DISSERTATION

The epidemiology of the 2016-2017 African Swine Fever outbreaks in the North West and Free State provinces of South Africa.

By

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STUDENT NUMBER: 36851728

Submitted in partial fulfilment of the requirements for the degree of

MASTER OF SCIENCE IN AGRICULTURE

in the Department of Agriculture and Animal Health

UNIVERSITY OF SOUTH AFRICA

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November 2022

A. DECLARATION

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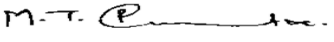
I hereby certify that I am the sole author of this dissertation and that no part of this dissertation or the dissertation has been submitted for a degree to any other university or institute.

I declare that this is a true copy of my dissertation, including any final revisions, as approved by my dissertation review committee.

SIGNATURE:  **DATE:** 21 November 2022

B. DEDICATION

I would like to dedicate this dissertation to my husband Graham Rametse and my two beautiful daughters, Oarabile and Bokang Rametse. It has not been an easy journey. We have missed so many bonding sessions for the sake of my studies and for that, I just want to say thank you for all the sacrifices. Your presence in my life has given me courage whenever I felt like giving up.

To my parents, my father, Thomas Maserumule, and my mother, Dolly Maserumule, thank you for believing in me and for making me realize how important education is. Your words of encouragement have kept me focused and I could not afford to disappoint you. Lastly, thank you for raising me in the fear of the Lord and for teaching me faith for the past 40 years of my life.

C. ACKNOWLEDGEMENTS

Firstly, I would like to thank God my creator, Jesus Christ, for granting me life and allowing me to walk this uncertain path. The postgraduate program is a journey that cannot be undertaken alone. I wish to express my appreciation to the following individuals, Dr Donald Sibanda, my previous supervisor for beginning the journey with me. Thank you for all your words of encouragement and to Dr Livio Heath, I am grateful for the funding and for granting me the opportunity to pursue my degree. Thank you for your assistance with the back-and-forth tracing of African Swine Fever (ASF) outbreaks and for providing me with secondary data on the outbreaks when needed. I just want to appreciate your guidance, patience, and wisdom. Your expertise has truly built my confidence. Dr Prudence Kayoka-Kabongo, where do I begin, thank you for allowing me the opportunity to complete my studies. You took a role of a mother and assured me that I will complete my studies when you assumed the new role of being my supervisor and I just want to say thank you. Your drive and energy have put a big smile on my face. I consider myself a fortunate student to have met you. Thank you for your useful comments on this work.

I am grateful to my employer, Agricultural Research Council for granting me the opportunity to further my studies. I am grateful to Veterinary officials from the Department of Agriculture, Land Reform, and Rural Development (DALRRD) formerly known as the Department of Agriculture, Forestry and Fisheries (DAFF) in the North West, Free State, and Northern Cape Provinces for organizing farm visits during the back and forward tracing of African Swine Fever (ASF) outbreaks and tick sampling period. Department of Veterinary Tropical Diseases – Faculty of Veterinary Sciences - University of Pretoria (Professor Robert Swanepoel, Dr Jannie Crafford, and Mr Anthony Craig) I thank you for the days spent together during the field trips when we sampled ticks in the extremely hot weather conditions. Professor Swanepoel thank you for capturing great memories and taking beautiful pictures. A special thanks to Dr Juanita van Heerden for reviewing this dissertation. Lastly a special thanks to my fellow postgraduate students and colleagues at TADL/OVI for the great chats and encouragement.

D. ABSTRACT

African Swine Fever (ASF) is a viral disease, which is endemic in most sub-Saharan African countries with warthog and tick vector (*Ornithodoros*) acting as biological reservoirs of the virus. A high mortality rate is evident among domestic pigs, and international trade becomes negatively affected including swine products. The African Swine Fever outbreak was confirmed by laboratory results issued on the 6th of June 2016. It occurred in the North West Province where smallholder farmers reported deaths in the free-roaming pigs. During the same period, another suspected outbreak of ASF was reported on a farm near Koffiefontein in the Free State.

This project aimed at establishing the source of the ASF outbreak in the Free State and North West provinces by conducting an epidemiological assessment, investigating possible risk factors, and assessing the role played by the sylvatic cycle. Face-to-Face interviews and direct observations were used to collect primary data. Affected pigs (*n*=2 539) were recorded in both provinces. Pigs that succumbed to the ASF virus were (*n*=880) in Free State and (*n*=664) in North West province whereas pigs that were culled, in both provinces, were respectively (*n*=571) and (*n*=424). Blood samples from live pigs comprised sera (*n*=174), blood on ethylene diamine tetra-acetic acid (EDTA) (*n*=67), and from dead pigs, tissue samples (*n*=44) were submitted to the laboratory for analysis.

Samples from warthogs namely, EDTA blood (*n*=2), sera (*n*=9,) and tissue (*n*=10) were also submitted to the laboratory. Methods used included virus isolation, enzyme-linked immunosorbent assay (ELISA), and PCR. Antibody ELISA was used to assess exposure to the African Swine Fever virus (ASFV). The polymerase chain reaction test was used to quantify the prevalence of pig exposure to the ASFV using tissue and EDTA samples. Blood samples on EDTA from domestic pigs (*n*=67) yielded 23 out of 67 samples (34%) positive for ASFV which suggests that healthy pigs carrying ASFV existed in the affected area. Twenty-six out of 67 (39%) tested negative with the rest, 18 out of 67 (27%) not tested. The tissue samples (*n*=44) revealed that 23 out of 44 (52%) tested positive and 21 out of 44 (48%) were negative for ASFV. Of the serum samples (*n*=174), 18 out of 174 (10%) were positive for antibody detection and 138 out of 174 (79%) were negative.

From assessments, EDTA samples (*n*=2) revealed that both samples 2 out of 2 (100%) were positive for ASFV, and tissue samples from warthog's carcasses (*n* = 10) revealed that 9 out of 10 (90%) were positive while 1 out of 10 (10%) was negative for ASFV detection. Lastly, serum samples (*n* = 9) revealed that 7 out of 9 sera (78 %) tested positive while 2 out of 9 sera (22%) tested negative for antibodies against ASFV. To establish the involvement of the sylvatic cycle, sampling of warthog burrows with *Ornithodoros moubata* was carried out. A total of 88 ticks were recovered from the burrows and the laboratory results demonstrated that 10 out of 88 tick samples (11.4%) collected from warthog burrows in Koffiefontein in the Free State tested positive for ASFV deoxyribonucleic acid (DNA), 68 out 88 (77.3%) were negative, and 10 out of 88 (10.4%) yielded inconclusive results. The positive results indicated a possibility that the sylvatic cycle has contributed to the dissemination of the ASFV. In addition, the questionnaire and farm observations revealed a lack of biosecurity as a major concern.

Keywords: ***Pigs, warthogs, African Swine Fever, sylvatic cycle, biosecurity, virus isolation, PCR, ELISA.***

E. LIST OF ABBREVIATIONS

°C	Degree Celsius
%	Percent
≥	Greater than or equal to
≤	Less than or equal to
µL	microliter
µM	micrometer
AHT	Animal Health Technician
AHTs	Animal Health Technicians
ASF	African Swine Fever
ASFV	African Swine Fever Virus
ARC	Agricultural Research Council
Bp	Base position
BHK	Baby Hamster Kidney
CAES	College of Agriculture and Environmental Sciences
CCR	Central Conversed Region
Co-op	Cooperative
DAFF	Department of Agriculture, Fisheries, and Forestry
DALRRD	Department of Agriculture, Land Reform, and Rural Development
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra-acetic Acid
ELISA	Enzyme-Linked Immunosorbent Assay
ESHPF	Emerging Smallholder Pig Farmers
ESSF	Emerging Small-Scale Farmers

ESSPF	Emerging Small-Scale Pig Farmers
FAT	Fluorescent Antibody Test
FS	Free State
FSP	Free State Province
GPS	Global Positioning System
HREC	Human Research Ethics Committee
HAD	Haemadsorption
ISO	International Organization for Standardization
ITRs	Inverted Terminal Repeats
kb	Kilobase
Kbp	Kilobase pairs
Km	Kilometer
LSCPF	Large Scale Commercial Pig Farmers
mm	millimeter
ml	milliliters
MS	Microsoft
MSCPF	Medium Scale Commercial Pig Farmers
NC	Negative Cut
NCP	Northern Cape Province
NJ	Neighbour Joining
nm	Nanometer
NW	North West
NWP	North West Province
OD	Optical Density

OIE	Office International des Epizooties
OVI	Onderstepoort Veterinary Institute
PBS	Phosphate Buffered Saline
pmol	Picomole
PC	Positive Cut
PCR	Polymerase Chain Reaction
pH	Potential Hydrogen
PTY LTD	Proprietary Limited
qPCR	quantitative Polymerase Chain Reaction
rPCR	Real-time Polymerase Chain Reaction
SA	South Africa
SOP	Standard Operating Procedure
Spp	species
TADL	Transboundary Animal Disease Laboratory
UNISA	University of South Africa
VI	Virus Isolation
vp72	Virus Protein 72

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CHAPTER 1:

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

African Swine Fever (ASF) is a highly contagious viral disease that affects both wild and domestic pigs, whose mortality rate can reach 100%. It is a controlled and notifiable disease to South Africa and the World Organization for Animal Health (OIE); this disease has trade repercussions when it is reported. African Swine Fever is caused by a large and complex double-stranded DNA virus that replicates preferentially in monocyte and macrophage host cells, causing symptoms such as high fever, loss of appetite, and hemorrhages in the skin and internal organs, as well as a high mortality rate in previously unexposed pig herds (Blome *et al.,* 2013). Infections with this virus can cause clinical disease in domestic and feral pigs and wild boar (*Sus scrofa*), or an asymptomatic carrier state in wild swine in eastern and southern Africa, specifically the warthog (*Phacochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*). ASF is undoubtedly one of the most significant constraints to African pig production (www.dalrrd.gov.za).

The disease is not a danger to human health though it has devastating effects on pig populations and the farming economy. Currently, there is no vaccine or treatment for ASFV. The virus is highly resistant to the environment, which means it can survive on clothes, boots, wheels, and other materials. It can also survive in pork products such as ham, sausages, and bacon. As a result, if appropriate precautions are not taken, human behavior can play a significant role in the spread of this pig disease across borders. In many countries, pigs have become a primary source of income, and every time there is an ASFV outbreak, families are left devasted. ASF is still spreading around the world, threatening pig health and welfare (www.woah.org).

The wild suids are thought to be the original vertebrate host of the African Swine Fever virus (ASFV), acting as the virus' reservoir. However, warthogs are thought to be the most important reservoir due to their wide distribution and ease of contact with *Ornithodoros* genus soft ticks as well as domestic pigs to spread the disease (Costard *et al*., 2013). Soft ticks are an important vector for the ASFV because they share a sylvatic cycle with warthogs, making it impossible to eradicate the virus in nature

(Penrith *et al*., 2013). The disease can be successfully controlled and eradicated in domestic pig production systems by eliminating virus contact.

1.2. LITIRATURE REVIEW

1.2.1. History and geographical distribution

Since 1912, outbreaks displaying the same disease symptoms were recorded in the surrounding of Chipata, formally known as Fort Jameson, in the Eastern Province of Zambia (Wilkinson *et al.,* 1988). ASF was described for the first time in the 1920s in Kenya, causing a mortality rate of 100 percent. The virus carried by the warthogs was identified as the reason for the occurrence of the disease and no clinical signs were observed (Montgomery, 1921). In South Africa, ASF was first described in 1928 from pigs in Modimolle which is situated in the northern-eastern part, followed by Angola in 1932 and Malawi in 1934 (De Kock *et al.,* 1940; Scott, 1965). For a few years, several outbreaks continued to occur in the Modimolle area (Penrith, 2013).

Because of the sylvatic cycle maintained by the presence of warthogs in certain areas where the disease was endemic, South Africa declared those areas “controlled” in 1935. These areas included parts of the Limpopo, Mpumalanga, North West, and KwaZulu-Natal provinces (Penrith, 2013; Magadla *et al*., 2016). Various districts in the Northern Transvaal now called Limpopo reported a total of ten ASF outbreaks between 1935 and 1938 and these outbreaks were due to warthog contact or swill feeding (Pini and Hurter, 1975; DAFF Annual Reports, 2018a, 2018b). In South Africa, the disease remains controlled under the Animal Diseases Act, of 1984 (Act 35 of 1984) (Janse van Rensburg *et al.,* 2020a).

By the 1950s, the disease was known to exist in most countries in eastern and southern Africa including the southern parts of Central Africa (Plowright *et al.,* 1994). Until the late 1950s, when the invasion occurred in both Europe and West Africa, commencing the first international events in the history of ASF, the disease remained restricted to this region. In 1951, there were reports in South Africa of three outbreaks in Pietersburg, Soutpansberg, and Letaba districts. Other parts of Limpopo and Mpumalanga provinces, formerly known as the Northern and Eastern Transvaal reported seventeen more ASF outbreaks between 1953 and 1962. Until 1973, there

were no further outbreaks reported (Pini and Hurter, 1975; DAFF Annual Reports, 2018a).

Botswana reported ASF outbreaks in 1953, 1987, and 1999 and these outbreaks were likely due to warthog contact. The first case outside the boundaries of Africa occurred in Portugal in 1957, it was due to waste coming from an airline flight at Lisbon airport, and it was fed to the pigs (Costard *et al.,* 2009). At a later stage, the disease was successfully eradicated. In 1959, the capital of Senegal in West Africa experienced the first ASF outbreak (Bastos *et al.,* 2003). Other ASF reports were reported in Guinea Bissau as well as the Island nation of Cape Verde (Brown *et al.,* 2018). Sub-Saharan Africa had thirty-four countries experiencing at least one confirmed case of ASF outbreak and the most recent outbreak was confirmed in Sierra Leone (Wadoum *et al.,* 2020).

Lisbon experienced another outbreak in the year 1960, until the mid-1990s. Thereafter, they were several countries in Europe, which were affected by the ASF outbreaks, and those countries included France in 1964 and 1967. In the late 1970s, ASF continued to be reported in the Caribbean Island countries (Costard *et al.,* 2009). South Africa continued to experience more outbreaks and between 1973 and 1974, eighteen ASF outbreaks were reported. The first outbreak was from Letaba district, the second outbreak from a farm situated 35 km from the first focus area; the third outbreak was from Pietersburg District, the fourth outbreak was again reported in Letaba District, and the fifth outbreak was reported in White River District and the last outbreak was reported in Thabazimbi District. During the period of these outbreaks, almost 4,000 pigs were either culled due to ASF or succumbed to ASF (DAFF, 2018a, 2018b; Pini and Hurter, 1975).

Two more European countries continued to be affected by the ASF outbreaks namely, France in 1977 and Malta in 1978, and 1980. The African Swine Fever outbreaks, which occurred in Brazil, in the year 1978, was through food waste probably from Spain and Portugal, carried by transcontinental flights, and/or animal products, imported by tourists (Lyra, 2006). The date of the last reported case was in 1981. In 1982, the African Swine Fever virus was introduced into Sardinia (Italy) and it has since remained endemic (Plowright *et al.,* 1994). Europe continued to experience

further ASF outbreaks and the following two countries were affected namely, Belgium in 1985 and the Netherlands in 1986. The disease continued to spread and was later introduced to Indian Ocean Islands as well as Madagascar in 1998 (Roger *et al.,* 2001) and Mauritius in 2007 (OIE, 2009).

South Africa reported the first outbreak of ASF outside the controlled area in 1996 around the town of Bela-Bela, Warmbaths region in Limpopo Province (Magadla *et al*., 2016). It was an isolated event, suspected to have been caused by illegally moving domestic pigs from ASF-controlled areas; the outbreak did not spread beyond the index farm (Penrith and Vosloo, 2009). The region of Caucasus in Georgia had an introduction of ASF in the year 2007 and the introduction was due to ASF's continued transcontinental spread. The neighbouring countries were affected by the widespread ASF introduction, which was due to the delay in recognizing the disease and other countries including Armenia, Azerbaijan, and other several territories in Russia.

The reported epidemic in Russian is from the territories of Chechnya, North Ossetia-Alania, Ingushetia, Orenburg; the Stavropolskiy Kray (Stavropol), the Krasnodarskiy Kray (Krasnodar) which went further westwards into the Rostovskaya Oblast; these territories have common borders with Ukraine. Several occasions of infections in wild boar did complicate the eradication of the disease (Beltran-Alcrudo *et al.,* 2008; OIE, 2009). In 2009, Namibia reported ASF outbreaks in a backyard farm, prison, and villages in the Omusati and Oshana districts near the border with Angola (Simulundu *et al*., 2017). The spread of the virus could have possibly been due to infectious pigs and meat from pigs (pork). However, according to the reports, warthog involvement was suspected (Penrith, 2020).

There was an occurrence of two epidemic episodes in South Africa, which occurred outside the controlled area, the first occurrence was in 2012. It was linked to an illegal movement of pigs, which were coming from ASF controlled areas to an auction and the domestic cycle was subsequent spread (Geertsma *et al.,* 2012; Janse van Rensburg *et al.,* 2020b). The second epidemic was in 2016 and 2017 respectively (DAFF, 2018a). ASF outbreaks in domestic pigs from controlled areas in South Africa seem to be linked to the sylvatic cycle (Janse van Rensburg *et al*., 2020c).

In 2015, Zimbabwe experienced an outbreak of ASF in Mashonaland Central province in villages, which are close to the border with the Tete Province of Mozambique, and this was after the disease has been absent since 1992. These outbreaks were attributed to the movement of infected pigs and pork including disposing of the carcasses negligently (van Heerden *et al*., 2017). The Chinese government first reported in August 2018 that ASF outbreaks had occurred on a farm in Shenyang City, Liaoning Province (Ge *et al*., 2018; Zhou *et al*., 2018; Li and Tian, 2018). Within one month, all the pigs on the farm died of the first clinical signs leading to the farm, being abandoned (Zhou *et al.,* 2018). More than one million pigs were culled across china since the first report and 32 provinces were affected by over 160 outbreaks (FAO, 2019).

The culling strategy was implemented to lessen the spread of the disease and within three months, the whole country of China was affected by the outbreak (Yun, 2020). Related outbreaks were reported in free-ranging pigs in Manicaland Province in January 2019, bordering Manica Province in Mozambique. The Mashonaland Central experienced another ASF outbreak in August/September 2019 (OIE, 2019). African Swine Fever continued to spread across borders in 2019, spreading to Vietnam, Mongolia, Cambodia, the Democratic People's Republic of Korea, the Lao People's Democratic Republic, Myanmar, the Philippines, the Republic of Korea, Indonesia, Timor-Leste, as well as in 2020 to India and Papua New Guinea (Weaver and Habib, 2020).

African Swine Fever outbreaks occurred in several parts of the free areas in three provinces between 2016 and 2019 namely, Northern Cape, North West, and the Free State (Janse van Rensburg *et al.,* 2020b). An epidemic of ASF in domestic pigs was identified in controlled areas, it occurred in Mpumalanga and Gauteng in 2019/2020 respectively. South Africa continued receiving new ASF cases in free areas post 2019 outbreaks and the following provinces were affected in 2020, namely, Eastern Cape, Free State, and North West. During the last week of January 2020, East Siang and Papum Pare districts of Arunachal Pradesh State of India observed a disease outbreak in domestic pigs with unusual mortality. Subsequently, there were similar disease outbreaks in pigs with high mortality in five districts of the neighbouring state, Assam (Rajukumar *et al*., 2021). It was reported that the local people in the Pasighat region

saw dead wild boars in the drainage/rivulets suspecting they could have contaminated the habitat (Patil *et al.,* 2020).

In general, pig production and animal agriculture are globally important for economic activities (Roelofse, 2013; Mokoele *et al.,* 2015). In terms of the overall South African agricultural sector, pork is one of the smallest industries contributing around 2.1% of the primary agricultural sector. South Africa has about 4000 commercial producers, 100 smallholder farmers, and 19 stud breeders. Pig numbers were estimated at 1.512 million in the year 2016 and this is a decrease of 1.6% compared to 2015. In addition, in 2016, Statistic SA found in their community survey on agricultural households that the number of households keeping pigs in South Africa increased from 112 678 in 2011 to 210,504 (Lehohla, 2016).

By the year 2016, Limpopo and North West provinces were the largest producers of pork (Figure 1.1). The pig farmers in South Africa consist of the following categories namely, the back-yard pig farmers who are subsistence farmers keeping indigenous breeds, using swill for feeding, and at times with minimal or no supplementation. The emerging small-scale pig farmers (ESSPF) or emerging smallholder pig farmers (ESHPF) are focusing on keeping pigs both for subsistence and commercial purpose (FAO,2010). The term emerging small-scale farmers (ESSF) is a term used to define previously underprivileged/disadvantaged farmers who are determined to become semi-commercial/commercial farmers (National Department of Agriculture, 2006).

The ESSPF requires an extension service for skills development in agricultural project operations (Sekokotla, 2012). Chikazunga *et al*., (2007) stated that although this group of farmers consumes a portion of its produce, they produce mainly for commercialisation, breeding a specific type of pigs with the ability to grow them. In South Africa, the ESSPF may have 1 to 50 sow units. Through multifaceted transport and marketing systems, pork is supplied to local markets and distant urban markets. The other category of pig farmers is the medium-scale commercial pig farmers (MSCPF) who are breed specific and have over 50 and up to 250 sows. Lastly, there are large-scale commercial pig farmers (LSCPF) operating with the abattoirs on a contract basis having more than 250 sows within a unit (Mokoele, 2015).

