues of grower	Xiao *et al.*, 2013a
Porcine parvovirus 6	PPV6	2014	China	Aborted pig foetuses	Ni *et al.*, 2014
Porcine parvovirus 7	PPV7	2016	USA	Rectal swabs of healthy adult pigs	Palinski *et al.*, 2016

3.5.1 Porcine parvovirus type 2 (PPV2)

Hijikata *et al.* (2001) first detected the genome of PPV2 accidentally during an epidemiological survey for hepatitis E virus in Myanmar pigs. The viral genome was detected in 8/86 (10%) of the screened sera from Myanmar pigs in the study. About a decade later, highly related parvoviruses were detected from serum samples obtained from commercial farms with severe field outbreaks of "high fever" suggested to be as a result of PRRSV and PCV2 infection of pigs in Southeastern China (Wang *et al.*, 2010). The novel Chinese strains formed a clade together with the initial one from Myanmar and were designated as Cnvirus sub-lineage under *Parvovirinae.* However, they are presently grouped as *Ungulate tetraparvovirus 3* under the genus *Tetraparvovirus* (Cotmore *et al.*, 2014).

Subsequently, PPV2 has been reported in many other countries including Hungary, USA, Japan, Germany and Thailand with prevalence of 6%, 21%, 58% , 78% and 83% respectively (Csagola *et al.*, 2012; Xiao *et al.*, 2013c; Streck *et al.*, 2013; Saekhow *et al.*, 2014; Saekhow and Ikeda, 2015). Higher prevalence ranging from 78% (Germany) to 100% (Japan) were however obtained when tonsils samples were used in the PPV2 screening as compared to when other samples including blood, faeces and lungs were used (Streck *et al.*, 2013; Saekhow *et al.*, 2014). The prevalence rates that could be obtained for the virus was therefore assumed to be dependent on the type of organ assayed, though, the virus tissue tropism and infection routes are yet to be elucidated (Saekhow and Ikeda, 2015).

3.5.2 Porcine parvovirus type 3 (PPV3)

PPV3, also referred to as porcine hokovirus (PHoV) belongs to the *Ungulate tetraparvovirus 2* species and it is presently grouped together with PPV2 in the genus *Tetraparvovirus*, one of the

two genera that comprises human and pig pathogens together under the subfamily *Parvovirinae*. The virus was also accidentally detected together with bovine parvoviruses (BHoV) in Hong Kong when an effort to determine the relationship that exists between the newly identified human parvovirus types 4 and 5 (PARVs 4 and 5) and animal parvoviruses were made. The PHoV and BHoV showed a genetic similarity to the human parvovirus types 4 and 5 (Lau *et al*., 2008). The virus is believed to have global distribution status as it has been found in domestic pigs in Asia (Pan *et al*., 2012; Li *et al*., 2013; Saekhow and Ikeda, 2015), North America (Xiao *et al*., 2012), Europe (Csagola *et al*., 2012) and Africa (Adlhoch *et al*., 2013). It has also been found in wild boars in Germany and Romania with prevalence of 33% and 50% respectively (Adlhoch *et al*., 2010; Cadar *et al*., 2011).

3.5.3 *Porcine parvovirus type 4 (PPV4)*

The first detection of PPV4 occurred in North Carolina from lung lavage of PCV2-infected pigs (Cheung *et al*., 2010); ever since, the virus has been detected in other countries including China, Hungary, Germany and Thailand with prevalence of 2%, 6%, 7% and 44% respectively (Huang *et al*., 2010; Csagola *et al*., 2012; Streck *et al*., 2013; Saekhow and Ikeda, 2015). Although the virus was initially detected from samples obtained from pigs with multiple clinical manifestations including PRRS and PCV2-associated diseases, the involvement of the viral pathogen in the observed disease conditions is yet to be resolved (Cheung *et al*., 2010; Saekhow and Ikeda, 2015). Phylogenetically, PPV4 has a closer relationship with bovine parvovirus 2 (BPV2); however, in terms of genomic organization and structure, the virus is quite similar to other porcine viruses under the genus *Bocavirus* with its possession of an additional ORF3 gene situated at the center of its genome. The deduced amino acids of the PPV4 ORF3 gene are however quite different from that of bocaviruses (Cheung *et al*., 2010). This is suggested to be

the basis of its separate clustering outside the bocaviruses' group. The virus is presently assigned to the genus *Copiparvovirus* together with BPV2 in the ICTV latest report (Cotmore *et al*., 2014).

3.5.4 *Novel unassigned porcine parvoviruses*

In recent years, some other novel porcine parvoviruses have been detected, although yet to be assigned to species and genus in the latest ICTV classification report. The viruses have been tentatively designated as porcine parvovirus types 5, 6 and 7 (PPV5, PPV6 and PPV7). Xiao *et al*. (2013a) detected a novel porcine parvovirus with the closest genomic sequence identity of 64.1-67.3% to PPV4 from swine lung samples during a study to ascertain the prevalence of PPV4 in United States. The viral genome was further characterized and was found to be different from PPV4 as its genome lacks the extra ORF3 that is peculiar to PPV4 and bocaviruses (Xiao *et al*., 2013d). The findings of phylogenetic analyses on the virus depicted its grouping with PPV4 and BPV2 as it formed a separate cluster with them under the genus *Copiparvovirus*. The virus was provisionally named as PPV5 and its prevalence in US pigs was 6.6% (32/483) (Xiao *et al*., 2013d).

In the following year, Ni *et al*. (2014) for the first time identified another novel pig parvovirus tentatively named as PPV6 from aborted swine foetuses which showed negative results for other swine's reproductive failure pathogens in Chinese pigs. Subsequently, the virus has been detected through a high throughput sequencing of PRRSV positive serum samples from swine in the US and Mexico; with a very high similarity to the Chinese PPV6 (Schirtzinger *et al*., 2015). Reports from the two investigations have unanimously proposed the classification of the novel

PPV6 under the genus *Copiparvovirus* alongside the PPVs 4 and 5 as they all formed same cluster in their phylogenetic analyses (Figure3.2).

More recently, another novel porcine parvovirus was discovered through metagenomic sequencing of pooled samples which include serum, lung lavage, nasal swabs and rectal swab samples from pigs in USA; having an overall prevalence of 16/182 (8.6%) (Palinski *et al*., 2016). The viral pathogen has just been found in serum samples from Chinese pigs with an overall prevalence of 21/64 (32.8%) and higher value of 19/29(65.5%) in pigs from PCV2-positive farms compared with that of PCV2-negative farms 2/35 ((5.7%) samples (Xing *et al*., 2018). The phylogenetic analysis of the viral genomes in the latest study showed 98.7-99.7% nucleotide identity between the viral strains from the two countries (Xing *et al*., 2018), whereas the previous studies revealed closer identity of 42.4% and 37.9% at the NS1 proteins for fruit bat (EhPV2) and turkey parvovirus (TuPV) respectively (Palinski *et al*., 2016). The novel viral agent has been provisionally named as PPV7, and a proposal was made for the formation of another genus *Chapparvovirus* (coined from <u>Ch</u>iroptera, <u>A</u>vian and <u>P</u>orcine) under the subfamily *Parvovirinae* to contain PPV7, EhPV2 and TuPV as they have shown less than 30% identity to other species in subfamily *Parvovirinae* which is far below the stipulated species inclusion value of >85% identity in NS1.The phylogenetic analysis based on the NS1 gene of all the known porcine parvoviruses in this study also confirmed separate grouping and clustering for PPV7 and TuPV (Figure 3.2).
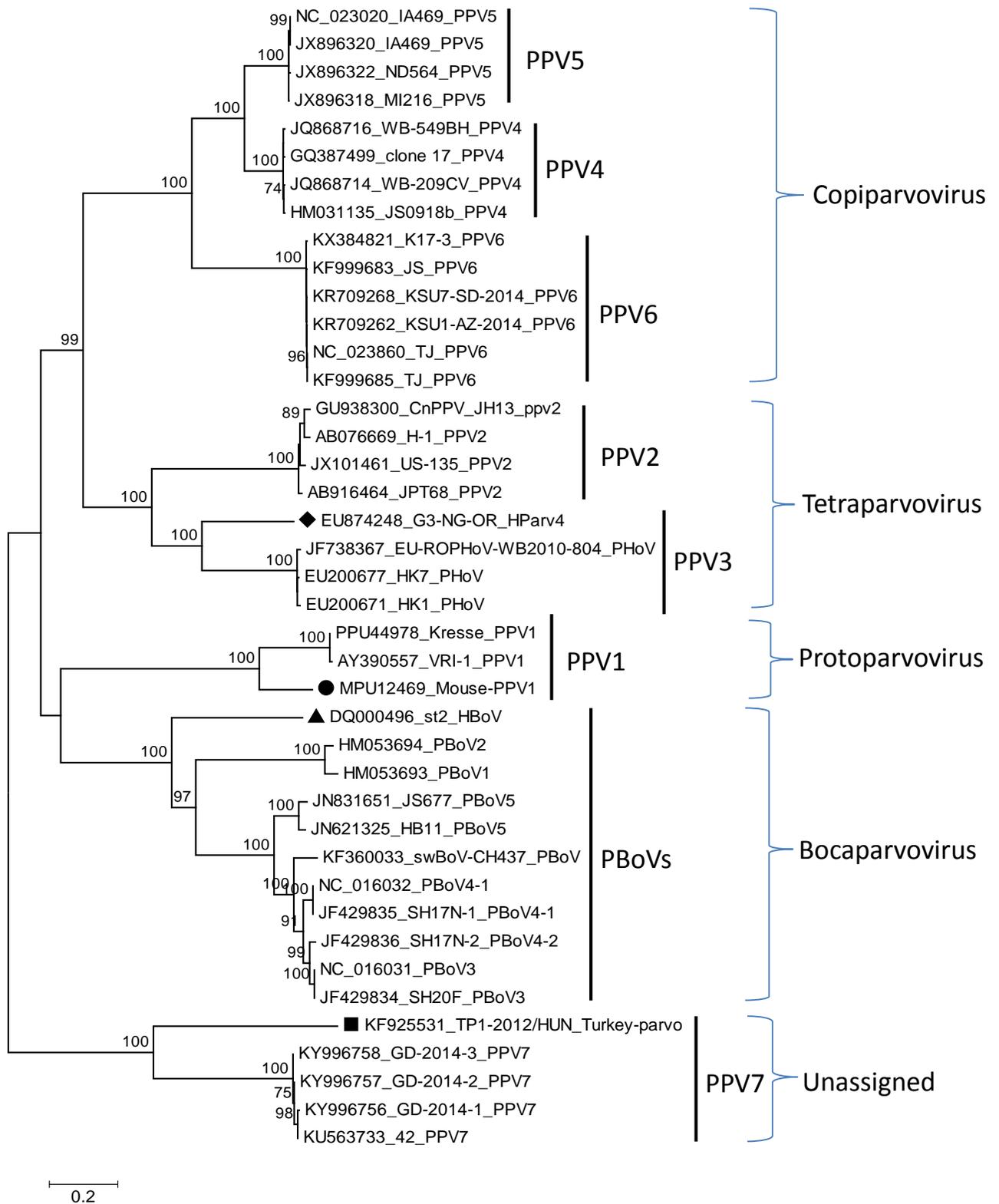
Figure 3.2: Phylogenetic analysis base on the NS1 gene of all porcine parvoviruses detected till date. While the black diamond sign indicates the human parvovirus 4 (*PARV4*) clustering with porcine hokovirus (PHoV), the black triangle depicts the reference genome of human bocavirus 2 (HBoV) clustering with other porcine bocaviruses. The black rectangle shows the turkey parvovirus (TuPV) that forms clade with the novel PPV7. Phylogenetic analysis was done in Mega 6 using the Neighbour-joining algorithms and 1000 bootstrap replicates (Tamura *et al*., 2013).

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3.5.6   *Porcine bocaviruses (PBoVs)*

Bovine parvovirus (BPV) is the first known member of the genus *Bocavirus* (now *Bocaparvovirus*) and the only virus in the *ungulate bocaparvovirus 1* species; it is the first member of the genus that was detected about six decades ago from calves with clinical symptoms of diarroea (Abixanti and Warfield, 1961). Presently, there are about eight porcine viruses and virus variants that have been grouped into four species namely u*ngulate bocaparvovirus 2, 3, 4* and *5* under the genus *Bocaparvovirus* with the BPV (Cotmore *et al*., 2014). The genus *Bocaparvovirus* is the second group in the subfamily *Parvovirinae* that consists of human viruses and swine together. The genus also comprises of feline bocavirus, canine bocaviruses, gorilla bocavirus and California sea lion bocaviruses (Cotmore *et al*., 2014).

The first porcine bocavirus was discovered in 2009 from lymph node samples collected from piglets diagnosed to have PCV2 wasting syndrome (Blomström *et al*., 2009). The viral pathogen was detected by using random amplification technique and high-throughput sequencing in a bid to study other co-infecting pathogens in PMWS-affected Swedish pigs. Partial genome (1879 bp) that comprises the entire NP1 and partial VP1/VP2 of the Swedish porcine boca-like virus (PBo-likeV) was amplified and analyzed alongside with genomes of Torque Teno virus (TTV) and PCV2; thereby confirming the presence of the viruses in the PCV2-associated wasting syndrome case, though the contribution of PBo-likeV to the development of PMWS is yet to be elucidated (Blomström *et al*., 2009).

Subsequently, detection of many other porcine bocaviruses which are different from the Swedish strain have been on the rise globally; and there have been passionate efforts to characterize and classify them (Cheng *et al*., 2010; McKillen *et al*., 2011; Li *et al*., 2012; Yang *et al*., 2012; Xiao

et al., 2013b). However, there have been a lot of disparities and confusions along the process (Gunn *et al*., 2015). In this review, the grouping according to the latest ICTV report is adopted in which eight porcine bocaviruses were identified and assigned into four species namely: *ungulate bocaparvovirus 2* (consisting of porcine bocavirus 1, 2 and 6 i.e. PBoV1, PBoV2 and PBoV6); *ungulate bocaparvovirus 3* (having only porcine bocavirus 5 i.e. PBoV5); u*ngulate bocaparvovirus 4* (consisting of porcine bocavirus 3 and 7 i.e. PBoV3 and PBoV7) and *Ungulate bocaparvovirus 5* (comprising of porcine bocavirus 4-1and 4-2 i.e. PBoV4-1 and PBoV4-2) (Cotmore *et al*., 2014).

PBoVs have been detected and characterized by using molecular techniques from array of samples which include faeces, serum, lung, lymph nodes and nasopharyngeal samples (Zhai *et al*., 2010; Lau *et al*., 2011; Cságola *et al*., 2012; Blomström *et al*., 2013; Choi *et al*., 2014). Though the viral agents have been detected in both symptomatic and asymptomatic pigs, many questions about their pathogenicity are yet to be answered (Gunn *et al*., 2015). However, more details about the epidemiology and pathogenesis of PBoVs have been previously reviewed (Zhou *et al*., 2014).

3.6 Epidemiology of porcine parvoviruses in African countries

Although pig production in African countries regarding the frequency of reared pigs is insignificant compared to other pig-producing countries of the world such as China, United States of America and Brazil; nonetheless, the importance of pig production cannot be overemphasized in the region considering its economic value in alleviating poverty and solving hunger challenge in developing countries of the world (FAO, 2012). However, little or no effort is geared towards detecting and studying most of the swine viral pathogens of global economic importance in the region. This assertion is strongly corroborated in a recent review analysis on

PCV2 and its associated diseases in the sub-Saharan Africa region in which only 2/49 (4%) of the countries represented in the region have meager documentation about the detection of the swine' viral pathogen in the region (Afolabi *et al*., 2017).

As is the case of PCV2 earlier stated, investigations on porcine parvoviruses in the entire African continent is relatively low when compared with vast research efforts that have been tailored towards the detection and characterization of the circulating viral strains in other countries of the world. Nevertheless, this section is dedicated to highlighting the past and current reported cases of porcine parvoviruses' infection in some African countries, in a bid to serve as awakening call for meaningful future proactive research activities in unraveling the occurrence and prevalence of the viral pathogens in the region. This will go a long way in ameliorating the plight of peasant farmers in the region whose livelihood is dependent on the piggery business.

3.6.1 *Porcine parvoviruses in South African pigs*

The first reported detection of porcine parvovirus in South African pig took place in 1975 in an outbreak of swine reproductive failure that occurred at a piggery in which PPV1 was isolated from aborted fetuses (Pini, 1975). Subsequently, the virus was detected as the etiologic agent of similar clinical features in some other occasions; this prompted Prozesky *et al*. (1980), to lunch a pathological investigation on the isolated local strain of PPV1, so as to elucidate its effect on foetus and pregnant sow for the purpose of comparing their findings with other documented cases. Experimental reproduction of typical PPV1-associated reproductive failure characterized by resorption, abortion and mummification of foetuses was successfully carried out through an utero inoculation of 15 sows at different levels of gestation with the local viral strain. Vascular

lesions were equally observed in the endometrium of the sows, which the authors assumed could contribute to the reproductive disorders observed in the inoculated sows (Prozesky *et al*., 1980).

Apart from the initial case study and the subsequent experimental evaluation carried out about four decades ago, there has been no documented research work on porcine parvoviruses in South African swine till date except a very recent preliminary epidemiological study carried out in this study to unravel the prevalence of some porcine parvoviruses in selected swine herds with the background of PCV2 infection. The prevalence of PPV1-4, PBo-likeV and PBoV1/2 were showed to be: 32/110 (29.1%), 24/110 (21.8%), 6/110 (5.5%), 48/110 (43.6%), 24/110 (21.8%) and 49/110 (44.6%) respectively in this current research . All the screened porcine parvoviruses were present in the studied pigs from confirmed PCV2-infected farms, indicating that South African pigs are not free from parvoviruses' infection. The insightful findings call for urgent large scale epidemiological survey in the country and the entire Southern Africa region for effective control of the viral pathogens.

3.6.2   *Detection of porcine parvoviruses in East African countries of Uganda and Kenya*

Relatively, more reported cases of porcine parvoviruses are available from the East African countries of Uganda and Kenya. In a study aimed at determining the presence of viral pathogens from serum samples obtained from bush pigs that roam around between the national park and farmland in Uganda; PPV4 alongside Torque teno sus viruses 1 and 2 (TTSuV1 and TTSuV2) were detected through metagenomic approach (Blomström *et al*., 2012). The PPV4 from the serum of bush pigs showed a sequence identity of 75.9-77.1% with other PPV4 sequences in the GenBank which cluster together with them in the phylogenetic analysis, with some display of divergence from other reference sequences used. The detection was the first report on PPV4 in

bush pig from Africa (Blomström *et al.*, 2012). Related effort was made to screen for the virus in samples from the domestic pigs using the primers designed from PPV4 sequence from the bush pig, however, no positive result was obtained (Brink, 2011).

Also, with the use of conventional PCR specific for PBoV types previously detected in Sweden and China, Blomström *et al.* (2013) went further to screen for the possible presence of porcine bocaviruses in 95 serum samples collected from six different districts previously used for the detection of TTSuV-1 and 2 in Ugandan pig (Brink *et al.*, 2012). They recorded a very low detection rate of 2/95 (2.1%) compared to 16.8% and 48.4% previously obtained for TTSuV-1 and TTSuV-2 respectively using the same samples. However, from the study, the first PBoV genome from Africa was assembled; having all the coding regions of the virus. The viral strain BuK8 (**JX854557**) from Uganda has amino acid sequence similarity of 98-99% to the two Chinese strains H18 (**HQ291308**) and SX (**HQ223038**) from only one group of presently known porcine bocaviruses. Future elaborate investigation was suggested to determine the occurrence of other PBoV species in the country (Blomström *et al.*, 2013).

Subsequently, in a more recent study, Amimo *et al.* (2016) screened for the presence of viral pathogens from faecal samples obtained from healthy pigs from Kenya and Uganda using metagenomics approach. Virome from 12 pigs were analyzed and many viral sequences were identified including 11% of the family *Parvoviridae.* Further analyses of the contigs showed very high presence of astroviruses of about 83.3% (10/12) followed by 7/12 (58.3%) of porcine bocaviruses. Subsequently, a commendable effort was made by the authors to sequence the full genomes of the two porcine bocavirus strains JOA_011 and JOA_015 obtained from healthy pigs in farms from the two countries. The two sequences were further analyzed and showed high

relatedness with the strain KU14 from South Korea (Amimo *et al*., 2017). It is expected that future large scale epidemiological studies that will unravel the occurrence and prevalence of porcine parvoviruses in pigs from the region will be conducted, considering the fact that two (PPV4 and PBoV) out of the numerous members of the viral group have already been found in the reported studies (Blomström *et al*., 2012; 2013; Amimo *et al*., 2017).

3.6.3 *Porcine parvoviruses in West African countries of Cameroun and Nigeria*

Interestingly, when the detection of first PBoV was reported in Uganda (Blomström *et al*., 2013), Ndze *et al*. (2013) in a separate study to unravel the occurrence and diversity of selected DNA viruses from about four viral families including those from *Parvoviridae* reported the first detection and prevalence findings on porcine bocaviruses in Cameroonian pigs. While other viral groups were absent in the samples from the asymptomatic pigs, some porcine parvoviruses in the subfamily *Parvovirinae* were detected including PPV4 with prevalence of 10/50 (20%). Others are bocaviruses including PBoV1/2 with prevalence of 3/50 (6%), 9/50 (18%) for PBoV3, 9/50 (18%) for PBoV4 and 8/50 (16%) for the combination of PBoV5 and PBoV6V/7V (Ndze *et al*., 2013). High co-infection rates between PPV4 and the various species of bocaviruses were equally noted in the study. Future efforts are required to amplify and sequence the complete genomes of the detected PBoVs for an adequate genetic characterization of the viral strains in circulation within the country.

Also in another study, Adlhoch *et al*. (2013) used samples collected in the year 2012 for the purpose of investigating the presence of hepatitis E virus (HEV) in Cameroonian pigs to screen for the presence of porcine hokovirus (PPV3), a related virus to human parvovirus 4 (PARV4), in the swine herds of the country. The outcome of the screening showed that 65/94 (69%) of the

pooled samples from Doula, Yaoundé, and Bamenda districts of Cameroon were positive for PHoV. From the 94 pooled samples, about 184 samples were used for individual testing to determine prevalence based on the individual samples apart from the one obtained for the pooled samples. When the results from the negative tested pools and the individual testing were used in their estimation, a general prevalence of 128/270 (47%) was obtained for the virus in the Cameroonian pigs. Phylogenetic analysis was subsequently performed on partial genomes generated from some of the pooled samples and the Cameroonian sequences had close homology of 98-99% to other reference sequences from Europe, USA and China. The authors, however, suggested further studies on the virus at other premises of Africa in other to determine the circulating viral strains in the region (Adlhoch *et al*., 2013).

In Nigeria, according to Aiki-Raji *et al*. (2017), the status of PPV1 (which is just one out of the numerous porcine parvoviruses in the subfamily P*arvovirinae*) in Nigerian pigs is largely unknown. In their sero-prevalence study recently done to unravel the prevalence of PRRSV and PPV1 in pigs from the South-Western region of the country, a prevalence of 36.1% was obtained for the classical porcine parvovirus in the studied area that comprises of only two states of Oyo and Lagos (Aiki-Raji *et al*., 2017). This finding also calls for proactive surveillance of the pathogen in pigs from the country in order to determine its true status in swine herds of the country so as to facilitate effective control measures.

3.7 Conclusion

The detection of parvoviruses in pigs has vividly been on the increase in recent years. Although the classical porcine parvovirus has been the most studied member of the viral group globally, however, efforts in various part of the world in detecting and analyzing the prevalence of other

novel porcine parvoviruses is highly commendable compared to a lethargic approach that is being used in African region. Considering the economic importance of parvoviruses to piggery business as highlighted in this review, most especially the continuous emergence of new strains/species with zoonotic potentials, the need for effective large-scale surveillance and characterization of porcine parvoviruses and many other neglected swine pathogens in the region becomes imperative. This will go a long way in enhancing the implementation of the globally acclaimed "One Health, One World" initiative for combating zoonotic diseases in the region.

References

Abixanti, F.R. and Warfield, M.S., 1961. Recovery of a hemadsorbing virus (HADEN) from the gastrointestinal tract of calves. *Virology*, *14*(2), pp.288-289.

Adlhoch, C., Kaiser, M., Ellerbrok, H. and Pauli, G., 2010. High prevalence of porcine Hokovirus in German wild boar populations. *Virology Journal*, *7*(1), p.171.

Adlhoch, C., Kaiser, M., Kingsley, M.T., Schwarz, N.G., Ulrich, M., de Paula, V.S., Ehlers, J., Löwa, A., Daniel, A.M., Poppert, S. and Schmidt-Chanasit, J., 2013. Porcine hokovirus in domestic pigs, Cameroon. *Emerging Infectious Diseases*, *19*(12), p.2060.

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964.

Aiki-Raji, C.O., Adebiyi, A.I., Abiola, J.O. and Oluwayelu, D.O., 2017. Prevalence of porcine reproductive and respiratory syndrome virus and porcine parvovirus antibodies in commercial pigs, southwest Nigeria. *Beni-Suef University Journal of Basic and Applied Sciences*, In press.

Allan, G.M., Kennedy, S., McNeilly, F., Foster, J.C., Ellis, J.A., Krakowka, S.J., Meehan, B.M. and Adair, B.M., 1999. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *Journal of Comparative Pathology*, *121*, pp.1–11.

Almond, G.W., Flowers, W.L., Batista, L. and D'Allaire, S., 2006. Diseases of the reproductive system. In: Straw, B.E., Zimmerman, J.J., D'Allaire, S. and Taylor, D.J. 9th Eds., *Diseases of Swine*. Ames, Iowa: Blackwell Publishing, pp.113–147.

Amimo, J.O., El Zowalaty, M.E., Githae, D., Wamalwa, M., Djikeng, A. and Nasrallah, G.K., 2016. Metagenomic analysis demonstrates the diversity of the fecal virome in asymptomatic pigs in East Africa. *Archives of Virology*, *161*(4), pp.887-897.

Amimo, J.O., Njuguna, J., Machuka, E., Okoth, E. and Djikeng, A., 2017. First Complete Genome Sequences of Porcine Bocavirus Strains from East Africa. *Genome Announcements*, *5*(14), p.e00093-17.

Belák, S., Karlsson, O.E., Blomström, A.L., Berg, M. and Granberg, F., 2013. New viruses in veterinary medicine, detected by metagenomic approaches. *Veterinary Microbiology*, *165*(1), pp.95-101.

Bergeron, J., Hebert, B. and Tijssen, P., 1996. Genome organization of the Kresse strain of porcine parvovirus: identification of the allotropic determinant and comparison with those of NADL-2 and field isolates. *Journal of Virology*, *70*(4), pp.2508-2515.

Bergeron, J., Menezes, J. and Tijssen, P., 1993. Genomic organization and mapping of transcription and translation products of the NADL-2 strain of porcine parvovirus. *Virology*, *197*(1), pp.86-98.

Blomström, A.L., Belák, S., Fossum, C., McKillen, J., Allan, G., Wallgren, P. and Berg, M., 2009. Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. *Virus Research*, *146*(1), pp.125-129.

Blomström, A.L., Fossum, C., Wallgren, P. and Berg, M., 2016. Viral Metagenomic Analysis Displays the Co-Infection Situation in Healthy and PMWS Affected Pigs. *PloS One*, *11*(12), p.e0166863.

Blomström, A.L., Ståhl, K., Masembe, C., Okoth, E., Okurut, A.R., Atmnedi, P., Kemp, S., Bishop, R., Belák, S. and Berg, M., 2012. Viral metagenomic analysis of bushpigs (Potamochoerus larvatus) in Uganda identifies novel variants of Porcine parvovirus 4 and Torque teno sus virus 1 and 2. *Virology Journal*, *9*(1), p.192.

Blomström, A.L., Ståhl, K., Okurut, A.R., Masembe, C. and Berg, M., 2013. Genetic characterisation of a porcine bocavirus detected in domestic pigs in Uganda. *Virus Genes*, *47*(2), pp.370-373.

Bolt, D.M., Häni, H., Müller, E. and Waldvogel, A.S., 1997. Non-suppurative myocarditis in piglets associated with porcine parvovirus infection. *Journal of Comparative Pathology*, *117*(2), pp.107-118.

Brink, M., 2011. Porcine viruses in Uganda: a study of TTSuV and PPV4 in wild and domestic pigs. [Dissertation]. *Uppsala: Swedish University of Agricultural Sciences*. http://urn.kb.se/resolve?urn=urn:nbn:se:slu:epsilon-s-341.

Brink, M., Ståhl, K., Masembe, C., Okurut, A.R., Berg, M. and Blomström, A.L., 2012. First time molecular detection and phylogenetic relationships of torque teno sus virus 1 and 2 in domestic pigs in Uganda: further evidence for a global distribution. *Virology Journal*, *9*(1), p.39.

Cadar, D., Cságola, A., Lőrincz, M., Tombácz, K., Spînu, M. and Tuboly, T., 2011. Distribution and genetic diversity of porcine hokovirus in wild boars. *Archives of Virology*, *156*(12), pp.2233-2239.

Cadar, D., Dán, Á., Tombácz, K., Lőrincz, M., Kiss, T., Becskei, Z., Spînu, M., Tuboly, T. and Cságola, A., 2012. Phylogeny and evolutionary genetics of porcine parvovirus in wild boars. *Infection, Genetics and Evolution*, *12*(6), pp.1163-1171.

Cartwright, S.F. and Huck, R.A., 1967. Viruses isolated in association with herd infertility absortions and stillbirths in pigs. *Veterinary Record*, *81*(8), pp.196-197.

Cartwright, S.F., Lucas, M. and Huck, R.A., 1971. A small haemagglutinating porcine DNA virus: II. Biological and serological studies. *Journal of Comparative Pathology*, *81*(1), pp.145-155.

Chen, H.T., Zhang, J., Yang, S.H., Ma, L.N., Ma, Y.P., Liu, X.T., Cai, X.P., Zhang, Y.G. and Liu, Y.S., 2009. Rapid detection of porcine parvovirus DNA by sensitive loop-mediated isothermal amplification. *Journal of Virological Methods*, *158*(1), pp.100-103.

Cheng, W.X., Li, J.S., Huang, C.P., Yao, D.P., Liu, N., Cui, S.X., Jin, Y. and Duan, Z.J., 2010. Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PloS One*, *5*(10), p.e13583.

Cheung, A.K., Wu, G., Wang, D., Bayles, D.O., Lager, K.M. and Vincent, A.L., 2010. Identification and molecular cloning of a novel porcine parvovirus. *Archives of Virology*, *155*(5), pp.801-806.