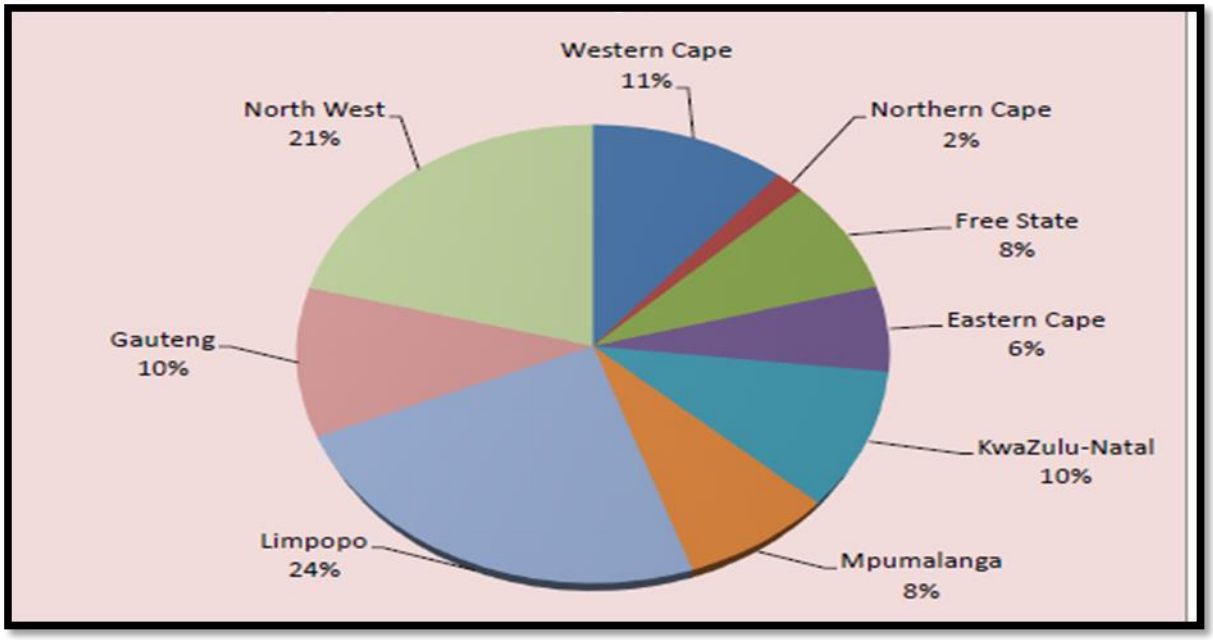

Figure 1.1: Distribution of Pigs per Province in 2016.

Source: courtesy of www.daff.gov.za

In sub-Saharan Africa, the major limiting factor for pig producers that has been recognised is ASF, though there are more serious constraints that have been discovered like the lack of proper and suitable feed at an affordable price. The disease challenge has been seen in many countries, which also includes poor quality or inaccessibility to animal health services, the value of housing pigs, poor genetic breeding stock, and a lack of understanding of pig production (Mashatise *et al*., 2005; Nwanta *et al*., 2011).

1.2.2. Aetiology of African Swine Fever virus disease or causative agent of African Swine Fever virus disease.

Figure 1.2 describes the structure of the African Swine Fever virus, ASFV is a large, enveloped virus with a genome of approximately 190 kbp of linear double-stranded DNA. ASFV belongs to the Asfivirus genus and the Asfarviridae family. The ASF virion has a complex icosahedral structure surrounded by membrane layers and measures about 200 nm in size. The viral core is made up of a nucleoprotein surrounded by a matrix protein. An inner membrane capsid layer surrounds the core and matrix. The capsid layer, made up of capsid (p72) protein, has icosahedral symmetry and is surrounded by an external membrane derived from the infected cell's plasma

membrane. ASFV is infectious even when the external membrane is absent (Yoo *et al.,* 2020)

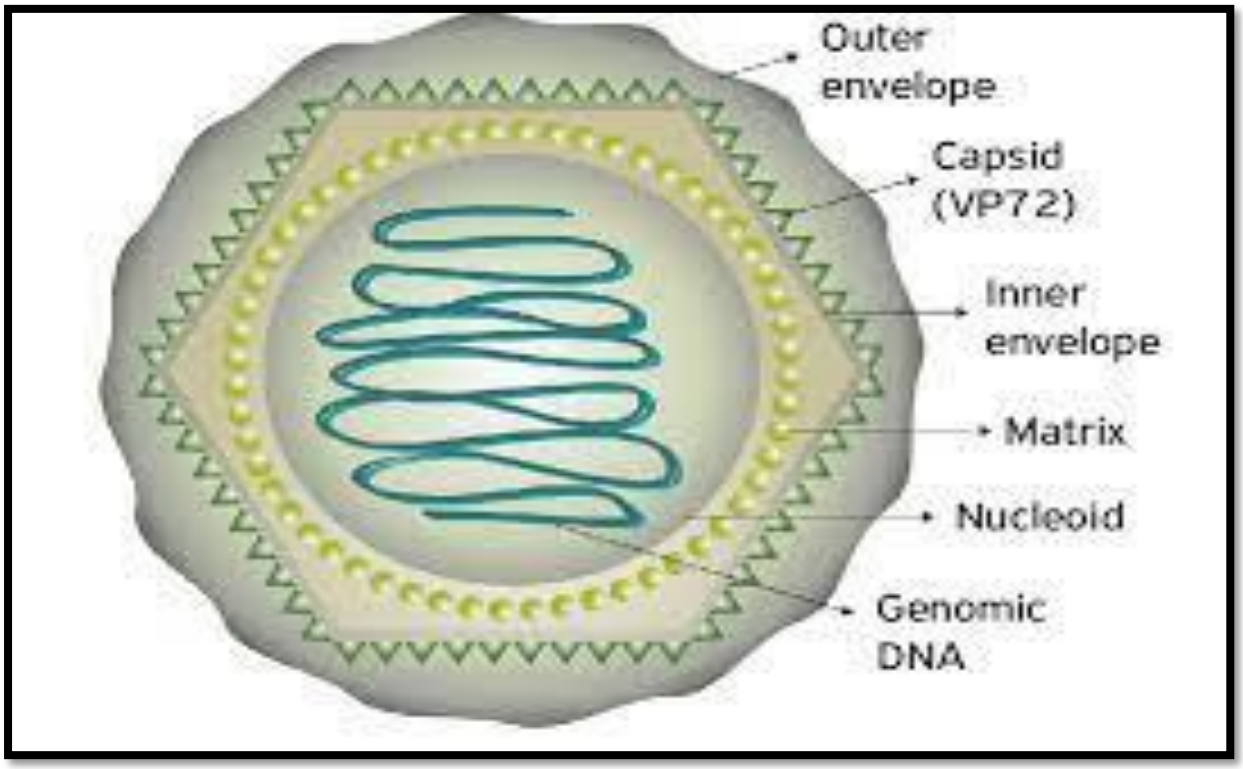

Figure 1.2: Schematic composition and structure of the African Swine Fever virus (Freitas and Lyra, 2018).

Source: courtesy of www.researchgate.net/figure

1.2.3. VIRUS SURVIVAL

1.2.3.1. In the environment

McVicar, (1984) stated that a large amount of the virus is contained in faeces coming from ASFV-infected pigs. The virus may remain viable in faeces for at least 11 days (FAO, 2000). ASFV may continue to be present in the blood, surviving at a temperature of 37°C for a month and in tissues for longer periods, such as excretions and secretions of infected pigs namely blood, urine, or saliva (Gallardo *et al.,* 2015). Davies *et al*. (2017) stated that urine might contain the viable virus for up to 15 days at 4°C, 5 days at 21°C, and 2 to 3 days at 37°C. To reduce the risk of contaminating the environment, the following disinfectants can be used for ASFV inactivation namely, 2% sodium hydroxide, detergents and phenol substitutes, sodium or calcium hypochlorite (2 to 3% chlorine), and iodine compounds (FAO, 1999).

Maintenance of ASFV can be prolonged in areas with large pig populations because of a constant supply of susceptible pigs (Penrith and Vosloo, 2009). Other maintenance of the virus can be infected pork meat moved illegally, pigs held with poor biosecurity, and wild boars gathering around feeding sites (Estrada-Pena *et al.,* 2010). EFSA *et al*. (2018) and Nurmoja *et al.* (2018) stated that the introduction of the virus into pig farms can occur through virus-contaminated materials such as grass, clothing, bedding, and vehicles and it has widely been reported in the spread of ASFV (Kleiboeker, 2008).

1.2.3.2. In animal products

Infective quantities of ASFV may be found in raw and frozen pork, as well as smoked, salted, and dried pork (Mebus *et al.,* 1997). In heat-treated products (at least 60°C for 30 min), the virus becomes inactivated. After the processing of the fresh meat has started, 140 days after processing commercial products (such as ham or cured pork loin) they will contain an ineffective virus (Sánchez-Vizcaíno, 2010). Undercooked pork, dried and, smoked pork, and carcass meal derived from pigs are considered potentially dangerous if fed to pigs.

1.2.3.3. In the host

Before any clinical signs can appear, there will be an infective amount of the virus shed by the domestic pigs and it occurs between the periods of 24 to 48 hours after infection with the ASF virus. During the acute stages of the disease, a high level of the virus become present in the blood and tissues whereby the extent amount of the virus gets shed in all secretions and excretions. Pigs that survive the acute phase of the disease remain infected for several months however, they do not shed the virus for more than 30 days (Beltrán-Alcrudo *et al.,* 2008).

In wild suids, the infective levels of the virus are found only in the lymph nodes. Other tissues are unlikely to contain infective levels of the virus for more than a period of two months after infection. The exact length of time, in which the infective levels of the virus are maintained in the lymphoid tissues of either the wild suids or domestic pigs, is not known and it might be due to individual variation (Geering *et al.,* 2001).

1.2.4. AFRICAN SWINE FEVER CONTROL MEASURES

1.2.4.1. General control measures

Factors, which contribute to the control of ASF being a challenge is the lack of effective vaccines and treatment including the presence of arthropods (Penrith *et al*., 2004). The existence of the sylvatic cycle prevents an effective eradication of ASF. Countries that have a high likelihood of eradicating the disease are those with sporadic outbreaks and no arthropod vectors as part of the transmission cycle. The enforcement of strict sanitary measures, speedy laboratory diagnosis, stamping out procedures, and rigorous movement control of both live pigs and pig products are the basis of controlling ASF (Agüero *et al.,* 2004; Lubisi *et al.,* 2009).

The spread of ASF can be avoided by a swift detection of the infected animals, which also reduces the possible transmission of the virus to uninfected animals (Agüero *et al.,* 2004). The spread of the disease can be limited by preventing contact between the warthogs, their burrows, and domestic pigs; this approach has proven to be successful (Penrith and Vosloo, 2009). Sporadic outbreaks may develop in endemic areas, where the virus spreads from infected ticks or warthogs to domestic pigs (Blood and Rodastitis, 1989), and control measures such as quarantine, culling the infected and in-contact pigs, and proper disposal of carcasses could be implemented.

Penrith and Vosloo, (2009), stated that South Africa has been implementing the control of ASF in the controlled areas as per the current animal diseases Act 35 of 1984 and commercial farming in the controlled zones is discouraged. In areas where ASF occurs, movement control, which includes control of the movement of animals, and products, which are within the control zone (Figure 1.3), and from the control zone to the free areas, strict infrastructure requirements, husbandry, and practices should be adhered to. To prevent the exposure of pigs to wild pigs or ticks, enclosures with pig proof and a double perimeter fence or concrete wall consisting of concrete floors should be implemented. Veterinary authorities are to be informed of all sicknesses and mortalities immediately. A study was carried out to examine the occurrence of ASF in domestic pigs in the controlled area of South Africa starting from 1977 to 2017 (Janse van Rensburg *et al.,* 2020a). It revealed that there were 59 reported ASF outbreaks in pigs which included farmed European wild boars that are susceptible to ASF, and

excluded African wild suids, which are resistant to the pathogenic effects of ASFV. It was reported that 4,031 pigs were at least affected by the outbreaks (Janse van Rensburg *et al.,* 2020a). Of the 59 reported outbreaks, 55 (93%) were reported in Limpopo, 3 (5%) were in North West, and only one (2%) was in Mpumalanga Province. ASF outbreaks were reported in domestic pigs within the controlled area by fifteen local municipalities. Most of these outbreaks happened in the north-western part of the controlled area, with the local municipalities of Thabazimbi, Lephalale, and Musina most affected, with more than half of the outbreaks occurring within these municipalities (Janse van Rensburg *et al.,* 2020a).

There are control measures for pigs, which are kept in ASF-controlled areas. The measures are prescribed in terms of the Animal Diseases Act, 1984 (Act 35 of 1984), pigs are required to be kept in pig-proof housing, and should an ASF outbreak occur the property is quarantined, and no pigs or pig products are allowed to leave the property. Janse van Rensburg *et al*. (2020b) further mention that a biosecurity breach can result in transmission to domestic pigs. Successful eradication of the disease has been seen in Portugal (1993) and Spain (1995) (Costard *et al.,* 2009). The success was due to rigorous detection and slaughter programmes which were accompanied by compensation.

1.2.4.2. Surveillance

Disease control needs epidemiological understanding through disease surveillance systems (Doherr and Audige, 2001; Hasler *et al.,* 2011). Surveillance systems in countries with low income where the disease is endemic are often dysfunctional and inefficient (Perry and Grace, 2009; de Balogh *et al.,* 2013). These failures are due to contributing factors such as deteriorated administrative services, continuous cuts in the budget, and shortage of veterinary professionals (Bendali, 2006). The much more considered, appropriate method of surveillance for acute infectious diseases with high mortality rate like ASF is passive surveillance and to be effective, compliance on reporting from everyone involved is very important, from the farmer to the concerned authority. High levels of participation from the community members and the benefits of surveillance at the first level of the chain improve reporting (Goutard *et al.,* 2015; Brookes *et al.,* 2017). Detailed instructions for dealing with reported outbreaks were

provided in the laws and regulations on how to handle reported outbreaks for low-income countries, including compulsory culling, quarantine, and trade regulations; however, compensation to farmers for losses incurred as a result of outbreaks or control efforts is rarely or never included. (Perry and Grace, 2009; Halliday *et al.,* 2012). Enforcing existing regulations due to lack of capacity is common (Halliday *et al.,* 2012). Some of the contributing factors include peer stigmatization, distrust of government officials, and a lack of disease awareness are other frequent disincentives for reporting (Halliday *et al.,* 2012; de Balogh *et al.,* 2013). True incentives in reporting disease outbreaks are rare in these settings. To overcome the challenge of ASF surveillance in low-income countries, alternative surveillance methods are required (Doherr and Audige, 2001).

1.2.4.3. Biosecurity

Currently, biosecurity is the only option farmers must implement to protect their pigs against ASF (Sanchez-Vizcaino *et al.,* 2015b). In some instances, there are largely non-existent farm biosecurity measures within the smallholder subsistence farming systems, which dominate in countries with low income. Even when the enclosure of the pigs exists, pigs will at least for part of the year still be found roaming freely. Pigs can be allowed to freely move without any physical restrictions during the day or even scavenge for days or months, this will only depend on the country and local practices. Piglets are frequently discovered outside the pens (Dione *et al.,* 2014; Ikwap *et al*., 2014).

Domestic pigs roam freely in Kenya, covering up to 10,000 m^2 in 24 hours, and they spend a lot of time outside the farmhouses (Thomas *et al*., 2013). Apart from the immediate measures of on-farm biosecurity in the smallholder pig production value chain, there are many critical points regarding biosecurity. The critical point includes the middleman who enters farms to buy pigs from farmers to sell immediately or later for slaughter, pigs being resold as live pigs, middlemen, and butchers maintaining their pig herd, unregulated and uncontrolled transportation, trade, slaughter, and inappropriate waste disposal, insufficient slaughter facilities, as well as a lack of veterinary control over live pigs, meat, and slaughter (Dione *et al.,* 2014; Barongo *et al.,* 2015).

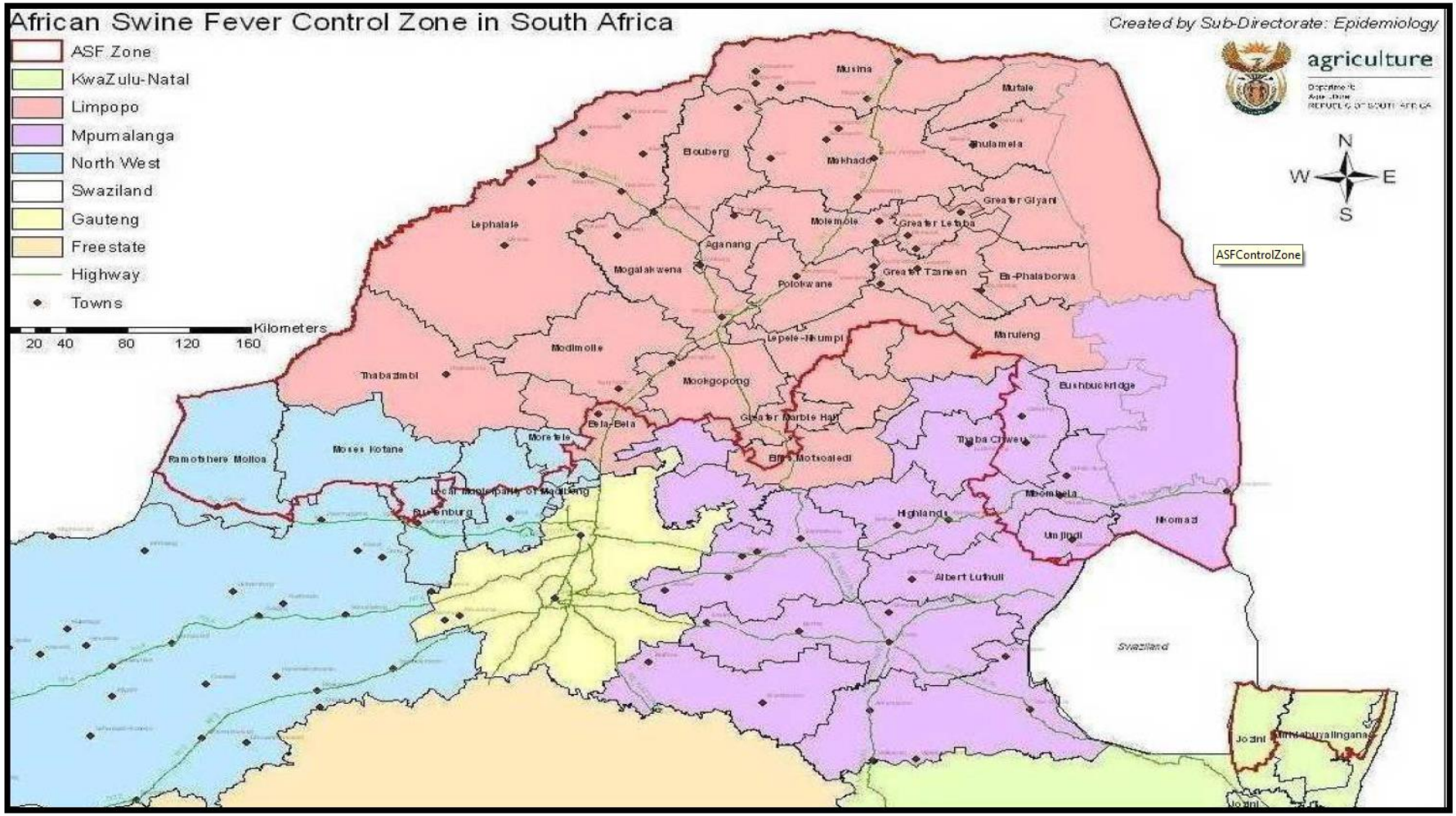

Figure 1.3: African Swine Fever Control Zone in South Africa

Source: courtesy of www.dalrrd.gov.za

1.2.5. EPIDEMIOLOGICAL FEATURES

1.2.5.1. *Ornithodoros* ticks

There are seven species of *Ornithodoros* ticks which comprise *O.savignyi, O. moubata, O. coriaceus, O. turicata, O. puertoricensis, O. parkeri, and former O. erraticus (Carios erraticus)*. Roger *et al.* (2001) state that the *Ornithodoros are* widely distributed in central, southern, and eastern Africa, and the Islands of Madagascar. *Ornithodoros moubata* is classified into four species with different hosts namely, *O. campactus* (tortoises), *O. apertus* (porcupines), *O. moubata* (Various hosts)*, and O. porcinus* (Warthogs) (Walton, 1967). *O. moubata and O. porcinus* are denoted by a sylvatic cycle. These species have a strong resistance to starvation with a life span of up to 15 years and persistence of infection for up to 5 years (Rennie *et al*., 2001).

The *Ornithodoros moubata* is found throughout southern Africa, most notably in South Africa, with northward extensions through Mozambique to central Tanzania in the east and southwest Africa to west of Angola. Walton (1967) stated that these species are frequently found in the burrows of warthogs and porcupines, but there is also a domestic form that lives in human settlements. Walton further suggested that the domestic fowl houses in South Africa were probably infested by this species.

African Swine Fever virus was isolated from the first tick specie namely, *O. erraticus* in Spain. It was identified as a biological vector and reservoir for ASFV (Sánchez-Botija, 1963) and this led to learning that ticks from the *O. moubata* complex play a role in the epidemiology of the disease in Africa (Plowright *et al*., 1969). Furthermore, the *O. moubata* ticks are a source of infection with ASFV for both domestic and wild pigs in Africa. The ASF virus is maintained in a sylvatic cycle in southern and eastern Africa between argasid ticks (tampans) of the *Ornithodoros moubata* complex and the common warthog (*Phacochoerus africanus).*

During a blood meal, transmission occurs (Plowright, 1981; Yadav *et al.,* 2020). The infected ticks can maintain the virus for long periods and further transmit it to hosts, which are susceptible. When the infectious vertebrate blood meal is not present, the ASF virus can be maintained for up to 15 months in the sylvatic *Ornithodoros* tick (Plowright *et al*.,1970) and possibly even indefinitely (Plowright, 1977).

The persistence of African Swine Fever virus (ASFV) infection in the absence of viraemic hosts is due to transstadial, transovarial, and sexual transmission in *O. moubata* ticks (Hess *et al*., 1989; Rennie *et al*., 2001). Mortality in ticks can also occur due to ASFV infection (Kleiboeker and Scoles, 2001). The persistence of the ASF infection depends on the initial infection titre, and hence the level of viraemia in infected pigs (Plowright, 1981; Haresnape *et al.,* 1988). The *O. moubata* ticks (also called the eyeless tampans) are present in Madagascar and they are widely distributed in South Africa (Plowright *et al.,* 1994; Roger *et al.,* 2001). Central Africa has limited evidence of its distribution (De Glanville *et al.,* 2010). The warthog does not show clinical indications of ASF, and it is thought that the virus coexists with it. (Plowright *et al*., 1994).

1.2.5.2. The suids

African Swine Fever virus infection affects domestic pigs, Eurasian wild boars, warthogs, bush pigs, and giant forest hogs. During the sylvatic cycle, warthogs and bush pigs develop asymptomatic illnesses and serve as a viral reservoir (Kimberling and Teegarden, 1979). The African wild suids, whereby the most important suids being the warthog (*Phacochoerus africanus*) are the natural host of the ASFV (Sánchez-Vizcaíno *et al.,* 2012.) In the Epidemiology of ASF, the bush pig (*Potamochoerus larvatus*) and the red river hog (*Potamochoerus porcus*) are of lesser importance as they are only infected sporadically (Anderson *et al.,* 1998). However, in the giant forest hog (*Hylochoerus meinertzhageni*) there is a single case of ASF disease being reported (Jori *et al.,* 2013).