Choi, C.S., Molitor, T.W., Joo, H.S. and Gunther, R., 1987. Pathogenicity of a skin isolate of porcine parvovirus in swine fetuses. *Veterinary Microbiology*, *15*(1-2), pp.19-29.

Choi, M.G., Park, S.J., Nguyen, V.G., Chung, H.C., Kim, A.R. and Park, B.K., 2014. Molecular detection and genetic analysis of porcine bocavirus in Korean domestic swine herds. *Archives of Virology*, *159*(6), pp.1487-1492.

Cotmore, S.F., Agbandje-McKenna, M., Chiorini, J.A., Mukha, D.V., Pintel, D.J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D. and Davison, A.J., 2014. The family parvoviridae. *Archives of Virology*, *159*(5), pp.1239-1247.

Cságola, A., Lőrincz, M., Cadar, D., Tombácz, K., Biksi, I. and Tuboly, T., 2012. Detection, prevalence and analysis of emerging porcine parvovirus infections. *Archives of Virology*, *157*(6), pp.1003-1010.

Cui, Y., Wang, Z., Ma, X., Liu, J. and Cui, S., 2014. A sensitive and specific nanoparticle-assisted PCR assay for rapid detection of porcine parvovirus. *Letters in Applied Microbiology*, *58*(2), pp.163-167.

Dea, S., Elazhary, M.A., Martineau, G.P. and Vaillancourt, J., 1985. Parvovirus-like particles associated with diarrhea in unweaned piglets. *Canadian Journal of Comparative Medicine*, *49*(3), p.343.

Devillers, N., Le Dividich, J. and Prunier, A., 2011. Influence of colostrum intake on piglet survival and immunity. *Animal*, *5*(10), pp.1605-1612.

Drolet, R., D'Allaire, S., Larochelle, R., Magar, R., Ribotta, M. and Higgins, R., 2002. Infectious agents identified in pigs with multifocal interstitial nephritis at slaughter. *Veterinary Record*, *150*(5), pp.139-142.

Duhamel, G.E., Bargar, T.W., Schmitt, B.J., Molitor, T.W. and Lu, W., 1991. Identification of parvovirus-like virus particles in intestinal crypt epithelial cells of pigs with diarrhea. *Journal of Veterinary Diagnostic Investigation*, *3*(1), pp.96-98.

Dunne, H.W., Gobble, J.L., Hokanson, J.F., Kradel, D.C. and Bubash, G.R., 1965. Porcine reproductive failure associated with a newly identified" SMEDI" group of picorna viruses. *American Journal of Veterinary Research*, *26*(115), p.1284.

Eterpi, M., McDonnell, G. and Thomas, V., 2009. Disinfection efficacy against parvoviruses compared with reference viruses. *Journal of Hospital Infection*, *73*(1), pp.64-70.

FAO, 2012. Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3, Rome.

Foerster, T., Streck, A.F., Speck, S., Selbitz, H.J., Lindner, T. and Truyen, U., 2016. An inactivated whole-virus porcine parvovirus vaccine protects pigs against disease but does not prevent virus shedding even after homologous virus challenge. *Journal of General Virology*, *97*(6), pp.1408-1413.

Gava, D., Souza, C.K., Mores, T.J., Argenti, L.E., Streck, A.F., Canal, C.W., Bortolozzo, F.P. and Wentz, I., 2017. Dynamics of vanishing of maternally derived antibodies of Ungulate protoparvovirus 1 suggests an optimal age for gilts vaccination. *Tropical Animal Health and Production*, *49*(5), pp.1085-1088.

Gunn, L., Collins, P.J., Fanning, S., McKillen, J., Morgan, J., Staines, A. and O'Shea, H., 2015. Detection and characterisation of novel bocavirus (genus Bocaparvovirus) and gastroenteritis viruses from asymptomatic pigs in Ireland. *Infection Ecology and Epidemiology*, *5*(1), p.27270.

Hijikata, M., Abe, K., Win, K.M., Shimizu, Y.K., Keicho, N. and Yoshikura, H., 2001. Identification of new parvovirus DNA sequence in swine sera from Myanmar. *Japanese Journal of Infectious Diseases*, *54*(6), pp.244-245.

Hohdatsu, T., Baba, K., Ide, S., Tsuchimoto, M., Nagano, H., Yamagami, T., Yamagishi, H., Fujisaki, Y. and Matumoto, M., 1988. Detection of antibodies against porcine parvovirus in swine sera by enzyme-linked immunosorbent assay. *Veterinary Microbiology*, *17*(1), pp.11-19.

Huang, J., Mor, S.K., Erber, J., Voss, E. and Goyal, S.M., 2014. Detection and characterization of porcine bocavirus in the United States. *Archives of Virology*, *159*(7), pp.1797-1801.

Huang, L., Zhai, S.L., Cheung, A.K., Zhang, H.B., Long, J.X. and Yuan, S.S., 2010. Detection of a novel porcine parvovirus, PPV4, in chinese swine herds. *Virology Journal*, *7*(1), p.333.

Joo, H.S., Donaldson-Wood, C.R. and Johnson, R.H., 1976. A standardised haemagglutination inhibition test for porcine parvovirus antibody. *Australian Veterinary Journal*, *52*(9), pp.422-424.

Jóźwik, A., Manteufel, J., Selbitz, H.J. and Truyen, U., 2009. Vaccination against porcine parvovirus protects against disease, but does not prevent infection and virus shedding after challenge infection with a heterologous virus strain. *Journal of General Virology*, *90*(10), pp.2437-2441.

Kailasan, S., Agbandje-McKenna, M. and Parrish, C.R., 2015. Parvovirus family conundrum: what makes a killer?. *Annual Review of Virology*, *2*, pp.425-450.

Krakowka, S., Ellis, J.A., Meehan, B., Kennedy, S., McNeilly, F. and Allan, G., 2000. Viral wasting syndrome of swine: experimental reproduction of postweaning multisystemic wasting syndrome in gnotobiotic swine by coinfection with porcine circovirus 2 and porcine parvovirus. *Veterinary Pathology*, *37*(3), pp.254-263.

Kresse, J.I., Taylor, W.D., Stewart, W.W. and Eernisse, K.A., 1985. Parvovirus infection in pigs with necrotic and vesicle-like lesions. *Veterinary Microbiology*, *10*(6), pp.525-531.

Lager, K.M. and Mengeling, W.L., 1994. Porcine parvovirus associated with cutaneous lesions in piglets. *Journal of Veterinary Diagnostic Investigation*, *6*(3), pp.357-359.

Lau, S.K., Woo, P.C., Tse, H., Fu, C.T., Au, W.K., Chen, X.C., Tsoi, H.W., Tsang, T.H., Chan, J.S., Tsang, D.N. and Li, K.S., 2008. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *Journal of General Virology*, *89*(8), pp.1840-1848.

Lau, S.K., Woo, P.C., Yip, C.C., Li, K.S., Fu, C.T., Huang, Y., Chan, K.H. and Yuen, K.Y., 2011. Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family Parvoviridae, and evidence for inter-and intra-host genetic diversity and recombination. *Journal of General Virology*, *92*(9), pp.2047-2059.

Lawson, J.R., 1961. Infectious infertility of swine. *Reports of the Meeting of the Expert Panel of Livestock Infertility*, Rome, Italy, p.67.

Lenghaus, C., Forman, A.J. and Hale, C.J., 1978. Experimental infection of 35, 50 and 60 day old pig foetuses with porcine parvovirus. *Australian Veterinary Journal*, *54*(9), pp.418-422.

Li, B., Ma, J., Xiao, S., Fang, L., Zeng, S., Wen, L., Zhang, X., Ni, Y., Guo, R., Yu, Z. and Zhou, J., 2012. Complete genome sequence of a novel species of porcine bocavirus, PBoV5. *Journal of Virology*, *86*(2), pp.1286-1287.

Li, S., Wei, Y., Liu, J., Tang, Q. and Liu, C., 2013. Prevalence of porcine hokovirus and its co-infection with porcine circovirus 2 in China. *Archives of Virology*, *158*(9), pp.1987-1991.

Mayr, A. and Mahnel, H., 1964. Cultivation of hog cholera virus in pig kidney cultures with cytopathogenic effect. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und*

Hygiene. 1. Abt. Medizinisch-hygienische Bakteriologie, Virusforschung und Parasitologie. Originale, *195*(2), p.157.

Mayr, A., Bachmann, P.A., Siegl, G., Mahnel, H. and Sheffy, B.E., 1968. Characterization of a small porcine DNA virus. *Archives of Virology*, *25*(1), pp.38-51.

McKillen, J., McNeilly, F., Duffy, C., McMenamy, M., McNair, I., Hjertner, B., Millar, A., McKay, K., Lagan, P., Adair, B. and Allan, G., 2011. Isolation in cell cultures and initial characterisation of two novel bocavirus species from swine in Northern Ireland. *Veterinary Microbiology*, *152*(1), pp.39-45.

Mengeling, W.L., Lager, K.M. and Vorwald, A.C., 2000. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. *Animal Reproduction Science*, *60*, pp.199-210.

Mengeling, W.L., Lager, K.M., Zimmerman, J.K., Samarikermani, N. and Beran, G.W., 1991. A current assessment of the role of porcine parvovirus as a cause of fetal porcine death. *Journal of Veterinary Diagnostic Investigation*, *3*(1), pp.33-35.

Mengeling, W.L., Paul, P.S. and Brown, T.T., 1980. Transplacental infection and embryonic death following maternal exposure to porcine parvovirus near the time of conception. *Archives of Virology*, *65*(1), pp.55-62.

Mengeling, W.L., Pejsak, Z. and Paul, P.S., 1984. Biological assay of attenuated strain NADL-2 and virulent strain NADL-8 of porcine parvovirus. *American Journal of Veterinary Research*, *45*(11), pp.2403-2407.

Molitor, T.W., Joo, H.S. and Collett, M.S., 1984. Porcine parvovirus DNA: characterization of the genomic and replicative form DNA of two virus isolates. *Virology*, *137*(2), pp.241-254.

Ndze, V.N., Cadar, D., Cságola, A., Kisfali, P., Kovács, E., Farkas, S., Ngu, A.F., Esona, M.D., Dán, Á., Tuboly, T. and Bányai, K., 2013. Detection of novel porcine bocaviruses in fecal samples of asymptomatic pigs in Cameroon. *Infection, Genetics and Evolution*, *17*, pp.277-282.

Ni, J., Qiao, C., Han, X., Han, T., Kang, W., Zi, Z., Cao, Z., Zhai, X. and Cai, X., 2014. Identification and genomic characterization of a novel porcine parvovirus (PPV6) in china. *Virology Journal*, *11*(1), p.203.

Palinski, R.M., Mitra, N. and Hause, B.M., 2016. Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. *Virus Genes*, *52*(4), pp.564-567.

Pan, Y., Zeng, Q., Zhu, C., Hua, X., Wang, M., Pan, K. and Cui, L., 2012. Frequency and characterization of porcine hokovirus (PHoV) in domestic pigs in eastern China. *Archives of Virology*, *157*(9), pp.1785-1788.

Parke, C.R. and Burgess, G.W., 1993. An economic assessment of porcine parvovirus vaccination. *Australian Veterinary Journal*, *70*(5), pp.177-180.

Paul, P.S. and Mengeling, W.L., 1984. Oronasal and intramuscular vaccination of swine with a modified live porcine parvovirus vaccine: multiplication and transmission of the vaccine virus. *American Journal of Veterinary Research*, *45*(12), pp.2481-2485.

Paul, P.S., Mengeling, W.L. and Brown, J.T., 1980. Effect of vaccinal and passive immunity on experimental infection of pigs with porcine parvovirus. *American Journal of Veterinary Research*, *41*(9), pp.1368-1371.

Pini, A., 1975. Porcine parvovirus in pig herds in Southern Africa. *Journal of the South African Veterinary Association*, *46*(3), pp.241-244.

Prozesky, L., Thomson, G.R., Gainaru, M.D., Herr, S. and Kritzinger, L.J., 1980. Lesions resulting from inoculation of porcine foetuses with porcine parvovirus. *The Onderstepoort Journal of Veterinary Research*, *47*(4), pp.269-274.

Pye, D., Bates, J., Edwards, S.J. and Hollingworth, J., 1990. Development of a vaccine preventing parvovirus-induced reproductive failure in pigs. *Australian Veterinary Journal*, *67*(5), pp.179-182.

Qing, L., Lv, J., Li, H., Tan, Y., Hao, H., Chen, Z., Zhao, J. and Chen, H., 2006. The recombinant nonstructural polyprotein NS1 of porcine parvovirus (PPV) as diagnostic antigen in ELISA to differentiate infected from vaccinated pigs. *Veterinary Research Communications*, *30*(2), pp.175-190.

Ren, X., Tao, Y., Cui, J., Suo, S., Cong, Y. and Tijssen, P., 2013. Phylogeny and evolution of porcine parvovirus. *Virus Research*, *178*(2), pp.392-397.

Saekhow, P. and Ikeda, H., 2015. Prevalence and genomic characterization of porcine parvoviruses detected in Chiangmai area of Thailand in 2011. *Microbiology and Immunology*, *59*(2), pp.82-88.

Saekhow, P., Mawatari, T. and Ikeda, H., 2014. Coexistence of multiple strains of porcine parvovirus 2 in pig farms. *Microbiology and Immunology*, *58*(7), pp.382-387.

Salmon, H., Berri, M., Gerdts, V. and Meurens, F., 2009. Humoral and cellular factors of maternal immunity in swine. *Developmental and Comparative Immunology*, *33*(3), pp.384-393.

Schirtzinger, E.E., Suddith, A.W., Hause, B.M. and Hesse, R.A., 2015. First identification of porcine parvovirus 6 in North America by viral metagenomic sequencing of serum from pigs infected with porcine reproductive and respiratory syndrome virus. *Virology Journal*, *12*(1), p.170.

Segalés, J., Allan, G.M. and Domingo, M., 2005. Porcine circovirus diseases. *Animal Health Research Reviews*, *6*(2), pp.119-142.

Simpson, A.A., Hébert, B., Sullivan, G.M., Parrish, C.R., Zádori, Z., Tijssen, P. and Rossmann, M.G., 2002. The structure of porcine parvovirus: comparison with related viruses. *Journal of Molecular Biology*, *315*(5), pp.1189-1198.

Streck, A.F., Canal, C.W. and Truyen, U., 2015a. Molecular epidemiology and evolution of porcine parvoviruses. *Infection, Genetics and Evolution*, *36*, pp.300-306.

Streck, A.F., Hergemöller, F., Rüster, D., Speck, S. and Truyen, U., 2015b. A TaqMan qPCR for quantitation of Ungulate protoparvovirus 1 validated in several matrices. *Journal of Virological Methods*, *218*, pp.46-50.

Streck, A.F., Homeier, T., Foerster, T., Fischer, S. and Truyen, U., 2013. Analysis of porcine parvoviruses in tonsils and hearts from healthy pigs reveals high prevalence and genetic diversity in Germany. *Archives of Virology*, *158*(6), pp.1173-1180.

Szelei, J., Zadori, Z. and Tijssen, P., 2006. Porcine parvovirus. In: Kerr, J.R., Cotmore, S.F., Bloom, M.E., Linden, R.M. and Parrish, C.R. Eds., *Parvoviruses*, Hodder Arnold, London, UK, pp.435–445.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), pp.2725-2729.

Thomson, G.R. and Prozesky, L., 1994. Porcine parvovirus infection. In: Coetzer, J.A.W., Thomson, G.R., Tustin, R.C. Eds., *Infectious Diseases of Livestock,* South Africa, Oxford University Press, pp.888-894.

Tijssen, P., Agbandje-McKenna, M., Almendral, J.M., Bergoin, M., Flegel, T.W., et al. 2011. Family Parvoviridae. In: King, A.M.Q, Adams, M.J., Carstens, E.B., Lefkowitz, E.J. eds., *Virus Taxonomy 9th report of the International Committee on Taxonomy of Viruses*. London: Academic Press. pp.405–425.

Tijssen, P., Bergeron, J., Dubuc, R. and Hébert, B., 1995, October. Minor genetic changes among porcine parvovirus groups are responsible for major distinguishing biological properties. In *Seminars in Virology* (Vol. 6, No. 5, pp. 319-328). Academic Press.

Truyen, U. and Streck, A. F., 2012. Porcine parvovirus. In: Zimmerman, J. K., Karriker, L., Ramirez, A., Schwartz, K. and Stevenson, G. 10th Eds., *Diseases of Swine*, John Wiley and Sons, Chichester, UK, pp.447–455.

Tse, H., Tsoi, H.W., Teng, J.L., Chen, X.C., Liu, H., Zhou, B., Zheng, B.J., Woo, P.C., Lau, S.K. and Yuen, K.Y., 2011. Discovery and genomic characterization of a novel ovine partetravirus and a new genotype of bovine partetravirus. *PLoS One*, *6*(9), p.e25619.

Wang, F., Wei, Y., Zhu, C., Huang, X., Xu, Y., Yu, L. and Yu, X., 2010. Novel parvovirus sublineage in the family of Parvoviridae. *Virus Genes*, *41*(2), pp.305-308.

Westenbrink, F., Veldhuis, M.A. and Brinkhof, J.M.A., 1989. An enzyme-linked immunosorbent assay for detection of antibodies to porcine parvovirus. *Journal of Virological Methods*, *23*(2), pp.169-178.

Whitaker, H.K., Neu, S.M. and Pace, L.W., 1990. Parvovirus infection in pigs with exudative skin disease. *Journal of Veterinary Diagnostic Investigation*, *2*(3), pp.244-246.

Wrathall, A.E., Wells, D.E., Cartwright, S.F. and Frerichs, G.N., 1984. An inactivated, oil-emulsion vaccine for the prevention of porcine parvovirus-induced reproductive failure. *Research in Veterinary Science*, *36*(2), pp.136-143.

Xiao, C.T., Gerber, P.F., Giménez-Lirola, L.G., Halbur, P.G. and Opriessnig, T., 2013c. Characterization of porcine parvovirus type 2 (PPV2) which is highly prevalent in the USA. *Veterinary Microbiology*, *161*(3), pp.325-330.

Xiao, C.T., Giménez-Lirola, L.G., Halbur, P.G. and Opriessnig, T., 2012. Increasing porcine PARV4 prevalence with pig age in the US pig population. *Veterinary Microbiology*, *160*(3), pp.290-296.

Xiao, C.T., Gimenez-Lirola, L.G., Jiang, Y.H., Halbur, P.G. and Opriessnig, T., 2013d. Characterization of a novel porcine parvovirus tentatively designated PPV5. *PLoS One*, *8*(6), p.e65312.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2013a. Complete genome sequence of a novel porcine parvovirus (PPV) provisionally designated PPV5. *Genome Announcements*, *1*(1), pp.e00021-12.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2013b. Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. *Infection, Genetics and Evolution*, *16*, pp.369-376.

Xing, X., Zhou, H., Tong, L., Chen, Y., Sun, Y., Wang, H. and Zhang, G., 2018. First identification of porcine parvovirus 7 in China. *Archives of Virology*, *163*(1), pp.209-213.

Yang, Y., Qin, X., Zhang, W., Li, Y. and Zhang, Z., 2016. Rapid and specific detection of porcine parvovirus by isothermal recombinase polymerase amplification assays. *Molecular and Cellular Probes*, *30*(5), pp.300-305.

Yang, W.Z., Yu, J.M., Li, J.S., Cheng, W.X., Huang, C.P. and Duan, Z.J., 2012. Genome characterization of a novel porcine bocavirus. *Archives of Virology*, *157*(11), pp.2125-2132.

Zeeuw, E.J.L., Leinecker, N., Herwig, V., Selbitz, H.J. and Truyen, U., 2007. Study of the virulence and cross-neutralization capability of recent porcine parvovirus field isolates and vaccine viruses in experimentally infected pregnant gilts. *Journal of General Virology*, *88*(2), pp.420-427.

Zhai, S.L., Yue, C., Wei, Z.Z., Ran, D.L., Long, J.X., Lin, T., Deng, Y., Sun, L.C., Huang, L. and Yuan, S.S., 2010. Development and application of a PCR assay for detection of porcine bocavirus. *Chinese Journal of Animal Infectious Diseases*, *2*, pp.14-17.

Zhou, F., Sun, H. and Wang, Y., 2014. Porcine bocavirus: achievements in the past five years. *Viruses*, *6*(12), pp.4946-4960.

CHAPTER FOUR

Molecular Detection of *Porcine circovirus type 2* in Swine Herds of Eastern Cape Province, South Africa.

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Abstract

Porcine circovirus type 2 (PCV2) remains the main causative viral pathogen of porcine circovirus-associated diseases (PCVAD) of great economic importance in pig industry globally. This present study aims at determining the occurrence of the viral pathogen in swine herds of the Province. The data obtained revealed that 15.93% of the screened samples (54/339) from the swine herds of the studied areas were positive for PCV2; while the severity of occurrence of the viral pathogen as observed at farm level ranges from approximately 5.6% to 60% in the studied farms. The majority (15 out of 17 = 88%) of the analyzed sequences were found clustering with other PCV2b strains in the phylogenetic analysis. More interestingly, two other sequences obtained were also found clustering within PCV2d genogroup, which is presently another fast-spreading genotype with observable higher virulence in global swine herds. This is the first report of PCV2 in swine herds of the Province and the first detection of PCV2b and PCV2d in South African swine herds. It follows the first reported case of PCV2a in an outbreak of porcine multisystemic wasting syndrome (PMWS) in Gauteng Province, South Africa more than one decade ago. This finding confirmed the presence of this all-important viral pathogen in pigs of the region; which could result in a serious outbreak of PCVAD and huge economic loss at the instances of triggering factors if no appropriate measures are taken to effectively curb its spread.

Keywords: Porcine circovirus type 2, Eastern Cape Province, South Africa

4.1   Introduction

Porcine circoviruses (PCVs) are of the genus *Circovirus* in the family *Circoviridae*. They are non-enveloped viruses with a single-stranded circular DNA genome (Tischer *et al*., 1982). They are the smallest known animal viruses and include porcine circovirus type 1 (PCV1) and porcine circovirus type 2 (PCV2), with genome sizes of 1759 and 1767/1768 nucleotides, respectively (Meehan *et al*., 1998). PCV1 was first detected in 1974 as a contaminant of the porcine kidney cell line PK-15 (ATCC CCL-33) and was determined to be non-pathogenic (Tischer *et al*., 1974). However, PCV2 was found in pigs more than two decades later and proved to have the clinical manifestation of post-weaning multi-systemic wasting syndrome (PMWS) (Allan and Ellis, 2000)

Globally, PCV2 is recognized as an emerging swine pathogen of great economic importance, causing huge losses in the piggery business. The viral pathogen has been the major culprit in cases of PMWS and other clinical disease manifestations in pigs that are generally regarded as porcine circovirus-associated diseases (PCVADs) (Opriessnig *et al*., 2007). These include porcine dermatitis and nephropathy syndrome (PDNS), porcine circovirus reproductive disorders, porcine respiratory disease complex, enteritis, acute pulmonary oedema, nervous system lesions, proliferative and necrotizing pneumonia, and a recently resurfacing neonatal congenital tremor (Opriessnig *et al*., 2007; Tummaruk and Pearodwong, 2016).

The viral genome of PCV2 is composed of at least five open reading frames (ORFs) that could be transcribed; however, ORF1 (coding for replication proteins) and ORF2 (coding for structural capsid protein) remain the widely sequenced and studied regions (Franzo *et al*., 2016a). PCV2 generally infects 7−16-week old weaners and growers, as younger pigs are protected by passive

immunity conferred by acquired maternal antibodies (McKeown *et al*., 2005). PCV2 infection normally affects the immune system of infected pigs by causing lymphoid tissue depletion, which leads to observable histological lesions. Co-infection with other pathogens normally result in worsened conditions in the infected pigs and is made possible due to the immunosuppression and reduced immunity that result from the PCV2 attacks on the protection system (Opriessnig *et al*., 2007).

PCV2 strains have been classified into four main genotypes (PCV2a, PCV2b, PCV2c, and PCV2d) based on phylogenetic analyses performed with their full genomes and ORF2 sequences (Franzo *et al*., 2015a). Previously, PCV2c was detected only in Denmark from archived materials; however, it was recently found in live feral pigs in Brazil and from field samples taken from sick pigs in China (Dupont *et al*., 2008; Franzo *et al*., 2015b; Liu *et al*., 2016). The duo of PCV2a and PCV2b have a worldwide distribution; since 2003 however, there has been an observable worldwide genotypic shift in occurrence from PCV2a to PCV2b in pig herds, which has since made PCV2b the predominant genotype (Franzo *et al*., 2016b). PCV2d (previously regarded as a mutant PCV2b) is the newly emerging genotype that is currently circulating the globe in swine herds and has an apparent higher virulence (Guo *et al*., 2012; Xiao *et al*., 2015).

PCV2-infected pigs normally shed the virus through many routes, including urine, faeces, milk, oronasal secretions, colostrum, and semen (Segalés *et al*., 2005; Patterson *et al*., 2011), thereby enhancing the transmission and spread of PCV2 infections among local herds. The global transmission of PCV2 infections is also greatly enhanced through the international trade of live pigs and pig products due to the subtle nature of the disease, which can be in the form of a subclinical infection (Vidigal *et al*., 2012; Franzo *et al*., 2015c; Franzo *et al*., 2016b). Hence,

proper diagnostic measures to check for the wild spread of the viral pathogen become imperative. There are two major ways of diagnosing PCV2 infections. Preliminarily, this can be done by observing for clinical signs, but this may not be accurate or reliable in cases of subclinical infections (Gillespie *et al*., 2009). The confirmatory detection of PCV2 nucleic acids or antigens in samples from infected animals is therefore imperative. This has been achieved over the years through polymerase chain reaction (PCR), immunohistochemistry (IHC), and *in-situ* hybridization (ISH) techniques (Opriessnig *et al*., 2007).

Although PCV2 is rampant in domestic pig populations globally, severe PCVAD may not occur on very many occasions. Not all pigs within the affected herd develop PCVAD because PCV2 is not the only factor required for disease expression (Gillespie *et al*., 2009). As a multifactorial disease, there have been numerous studies on infectious and non-infectious cofactors in PCV2 infections, which include the co-infection of PCV2 and other pathogens (Gillespie *et al*., 2009). Non-infectious cofactors of PCV2 infection include the genetic background of the pig, management practices such as high stocking density, and prevailing environmental conditions such as temperature fluctuations within the pen (Patterson *et al*., 2015; Li *et al*., 2016).

Being a multifactorial disease, the prevention and control of PCVAD have been achieved over time through all-encompassing measures that consider both infectious and non-infectious contributing agents. Although vaccination has proven effective in preventing PCV2 infection and spread within the herds, the effectiveness is better achieved by combining it with good management practices such as preventing cross-fostering, strict biosecurity practice, careful breed selection, maintaining high standards of hygiene through the effective use of disinfectants, and good housing conditions (Madec *et al*., 2001; Rose *et al*., 2003).

Despite the ubiquitous status of the virus, it has recently been revealed that the virus is grossly understudied in sub-Saharan Africa and on the African continent at large (Afolabi *et al*., 2017). In South Africa, PCV2 was first detected on a commercial breeding farm in 2001 in pigs with clinical manifestations of PDNS and PMWS (Drew *et al*., 2004). To date, however, no large-scale study has been conducted on the prevalence of PCV2 and its associated diseases to ascertain the true infection status of the pigs in the country. The recommendations by Drew *et al*. (2004) and An *et al*. (2007) for further molecular epidemiological studies on PCV2 strains at other premises in South Africa have been grossly neglected.

Therefore, the focus of this study was to validate the current PCV2 status of pigs in South Africa by surveilling for its presence in the swine herds of Eastern Cape Province. This serves as the first surveillance on the pathogen's occurrence in the swine herds of this region and helps to contribute more South African-generated PCV2 sequences to GenBank, as there are currently very few from the country. In addition, it serves as motivation to conduct further studies in the near future entailing wider geographic regions to accurately document the virus genogroups circulating in the country and the entire sub-Saharan African region.

4.2   Methods

4.2.1   *Sample collection*

A total number of three hundred and seventy five (375) field samples, consisting of blood, faecal and nasal swabs samples were collected from seven (commercial, semi-commercial and communal) farms from three District Municipalities of Eastern Cape Province, South Africa in 2015 and 2016. The fresh samples were randomly collected from both healthy and diseased pigs of the sampled farms; they were processed and kept in -80°C freezer until when used. A

Questionnaire was also designed (Appendix 4.1) and administered to each of the sampled farm to obtain information about some farm management practices and level of awareness on PCV2 from the farm managers. Ethical approval and permission to conduct the study were obtained from appropriate quaters prior to the commencement of the study (Appendix 4.2).

4.2.2 DNA extraction and PCV2 detection

Extraction of total genomic DNA was done from the processed samples by using ReliaPrep™ gDNA Tissue Miniprep System (Promega, Madison, USA) with strict adherence to the manufacturer procedures. Initial screening of the samples was performed through polymerase chain reaction (PCR) by using the primer pair P1 and P2, and subsequently, positive samples were again subjected to a second round of PCR amplification by using a primer pair P3 and P4 (Table 4.1). The first primer pair amplified a chunk of about 629 base pair (bp) long of ORF1 region (replicase gene) of the viral genome while the second overlapping primer pair amplified the remaining part of the ORF1 region and a portion of ORF2 (capsid gene) totaling about 630 bp in length.

Table 4.1: Primer pairs used for conventional PCR screening and subsequent nucleotide sequencing of partial PCV2 genomes

Primer identity	Primer sequence	Amplicon length	Nucleotide Position	Reference
P1Fw	5′-TAATCCTTCCGAAGACGAGC-3′	629	116–135	An *et al*. (2007)
P2Rv	5′-CGATCACACAGTCTCAGTAG-3′	629	726–745	An *et al*. (2007)
P3Fw	5′-CAGAAGCGTGATTGGAAGAC-3′	630	531–550	An *et al*. (2007)
P4Rv	5′-ATGTAGACCACGTAGGCCTC-3′	630	1142–1161	An *et al*. (2007)

The PCR amplification for PCV2 detection was carried out as earlier described by An *et al*. (2007) with some modifications. The PCR reaction mixtures were made by adding 5µL of extracted DNA to 45 µL of a reaction mixture containing a final concentration of 1.25mM MgCl$_2$, 5X PCR buffer, 0.2mM dNTPs, 10 pmol of each primer, and 2.5 U of Taq DNA polymerase. PCR amplification was done in a MyCycler™ (Thermer Cycler 1.065) machine (Bio-Rad, Apllied Biosytem, California) with amplification conditions of 95 $^{\circ}$C for 4 mins (initial denaturation); 35 cycles (final denaturation of 95 $^{\circ}$C for 30 s, annealing of 57 $^{\circ}$C for 30 s, elongation of 72 $^{\circ}$C for 1 min); final elongation of 72 $^{\circ}$C for 5 mins. PCR products were analyzed by electrophoresis on 1.5% Agarose gels stained with Ethidium bromide (EB) and visualized using an Alliance 4.7 transilluminator (UVitec, Cambridge, UK).