Even though the African wild suids are susceptible to ASF infection, they usually show no sign of the disease. Sánchez-Vizcaíno *et al.* (2012) stated that regardless of their breed and age, wild boars, domestic pigs, and feral pigs (all *sus scrofa*) are also susceptible to ASF infection manifested by a devastating haemorrhagic fever which causes up to 100% mortality caused by the virus. In some of the domestic pigs in parts of Africa where ASF is endemic, the pathogenic effects of the disease show an increased resistance, and a high proportion of pigs, which are healthy show antibodies to the ASFV. This was reported for the first time in the Mchinje District of Malawi (Haresnape *et al.,* 1985). In addition, Wilkinson *et al.* (1988) stated that Angola and eastern Zambia reported evidence of increased resistance to ASF. Endemic establishment of the disease in regions without the sylvatic cycle can therefore be

ascribed to pig populations with a high level of contact, for example in free-ranging populations that commonly occur in West Africa (Brown *et al.,* 2018).

1.2.6. TRANSMISSION AND SPREAD OF AFRICAN SWINE FEVER

1.2.6.1. Epidemiological cycles

Eastern and southern Africa comprise the sylvatic cycle between warthogs and soft ticks (Plowright *et al.,* 1969) and for several decades, in South Africa, the sylvatic cycle of ASF, with occasional spill over infections in domestic pigs has been described (Steyn, 1932; De Kock *et al.,* 1940; Magadla *et al.,* 2016). This transboundary animal disease has recently led to an increased number of countries affected by it, especially domestic pigs (Penrith *et al.,* 2019). There are four described epidemiological cycles for ASFV (Figure 1.4) namely, the sylvatic cycle, which occurs, between warthogs and soft ticks, the domestic pig-tick cycle, the domestic pig cycle, and recently a wild boar-habitat cycle (Haresnape and Wilkinson, 1989; Chenais *et al.,* 2018).

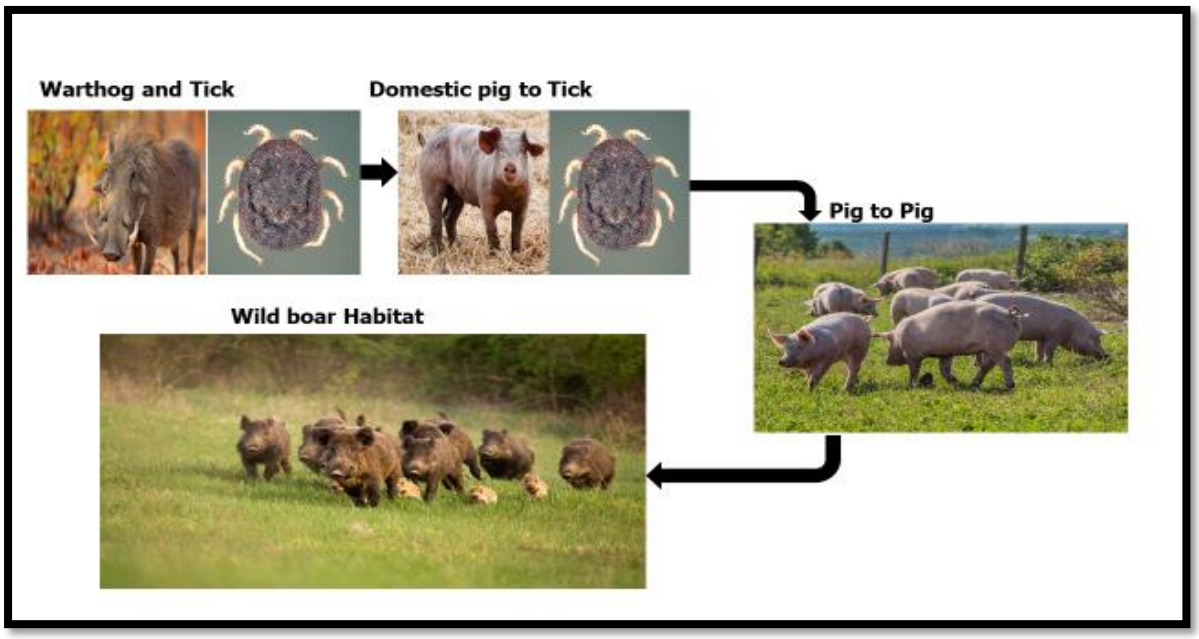

Figure 1.4: Schematic representation of African Swine Fever transmission.

(Source: courtesy of MT Rametse, 2021).

In addition, for the ASF virus to be maintained, it will depend on the sylvatic cycle. In the sylvatic cycle between warthogs and argasid ticks of the *Ornithodoros moubata* complex, transmission occurs between ticks and neonatal warthogs, among ticks, and between ticks. In southern and eastern Africa, whereby warthogs and ticks of the

Ornithodoros moubata complex are involved, the study of the sylvatic cycle has been well-described (Wilkinson and Pensaert, 1989, Thomson, 1985; Plowright *et al.,* 1994). Adult warthogs, even if they have infective levels of ASF virus in lymph nodes, they do not shed the virus or develop viraemia which is sufficient to cause infection by ticks that feed on their blood.

It is shown that transmission of the ASF virus between ticks and warthogs seems to occur during the first four to six weeks of life, and this is the time in which the young warthogs spend most of their time in the burrows and large numbers of resident infected *Ornithodoros moubata* ticks infect them. The infective levels of virus in the saliva of the ticks feeding on warthog piglets, which acts as an anticoagulant, cause viraemia, which will last two to three weeks in the warthogs and it is sufficient to infect other ticks. At this stage, the young warthogs show no signs of the disease (Thomson *et al.,* 1980). Studies in eastern and southern Africa showed that infection rates of free-living warthogs were rarely below 80 percent in areas where the tick vector was present (Plowright *et al.,* 1994).

In the Iberian Peninsula and Africa, there has been an infestation of *Ornithodoros spp* ticks, which were discovered that they infest pigpens regularly (Oleaga *et al.,* 1990) and they can be involved in the transmission and long-term maintenance of ASF when they continuously feed on the pigs. Looking at the occurrence of outbreaks in other areas like Spain it was discovered that the outbreaks were due to the association with the presence of the *Ornithodoros erraticus* (Perez-Sanchez *et al.,* 1994). Warthogs are infected asymptomatically for the rest of their lives and the maintenance of infection is dependent on *Ornithodoros moubata* (Jori and Bastos, 2009). The presence of both the warthogs and ticks in a region does not necessarily mean the existence of a sylvatic cycle as indicated in the review by Jori and Bastos (2009).

The continued presence of *Ornithodoros erraticus* ticks in Portugal mainly on farms, which were previously infected, by ticks, in the year 1999 ASF was thought to have emerged again (Sanchez-Vizcaino *et al.,* 2009; Boinas *et al.,* 2011). The ASFV was isolated on a farm in Madagascar, which for four years had no pigs being introduced (Ravaomanana *et al.,* 2010). In a situation like this, only when the tick population becomes extinct, then there will be a decrease in ASF infection and this will be because of the absence of the tick population over a long period (Oleaga *et al.,* 1990).

Recently, the domestic pig cycle of ASF has been reported to occur in South Africa (Geertsma *et al.,* 2012; Janse van Rensburg *et al.,* 2020c). Costard *et al.* (2009); Etter *et al.,* (2011), and Brown *et al.,* (2018) agree that the domestic pig cycle amongst free-ranging pigs has been described in areas such as West Africa. Penrith *et al.* (2007) stated that the rapid reproduction rate of pigs provides a constant supply of susceptible pigs to maintain the circulation of ASF virus in high-contact pig population example, where there are free-ranging pigs.

After there has been an infection of ASFV, the occurrence of the transmission through direct contact between domestic pigs can be up to 30 days, and in a situation where there was contact with blood products, the transmission can be for eight weeks (Costard *et al.,* 2009). ASFV becomes transmitted through direct contact and by fomites such as contaminated clothing, shoes, equipment, and vehicles (Mur *et al.,* 2012). This occurs only when introduced into the domestic pig population (Arias *et al.,* 2002; Arias and Sanchez-Vizcaino, 2012). Penrith *et al.* (2013) further state the occurrence of ASF virus circulation under conditions of low biosecurity that may include feeding of catering waste and amongst confined domestic pigs.

Pigs can experience infection if exposed to carcasses, which are poorly disposed of, or if they are feeding on frozen, cooked, or cured pork products, which are poorly cooked. ASFV is very resistant to inactivation and at a pH level of 4-10 it remains stable and is not affected by meat maturation; to be inactivated it will require a temperature of 60°C for 20 minutes. For the smoked sausages and air-dried hams to be inactivated, they will require smoking at 32-49°C for 12 hours and then drying for 25-30 days to be free of ASFV (Plowright *et al.,* 1994).

The continued cycle of ASF spread amongst the domestic pig population suggests the mortalities of ASF may not in all cases be as high as previously thought and it could be attributed to ASF strain differences in virulence or resistance on the part of the pig to the circulating strains (Haresnape *et al.,* 1985; Etter *et al.,* 2011). In addition, Penrith *et al.* (2004) and Penrith, (2013) stated that in cases where clinically healthy pigs demonstrate antibodies to ASF, this could indicate resistance to the ASFV, although the basis of this resistance is unknown.

1.2.6.2. Scavenging animals

Major concerns during an emergency disease outbreak are due to scavenging animals because they can easily spread and/or catch the disease. In addition, because of their far-reaching view, it has been shown that birds are more efficient in locating carcasses than earthbound mammals, which are probably distracted by the vegetation (Kane *et al.,* 2014). Scavenging of offal and remains of infected pork discarded during preparation for human consumption is probably more significant in areas where national dishes are subjected to lengthy cooking.

Swill feeding, particularly swill coming from aircraft and ships, has been viewed as a major source of infection. Swill that consists of or contains large amounts of infected pork has a high potential for spreading infection and has perhaps contributed too many of the outbreaks that have occurred. When an outbreak occurs and pigs die, large amounts of infected pork become available. Surplus meat may be dried or subjected to other processes that do not inactivate the virus and pigs are then, moved rapidly in an attempt to avoid disease and evade uncompensated compulsory slaughter. The incubation period differs from five to 15 days and clinical disease is usually peracute or acute. When less virulent strains are involved, subacute or chronic manifestations of ASF may occur however, they have been described rarely in Africa. The major indicator for ASF is high mortality among pigs of all ages.

1.2.7. CLINICAL SIGNS

The most common form of ASF is the peracute ASF. Pigs might show few clinical signs and sometimes no signs, the pigs will die rapidly. If the pigs show any clinical signs, the following symptoms will be observed, they can have a high fever, and cyanotic-red portions of the abdomen, ears, and legs. The position of recumbency will be seen (Penrith *et al.,* 2009). Pigs with acute ASF may exhibit the following symptoms: high fever, anorexia, lethargy, as well as diarrhoea, constipation, and nausea. The redness exhibited in the peracute form can also be visible in the acute form; however, the cyanotic location appears on the legs, ears, and ventral abdomen. Pregnant sows will usually abort. At the later stage of the disease, neurological signs like convulsions can appear and what can also appear is the loss of full control of bodily movements (ataxia), which is common. Death will occur within 2-7 days and some pigs may recover though is rare (Penrith *et al.,* 2009).

Subclinical persistent carriers of ASFV occur in pigs, which recover from acute or subacute ASF. If the virus is reactivated by stress, the transmission will occur through direct contact however, low levels of the virus in recovered pigs are considered not to transmit the virus via direct contact. Administering corticosteroids in carrier pigs, which was conducted during an experiment, has shown (corticosteroids) to cause new viremia. It is still unknown if persistently, infected sows are capable of transmitting ASFV to their piglets (Wilkinson, 1984).

When pigs are infected by a less virulent strain, subacute ASF will be seen and the symptoms will last between 3-4 weeks. Symptoms are fever, which fluctuates, emaciation, and cough which is due to pneumonia. Abortion will occur in pregnant sows. Death, recovery, or the chronic state of ASF can develop in pigs with subacute ASF (Penrith *et al.,* 2009). Diffuse symptoms such as emaciation, arthritis, pneumonia, and dermatitis are common in pigs with a chronic form of ASF. One common thing is secondary bacterial infections and within a couple of months, the pigs will usually die (Penrith *et al.,* 2009).

1.2.8. DIAGNOSIS OF ASF VIRUS

Anderson, (1986) stated that ASFV replicates primarily in cells of the reticuloendothelial system. All sorts of samples collected from domestic pigs can be used for ASF diagnosis; in wild swine, there were lower viral titres, which were reported in bush pigs compared to samples from domestic pigs in a study in which both species were experimentally infected (Oura *et al.,* 1998). Spleen and lymph nodes on ice but not frozen are the samples of choice. If maintenance of the cold chain is a problem or difficult to be maintained the samples may be preserved in 50% glycerol-saline.

For histopathological examination and immunohistochemistry, an additional set of samples from various organs (spleen, lymph nodes, lung, liver, kidney, brain) may be taken in 10% buffered formalin. If only live sick pigs are available, whole blood in anticoagulant (EDTA or purple top) may be submitted for PCR, and blood in heparin or green top for viral isolation. Fluorescent antibody (FAT) test and enzyme-linked immunosorbent assay (ELISA) can be used to detect viral antigen; real-time and conventional polymerase chain reaction assays can be used to detect the viral genome (OIE, 2012). Antibodies can be detected using an indirect fluorescent antibody test (ELISA).

1.2.8.1. Antibody Detection Test

Antibody detection has epidemiological value in endemic areas, and it is recommended to combine antibody detection with viral genome detection by PCR in such cases (OIE, 2012). Antibody detection has limited diagnostic value because it cannot distinguish between ongoing and older infections. It should be noted that serum is not a useful sample for diagnosis of acute or peracute ASF, as most pigs die before antibodies can be detected. Enzyme-Linked Immunosorbent Assay (ELISA) procedures are often inexpensive, quick, and easily automated; the OIE recommends combining ELISA with other tests for antibody detection, such as fluorescent antibody tests or indirect immunoperoxidase tests (OIE, 2012; Gallardo *et al.,* 2015).

1.2.8.2. Viral detection test using conventional and real-time PCR

Commonly used laboratory tests to detect the presence of viruses are polymerase chain reaction (PCR). Furthermore, the PCR test can be used to detect viral genome, it is the most sensitive technique, which is fast and can be performed on putrefied samples. The observation of haemadsorption or cytopathic effects remains the gold standard for the diagnosis of viral isolation (Oura *et al.,* 2013). Agüero *et al.* (2003) stated that various conventional PCR assays have been described for ASV detection, and Bastos *et al*. (2003), confirm it. In addition, these have now mostly been replaced by real-time polymerase chain reaction (rPCR) assays; however, conventional PCR assays are useful in less developed labs that do not have rPCR equipment.

The real-time polymerase chain reaction (rPCR) approach has several advantages over gel-based conventional PCR methods. It detects the amplification of target sequences by fluorescence signals from target-specific oligonucleotide probes. These include increased speed, sensitivity, reduced chances of cross-contamination and it is because of a closed system, and provision of a quantitative result. Portable rPCR machines are now becoming available, making it possible to utilise these molecular technologies in the field, with the possibility for radical changes in future diagnostic approaches. King *et al. (*2003) stated that several rPCR assays have been described for the detection of ASFV and this statement was later confirmed by McKillen *et al.,* 2010 and Fernández-Pinero *et al.,* 2013.

1.2.8.3. Viral detection test using FAT

Although the manual from the office international des epizooties (OIE) points out that the FAT is not as sensitive as PCR, in countries where FAT is used it will detect ASF virus in an outbreak, when large amounts are present in blood or tissue samples, and is a more robust test than PCR when laboratory conditions are not ideal. Bool *et al.* (1969) stated that the FAT can be used to detect ASFV antigen in tissue samples of suspect pigs. In addition, the test can detect ASFV antigen in leucocyte cultures with no HAD, allowing non-haemadsorbing strains of the virus to be identified. Furthermore, in cases of acute ASF, the FAT test is highly sensitive and shows a decreased sensitivity in subacute and chronic diseases. This may be due to the formation of antigen-antibody complexes in the tissues of infected pigs, which block the interaction between the ASFV antigen and ASF, the conjugate (Oura *et al.,* 2013).

1.3. PROBLEM STATEMENT

African Swine Fever is a controlled and notifiable disease that is endemic in South Africa. There are defined controlled areas in some of the provinces but, the outbreaks experienced in the North West and Free State occurred outside of the known controlled areas hence it was critical to investigate and find the source of introduction of the virus responsible for these outbreaks.

1.4. AIMS AND OBJECTIVES OF THE STUDY

The aim of this study was the identification of the source of the African Swine Fever (ASF) outbreak in the Free State and North West provinces.

The specific objectives were:

- To explore the extent of disease prevalence in the affected areas.
- To establish the risk factors which led to the disease outbreak focusing on farming practices and systems.
- To establish the role of the sylvatic cycle in the dissemination of the virus in the affected areas.

1.5. HYPOTHESIS

The free movement of pigs, swill feeding, and lack of biosecurity in the informal farming setting may have contributed to the outbreak.

CHAPTER 2: MATERIAL AND METHODS

2.1. Outbreak investigation on farm level to assess ASF risk factors

The Veterinary authorities under the Department of Agriculture, Land Reform and Rural Development (DALRRD) previously known as the Department of Agriculture Fishers and Forestry (DAFF) have a mandate to investigate disease outbreaks. The first two outbreaks in the North West (NW) and Free State (FS) provinces were reported around the same time. The first outbreak in the NW was reported from the communal township called Ipelegeng where there are free roaming pigs owned by various farmers. The high number of mortalities occurred from May 2016; the State Veterinarian suspected septicaemia.

2.1.1. Study area

From 2016 to 2017, ASF outbreaks occurred outside of the ASF-controlled area in North West and Free State Provinces. The study area in North West was Schweizer-Reneke Township, and in the Free State, the areas included Bloemfontein, Koffiefontein, Botshabelo, and Thaba Nchu (Figure 2.1). These are two of South Africa's nine provinces; North West is on Latitude 26° 39' 49.896" S and Longitude 25° 17' 1.529" E while Free State province is bordered on the North West province with Latitude 28°27'14.8"S, Longitude 26°47'48.43"E. Esri's ArcGIS® software was used to map the geographical distribution of outbreaks.

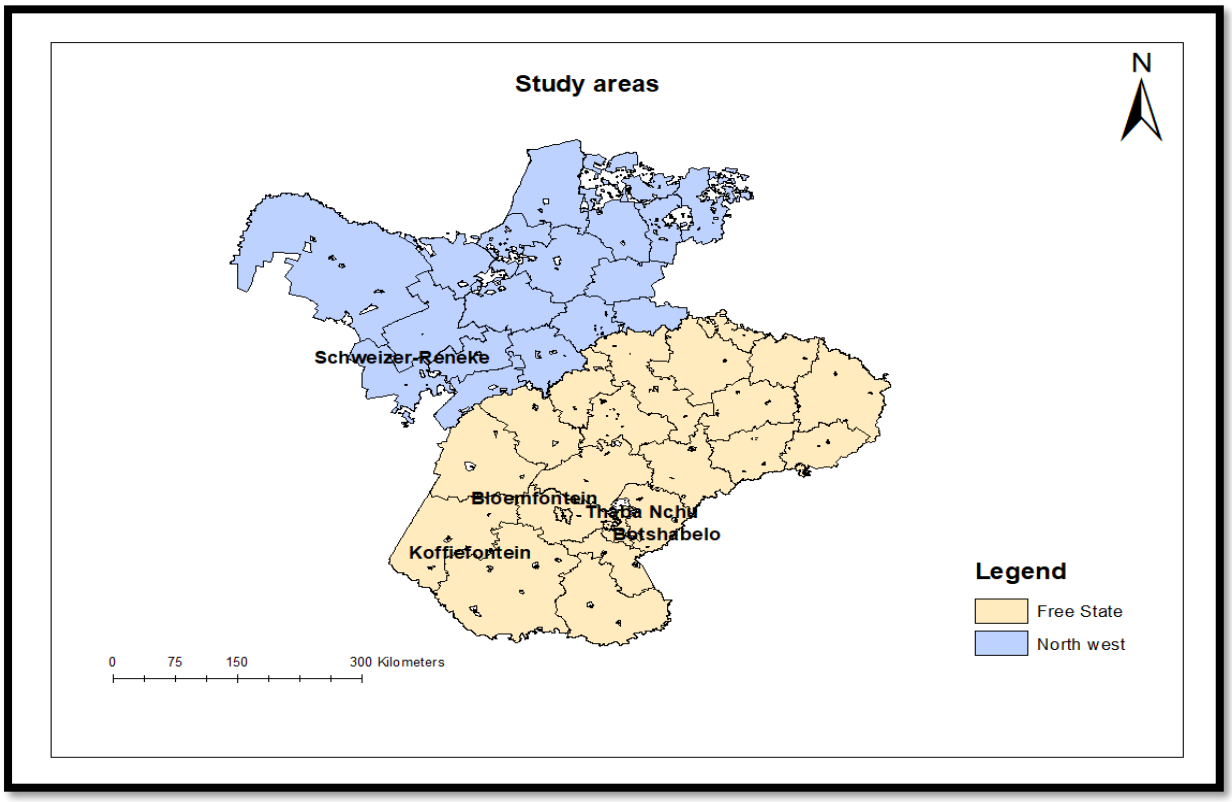

Figure 2.1: Map of North West and Free State provinces showing study sites *(Source: courtesy of www.esri.com).*

2.1.2 Sampling methods

The study was divided into the following phases:

Phase 1: Plenary meetings with representatives from the Department of Agriculture, Land Reform, and Rural Development (DALRRD). The status of the outbreaks was discussed at the meeting.

Phase 2: Farmers affected by the outbreaks were interviewed (face-to-face) using a semi-structured questionnaire for data collection. Famers were interviewed in their preferred language, namely Setswana and Sesotho. The semi-structured questionnaire was to gather both qualitative and quantitative data. According to Bless and Smith (2000), an interviewer-administered interview is an important tool for data collection because it lessens word problems or the possibility of misinterpretation (misunderstanding) by respondents and can be administered to farmers who cannot read or write. Moreover, the presence of the interviewee improves the quality of the

response because the interviewer can probe for more specific responses (Leedy *et al.,* 2004).

Phase 3: Farmers' questionnaire and observation sheet data were captured in Excel during this phase for further analysis.

2.1.3 Ethical approval

The sampling was conducted under the approval of section 20 by the Department of Agriculture, Land Reform and Rural Development (DALRRD) former DAFF with Ref: 12/11/1/1/15 and ethical clearance (Ref: 2019/CAES_HREC/126) from the University of South Africa (refer to appendices).

2.1.4 Data collection

Only biological samples from live and dead animals as approved by the Department of Agriculture, Land Reform, and Rural Development (DALRRD), were tested. Information related to field samples was extracted from submission forms submitted by veterinary officials to the laboratory. Primary data was gathered through face-to-face interviews using the questionnaire and direct observations by the author. The questionnaire (Appendix 1) was designed based on ASF experts and addressed the primary objectives of the study. All questions were prepared in English, but the interviews were conducted in Setswana and Sesotho with the help of veterinary officials.