4.2.3 *Sequencing of amplified nucleotide sequences, analysis and construction of phylogenetic tree*

Positive PCR products of high quality were selected for sequencing at University of Stellenbosch Central DNA Sequencing Facility using the forward and reverse primers earlier used in PCR amplification. Post PCR clean-ups were performed using Nucleofast 96 well PCR plate (Macherey-Nagel, Düren, Germany) with adherence to the manufacturer's instructions on a Tecan EVO150 robotic workstation (Tecan Group, Männedorf, Switzerland). The purified products were sequenced with standard Sanger sequencing using the BigDye Terminator V3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA) in line with the manufacturer's instructions with slight modifications. Sequenced DNA were edited, blasted and assembled using Geneious 10.1.2 (Kearse *et al*., 2012).

The execution of Basic Local Alignment Search Tool (BLAST) was carried out on the DNA sequences as an initial measure to ascertain that all the sequences were truly PCV2 in comparison with other sequences present in the GenBank. Nucleotide sequence alignment was performed by using Bioedit software (Hall *et al*., 1999) while amino acid alignment was done by using ClustalW as implemented in Geneious 10.1.2 software (Kearse *et al*., 2012). The Phylogenetic tree was reconstructed using the distance-based neighbor joining algorithm as implemented in Mega 6 (Tamura *et al*., 2013). Reliability was evaluated by the bootstrapping method on 1000 replicate of the alignment. All sequences that were obtained from GenBank and used for reconstructing the phylogenetic tree are as follow: **PCU49186, AY556474, AY325495, AY322004, AJ223185, AF408635, AF381176, KM924366, KM924364, FJ870968, FJ870967, DQ104423, AF055392, AY691169, AY424405, AY322003, AY321985, KU041850, KU041849, HQ202970, HM038016, FJ870974, FJ870969, AF055394, KX247842, JX406426, EU418626, HQ395035, GU247990, KX247844, AF201311, EU148503, EU148504, EU148505, KU041859, KU041855, KU041851, KJ680361, KJ680354, KJ680353, KJ511876, KX828241, AY181946, FJ712215, JX535296, KY425815 and KU311021** (see details in Appendix 4.2).

4.3   Results

4.3.1   Characteristics and observable farm management practices on the sampled farms

Three hundred and seventy-five blood, faecal, and nasal-swab samples were collected from seven commercial and communal pig farms in three district municipalities of Eastern Cape Province in the years 2015 and 2016. From the administered questionnaire, it was obvious that virtually all the managing personnel of the sampled farms were ignorant of PCV2 and its

associated diseases. Moreover, the best practices that are required in a pig farming operation to prevent the transmission and outbreak of infectious diseases were essentially absent on most of the communal farms. Only one of the seven sampled farms employed high biosecurity measures in their piggery operations. It was also highly notable that none of the farms vaccinated their pigs against PCV2, not even the sampled commercial farm. More than half (57%) of the sampled farms were not using disinfectants for routine cleaning of their pens. Furthermore, a majority of the sampled farms had experienced some PCVAD symptoms such as wasting, abortion, and respiratory distress (Table 4.2).

4.3.2   *Molecular detection, characterization, and analysis of PCV2 DNA sequences from the field samples*

Using PCV2-specific primers, the screened samples showed that the farm-level occurrence of PCV2 ranged from 5.6% to 60%, while overall, 54 of the 339 screened samples were positive for PCV2, representing 15.93% (Table 4.3). The assembly of the two amplified and sequenced PCV2 DNA fragments yielded nucleotide sequences of ~1041 nucleotides, which spanned a large portion of the replicase gene region of the viral genome and small portion of the structural (capsid) gene region.

Table 4.2: Farms features and some management practices of sampled farms

Farm code	Farm one (2FTP)	Farm two (TSO)	Farm three (MTH)	Farm four (CHA)	Farm five (CHB)	Farm six (CHC)	Farm seven (CHD)
Herds population	>5,000	100	80	200	250	50	70
Herds composition	Weaners and growers	Sows, weaners, growers and boars	Sows, weaners, growers and boars	Sows, weaners, growers and boars	Sows, weaners, growers and boars	Sows, weaners, growers and boars	Sows, weaners, growers and boars
Breeds	Large white and Landrace	Large white	Large white	Large white and Landrace	Large white and Landrace	Large white	Large white
Biosecurity measures	High	Very low	None	Very low	Low	None	None
Biocide use	Virocide	Nil	Nil	Dazzel dip	Dazzel dip	Nil	Nil
Vaccination regime	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Antibiotics use	Applied in cases of infections	Occasionally in cases of infections	Applied in cases of infections	Applied in cases of infections	Occasionally in cases of infections	Occasionally in cases of infections	Occasionally in cases of infections
Antibiotic/ drugs use	Norotrim, Depomycin, Lantrax, Kyroligo, Advocin UltaJet,	Iron, Ivomec / dectomaxn	Iron, Ivomec / dectomax	Iron, Ivomec / dectomax	Iron, Ivomec / dectomax	Iron, Ivomec / dectomax	Iron, Ivomec / dectomax
All-in/All-out practice	Yes	No	No	Yes, but not always	Yes, but not always	No	No
Disease symptoms and occurrence	Swollen lymph nodes, meningitis, respiratory disorders, red cat, gastric ulcers, scrotal and navel hernia	Swollen lymph nodes, respiratory disorders	Meningitis, respiratory disorders, gastric ulcers, hernia	Wasting, Swollen lymph nodes, respiratory disorders, dysentery	Respiratory disorders	Swollen lymph nodes	Swollen lymph nodes, meningitis, respiratory disorders
Prior knowledge of PCV2 infections	Yes	No	No	No	No	No	No
Cases of abortion	Not applicable	Occasionally	Occasionally	Occasionally	Occasionally	Occasionally	Occasionally

Table 4.3: Farm level occurrence of PCV2

District Municipalies	Farms (Codes)	Farm types	Total Samples collected	Sampling Year	Samples screened	Positive samples	Positive samples (%)
AMATHOLE	2FTP	Commercial	206	2015	206	19	9.22
O.R. TAMBO	TSO	Communal	24	2015	24	2	8.33
	MTH	Communal	14	2015	14	2	14.29
CHRIS-HANI	CHA	Communal	35	2016	35	21	60.00
	CHB	Communal	59	2016	36	2	5.56
	CHC	Communal	13	2016	ND	ND	ND
	CHD	Communal	24	2016	24	8	33.33

ND- Not Determined

Using the Basic Local Alignment Search Tool (BLAST) analysis from the National Center for Biotechnology Information (NCBI) database, a homology search of the generated sequences confirmed that all 17 sequences were PCV2. Furthermore, a molecular analysis of the selected sequences revealed that 15 (88.2%) of the 17 PCV2-positive sequences clustered with other PCV2b sequences from different parts of the world, while the remaining two (11.8%) were grouped with the PCV2d reference sequences (Figure 4.1). Both the nucleotide and amino acid sequence alignments of the PCV2 sequences from this study in comparison with the representative PCV2 reference sequences of the four major genogroups of importance in the global pig industry showed that the PCV2 sequences from this study belonged to the PCV2b and PCV2d genotypes (Figure 4.2 and 4.3).
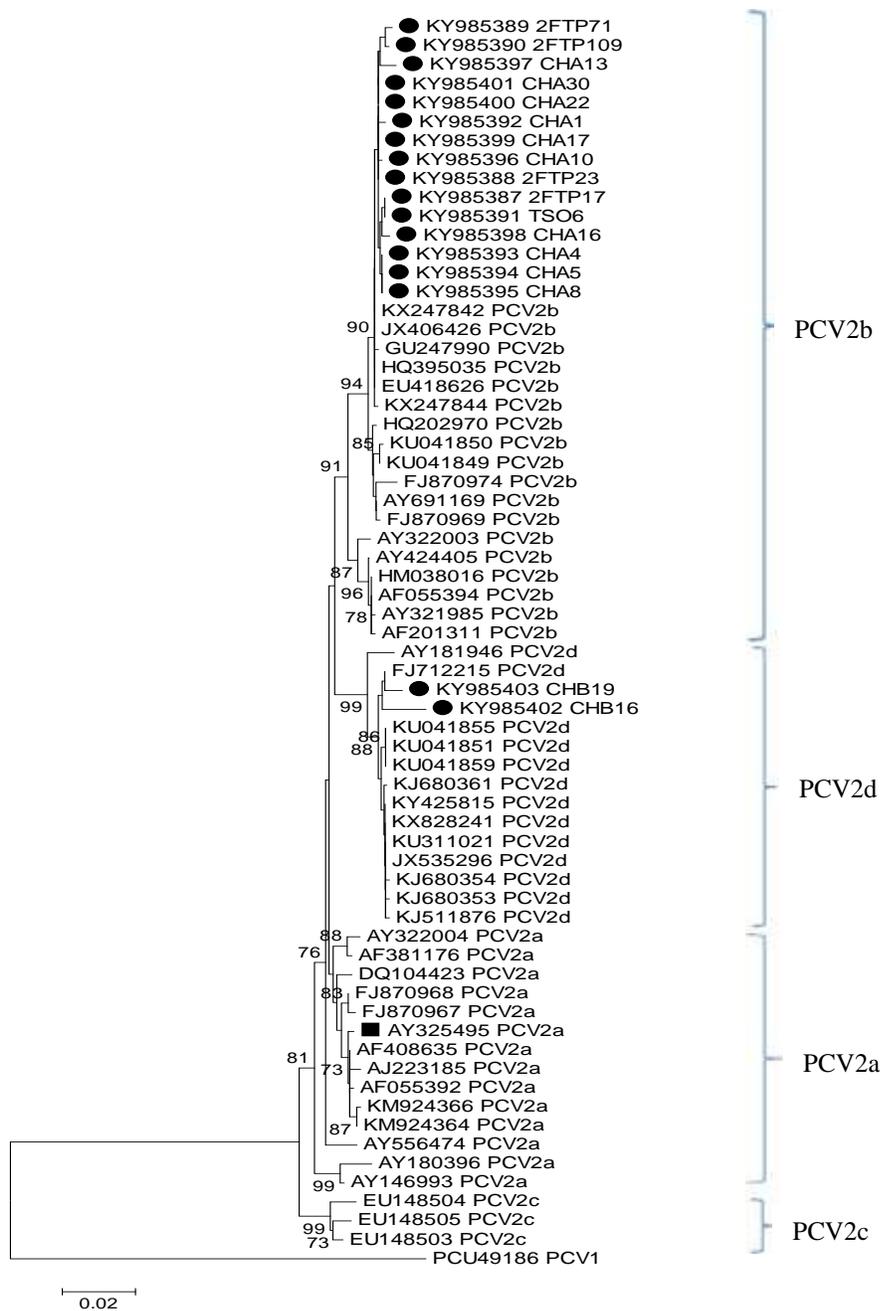
Figure 4.1: Phylogenetic analysis of the partial genomes of the PCV2 from this study with other sequences from GenBank. To further compare the derived sequences in this study with globally reported PCV2 sequences curated in GenBank, a phylogeny was performed using reference sequences from different geographic regions of the world in order to determine the evolutionary origins of the study sequences. The tree was constructed using 64 partial genomes of PCV2 (1041nt) which comprised of 17 strains from this study and 47 other sequences obtained from GenBank while PCV1 sequence served as an out-group. The construction was done by using a Neighbor Joining algorithm and the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The only PCV2 strain previously submitted to the GenBank from South Africa is indicated with the black square box. Analyses were conducted in MEGA6 (Tamura *et al.*, 2013).
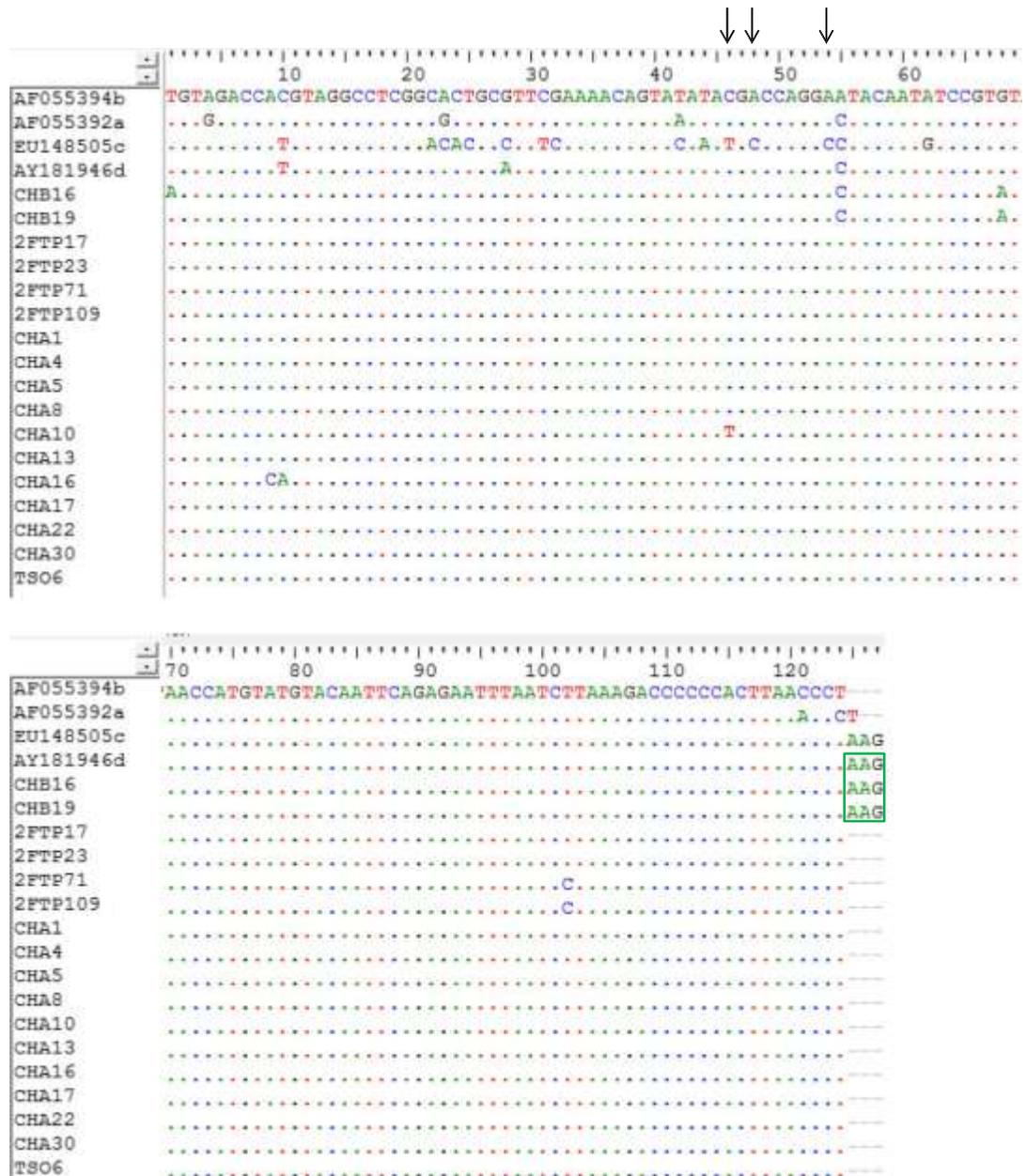
Figure 4.2: Nucleotide sequence alignment of a fragment of ORF2 genes of the 17 PCV2 strains. A segment of aligned PCV2 nucleotide sequences from this study and homologous reference sequences of four major genogroups of PCV2 obtained from GenBank. The green box shows the 3′ end of ORF2 gene where the stop codon mutation (TAA ⟶ AAG) gives rise to longer ORF2 gene (705nt) that characterizes PCV2d capsid gene (Guo *et al*., 2010). The black arrows show some of the marker nucleotides that differentiate PCV2c from PCV2d (Franzo *et al*., 2015a).
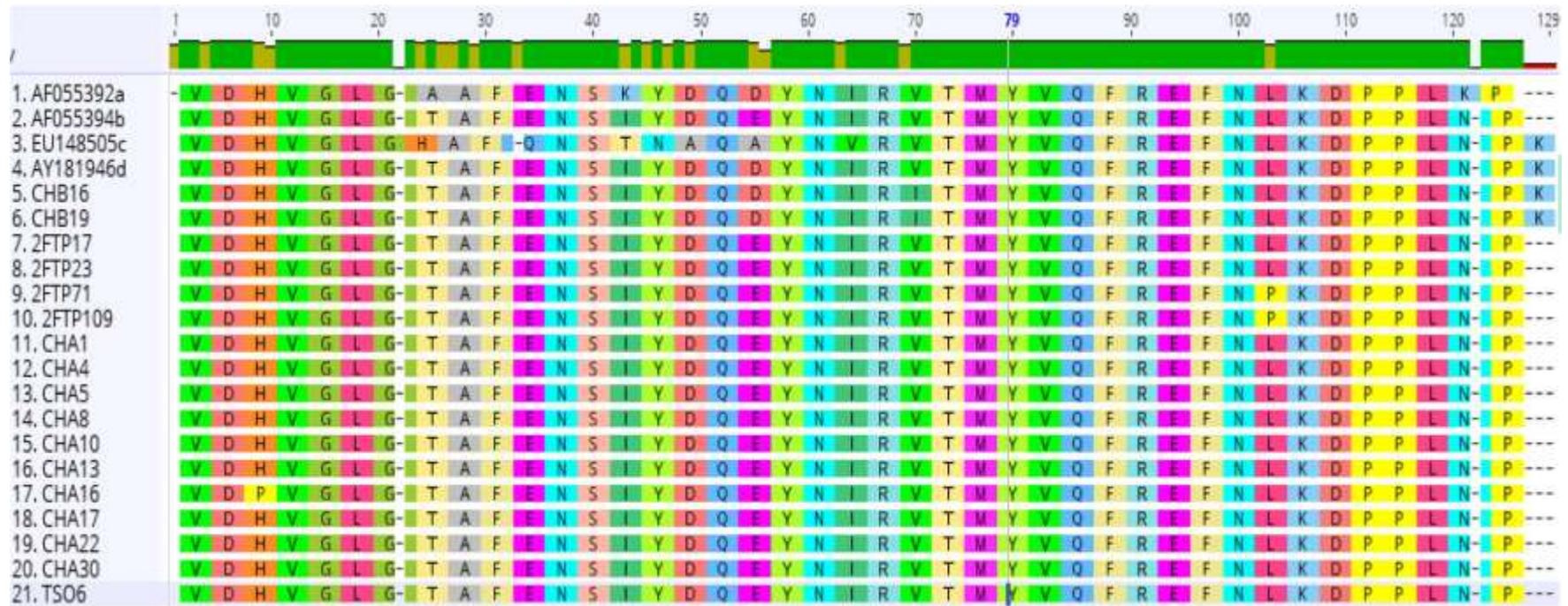
Figure 4.3: Amino acid sequence alignment of the partial capsid protein of the PCV2 strains. A segment showing alignment of the deduced amino acid (aa) sequences of the 17 PCV2 from this study and the reference sequences of four major genogroups of PCV2. Mutations at the C terminus of the capsid protein are shown, resulting to elongation of the ORF2 protein by one Lysine (K) residue in sample CHB16, CHB19 and PCV2d reference sequence (AY181946) as indicated by the green box compared to other 15 sequences in this study that formed the same pattern with reference PCV2b sequence (AF055394).

4.4   Discussion

Infectious swine pathogens such as PCV2 have greatly attracted the attention of researchers since the early 1990s due to the economic havoc they produced in many pig-producing countries of the world (Vidigal *et al*., 2012). Since 1997, PCV2 has been one of the most studied pig pathogens and has been referred to as an everlasting, worldwide, endemic pathogen in swine (Segalés *et al*., 2013). Since its first detection, there has been a notable successive change in the predominance of the circulating viral pathogen genogroup at the global level, changing from PCV2a before the year 2003 to PCV2b after that year (Franzo *et al*., 2016b). Presently, PCV2d is becoming the most rampant and ravaging group with its observably higher virulence (Xiao *et al*., 2015).

Despite the concern and global efforts to study the epidemiology of the viral pathogen, there has been a seemingly lethargic approach to its study in South Africa and the entire sub-Saharan African countries (Afolabi *et al*., 2017). However, the scant information available has confirmed the presence of different PCV2 genogroups in the region, as the previously called North American-like strain [PCV2 group 2 (PCV2a)] was initially detected in South Africa in 2001 (Drew *et al*., 2004), and the European cluster [PCV2 group 1 (PCV2b)] was found in Ugandan pigs over a decade later (Ojok *et al*., 2013). Considering the economic importance of pig production in the developing countries of the world (FAO, 2012), the importance of this study in determining the PCV2 status of pigs on a relatively larger scale cannot be overemphasized.

In this study, 54 of the 339 samples screened for the presence of PCV2 were positive for the pathogen, confirming its presence on all of the tested farms. In addition, the farm level severity of occurrence was as high as 60% on one of the surveyed farms. This supports the ubiquitous status of PCV2 in swine herds globally (Gillespie *et al*., 2009). Some selected high-quality

amplicons were sequenced and processed to obtain 17 partial PCV2 genomes that were then analysed. The phylogenetic analyses confirmed that 15 of the PCV2 sequences formed a group together with the PCV2b reference sequences and were closely clustered with the Chinese strains NQY1 (**KX247842**), FX1102 (**JX406426**), FJMH0508 (**GU247990**), 09HuB (**HQ395035**), RUZHOU (**EU418626**), and XT1 (**KX247844**). The remaining two PCV2 sequences were grouped with the PCV2d GenBank sequences, being closest to the Xuancheng (**FJ712215**) strain from China. The only available reference sequence from South Africa, strain SA1 (**AY325495**), maintained its position among the other PCV2a reference strains used in this analysis, thus confirming the findings of Drew *et al*. (2004) (Figure 4.1).

The topography observed in the phylogenetic clustering was further confirmed through the nucleotide and amino acid sequence alignments (Figures 4.2 and 4.3), in which CHB16 and CHB19 were found to have mutations in the stop codon at the 3′ end of ORF2, leading to an amino acid (Lysine-K) extension. This is a typical feature that distinguishes the mutant PCV2b (mPCV2b, now categorized as PCV2d), which is known to be more virulent than PCV2a and 2b (Guo *et al*., 2012). This outcome agrees with the observed global PCV2 infection pattern, which has had a notable genotypic shift around the world, first from PCV2a to PCV2b, and recently to PCV2d (Cortey *et al*., 2008; Xiao *et al*., 2015; Huang *et al*., 2016). The same incidence of genotypic shift may have also occurred in South African pigs since Drew *et al*. (2004) first reported the case of a PCV2a outbreak in 2001, which was traced to inseminating the affected gilts with imported semen from Iowa, North America. This assertion was made due to the clustering of the first South African PCV2 strain (SA1) with many other sequences from there and Asia.

Alternatively, it is possible that the PCV2b and 2d strains detected in this study have been in circulation within the country all this time, but undetected. This claim might be substantiated on the basis that the initial detection in 2001 was from a case study of an outbreak in a commercial breeding unit that supplied breeding stock to other smaller farms (Drew *et al*., 2004) rather than from a large-scale surveillance study. In that case study, only 4 tissue samples from affected pigs were sent to the United Kingdom for analysis. Hence, an unnoticed transmission of different PCV2 genotypes from one herd to another could have been occurring within the country for many years prior to their being detected. Considering it was present at all of the tested farms, another scenario is that the PCV2b strains detected in this study entered through another route and are now spreading rapidly within the eastern region of the country. The occurrence of PCV2d in this study is highly significant and may represent the generally believed genotype shift from PCV2b to 2d, as has been observed in swine herds elsewhere in the world (Xiao *et al*., 2015). However, achieving a better understanding of this requires further prevalence studies and an in-depth molecular characterization of the PCV2 genotypes in circulation within the country.

The findings from this study suggest that the probable cost of neglecting proper PCV2 surveillance in South African pigs, as stated by Mokoele *et al*. (2015), could be grievous and devastating with any instance of a large-scale PCV2 infection outbreak in the country. It is noteworthy that PCV2 was detected in all the sampled farms in the province, indicating a significant level of occurrence of the 'small but powerful' viral pathogen in the swine herds. The claim of negligence on the part of stakeholders in the country's swine industry is further corroborated by the findings of our investigation. The pig farmers in the area had essentially no level of awareness (Table 4.2), and hence, were currently implementing no preventive measures.

Good management practice has been considered paramount in the prevention and control of PCV2 infections (Rose *et al*., 2003). It is therefore not surprising that PCV2 was detected on all the sampled farms in this study, owning to the fact that the management practices of the majority of the farms was very poor. For example, a large percentage of the farms had little or no biosecurity measures in place that could forestall the farm-to-farm transmission of infectious agents such as PCV2. Furthermore, other important recommendations made in 1997 in Madec's proposed 20-point plan to lower the impact of the disease (Madec *et al*., 2001) were grossly absent on the farms studied, such as the maintenance of good hygiene through the effective use of disinfectants and an all-in/all-out stocking practice. It is noteworthy, however, that despite the observance of good management practices, PCV2 was still present at the commercial farm (farm one) in the study. This observation further stresses the importance of an effective vaccination regime against PCV2 as part of an all-encompassing strategy in preventing infection (Madec *et al*., 2001; Rose *et al*., 2003).

As a multifactorial disease having both infectious and non-infectious co-factors, PCVAD prevention and control rely on a prompt and adequate diagnosis, which is a function of efficient surveillance to ascertain the status of circulating PCV2 genotypes in the swine population. This normally informs the adoption of an effective vaccination regime and good management procedures to forestall rapid spread and the incidence of large-scale outbreaks. According to Segalés (2015), continuous surveillance for new PCV2 variants is important, being a DNA virus that has evolutionary rates comparable to that of RNA viruses based on findings of Firth *et al*. (2009). The observable gross negligence by the stakeholders regarding PCV2 infection in South Africa and sub-Saharan African countries (Afolabi *et al*., 2017) at large could suppress the huge

potential suggested for pig production to alleviate poverty and help solve hunger issues in developing countries (FAO, 2012).

4.5   Conclusion

The detection of PCV2b and its mutant strain (PCV2d) in the swine herds of Eastern Cape Province after the initial detection of PCV2a in South African pigs more than a decade and half ago is very disturbing considering the economic importance of this viral pathogen. It is therefore extremely important that a rigorous enlightenment of the farmers about PCV2 and its associated diseases be undertaken in the country. This is highly imperative, as our survey revealed that virtually all of the farmers in the region were ignorant of the pathogen. An effective large-scale vaccination regime should also be initiated to curtail the rapid circulation of the virus and prevent impending future outbreaks. This should be done *pari passu* with educating the farmers (especially the communal ones) on the biosecurity measures required in piggery operations for optimal performance. Further studies on the prevalence of PCV2 and its co-infecting pathogens in the pigs of Eastern Cape Province and other South African provinces should also be conducted without further delay, with a candid effort geared towards obtaining more of the viral genomes (preferably complete) in circulation within the country. This will provide a thorough and immediate molecular characterization of the circulating strains and better our understanding of the epidemiology of this viral pathogen, both within the country and beyond.

References

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964.

Allan, G.M. and Ellis, J.A., 2000. Porcine circoviruses: a review. *Journal of Veterinary Diagnostic Investigation*, *12*(1), pp.3-14.

An, D.J., Roh, I.S., Song, D.S., Park, C.K. and Park, B.K., 2007. Phylogenetic characterization of porcine circovirus type 2 in PMWS and PDNS Korean pigs between 1999 and 2006. *Virus Research*, *129*(2), pp.115-122.

Cortey, M., Pileri, E., Sibila, M., Pujols, J., Balasch, M., Plana, J. and Segalés, J., 2011. Genotypic shift of porcine circovirus type 2 from PCV-2a to PCV-2b in Spain from 1985 to 2008. *The Veterinary Journal*, *187*(3), pp.363-368.

Drew, T.W., Grierson, S.S., King, D.P., Hicks, D., Done, S., Banks, M., Neser, J.A., Evans, D.P.B. and Grimbeek, P., 2004. Genetic similarity between porcine circovirus type 2 isolated from the first reported case of PMWS in South Africa and North American isolates. *Veterinary Record*, *155*(5), pp.149–151.

Dupont, K., Nielsen, E.O., Baekbo, P. and Larsen, L.E., 2008. Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case–control study supports a shift in genotypes with time. *Veterinary Microbiology*, *128*(1), pp.56-64.

FAO, 2012. Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3, Rome.

Firth, C., Charleston, M.A., Duffy, S., Shapiro, B. and Holmes, E.C., 2009. Insights into the evolutionary history of an emerging livestock pathogen: porcine circovirus 2. *Journal of Virology*, *83*(24), pp.12813-12821.

Franzo, G., Cortey, M., de Castro, A.M.M.G., Piovezan, U., Szabo, M.P.J., Drigo, M., Segalés, J. and Richtzenhain, L.J., 2015b. Genetic characterisation of Porcine circovirus type 2 (PCV2) strains from feral pigs in the Brazilian Pantanal: an opportunity to reconstruct the history of PCV2 evolution. *Veterinary Microbiology*, *178*(1), pp.158-162.

Franzo, G., Cortey, M., Olvera, A., Novosel, D., De Castro, A.M.M.G., Biagini, P., Segalés, J. and Drigo, M., 2015a. Revisiting the taxonomical classification of Porcine Circovirus type 2 (PCV2): still a real challenge. *Virology Journal*, *12*(1), p.131.

Franzo, G., Cortey, M., Segalés, J., Hughes, J. and Drigo, M., 2016b. Phylodynamic analysis of porcine circovirus type 2 reveals global waves of emerging genotypes and the circulation of recombinant forms. *Molecular Phylogenetics and Evolution*, *100*, pp.269-280.

Franzo, G., Tucciarone, C.M., Cecchinato, M. and Drigo, M., 2016a. Porcine circovirus type 2 (PCV2) evolution before and after the vaccination introduction: A large scale epidemiological study. *Scientific Reports*, *6*, p.39458.