To investigate ASF outbreaks on the farm level, (n=42) questionnaires and observation data were collected in the field from both provinces. Questions in the questionnaire covered swill feeding, auctions, biosecurity, disease reporting, housing, and the presence of ticks. Face-to-face interviews were conducted to understand the impact of the disease outbreak on the farmers and to establish the possible source of the outbreak. Farmers, Veterinarians, and Animal Health Technicians who were directly impacted by the outbreak were included among those interviewed. The veterinarians and Animal Health Technicians (AHTs) were interviewed on the disease action plan. In addition, the ASF action plan and eradication measures implemented by North West province were also used to assess the risk factors

2.1.5 Sampling frame and strategy

The farms were chosen purposively since it was an outbreak investigation, and the criteria was an ASF case being reported on a farm or herd. The population size for the affected farms has been reported to be 1088 for North West, and 1451 for Free State, making 2539 affected pigs. Although, DALRRD reported two ASF outbreaks in Schweizer-Reneke, North West, and 12 in the Free State province. These included 11 farms in North West and 31 farms in Free State, which were sampled by the Veterinary officials and samples were collected based on available specimens. Biological samples were collected from live animals and post-mortem cases on the pig farms and included whole blood in EDTA, sera, and tissues from the necropsy. In addition, opportunistic samples (whole blood in EDTA, whole blood for serum, tissues) were collected from warthogs submitted by hunters.

2.1.6 Sampling of domestic pig farms

Purposive sampling was used in this study based on the ASF outbreak in the areas investigated in order to reduce the risk of the spread of the disease (Mariner and Paskin, 2000). Therefore, the inclusion criteria were pig farmers directly affected by the ASF outbreaks during 2016 and 2017. The number of domestic pigs from 42 farms sampled in the North West and Free State provinces is shown in Table 2.1. The laboratory information included state veterinarian details, registered farm name, animal species, age, and type of specimens, the test required, owner's details, necropsy and clinical findings, and address of the sender.

Not all samples harvested in the field by the veterinary officials were properly stored, this can be due to a lack of cold chain and required equipment needed to store samples. This was outside our control as the samples were collected by field officers and dispatched to the laboratory for diagnosis of ASF. This was a limitation of the study. The properly stored samples were transported on ice (+4°C) before being sent to the laboratory for further testing. The samples comprised blood on EDTA in 10 ml tubes (*n*=67), sera in 10 ml Vacutainer® tubes without anticoagulant (*n*=174), and (*n*=44) pooled tissue samples collected during post-mortem consisting of one or more of the following namely lymph nodes, liver, lungs, kidney, and spleen. Furthermore, reports on clinical and necropsy findings were analysed to understand the pathogenesis of ASFV.

Table 2.1: Farm data from ASF outbreaks in North West and Free State Provinces in 2016/2017.

Detailed farm data from ASF outbreaks in North West Province in 2016/2017								
Farm location	Specie	Breed	Age	Sex	No. of pigs sampled	Sample type		
						Tissue	Serum	EDTA blood
Ipelegeng Farm 1	Domestic Pigs	Not specified	Not specified	Not specified	10	0	10	0
Ipelegeng Farm 2	Domestic Pigs	Large white	Not specified	Male and Female	2	2 (pooled)	0	0
Ipelegeng Farm 3	Domestic Pigs	Not specified	Not specified	Mixed	13	0	13	13
Ipelegeng Farm 4	Domestic Pigs	Not specified	Not specified	Not specified	7	7 (pooled)	0	0
Ipelegeng Farm 5	Domestic Pigs	Not specified	Not specified	Mixed	33	0	33	0
Ipelegeng Farm 6	Domestic Pigs	Not specified	Not specified	Not specified	1	1 (pooled)	0	0
Ipelegeng Farm 7	Domestic Pig	Not specified	Not specified	Not specified	1	1 (pooled)	1	0
Zanfontein Farm 8	Domestic Pigs	Not specified	Not specified	Mixed	7	0	7	0
Delareyville Farm 9	Domestic Pigs	Large white	2 years	Females	3	3 (pooled)	0	0
Delareyville Farm 10	Domestic Pigs	Not specified	6 months	Males	2	2 (pooled)	0	2
Migdol Farm 11	Domestic Pigs	Not specified	Not specified	Not specified	26	1 (pooled)	26	0
					105	17	90	15

Detailed farm data from ASF outbreaks in Free State Province in 2016/2017								
Farm location	Specie		Age	Sex	No. of pigs sampled	Sample type		
						Tissue	Serum	EDTA blood
Koffiefontein Farm 1	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 2	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 3	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 4	Domestic Pigs	Not specified	Weaners	Females	4	0	4	0
Koffiefontein Farm 5	Domestic Pigs	Not specified	Weaners	Females	3	0	3	0
Koffiefontein Farm 6	Domestic Pigs	Not specified	Weaners	Females	3	0	3	0
Koffiefontein Farm 7	Domestic Pigs	Not specified	Weaners	Females	3	3 (pooled)	0	0
Koffiefontein Farm 8	Domestic Pigs	Not specified	Weaners	Females	4	0	4	0
Koffiefontein Farm 9	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 10	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 11	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 12	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 13	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Koffiefontein Farm 14	Domestic Pig	Not specified	Weaners	Female	6	0	6	0
Koffiefontein Farm 15	Domestic Pigs	Not specified	Weaners	Females	2	0	2	0
Fauresmith Farm 16	Domestic Pigs	Large white	Various Ages	Mixed	2	1 (pooled)	2	1
Fauresmith Farm 17	Domestic Pigs	Not specified	Not specified	Not specified	9	0	8	9
Thaba Nchu Farm 18	Domestic Pigs	Not specified	Not specified	Not specified	6	0	6	0
Thaba Nchu Farm 19	Domestic Pigs	Large white	Adult, sub-adult, piglet	One male and two females	3	3 (pooled)	2	2
Bloemfontein Farm 20	Domestic Pig	Not specified	Weaner	Male	1	1(pooled)	0	1
Bloemfontein Farm 21	Domestic Pigs	Large white	6-12 months	Males	2	0	2	2
Bloemfontein Farm 22	Domestic Pigs	Not specified	Weaners	Not specified	4	0	0	4
Bloemfontein Farm 23	Domestic Pig	Not specified	Weaner	Not specified	1	1 (pooled)	0	0
Bloemfontein Farm 24	Domestic Pigs	Not specified	Not specified	Not specified	4	0	4	4
Bloemfontein Farm 25	Domestic Pigs	Large white	Various Ages	Mixed	20	15 (pooled)	15	20
Bloemfontein Farm 26	Domestic Pig	Large white	Sub-adult	Male	1	1 (pooled)	0	0
Botshabelo Farm 27	Domestic Pigs	Not specified	Not specified	Mixed	7	0	3	4
Botshabelo Farm 28	Domestic Pigs	Not specified	Weaners	Mixed	5	0	0	5
Botshabelo Farm 29	Domestic Pigs	Not specified	Weaners	Mixed	4	0	4	0
Smithfield Farm 30	Domestic Pig	Not specified	Weaner	Not specified	1	1 (pooled)	0	0
Dewetsdorp Farm 31	Domestic Pig	Not specified	Not specified	Male	1	1 (pooled)	0	0
					112	27	84	52

2.1.7 Sampling of warthog farms

During the ASF outbreaks, veterinary officials received samples from warthogs (*n*=13), which were collected during hunting by local farmers from the Free State (Bloemfontein 1, Bloemfontein 2, and Fauresmith) and North West provinces (Koffiefontein) (Table 2.2). The following samples were collected namely, two EDTA blood in 10 ml tubes, nine sera in plain Vacutainer® tubes, and 10 tissue samples namely lymph nodes, liver, lungs, kidney, and spleen.

Table 2.2: Wild pigs' data from ASF outbreaks in North West and Free State Provinces in 2016-2017.

Detailed wild pigs data from ASF outbreaks in Free State Province in 2016/2017							
Farm location	Specie	Age	Sex	No. of pigs sampled	Sample type		
					Tissue	Serum	EDTA blood
Bloemfontein 1	Wild Pigs	Adults	Females	2	2 (pooled)	0	0
Bloemfontein 2	Wild Pigs	Not specified	Not specified	4	4 (pooled)	0	0
Fauresmith	Wild Pigs	Young	Mixed	5	2 (pooled)	6	0
			Total	11	8	6	0
Detailed wild pigs data from ASF outbreaks in North West Province in 2016/2017							
Farm location	Specie	Age	Sex	No. of pigs sampled	Sample type		
					Tissue	Serum	EDTA blood
Koffiefontein	Wild Pigs	Not specified	Not specified	2	2 (pooled)	3	2
			Total	2	2	3	2

2.1.8 Sampling of warthog burrows

The farms were visited in January 2020 for the collection of *Ornithodoros moubata* ticks. The global positioning system (GPS) coordinates of all located and sampled warthog burrows were recorded with the assistance of Animal Health Technicians (AHTs) working in the areas. The sampling units were farms in Koffiefontein (Free State) where there were warthog dwellings and the presence of warthogs. Ten burrows from 10 sites, representing three farms, were sampled and (*n*=88) ticks were recovered (Table 2.3). A direct sampling technique was employed with a manual collection method (Jori *et al.,* 2013). The number of scrapings and time spent in each burrow were equalized to ensure consistency in the tick sampling technique. Each hole was scraped ten times using a long spade that had been particularly modified for this purpose, taking between 30 and 45 minutes per burrow (Figure 2.2).

To make it easier to identify tick movement, a black plastic sheet was placed close to the burrow, and the collected soil scraping was spread on the sheet in direct sunlight (Figure 2.3). Loose soil removed from the burrows was sieved directly into the white trays for better detection of more ticks particularly the small ticks that passed through the sieve. The scraping was done on the sides, bottom, top, and deep areas of the burrows. Specific identification was performed under direct sunlight and the ticks (*Ornithodoros moubata)* were placed into an airtight acrylic plastic container to protect the ticks during transportation.

Entomological forceps were used to transfer the ticks from sand scraps into the container (Jori *et al.,* 2013). The ticks were pooled according to the sampling site. For further laboratory analysis, the specimens were transported to Transboundary Animal Diseases Laboratory (TADL), ARC-Onderstepoort Veterinary Institute (OVI), under a Red Cross permit issued by DALRRD. The containers were big enough to hold the ticks without causing any damage, between 30-40 ml and they were labelled with the date, specie, quantity, farm name, and province. The containers were covered with bubble wrap to prevent them from breaking.

Table 2.3: Number of recovered *Ornithodoros moubata* ticks from Koffiefontein

Location	Farm	Site	Warthog Burrows (WB)	Ticks collected	Time spent	Tick species
Koffiefontein	KFS1	1	WB No.1	0	40 minutes	
Koffiefontein	KFS1	2	WB No.2	0	30 minutes	
Koffiefontein	KFS1	3	WB No.3	10	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS1	4	WB No.4	10	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS2	1	WB No.5	10	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS2	2	WB No.6	10	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS2	3	WB No.7	0	37 minutes	
Koffiefontein	KFS2	4	WB No.8	20	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS3	1	WB No.9	10	45 minutes	*Ornithodoros moubata*
Koffiefontein	KFS3	2	WB No.10	18	45 minutes	*Ornithodoros moubata*
				Total 88		

Figure 2.2: Sieve (A) and Spade (B) and (C) used during sampling.

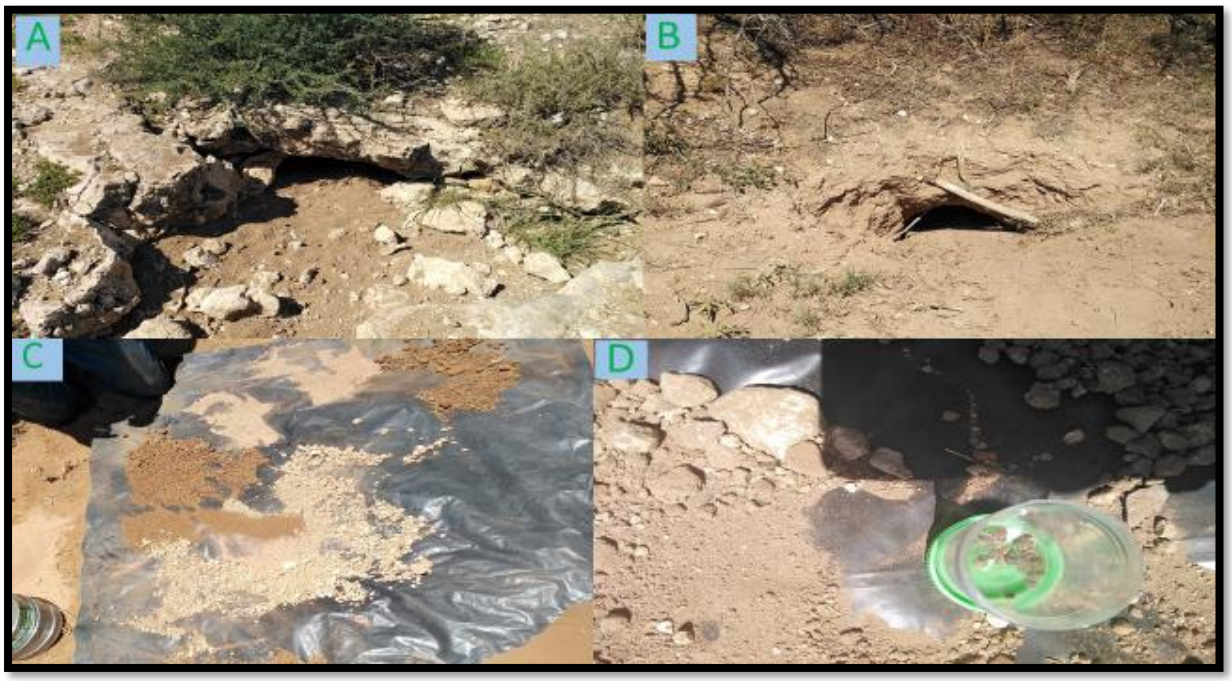

Figure 2.3: Method used for tick collection (A) and (B) Warthog burrows (C) Sampled soil on the black plastic sheet for exposure of ticks, (D) Collected ticks.

2.1.9 Clinical and necropsy data from domestic pigs from the provinces of North West and Free State.

Veterinary officials submitted laboratory forms with general information on clinical and necropsy data (Table 2.4). Post-mortem examinations were performed on 44 carcasses from seven farms in the North West province and nine farms in the Free State province. One farm in the North West province had no necropsy data report, and two farms in the Free State province had neither clinical nor necropsy report.

Table 2.4: Clinical and necropsy data submitted to the laboratory

Clinical and necropsy data from ASF outbreaks in North West Province in 2016/2017		
Farm location	**Clinical findings**	**Necropsy findings**
Ipelegeng Farm 2	Breathing difficulty, lethargy, and recumbency, listlessness	Enlarged haemorrhagic lymph nodes,ecchymotic haemorrhages under the skin,congestion of the lungs
Ipelegeng Farm 4	Breathing difficulty, fever >40 °C, lethargy,and recumbency,reddening of ventral chest and abdomen.	Ecchymotic haemorrhages under the skin,petechiae in kidney cortex, severe chronic enteritis, and enlarged haemorrhagic lymph nodes.
Ipelegeng Farm 6	Breathing difficulty, lethargy, and recumbency, listlessness	Enlarged haemorrhagic lymph nodes,bleeding into pleural and abdominal cavities,enlarged spleen
Ipelegeng Farm 7	Breathing difficulty, lethargy, and recumbency, listlessness	Enlarged haemorrhagic lymph nodes,ecchymotic haemorrhages under the skin,petechiae in kidney cortex and congestion of the lungs
Delareyville Farm 9	Breathing difficulty, lethargy, ataxia, and recumbency	Pneumonia,severe chronic enteritis,ecchymotic haemorrhages under the skin,enlarged and haemorrhagic lymph nodes
Delareyville Farm 10	Reddening of ventral chest and abdomen	No report
Migdol Farm 11	Breathing difficulty, lethargy, and recumbency, listlessness,reddening of ventral chest and abdomen	Enlarged haemorrhagic lymph nodes and severe chronic enteritis
Clinical and necropsy data from ASF outbreaks in Free State Province in 2016/2017		
Farm location	**Clinical findings**	**Necropsy findings**
Koffiefontein Farm 7	Breathing difficulty, lethargy, dark diarrhoea,ataxia, coughing and recumbency	Enlarged haemorrhagic lymph nodes,enlarged spleen, ecchymotic haemorrhages under the skin,and congestion of the lungs
Fauresmith Farm 16	Breathing difficulty,lethargy,and recumbency,reddening of ventral chest and abdomen.	Enlarged haemorrhagic lymph nodes,ecchymotic haemorrhages under the skin, and petechiae in kidney cortex.
Thaba Nchu Farm 19	Breathing difficulty, lethargy, and recumbency, listlessness	Enlarged haemorrhagic lymph nodes,bleeding into pleural and abdominal cavities,enlarged spleen
Bloemfontein Farm 20	Breathing difficulty, lethargy, and recumbency, listlessness	Enlarged haemorrhagic lymph nodes,ecchymotic haemorrhages under the skin,petechiae in kidney cortex and congestion of the lungs
Bloemfontein Farm 23	Breathing difficulty, lethargy, and recumbency	Pneumonia,severe chronic enteritis,petechial haemorrhage on both small, large intestines and the lungs,enlarged and haemorrhagic lymph nodes
Bloemfontein Farm 25	Reddening of ventral chest and abdomen	No report
Bloemfontein Farm 26	Breathing difficulty, lethargy, and recumbency, listlessness,reddening of ventral chest and abdomen	Enlarged haemorrhagic lymph nodes and severe chronic enteritis
Smithfield Farm 30	No report	Pneumonia,severe chronic enteritis,congestion of the lungs, petechial haemorrhage,enlarged and haemorrhagic lymph nodes
Dewetsdorp Farm 31	Breathing difficulty, lethargy, and recumbency, listlessness	Ecchymotic haemorrhages under the skin,petechiae in kidney cortex, severe chronic enteritis, and enlarged haemorrhagic lymph nodes.

2.1.10 Data Management and Analyses

All data and samples related to the research project were submitted to TADL at the ARC-OVI for analysis and testing. The data were analysed using descriptive statistics because the research is descriptive in nature. Counts, percentages, and frequency distribution were among the frequency measures employed. Data were captured in Microsoft Excel. Raw data and later, results were also entered into the ARC laboratory management system (Labware 8) and records will be kept for at least 10 years according to the International Organization for Standardization (ISO) 17025.

2.1.11 Limitations of the Study

Time and weather were the major challenges of this study. Accessibility to some of the farms (Figure 2.4) by vehicles was very difficult and therefore the sampling team had to walk long distances to reach warthog burrows identified by the team with the help of animal health technicians in the field.

Figure 2.4: Accessing Warthogs Farms.

(*Source: courtesy of Professor Robert Swanepoel, University of Pretoria, 2020)*

2.2. ASFV DETECTION IN THE LABORATORY

2.2.1. Antibody detection using ELISA

The enzyme-linked immunosorbent assay (ELISA) was performed according to the method described by (Pastor *et al.,* 1990). It is a direct test that can detect antibodies to ASFV in pigs, which have been infected by viruses of low or moderate virulence. A total number of domestic pig samples (*n*=174) was tested that comprised serum samples (*n*=90) and (*n*=84) from the North West and Free State provinces, respectively. Warthog serum samples (*n*=9) that included North West (*n*=3) and Free State (*n*=6) were received and tested. The serum samples were tested by the TADL-OVI laboratory technicians for antibodies using a commercial Ingezim compact ELISA kit according to the manufacturer's instructions (INGENASA, Madrid, Spain). A report with results from the analysis was provided for further analysis in this study. The tests were valid if the Optical Density (OD) of the negative control (NC) was at least four times more than the OD of the positive control (PC), therefore NC/PC ≥ 4. Positive cut-off = NC-[(NC-PC) × 0.5], Negative cut-off = NC-[(NC-PC) × 0.4]. The following formula calculation was followed when calculating for blocking % of samples:

$$X\% = \frac{NC - \text{sample OD}}{NC - PC} \times 100$$

The results were interpreted as positive, negative, or ambiguous. Blocking % of ≥50 was considered positive. Blocking % of ≤ 40 was considered negative and the results which were between both values were considered ambiguous.

2.2.2. PCR assay screening using EDTA blood and tissue samples.

The polymerase chain reaction (PCR) was used by the laboratory technicians to detect the ASFV genome using homogenised tissue and EDTA blood samples. A total of (*n*=4) tissue samples from domestic pigs were tested. The number of samples per province was (*n*=17) from North West and (*n*=27) from Free State. In addition, EDTA blood samples (*n*=67) were tested. From these 67 samples, (*n*=15) blood samples were from North West and (*n*=52) from the Free State province. A total of warthog samples (*n*=12) were also tested. These samples included EDTA blood (*n*=2), tissue samples (*n*=2) from North West, and (*n*=8) tissue samples from the Free State.

A 278 bp region corresponding to the central portion of the p72 gene was amplified using the diagnostic primers, primer 1 (5'-ATGGATACCGAGGGAATAGC-3') and primer 2 (5'-CTTACCGATGAAAATGATAC-3') to confirm the presence of ASFV DNA (Wilkinson, 2000). To amplify this segment, GoTaq Hot Start Green Master mix DNA polymerase (Promega) in a 50 µL reaction was used. In addition, the template was amplified following 40 cycles, with the first denaturation at 96°C for two minutes, denaturation at 96°C for 12 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 40 seconds. To address the possibility of false negative and false positive results, positive (Spec/57 genotype VIII) and negative Baby Hamster Kidney cell line (BHK) controls were included.

2.2.3. PCR assay using ticks for DNA extraction

2.2.3.1. DNA extraction

Eighty-eight *Ornithodoros* spp. ticks (were placed in 2 ml screw cap microcentrifuge tubes pre-loaded with 1.4 mm Zirconium Silicate grinding beads (Biotechnology Hub Africa) and 1 ml of Phosphate Buffered Saline (PBS). To homogenize the samples, a Spex SamplePrep 1600 MiniG tissue homogenizer was used. A total of 200 µL of the tick pool homogenate was added to 200 µL of AL buffer for cell lysis, and heat treated at 70°C for 10 min. Automated nucleic acid extraction followed using the IndiMag Pathogen kit (WhiteSci, South Africa) on a MagMAX-96 express magnetic particle processor following the manufacturer's instructions. DNA was eluted in 100 µL of elution buffer. In addition, positive controls were included with each extraction.

2.2.3.2. Real-Time PCR

The real-time quantitative PCR (qPCR) assay described by Zsak *et al.,* (2005) was used to test eluates for ASFV nucleic acid, with modifications by Sunwoo *et al.,* (2019). A 5 µl of DNA was amplified in 20-µl reactions using 20 pmol and 7 pmol of probe in Perfecta Fastmix II on a CFX96 real-time system (Quanta Biosciences, Beverly, MA). Each reaction was performed in duplicate. The location and sequence of the primers and probe were as follows: forward primer, starting at base position 1466, 5'CCTCGGCGAGCGCTTTATCAC 3', reverse primer, starting at base position 1528, 5' GGAAACTCATTCACCAAATCCTT 3', probe, starting at base position 1486, 5' CGATGCAAGCTTTAT 3' (Zsak *et al.,* 2005). Positive and no template controls were included during each PCR run.

2.2.3.3. Conventional PCR for confirmation of positive and doubtful results

To confirm the positive and doubtful results, Conventional PCR was employed. Genomic regions of the C-terminus end of p72 gene, where a 478 bp region was amplified for the confirmation of the presence of virus using primer p72-U; primer sequence 1: 5’ GGCACAAGTTCGGACATGT 3’ and p72-D; primer sequence 2: 5’ GTACTGTAACGCGCAGCACAG 3’ (Bastos *et al*., 2003; Lubisi *et al*., 2005). To run the amplification, GoTaq Hot Start Green Master mix DNA polymerase (Promega) in a 50 µL reaction was used. In addition, the template was amplified following 40 cycles, with the first denaturation at 96°C for two minutes, denaturation at 96°C for 12 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 40 seconds. To address the possibility of false negative and false positive results, positive (Spec/57 genotype VIII) and negative Baby Hamster Kidney cell line (BHK) controls were included.

2.2.4. Purification and nucleotide sequencing of PCR products

The amplicons of the expected size for p72 were excised from the gel and purified using QIAquick gel extraction and PCR purification kit (Qiagen) according to manufacturers' instructions. The nucleotide sequences were determined by automated cycle sequencing at Inqaba Biotechnical Industries (PTY) Ltd, South Africa.

2.2.5. Phylogenetic Analysis

The evolutionary history of 73 ASF viruses from Southern Africa obtained from GenBank, including all isolates from the 2016/2017 outbreaks in the North West and Free State provinces, was inferred in MEGA X (Kumar *et al.,* 2018), using the Neighbour-Joining (NJ) method (Saitou and Nei, 1987). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Tamura 3-parameter method (Tamura, 1992) and are in the units of the number of base substitutions per site.

CHAPTER 3: RESULTS

3.1 Outbreak investigation on farm level to assess ASF risk factors

3.1.1 African Swine Fever 2016/2017 outbreaks in the North West and Free State Province.

Table 3.1 shows the dates, and location of the outbreaks reported in 2016/2017 by DALRRD, with Schweizer-Reneke in North West being the first area to be affected, only two outbreaks were reported. Free State Province reported a total of (*n*=12) outbreaks.

Table 3.1: Frequency of ASFV outbreak reports in Northwest and Free State Province.

Date	Area	No. of outbreaks	Province
06 June 16	Schweizer-Reneke	1	Northwest
09 June 16	Koffiefontein	1	Free State
27 June 16	Botshabelo	2	Free State
05 July 16	Bloemfontein	1	Free State
18 July 16	Thaba Nchu	1	Free State
25 July 16	Dewetsdorp	1	Free State
26 July 16	Smithfield	1	Free State
11 August 16	Bloemfontein	1	Free State
29 August 16	Thaba Nchu	1	Free State
16 September 16	Bloemfontein	1	Free State
16 September 16	Fauresmith	1	Free State
01 November 16	Delareyville	1	Northwest
09 December 16	Thaba Nchu	1	Free State

Source: field data from 2016/2017 ASF outbreaks

Figure 3.1 depicts the distribution of ASF outbreaks, with 14 outbreaks reported in total. There were no outbreaks, which had an impact on the commercial pig industry. Through personal communication, the Animal Health Technicians reported illegal hunting and movements of warthogs in the Free State, Koffiefontein area.

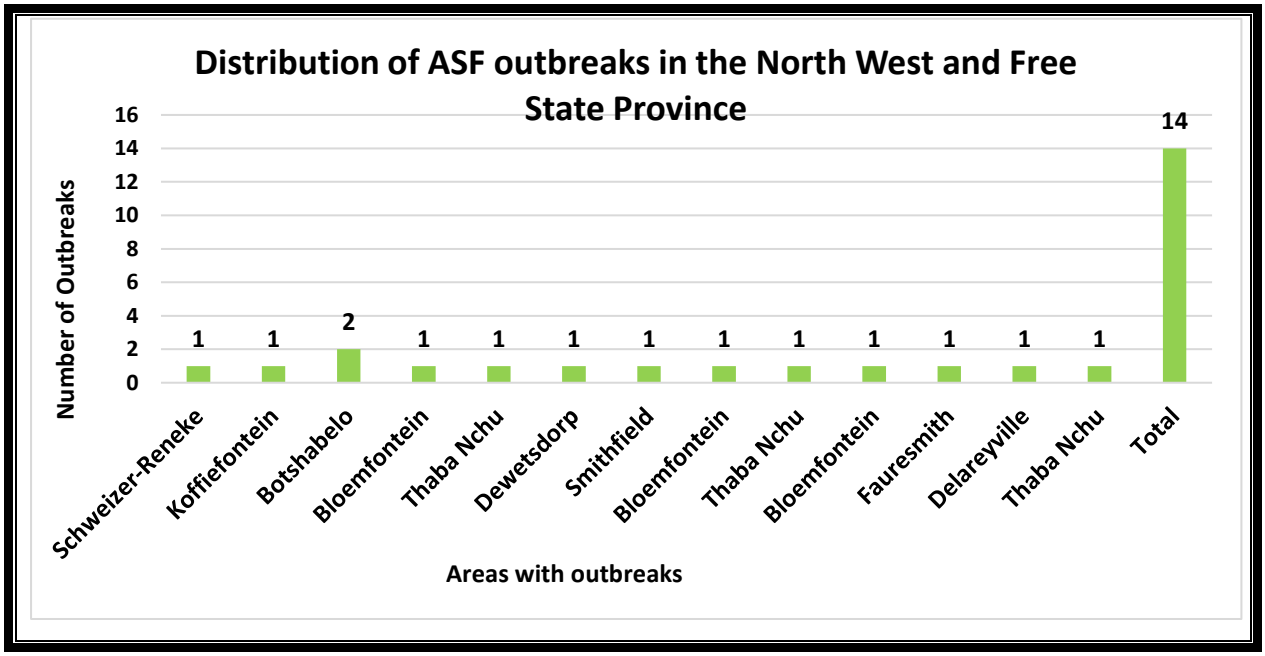

Figure 3.1: Distribution of ASF 2016/2017 outbreaks in the North West and Free State Province.

Source: field data from 2016/2017 ASF outbreaks

Table 3.2 shows that a total of 664 out of 1088 (61.02%) pigs succumbed to ASFV in North West and 424 out of 1088 (38.97%) were culled, 880 out of 1451 (60.64%) pigs succumbed to an ASFV while a total of 571 out of 1451 (39.35%) were culled in Free State, symptoms were consistent with ASF. In addition (*n*=2539) pigs were at risk.

Table 3.2: Summary of morbidity and mortality data during the ASF outbreaks per province

Province	No of outbreaks	Pigs died	Pigs culled	Population at risk
North West	**2**	**664 (61.02%)**	**424 (38.97%)**	**1088**
Free State	**12**	**880 (60.64%)**	**571 (39.35%)**	**1451**
Total	**14**	**1544 (60.81%)**	**995 (39.18%)**	**2539**

Source: field data from 2016/2017 ASF outbreaks

3.1.2. Possible factors that could have led to the ASF outbreak looking at farm practices, systems, and farmers' attitudes.

The analysis of the questionnaires revealed that pigs were allowed to scavenge for food on (*n*=7) 63.63% out of (*n*=11) farms in the North West and (*n*=19) 61.29% out of (*n*=31) farms in the Free State provinces (Table 3.3). Nine out of eleven (81.81%) interviewed farmers who reported sick pigs six months before ASFV was confirmed by the laboratory were from the North West and 5 out of 31 (16.12%) were from the Free State province. As soon as the outbreak was confirmed veterinary officials, implemented control measures on 11 out of 11 (100%) farms in the North West and 31 out of 31 farms in the Free State province. It was further noted that 100% of farms from both provinces had no ticks on their farms.

Table 3.3: Outbreak details and animal health management

North West Province				Free State Province			
Questionnaire variables		No. of incidents	Proportion of farms (%)	Questionnaire variables		No. of incidents	Proportion of farms (%)
Farms with pigs scavanging for food	Yes	7	63,63	Farms with pigs scavanging for food	Yes	19	61,29
	No	4	36,36		No	12	38,70
Farmers reporting health problems in their livestock during the past 6 months	Yes	9	81,81	Farmers reporting health problems in their livestock during the past 6 months	Yes	5	16,12
	No	2	18,18		No	26	83,87
Any control measures taken	Yes	11	100	Any control measures taken	Yes	11	35,48
	No	0	0		No	20	64,51
Are farmers experiencing ticks on their pigs	Yes	0	0	Are farmers experiencing ticks on their pigs	Yes	0	0
	No	11	100		No	31	100

The results on biosecurity (Table 3.4) revealed that all farms 11 out of 11 (100%) in the North West and 30 out of 31 (96.77%) farms in the Free State province were fenced. Only 1 out of 11 (9.09%) farms practiced routine cleaning in North West and 18 out of 31 (58.06%) farms from Free State practiced routine cleaning. Disinfectants were used by 1 out of 11 (9.09%) farmers in the North West and 4 out of 31 (12.90%) in the Free State province to reduce infections. Only one farmer out of eleven (9.09%) from the North West province quarantined new pigs on arrival and in the Free State only 21 out of 31 (67.74%). There were no warthogs or bush pigs spotted in nearby North West communities and only 1 out of 31 (3.22%) farmers confirmed seeing warthogs near his farm in the Free State province. In the Free State, bones were

discovered inside a pig enclosure during an interview with 1 out of 31 (3.22%) farmers, and there was no evidence of bones inside the enclosures in any of the farms visited in the North West province. Figures 3.2 and 3.3 depict farm layouts in the Free State and North West provinces, respectively.

Table 3.4: Biosecurity

North West Province				Free State Province			
Questionnaire variables		No. of incidents	Proportion of farms (%)	Questionnaire variables		No. of incidents	Proportion of farms (%)
Are Farmers premises fenced	Yes	11	100	Are Farmers premises fenced	Yes	30	96.77
	No	0	0.00		No	1	3.22
Is routine cleaning practiced	Yes	1	9.09	Is routine cleaning practiced	Yes	18	58.06
	No	10	90.90		No	13	41.93
Are disinfectants used	Yes	1	9.09	Are disinfectants used	Yes	4	12.90
	No	10	90.90		No	27	87.09
Are new pigs quarantined on arrival	Yes	1	9.09	Are new pigs quarantined on arrival	Yes	21	67.74
	No	10	90.9		No	10	32.25
Are there warthogs or bushpigs nearby	Yes	0	0	Are there warthogs or bushpigs nearby	Yes	1	3.22
	No	11	100		No	30	96.77
Any evidence of warthogs or bush meat fed to the pigs	Yes	0	0	Any evidence of warthogs or bush meat fed to the pigs	Yes	1	3.22
	No	11	100		No	30	96.77

Figure 3.2: Two Farm setups in Free State Province during 2016/2017 ASF outbreaks

Figure 3.3: Two Farm setups in North West Province during 2016/2017 ASF outbreaks

Table 3.5 shows that 9 out of 11(81.81%) farmers in the North West province and 31 out of 31 (100%) farmers in the Free State province cleaned the vehicles used to transport their pigs. Furthermore, 2 out of 11 (18.18%) farmers in North West province did not know whether their vehicles had been cleaned. Farmers in the North West province, 1 out of 11 (9.09%) and 29 out of 31 (93.54%) in the Free State province cleaned the external parts of their trucks.

The majority of farmers in the North West province, 7 out of 11 (63.63%) reported using domestic (kitchen) waste as pig feed, while only 3 out of 11 (27.27%) reported using restaurant waste, and 1 out of 11 (9.09%) reported using the compound feed. Furthermore, 3 out of 31 (9.67%) farmers in the Free State province used compound feed, 13 out of 31 (41.93%) used domestic (kitchen) waste, and 18 out of 31 (58.06%) used restaurant waste as feed for their pigs. It was also found that none of the farmers in both the North West and Free State provinces shared workers. Only 3 out of 11 (27.27%) farms in the North West province had dead pigs confiscated, while the Free State province had none.

Table 3.5: Introduction routes

North West Province				Free State Province			
Questionnaire variables		No. of incidents	Proportion of farms (%)	Questionnaire variables		No. of incidents	Proportion of farms (%)
Do farmers clean their vehicles before and after transporting their pigs?	Yes	9	81.81	Do farmers clean their vehicles before and after transporting their pigs?	Yes	31	100
	No	0	0		No	0	0
	Unknown	2	18.18		Unknown	0	0
Do farmers clean the external part of the truck	Yes	1	9.09	Do farmers clean the external part of the truck	Yes	29	93.54
	No	7	63.63		No	2	6.45
	Unknown	2	18.18		Unknown	0	0
What do they feed their pigs with	Compound feed	1	9.09	What do they feed their pigs with	Compound feed	3	9.67
	Industrial and agricultural by -product	0	0		Industrial and agricultural by -product	0	0
	Fish meal,blood meal, meat meal	0	0		Fish meal,blood meal, meat meal	0	0
	Domestic (Kitchen) waste	7	63.63		Domestic (Kitchen) waste	13	41.93
	Restaurant waste	3	27.27		Restaurant waste	18	58.06
	Other	0	0		Other	0	0
Do farmers share their workers with other farms close by?	Yes	0	0	Do farmers share their workers with other farms close by?	Yes	0	0
	No	11	100		No	31	100
Any confiscated dead/live pigs during the outbreak	Yes	3	27.27	Any confiscated dead/live pigs during the outbreak	Yes	0	0
	No	8	72.72		No	31	100

Table 3.6 demonstrate the results which were gathered through observations to further establish the risk of African Swine Fever ASFV in the province and the results revealed that 11 out of 11 (100%) of the farms were located in the township. The majority of farms, 5 out of 11 (45.45%) had pig housing made of brick construction, 2 out of 11 (18.81%) used corrugated material, 3 out of 11 (27.27%) used a fence, and only 1 out 11 (9.09%) used planks. The results further revealed that 7 out of 11 (63.63%) of the outside appearance of the farms was tidy.

A total of 3 out of 11 (27.27%) of the farms disposed of the manure or slurry on the farm while 8 out of 11 (72.72%) did not remove the manure or slurry from the enclosures. No farmer used stainless steel feeding troughs; however, cement or concrete feeding troughs were used on 3 out of 11 (27.27%) of the farms, and no feeding troughs were used on 8 out of 11 (72.72%) of the farms. In addition, all the farms had a clear view of fencing as a barrier around the premises.

Table 3.6: Farm observations in North West Province

Farm observation in North West Province			
Observation variables		Number of incidents	Proportion of farms (%)
Farm location	In the township	11	100
	Outside the township	0	0
Type of housing	Corrugated	2	18.81
	Fence	3	27.27
	Brick construction	5	45.45
	Planks	1	9.09
The farm's outside appearance	Tidy	7	63.63
	Untidy	4	36.36
Disposal of manure/slurry	On the farm	3	27.27
	Outside the farm premises	0	0
	No disposal	8	72.72
Feeding method	Stainless steel feeding troughs	0	0
	Cement/ concrete feeding troughs	3	27.27
	On the floor/no feeding troughs	8	72.72
Type of fencing on the farm	Clear view fencing	11	100
	Wood fencing	0	0
	Brick wall	0	0
	No fencing	0	0

Table 3.7 reports on the observations gathered from the Free State Province. The results revealed that most of the farms, 29 out of 31 (93.54%) are located in the township and only 2 out of 31 (6.54%) are outside the township. The results further revealed that 17 out of 31 (54.83%) farms used a fence for housing pigs and only 1 out of 31 (3.22%) used planks. The outside farm appearance demonstrated 16 out of 31 (51.61%) to be tidy and 15 out of 31 (48.38%) to be untidy. A total of 24 out of 31 (77.41%) farms disposed of manure or slurry on the premises and 7 out of 31 (22.58%) did not dispose of it. The majority of the farms, 26 out of 31 (83.87%) did not have any feeding troughs inside the enclosures. The results revealed that 30 out of 31 (96.77%)

of the farms used a clear-view fence as a barrier on the premises and only 1 out of 31 (3.22%) had no fencing.

Table 3.7: Farm observations in Free State Province

Farm observation in Free State Province			
Observation variables		Number of incidents	Proportion of farms (%)
Farm location	In the township	29	93.54
	Outside the township	2	6.54
Type of housing	Corrugated	8	25.80
	Fence	17	54.83
	Brick construction	5	16.12
	Planks	1	3.22
The farm's outside appearance	Tidy	16	51.61
	Untidy	15	48.38
Disposal of manure/slurry	On the farm	24	77.41
	Outside the farm premises	0	0
	No disposal	7	22.58
Feeding method	Stainless steel feeding troughs	1	3.22
	Cement/ concrete feeding troughs	4	12.90
	On the floor/no feeding troughs	26	83.87
Type of fencing on the farm	Clear view fencing	30	96.77
	Wood fencing	0	0
	Brick wall	0	0
	No fencing	1	3.22

3.1.3 African Swine Fever (ASF) action plan and eradication measures.

Figure 3.4 shows the burial sites in the North West province. As part of disease surveillance, the province established a five km quarantine radius, sampling of wild pigs, and sero-surveillance. Free-roaming pigs were also found during the disease surveillance. Figure 3.5 shows the schematic view of the ASF action plan and eradication measures implemented by North West province. Movement controls, quarantine restriction, forward and backward tracing, suspension of slaughter at a

local abattoir, and auction monitoring were implemented, in addition, tyre bath, culling, roadblocks, and control points were also implemented (Figure 3.6). The findings also show that the carcasses were burnt, covered with lime, and buried according to the relevant regulation for the control of African Swine Fever disease. The disease action plans derived from the questionnaire results in North West Province demonstrated that veterinary officials engaged with the community members during the ASF outbreaks to bring awareness through radio interviews, newspapers, and media statements (Table 3.8). In terms of disinfection and mop-up in North West province, 11/11 pig farms were disinfected three days after culling using an F10®SC product with a concentration of 1:150, and pressure sprayers were used.

Figure 3.4: Burial site in Schweizer-Reneke-outside Ipelegeng Township during 2016/2017 ASF outbreaks.

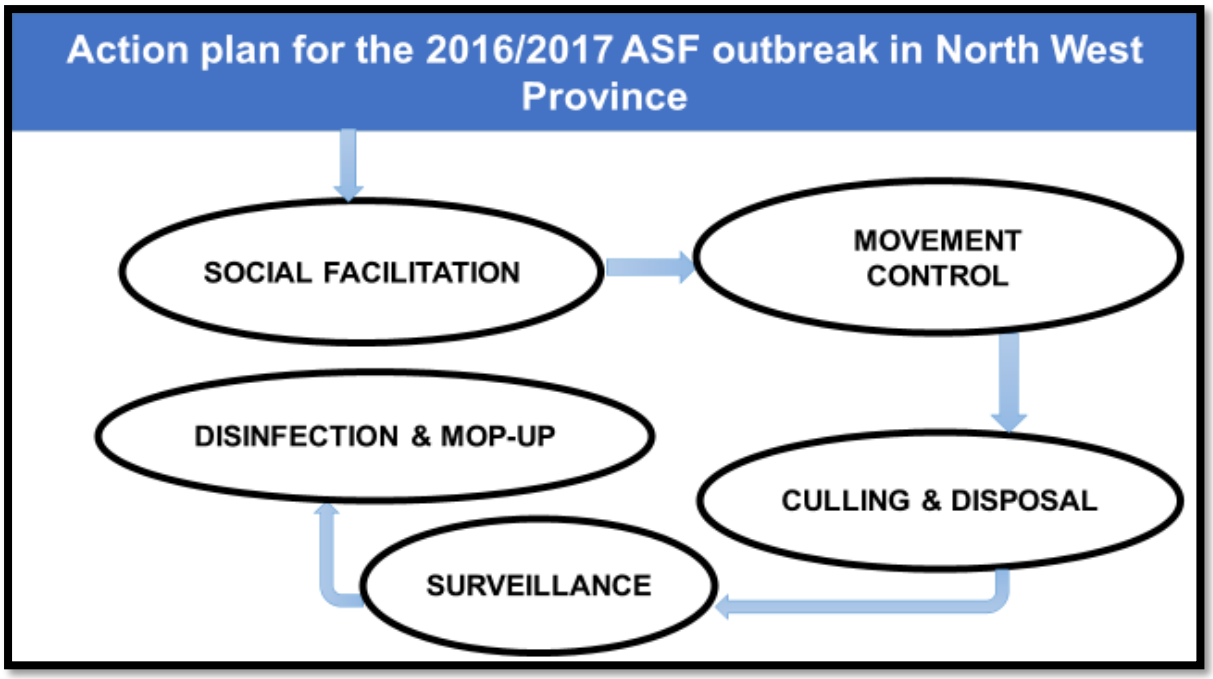

Figure 3.5: Schematic view of ASF action plan and eradication measures implemented by North West province.

(Source: courtesy of MT Rametse, 2022).

Figure 3.6: ASF control plan in the Northwest province Schweizer-Reneke during 2016/2017 outbreaks.

(*Source: courtesy of Dr ME Machedi*).

The Animal Health Technicians mentioned that during the outbreaks, two warthogs were found dead along the dam, which has been dry since June 2016 (Figure 3.7), and unfortunately, they were not sampled to establish the cause of death.

Figure 3.7: Dry dam on a Farm in Koffiefontein during the ASFV 2016/2017 outbreak.

Further data was gathered using a questionnaire on disease action plans in Free State Province. The results showed that veterinary officials engaged only with farmers to raise awareness during ASF outbreaks, with no radio interviews, media statements, or print media reporting (Table 3.8). Movement controls were implemented namely, quarantine restriction, forward and backward tracing, suspension of slaughter at a local abattoir, and auction monitoring. There was no implementation of tyre baths, roadblocks, and control points. The findings also indicate that the carcasses were burnt and buried. As part of disease surveillance, the province established a five-kilometer quarantine radius, sampling of wild pigs, and sero-surveillance. Free-roaming pigs were also found during the disease surveillance.