Franzo, G., Tucciarone, C.M., Dotto, G., Gigli, A., Ceglie, L. and Drigo, M., 2015c. International trades, local spread and viral evolution: The case of porcine circovirus type 2 (PCV2) strains heterogeneity in Italy. *Infection, Genetics and Evolution*, *32*, pp.409-415.

Gillespie, J., Opriessnig, T., Meng, X.J., Pelzer, K. and Buechner-Maxwell, V., 2009. Porcine circovirus type 2 and porcine circovirus-associated disease. *Journal of Veterinary Internal Medicine*, *23*(6), pp.1151-1163.

Guo, L., Fu, Y., Wang, Y., Lu, Y., Wei, Y., Tang, Q., Fan, P., Liu, J., Zhang, L., Zhang, F. and Huang, L., 2012. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. *PLoS One*, *7*(7), p.e41463.

Guo, L.J., Lu, Y.H., Wei, Y.W., Huang, L.P. and Liu, C.M., 2010. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virology Journal*, *7*(1), p.273.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series, 41*(41), pp. 95-98.

Huang, L., Wang, Y., Wei, Y., Chen, D., Liu, D., Du, W., Xia, D., Wu, H., Feng, L. and Liu, C., 2016. Capsid proteins from PCV2a genotype confer greater protection against a PCV2b strain than those from PCV2b genotype in pigs: evidence for PCV2b strains becoming more predominant than PCV2a strains from 2000 to 2010s. *Applied Microbiology and Biotechnology*, *100*(13), pp.5933-5943.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. and Thierer, T., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), pp.1647-1649.

Li, Y., Liu, H., Wang, P., Wang, L., Sun, Y., Liu, G., Zhang, P., Kang, L., Jiang, S. and Jiang, Y., 2016. RNA-seq analysis reveals genes underlying different disease responses to porcine circovirus type 2 in pigs. *PloS One*, *11*(5), p.e0155502.

Liu, X., Wang, F.X., Zhu, H.W., Sun, N. and Wu, H., 2016. Phylogenetic analysis of porcine circovirus type 2 (PCV2) isolates from China with high homology to PCV2c. *Archives of Virology*, *161*(6), pp.1591-1599.

Madec, F., Rose, N., Eveno, E., Morvan, P., Larour, G., Jolly, J.P., Le Diguerher, G., Cariolet, R., Le Dimna, M., Blanchard, P. and Jestin, A., 2001, September. PMWS: on-farm observations and preliminary analytic epidemiology. In *Proceedings of the ssDNA Viruses Plants, Birds, Pigs and Primates (ESVV) Meeting* (pp. 86-87).

McKeown, N.E., Opriessnig, T., Thomas, P., Guenette, D.K., Elvinger, F., Fenaux, M., Halbur, P.G. and Meng, X.J., 2005. Effects of porcine circovirus type 2 (PCV2) maternal antibodies on experimental infection of piglets with PCV2. *Clinical and Diagnostic Laboratory Immunology*, *12*(11), pp.1347-1351.

Meehan, B.M., McNeilly, F., Todd, D., Kennedy, S., Jewhurst, V.A., Ellis, J.A., Hassard, L.E., Clark, E.G., Haines, D.M. and Allan, G.M., 1998. Characterization of novel circovirus DNAs associated with wasting syndromes in pigs. *Journal of General Virology*, *79*(9), pp.2171-2179.

Mokoele, J.M., van Rensburg, L.J., Van Lochem, S., Bodenstein, H., Du Plessis, J., Carrington, C.A., Spencer, B.T. and Fasina, F.O., 2015. Overview of the perceived risk of transboundary pig diseases in South Africa. *Journal of the South African Veterinary Association*, *86*(1), pp.1-9.

Ojok, L., Okuni, J.B., Hohloch, C., Hecht, W. and Reinacher, M., 2013. Detection and characterisation of porcine circovirus 2 from Ugandan pigs. *Indian Journal of Veterinary Pathology*, *37*(1), pp.77-80.

Opriessnig, T., Meng, X.J. and Halbur, P.G., 2007. Porcine circovirus type 2–associated disease: update on current terminology, clinical manifestations, pathogenesis, diagnosis, and intervention strategies. *Journal of Veterinary Diagnostic Investigation*, *19*(6), pp.591-615.

Patterson, A.R., Madson, D.M., Halbur, P.G. and Opriessnig, T., 2011. Shedding and infection dynamics of porcine circovirus type 2 (PCV2) after natural exposure. *Veterinary Microbiology*, *149*(1), pp.225-229.

Patterson, R., Nevel, A., Diaz, A.V., Martineau, H.M., Demmers, T., Browne, C., Mavrommatis, B. and Werling, D., 2015. Exposure to environmental stressors result in increased viral load and further reduction of production parameters in pigs experimentally infected with PCV2b. *Veterinary Microbiology*, *177*(3), pp.261-269.

Rose, N., Larour, G., Le Diguerher, G., Eveno, E., Jolly, J.P., Blanchard, P., Oger, A., Le Dimna, M., Jestin, A. and Madec, F., 2003. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. *Preventive Veterinary Medicine*, *61*(3), pp.209-225.

Segalés, J., 2015. Best practice and future challenges for vaccination against porcine circovirus type 2. *Expert Review of Vaccines*, *14*(3), pp.473-487.

Segalés, J., Calsamiglia, M., Olvera, A., Sibila, M., Badiella, L. and Domingo, M., 2005. Quantification of porcine circovirus type 2 (PCV2) DNA in serum and tonsillar, nasal, tracheo-bronchial, urinary and faecal swabs of pigs with and without postweaning multisystemic wasting syndrome (PMWS). *Veterinary Microbiology*, *111*(3), pp.223-229.

Segalés, J., Kekarainen, T. and Cortey, M., 2013. The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?. *Veterinary Microbiology*, *165*(1), pp.13-20.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), pp.2725-2729.

Tischer, I., Gelderblom, H., Vettermann, W. and Koch, M.A., 1982. A very small porcine virus with circular single-stranded DNA. *Nature*, *295*(5844), pp.64-66.

Tischer, I., Rasch, R. and Tochtermann, G., 1974. Characterization of papovavirus and picornavirus-like particles in permanent pig kidney cell lines. *Zentralblatt fuer Bakteriologie Mikrobiologie und Hygiene series A*, *226*(2), pp.153-167.

Tummaruk, P. and Pearodwong, P., 2016. Porcine circovirus type 2 expression in the brain of neonatal piglets with congenital tremor. *Comparative Clinical Pathology*, *25*(4), pp.727-732.

Vidigal, P.M., Mafra, C.L., Silva, F.M., Fietto, J.L., Júnior, A.S. and Almeida, M.R., 2012. Tripping over emerging pathogens around the world: a phylogeographical approach for determining the epidemiology of Porcine circovirus-2 (PCV-2), considering global trading. *Virus Research*, *163*(1), pp.320-327.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2015. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. *Journal of General Virology*, *96*(7), pp.1830-1841.

CHAPTER FIVE

Genetic characterization of the first complete genomes of porcine circovirus type 2b and 2d from South African pigs

(Under review in *Transboundary and Emerging Diseases)*

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Abstract

Porcine circovirus type 2 (PCV2) is a swine infectious viral pathogen of great significance in global swine herds. The virus was recently detected in a South African pig population sequel to its first detection in 2001, yet there is a dearth of information about the genomic features of the viral strains in circulation within the country. Till date, only one complete genome of the virus from South Africa is available on global data base. This current effort was therefore geared towards the full-genome amplifications, sequencing and characterization of the circulating PCV2 strains in the Eastern Cape Province. With the use of conventional polymerase chain reaction method, fifteen complete PCV2 genomes were successfully amplified, sequenced and assembled. Neighbour Joining and Maximum Likelihood phylogenetic analysis of the ORF2 and full genomes unanimously showed that most of the assembled genomes (11) belong to genotype PCV2b. Furthermore, three of the characterized sequences formed clade with other reference mutant PCV2b and PCV2b subtype 1C (i.e. PCV2d) strains from different parts of the world. The last sequence however, clustered with other reference strains belonging to PCV2 intermediate clade 2 (PCV2-IM2) recently identified in a global PCV2 strains phylogenetic analysis. This study reports the first complete genome sequences of PCV2b, PCV2d and PCV2-IM2 in pigs from South Africa; and a possible insight to the genetic characteristics and variability of the viral strains presently in circulation within the country.

Keywords: Porcine circovirus type 2b, full-genomes, porcine circovirus type 2d, South Africa

5.1   Introduction

Since the early 1990's when porcine circovirus type 2 (PCV2) was first discovered as a primary causative viral pathogen of porcine multisystemic wasting syndrome (PMWS) in North America (Clark, 1996; Harding, 1996); the virus has become an important swine pathogen of great economic importance in global pig industry. Apart from PMWS, it has been associated with many other disease manifestations generally called porcine circovirus associated diseases (PCVAD) (Chae, 2005). PCV2, alongside the avirulent porcine circovirus type 1 (PCV1), belongs to the family *Circoviridae* and genus *Circovirus* with a circular, single-stranded and ambisense genome of about 1767-1768 base pair (bp) long; having two main open reading frames (ORFs 1 and 2), which code for non-structural replicase and structural capsid proteins respectively (Mankertz *et al*., 1998; Nawagitgul *et al*., 2000). Presently, another novel porcine circovirus pathogenic strain has been found circulating in North America and Asia in cases of reproductive failure and porcine dermatitis and nephropathy syndrome (PDNS) with a genome size of about 2000 bp; and has been designated: porcine circovirus type 3 (PCV3) (Fan *et al*., 2017; Palinski *et al*., 2017)

PCV2 is the smallest known DNA virus of animals, which is expected to have a highly conserved genome; however, the rate of nucleotide substitutions in the viral genome per site and year is noted to be the highest of its kind for a typical single-stranded DNA (ssDNA) virus (Xiao *et al*., 2015). It has been estimated to be in order of 1.2 x 10^{-3}; thus making PCV2 to have an evolutionary dynamics that is much closer to single-stranded RNA (ssRNA) viruses rather than ssDNA viruses (Firth *et al*., 2009). Strains of PCV2 have therefore displayed substantial genetic variations that warranted creating a unified nomenclature for them; and this has been widely achieved by using Neighbour Joining (NJ) phylogeny among many other distance-matrix

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methods with pairwise proportional difference of nucleotides (p-distance) model. They were initially subdivided into three phylogenetic groups or genotypes using the capsid gene or the whole PCV2 genome as target sequences based on the proportion of nucleotide sites where two compared sequences differ, using the p-distance cut-off value of 0.035 and 0.020 for the capsid gene and whole genome sequences respectively (Segalés *et al*., 2008).

The three major initial genotypes include PCV2a, b and c; with PCV2a having five clusters (2A-E) and PCV2b having three clusters (1A-C) (Olvera *et al*., 2007). Subsequently, two new PCV2 genotypes namely: PCV2d and PCV2e were detected in Chinese pigs (Wang *et al*., 2009). The proposed PCV2d group members have been confirmed to have very close identity with other PCV2b strains in cluster 1C, with a distinguishing feature of elongated ORF2 gene of 705 nt instead of 702 nt that characterizes a typical PCV2b with some few exceptions (Xiao *et al*., 2015). This resulted from an observable mutation of the stop codon at the C terminus end of their capsid genes. The mutation brought about an amino acid (Lysine-K) extension of the structural protein to 234 amino acids instead of 233 (Guo *et al*., 2010). Presently according to Xiao *et al*. (2015), the PCV2d genotype now consists of PCV2 strains that were previously grouped as PCV2b subtype 1C and those that were designated as mutant PCV2b (mPCV2b); obtained from pigs with clinical manifestation of PCVAD in cases of suspected vaccine failure (Guo *et al*., 2012; Opriessnig *et al*., 2013).

Furthermore, identification of different signature motifs in the ORF2 genomes of PCV2 strains has also helped extensively in genetic characterization of the viral strains. The PCV2a are generally known to have signature motif 1 of 5′-ACCAACAAAATCTCTATA-3′ that corresponds to amino acid sequence 'TNKISI' while PCV2b strains have 5′-

TCAAACCCCCGCTCTGTG-3′ signifying 'SNPRSV' amino acid sequence in their capsid protein (Cheung *et al*., 2007). In the case of PCV2d strains, the motif 1 consists of 5′-TCAAACCCCCTCACTGTG-3′ with corresponding 'SNPLTV' amino acid sequence; however, the motif has also been found in PCV2c strains capsid genes (Xiao *et al*., 2015). A recent taxonomical classification effort by Franzo *et al*. (2015a), based on identification of more marker nucleotide positions in the capsid gene among the viral strains; has however, provided means of differentiating between PCV2c and PCV2d strains. Apart from the four presently recognized phylogenetic groups of PCV2, four major intermediate (IM) clades (IM1-IM4) have been identified. This was done through NJ phylogeny of ORF2 sequences of global PCV2 strains, with confirmation by using more accurate and outperforming methods such as Maximum Likelihood (ML); and about 64 PCV2 strains which fell within the intermediate clades (Xiao *et al*., 2015).

Prior to 2003, PCV2a was the predominant genotype in global swine herds; however, this has changed since then as there has been an unprecedented drastic spread of PCV2b in global swine, thereby making it the most widespread genotype presently with an observable severe increase in outbreaks of associated diseases (Beach and Meng, 2012). The third genotype, PCV2c, has not been of serious economic importance as it was initially found only in Danish archived swine samples obtained from healthy herds and has been found recently in samples from wild pigs in Brazil (Dupont *et al*., 2008; Franzo *et al*., 2015b). Similarly, there has been a recent noticeable world-wide spread of the fourth genotype PCV2d in swine herds of many countries, an event which is being currently termed as another genotype shift from PCV2b to PCV2d globally (Xiao *et al*., 2015).

In South Africa, PCV2 and PMWS was first identified in 2001 in a well-managed commercial breeding facility in Gauteng Province. The assembled genome of the isolated PCV2 strain from the published case report was characterized and found to be PCV2a of very high homology with other PCV2a strains from North America and Asia which was later traced to the semen obtained from America for artificial insemination purposes (Drew *et al*., 2004). The full genome obtained was named PCV2a strain SA1 with an accession number **AY325495** and it has since then remained the only PCV2 complete genome on GenBank from South Africa and the entire sub-Saharan Africa region (Afolabi *et al*., 2017a). Full genome amplification and characterization of PCV2 strains is important for better understanding of its genetic make-up and variability, in order to recommend the most appropriate vaccines and vaccination regime for its prevention and control. Therefore, this present study is focused on full genome amplifications and genetic characterization of PCV2 sequences obtained from pigs in some selected commercial and communal swine herds of Eastern Cape, South Africa.

5.2   Materials and methods

5.2.1   *DNA extraction and conventional PCR amplification of PCV2 complete genome*
A total of fifty-four previously confirmed PCV2-positive samples obtained from pigs (age range between 1 to 6 months) with some clinical manifestations of PMWS such as wasting, enlarged lymph nodes, respiratory disorder, dermatitis and some pigs without any clinical signs were used in this study. The samples were selected from archived field samples (blood, faecal and nasal swabs) that were obtained between the year 2015 and 2016 from some selected farms in Eastern Cape, South Africa; previously used in a molecular epidemiological survey of some RNA and DNA viral swine pathogens in the region.

Total viral genomic DNA was extracted from the processed samples and the whole genome PCR amplification was done as earlier described (Afolabi *et al*., 2017b) with some modifications. Briefly, four overlapping primer pairs (Table 5.1) were used to carry out the PCR amplification of the complete PCV2 genomes according to Franzo *et al*. (2015b) and the optimized PCR conditions were as follows: 94 °C for 5 mins as initial denaturation, followed by 35 cycles that consists final denaturation step of 94 °C for 30 s, annealing step of 50-57 °C for 30 s and initial elongation of 72 °C for 1 min; the amplification process ended with final elongation of 72 °C for 5mins. Generated amplicons were subsequently analyzed by electrophoresis using 1.2% agarose gels stained with ethidium bromide, amplified DNA were visualized in the stained gel using an Alliance 4.7 transilluminator. PCR products with clear target bands on visualized gel were selected for further sequencing.

Table 5.1: The oligonucleotide primers used in amplification and sequencing of PCV2 complete genomes

Primer identity	Primer sequence (5′-3′)	Amplicon size	Reference
P1Fw	5′-TAATCCTTCCGAAGACGAGC-3′	629	An *et al*., 2007
P1Rv	5′-CGATCACACAGTCTCAGTAG-3′	629	An *et al*., 2007
P2Fw	5′-CAGAAGCGTGATTGGAAGAC-3′	630	An *et al*., 2007
P2Rv	5′-ATGTAGACCACGTAGGCCTC-3′	630	An *et al*., 2007
P3Fw	5′-AGAAGCTCTTTATCGGAGGA-3′	598	An *et al*., 2007
P3Rv	5′-CCTTTGAATACTACAGAATAAG-3′	598	Cortey M (unpublished data)
P4Fw	5′- TATGGCGGGAGGAGTAGTT-3′	692	Cortey M (unpublished data)
P4Rv	5′- GTTCGTCCTTCCTCATTACC-3′	692	An *et al*., 2007

5.2.2   Nucleotide sequencing and assembly of sequenced PCV2 genomes

Post PCR clean-ups and Sanger sequencing of selected PCR products were done at a commercial DNA sequencing facility. Briefly, two-way sequencing reactions were conducted on the purified products using the Big Dye Terminator V 3.1 sequencing kit (Applied Biosystems, Foster City, CA, USA) in line with the instructions from the manufacturer; and the reaction was run on 3500 genetic Analyzers (Applied Biosystems, USA). Sequenced DNA segments were edited and aligned to generate consensus sequences which were subsequently subjected to De-novo assembly in Geneious 10.2.3 (Kearse *et al.*, 2012) by using default settings. Blast search for homology was performed on the assembled genomes using BLASTN programme as implemented in Geneious 10.2.3 software (Kearse *et al.*, 2012).

5.2.3   Nucleotide sequence analyses

In order to further characterise the sequenced and assembled PCV2 genomes, forty-two complete genomes and capsid gene reference sequences were retrieved from GenBank to constitute data set of fifty-seven each with the addition of fifteen sequences obtained from this study. Multiple sequence alignment of the reference sequences and those that were obtained in this study was done using MAFFT-v7.311 (http://mafft.cbrc.jp/alignment/server/) and aligned sequences were analyzed in Bioedit-v7.2.3 (Hall, 1999). Phylogenetic analysis was done through the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods as implemented in MEGA6 (Tamura *et al.*, 2013) on the data sets. Reliability of the tree was done using the bootstrapping method calculated on 1000 replicates of the alignment and Jukes-cantor parameter was employed as a nucleotide substitution model. Furthermore, a p-distance analysis of the sequences from this study and selected reference strains representing four genotypes of PCV2 as identified previously by phylogenetic analyses, were calculated in MEGA 6 using Bootstrap variance estimation

method of 1000 replicates and the same nucleotide substitution model as earlier stated (Tamura *et al*., 2013). Analyses were carried out at both level of capsid gene and complete genomes of the PCV2 strains.

5.3   Results

A total of fifteen (15) full genomes of PCV2 were successfully amplified, sequenced and assembled in this study (Table 5.2). The assembled circular whole genome sequences are 1,767 nt in sizes with their capsid gene ranging from 702 to 705 nt in length and replicase gene of 945 nt (Figure 5.1). Based on the phylogenetic analysis of capsid gene and PCV2 complete genomes from this study and other reference sequences using Neighbour Joining (NJ) method, three different groups of new South African PCV2 sequences were identified namely: Groups 1, 2 and 3 (Figure 5.2a). The phylogenetic analysis of the capsid gene and complete genomes of sequences from this study and other reference sequences from GenBank showed little or no differences in their clustering patterns (Figure 5.2a and 5.2b). The topology was further confirmed by using a more accurate Maximum Likelihood (ML) method with little or no difference (Appendix 5.1).

Eleven sequences (Group 1) from this study clustered distinctly with other reference PCV2b-1b strains (Figure 5.2a). The sequence identity analysis of their ORF2 gene further showed that they have sequence homology ranging from 99.8% - 100% within themselves. Also, they have the highest homology (at the level of ORF2 gene) of 99.7% with **AY321987** (France) followed by 99.4% with **DQ923524** from Brazil (Appendix 5.2). Similarly, three sequences (belonging to Group 3) from this study, formed a clade with other reference mPCV2b, PCV2b-1c and PCV2d. Strains that were formerly known as mPCV2b and PCV2b-1c have been re-grouped as PCV2d in a recent global analysis, forming a clade with other reference PCV2d strains (Xiao *et al*., 2015).

Table 5.2: The features and origin of PCV2 complete genomes obtained and analyzed

Clustering Groups	Sequence identity	Place of collection	Clinical manifestation	Age (Weeks)	Sample type	Year of collection	Genome length	Accession number
	SA1-BA	Alice	Wasting	4	Nasal swab	2015	1,767	MG846653
	SA2-BB	Alice	Respiratory distress	9	Faecal swab	2015	1,767	MG846654
	SA4-BD	Queestown	Wasting, respiratory distress	4	Faecal swab	2016	1,767	MG846656
	SA5-BE	Queestown	No symptom	4	Faecal swab	2016	1,767	MG846657
	SA6-BF	Queestown	Swollen lymph node	5	Faecal swab	2016	1,767	MG846658
Group 1	SA7-BG	Queestown	Respiratory distress	7	Faecal swab	2016	1,767	MG846659
	SA8-BH	Queestown	Wasting, Swollen lymph node	7	Faecal swab	2016	1,767	MG846660
	SA9-BI	Queestown	Dermatitis	6	Faecal swab	2016	1,767	MG846661
	SA10-BJ	Queestown	Wasting	12	Faecal swab	2016	1,767	MG846662
	SA11-BK	Queestown	Respiratory distress	11	Faecal swab	2016	1,767	MG846663
	SA12-BL	Queestown	No symptom	10	Faecal swab	2016	1,767	MG846664
Group 2	SA3-BC	Alice	No symptom	8	Faecal swab	2015	1,767	MG846655
	SA1-DA	Whittlesea	Swollen lymph node	5	Faecal swab	2016	1,767	MG846665
Group 3	SA2-DB	Whittlesea	Wasting,	5	Faecal swab	2016	1,767	MG846666
	SA3-DC	Tsolo	Wasting	6	Blood	2015	1,767	MG846667
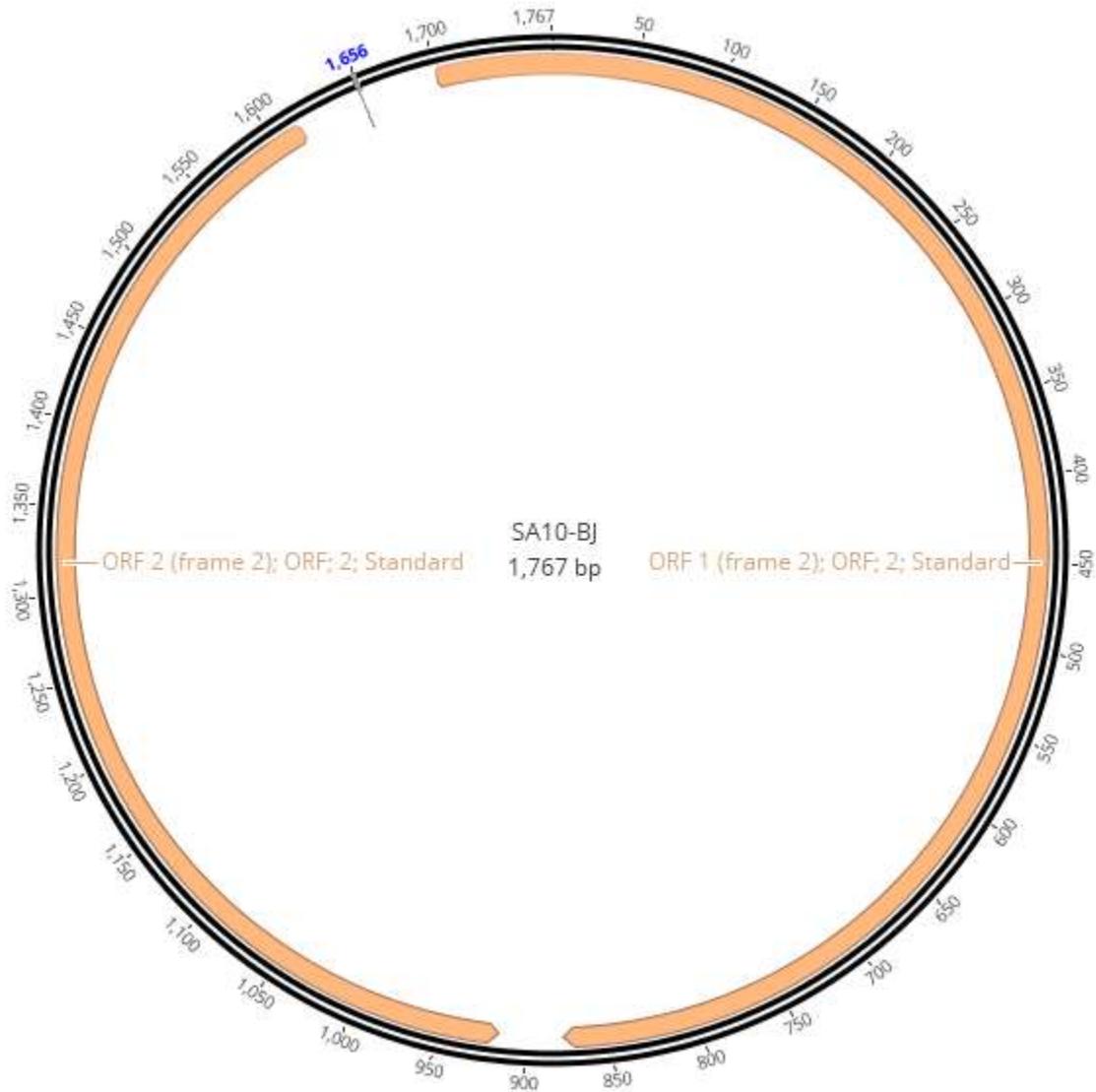
Figure 5.1: Typical PCV2 complete genome assembled (Strain SA10-BJ). The two major ORFs are shown with the brown arrows within the circular genome, with the ORF1 (replicase gene) in the forward direction and ORF2 (capsid gene) in the reverse direction. The full genome was assembled from four sequenced overlapping fragments with the aid of De-Novo Assembler as implemented in Geneious 10.2.3 software (Kearse *et al*., 2012).
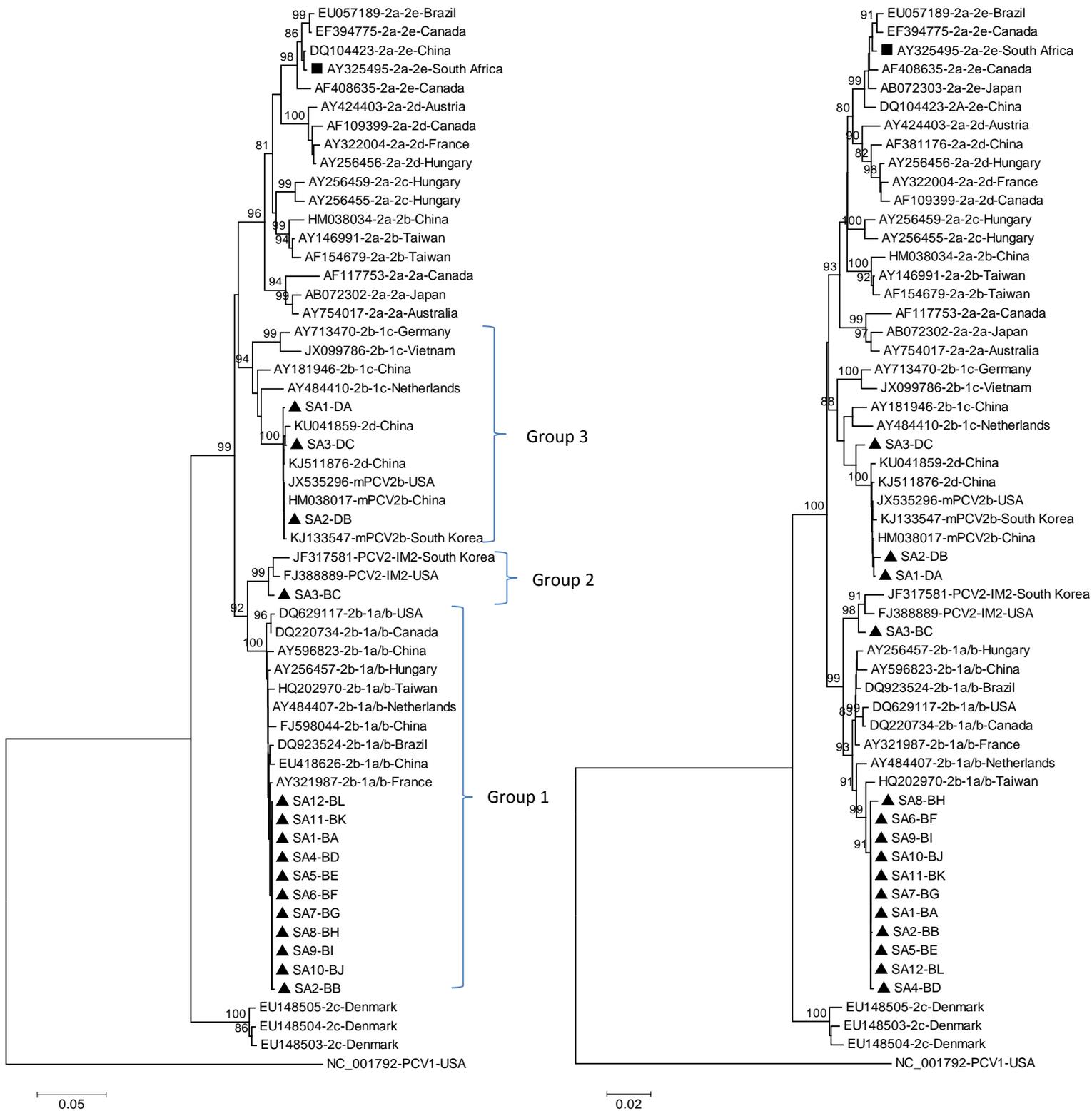
Figure 5.2: Phylogenetic analysis based on (a) ORF2 genes and (b) complete genome sequences. The tree was drawn with 15 (▲) PCV2 sequences from this study and 42 reference sequences; including the previous strain SA1 (■) and one PCV1 strain as an out-lier. GenBank accession numbers, genotypes and country of origin are indicated. Neighbour-Joining method was used and Bootstrap values ≥80% are shown.