Table 3.8: Disease action plan per province

North west Province			Free State Province	
Disease action plan				
Social Facilitation			**Social Facilitation**	
Meeting with farmers	YES OR NO		Meeting with farmers	YES OR NO
	Yes			Yes
Radio Interviews	Yes		Radio Interviews	No
Print media	Yes		Print media	No
Media statement	Yes		Media statement	No
Movement control			**Movement control**	
Quarantine	Yes		Quarantine	Yes
Forward and backward tracing	Yes		Forward and backward tracing	Yes
Roadblocks and control points	Yes		Roadblocks and control points	No
Tyre bath	Yes		Tyre bath	No
Suspension of slaughter at local abattoir	Yes		Suspension of slaughter at local abattoir	Yes
Auction monitoring	Yes		Auction monitoring	Yes
Culling			**Culling**	
Burn	Yes		Burn	Yes
Bury	Yes		Bury	Yes
Other	Carcasses were also covered with lime		Other	Carcasses were not covered with lime
Surveillance			**Surveillance**	
Quarantine radius	5km		Quarantine radius	5 km
Sero-surveillance	Yes		Sero-surveillance	Yes
Free roaming pigs	Yes		Free roaming pigs	Yes
Wild pigs sampled	Yes		Wild pigs sampled	Yes

3.1.4 Clinical and Necropsy findings of the 2016/2017 ASFV outbreak in the Free State and North West Province

Figure 3.8 demonstrates analysed clinical findings data extracted from submission forms. The data was gathered from (*n*=7) out of (*n*=11) farms in North West and (*n*=9) out of (*n*=31) farms in the Free State province. Data from farms in the North West province revealed that (*n*=6) farms had pigs exhibiting symptoms of recumbency, lethargy, and difficulty breathing. There were two farms, one farm had pigs showing a fever higher than 40°C, whereas the other had pigs with ataxia. Pigs from three farms had reddening of the ventral chest and abdomen. In addition, four farms had pigs displaying listlessness. Information extracted from submission forms revealed that in the Free State Province, a total of (*n*=9) farms were covered out of (*n*=31). Coughing, dark diarrhoea, and ataxia was seen from (*n*=1) farm, recumbency, lethargy, and breathing difficulty were further seen from seven farms. Similar to farms in the North West province, pigs on three farms in the Free State province displayed reddening of the ventral chest and abdomen. The results showed that the pigs from both provinces had either acute or subacute forms of the disease.

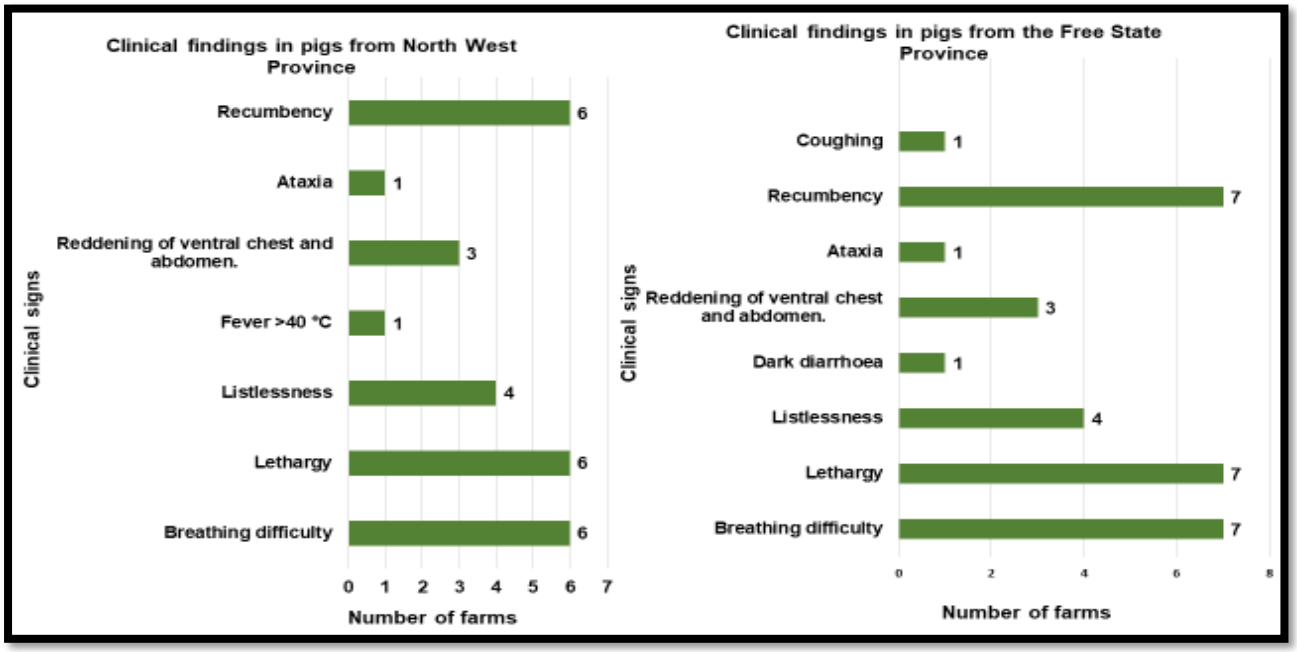

Figure 3.8: Analysed data on clinical findings from domestic pigs in the North West and Free State province.

Figure 3.9 depicts an analysis of necropsy data extracted from submission forms. Post mortems were conducted from 7 out of 11 (63.63%) farms in the North West and 9 out of 31 (29.03%) farms in the Free State province. Data from farms in the North West province revealed 6 out of 11 farms with carcasses exhibiting enlarged haemorrhagic lymph nodes, three with severe chronic enteritis and ecchymotic haemorrhages under the skin. Petechiae in the kidney cortex and congestion of the lungs were discovered in carcasses from two farms. In addition, there was a farm *(n=1)* that had a carcass with an enlarged spleen and another farm had a carcass with bleeding into pleural and abdominal cavities.

Pneumonia, an enlarged spleen, and lung congestion were found in carcasses from 2 out of 31 farms in the Free State province. Most of the farms (*n*=8) had carcasses with enlarged haemorrhagic lymph nodes. Carcasses with severe chronic enteritis and ecchymotic haemorrhages under the skin were discovered from (*n*=4) farms. In addition, there were two separate farms, (*n*=1) that had carcasses with petechial haemorrhage on both small, and large intestines and the lungs, and another with bleeding into pleural and abdominal cavities.

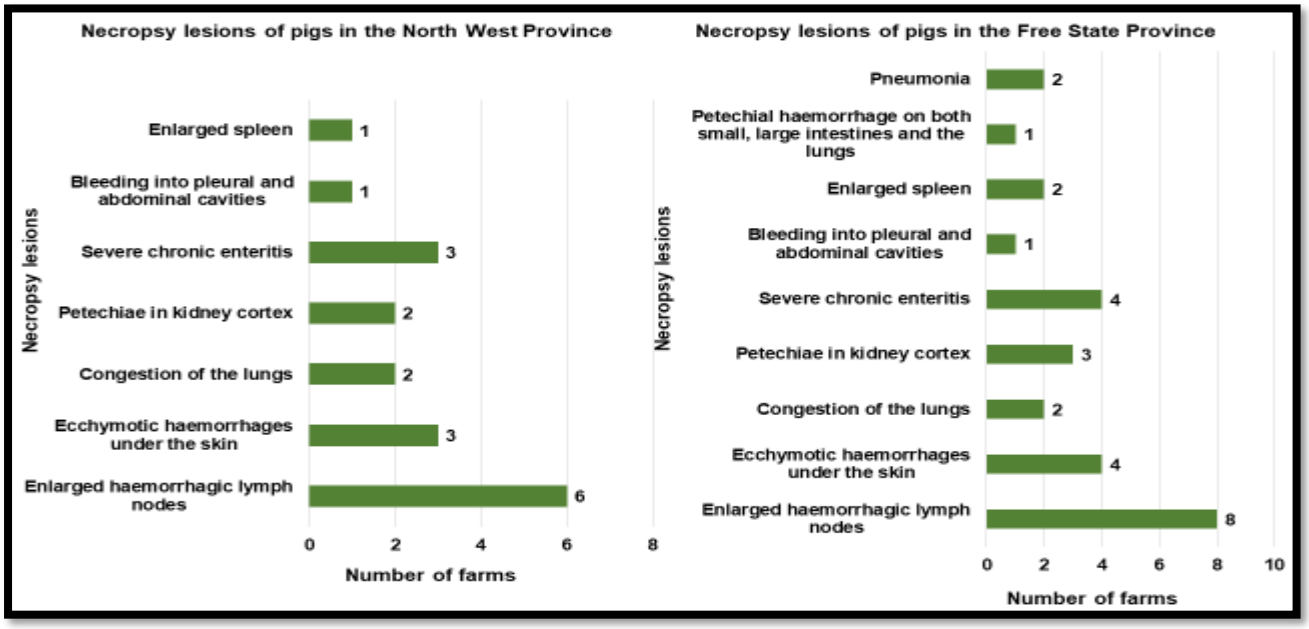

Figure 3.9: Analysed data on necropsy lesions from domestic pigs in the North West and Free State province.

3.2. DETECTION OF AFRICAN SWINE FEVER VIRUS.

3.2.1. Laboratory results from PCR and ELISA assays

Table 3.9 shows a summary of the collected samples. This sero-prevalence indicates that the virus has been spreading in these areas and that subclinical cases may occur. Of the 67 EDTA blood samples submitted for PCR test, 23 out of 67 (34%) tested positive with the correct band size and 26 out of 67 (39%) tested negative whereby 27% of the samples were not tested. Of the 44 tissue samples submitted for PCR test, only 23 out of 44 (52%) tested positive and 21 out of 44 (48%) tested negative. In addition, warthog tissue samples submitted for PCR test, only 9 out of 10 (90%) tested positive and 1 out of 10 (10%) tested negative.

Table 3.9: Summary of results for the samples collected during the ASF outbreak in the domestic and wild pigs from North West and Free State Province.

Species							
Domestic Pigs				Warthogs			
		Results				Results	
Sample type	No. of samples	Pos^+	Neg^-	Sample type	No. of samples	Pos^+	Neg^-
EDTA	67	23	26	EDTA	2	2	0
Serum	174	18	138	Serum	9	7	2
Tissue	44	23	21	Tissue	10	9	1

Table 3.10 shows a 52.3% proportion of pigs having positive PCR results using tissue samples from the domestic pigs in Northwest and Free State provinces and Table 3.11 shows a 10.3% proportion of seropositive pigs by ELISA from North West and Free State province. Table 3.12 shows 20% and 38.5% proportion of samples testing positive on PCR from Northwest and Free State provinces respectively.

Table 3.10: Prevalence of ASFV by diagnostic PCR using tissue samples from the domestic pigs

Province sampled	Total sampled	Total not tested	Doubtful	Number of PCR positive	Number of PCR negative	The proportion of positive PCR pigs (%)
North-West	17	0	0	10	7	58.8%
Free State	27	0	0	13	14	48.1.%
Total	**44**	**0**	**0**	**23**	**21**	**52.3%**

Table 3.11: Proportion of domestic pigs with ASFV antibodies during field surveillance by ELISA in North West and Free State province

Province sampled	Total sampled	Total not tested	Doubtful	Number positive	Number negative	The proportion of seropositive pigs (%)
North-West	90	10	5	0	75	0%
Free State	84	0	3	18	63	21.4%
Total	**174**	**10**	**8**	**18**	**138**	**10.3%**

Table 3.12: Prevalence of ASFV by diagnostic PCR using EDTA blood of the domestic pigs

Province sampled	Total sampled	Total not tested	Doubtful	Number of PCR positive	Number of PCR negative	The proportion of positive PCR pigs (%)
North-West	15	0	0	3	12	20%
Free State	52	18	0	20	14	38.5%
Total	**67**	**18**	**0**	**23**	**26**	**34%**

Figure 3.10 (a and b) shows the analysed laboratory results in domestic pigs, 174 serum samples were sent in for antibody detection and the results revealed that antibodies were positively detected only in 18 out of 174 (10%) of the pig population and 138 out of 174 (79%) tested negative while 10 out 174 (6%) were not tested and 8 out of 174 (5%) of the results were doubtful. For the detection of ASFV, EDTA samples were sent and only 23 out of 67 (34%) tested positive. Moreover, 23 out of 44 (52%) organ samples tested positive for ASFV.

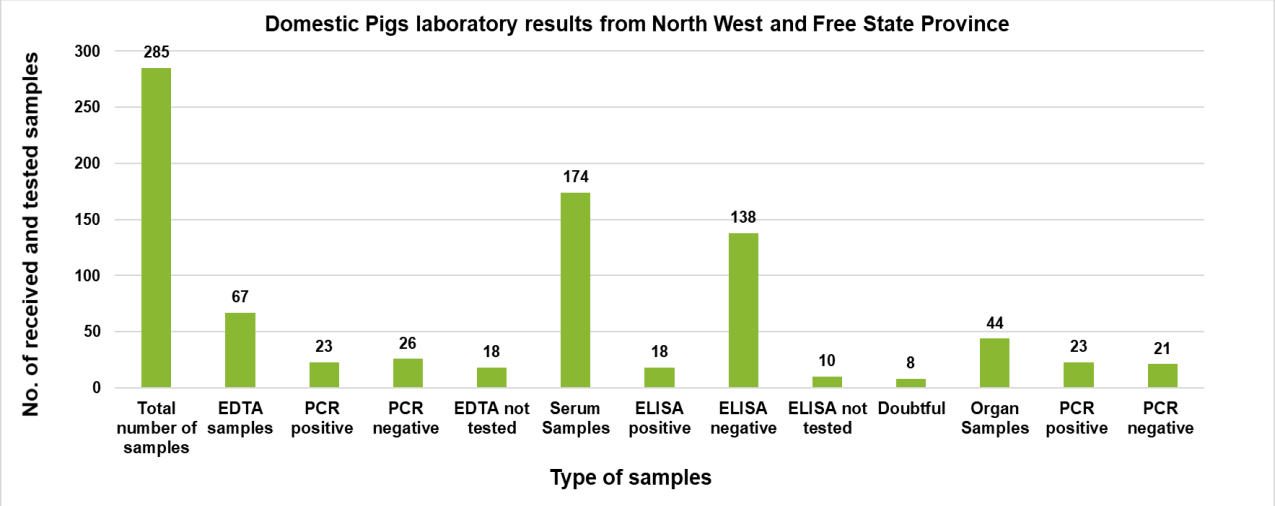

Figure 3.10 (a) ASFV analysed Laboratory results from the domestic pigs in the North West and Free State Province.

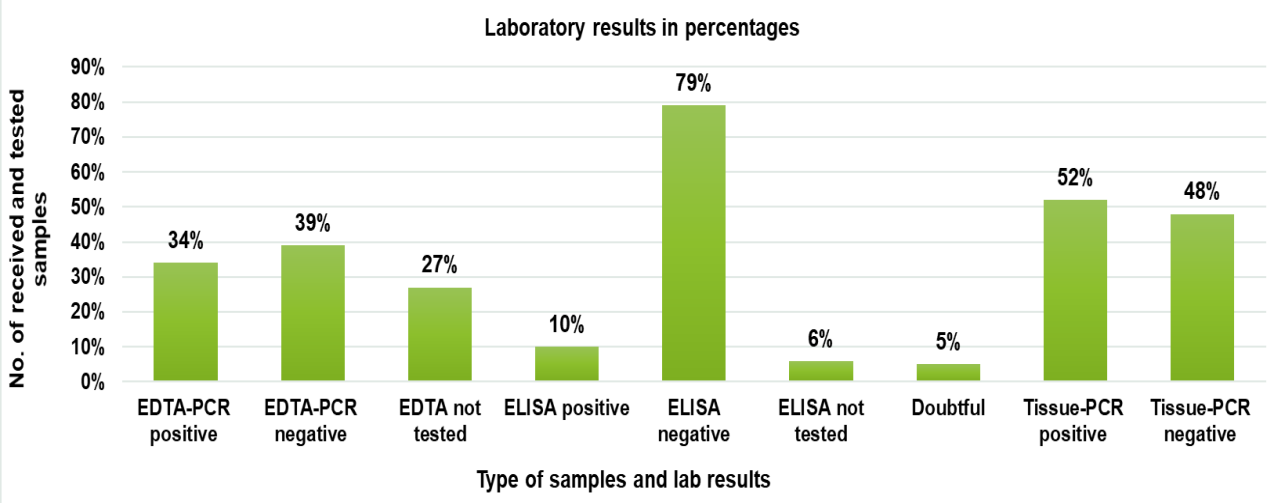

Figure 3.10 (b): ASFV analysed Laboratory results from the domestic pigs in the North West and Free State Province.

Ninety percent of the warthog EDTA blood samples from the FSP tested positive for ASFV detection and 100% were from the NWP. For antibody detection, 100% from FSP tested positive for antibodies while 1 out of 3 (33%) from the NWP tested positive in serum samples. All the organ samples from the NWP tested positive for ASFV, and 7 out of 8 (90%) were from the FSP (Figure 3.11).

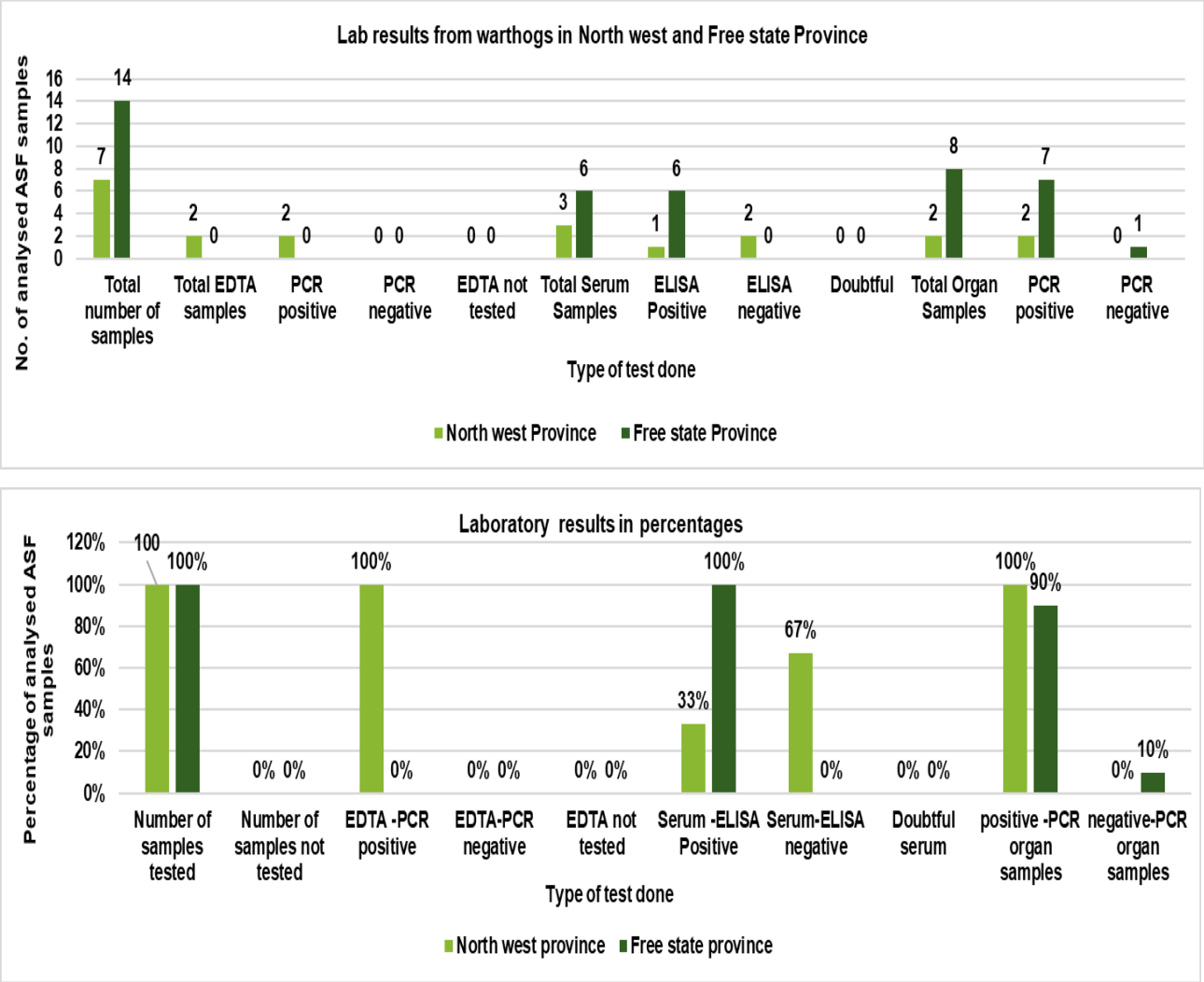

Figure 3.11: ASFV analysed Laboratory results from wild pigs in the North West and Free State Province.

3.2.2. Genetic characterization of the outbreak virus in domestic pigs and *Ornithodoros moubata* ticks.

The laboratory results revealed that the 2016/2017 ASF outbreaks were caused by genotype I (Figure 3.12). The positive PCR test results obtained from the outbreak samples revealed that the strains from the Koffiefontein in the Free State Province were designated RSA 02/2016, RSA 08/2016, RSA 12/2016, and RSA 16/2016. Furthermore, the strains from other outbreaks within the Free State from Bloemfontein were designated, RSA 06/2016, RSA 07/2016, RSA 13/2016, and RSA 15/2016. Mangaung strain was designated RSA 03/2016, Botshabelo strain was designated RSA 11/2016, Thaba Nchu strains were designated RSA 09/2016, and RSA 10/2016 lastly Khotsong strain was designated RSA 04/2016. The strains from Ipelegeng in the North West Province outbreaks were designated RSA 01/2016 and RSA 14/2016 and Zeerust was designated RSA 17/2016.