The sequence SA1-DA, SA2-DB and SA3-DC have similarity ranging from 99.5% to 99.8% within themselves at capsid gene level. With other PCV2d reference strains, SA1-DA and SA3-DC have homology of 99.8% and 99.7% with **JX535296** (USA) and **HM038017** (China) respectively; while SA2-DB has similarity of 100% with the two reference strains (Appendix 5.2). Interestingly, one of the sequences (sole member of Group 2) from this study constantly stood aloof outside the PCV2b cluster that contains the majority of new sequences from the South African swine population with both NJ and ML phylogeny; forming a clade with other reference sequences that were recently identified as PCV2-IM2 (Xiao *et al*., 2015). The ORF2 gene of the sequence (SA3-BC) precisely has homology of 98.2% with **JF317581** (South Korea) and 98.7% with **FJ388889** from the USA; whereas with other PCV2b and PCV2d strains from this study, it has the highest similarity of 96.4% and 92.6% respectively (Appendix 5.2).

The p-distance analysis of the viral genomes and capsid gene sequences gives more insights to the initial phylogenetic findings. The sequence SA3-BC (in Group 2) has sequence divergence of 0.022 ± 0.004 and 0.037 ± 0.007 with the other eleven South African PCV2 sequences that formed same clade with reference PCV2b strains at complete genome and ORF2 levels respectively (Table 5.3). The values are slightly higher than the 0.020 and 0.035 cut off p-distance values previously stipulated for the complete genomes and capsid gene of PCV2 respectively (Segalés *et al*., 2008). Although, slightly lower values of 0.018 ± 0.003 and 0.034 ± 0.007 (for complete genome and capsid gene) were obtained between the SA3-BC and the reference PCV2b used in the analysis; however, a far much lower value of 0.010 ± 0.001 and 0.013 ± 0.002 were obtained for the complete genomes and capsid gene respectively, when compared with the reference PCV2-IM2 (Table 5.4). The eleven sequences (Group 1) that formed a cluster with reference PCV2b strains and the three sequences (Group 3) that formed a cluster with reference PCV2d

strains, showed a very small p-distance values within themselves in their group (Table 5.3); thus indicating their high degree of homology.

The alignment of the deduced amino acids of the capsid gene sequences generated showed that the PCV2 sequences in Group 1 from this study have the previously reported (Cheung *et al.*, 2007; Cheung, 2009) typical signature motif 1of S_NPR_SV (position 86-91) and signature motif 2 of AGIE (position 190-191-206-210); while the three other sequences in Group 3 displayed S_NPL_TV and TGID at the same positions of the capsid protein respectively (Figure 5.3). The standing-alone sequence, SA3-BC, however, displayed different patterns having S_NPR_SV at position 86-91 for motif 1 (atypical of PCV2b strains) and SRIE at position 190-191-206-210 for signature motif 2 instead of AGIE (Figure 5.3).

Table 5.3: Genetic divergence within and between the new South African (SA) PCV2 sequences.

New SA PCV2 Sequences (n)	Proposed Genotypes	p-distance within SA sequences (mean ± SE)		p-distance between the new SA sequences (mean ± SE)		
		Capsid gene	Complete genome	PCV2b	PCV2-IM2	PCV2d
Group 1 (11)	PCV2b	0.0003±0.0003	0.0010±0.0003	-	0.022 ± 0.004	0.037 ± 0.004
Group 2 (1)	PCV2-IM2	n/c	n/c	0.037 ± 0.007	-	0.041 ± 0.004
Group 3 (3)	PCV2d	0.0030±0.0020	.0100±0.0020	0.063 ± 0.009	0.074 ± 0.010	-

NB: n/c means not computed.

This was analyzed by the neighbor-joining method using capsid gene and complete genomes sequences with 1000 bootstrap pseudo replication (implemented in Mega 6).

Table 5.4: Genetic divergence of South African (SA) sequences and different PCV2 reference genotypes.

New SA PCV2 Sequences (n)	Between new SA sequences and other reference strains representing four main genotypes (mean±SE)							
	Capsid gene				Complete genome			
	PCV2a	PCV2b	PCV2-IM2	PCV2d	PCV2a	PCV2b	PCV2-IM2	PCV2d
	EF394775	AY321987	FJ388889	HM038017	EF394775	AY321987	FJ388889	HM038017
Group 1 (11)	0.084±0.011	0.003±0.002	0.041±0.008	0.062±0.009	0.048±0.005	0.011±0.002	0.029±0.004	0.041±0.005
Group 2 (1)	0.046±0.008	0.034±0.007	0.013±0.004	0.073±0.010	0.030±0.004	0.018±0.003	0.010±0.003	0.044±0.005
Group 3 (3)	0.103±0.012	0.060±0.009	0.077±0.011	0.001±0.001	0.054±0.005	0.039±0.005	0.047±0.005	0.006±0.001

This was determined by using the neighbour-joining method with 1000 bootstrap pseudo replication (implemented in Mega 6).

Analyses involved capsid genes and complete genome sequences from this study and selected reference strains.
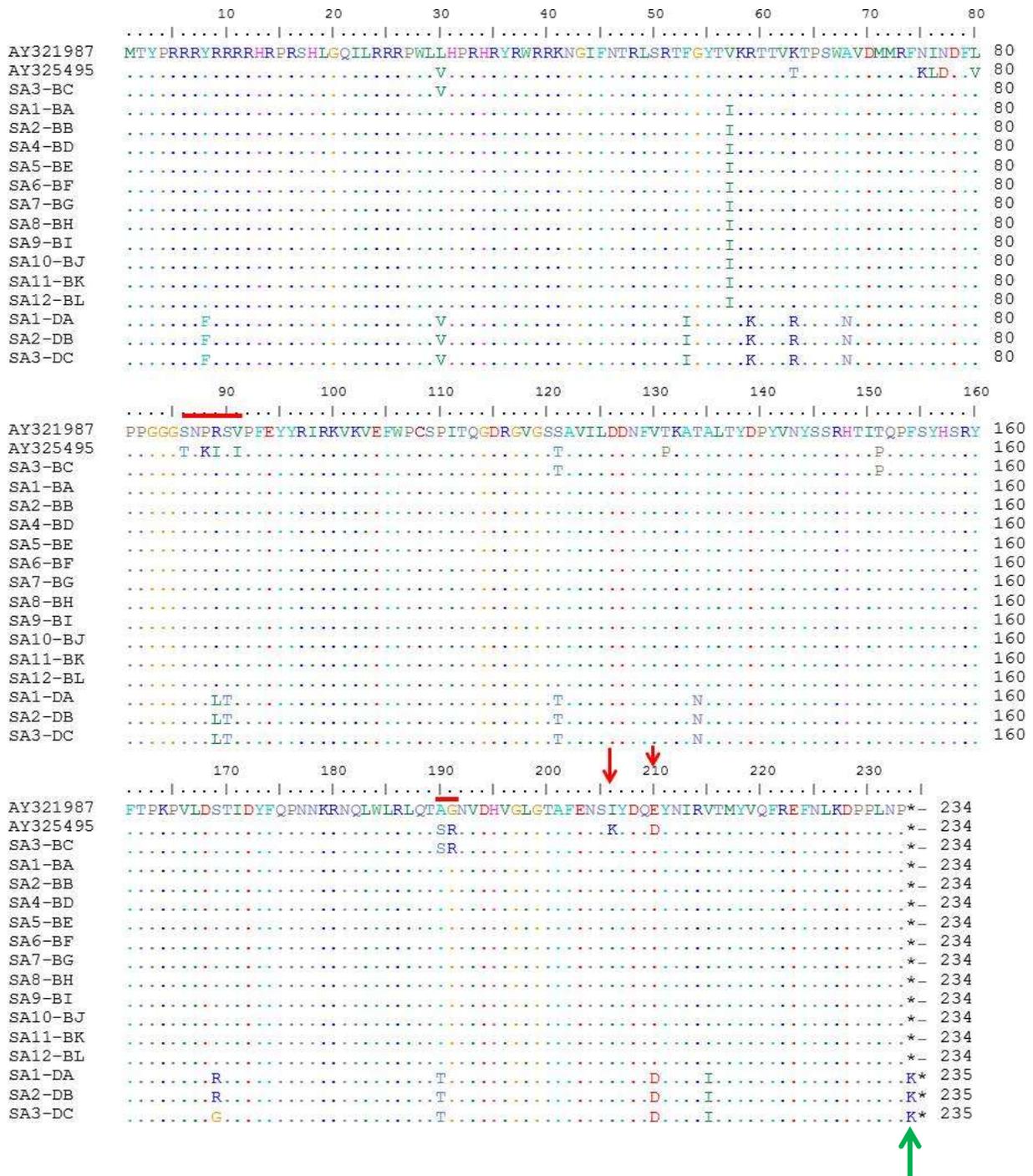
Figure 5.3: The alignment of the deduced capsid amino acid sequences of the PCV2 genomes obtained from this study and the previous strain SA1. The reference strain **AY321987** (PCV2b) from France was used as a majority sequence. The red bars and arrows are showing the distinguishing motif sequences as stipulated by Cheung (2009), while the green arrow shows the lysine (K) extension at the C terminus of mPCV2b capsid protein (Guo *et al*., 2012).

5.4 Discussion

PCV2 has become one of the most extensively investigateded viral agents of pigs globally. Since its detection in early 1990's, concerted efforts have been made all over the world to characterize the pathogen in order to solve the problem of its numerous associated diseases of high economic value to swine industry worldwide. It is however amazing that there is negligible record of PCV2 genetic resources from pigs of sub-Sahara African countries as was recently revealed (Afolabi *et al*., 2017a). Over the years, various studies which have been focused on evolution and epidemiology of PCV2 in global swine have suffered lack of reasonable number of PCV2 genomes from the region of Africa. Only one complete PCV2 genome from South Africa (strain SA1) is currently available as representative reference PCV2 genome from the African continent that could be enrolled in such various analysis globally (An *et al*., 2007; Olvera *et al*., 2007; Vidigal *et al*., 2012; Franzo *et al*., 2016).

According to Jaganathan *et al*. (2011), "publication of whole genome sequences allows researchers to trace the presence and the spread of economically important viruses, determine their genetic distribution and assist in making priority decisions for good control programs". Hence, this present effort becomes highly imperative for the purpose of characterising the complete genomes of circulating PCV2 strains in the country and to populate the viral database from the region. This study serves as a confirmation of the occurrence of other PCV2 genotypes apart from genotype 2a that was earlier detected in South African swine more than a decade ago. The outcome further corroborates findings from a recent epidemiological survey in which the presence of other PCV2 genotypes apart from the earlier detected type 2a was observed when partial PCV2 genomes were analyzed (Afolabi *et al*., 2017b).

Fifteen complete genomes of PCV2 were successfully sequenced and assembled in this study from 54 PCV2-positive archived samples obtained from six commercial and communal farms earlier described (Afolabi *et al*., 2017b). Eleven out of the fifteen sequences, belonging to Group 1 in this study, were identified as PCV2b through phylogenetic analysis. A very high nucleotide similarity was observed within them and with negligible divergence, this imply a high level of homogeneity in the PCV2 genogroup that is currently in circulation within the region. This could indicate the possibility of farm to farm transmission of the PCV2 genotypes through pig stocking or other farm management practices within the studied region.

The clustering of the new sequences and their close identity with other PCV2b reference strains from Europe, Asia, South and North America speaks volume about the origin of the viral strain. Their transmission to this region of South Africa could still be associated with the first witnessed PCV2 disease outbreak in Gauteng province that was earlier linked to semen obtained from America in 1999 by a commercial well-managed breeding facility. This is because; the strain SA1 that was obtained from the case report (Drew *et al*., 2004) had a high homology with other reference strains from America and Asia when it was characterized.

More interestingly, the genetic characterization performed on complete genomes and ORF gene of PCV2 sequences in this study showed that the three sequences in Group 3 are highly related to the mutant strains of PCV2b which now forms PCV2d genotypes together with former strains belonging to PCV2b subtype 1c (Xiao *et al*., 2015). The strains that were previously known as mPCV2b were first observed in cases of "vaccine escape" in America and Asia (Guo *et al*., 2012, Xiao *et al*., 2012; Opriessnig *et al*., 2013); but they are rapidly spreading globally (Xiao *et al*., 2015). It is however noteworthy that there is no vaccination programme currently in place

against PCV2 in Eastern Cape Province and in the entire South Africa possibly. This suggests that other factors could be responsible for the evolution of the PCV2b mutant strain in this region other than the previously known vaccine selection; this assertion however requires further study and evaluation.

Furthermore, the outcome of this study revealed the possibility of a natural inter-genotypic recombination event of PCV2 strains in swine from the region. This becomes evident with the first detection of a PCV2 sequence (SA3-BC) that constantly clustered separately from the two main clades (Group 1 and Group 3) observed among the new PCV2 sequences in this study. The genetic characterization of the sequence showed that it has higher homology with other reference PCV2-IM2 strains from South Korea and America, thereby confirming its identity as one of the intermediate strains recently identified globally (Xiao *et al*., 2015). It was observed that the SA3-BC possesses sequence motif 1 of PCV2b (SNPRSV) as identified by Cheung (2009); however, the sequence motif 2 (SRIE) within the capsid gene of the PCV2 sequence, constitutes a combination of the one that is found in the ORF2 gene of the first South African PCV2 strain SA1 (SR at position 190/191and IE (position 206/210) which is normally found in PCV2b strains (Figure 5.3). This is probably an indication of the presence of the PCV2a strain in the studied region; however, this might not have been detected due to the relatively small number of farms that were initially sampled in the previous study.

It is however noteworthy that a base by base examination of the capsid gene of the sequence SA3-BC showed that majority of the nucleotide motifs recently identified by Franzo *et al*. (2015a) for PCV2b are present in it. This further explains the reason why the p-distance value obtained between the sequence and the reference PCV2b strain of 0.018±0.003 for complete

genome and 0.034±0.007 for capsid gene as shown in Table 5.4, falls below the cut-off values of 0.020 and 0.035 for PCV2 complete genomes and capsid gene respectively (Segalés *et al*., 2008).

According to Xiao *et al*. (2015), some of the PCV2 strains presently falling within the intermediate clades may be antiquity of intermediate viral strains during the evolution of PCV2 or could represent un-natural recombinants, which could be artefacts of sequence amplification process during PCR reaction using Taq polymerase as suggested by Martin *et al*. (2011). Nevertheless, according to Huynh *et al*. (2014), recombination events are usual occurrence in natural populations of PCV2 and observable changes in some important region such as the immuno-reactive sites of PCV2 capsid gene as identified by Trible *et al*. (2011), can result to alterations in the virulence of the PCV2 strains. Hence, the genetic implication of the observed features (in terms of virulence and otherwise) of the PCV2 sequence SA3-BC from this study which is proposed to be a typical PCV2-IM2 strain requires further critical study and evaluation, much more that it was found in pigs with no symptom of PCVAD (Table 5.2).

5.5   Conclusion

The occurrence of PCV2 in the South African pigs is no more a tale but a mind boggling reality, and it calls for an urgent attention of every stakeholder within the agricultural sectors and the government. This becomes imperative considering the economic value of piggery with its numerous potentials of providing cheaper animal protein for the vast South African population and as means of livelihood for many people. This study reports the genetic characterization of first PCV2b, PCV2d and PCV2-IM2 complete genomes from South African pigs and shed more light into the diversity of PCV2 strains that could currently be in circulation within the country. Hence, a country-wide vaccination regime should be adopted with immediate effect to control

the pathogen and also to curb its wild spread. Also, there is need for further major epidemiological survey of other premises of South Africa to know the extent of the viral spread and have detailed information about the evolution and transmission of the viral pathogen within the country.

References

Afolabi, K.O., Iweriebor, B.C., Obi, L.C. and Okoh, A.I., 2017b. Molecular detection of Porcine circovirus type 2 in swine herds of Eastern Cape Province South Africa. *BMC Microbiology*, *17*(1), p.212.

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017a. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964.

An, D.J., Roh, I.S., Song, D.S., Park, C.K. and Park, B.K., 2007. Phylogenetic characterization of porcine circovirus type 2 in PMWS and PDNS Korean pigs between 1999 and 2006. *Virus Research*, *129*(2), pp.115-122.

Beach, N.M. and Meng, X.J., 2012. Efficacy and future prospects of commercially available and experimental vaccines against porcine circovirus type 2 (PCV2). *Virus Research*, *164*(1), pp.33-42.

Chae, C., 2005. A review of porcine circovirus 2-associated syndromes and diseases. *The Veterinary Journal*, *169*(3), pp.326-336.

Cheung, A.K., 2009. Homologous recombination within the capsid gene of porcine circovirus type 2 subgroup viruses via natural co-infection. *Archives of Virology*, *154*(3), pp.531-534.

Cheung, A.K., Lager, K.M., Kohutyuk, O.I., Vincent, A.L., Henry, S.C., Baker, R.B., Rowland, R.R. and Dunham, A.G., 2007. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. *Archives of Virology*, *152*(5), pp.1035-1044.

Clark, E.G., 1996. Post-weaning multisystemic syndrome. *In: Proceedings of Western Canadian Association of Swine Practitioners*, pp. 22–25.

Drew, T.W., Grierson, S.S., King, D.P., Hicks, D., Done, S., Banks, M., Neser, J.A., Evans, D.P.B. and Grimbeek, P., 2004. Genetic similarity between porcine circovirus type 2 isolated from the first reported case of PMWS in South Africa and North American isolates. *Veterinary Record*, *155*(5), pp.149–151.

Dupont, K., Nielsen, E.O., Baekbo, P. and Larsen, L.E., 2008. Genomic analysis of PCV2 isolates from Danish archives and a current PMWS case–control study supports a shift in genotypes with time. *Veterinary Microbiology*, *128*(1), pp.56-64.

Fan, S., Ku, X., Chen, F., Wang, Y., Yu, X. and He, Q., 2017. Complete Genome Sequence of a Novel Porcine Circovirus Type 3 Strain, PCV3/CN/Hubei-618/2016, Isolated from China. *Genome Announcements*, *5*(15), pp.e00100-17.

Firth, C., Charleston, M.A., Duffy, S., Shapiro, B. and Holmes, E.C., 2009. Insights into the evolutionary history of an emerging livestock pathogen: porcine circovirus 2. *Journal of Virology*, *83*(24), pp.12813-12821.

Franzo, G., Cortey, M., de Castro, A.M.M.G., Piovezan, U., Szabo, M.P.J., Drigo, M., Segalés, J. and Richtzenhain, L.J., 2015b. Genetic characterisation of Porcine circovirus type 2 (PCV2) strains from feral pigs in the Brazilian Pantanal: an opportunity to reconstruct the history of PCV2 evolution. *Veterinary Microbiology*, *178*(1), pp.158-162.

Franzo, G., Cortey, M., Olvera, A., Novosel, D., De Castro, A.M.M.G., Biagini, P., Segalés, J. and Drigo, M., 2015a. Revisiting the taxonomical classification of Porcine Circovirus type 2 (PCV2): still a real challenge. *Virology Journal*, *12*(1), p.131.

Franzo, G., Cortey, M., Segalés, J., Hughes, J. and Drigo, M., 2016. Phylodynamic analysis of porcine circovirus type 2 reveals global waves of emerging genotypes and the circulation of recombinant forms. *Molecular Phylogenetics and Evolution*, *100*, pp.269-280.

Guo, L., Fu, Y., Wang, Y., Lu, Y., Wei, Y., Tang, Q., Fan, P., Liu, J., Zhang, L., Zhang, F. and Huang, L., 2012. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. *PLoS One*, *7*(7), p.e41463.

Guo, L.J., Lu, Y.H., Wei, Y.W., Huang, L.P. and Liu, C.M., 2010. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virology Journal*, *7*(1), p.273.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In *Nucleic Acids Symposium Series, 41*(41), pp. 95-98.

Harding, J.C., 1996. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. *In: Proceedings of Western Canadian Association of Swine Practitioners*, p. 21.

Huynh, T.M.L., Nguyen, B.H., Nguyen, V.G., Dang, H.A., Mai, T.N., Tran, T.H.G., Ngo, M.H., Le, V.T., Vu, T.N., Ta, T.K.C. and Vo, V.H., 2014. Phylogenetic and phylogeographic analyses of porcine circovirus type 2 among pig farms in Vietnam. *Transboundary and Emerging Diseases*, *61*(6). pp.e25–e34.

Jaganathan, S., Toung, O.P., Yee, P.L., Yew, T.D., Yoon, C.P. and Keong, L.B., 2011. Genetic characterization of Porcine Circovirus 2 found in Malaysia. *Virology Journal*, *8*(1), p.437.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. and Thierer, T., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), pp.1647-1649.

Mankertz, A., Mankertz, J., Wolf, K. and Buhk, H.J., 1998. Identification of a protein essential for replication of porcine circovirus. *Journal of General Virology*, *79*(2), pp.381-384.

Martin, D.P., Lemey, P. and Posada, D., 2011. Analysing recombination in nucleotide sequences. *Molecular Ecology Resources*, *11*(6), pp.943-955.

Nawagitgul, P., Morozov, I., Bolin, S.R., Harms, P.A., Sorden, S.D. and Paul, P.S., 2000. Open reading frame 2 of porcine circovirus type 2 encodes a major capsid protein. *Journal of General Virology*, *81*(9), pp.2281-2287.

Olvera, A., Cortey, M. and Segalés, J., 2007. Molecular evolution of porcine circovirus type 2 genomes: phylogeny and clonality. *Virology*, *357*(2), pp.175-185.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2013. Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the US concurrently infected with PPV2. *Veterinary Microbiology*, *163*(1), pp.177-183.

Palinski, R., Piñeyro, P., Shang, P., Yuan, F., Guo, R., Fang, Y., Byers, E. and Hause, B.M., 2017. A novel porcine circovirus distantly related to known circoviruses is associated with porcine dermatitis and nephropathy syndrome and reproductive failure. *Journal of Virology*, *91*(1), pp.e01879-16.

Segalés, J., Olvera, A., Grau-Roma, L., Charreyre, C., Nauwynck, H., Larsen, L., Dupont, K., McCullough, K., Ellis, J., Krakowka, S. and Mankertz, A., 2008. PCV-2 genotype definition and nomenclature. *Veterinary Record*, *162*(26), pp.867-868.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), pp.2725-2729.

Trible, B.R., Kerrigan, M., Crossland, N., Potter, M., Faaberg, K., Hesse, R. and Rowland, R.R., 2011. Antibody recognition of porcine circovirus type 2 capsid protein epitopes after vaccination, infection, and disease. *Clinical and Vaccine Immunology*, *18*(5), pp.749-757.

Vidigal, P.M., Mafra, C.L., Silva, F.M., Fietto, J.L., Júnior, A.S. and Almeida, M.R., 2012. Tripping over emerging pathogens around the world: a phylogeographical approach for determining the epidemiology of Porcine circovirus-2 (PCV-2), considering global trading. *Virus Research*, *163*(1), pp.320-327.

Wang, F., Guo, X., Ge, X., Wang, Z., Chen, Y., Cha, Z. and Yang, H., 2009. Genetic variation analysis of Chinese strains of porcine circovirus type 2. *Virus Research*, *145*(1), pp.151-156.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2012. Complete genome sequence of a novel porcine circovirus type 2b variant present in cases of vaccine failures in the United States. *Journal of Virology*, *86*(22), pp.12469-12469.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2015. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. *Journal of General Virology*, *96*(7), pp.1830-1841.

CHAPTER SIX

Prevalence of porcine parvoviruses in some South African swine herds with background of porcine circovirus type 2 infection

(Under review in BMC Microbiology)

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Abstract

The classical porcine parvovirus is an important pathogen of reproductive disorders in pigs with a confirmed history of global distribution. The detection of many novel porcine parvoviruses has however been on the increase for the past few years, but there is a dearth of information on the occurrence and prevalence of these viruses in South Africa. Molecular detection of some known parvoviruses namely, porcine parvoviruses (PPVs) - 1, 2, 3 and 4; porcine bocavirus-like virus (PBo-likeV) and porcine bocaviruses (PBoV1/2) was carried out from 110 randomly selected archived swine samples collected in the year 2015 and 2016. Samples were drawn from previously screened and confirmed PCV2-infected farms with farm-level occurrence ranged from 5-60%. All the amplified parvoviruses' genomes were sequenced with the exception of PPV3 due to low quality amplicons obtained. Sequenced partial genomes were analysed using Neighbour-Joining phylogeny. The findings showed that all the screened parvoviruses were present as follow: PPV (29.1%), PPV2 (21.8%), PPV3 (5.5%), PPV4 (43.6%), PBo-likeV (21.8%) and PBoV1/2 (44.6%). While the PPV4 sequences were of low quality, the PBo-likeV sequences were found to be highly conserved with little or no nucleotide differences compared to the reference strains. However, other parvovirus sequences revealed varying degrees of variability. Phylogenetic analyses of PPV1, 2 and PBoV1/2 were executed with two major clades observed for each. This study reports the first epidemiological and molecular characterisation of emerging porcine parvoviruses in South African swine herds. It gives an insight into the distribution of these viral pathogens within the herds of the study area and brings to the fore, the urgent need for a large scale epidemiological survey of the circulating parvoviruses among pigs for mitigation purposes.

Keywords: Porcine parvoviruses, prevalence, South Africa

6.1   Introduction

Pig is one of the most economically viable red meat-producing livestock globally. Its economic potentials are pivoted on some of its unique features compared to other farm animals. It has a very short life cycle and high feed to flesh conversion rate. Its fecundity is second to none, as a sow can farrow twice in a year with average litters ranging from 6-8 per time. Despite the huge fertility potential, swine infertility infectious pathogens of economic importance are numerous. Classical porcine parpovirus type 1 (PPV1) is one of the most important infertility pathogen of swine, with its widely established reproductive disorders acronymed: SMEDI, meaning "stillbirth, mummification, embryonic death and infertility" (Mengeling *et al*., 2000; Truyen and Streck, 2012).

The virus belongs to the genus *Parvovirus* of the family *Parvoviridae*. The family consists of two subfamilies namely: *Densovirinae* (consist a group of arthropods infecting viruses) and *Parvovirinae* (a group of viruses that infect vertebrates). In a latest proposed classification, the sub-family *Parvovirinae* consists of eight genera with the addition of a lot of new species, viruses and their variants as members of the group. These include the *Amdoparvovirus, Aveparvovirus, Dependoparvovirus, Erythroparvovirus, Copiparvovirus, Bocaparvovirus, Protoparvovirus (Parvovirus)* and *Tetraparvovirus*; the last four genera are presently containing the porcine viruses (Cotmore *et al*., 2014). Parvoviruses are non-enveloped, single-stranded, linear DNA viruses with genomes size ranging from about 4-6 kb. Their genomes consist of two major open reading frames (ORFs) that code for structural (capsid) and non-structural (NS) viral proteins.

The classical porcine parvovirus belongs to the genus *Protoparvovirus* with virulent PPV-Kresse and avirulent PPV-NADL2 as the main variants in the newly proposed classification. PPV1 was

initially identified in the early 1960 (Cartwright and Huck, 1967), but presently, it is endemic in most swine-producing countries of the world; especially in herds where vaccines are not properly administered or in cases where immunosuppressing factors give rise to vaccine inefficiency (Csagola *et al*., 2012). Apart from PPV1, many other new porcine parvoviruses have been detected globally with the recent advancements in molecular techniques.

Porcine parvovirus type 2 (PPV2), otherwise called Cnvirus (CnP-PARV4), belongs to the genus *Tetraparvovirus* alongside with porcine parpovirus type 3 (PPV3 i.e. P-PARV4). It was initially detected by accident in serum of pigs from Myanmar during a hepatitis E survey (Hijikata *et al*., 2001). Subsequently, it was also detected from serum samples obtained from "feverish" and PMWS-affected pigs in China (Wang *et al*., 2010) and more recently in the USA (Xiao *et al*., 2013a). PPV3 was first detected in Hong Kong in 2008 from porcine samples including lymph nodes, serum, liver and faeces, earlier called porcine hokovirus (PHoV). It was further analysed alongside with bovine hokovirus (BHoV) and were found to be closely related to human parvovirus 4 (PARV4) (Lau *et al*., 2008). The virus is thought to have universal distribution due to its detection in different parts of the world (Csagola *et al*., 2012).

Also, porcine parvovirus type 4 (PPV4) is currently grouped together with bovine parvovirus 2 under the genus *Copiparvovirus*. It was detected for the first time in 2005 from swine herds with porcine circovirus type 2 (PCV2) infection and clinical signs of porcine circovirus associated disease (PCVAD) at North Carolina, USA; having high resemblance with viruses in the genus *Bocavirus* such as human bocavirus with an additional ORF3 gene in between the ORFs 1 and 2 (Cheung *et al*., 2010). Recently, through metagenomics, more novel but unclassified porcine parvoviruses have been detected and tentatively designated as PPV5 in the USA (Xiao *et al*., 2013b); PPV6 in North America (Schirtzinger *et al*., 2015) and Poland (Cui *et al*., 2017); and

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PPV7 from rectal swabs of pigs from the US (Palinski *et al*., 2016) and more recently in China (Xing *et al*., 2018).

Other classified emerging porcine parvoviruses are grouped under the genus *Bocaparvovirus* together with human bocaviruses among others. In 2009, a porcine boca-like virus (PBo-likeV) was first detected in Sweden from the lymph nodes of pigs with clinical manifestation of PMWS (Blomstrom *et al*., 2009). Ever since then, many other porcine bocaviruses with a very low similarity to the Swedish strain have been identified in eleven countries of the world belonging to four continents namely: Europe, North America, Asia and Africa (Zhou *et al*., 2014). This includes PBoV1/2, 6V and 7V from China (Cheng *et al*., 2010), PBoV3/4 from Northern Ireland and China (McKillen *et al*., 2011; Lau *et al*., 2011) and PBoV5 from China (Li *et al*., 2012).

In Africa, porcine bocaviruses were detected for the first time in two different studies simultaneously in Uganda (Blomstrom *et al*., 2013) and Cameroon (Ndze *et al*., 2013). The former in their study, screened for only the Swedish porcine bocavirus strain (i.e. PBo-likeV) from 95 serum samples from Uganda; whereas the latter carried out a relatively extensive study on the occurrence and genetic diversity of some selected DNA viruses from faecal samples of asymptomatic pigs in Cameroon, although with relatively small samples. Prevalence of novel parvovirus PPV4 was determined to be 20% and some porcine bocaviruses were PBoV3 (18%), PBoV4 (18%), PBoV5 plus 6V/7V (16%), and PBoV1/PBoV2 (6%) among the Cameroonian pigs.