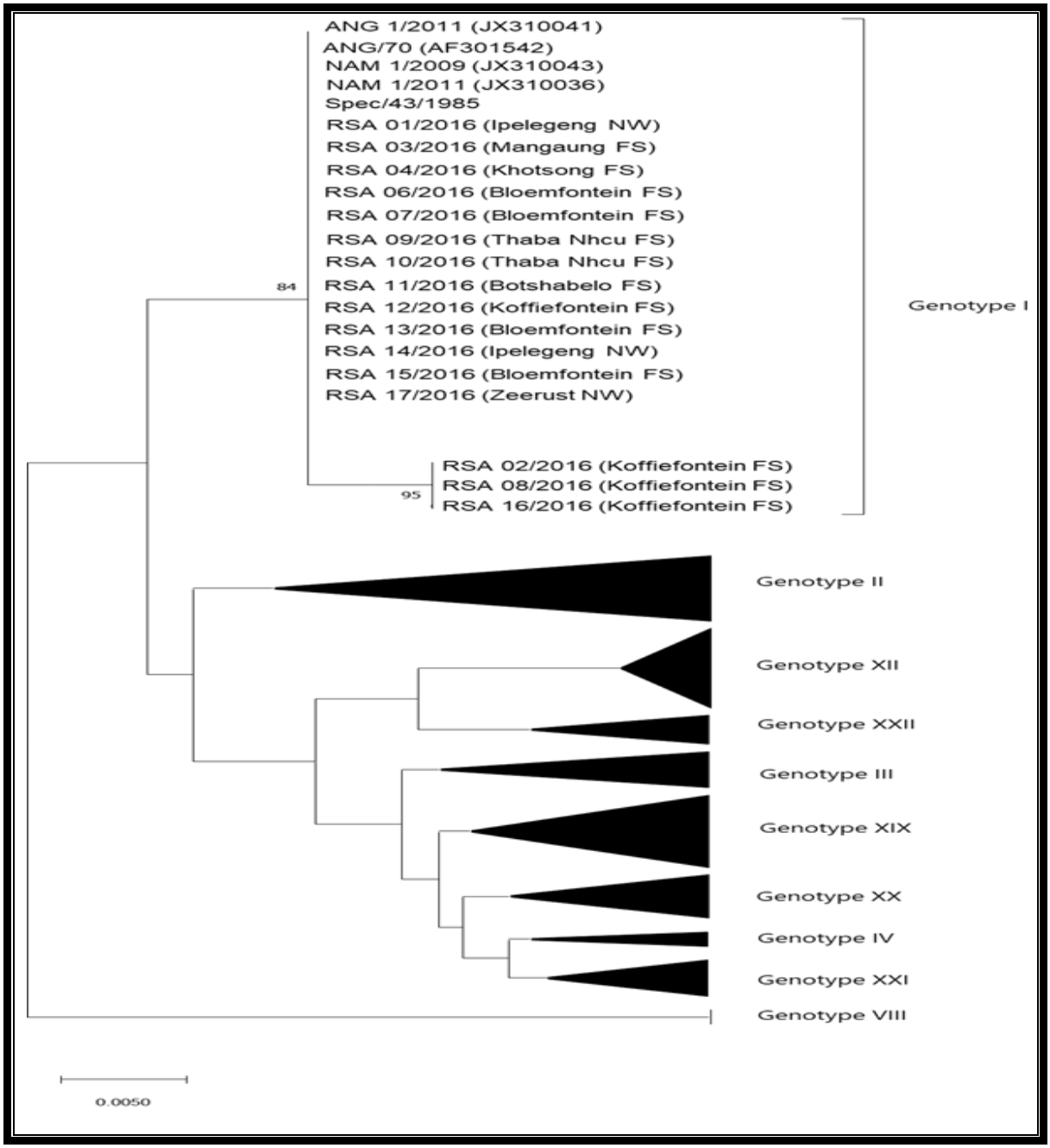

Figure 3.12: Neighbour-Joining tree depicting p72 gene relationships of African Swine Fever viruses from outbreaks in domestic pigs in southern Africa, including all isolates from the 2016/2017 outbreaks in the North West and Free State provinces.

Bootstrap values >60% obtained following 1,000 replications and are indicated next to the Nine genotypes were designated based on previous studies (I–X, Bastos *et al.,* 2003 and II–XVI, Lubisi *et al.,* 2005; Boshoff *et al.*, 2007 XVII-XXII).

In Koffiefontein, Free State, 88 *Ornithodoros moubata* ticks were recovered and collected from seven of ten burrows (Table 3.13). All of the recovered ticks were nymphs with no adults. The burrows were found in savannah grassland areas with sandy soil. Using real-time PCR, the laboratory results demonstrated that 10 out of 88 (11%) of the samples collected from warthog burrows in Koffiefontein tested positive for ASFV DNA with 68 out of 88 (77%) testing negative, and 10 out of 88 (11%) doubtful. Table 3.13 displays the results from Koffiefontein, where two warthog burrows had a significant number of ticks recovered (18 and 20) that tested negative for ASFV, and three other warthog burrows had ticks recovered that also tested negative. The three remaining warthog burrows had no ticks recovered. In addition, the laboratory results from one warthog burrow were doubtful, leaving only one burrow testing positive for ASFV.

Table 3.13: Virus detection of ASFV in ticks (*Ornithodoros moubata*) in Koffiefontein.

Warthog Burrows (WB)	Number of ticks	Laboratory results
WB No.1	0	
WB No.2	0	
WB No.3	10	Doubtful
WB No.4	10	Negative
WB No.5	10	Positive
WB No.6	10	Negative
WB No.7	0	
WB No.8	20	Negative
WB No.9	10	Negative
WB No.10	18	Negative
	Total 88	

Phylogenetic analysis confirmed that the virus recovered from the ticks was genetically identical to viruses that caused the outbreak of ASF in domestic pigs on the farm in 2016. Ticks collected from the Koffiefontein area, shown in blue (Figure 3.13), compared to viruses recovered from 2016/2017 outbreaks in the North West and Free State provinces.

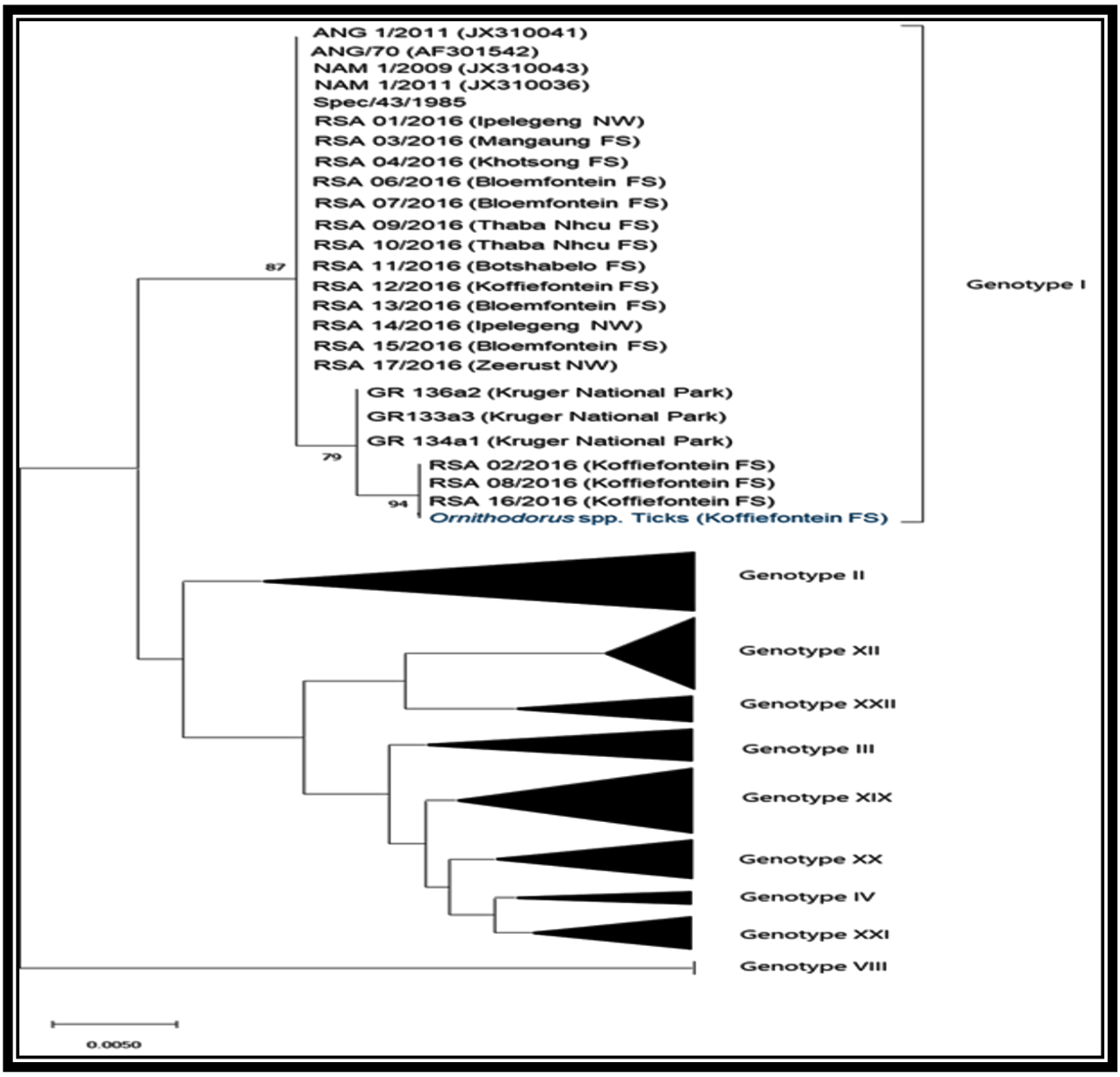

Figure 3.13: Neighbour-Joining tree depicting p72 gene relationships of African Swine Fever viruses from *Ornithodoros ssp.* Isolates GR133a3, GR134a1, and GR136a3 were isolated from ticks in the Kruger National Park in 1981. Bootstrap values >60% obtained following 1,000 replications and are indicated next to the Nine genotypes were designated based on previous studies (I–X, Bastos *et al.,* 2003 and II–XVI, Lubisi *et al*., 2005; Boshoff *et al*., 2007 XVII-XXII).

CHAPTER 4: DISCUSSION

4.1 Outbreak investigation on farm level to assess ASF risk factors

4.1.1 Farm practices, systems, and farmers' attitudes evaluated.

Pig production creates economic opportunities for many families by alleviating the burden of poverty, owing to the fact that pigs are an inexpensive source of protein that requires little space to manage. ASF outbreaks' high morbidity and mortality rates have a devastating effect, threatening food security and increasing the burden of poverty on families who rely on pig farming for a living. The index case was in pigs from Schweizer-Reneke in the North West Province and septicaemia was suspected. Index cases from the Free State province occurred in Koffiefontein. The commercial pig industry was not affected by any outbreaks, which could be attributed to the fact that most commercial farms have strict access controls or practice a reasonable level of biosecurity, which makes a significant contribution to the pig industry's safety.

The outbreaks, confirmed in both provinces were reported outside the control zone area and they were confirmed by the laboratory at almost the same time. The study further revealed risk factors for ASF maintenance and dissemination such as informal marketing systems and the movement of pigs from Bloemfontein in the Free State province for auction. Pigs were sold locally in both the Free State and North West provinces for religious and other ceremonial purposes. On the other hand, tracing the exact source of a pig-related outbreak is usually difficult, if not impossible, because the movements involved are frequently illegal (Penrith and Vosloo, 2009). Other risk factors included free-roaming pigs and uncooked swill feeding; failure to monitor such activities increases the risk of the virus spreading to other areas, resulting in a biosecurity breach. Veterinary reports and in-person interviews suggested that the virus was circulating for at least six months before samples were submitted followed by ASF being confirmed and detected by the laboratory. The delay in detecting and confirming the disease led to the delayed implementation of contingency plans therefore infected pigs posed a risk of shedding the virus on other pigs.

This study focused on establishing risk factors that, could have led to ASF outbreaks. There were no new introductions of pigs before the outbreaks. This work and analysis revealed some deficiencies in record keeping. It becomes a significant challenge when there are no or limited records to establish risk factors at the farm level. In this study,

an attempt was made to validate some of the farmers' data collected through observational analyses conducted by the interviewer. Despite this difficulty, the study revealed that veterinary officials worked tirelessly to implement control measures to contain the virus. The major concern is how certain farmers (16.12%) were not willing to report health problems experienced by their pigs, due to assumptions that the pigs were dying of cold weather, they further mentioned that they had a challenge with transport to reach the veterinary offices and such behaviour may favour disease occurrence.

During the farm visit, one farmer privately mentioned to us that they do not report sick animals because they are afraid of being victimized by their fellow farmers. This kind of attitude could indicate that the farmers are fully aware that any abnormalities observed on their pigs should be reported, but they deliberately choose not to report them to their local veterinary officials. The dead pigs were dumped at the gravesite, and this would be another mode of ASFV transmission even if the pigs were not yet clinically ill. Moving carcasses around proved that there was no good record-keeping system. Farmers knew that no one would question a decreasing trend in the pig population on their farms. The farmers further mentioned that they were at a later stage compelled to inform the veterinary officials as they started experiencing high mortalities. Following ASF outbreaks in other developing countries, a similar attitude by farmers has been shown to contribute to the spread of ASFV (Nana-Nukechap and Gibbs, 1985; Costard *et al.,* 2009; Fasina *et al.,* 2010; Penrith *et al.,* 2013).

African Swine Fever (ASF) disease awareness and willingness to report it for proper government action to be taken will go a long way toward controlling the disease's spread and, eventually, getting rid of the disease (Ebwanga *et al.,* 2021). Awareness campaigns by veterinary officials may assist in establishing relationships with their farmers and building trust. This kind of practice may improve better reporting of health issues on their farms. A pig farm is a valuable asset, but it is critical to implement adequate biosecurity measures. Except for the large commercial farms with quarantine areas, the farmers in NWP and FSP affected by the ASF outbreaks were smallholder farms owned by families that lacked quarantine areas. Pig-keeping units in communal or small-scale settings may practice poor biosecurity, making them more vulnerable to ASF transmission (Chenais *et al*., 2017; Simulundu *et al.*, 2017; Penrith *et al.*, 2019).

The farmers confirmed the availability of free-roaming pigs in the areas. Animals from different herds can share the same grazing areas, and they can also mix with wild pigs, facilitating virus transmission (Mur *et al.,* 2016). There were farmers who were personally involved in the daily running of the farms with no help because they could not afford to employ. Due to low levels of biosecurity, during the ASF outbreak investigation in 2016/2017, the AHTs did report seeing warthog pigs in Koffiefontein scavenging crop fields together with the domestic pigs (Figure 3.2 above). In many areas of Africa, warthogs are known to scavenge crop fields and are identified as major contributors in terms of destroying maize and bean fields (FAO, 2010). One farm had evidence of meat and bones being fed to the pigs, and veterinary officials confirmed that warthog hunting is very common in the area. This farmer could not agree or disagree that the meat and the bones belonged to warthogs.

During the period of tick sampling in January 2020, no live warthogs were spotted in Koffiefontein except the carcass remains, which confirms the presence of warthogs in the area. Since the 2016/2017 ASF outbreaks, there were no further outbreaks reported in this area but this should not be ignored, as an intensive study is required mainly where different suid species tend to co-exist, and the establishment of several warthog populations in this area will be of utmost importance. The discovery of illegal traps used for warthogs in Koffiefontein is of great concern and this proves that domestic pigs can easily gain access to warthog meat through human beings, who further play a role in the dissemination of ASFV due to illegal hunting by transporting warthog carcasses to the vicinity of domestic pigs. Further risk will be ticks, which dislodge from the warthog carcasses, further remaining a source of infection for a long period.

Though no signs of ASF can be seen in warthogs, substantial viral replication and viremia can be detected in young animals (Thomson *et al*., 1980). There was clear evidence that the farmers were inexperienced in areas of biosecurity measures, biosecurity was non-existent because new pigs were not quarantined on arrival, routine cleaning was not practiced and disinfectants were not used. Lack of understanding and acknowledgment of the importance of biosecurity meant that their farms were exposed to unknown pathogens. Decontamination of animal houses, sheds, pens, yards, water troughs, and surrounding areas is therefore critical to reducing the risk of ASFV contamination of the environment. ASFV-inactivating

disinfectants include 2% sodium hydroxide, detergents, phenol substitutes, sodium or calcium hypochlorite (2-3% chlorine), and iodine compounds (FAO, 1999; DADF, 2020).

However, two farmers, one in NWP and another in Thaba Nchu, FSP, implemented rudimentary biosecurity measures to great effect. The mentioned farmer in FSP (Figure 3.2 above) kept pig production within proximity of other farmers but did not experience any mortalities during the outbreak. When visiting the farm, it was discovered that his pigs were produced in a low biosecurity subsistence husbandry system, which could have possibly led to his farm being protected from ASF infection. He mentioned that he had two employees who worked on the farm and that he used F10®SC and other store-bought disinfectants for the footbath and cleaning to minimise contamination. He also uses formulated feed which he sources from the Co-op in Bloemfontein. A study on pig productivity in south-eastern Nigeria found that a reasonable implementation of biosecurity protected farmers from ASF (Nwanta *et al.,* 2011).

The farmers in NWP (*n*=10) and FSP (*n*=31) confirmed using uncooked swill for their pigs, which they source from local restaurants. The spread of ASF has frequently been linked to swill feeding (European Food Safety Authority *et al.,* 2017). One farmer mentioned that he only uses commercial feed when the auction period is approaching for the pigs to grow faster to generate a good price; this clearly indicates that the farmer was aware of the benefits, which come with commercial feed. This similar behaviour was seen in a study, which was conducted in South Dagon Township in Myanmar (Ebata *et al.,* 2020). Phiri *et al.* 2003 stated that pig farming is an appealing alternative to ruminant farming because it requires less investment, does not contend for pastureland, can be used to transform kitchen waste into food, and has a relatively short reproduction cycle, allowing for a better financial return.

One farmer in NWP confirmed that he does not use swill for his pigs (Figure 3.3 above), and there was evidence of bags of commercial feed on the premises. This behaviour can minimise the risk of ASF. He practiced biosecurity, he had only two employees, and every person leaving the premises will have their equipment disinfected. His weaners (*n*=29) started dying during the ASFV outbreak, the veterinary officials tested all live and dead pigs for ASFV, and they all tested negative.

This does not necessarily imply that the pigs were not infected with the ASFV; perhaps the animal had just been infected and has not yet seroconverted and again clinical signs may not be evident.

Observations revealed risk factors and we identified pigs from 17 farms in the Free State province that were confined in enclosures constructed with a fence structure. These structures do supply the animals with ventilation but they are not good structures as they expose the animals to poor weather conditions and, can easily expose the pigs to unknown pathogens through direct or indirect contact, particularly since we discovered that biosecurity was compromised. This type of structure could be motivated by the high cost of building materials. Manure and slurry, are risk factors, they can play a role in the dissemination of the ASFV and it was clear that the farmers from both provinces had no knowledge of this. The disease transmission pathways of ASF are based on its epidemiological characteristics; manure and slurry are also pathways (Gallardo *et al.,* 2015).

Feeding the pigs directly on the ground is another risk factor because pigs can easily consume infected urine, faeces, and feed. It is now clear that ASFV can be transmitted directly through contact between infected and susceptible pigs, through consumption of infected pig meat, bites of infected *Ornithodoros spp*., and through contact with virus contaminated materials or objects (bedding, feed, equipment, clothes, and footwear, and vehicles) and fluids such as blood, faeces, urine, or saliva from infected pigs (Penrith and Vosloo, 2009). Farmers should be encouraged to use feeding troughs because they can be easily cleaned and, disinfected to reduce the risk of disease pathogens. In the FSP, there was one farmer in Koffiefontein who had no fencing at all on his farm (Figure 3.2 above); hence, his pigs co-existed with the warthogs on the crop field.

The observation gave us the impression that farmers prioritised the economic and social benefits of pig farming over disease risk. Observations further revealed that the farmers conducted their small-scale farming business in the townships, with the exception of one farmer in FSP who was 15 kilometers outside the township. His farm was on agricultural land and that meant that he had the advantage to have more pigs by expanding the size of his pens. The township farming could be encouraged by the fact that pigs require little labour and land, which allowed families with limited land to

diversify their income sources. Households engage in pig farming to supplement other, more resource-intensive sources of income (Ebata *et al.,* 2020). The North West province's implementation of the ASF action plan and eradication measures demonstrated that veterinary officials had a good understanding of disease epidemiology. Actions to prevent ASF introduction and spread should consider the disease's epidemiology, with a particular focus on virus resistance in the environment, routes of transmission and excretion, and the characteristics of the farming systems in place (Arias *et al.,* 2018).

4.1.2 Clinical presentation and Necropsy findings.

The symptoms of ASF can vary depending on the virus isolate, infection route, dose, and characteristics of the host (Sanchez-Vizcaino *et al.,* 2015a). African Swine Fever can be acute, subacute, or chronic, and some animals may seroconvert without becoming ill. Subacute or chronic ASF infection is caused by less virulent strains. In this study, the disease was suspected where clinical symptoms and necropsy findings, were suggestive of ASF. Acute forms of the disease were observed in North West province according to the veterinary reports analysed during the outbreaks. Pigs were lethargic developing a fever above 40°C and the high fever indicated that a highly virulent strain was circulating further putting other pigs at risk of being exposed to ASFV. Sanchez-Vizcaino *et al.* (2015a) state that this is the most usual form of the disease.

Furthermore, the pigs had difficulty breathing, confirming the spread of a highly virulent strain. Severe pulmonary oedema, accompanied by respiratory changes, is a common finding in pigs infected with highly virulent strains of ASF (Sanchez-Vizcaino *et al.,* 2015a). The pigs displayed erythema on their skin; the reddening was visible on their ventral chest and abdomen. Erythema (most apparent in white pigs) can also manifest clinically in the ears, tail, extremities, and perianal areas. As the symptoms progressed pigs developed ataxia which occurs due to hind-limb weakness. Similar clinical presentations which conformed to an acute form were observed in pigs from the FSP; they had recumbency, dark diarrhoea, ataxia, reddening of the ventral chest and abdomen, listlessness, lethargy, and difficult breathing indicative of the lung oedema that is often the primary cause of death. Coughing was also reported and it can be associated with chronic disease. Chronic ASF can be fatal (Spickler and Roth, 2019).

Necropsy findings in NWP pigs confirmed the acute form, and the findings were enlarged spleen, bleeding into pleural and abdominal cavities, severe chronic enteritis, petechiae in the kidney cortex, congestions of the lungs, ecchymotic haemorrhages under the skin, and enlarged haemorrhagic lymph nodes. Gómez-Villamandos *et al.* (1995) state that during necropsy, most observed lesions are haemorrhages, oedema, and infarcts in the lymph nodes. It can also be observed in the spleen, which is frequently dark in colour and enlarged. Other organs, which are frequently affected, are the kidneys, liver, gall bladder, stomach, and lungs, which will frequently have petechial and haemorrhages. Sánchez-Vizcaíno *et al.* (2015a) confirm that the pigs survive longer in acute and subacute forms and there is a possibility of observing the presence of haemorrhagic lesions on the skin, also the haemorrhagic excretions.