In South Africa, the first reported case of PPV1 was in 1975 from an outbreak of reproductive failure in a piggery in which the virus was successfully isolated and implicated (Pini, 1975). The virus was subsequently identified on a number of occasions as the cause of similar problems, and this prompted further investigations by Prozesky *et al*. (1980) in which validating in-vivo

experiments were made to study the effect of the local strain of PPV1 on the foetus and pregnant sow in order to compare their results with previous reports relating to the pathology of the virus. Their findings showed that *in-utero* inoculation of sows at various stages of gestation with the local strain of PPV1 brought about resorption, abortion or farrowing of weak, dead or mummified foetuses, all of which are typical reproductive disorders associated with PPV1 infection (Prozesky *et al*., 1980). Despite these insightful findings that are expected to trigger large scale epidemiological study to know the extent of the spread of the virus in the country, nothing has been done to that effect on even the old classical parvovirus which has been established to be the culprit in swine reproductive failure and one of the important cofactors of PCVAD. Although the actual pathogenicity mechanisms of most newly detected porcine parvoviruses have not been well explained, however, their detection has also been reported to be common in PCVAD cases (Opriessnig *et al*., 2014). This present study, therefore, is aimed at determining the prevalence of emerging and re-emerging porcine parvoviruses in a South African swine population with a confirmed background of PCV2 infections.

6.2   Materials and methods

6.2.1   Samples description and DNA extraction

To study the prevalence of some designated porcine parvoviruses in a South African swine population with a confirmed background of PCV2 infection (with farm level occurrence of about 6 to 60%) according to a previous study (Afolabi *et al*., 2017a), a total of 110 samples were randomly selected from archived samples collected from healthy and sick pigs with clinical symptoms related to PCVAD; from some communal and commercial farms in Eastern Cape Province in the year 2015 and 2016. Previously, a molecular epidemiological survey for the detection of PCV2 was carried out to ascertain the actual status of pigs in the region as regarding

PCV2 and associated diseases. Viral DNA was extracted from the processed samples by using a Quick-DNA™ Universal Kit (Zymo Research, California USA) with strict compliance to the manufacturer's guidelines. Extracted DNA was constantly maintained at -20 °C when not in use.

6.2.2 PCR amplification of viral DNA and sequencing

The nucleic acids of different porcine parvoviruses were detected and amplified using conventional PCR procedures according to Cságola *et al*. (2012) with slight modifications. Briefly, designed primer according to Ogawa *et al*. (2009) was used for PPV1 whereas primers for other parvoviruses were adopted from Cságola *et al*. (2012) and Zhai *et al*. (2010) (Table 6.1). The specific PCR methods for each of the viruses were carried out as previously described (Cságola *et al*., 2012) with some modifications. The PCR mixture contained 2 μl of each primer (25pM), 0.25 μl of GoTaq®G2 Flexi DNA polymerase (5U/μl), 10 μl of 5X Green GoTaq®G2 Flexi Buffer, 6 μl of 25mM $MgCl_2$ solution (Promega, Madison USA), 1 μl of a PCR nucleotide mix (10mM each) and 5 μl of the DNA template; nuclease-free water was added to make-up 50 μl reaction mixture. Optimized annealing temperatures ranging from 50 to 55 °C were used as thermal cycler condition for the viral DNA amplification. All the PCR products were analyzed by electrophoresis on 1.5% Agarose gels stained with ethidium bromide and visualized using an Alliance 4.7 transilluminator (UVitec, Cambridge, UK).

Table 6.1: Oligonucleotide primers used for the amplification and sequencing parvoviruses partial genomes

Primer identity	Primer sequence	Amplicon size (bp)	Virus type/Region amplified	Reference
PPV1F	5'-CACAGAAGCAACAGCAATTAGG-3'	203	PPV1/ORF2	Ogawa *et al.*, 2009
PPV1R	5'-CTAGCTCTTGTGAAGATGTGG-3'			
PPV2AF	5'-ACACGATGAGCGGTACGA-3'	279	PPV2/ORF2	Csagola *et al.*, 2012
PPV2AR	5'-TCCTCACGAGGTCTCTTCTG-3'			
PPV3DF	5'-GCAGTCTGCGCTTAACTT-3'	392	PPV3/ORF2	Csagola *et al.*, 2012
PPV3DR	5'-CTGCTTCATCCACTGGTC-3'			
PPV4DF	5'-TCATAGCACTATGGCGAGC-3'	284	PPV4/ORF2	Csagola *et al.*, 2012
PPV4DR	5'-AGCATTCTGCGTTGGACA-3'			
SbocaF	5'-GGGCGAGAACATTGAAGAGGT-3'	495	PBo-likev/ORF2	Zhai *et al.*, 2010
SbocaR	5'-TTGTGAGTATGGGTATTGGTG-3'			
PBoVF	5'-TGGTGGAACGTCTCTCTGACA-3'	466	PBoV1 and 2/ NS1 & NP1	Csagola *et al.*, 2012
PBoVR	5'-GAGTCATTCGGTCTCCTCCAT-3'			

6.2.3 *Nucleotide sequencing and analyses*

The amplified partial genomes of the parvoviruses were sequenced using the same primer pairs as used for the PCR amplification at a commercial DNA sequencing facility at Stellenbosch University, South Africa. Sequenced viral DNA were edited, blasted, aligned (ClustalW) and subjected to nucleotide sequence identity analysis using Geneious 11.0.2 (Kearse *et al*., 2012). Phylogenetic analyses were conducted using the neighbor joining method as implemented in Mega 6 (Tamura *et al*., 2013). The GenBank Accession numbers of the parvovirus sequences obtained in this study and other reference sequences from the NCBI GenBank used in the phylogenetic analyses and other molecular characterizations are provided in Appendix 6.1.

6.3   Results

6.3.1 *Occurrence and prevalence of porcine parvoviruses in pigs*

In this study, the occurrence and prevalence of seven porcine parvoviruses were determined using PCR technique. Exactly 110 samples were randomly selected from 339 archived samples obtained from six swine herds of the Province in 2015 and 2016. Initially, the farm level occurrence of PCV2 in the swine herds was confirmed to be about 6 to 60% (Afolabi *et al*., 2017a). All the screened parvoviruses were present in the samples and their prevalence ranges from 5.5% in PPV3 to 44.6% in PBoVs (Table 6.2). A double infection of parvoviruses was observed to be rampant, as high as 20/110 (18.2%) of the screened pig samples for PPV2/PPV4 and PPV4/PBoV; keenly followed by 19/110 (17.3%) of the samples for PPV1/PPV4 and PPV1/PBoV (Table 6.3). Exactly 19/110 (17.3%) of the samples were positive for three of the viruses simultaneously, whereas 8/110 (7.3%) samples were positive for four of the viruses.

Table 6.2: The prevalence of porcine parvoviruses in swine herds of the Eastern Cape Province

Virus type	Number of positive / Total sample	Prevalence
PPV1	32/110	29.1%
PPV2	24/110	21.8%
PPV3	6/110	5.5%
PPV4	48/110	43.6%
PBo-likeV	24/110	21.8%
PBoV1 and PBoV2	49/110	44.6%

Table 6.3: The summary of double infections in the screened samples

Virus type	PPV1	PPV2	PPV3	PPV4	PBo-likeV	PBoV
PPV1	32					
PPV2	3	24				
PPV3	2	2	6			
PPV4	19	20	2	48		
PBo-likeV	5	5	1	13	24	
PBoV	19	8	5	20	12	49

6.3.2 *Genetic analyses of the sequenced genomes*

Sequenced PCR products of the screened parvoviruses yielded partial ORF2 genomes of the viruses as previously stated (Csagola *et al*., 2012). However, with the exception of PBoV in which the primer used amplified partial NS1 and NP1 genes of PBoV1 and PBoV2 as observed when aligned with other reference sequences from the GenBank using the Geneious software. Amplicons of the only six positive samples of PPV3 were of low quality when analyzed through agarose gel electrophoresis; hence, not subjected to sequencing. Sequenced partial genomes of PPV4 also yielded chromatogram of low quality and therefore excluded from further analysis. Thirteen PPV1 partial VP2 genomes were obtained in this study, having nucleotide sequence identity ranging from 98.5 – 100% within themselves and 97.3–100% with other reference sequences. The phylogenetic analysis of the sequences and other references formed two distinct clades in which all the PPV1 sequences from this study grouped together in clade 1 with virulent PPV-Kresse strain while the NADL-2 strains formed a separate cluster (Figure 6.1A).

Four PPV2 sequences from this study clustered separately with other PPV2 reference sequences from China and Japan, precisely having a relatively close branching with strain JPT68 from Japan in clade 2 apart from the original PPV2 strain H-1 from Myanmar in clade 1(Figure 6.1B). They have sequence homology of 98.2-99.3% with themselves and 91.9-97.5% with other reference PPV2 sequences from the GenBank. Six amplified and sequenced PBo-likeV sequences from this study also showed a high level nucleotides sequence similarity with each other (99.4-100%) and with other reference sequences (99.0-99.8%) as earlier noted by Csagola (2012). However, all of them possessed another nucleotide sequence difference of Cytosine instead of Guanine at position 3965 when aligned with the whole genome of the Chinese reference strain SX (**HQ223038**)
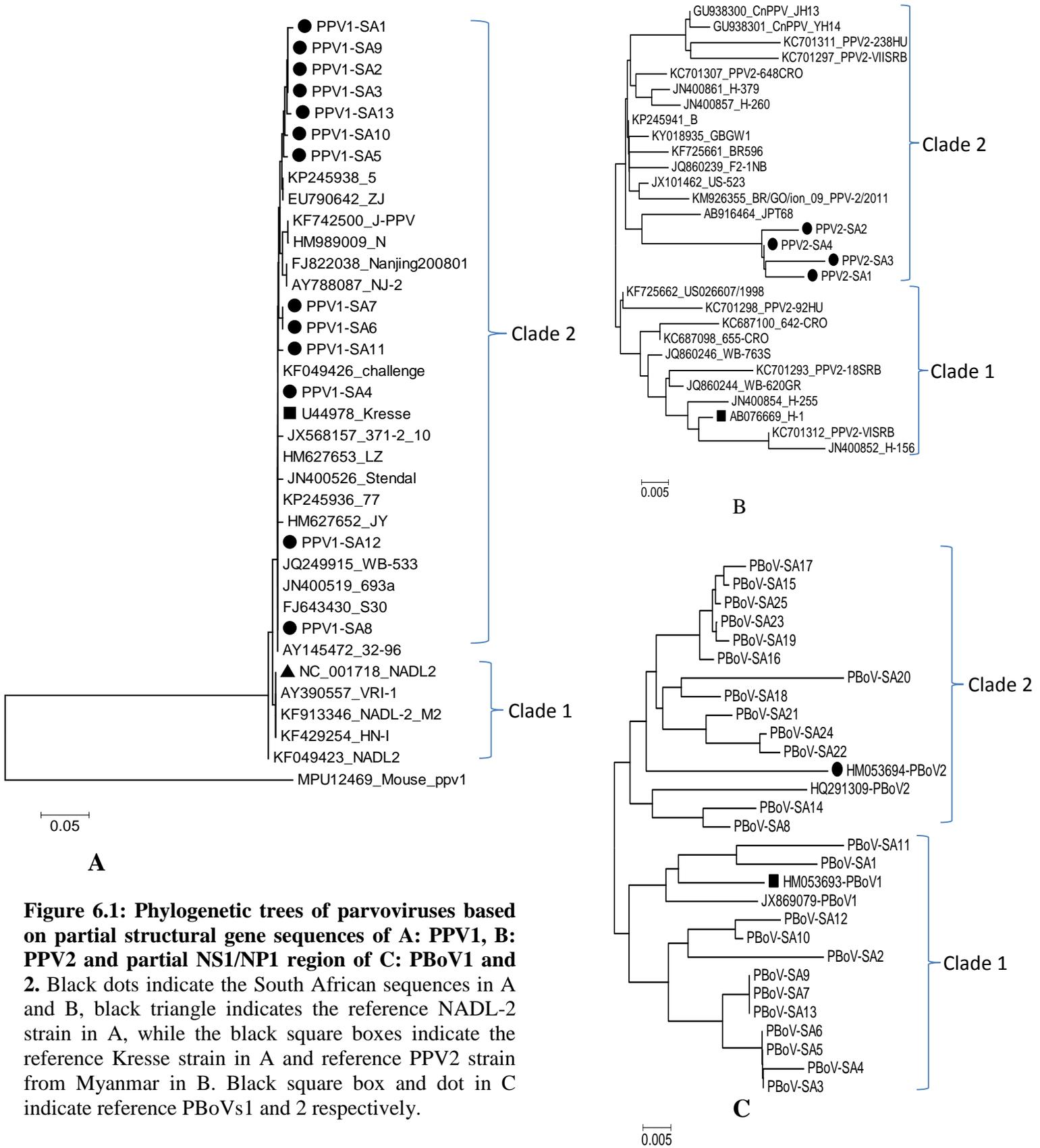
Figure 6.1: Phylogenetic trees of parvoviruses based on partial structural gene sequences of A: PPV1, B: PPV2 and partial NS1/NP1 region of C: PBoV1 and 2. Black dots indicate the South African sequences in A and B, black triangle indicates the reference NADL-2 strain in A, while the black square boxes indicate the reference Kresse strain in A and reference PPV2 strain from Myanmar in B. Black square box and dot in C indicate reference PBoVs1 and 2 respectively.

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Furthermore, two distinct clades were also observed in the phylogenetic analysis of 25 PBoVs sequences in this study; of which 12 sequences clustered together with PBoV1 reference sequence from China (**HM053693**). The remaining thirteen sequences grouped together in the clade 2 with the reference PBoV2 sequence, thereby giving the South African porcine bocavirus sequences a kind of preliminary grouping (Figure 6.1C). Two clustering patterns were equally noticed within each clade that was formed. In clade 1, PBoV-SA1 and PBoV-SA11 clustered separately with the reference PBoV1 from China and the Cameroonian strain (**JX869079**); while the remaining ten sequences (PBoV-SA2 to PBoV-SA7, PBoV-SA9 to PBoV-SA10 and PBoV-SA12 to PBoV-SA13) in the clade 1 clustered separately. Moreover in the clade 2, two sequences (PBoV-SA8 and PBoV-SA14) grouped separately with Chinese strain A6 (**HQ291309**); whereas the remaining eleven sequences clustered separately with the PBoV2 reference strain (**HM053694)** (Figure 6.1C)**.**

6.4   Discussion

Timely detection and the molecular profiling of pigs infectious pathogens has become imperative for optimum profitability in any serious pig-producing nations of the world; most especially as the inevitable global swine business has been grossly implicated in the transmission of infectious diseases all over the world. Despite the huge potentials of the business in meeting hunger and unemployment challenges in developing countries, stakeholders are expected to be resilient in combating various challenges that face the business; especially those from numerous associated infectious diseases. Undoubtedly, effective surveillance on the emerging and re-emerging swine pathogens is paramount for economic sustainability, stability and growth of the business. Thus, this current investigation into the occurrence and prevalence of porcine parvoviruses in a South African swine population cannot be overemphasized, being the first of its kind in the Republic.

In this study we reported a relatively high occurrence and the prevalence of porcine parvoviruses in swine herds of one of the provinces of South Africa where communal pig farming is common. This is not surprising as our previous findings have earlier established high prevalence of PCV2 in the swine population. Also, the attendant negligence on the prevention and control as a result of non-availabilty of large scale vaccination programme in the country has become evident (Afolabi *et al.*, 2017a, b). In many occasions, parvoviruses' infections and infiltrations have been implicated in swine herds with background history of PCV2 infections; this probably could be attributable to immunosuppressive potential of the virus making the host vulnerable to multiple infections (Saekhow and Ikeda, 2015). Most of the reported cases of classical porcine parvovirus infections and other novel porcine parvoviruses have been coincident in PWMS and other PCVADs (Opriessnig *et al.*, 2014). Although most of the amplified, sequenced and analyzed PPVs sequences in this study are relatively small, however, they provide preliminary insights into the viral strains and species presently in circulation within the region and the extent of their occurrence.

In the present study, all the PPV1 sequences clustered with the virulent strain Kresse as observed in the phylogenetic analysis. This could be the reason why there were observable clinical manifestations of PMWS in some of the sampled farms as previously established (Afolabi *et al.*, 2017a, b). PPV1 is a confirmed co-infecting pathogens with PCV2 in many PMWS cases both on the field and experimentally (Kennedy *et al.*, 2000; Csagola *et al.*, 2012). Although a commercial breeding facility which was one of the sampled farms in the previous study claimed vaccination of their pigs against PPV1, however, some of the samples from their weaners and growers were positive for the virus. This could be as a result of ineffective vaccination regime. The present detection of PPV1 was not new in the South African swines; Pini as far back as 1975

reported the first outbreak of reproductive failure due to PPV1 infection in the country. It is therefore highly imperative that further epidemiological study should be done most especially on samples from sows with observable reproductive disorders from the area and other premises of South Africa. This will help in re-confirming its probable roles in cases of reproductive failures (apart from PCVADs) in pigs from the country as earlier observed (Pini, 1975).

The prevalence of PPV2 (21.8%) observed in the present investigation is notable as it falls between the overall global range of 1.8-55% in faeces, sera, lung and other organs of domestic pigs and wild boars as stated by Xiao *et al*. (2013c). Higher prevalence of PPV2 has however been observed in tonsils samples of pigs from Germany (78%) (Streck *et al*., 2013), Thailand (83%) (Saekhow and Ikeda, 2015) and Japan healthy pigs (58%) and sick pigs (100%) (Saekhow *et al*., 2014); thereby creating the notion that the observed differences in prevalence may be related to the type of organs assayed. Though the initial detection of PPV2 was accidental during a survey for hepatitis E virus in Myanmar (Hijikata *et al*., 2001), it has been subsequently detected in pigs with Porcine Respiratory and Reproductive Syndrome and PCV2 associated diseases in China and North America, which is in agreement with this present report (Wang *et al*., 2010; Opriessnig *et al*., 2014). Phylogenetic analysis on PPV2 sequences from this study and reference sequences from GenBank also showed two major clades; having the four PPV2 sequences clustering directly with strain JPT68 (**AB916464**) from Japan in the clade 2 together with Chinese strain CnPPVs that were obtained from pigs with high fever symptoms and PCVAD. Despite these, the role of PPV2 in the presently associated diseases still requires proper diagnostic investigations (Saekhow *et al*., 2014).

In the present investigation, PPV3 has the least prevalence (5.5%) and of low quality amplicons. The virus is known for its closer identity of 61.5-63% to human PARV4 (Lau *et al*., 2008), and

its overall prevalence according to Xiao *et al*. (2013c) varies from 3.1-44.4% in sera, lung, lymph nodes, nasopharyngeal, feces and other organ from pigs. More recently, the virus was detected in 58 of the 80 tonsils samples in Thailand and has been suggested alongside with PPV2, to have higher detection rate in tonsils (Saekhow and Ikeda, 2015); which could be responsible for its low detection in this study. Moreover, lower detection could be as a result of age of the screened pigs, as higher detection has also been attributed with age. According to Csagola *et al*. (2012), higher positivity has been detected in adult swine rather than in pigs of less than one year old. PPV4 obtained is equally high, though low quality sequences generated debarred further genetic characterization of strains in circulation in the studied area. Also, the virus was first detected from cases of PMWS-affected pigs in America (Cheung *et al*., 2010) as is the case in this study; subsequently in healthy and sick pigs in China (Huang *et al*., 2010) and more recently in asymptomatic pigs in Cameroon where prevalence of 20% was reported (Ndze *et al*., 2013). Though its global prevalence ranges from 0.76-10% according to Xiao *et al*. (2013c), a relatively higher value obtained follows the same pattern with a more recent detection of 44% (41/80) in Thai swine herds (Saekhow and Ikeda, 2015).

Sequel to the detection of first swine bocavirus (PBo-likeV) in Swedish pigs with background of PMWS about a decade ago (Blomstrom *et al*., 2009), several other genetically different porcine bocaviruses from PBo-likeV have been characterized from swine faeces in China and different part of the world through advancements in molecular epidemiology. In this study, the possible presence and prevalence of Swedish type porcine bocavirus in South African swine population was determined; and also, PBoV1 and PBoV2 that were subsequently detected a year after in Chinese pigs (Cheng *et al*., 2010). Earlier, PBo-likeV strain with 99% nucleotide sequence identity with the available 1890-nt region of Swedish strains was obtained in Ugandan pigs

which happened to be the first to be detected in Africa (Blomstrom *et al.*, 2013). The sequences from this study also have sequence identity of 99.4-99.8% with the Swedish reference strain and 99-99.6% with the Ugandan strain. Very low detection of the virus, about 2% (2/98), was observed in the study compared to this present study where prevalence of 21.8% (24/110) was obtained, which is comparable to 18% (23/80) that was obtained more recently in Thai pigs (Saekhow and Ikeda, 2015).

The PBoV1 and PBoV2 in this study have the highest prevalence of 44.6% and the phylogenetic analysis of the 25 sequences obtained gave insightful information about the distribution of the viruses in the South African swine herds. It was observed that twelve sequences from two district municipalities clustered with reference PBoV1 forming clade 1; whilee two sequences from the same region (PBoV-SA8 and PBoV-SA14) clustered with reference sequence PBoV2 (HQ291309) in clade 2 (Figure 6.1C). Other sequences from this study in clade 2 (PBoV-SA15 to PBoV-SA25) were incidentally obtained from pigs in the third district municipality of the studied location. This implies that the two groups of virus were transmitted to the province through different routes possibly; however, the clustering of two sequences from the other two district municipalities with those from the third district municipality in PBoV2 group requires further investigation with larger number of samples. This will help to ascertain whether the two groups (PBoVs1 and 2) were originally circulating within the two district municipality or recently introduced from the third district municipality.

6.5   Conclusion

This study reports the first major epidemiological study on the occurrence and prevalence of novel porcine parvoviruses in South African pigs. It gives preliminary insight into the presence

of the heterogeneous viral group in swine herds of the country. However, a larger epidemiological study is required to ascertain the extent of spread in the country. Also, future efforts should be geared towards isolating and sequencing the whole genomes of the viruses for adequate genetic characterization and selection of effective vaccines and vaccination regimes to curb their spread and control their presence. Furthermore, their isolation will facilitate thorough *in-vitro* and *in-vivo* studies on the pathogenicity of the viral pathogens in the South African swine and contribute to the global knowledge about the clinical relevance of most of the parvoviruses not currently identified globally.

References

Afolabi, K.O., Iweriebor, B.C., Obi, L.C. and Okoh, A.I., 2017a. Molecular detection of Porcine circovirus type 2 in swine herds of Eastern Cape Province South Africa. *BMC Microbiology*, *17*(1), p.212.

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017b. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964.

Blomström, A.L., Belák, S., Fossum, C., McKillen, J., Allan, G., Wallgren, P. and Berg, M., 2009. Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. *Virus Research*, *146*(1), pp.125-129.

Blomström, A.L., Ståhl, K., Okurut, A.R., Masembe, C. and Berg, M., 2013. Genetic characterisation of a porcine bocavirus detected in domestic pigs in Uganda. *Virus Genes*, *47*(2), pp.370-373.

Cartwright, S.F. and Huck, R.A., 1967. Viruses isolated in association with herd infertility absortions and stillbirths in pigs. *Veterinary Record*, *81*(8), pp.196-197.

Cheng, W.X., Li, J.S., Huang, C.P., Yao, D.P., Liu, N., Cui, S.X., Jin, Y. and Duan, Z.J., 2010. Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PloS One*, *5*(10), p.e13583.

Cheung, A.K., Wu, G., Wang, D., Bayles, D.O., Lager, K.M. and Vincent, A.L., 2010. Identification and molecular cloning of a novel porcine parvovirus. *Archives of Virology*, *155*(5), pp.801-806.

Cotmore, S.F., Agbandje-McKenna, M., Chiorini, J.A., Mukha, D.V., Pintel, D.J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D. and Davison, A.J., 2014. The family parvoviridae. *Archives of Virology*, *159*(5), pp.1239-1247.

Cságola, A., Lőrincz, M., Cadar, D., Tombácz, K., Biksi, I. and Tuboly, T., 2012. Detection, prevalence and analysis of emerging porcine parvovirus infections. *Archives of Virology*, *157*(6), pp.1003-1010.

Cui, J., Fan, J., Gerber, P.F., Biernacka, K., Stadejek, T., Xiao, C.T. and Opriessnig, T., 2017. First identification of porcine parvovirus 6 in Poland. *Virus Genes*, *53*(1), pp.100-104.

Hijikata, M., Abe, K., Win, K.M., Shimizu, Y.K., Keicho, N. and Yoshikura, H., 2001. Identification of new parvovirus DNA sequence in swine sera from Myanmar. *Japanese Journal of Infectious Diseases*, *54*(6), pp.244-245.

Huang, L., Zhai, S.L., Cheung, A.K., Zhang, H.B., Long, J.X. and Yuan, S.S., 2010. Detection of a novel porcine parvovirus, PPV4, in chinese swine herds. *Virology Journal*, *7*(1), p.333.

Kearse, M., Moir, R., Wilson, A., Stones-Havas, S., Cheung, M., Sturrock, S., Buxton, S., Cooper, A., Markowitz, S., Duran, C. and Thierer, T., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics*, *28*(12), pp.1647-1649.

Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S. and Allan, G.M., 2000. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *Journal of Comparative Pathology*, *122*(1), pp.9-24.

Lau, S.K., Woo, P.C., Tse, H., Fu, C.T., Au, W.K., Chen, X.C., Tsoi, H.W., Tsang, T.H., Chan, J.S., Tsang, D.N. and Li, K.S., 2008. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *Journal of General Virology*, *89*(8), pp.1840-1848.

Lau, S.K., Woo, P.C., Yip, C.C., Li, K.S., Fu, C.T., Huang, Y., Chan, K.H. and Yuen, K.Y., 2011. Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family Parvoviridae, and evidence for inter-and intra-host genetic diversity and recombination. *Journal of General Virology*, *92*(9), pp.2047-2059.

Li, B., Ma, J., Xiao, S., Fang, L., Zeng, S., Wen, L., Zhang, X., Ni, Y., Guo, R., Yu, Z. and Zhou, J., 2012. Complete genome sequence of a novel species of porcine bocavirus, PBoV5. *Journal of Virology*, *86*(2), pp.1286-1287.

McKillen, J., McNeilly, F., Duffy, C., McMenamy, M., McNair, I., Hjertner, B., Millar, A., McKay, K., Lagan, P., Adair, B. and Allan, G., 2011. Isolation in cell cultures and initial characterisation of two novel bocavirus species from swine in Northern Ireland. *Veterinary Microbiology*, *152*(1), pp.39-45.

Mengeling, W.L., Lager, K.M. and Vorwald, A.C., 2000. The effect of porcine parvovirus and porcine reproductive and respiratory syndrome virus on porcine reproductive performance. *Animal Reproduction Science*, *60*, pp.199-210.

Ndze, V.N., Cadar, D., Cságola, A., Kisfali, P., Kovács, E., Farkas, S., Ngu, A.F., Esona, M.D., Dán, Á., Tuboly, T. and Bányai, K., 2013. Detection of novel porcine bocaviruses in

fecal samples of asymptomatic pigs in Cameroon. *Infection, Genetics and Evolution*, *17*, pp.277-282.

Ogawa, H., Taira, O., Hirai, T., Takeuchi, H., Nagao, A., Ishikawa, Y., Tuchiya, K., Nunoya, T. and Ueda, S., 2009. Multiplex PCR and multiplex RT-PCR for inclusive detection of major swine DNA and RNA viruses in pigs with multiple infections. *Journal of Virological Methods*, *160*(1), pp.210-214.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2014. Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. *Veterinary Microbiology*, *173*(1), pp.9-16.

Palinski, R.M., Mitra, N. and Hause, B.M., 2016. Discovery of a novel Parvovirinae virus, porcine parvovirus 7, by metagenomic sequencing of porcine rectal swabs. *Virus Genes*, *52*(4), pp.564-567.

Pini, A., 1975. Porcine parvovirus in pig herds in Southern Africa. *Journal of the South African Veterinary Association*, *46*(3), pp.241-244.

Prozesky, L., Thomson, G.R., Gainaru, M.D., Herr, S. and Kritzinger, L.J., 1980. Lesions resulting from inoculation of porcine foetuses with porcine parvovirus. *The Onderstepoort Journal of Veterinary Research*, *47*(4), pp.269-274.

Saekhow, P. and Ikeda, H., 2015. Prevalence and genomic characterization of porcine parvoviruses detected in Chiangmai area of Thailand in 2011. *Microbiology and Immunology*, *59*(2), pp.82-88.

Saekhow, P., Mawatari, T. and Ikeda, H., 2014. Coexistence of multiple strains of porcine parvovirus 2 in pig farms. *Microbiology and Immunology*, *58*(7), pp.382-387.

Schirtzinger, E.E., Suddith, A.W., Hause, B.M. and Hesse, R.A., 2015. First identification of porcine parvovirus 6 in North America by viral metagenomic sequencing of serum from pigs infected with porcine reproductive and respiratory syndrome virus. *Virology Journal*, *12*(1), p.170.

Streck, A.F., Homeier, T., Foerster, T., Fischer, S. and Truyen, U., 2013. Analysis of porcine parvoviruses in tonsils and hearts from healthy pigs reveals high prevalence and genetic diversity in Germany. *Archives of Virology*, *158*(6), pp.1173-1180.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution*, *30*(12), pp.2725-2729.

Truyen, U. and Streck, A. F., 2012. Porcine parvovirus. In: Zimmerman, J. K., Karriker, L., Ramirez, A., Schwartz, K. and Stevenson, G. 10th Eds., *Diseases of Swine*, John Wiley and Sons, Chichester, UK, pp.447–455.

Wang, F., Wei, Y., Zhu, C., Huang, X., Xu, Y., Yu, L. and Yu, X., 2010. Novel parvovirus sublineage in the family of Parvoviridae. *Virus Genes*, *41*(2), pp.305-308.

Xiao, C.T., Gerber, P.F., Giménez-Lirola, L.G., Halbur, P.G. and Opriessnig, T., 2013a. Characterization of porcine parvovirus type 2 (PPV2) which is highly prevalent in the USA. *Veterinary Microbiology*, *161*(3), pp.325-330.

Xiao, C.T., Gimenez-Lirola, L.G., Jiang, Y.H., Halbur, P.G. and Opriessnig, T., 2013b. Characterization of a novel porcine parvovirus tentatively designated PPV5. *PLoS One*, *8*(6), p.e65312

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2013c. Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. *Infection, Genetics and Evolution*, *16*, pp.369-376.

Xing, X., Zhou, H., Tong, L., Chen, Y., Sun, Y., Wang, H. and Zhang, G., 2018. First identification of porcine parvovirus 7 in China. *Archives of Virology*, *163*(1), pp.209-213.

Zhai, S., Yue, C., Wei, Z., Long, J., Ran, D., Lin, T., Deng, Y., Huang, L., Sun, L., Zheng, H. and Gao, F., 2010. High prevalence of a novel porcine bocavirus in weanling piglets with respiratory tract symptoms in China. *Archives of Virology*, *155*(8), pp.1313-1317.

Zhou, F., Sun, H. and Wang, Y., 2014. Porcine bocavirus: achievements in the past five years. *Viruses*, *6*(12), pp.4946-4960.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

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7.1   General Discussion

As the global human population is increasing at an alarming rate, the need for sustainable means to meet up their feeding demand and livelihood has become so evident. Despite the global technological advancement and industrialisation, an unimaginable percentage of human population is still faced with the challenges of hunger and poverty. This has brought about the global quest for food security over time, however with time, greater need for nutrition security has become a reality as it is not just enough to have something to eat, but to eat quality food for healthy living. Agriculture remains the only means of feeding people and the basic source of livelihood for man than any other sector. Livestock farming, however, takes a greater percentage of total agricultural output globally and remains the major source of protein for the teeming human population (Upton, 2004).

Pig production is one of the cheapest sources of animal protein due to the inherent potentials of the animal to reproduce and grow very fast within a short period of time (FAO, 2012; Ironkwe and Amefule, 2008). Challenges of swine infectious diseases have seriously brought about huge set back to the promising potential of the piggery business over the years, and this has specially caught the attention of researchers since around 1990's when many pig-producing countries of the world had various magnitude of economic losses due to swine diseases' outbreak including PCV2-associated diseases such as PMWS (Vidigal *et al*., 2012; Segalés *et al*., 2013). This present study therefore gives insight into the status of porcine circovirus type 2 and porcine parvoviruses in swine herds of some selected communities in Eastern Cape Province, South Africa; through molecular epidemiological survey for the detection of the viruses, and subsequent genetic characterisation of the detected viral pathogens presently in circulation within the region.

PCV2 was discovered for the first time in Canada in the early 1990's (Clark, 1996; Harding, 1996). Since then, the virus has become widespread and has been found in virtually all pig-producing countries of the world. First reported outbreak of a PCV2 infection (PMWS) in South Africa was at Gauteng Province in 2001 at a well-managed breeding farm (Drew *et al*., 2004). It was suggested that major epidemiological survey should be carried out in other premises of South Africa, but nothing was done to that effect. At the beginning of this study, the occurrence of PCV2 was investigated in a South African Province of Eastern Cape from swine's blood, faecal and nasal swab samples obtained in 2015 and 2016 from some selected farms using molecular approach. An overall prevalence of 54/339 (15.9%) was obtained for the virus in the six sampled farms. The viral pathogen was detected in all the sampled farms, with farm level prevalence ranging from 5.6 – 60%; thereby confirming the ubiquity of PCV2 (Gillespie *et al*., 2009; Afolabi *et al*., 2017a).

Amplified genomes of the virus from the initial molecular screening of swine samples were subsequently sequenced and analysed. Seventeen (17) partial genomes of PCV2 were successfully assembled and subjected to further phylogenetic analysis. The preliminary findings showed that 15/17 (88%) of the obtained sequences clustered with other reference (PCV2b) sequences obtained from the GenBank, whereas the remaining two sequences formed a clade with PCV2d reference sequences. PCV2a was initially prevalent in the global swine herds until 2003 when a notable genotypic shift to PCV2b was experienced (Beach and Meng, 2012). The initial detection of PCV2a with closer identity to other strains from North America and Asia in Gauteng Province in 2001 (Drew *et al*., 2004) and this subsequent detection of PCV2b, portrays a seeming genotypic shift as it has been experienced globally. However, this claim cannot be substantiated since the first reported detection was just from a case study in which very small

number of samples from pigs with clinical symptoms of PMWS were analysed. In other hand, there is the possibility that PCV2b has been spreading alongside with PCV2a ever since the first introduction of the exotic swine pathogen into the country; and has spread to distant Provinces of South Africa unknowingly due to the initial negligence to carry out large scale epidemiological survey on the virus after its first detection.

Noteworthy is the detection of two sequences with a very close homology with reference sequences of the third common genotype, PCV2d, in South African swine. The PCV2d viral strain is presently becoming the predominant genotype in global swine herds with an observable higher virulence, thereby representing another observable genotypic shift as earlier presumed (Guo *et al*., 2012; Xiao *et al*., 2015). This viral strain was first detected in cases of vaccine failure and was initially named as mutant PCV2b (mPCV2b) (Guo *et al*., 2012; Opriessnig *et al*., 2013) and subsequently as PCV2d (Xiao *et al*., 2015). The detection of this newest variant in one of the six screened farms in this study despite non vaccination against the virus in the region based on the demographical information obtained in this study (Afolabi et el., 2017a), showed possible introduction and subsequent spread of the exotic swine pathogen in the Eastern Cape region via another route. This assertion however needs further confirmation through large scale surveillance in the country to determine the actual status of the South African pigs as regarding the identified viral genotypes.

Furthermore, the level of awareness among the farmers on the viral pathogen in the sampled regions is almost zero and there is no vaccination protocol in place against the virus as earlier mentioned (Afolabi *et al*., 2017a). The scourge of PCV2 and its associated diseases has been greatly abated in other parts of the world through effective vaccination procedure against the

virus and its other co-infecting pathogens in addition with observance of good farm management practices including effective biosecurity controls (Rose *et al*., 2003). It is therefore not a surprise that clinical signs of PMWS were observed in some of the farm investigated in the Province since there are no prevention and control measures in place to combat its menace.

Complete viral genome amplification and sequencing is imperative for a meaningful genetic characterisation; which invariably gives better understanding on the genotypes of the viral pathogen in circulation at a particular place and helps in adopting effective control and prevention measures (Jaganathan *et al*., 2011). Much more, there is dearth of available PCV2 genetic materials from South Africa and African continent at large (Afolabi *et al*., 2017b). This informed the subsequent efforts in this study to carry out whole-genome amplification and sequencing of detected local strains of PCV2 from the studied area. With the aid of four overlapping primers and conventional PCR techniques, 15 complete PCV2 genomes were successfully amplified, sequenced, assembled and genetically characterised from 54 PCV2-positive samples earlier obtained in this study. The phylogenetic analysis of the complete ORF2 genes and full-genomes of the PCV2 sequences in this study through a faster Neighbour-Joining method and the subsequent confirmation analysis through a slower but more accurate Maximum Likelihood method gave a unanimous outcome, with PCV2 sequences from this study forming three distinct groups with other PCV2 reference sequences from the GenBank.

Precisely, 11 sequences from two District Municipalities of the three that represent the sampled area in this study, clustered together with other PCV2b-1b reference strains to form the first group of PCV2 sequences. The sequences showed a very high nucleotide sequence (ORF2 gene) identity ranging from 99.8-100% within themselves and high homology of 99.7% with reference

strain **AY321987** from France and 99.4% with **DQ923524** from Brazil. Also, their p-distance values with the reference PCV2b strain were 0.003±0.002 and 0.011±0.002 at capsid gene and complete genome levels respectively. The values are quite lower than the stipulated cut-off p-distance values of 0.020 and 0.035 for complete genomes and capsid gene respectively, earlier set for the grouping of PCV2 sequences into a genotype (Segalés *et al*., 2008); thereby confirming their status as PCV2b genotype. Further analysis of their complete capsid gene through multiple nucleotide sequence alignment indicated that they all shared typical signature motifs 1 of S_NPR_SV (position 86-91) and signature motif 2 of AGIE (position 190-191-206-210) as stipulated for PCV2b genotypes (Cheung *et al*., 2007; Cheung, 2009).

The consistent clustering of the eleven sequences from this study with other PCV2b reference strains from Asia, Europe, South and North America could depict the likelihood of their possible connection with the first PMWS outbreak in Gauteng. The isolated PCV2 strain SA1 from the case study showed a very high homology with reference strains from North America and Asia, and its entry was connected with semen that was bought from Iowa by the farm for insemination purpose. Trade in live pigs and their products, including semen for breeding, has been implicated in the transmission and spread of swine infectious agents most especially in cases of trading animals with subclinical infections with no symptoms of disease (Rose *et al*., 2003; Drew *et al*., 2004; Vidigal *et al*., 2012;).

Furthermore, three sequences that formed group three of PCV2 detected in this study showed a very high nucleotide sequence homology ranging from 99.7-100% with reference mPCV2b from USA (**JX535296**) and China (**HM0338017**). Their p-distance values at the capsid gene and complete genome level with reference mPCV2b were significantly low compared to the cut-off

values, having 0.001±0.001 and 0.006±0.001 respectively. It is therefore not surprising that the three sequences consistently formed a clade with other reference mPCV2b and PCV2b-1C that have been recently categorized as PCV2d genotype in a recent global analysis (Xiao *et al*., 2015). Strains belonging to PCV2d genotype usually have a distinguishing feature of an elongated ORF2 gene due to the mutation of a stop codon at the C terminus end of their capsid genes resulting to capsid protein of 234 amino acids (aa) with an additional Lysine (K) instead of 233 aa, and they exhibited a higher level of virulence from the onset (Guo *et al*., 2010; 2012). The three sequences from this study likewise possessed the distinguishing feature of ORF2 elongation and also have the stipulated signature motifs of S_NPL_TV and TGID that characterize PCV2d strains compared to S_NPR_SV and AGIE found in capsid gene of typical PCV2b strains. The mPCV2b strains which have now become member of PCV2d group were subsequently detected in seeming cases of vaccine failure in the USA and Brazil after its initial detection in China (Guo *et al*., 2010; Opriessnig *et al*., 2013; Salgado *et al*., 2014).

Presently, its rapid spread and detection globally has brought about the assertion of another possible genotypic shift, that is, from PCV2b to PCV2d in global swine due to likelihood of vaccine selection (Xiao *et al*., 2015). However, the fact that vaccination programme against PCV2 is grossly lacking in South Africa based on recent findings (Afolabi *et al*., 2017a) raises a question on the likely source of mPCV2b in the country. It could either be that the viral genogroup was recently introduced into the country through the Eastern Cape region and presently limited to the area; or that there are other factors responsible for the evolution of mPCV2b other than the previously identified vaccine selection pressure. These assertions, however, require further investigation through a large scale epidemiological study in the country to better determine the status of PCV2b mutant strains in South African swine.

The molecular characterization of PCV2 sequences from this study further revealed the likelihood of natural inter-genotypic recombination event going on in the swine herds of the region. The remaining one sequence from this study formed a clade in the phylogenetic analysis with recently identified PCV2 intermediate strains 2 (PCV2-IM) to form group 2 of PCV2 sequences that was characterized. It clustered separately from the other two main groups observed, and showed a very high identity with the PCV2-IM2 strains from South Korea (98.2%) and United States of America (98.7%). The p-distance analysis revealed that it has sequence divergence values of 0.022±0.004 and 0.037±0.007 (complete genome and ORF2 gene respectively) with the other eleven sequences (in group 1) that formed same cluster with reference PCV2b strains from GenBank. The observed values are slightly higher than the stipulated cut-off p-distance value of 0.020 and 0.035 for PCV2 classification into a genotype both at complete genomes and capsid gene levels respectively (Segalés *et al*., 2008). The sequence however has p-distance value of 0.010±0.001 and 0.013±0.002 (complete genomes and capsid gene respectively) with the PCV2-IM2 reference strain, thereby showing their high level of relatedness.

Further analysis on the capsid gene of the sequence showed that it has sequence motif 1 of PCV2b (SNPRSV) as identified by Cheung (2009); however, the sequence motif 2 (SRIE) within the capsid gene of the sequence, constitutes a combination of the one that is found in the ORF2 gene of the first South African PCV2a strain SA1 (SR at position 190/191) and IE at position 206/210 which is normally found in PCV2b strains. This is probably an indication of the presence of the PCV2a strain in the studied region; however, this might not have been detected due to the relatively small number of farms that were initially sampled. Xiao *et al*. (2015) in the

recent phylogenetic analysis that employed globally available PCV2 sequences identified four major intermediate clades (IM1 – IM4) apart from the main phylogenetic groups of PCV2. Based on the study, it was asserted that PCV2 sequences that presently fall within the clades could be relics of intermediate virus strains during the evolution of PCV2; however, according to Martin *et al*. (2011), it could represent un-natural recombinants formed as sequence amplification artefacts during PCR reaction using Taq polymerase. Nevertheless, recombination events are common in natural populations of PCV2 (Huynh *et al*., 2014), and observable changes in some important region such as the immuno-reactive sites of PCV2 capsid gene as identified by Trible *et al*. (2011), can result to alterations in the virulence of the PCV2 strains. Hence, the genetic implication of the observed features (in terms of virulence and otherwise) of the intermediate PCV2 sequence from this study, which precisely expressed genomic relatedness with PCV2-IM2 reference strains, requires further critical study and evaluation, much more that it was found in pigs with no symptom of PCVAD.

Furthermore, a significant effort was made to determine the occurrence and prevalence of some porcine parvoviruses (PPVs) in selected samples earlier used for the screening of PCV2 in the study area. Specific PCR methods for seven different PPVs including porcine parvovirus 1 (PPV1), porcine parvovirus 2 (PPV2), porcine parvovirus 3 (PPV3), porcine parvovirus 4 (PPV4), porcine bocavirus-like virus (PBo-likeV), porcine bocavirus 1 and 2 (PBoV1 & PBoV2); were used to determine the prevalence of the viruses from 110 randomly selected archived swine samples. Findings showed that all the screened PPVs had the prevalences in the order 5.5% (PPV3), 21.8% (PPV2), 21.8% (PBo-likeV), 29.1% (PPV), 43.6% (PPV4) and 44.6% for PBoV1 and PBoV2.

Among all the presently known PPVs, PPV1 is a renowned co-infecting pathogen in PMWS which is one of the important PCV2-associated diseases of global economic importance. Both the field and experimental cases of PMWS in swine have confirmed the involvement of PPV1 as one of the co-factors that exacerbate the disease conditions in PCV2-infected pigs with clinical features of wasting syndrome (Allan *et al*., 1999; Kennedy *et al*., 2000; Sun *et al*., 2015). It is therefore not surprising that clinical signs of PMWS were observed earlier, which could depict co-infection effects of PCV2 and PPV1 in the swine herds. The virus is also a major etiologic agent of reproductive failure in breeding sows, characterized by stillbirth, mummified foetuses, early embryonic and foetal mortality, delayed return to estrus and infertility; which are abbreviated as SMEDI (Szelei, *et al*., 2006, Truyen and Streck, 2012). The sampled pigs in this study were all weaners and growers; hence, the likely contribution of PPV1 to possible cases of swine reproductive failure in the region could not be determined. This could however be the focus of future epidemiological studies since the involvement of the virus in impairing the advantageous reproductive ability in pigs cannot be overemphasized as observed globally.

Apart from PPV1 that has been previously reported in South African pigs as far back as 1975 in a case study on outbreak of reproductive failure (Pini,1975), the detection of many other PPVs is being reported for the first time in the country. Many novel PPVs have been detected globally within the last decades apart from PPV2 that was accidentally detected in 2001 when screening for the presence of hepatitis E virus in Myanmar pigs (Hijikata *et al*., 2001). The viral pathogen was subsequently detected in Southeastern China about a decade after from severe outbreaks of so called "high fever" in which PCV2 and PRRSV were implicated as causative agents (Wang *et al*., 2010). The detection of the virus in this study coupled with the initial detection of PCV2 in the sampled farms is in agreement with what was observed in the Chinese pigs, though the

involvement of the virus in the observed disease condition in pigs from China and many other clinical cases as reported globally is yet to be well elucidated (Wang *et al*., 2010; Saekhow and Ikeda, 2015).

The detection of PPV3 for the first time in South African swine as reported in this present study is also very significant as the virus is assumed to have zoonotic potential. The virus was equally detected for the first time (in 2008) by accident alongside with bovine parvovirus (i.e. bovine hokovirus – BhoV) in Hong Kong when an effort to determine the relationship that exists between the newly identified human parvovirus type 4 and 5 (*PARV4* and *5*) and animal parvoviruses was made (Lau *et al*., 2008). The two viruses showed a genetic similarity to the human parvovirus type 4 and 5; presently, PPV3 is thought to be ubiquitous as it has been found virtually in every region of the world including Cameroon, a West African country (Adlhoch *et al*., 2013; Xiao *et al*., 2013). Although the human *PARV4* has been identified in cases of people co-infected with hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV) (Panning *et al*., 2010; Simmons *et al*., 2012; Yu *et al*., 2012); its pathogenicity and that of PP3 is yet to be well explained. Larger epidemiological survey on the presence of the viral pathogen and its likely association with human parvoviruses in South Africa should however be given proper attention in the nearest future.

Also, PPV4 was initially detected from pigs suffering from PRRS and PCV2-associated diseases in North Carolina, USA; however, its involvement in the observed disease conditions is yet to be resolved (Cheung *et al*., 2010; Saekhow and Ikeda, 2015). Prior to its current detection in this study, the virus was recently found in bush pigs in Uganda (Blomström *et al*., 2012) and more recently in asymptomatic Cameroonian pigs with prevalence of 10/50 (20%) (Ndze *et al*., 2013).

Prevalence of higher value of 48/110 (43.6 %) was obtained in this study for PPV4 as against that of Cameroon; however, the value follows the same pattern with 41/80 (44 %) that was recently obtained in Thailand (Saekhow and Ikeda, 2015). As earlier mentioned, the virus was first detected from cases of PMWS-affected pigs in America (Cheung *et al*., 2010). However, it has also been found in healthy and sick pigs in China (Huang *et al*., 2010) and more recently in asymptomatic pigs in Cameroon (Ndze *et al*., 2013). Though the clinical relevance of PPV4 in the implicated disease condition is yet to be well explained as stated by Xiao *et al*. (2013), further study on its likely roles in connection with PCV2 infection in South African swine herds as shown in this study should be given utmost priority.

The first porcine bocavirus strain (PBo-likeV) that was detected in Sweden from pigs with background of PMWS (Blomstrom *et al*., 2009) was also detected in this present investigation with prevalence of 24/110 (21.8%) comparable with slightly lower value of 23/80 (18%) obtained in Thai pigs recently (Saekhow and Ikeda, 2015). The prevalence value obtained for PBo-likeV strain in this study was however significantly higher than the 2/95 (2.1%) previously obtained in pigs at Uganda (Blomstrom *et al*., 2013). It is however noteworthy that sequence identity of 99.4-99.8% shown by the PBo-likeV sequences in this study with the Swedish reference strain, is highly comparable with 99-99.6% earlier showed by the Ugandan PBo-likeV sequences (Blomstrom *et al*., 2013). Other than the Swedish-type porcine bocavirus that was detected, the PBoV1 and PBoV2 of Chinese origin (Cheng *et al*., 2010) were collectively screened in swine samples from the study area. Both of them showed highest prevalence of 49/110 (44.6%) and were almost equally distributed in the studied area based on the findings from phylogenetic analysis. Prevalence of PBoV1/2 obtained in this study was equally higher than the collective prevalence of 3/50 (6%) previously obtained in Cameroonian pigs as reported

by Ndze *et al*. (2013). The occurrence and prevalence of only three out of the eight presently identified porcine bocaviruses were determined in this study. Future large scale surveillance for the presence of other members of the group is highly warranted in South Africa; this becomes imperative as some of the novel porcine bocaviruses also share some level of relatedness to human bocaviruses, and their possibility of overcoming the inter-species barrier through mutation or recombination and re-emergence as human pathogen has been presumed (Lau *et al*., 2011).

Presently, majority of the novel porcine parvoviruses are yet to be thoroughly investigated. Very limited information on the relevance or importance of these viruses to global swine population and pathogenicity potentials of the viruses is available (Xiao *et al*., 2013). However, their detection has been reported to be common in PCVAD cases (Opriessnig *et al*., 2014). The general observation is synonymous to what was obtained in this study, as relatively high prevalence of the porcine parvoviruses was observed in swine herds from the study area with confirmed PCV2 farm level prevalence of 5.6-60%. These present findings therefore lay more emphasis on the need for further investigations on the likely roles of all currently identified novel porcine parvoviruses in the PCV2-associated diseases as it has been earlier suggested globally.

7.2   General Conclusion

As much as pig production owns a unique potential of serving as good source of cheap animal proteins for ever-increasing human population, transmission and spread of trans-boundary emerging and re-emerging swine diseases will continue to pose enormous challenges to the industry as efforts are being made to increase its production and trading within countries of the world. However, timely and effective surveillance for the detection of such diseases becomes

highly imperative for efficient prevention and control in order to avert undue economic woes that may ensue due to an unwarranted negligence of the stakeholders. This study has revealed the presence of porcine circovirus type 2 and porcine parvoviruses in swine herds of Eastern Cape Province, South Africa. It is invariably a good pointer to the true health status of swine population in the country. Within the space of about two decades that PCV2a was detected for the first time in the country, this study reports the occurrence of three additional genotypes in swine herds of the studied area. Interestingly, an additional effort to determine the presence of porcine viruses in the subfamily *Parvovirinae* which have been largely implicated in many PCV2-related diseases' condition also revealed the occurrence of the entire screened viral group in the studied area. Therefore, there is need for proactive measures by the government and every stakeholder in rising up to the task of combating swine infectious diseases through all-encompassing measures.

7.3   General Recommendations

i.   Considering the unprecedented level of ignorance shown by majority of farmers in the studied area about the viral pathogens of interest, most especially PCV2; it is therefore extremely important that a rigorous awareness and enlightenment programmes should begin with immediate effect in order to inform and educate the farmers about the viral pathogens and their associated diseases in the country.

ii.   Simultaneously, an effective large-scale vaccination programmes should also be initiated to curtail the rapid circulation of the viral pathogens and prevent impending future outbreaks. This should be done *pari passu* with educating the farmers on the biosecurity measures required in piggery operations for optimal performance.

iii.   There is an urgent need for effective large-scale surveillance and characterization of the viral pathogens and many other swine pathogens including PRRSV, swine influenza virus, swine hepatitis E virus, classical swine fever to mention but few, in the region and the country at large. This will go a long way in enhancing the implementation of the global "One Health, One World" initiative for combating swine zoonotic diseases in the region.

iv.   Future additional sequencing studies on the viral pathogens in South Africa and other African countries becomes imperative in order to populate the data base with sequences from the region.

v.   Isolation of the detected viral pathogens should be carried out in order to facilitate thorough *in-vitro* and *in-vivo* studies for validating pathogenicity of the local viral strains in the South African swine population and foster development of local vaccines for future use in the country and other African countries. Also, it will contribute to the world of knowledge about the clinical relevance of most of the parvoviruses that are grossly lacking globally.

vi.   There is need to legislate and enforce all-encompassing regulatory measures for preventing the transmission of exotic swine infectious diseases into the country, not selective one that gives priority to the prevention of one particular pathogen per time as it is the case of PRRSV.

References

Adlhoch, C., Kaiser, M., Kingsley, M.T., Schwarz, N.G., Ulrich, M., de Paula, V.S., Ehlers, J., Löwa, A., Daniel, A.M., Poppert, S. and Schmidt-Chanasit, J., 2013. Porcine hokovirus in domestic pigs, Cameroon. *Emerging Infectious Diseases*, *19*(12), p.2060.

Afolabi, K.O., Iweriebor, B.C., Obi, L.C. and Okoh, A.I., 2017a. Molecular detection of Porcine circovirus type 2 in swine herds of Eastern Cape Province South Africa. *BMC Microbiology*, *17*(1), p.212.

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017b. Global Status of Porcine circovirus Type 2 and Its Associated Diseases in Sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964.

Allan, G.M., Kennedy, S., McNeilly, F., Foster, J.C., Ellis, J.A., Krakowka, S.J., Meehan, B.M. and Adair, B.M., 1999. Experimental reproduction of severe wasting disease by co-infection of pigs with porcine circovirus and porcine parvovirus. *Journal of Comparative Pathology*, *121*, pp.1–11.

Beach, N.M. and Meng, X.J., 2012. Efficacy and future prospects of commercially available and experimental vaccines against porcine circovirus type 2 (PCV2). *Virus Research*, *164*(1), pp.33-42.

Blomström, A.L., Belák, S., Fossum, C., McKillen, J., Allan, G., Wallgren, P. and Berg, M., 2009. Detection of a novel porcine boca-like virus in the background of porcine circovirus type 2 induced postweaning multisystemic wasting syndrome. *Virus Research*, *146*(1), pp.125-129.

Blomström, A.L., Ståhl, K., Masembe, C., Okoth, E., Okurut, A.R., Atmnedi, P., Kemp, S., Bishop, R., Belák, S. and Berg, M., 2012. Viral metagenomic analysis of bushpigs

(Potamochoerus larvatus) in Uganda identifies novel variants of Porcine parvovirus 4 and Torque teno sus virus 1 and 2. *Virology Journal*, *9*(1), p.192.

Blomström, A.L., Ståhl, K., Okurut, A.R., Masembe, C. and Berg, M., 2013. Genetic characterisation of a porcine bocavirus detected in domestic pigs in Uganda. *Virus Genes*, *47*(2), pp.370-373.

Cheng, W.X., Li, J.S., Huang, C.P., Yao, D.P., Liu, N., Cui, S.X., Jin, Y. and Duan, Z.J., 2010. Identification and nearly full-length genome characterization of novel porcine bocaviruses. *PloS One*, *5*(10), p.e13583.

Cheung, A.K., 2009. Homologous recombination within the capsid gene of porcine circovirus type 2 subgroup viruses via natural co-infection. *Archives of Virology*, *154*(3), pp.531-534.

Cheung, A.K., Lager, K.M., Kohutyuk, O.I., Vincent, A.L., Henry, S.C., Baker, R.B., Rowland, R.R. and Dunham, A.G., 2007. Detection of two porcine circovirus type 2 genotypic groups in United States swine herds. *Archives of Virology*, *152*(5), pp.1035-1044.

Cheung, A.K., Wu, G., Wang, D., Bayles, D.O., Lager, K.M. and Vincent, A.L., 2010. Identification and molecular cloning of a novel porcine parvovirus. *Archives of Virology*, *155*(5), pp.801-806.

Clark, E.G., 1996. Post-weaning multisystemic syndrome. *In: Proceedings of Western Canadian Association of Swine Practitioners*, pp. 22–25.

Drew, T.W., Grierson, S.S., King, D.P., Hicks, D., Done, S., Banks, M., Neser, J.A., Evans, D.P.B. and Grimbeek, P., 2004. Genetic similarity between porcine circovirus type 2 isolated from the first reported case of PMWS in South Africa and North American isolates. *Veterinary Record*, *155*(5), pp.149–151.

FAO, 2012. Pig Sector Kenya, FAO Animal Production and Health Livestock Country Reviews, No. 3, Rome.

Gillespie, J., Opriessnig, T., Meng, X.J., Pelzer, K. and Buechner-Maxwell, V., 2009. Porcine circovirus type 2 and porcine circovirus-associated disease. *Journal of Veterinary Internal Medicine*, *23*(6), pp.1151-1163.

Guo, L., Fu, Y., Wang, Y., Lu, Y., Wei, Y., Tang, Q., Fan, P., Liu, J., Zhang, L., Zhang, F. and Huang, L., 2012. A porcine circovirus type 2 (PCV2) mutant with 234 amino acids in capsid protein showed more virulence in vivo, compared with classical PCV2a/b strain. *PLoS One*, *7*(7), p.e41463.

Guo, L.J., Lu, Y.H., Wei, Y.W., Huang, L.P. and Liu, C.M., 2010. Porcine circovirus type 2 (PCV2): genetic variation and newly emerging genotypes in China. *Virology Journal*, *7*(1), p.273.

Harding, J.C., 1996. Post-weaning multisystemic wasting syndrome (PMWS): preliminary epidemiology and clinical presentation. *In: Proceedings of Western Canadian Association of Swine Practitioners*, p. 21.

Hijikata, M., Abe, K., Win, K.M., Shimizu, Y.K., Keicho, N. and Yoshikura, H., 2001. Identification of new parvovirus DNA sequence in swine sera from Myanmar. *Japanese Journal of Infectious Diseases*, *54*(6), pp.244-245.

Huang, L., Zhai, S.L., Cheung, A.K., Zhang, H.B., Long, J.X. and Yuan, S.S., 2010. Detection of a novel porcine parvovirus, PPV4, in chinese swine herds. *Virology Journal*, *7*(1), p.333.

Huynh, T.M.L., Nguyen, B.H., Nguyen, V.G., Dang, H.A., Mai, T.N., Tran, T.H.G., Ngo, M.H., Le, V.T., Vu, T.N., Ta, T.K.C. and Vo, V.H., 2014. Phylogenetic and phylogeographic analyses of porcine circovirus type 2 among pig farms in Vietnam. *Transboundary and Emerging Diseases*, *61*(6). pp.e25–e34.

Ironkwe, M.O. and Amefule, K.U., 2008. Appraisal Of Indigenous Pig Procution And Management Practices In Rivers State, Nigeria. *Journal of Agriculture and Social Research*, *8*(1), pp.1-7.

Jaganathan, S., Toung, O.P., Yee, P.L., Yew, T.D., Yoon, C.P. and Keong, L.B., 2011. Genetic characterization of Porcine Circovirus 2 found in Malaysia. *Virology Journal*, *8*(1), p.437.

Kennedy, S., Moffett, D., McNeilly, F., Meehan, B., Ellis, J., Krakowka, S. and Allan, G.M., 2000. Reproduction of lesions of postweaning multisystemic wasting syndrome by infection of conventional pigs with porcine circovirus type 2 alone or in combination with porcine parvovirus. *Journal of Comparative Pathology*, *122*(1), pp.9-24.

Lau, S.K., Woo, P.C., Tse, H., Fu, C.T., Au, W.K., Chen, X.C., Tsoi, H.W., Tsang, T.H., Chan, J.S., Tsang, D.N. and Li, K.S., 2008. Identification of novel porcine and bovine parvoviruses closely related to human parvovirus 4. *Journal of General Virology*, *89*(8), pp.1840-1848.

Lau, S.K., Woo, P.C., Yip, C.C., Li, K.S., Fu, C.T., Huang, Y., Chan, K.H. and Yuen, K.Y., 2011. Co-existence of multiple strains of two novel porcine bocaviruses in the same pig, a previously undescribed phenomenon in members of the family Parvoviridae, and evidence for inter-and intra-host genetic diversity and recombination. *Journal of General Virology*, *92*(9), pp.2047-2059.