Necropsy findings in FSP pigs did not only confirm the acute form namely, enlarged spleen, bleeding into pleural and abdominal cavities, severe chronic enteritis, petechiae in the kidney cortex, congestions of the lungs, ecchymotic haemorrhages under the skin, enlarged haemorrhagic lymph nodes, and petechial haemorrhages on both small, large intestines. Spickler and Roth (2019) confirm that haemorrhages, petechiae are sometimes detected in other organs including the stomach and intestines. The spleen and lymph nodes typically contain the highest concentrations of the virus, and viral DNA may remain in the spleen longer after death than in other internal organs (Spickler and Roth, 2019). The subacute form was also observed which was characterised by pneumonia and petechial haemorrhages of the lungs. Multifocal pneumonia can also be seen with patches of consolidation and dark colour in the lung (Salguero, 2020). This lesion can also be attributed to secondary infections caused by ASFV's immunosuppression (Moulton *et al.,* 1975; Gomez-villamandos *et al.,* 2003; Salguero *et al.,* 2005).

4.1.3 Action plan and eradication measures.

Prevention of ASF is based on avoiding disease transmission. The delay in detecting and confirming the disease led to the delayed implementation of contingency plans therefore infected pigs posed a risk of shedding the virus on other pigs. The most effective method of controlling ASF is early disease detection, which is critical to maintaining good animal health (Gervasi *et al.,* 2019). Both NWP and FSP areas were

immediately placed under quarantine to implement control measures to contain the disease by removing the source of the virus. They monitored and banned all pig movements. Before veterinary officials engaged in mass culling and burial one farmer in the FSP mentioned that they were verbally instructed to burn the carcasses on their premises without supervision.

Pork meat is the main source of protein in families farming with pigs and instruction without any supervision can lead to farmers not implementing what they have been told, instead, they can decide to sell or keep the carcass for consumption, which further poses a risk of disseminating the virus. Cultural habits, taboos (such as throwing away food), and poverty can all play a role in the consumption and trading of infected pigs (Chenais *et al.,* 2017). The site where the carcasses were disposed of was visited during farm visits in NWP and was a 1.5 m hole. The carcasses were burned and limed before burial to avoid scavenging. The veterinary official visited the site three days after the burial, and there was no sign of illegal activity. These results could indicate one of two things, community members or farmers did not exhume the carcasses because they understood what ASFV was, or because the carcasses were limed (making it not safe for consumption), hence they could not engage in illegal activities.

In the Free State province, pigs were culled and only burned before burial. Veterinary officials visited the burial site on the following day after burial and they discovered that carcasses were being removed illegally. This behaviour explains that the community members were aware that it was still safe to consume carcasses, which succumbed or were culled due to ASF. The other contributing factor could be that no chemicals were used on carcasses, which made the carcasses safe for consumption. Lack of cooperation from farmers and community members delays disease eradication, and it will be a good thing if veterinary officials could continue with ASF awareness workshops even in the absence of ASF outbreaks. This action may make farmers feel important and aware that veterinary officials care about them and their animals. Furthermore, one farmer in the Koffiefontein area was feeding his surviving pigs with meat from dead pigs, as evidenced by the bones we discovered. This further poses a risk to surviving pigs. Areas in which there is a challenge in controlling the movements of pigs, pig products, and slaughtering of all pigs should at least be identified as hot spots for the dissemination of ASV and they should be monitored closely.

Carcasses should be disposed of in such a way that they no longer pose a risk of further pathogen transmission to susceptible animals, either directly or indirectly. In the case of ASF, safe disposal methods include, rendering, incineration, burning, or deep burial on the spot (Davies *et al.,* 2017). In addition, Guberti *et al.* (2018) stated that outdoor carcass burning could also be accomplished in a variety of ways, including pyre burning, pit burning, aboveground incineration, or a combination of the aforementioned methods. However, deep burial is a better option, which can be accomplished through trench burial or mass burial, with the carcasses disinfected in both cases. Burial pits should be dug deep enough to ensure a soil layer at least one meter above the carcass to prevent scavenging and contamination.

4.2 Detection of African Swine Fever virus.

4.2.1 Laboratory results from ELISA and PCR assay.

Because no vaccine is available to prevent ASF, the presence of antibodies indicates prior infection (OIE, 2008). In endemic areas, antibody detection can be of epidemiological value, and it is recommended to combine antibody detection with viral genome detection by PCR in such cases (OIE, 2012). Pigs that were sampled in NWP did not show any antibody detection for ASF using the OIE- recommended ELISA assay and it cannot be concluded that the pigs were not infected or the virus was not circulating because five samples were doubtful while 10 were not tested. In addition, the lack of antibodies could have been because of adult pigs, which developed antibodies earlier in life and disappearing at a later stage. However, there are studies, which have indicated that antibodies can persist for longer periods, maybe even for life (Sánchez-Vizcaíno *et al.,* 2009).

In addition, it might be due to antigen ELISA essay having a reduced sensitivity because of samples, which were poorly kept during transit and it could be that when the pigs were sampled, they have not yet seroconverted because of a lack of good immune response to the virus. The other factor, which needs to be considered is the high number of pigs that died (n=664) 61.02% before the province could remove the source of infection. Except for the high virulence strain circulating in the NWP, the other contributing factor could have been due to farmers' illegally selling or keeping carcasses during the outbreak because 19 carcasses were confiscated by the veterinary officials. The carcasses posed a high risk of disseminating the virus or

making it more difficult in removing the source of infection. Because ELISA is used in the detection of ASF antibodies in animals that have survived the infection, both provinces continued with clinical surveillance until May 2017 to determine whether the disease is endemic in the affected areas and laboratory results revealed that the antibodies could no longer be detected.

The study revealed that 18 of the pigs in Free State Province tested positive for antigen detection using ELISA assay further showing that the pigs were exposed to ASFV. A total of (*n*=880) pigs that died of ASFV could also confirm that a highly virulent strain was circulating. A recommended OIE diagnostic PCR was used to analyse 67 EDTA blood samples, which were collected during ASF outbreaks from healthy pigs in North West and Free State Provinces. The samples were collected over 3 months from May 2016 to July 2016. A total of 23 tested positive for ASFV, 26 tested negative and 18 samples were not tested. A proportion of PCR positives (34%) was obtained from both provinces, ASFV DNA was detected in apparently healthy pigs suggesting that circulation of ASFV in the pig population from NW, and FSP was a critical issue as these particular pigs could play a major role in maintaining the virus. Furthermore, they could spread the virus to other uninfected areas by carriers.

Twelve outbreaks were confirmed in the FSP, compared to NWP with only two outbreaks. This could also confirm that the FSP had a challenge in removing the source of infection, putting more areas at risk of being affected. The low proportion of ASFV antigen-positive pigs (10%), seen in both provinces could also be caused by the effect of temperature fluctuations during transit from the collection site in the field to the laboratory. This could indicate that the field veterinarian and AHTs should be trained on the importance of a cold chain; it maintains the appropriate quality of the samples for further analysis.

Penrith *et al.* (2004) stated that because of exposure to ASFV, antibodies should last for at least two years. Therefore, eighteen months post-2016/17 ASF outbreaks a further study was conducted to establish whether the domestic pig cycle had been eradicated in the areas through control measures, which were implemented. Serological surveillance was conducted and all collected serum samples tested negative for antibodies against ASF, and the results strengthened the assertion that the clinical surveillance together with the culling policy implemented was successful in

eradicating domestic pigs which were exposed to the ASFV in the affected areas following the 2016/17 outbreaks (Janse van Rensburg *et al.,* 2020c).

The African wild suids, whereby the most important suids being the warthog (*Phacochoerus africanus*), are the natural host of the ASF Virus (Sánchez-Vizcaíno *et al.,* 2012). Warthogs were sampled and ELISA results were positive, the presence of anti-ASFV antibodies in these warthogs indicated infection. PCR samples were also positive meaning that ASFV was present in warthogs found in the FSP and NWP. The findings confirm that domestic pigs in these areas are at risk of being infected with ASFV, particularly in the FSP, where warthog hunting is prevalent. Guinat *et al.* (2016) confirm that the presence of ASFV increases the risk of virus transmission from the wild to domestic pigs through direct exposure to infected animals or the meat obtained from these wild animals. This can be prevented by applying biosecurity measures without compromise.

4.2.2 Genetic characterization of the outbreak virus in domestic pigs, and *Ornithodoros moubata* ticks.

Phylogenetic analysis of the p72 gene sequences from this study confirmed that the 2016/2017 ASF outbreaks for North West and Free State Provinces belonged to genotype I. A wide geographical distribution of genotype I ASFVs correlate with Southern Africa, Central, and East Africa, West Africa, Europe, the Caribbean, and South America (Lubisi *et al*., 2005). In southern Africa genotype I is typically associated with Northern Zimbabwe, Botswana, Namibia, and Angola. However, viruses belonging to this genotype were also found in *Ornithodoros spp*. ticks collected in 1981 during a survey conducted in the Kruger National Park. In 1985, an outbreak of domestic pigs occurred in the Waterberg area also caused by a genotype I virus (Janse van Rensburg *et al.,* 2020a). The information available at the time of this study was not sufficient to confirm the origin of the 2016/2017 outbreaks in the North West and Free State provinces. The nucleotide sequences of the p72 genes of ASFV obtained from both provinces were 100% similar revealing that the same virus caused these outbreaks.

4.2.3 Role of the sylvatic cycle in affected areas

The main farming activity from the sampled farm was livestock other than domestic pigs and crops. The illegal traps we found along the fences seemed to have contributed to the death of the warthogs. The AHT had no actual count of warthogs in the areas. There is little information on the role played by wild pigs in the epidemiology of ASF in other African locations (Jori *et al.,* 2007). Fortunately, it is well-described for warthogs in East and Southern Africa (Thomson, 1985; Plowright *et al.,* 1994). Studies have further revealed infection rates of free-living warthogs to be below 80% in areas with the existence of a tick vector (Plowright *et al.,* 1994). The African wild suids, whereby the most important suids being the warthog (*Phacochoerus africanus*), are the natural host of the ASFV (Sánchez-Vizcaíno *et al.,* 2012). Adult warthogs, even if they have infective levels of ASFV in lymph nodes, they do not shed the virus or develop viraemia which is sufficient to cause infection of ticks that feed on their blood. To infect ticks with ASFV, titres of at least 10^3 HAD_{50}/mL are required and it is achieved in young warthogs compared to adults, which rarely have ASFV titres above 10^2 HAD_{50}/mL (Jori and Bastos, 2009).

The presence of warthogs and their burrows may influence the likelihood of contact between domestic pigs, warthogs, and infected soft ticks (*Ornithodoros moubata*), potentially leading to ASFV transmission through the sylvatic cycle. In addition, the risk factors for the ASF outbreak existed at the farm before the outbreak because of the presence of warthogs in and around Koffiefontein. Table 3.13 above shows data resulting from the assessment of the presence of *Ornithodoros moubata* from warthog burrows with tick infestation of 70% and 30% with no infestation. Jori *et al*. (2013), stated that tick sampling in warthog burrows is time-consuming and labour-intensive, further posing a risk to the sampling team whilst attempting to enter the burrows for scraping.

There was a recovery of a few ticks in January 2020. The seropositive warthogs which were tested in July 2016 and the PCR-positive ticks tested in July 2020 came from the same area, Koffiefontein and this confirms a possibility of transmission between warthogs and ticks. In addition, this discovery of positive ticks should be considered a potential reservoir for ASFV, therefore, enabling the virus to persist locally in the environment for a long period. Hess *et al*. (1989) stated that ASFV can persist in the

absence of a viraemic host because of the ability of *Ornithodoros spp.* of transmitting ASFV from tick to tick through transstadial and, Plowright *et al*. (1970) further stated that transmission could be through sexual and transovarial transmission. It is important to mention that the Free State province was selected for tick sampling due to the recent occurrence of ASF outbreaks in domestic pigs and the presence of warthogs.

CHAPTER 5: CONCLUSION

The background of all recorded outbreaks has shown that there is a need to improve continuous awareness among pig farmers at all levels from backyard/small-scale to large-scale farmers. The prompt response of North West province to contain the spread of ASFV is commendable, indeed, inviting other role players timeously in the implementation of control measures is crucial, here the Department of Environmental Affairs was involved in the burial of carcasses. The province was also engaged in social facilitation by organising meetings with farmers for three days before the start of the operation. They conducted radio interviews and had a joint media statement with the local municipality. For surveillance, the affected areas were put under quarantine within a five km radius, the pig farms in the area were also identified and sero-surveillance was conducted in the North West and the Free State provinces. In addition, necropsy samples were collected from culled pigs, and they were sent to the laboratory to confirm ASFV.

The movement control included quarantine with clear conditions, forward and backward tracing, roadblocks and control points, tyre baths, suspension of slaughter at a local abattoir, auction monitoring, and monitoring of any illegal trade of pig products. Local farmers were also requested to provide samples from the wild pigs during hunting and the samples tested positive for ASFV. Early detection, movement controls, and biosecurity measures should be improved and prioritised. The farmers and veterinary officials should be informed about ASF disease, be able to recognise it, and know what they need to do when they suspect ASF. It is crucial that pig farmers understand how ASFV is transmitted and when they can cooperate with the veterinary officials, they will be protecting their pigs from ASF infections. To continue minimising the spread of ASF, the disposal should be carried out in such a way that the carcasses no longer constitute a risk of spreading the virus through direct or indirect contact.

Warthogs (*Phacochoerus Africanus*) play a major role in the sylvatic cycle as a reservoir of the virus. The risk of spill over to domestic pigs is highly possible due to illegal hunting in the Koffiefontein area, posing a risk of contamination of the environment, which may occur if there are any existing infected warthog carcasses. This irresponsible behaviour by illegal hunters can act as an effective route for the transmission of ASFV between wild and domestic pigs. The introduction of new animals and the use of contaminated swill can introduce several diseases into a

healthy herd. Therefore, farmers should be encouraged to boil the swill for 60 minutes as stated in Regulation 24 of the Animal Disease Act, 1984 (Act 35 of 1984). Farmers should further be taught to separate new pigs from the old herd. Before the new animals can be introduced to the old herd, farmers should at least engage their local veterinary officials to conduct general health checks on their new herd. This approach will prevent the introduction of new infections.

Properly constructed pig structures should be encouraged particularly in areas located in the proximity of warthogs' areas to reduce the possibility of contact with the domestic pig population. During tick sampling, adult ticks were not found and ASFV was only detected in the nymph's stage collected from a small number of warthogs' burrows therefore, further investigation using molecular screening of adult ticks to assess the presence of ASFV can be conducted. Submission forms with a detailed and accurate history should accompany samples submitted to the laboratory for testing; this will improve ASF risk assessment in the field. The lack of adequate information in the submission forms limits the possibility of thorough investigation during outbreaks and further delays the implementation of disease control measures.

The veterinary reports for 2016/2017 show that an in-depth, practical knowledge of biosecurity and animal movement is required. Lack of proper biosecurity increases the risk of ASF outbreaks. The introduction of ASF can be limited when the information is imparted, and farmers are willing to cooperate with veterinary officials and this can further lead to successful farming practices. Continuous awareness campaigns with small-scale farmers and auctioneers should be encouraged by communicating correct information; their attitude and belief in biosecurity measures could play a role in the prevention of ASF outbreaks such as quarantining new pigs delivered on the farm, changing clothes, and disinfecting equipment. Overall, the questionnaire and farm observations revealed a lack of biosecurity as a major concern.

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APPENDICES

ASF BACK AND FORWARDTRACING INTERVIEW

Veterinary Officials Questionnaire

This questionnaire must be filled during the interview of the Veterinary official i.e. Animal Health Technician or Veterinarian.

DATE & MONTH: _____

NAME OF INTERVIEWER: _____

Veterinary office:	District:

Note: Information will be handled as confidential.

Q.1 LOCATION OF THE OUTBREAK

1.1 Province: _____

1.2 Local Municipality: _____

1.3 Farm Name: _____

1.4 Type of farming system: ☐ Extensive ☐ Intensive ☐

Q.2 OUTBREAK DETAILS

2.1 Specie:

Porcine ☐

2.2 Affected Age:

Piglets ☐ Weaners ☐ Adults ☐

2.3 Affected Sex:

Males ☐ Females ☐

2.4 Do pigs in the area scavenge or search for food in the field?

Yes ☐ No ☐

2.5 Estimated population at risk: _____

2.6 When was the first case reported? _____

2.7 Number of deaths: _____

2.8 Number of pigs culled: _____

2.9 Estimated radius affected by the outbreak: _____

2.10 Do farmers declare to the state vet when they are moving animals?

Yes ☐ No ☐

2.11 Epidemiological comments on the outbreaks?

_____
_

_____
_

_____
_

_____
_

2.13 Which samples have been collected for testing?

Blood ☐ Tissue ☐ None ☐

Q.3 ANIMAL HEALTH MANAGEMENT

3.1 Did farmers report any health problems in their livestock during the past 6 months?

Yes ☐ No ☐

3.2 Describe Health problem and action implemented to treat sick pig's

_____

_____

___

3.3 Where there any control measures taken thus far?

Yes ☐ No ☐

If yes, please explain

_____

_

_____

_

_____

_

_____

_

3.4 Are farmers experiencing ticks on their pigs?

Yes ☐ No ☐

Q.4 BIOSECURITY

4.1 Are the farmer's premises fenced?

Yes ☐ No ☐

4.2 Do they practice routine cleaning of the area where pigs are kept?

Yes ☐ No ☐

How often? _____

4.3 Which disinfectants are they using? _____

4.4 Do farmers quarantine new pigs on arrival?

Yes ☐ No

4.5 Are there warthogs'/bush pigs nearby?

Yes ☐ No ☐

4.6 How often are the warthogs'/bush pigs seen in the surrounding areas?

_____

_____

_____

_____

4.7 Has there any evidence of bush meat being fed to the pigs?

Yes ☐ No ☐

Q.5 INTRODUCTION ROUTES

5.1 How do farmers transport their pigs? _____

Do they clean their means of transportation before/after transport?

Yes ☐ No ☐ not known ☐

Do they clean the external part of the truck?

Yes ☐ No ☐ not known ☐

5.2 What do they feed their pigs?

Compound feed ☐

Industrial and agricultural by-products ☐

Fish meal, blood meal, meat meal ☐

Domestic (kitchen) waste (vegetables peels, etc.) ☐

Restaurant waste ☐

Other _____

5.3 How often do farmers purchase/sell their pigs?

_____

5.4 Do they share their workers with other farms close by?

Yes ☐ No ☐

5.5 Confiscated dead/live pigs during the outbreak?

Yes ☐ No ☐

Q.6 DISEASE CONTROL ACTION PLAN

6.1 Social Facilitation:

a. Meetings with Farmers Yes ☐ No ☐

b. Radio Interviews Yes ☐ No ☐

c. Print Media Yes ☐ No ☐

d. Media Statement Yes ☐ No ☐

6.2 Movement Control:

a. Quarantine Yes ☐ No ☐

b. Forward and backward tracing Yes ☐ No ☐

c. Roadblocks and Control points Yes ☐ No ☐

d. Tyre bath Yes ☐ No ☐

e. Suspension of slaughter at local abattoir Yes ☐ No ☐

f. Auction Monitoring Yes ☐ No ☐

6.3 Culling:

a. Burn ☐

b. Bury ☐

c. Other ☐

If other, please specify _____

6.4 Surveillance:

a. Quarantine radius _____

b. Sero-surveillance Yes ☐ No ☐

c. Farms Identified Yes ☐ No ☐

d. Free roaming pigs Yes ☐ No ☐

e. Suspension of slaughter at local abattoir Yes ☐ No ☐

f. Wild pigs sampled Yes ☐ No ☐

Farm level observation sheet	
Name of province: **Date:** **(DDMMMYY)**	**Name of location:**
Farm location	In the township
	Outside the township
Type of housing	Corrugated
	Fence
	Brick construction
	Planks
The farm's outside appearance	Tidy
	Untidy
Disposal of manure	On the farm
	Outside the farm premises
	No disposal
Feeding method	Stainless steel feeding troughs
	Cement/ concrete feeding troughs
	On the floor/no feeding troughs
Type of fencing on the farm	Clear view fencing
	Wood fencing
	Brick wall
	No fencing

UNISA university of south africa

UNISA-CAES HEALTH RESEARCH ETHICS COMMITTEE
UNISA-CAES ANIMAL RESEARCH ETHICS COMMITTEE

Date: 04/10/2019

NHREC Registration # : REC-170616-051
REC Reference # : 2019/CAES_HREC/126
Name : Ms MT Rametse
Student #: 36851728

Dear Ms Rametse

Decision: Ethics Approval from 04/10/2019 to completion

Researcher(s): Ms MT Rametse
36851728@mylife.unisa.ac.za

Supervisor (s): Dr D Sibanda
donvet@gmail.com; +61484256126

Dr LE Heath
HeathL@arc.agric.za; 012-529-9501

Working title of research:

The epidemiology of the 2016-2017 African swine fever outbreaks in the North West and Free State provinces of South Africa

Qualification: MSc Agriculture

Thank you for the application for research ethics clearance by the Unisa-CAES Health and Animal Research Ethics Committees for the above mentioned research. Ethics approval is granted until the completion of the project, **subject to submission of yearly progress reports. Failure to submit the progress report will lead to withdrawal of the ethics clearance until the report has been submitted.**

Due date for progress report: 30 September 2020

*The **medium risk application** was **reviewed** by the UNISA-CAES Health and Animal Research Ethics Committees on 03 and 04 October 2019 respectively in compliance with the Unisa Policy on Research Ethics and the Standard Operating Procedure on Research Ethics Risk Assessment.*

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Enquiries : Dr KJ Mojapelo
Date 14 March 2019

Director Animal Health
Department of Agriculture, Forestry & Fisheries
Pretoria
0001

Supporting letter for Section 20 application for "The epidemiology of the 2015-2017 African Swine Fever outbreaks in the North West and Free State provinces of South Africa."

Dear Dr Kipho Maja,

I hereby submit this letter as an indication of supporting the project "The epidemiology of the 2016-2017 African Swine fever outbreaks in the North West and Free State provinces of South Africa" relating to the survey of the study area and collection of ticks.

The exact location for the research is not yet identified. The support is given because there are no known outbreaks of African Swine Fever in the Free State province and there are no restrictions.

.....
DR KJ MOJAPELO
DIRECTOR VETERINARY SERVICES

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Reference: 12/11/1/1/15

Ms Mantoane Thapelo Rametse
Onderstepoort Veterinary Research
100 Old Southpan Road
Onderstepoort 0110

Dear Ms Rametse,

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Your application, submitted on 31 January 2019, requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study "The epidemiology of the 2016-2018 African Swine fever outbreaks in the North West and Free State Provinces of South Africa", with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. All potentially infectious material utilised or collected during the study is to be destroyed at the completion of the study. Records must be kept for five years for audit purposes. A dispensation application may be made to the Director Animal Health in the event that any of the above is to be stored or distributed;
3. Samples to be transported must be packaged in compliance with the Regulations of the National Road Traffic Act, 1996 (Act No 93 of 1996) or IATA requirements;