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Martin, D.P., Lemey, P. and Posada, D., 2011. Analysing recombination in nucleotide sequences. *Molecular Ecology Resources*, *11*(6), pp.943-955.

Ndze, V.N., Cadar, D., Cságola, A., Kisfali, P., Kovács, E., Farkas, S., Ngu, A.F., Esona, M.D., Dán, Á., Tuboly, T. and Bányai, K., 2013. Detection of novel porcine bocaviruses in fecal samples of asymptomatic pigs in Cameroon. *Infection, Genetics and Evolution*, *17*, pp.277-282.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2013. Emergence of a novel mutant PCV2b variant associated with clinical PCVAD in two vaccinated pig farms in the US concurrently infected with PPV2. *Veterinary Microbiology*, *163*(1), pp.177-183.

Opriessnig, T., Xiao, C.T., Gerber, P.F. and Halbur, P.G., 2014. Identification of recently described porcine parvoviruses in archived North American samples from 1996 and association with porcine circovirus associated disease. *Veterinary Microbiology*, *173*(1), pp.9-16.

Panning, M., Kobbe, R., Vollbach, S., Drexler, J.F., Adjei, S., Adjei, O., Drosten, C., May, J. and Eis-Hübinger, A.M., 2010. Novel human parvovirus 4 genotype 3 in infants, Ghana. *Emerging Infectious Diseases*, *16*(7), p.1143.

Pini, A., 1975. Porcine parvovirus in pig herds in Southern Africa. *Journal of the South African Veterinary Association*, *46*(3), pp.241-244.

Rose, N., Larour, G., Le Diguerher, G., Eveno, E., Jolly, J.P., Blanchard, P., Oger, A., Le Dimna, M., Jestin, A. and Madec, F., 2003. Risk factors for porcine post-weaning multisystemic wasting syndrome (PMWS) in 149 French farrow-to-finish herds. *Preventive Veterinary Medicine*, *61*(3), pp.209-225.

Saekhow, P. and Ikeda, H., 2015. Prevalence and genomic characterization of porcine parvoviruses detected in Chiangmai area of Thailand in 2011. *Microbiology and Immunology*, *59*(2), pp.82-88.

Salgado, R.L., Vidigal, P.M., de Souza, L.F., Onofre, T.S., Gonzaga, N.F., Eller, M.R., Bressan, G.C., Fietto, J.L., Almeida, M.R. and Júnior, A.S., 2014. Identification of an emergent porcine circovirus-2 in vaccinated pigs from a Brazilian farm during a postweaning multisystemic wasting syndrome outbreak. *Genome Announcements*, *2*(2), p.e00163-14.

Segalés, J., Kekarainen, T. and Cortey, M., 2013. The natural history of porcine circovirus type 2: from an inoffensive virus to a devastating swine disease?. *Veterinary Microbiology*, *165*(1), pp.13-20.

Segalés, J., Olvera, A., Grau-Roma, L., Charreyre, C., Nauwynck, H., Larsen, L., Dupont, K., McCullough, K., Ellis, J., Krakowka, S. and Mankertz, A., 2008. PCV-2 genotype definition and nomenclature. *Veterinary Record*, *162*(26), pp.867-868.

Simmons, R., Sharp, C., McClure, C.P., Rohrbach, J., Kovari, H., Frangou, E., Simmonds, P., Irving, W., Rauch, A., Bowness, P. and Klenerman, P., 2012. Parvovirus 4 infection and clinical outcome in high-risk populations. *Journal of Infectious Diseases*, *205*(12), pp.1816-1820.

Sun, J., Huang, L., Wei, Y., Wang, Y., Chen, D., Du, W., Wu, H. and Liu, C., 2015. Prevalence of emerging porcine parvoviruses and their co-infections with porcine circovirus type 2 in China. *Archives of Virology*, *160*(5), pp.1339-1344.

Szelei, J., Zadori, Z. and Tijssen, P., 2006. Porcine parvovirus. In: Kerr, J.R., Cotmore, S.F., Bloom, M.E., Linden, R.M. and Parrish, C.R. Eds., *Parvoviruses*, Hodder Arnold, London, UK, pp.435–445.

Trible, B.R., Kerrigan, M., Crossland, N., Potter, M., Faaberg, K., Hesse, R. and Rowland, R.R., 2011. Antibody recognition of porcine circovirus type 2 capsid protein epitopes after vaccination, infection, and disease. *Clinical and Vaccine Immunology*, *18*(5), pp.749-757.

Truyen, U. and Streck, A. F., 2012. Porcine parvovirus. In: Zimmerman, J. K., Karriker, L., Ramirez, A., Schwartz, K. and Stevenson, G. 10th Eds., *Diseases of Swine*, John Wiley and Sons, Chichester, UK, pp.447–455.

Upton, M., 2004. *The role of livestock in economic development and poverty reduction*. PPLPI Working paper No. 10. FAO, Roma, Italy.

Vidigal, P.M., Mafra, C.L., Silva, F.M., Fietto, J.L., Júnior, A.S. and Almeida, M.R., 2012. Tripping over emerging pathogens around the world: a phylogeographical approach for determining the epidemiology of Porcine circovirus-2 (PCV-2), considering global trading. *Virus Research*, *163*(1), pp.320-327.

Wang, F., Wei, Y., Zhu, C., Huang, X., Xu, Y., Yu, L. and Yu, X., 2010. Novel parvovirus sublineage in the family of Parvoviridae. *Virus Genes*, *41*(2), pp.305-308.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2013. Molecular evolutionary genetic analysis of emerging parvoviruses identified in pigs. *Infection, Genetics and Evolution*, *16*, pp.369-376.

Xiao, C.T., Halbur, P.G. and Opriessnig, T., 2015. Global molecular genetic analysis of porcine circovirus type 2 (PCV2) sequences confirms the presence of four main PCV2 genotypes and reveals a rapid increase of PCV2d. *Journal of General Virology*, *96*(7), pp.1830-1841.

Yu, X., Zhang, J., Hong, L., Wang, J., Yuan, Z., Zhang, X. and Ghildyal, R., 2012. High prevalence of human parvovirus 4 infection in HBV and HCV infected individuals in shanghai. *PLoS One*, *7*(1), p.e29474.

RESEARCH OUTPUTS:

Presentations and Publications

CONFERENCE PRESENTATIONS

> April 4 – 7, 2018 - **Attendance and oral presentation** of paper titled: *"Genetic Characterization of the first complete genomes of porcine circovirus type 2b and 2d from South African pigs"* at the South African Society for Microbiology (SASM) Conference, held at Misty Hills Hotel and Conference Centre, Muldersdrift, Johannesburg, South Africa.

Authors: **Afolabi, K.O.,** Iweriebor, B.C., Obi, L.C. and Okoh, A.I.

> October 23, 2017 - **Attendance and oral presentation** of paper titled: *"Occurrence of porcine circovirus type 2 in pigs of Eastern Cape Province South Africa"* at the University of Fort Hare Research and Innovation Day, held at UFH Alice Campus.

Authors: **Afolabi, K.O.,** Iweriebor, B.C., Okoh, A.I. and Obi, L.C.

> June 1 – 5, 2017 - **Attendance and poster presentation** of paper titled: *"Molecular detection of porcine circovirus 2 in swine herds of Eastern Cape Province, South Africa"* at the American Society for Microbiology (ASM) Microbe 2017 congress, held at Ernest N. Morial Convention Center 900 Convention Center Blvd, New Orleans, LA 70130, USA.

Authors: **Afolabi, K.O.,** Iweriebor, B.C., Obi, L.C. and Okoh, A.I.

PUBLICATIONS

Afolabi, K.O., Iweriebor, B.C., Okoh, A.I. and Obi, L.C., 2017. Global status of porcine circovirus type 2 and its associated diseases in sub-Saharan Africa. *Advances in Virology*, *2017*, 2017:6807964. https://doi.org/10.1155/2017/6807964

Afolabi, K.O., Iweriebor, B.C., Obi, L.C. and Okoh, A.I., 2017. Molecular detection of porcine circovirus type 2 in swine herds of Eastern Cape Province South Africa. *BMC Microbiology*, *17*(1), p.212. https://doi.org/10.1186/s12866-017-1121-4

APPENDICES

Appendix 4.1

Title of data: Questionnaire on porcine circovirus type 2 (PCV2) study

Description of data: Structured questionnaire administered to obtain information regarding some farm management practices and level of awareness on PCV2 from the managers of the selected farms.

University of Fort Hare
Together in Excellence

QUESTIONNAIRE ON PORCINE CIRCOVIRUS TYPE 2 (PCV2) STUDY

1)   Location of farm (District municipality):…………………………………………..

…………………………………………………………...

2) Size of herd: ≤100   [   ]   101 – 1000   [   ]   1000 – 2000   [   ]

5000 and above   [   ]

3) Ages of pigs: piglets   [   ]   growers   [   ]   finisher   [   ]   boars   [   ]

Sows   [   ]

4) What breed of pigs do you have in your farm? Large white   [   ] Land race   [   ]

Duroc   [   ]   Yorkshire   [   ]

Others………………………………………………………………………..……..

5)   Observed sanitary/biosecurity measures: none   [   ]   low   [   ]   medium   [   ]

     High   [   ]   very high   [   ]

6) What biocide do you use in the farm?..

…………………………………………………………………………………………

7) Do you vaccinate your animals? If so against which diseases?....................................

…………………………………………………………………………………………

…………………………………………………………………………………………

…………………..

8) Types of antibiotics/drugs used and when?…………………………………………...

…………………………………………………………………………………………

…………………..

9)   Do you practice all-in/all-out pig rearing style in your farm?   Yes   [   ]   No   [   ]

10) Do you have veterinary personnel that attend to your animals?   Yes   [   ]   No   [   ]

11) How often does he/she visits your farm?..

12) What disease outbreaks have you ever experienced on your farm?...

……………………………………………………………………………………………..

……………………………………………………………………………………………..

……………...

13) How often do your pregnant sows have abortion?..

……………………………………………………………………………………………..

……………………………………………………………………………………………..

14)   If you experience abortion among your sows what do you do? Sell the sow, treat it and

leave it still in the farm?...

15)   Breeding procedure Artificial insemination   [   ]   Natural crossing   [   ]

16)   Do you have a sick bay for diseased animals on your farm? Yes [   ] No [   ]

17)   Have you heard of PCV2/post weaning multisystemic wasting syndrome? Yes   [   ]

No   [   ]

18)   If yes, have you noticed it among your piglets [   ] growers [   ] or finishers [   ]?

19)   Observable clinical features of PCV2 associated diseases………………..……………..

………………………………………………………………………………………………………

………………………………………………………………………………………………………

………………………………………………………………………………………………………

………………………………………………………………………………………………………

20)   What kind of feeds do you use in your farm and where do you usually purchase

it?...

....

....

Appendix 4.2: Ethical clearance and permission to conduct the study

University of Fort Hare
Together in Excellence

ETHICAL CLEARANCE CERTIFICATE
REC-270710-028-RA Level 01

Certificate Reference Number: OBI012 (Project)

Project title: **Porcine Circovirus: An emerging Enzootic Pathogen with huge economic impact on piggery business yet understudied in South Africa**

Nature of Project: Independent project

Principal Researcher: Prof LC Obi
Sub researchers   Prof A Okoh
                    Dr BC Iweribor

Supervisor: N/A

Co-supervisor: N/A

On behalf of the University of Fort Hare's Research Ethics Committee (UREC) I hereby give ethical approval in respect of the undertakings contained in the above-mentioned project and research instrument(s). Should any other instruments be used, these require separate authorization. The Researcher may therefore commence with the research as from the date of this certificate, using the reference number indicated above.

Please note that the UREC must be informed immediately of

- Any material change in the conditions or undertakings mentioned in the document

- Any material breaches of ethical undertakings or events that impact upon the ethical conduct of the research

The Principal Researcher must report to the UREC in the prescribed format, where applicable, annually, and at the end of the project, in respect of ethical compliance.

Special conditions: Research that includes children as per the official regulations of the act must take the following into account:

Note: The UREC is aware of the provisions of s71 of the National Health Act 61 of 2003 and that matters pertaining to obtaining the Minister's consent are under discussion and remain unresolved. Nonetheless, as was decided at a meeting between the National Health Research Ethics Committee and stakeholders on 6 June 2013, university ethics committees may continue to grant ethical clearance for research involving children without the Minister's consent, provided that the prescripts of the previous rules have been met. This certificate is granted in terms of this agreement.

The UREC retains the right to

- Withdraw or amend this Ethical Clearance Certificate if
    - Any unethical principal or practices are revealed or suspected
    - Relevant information has been withheld or misrepresented
    - Regulatory changes of whatsoever nature so require
    - The conditions contained in the Certificate have not been adhered to

- Request access to any information or data at any time during the course or after completion of the project.

- In addition to the need to comply with the highest level of ethical conduct principle investigators must report back annually as an evaluation and monitoring mechanism on the progress being made by the research. Such a report must be sent to the Dean of Research's office

The Ethics Committee wished you well in your research.

Yours sincerely

Professor Gideon de Wet
Dean of Research

27 April 2015

Province of the
EASTERN CAPE
DEPARTMENT OF RURAL DEVELOPMENT AND AGRARIAN REFORM
VETERINARY SERVICES
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TO WHOM IT MAY CONCERN

Professor Anthony Okoh from the University of Fort Hare is currently conducting a scientific research in the Eastern Cape Province on the Porcine Circovirus : An emerging enzootic pathogen with huge economic impact on piggery business yet understudied , in the Eastern Cape province. The department would be grateful if Professor A Akoh would share with it his research findings.

We wish to confirm that Eastern Cape : Animal Health has no objection to Professor Okoh collecting field samples for the purposes of this research throughout the province of Eastern Cape.

The collection of the samples by Prof Okoh should comply to the Animal Disease Act 35 of 1984, the Veterinary and Paraveterinary Act 19 of 1982 and the Animal Protection Act of 1962 prescripts and regulations. The staff of Animal Health will not be responsible for collection of specimen for Prof A Okoh's research but if during the course of performing their normal duties porcine samples are collected for disease surveillance, they may share the specimen.

The department would be grateful if Professor A Akoh would share with it his research findings.

Dr C.L . Mnqeta
Director: Animal Health Eastern Cape
Date: 24/07/2015

Vibrant, equitable, sustainable rural communities and food security for all.   Page 1

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Appendix 4.3

Title of data: PCV2 sequences and other reference sequences previously reported, and used in the phylogenetic analysis.

Description of data: Details of PCV2 sequences used in Chapter four and other reference sequences obtained from GenBank

Genotype	Name	Geographical location	Source
PCV1	PCU49186	Northern Ireland UK	GenBank
PCV2a	AY556474	China	GenBank
	AY325495	South Africa	GenBank
	AY322004	France	GenBank
	AJ223185	USA	GenBank
	AF408635	Canada	GenBank
	AF381176	China	GenBank
	KM924366	South Korea	GenBank
	KM924364	South Korea	GenBank
	FJ870968	China	GenBank
	FJ870967	China	GenBank
	DQ104423	China	GenBank
	AF055392	Canada	GenBank
PCV2b	AY691169	China	GenBank
	AY424405	Austria	GenBank
	AY322003	France	GenBank
	AY321985	France	GenBank
	KU041850	China	GenBank
	KU041849	China	GenBank
	HQ202970	Taiwan	GenBank
	HM038016	China	GenBank
	FJ870974	China	GenBank

	FJ870969	China	GenBank
	AF055394	France	GenBank
	KX247842	China	GenBank
	JX406426	China	GenBank
	EU418626	China	GenBank
	HQ395035	China	GenBank
	GU247990	China	GenBank
	KX247844	China	GenBank
	AF201311	Germany	GenBank
	KY985387 (2FTP17; Strain AFOS1-FH1)	South Africa	This study
	KY985388 (2FTP23; Strain BEN1-FH2)	South Africa	This study
	KY985389 (2FTP71; Strain OBI1-FH3)	South Africa	This study
	KY985390 (2FTP109; Strain OKOH1-FH4)	South Africa	This study
	KY985391 (TSO6; Strain AFOS2-TSO)	South Africa	This study
	KY985392 (CHA1; Strain AFOS3-CHA1)	South Africa	This study
	KY985393 (CHA4; Strain AFOS4-CHA2)	South Africa	This study
	KY985394 (CHA5; Strain BEN2-CHA3)	South Africa	This study
	KY985395 (CHA8; Strain BEN3-CHA4)	South Africa	This study
	KY985396 (CHA10; Strain BEN4-CHA5)	South Africa	This study
	KY985397 (CHA13; Strain OBI2-CHA6)	South Africa	This study
	KY985398 (CHA16; Strain OBI3-CHA7)	South Africa	This study
	KY985399 (CHA17; Strain OBI4-CHA8)	South Africa	This study
	KY985400 (CHA22; Strain OKOH2-CHA9)	South Africa	This study
	KY985401 (CHA30; Strain OKOH3-CHA10)	South Africa	This study
PCV2c	EU148505	Denmark	GenBank
	EU148504	Denmark	GenBank
	EU148503	Denmark	GenBank
PCV2d	KU041859	China	GenBank
	KU041855	China	GenBank
	KU041851	China	GenBank
	KJ680361	China	GenBank

KJ680354	China	GenBank
KJ680353	China	GenBank
KJ511876	China	GenBank
KX828241	South Korea	GenBank
AY181946	China	GenBank
FJ712215	China	GenBank
JX535296	USA	GenBank
KY425815	China	GenBank
KU311021	China	GenBank
KY985402 (CHB16; Strain AFOS5-CHB1)	South Africa	This study
KY985403 (CHB19; Strain AFOS6-CHB2)	South Africa	This study

Appendix 5.1: Phylogenetic analysis based on (a) ORF2 genes and (b) complete genome sequences. The tree was drawn with 15 (▲) PCV2 sequences from this study and 42 reference sequences; including the previous strain SA1 (■) and one PCV1 strain as an out-group. GenBank accession numbers and country of origin are indicated. Maximum Likelihood method was used and Bootstrap values ≥80% are shown.

Appendix 5.2: Pairwise nucleotide sequence identity of ORF2 genes of South African PCV2 sequences and some reference strains

Seq->	SA3-BC	SA1-BA	SA2-BB	SA4-BD	SA5-BE	SA6-BF	SA7-BG	SA8-BH	SA8-BH	SA10-BJ	SA11-BK	SA12-BL	SA1-DA	SA2-DB	SA3-DC	EF394775	Seq->
SA3-BC	ID	0.964	0.962	0.964	0.964	0.964	0.964	0.964	0.964	0.964	0.964	0.964	0.926	0.926	0.924	0.955	
SA1-BA	0.964	ID	0.998	1	1	1	1	1	1	1	1	1	0.936	0.936	0.934	0.92	
SA2-BB	0.962	0.998	ID	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.998	0.934	0.934	0.933	0.918	
SA4-BD	0.964	1	0.998	ID	1	1	1	1	1	1	1	1	0.936	0.936	0.934	0.92	
SA5-BE	0.964	1	0.998	1	ID	1	1	1	1	1	1	1	0.936	0.936	0.934	0.92	
SA6-BF	0.964	1	0.998	1	1	ID	1	1	1	1	1	1	0.936	0.936	0.934	0.92	
SA7-BG	0.964	1	0.998	1	1	1	ID	1	1	1	1	1	0.936	0.936	0.934	0.92	
SA8-BH	0.964	1	0.998	1	1	1	1	ID	1	1	1	1	0.936	0.936	0.934	0.92	
SA9-BI	0.964	1	0.998	1	1	1	1	1	ID	1	1	1	0.936	0.936	0.934	0.92	
SA10-BJ	0.964	1	0.998	1	1	1	1	1	1	ID	1	1	0.936	0.936	0.934	0.92	
SA11-BK	0.964	1	0.998	1	1	1	1	1	1	1	ID	1	0.936	0.936	0.934	0.92	
SA12-BL	0.964	1	0.998	1	1	1	1	1	1	1	1	ID	0.936	0.936	0.934	0.92	
SA1-DA	0.926	0.936	0.934	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	ID	0.998	0.995	0.9	
SA2-DB	0.926	0.936	0.934	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.998	ID	0.997	0.9	
SA3-DC	0.924	0.934	0.933	0.934	0.934	0.934	0.934	0.934	0.934	0.934	0.934	0.934	0.995	0.997	ID	0.899	
EF394775	0.955	0.92	0.918	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.92	0.9	0.9	0.899	ID	
AY325495	0.96	0.924	0.923	0.924	0.924	0.924	0.924	0.924	0.924	0.924	0.924	0.924	0.899	0.899	0.897	0.991	
JF317581	0.982	0.952	0.951	0.952	0.952	0.952	0.952	0.952	0.952	0.952	0.952	0.952	0.924	0.924	0.923	0.954	
FJ388889	0.987	0.96	0.958	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.923	0.923	0.921	0.955	
AY321987	0.967	0.997	0.995	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.997	0.939	0.939	0.937	0.923	
HQ202970	0.962	0.992	0.991	0.992	0.992	0.992	0.992	0.992	0.992	0.992	0.992	0.992	0.934	0.934	0.933	0.918	
DQ923524	0.965	0.994	0.992	0.994	0.994	0.994	0.994	0.994	0.994	0.994	0.994	0.994	0.939	0.939	0.937	0.921	
DQ629117	0.96	0.99	0.988	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.934	0.934	0.933	0.917	
EU148505	0.892	0.903	0.902	0.903	0.903	0.903	0.903	0.903	0.903	0.903	0.903	0.903	0.899	0.899	0.899	0.868	
KU041859	0.921	0.931	0.93	0.931	0.931	0.931	0.931	0.931	0.931	0.931	0.931	0.931	0.994	0.995	0.995	0.896	
JX53529626	0.926	0.936	0.934	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.998	1	0.997	0.9	

HM038017	0.926	0.936	0.934	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.936	0.998	1	0.997	0.9
NC_001792	0.659	0.659	0.661	0.659	0.659	0.659	0.659	0.659	0.659	0.659	0.659	0.659	0.652	0.652	0.654	0.653

Appendix 5.2 (contd.): Pairwise nucleotide sequence identity of ORF2 genes of South African PCV2 sequences and some reference strains

Seq->	AY325495	JF317581	FJ388889	AY321987	HQ202970	DQ923524	DQ629117	EU148505	KU041859	JX535296	HM038017	NC_001792
SA3-BC	0.96	0.982	0.987	0.967	0.962	0.965	0.96	0.892	0.921	0.926	0.926	0.659
SA1-BA	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA2-BB	0.923	0.951	0.958	0.995	0.991	0.992	0.988	0.902	0.93	0.934	0.934	0.661
SA4-BD	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA5-BE	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA6-BF	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA7-BG	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA8-BH	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA9-BI	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA10-BJ	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA11-BK	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA12-BL	0.924	0.952	0.96	0.997	0.992	0.994	0.99	0.903	0.931	0.936	0.936	0.659
SA1-DA	0.899	0.924	0.923	0.939	0.934	0.939	0.934	0.899	0.994	0.998	0.998	0.652
SA2-DB	0.899	0.924	0.923	0.939	0.934	0.939	0.934	0.899	0.995	1	1	0.652
SA3-DC	0.897	0.923	0.921	0.937	0.933	0.937	0.933	0.899	0.995	0.997	0.997	0.654
EF394775	0.991	0.954	0.955	0.923	0.918	0.921	0.917	0.868	0.896	0.9	0.9	0.653
AY325495	ID	0.952	0.957	0.927	0.923	0.925	0.921	0.872	0.895	0.899	0.899	0.655
JF317581	0.952	ID	0.984	0.952	0.951	0.954	0.951	0.882	0.92	0.924	0.924	0.658

FJ388889	0.957	0.984	ID	0.96	0.958	0.961	0.961	0.885	0.919	0.923	0.923	0.658
AY321987	0.927	0.952	0.96	ID	0.992	0.994	0.99	0.906	0.934	0.939	0.939	0.659
HQ202970	0.923	0.951	0.958	0.992	ID	0.992	0.988	0.902	0.93	0.934	0.934	0.661
DQ923524	0.925	0.954	0.961	0.994	0.992	ID	0.99	0.906	0.934	0.939	0.939	0.661
DQ629117	0.921	0.951	0.961	0.99	0.988	0.99	ID	0.9	0.93	0.934	0.934	0.661
EU148505	0.872	0.882	0.885	0.906	0.902	0.906	0.9	ID	0.896	0.899	0.899	0.661
KU041859	0.895	0.92	0.919	0.934	0.93	0.934	0.93	0.896	ID	0.995	0.995	0.652
JX53529626	0.899	0.924	0.923	0.939	0.934	0.939	0.934	0.899	0.995	ID	1	0.652
HM038017	0.899	0.924	0.923	0.939	0.934	0.939	0.934	0.899	0.995	1	ID	0.652
NC_001792	0.655	0.658	0.658	0.659	0.661	0.661	0.661	0.661	0.652	0.652	0.652	ID

Appendix 6.1

Title of data: PPVs' sequences and other reference sequences previously reported, and used in

the molecular characterisation.

Description of data: Details of porcine parvoviruses' sequences used in Chapter six and other

reference sequences obtained from GenBank

Virus	Strain name	Accession number	Geographical location	Source
PPV1	PPV1-SA1	MG846605	South Africa	This study
	PPV1-SA2	MG846606	South Africa	This study
	PPV1-SA3	MG846607	South Africa	This study
	PPV1-SA4	MG846608	South Africa	This study
	PPV1-SA5	MG846609	South Africa	This study
	PPV1-SA6	MG846610	South Africa	This study
	PPV1-SA7	MG846611	South Africa	This study
	PPV1-SA8	MG846612	South Africa	This study
	PPV1-SA9	MG846613	South Africa	This study
	PPV1-SA10	MG846614	South Africa	This study
	PPV1-SA11	MG846615	South Africa	This study
	PPV1-SA12	MG846616	South Africa	This study
	PPV1-SA13	MG846617	South Africa	This study
	Kresse	U44978	Canada	GenBank
	LZ	HM627653	China	GenBank
	77	KP245936	China	GenBank
	Challenge	KF049426	Germany	GenBank
	NADL2	NC_001718	USA	GenBank
	VRI-1	AY390557	South Korea	GenBank
	5	KP245938	China	GenBank
	NADL-2_M2	KF913346	Hungary	GenBank

	J-PPV	KF742500	China	GenBank
	HN-I	KF429254	China	GenBank
	NADL2	KF049423	Germany	GenBank
	371-2_10	JX568157	China	GenBank
	WB-533	JQ249915	Romania	GenBank
	Stendal	JN400526	Germany	GenBank
	693a	JN400519	Germany	GenBank
	N	HM989009	China	GenBank
	JY	HM627652	China	GenBank
	Nanjing200801	FJ822038	China	GenBank
	S30	FJ643430	Brazil	GenBank
	ZJ	EU790642	China	GenBank
	NJ-2	AY788087	China	GenBank
	32-96	AY145472	Brazil	GenBank
	Mouse_PPV1	MPU12469	USA	GenBank
PPV2	PPV2-SA1	MG846618	South Africa	This study
	PPV2-SA2	MG846619	South Africa	This study
	PPV2-SA3	MG846620	South Africa	This study
	PPV2-SA4	MG846621	South Africa	This study
	GBGW1	KY018935	South Korea	GenBank
	B	KP245941	China	GenBank
	BR596	KF725661	Brazil	GenBank
	PPV2-18SRB	KC701293	Serbia	GenBank
	WB-620GR	JQ860244	Romania	GenBank
	CnPPV_JH13	GU938300	China	GenBank
	JPT68	AB916464	Japan	GenBank
	PPV2-238HU	KC701311	Hungary	GenBank
	H-1	AB076669	Myanmar	GenBank
	PPV2-648CRO	KC701307	Croatia	GenBank
	US-523	JX101462	USA	GenBank
	F2-1NB	JQ860239	Romania	GenBank

	US026607/1998	KF725662	USA	GenBank
	H-379	JN400861	Hungary	GenBank
	H-260	JN400857	Hungary	GenBank
	PPV2-VISRB	KC701312	Serbia	GenBank
	H-156	JN400852	Hungary	GenBank
	H-255	JN400854	Hungary	GenBank
	PPV2-92HU	KC701298	Hungary	GenBank
	642-CRO	KC687100	Croatia	GenBank
	BR/GO/ion_09_PPV-2/2011	KM926355	Brazil	GenBank
	CnPPV_YH14	GU938301	China	GenBank
	PPV2-VIISRB	KC701297	Serbia	GenBank
	655-CRO	KC687098	Croatia	GenBank
	WB-763S	JQ860246	Romania	GenBank
PBo-likeV	PBo-likeV-SA1	MG846627	South Africa	This study
	PBo-likeV-SA2	MG846626	South Africa	This study
	PBo-likeV-SA3	MG846625	South Africa	This study
	PBo-likeV-SA4	MG846624	South Africa	This study
	PBo-likeV-SA5	MG846623	South Africa	This study
	PBo-likeV-SA6	MG846622	South Africa	This study
	Swebo_1	FJ872544	Sweden	GenBank
	Buk8_1	JX854557	Uganda	GenBank
	SX	HQ223038	China	GenBank
PBoV	PBoV-SA1	MG846652	South Africa	This study
	PBoV-SA2	MG846651	South Africa	This study
	PBoV-SA3	MG846650	South Africa	This study
	PBoV-SA4	MG846649	South Africa	This study
	PBoV-SA5	MG846648	South Africa	This study
	PBoV-SA6	MG846647	South Africa	This study
	PBoV-SA7	MG846646	South Africa	This study
	PBoV-SA8	MG846645	South Africa	This study
	PBoV-SA9	MG846644	South Africa	This study

PBoV-SA10	MG846643	South Africa	This study
PBoV-SA11	MG846642	South Africa	This study
PBoV-SA12	MG846641	South Africa	This study
PBoV-SA13	MG846640	South Africa	This study
PBoV-SA14	MG846639	South Africa	This study
PBoV-SA15	MG846638	South Africa	This study
PBoV-SA16	MG846637	South Africa	This study
PBoV-SA17	MG846636	South Africa	This study
PBoV-SA18	MG846635	South Africa	This study
PBoV-SA19	MG846634	South Africa	This study
PBoV-SA20	MG846633	South Africa	This study
PBoV-SA21	MG846632	South Africa	This study
PBoV-SA22	MG846631	South Africa	This study
PBoV-SA23	MG846630	South Africa	This study
PBoV-SA24	MG846629	South Africa	This study
PBoV-SA25	MG846628	South Africa	This study
PBoV1B37	JX869079	Cameroon	GenBank
A6	HQ291309	China	GenBank
PBoV2 pig/ZJD/China/2006	HM053694	China	GenBank
PBoV1 pig/ZJD/China/2006	HM053693	China	GenBank