The prevalence of African swine fever determinants along the control zone in South Africa

BY

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B. Summary

The prevalence of African swine fever determinants along the control zone in South Africa

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African swine fever (ASF) has been reported and confirmed in South Africa since the early 20th century, which lead to the inception of the Swine Fever control zone in 1935. In the South African context, the sylvatic cycle is the main maintenance and transmission cycle that leads to sporadic outbreaks in the domestic pig population, particularly reported in the designated ASF control area.

ASF is endemic in sub-Saharan Africa and maintains itself through three different epidemiological cycles in different regions of the continent. The current outbreaks in the Caucasus and Russia have shown the ability of African swine fever virus (ASFV) to establish itself where low biosecurity conditions exist. In South Africa, the spread of ASF has been successfully controlled in the domestic pig populations with control based on the Animal Disease Act 35 of 1984. The act prohibits the movement of all

suid species and their products from the ASF control area in the north, except where special permission has been granted by the Provincial Veterinary Services.

One of the key uncertainties related to climate change is potential variations in the weather patterns and fluctuations in climatic conditions that could lead to alterations in production systems and land use patterns. These in turn raise the possibility of redistribution of both the arthropod vectors and wild suids to environmentally suitable areas. It is therefore critical for the zoning of ASF that patterns of distribution of the reservoir hosts are monitored in line with the possible variations in the weather patterns around and along the ASF control line. Nonetheless, there are no known records of the reassessment of the swine fever control line, which was instituted based on the distribution of previous outbreaks and the presence of warthogs and tampans, since its inception in 1935.

The objective of this study was to evaluate the distribution of the ASF disease determinants; warthogs and warthog burrows, *Ornithodoros moubata* and ASFV; along the ASF control line with the view of determining whether there was a need to re-align the trajectory of the line or not.

A total of 304 farms were randomly selected 20 km north and 20 km south of the ASF control line from the North West, Gauteng, Limpopo and Mpumalanga Provinces through proportional weighting. A total of 73 farms from the initial sample, distributed along the ASF control line, were sampled for the presence of warthogs, warthog burrows and soft ticks of the *Ornithodoros spp*. (tampans). One hundred and fifty seven warthog burrows were found, of which 92% were recently used by warthogs. Tampans were recovered from 22.2% of the 63 farms where warthog burrows were found and 12.74% of the total (157) warthog burrows. Of the infested

warthog burrows, only 5% (one of the twenty burrows) constituting 7.14% (one out of 14 farms) found south but in close proximity to the ASF control line, was positive for ASFV DNA. There were no warthog burrows found with PCR positive tampans north of the ASF control line. The spread of tampans beyond the ASF control line poses a question on whether the control line needs to be moved further south in the affected parts of the country.

The study confirmed that the reservoirs are found beyond the current ASF control line. Although the causes for this apparent re-distribution are unclear, changes in land use and the increase in wildlife farming may contribute to this finding. Examination of weather data along the control line between 1993 and 2012 found the maximum temperatures was increasing and humidity is decreasing. In the absence of previous data on warthog and tampan distribution along the control line, the present study cannot evaluate if these changes have had an impact on the distribution of warthogs and tampans in the vicinity of the control line. This study provides baseline data for future monitoring of the control line and concluded that there was currently no need to realign the trajectory of the ASF disease control line but to conduct scheduled monitoring of the *O. moubata* status in the future.

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F. Abbreviations

°C - Degrees Celsius

% - Percent

ARC - Agricultural Research Council

ASF - African swine fever

ASFV - African swine fever virus

CI - Confidence Interval

DAFF - Department of Agriculture Fisheries and Forestry

DNA - Deoxyribonucleic acid

EDD - Exotic Disease Division

GDARD - Gauteng Department of Agriculture and Rural Development

GIS - Geographic Information System

GPS - Global Positioning System

ISCW - Institute for Soil, Climate and Water

Km - Kilometer

KNP - Kruger National Park

max - Maximum

mm - Millimeters

min - Minimum

No. - Number

OIE - Office international des Epizooties

OVI - Onderstepoort Veterinary Institute

PCR - Polymerase Chain Reaction

PDVS - Provincial Department of Veterinary Services

SAWS - South African Weather Services

SV - State Veterinarian

TADP - Trans-boundary Animal Diseases Programme

Temp - Temperature

CHAPTER 1. LITERATURE REVIEW

1.1 Aetiology of African swine fever

African swine fever (ASF) is a highly contagious, in most of the situations, lethal and notifiable disease of domestic pigs caused by the African swine fever virus (ASFV), a deoxyribonucleic acid (DNA) arbovirus belonging to the genus Asfivirus of the family Asfarviridae (Dixon et al 2000). It is currently the only member of the family. ASFV affects the members of the Suidae family (Penrith 2013) and is the only known arthropod borne virus with a single molecule of linear double stranded DNA genome (Kleiboeker & Scoles 2001; Penrith *et al* 2004a). The DNA genome is between 170 and 190 kbp in size depending on the isolate (Dixon et al 2004).

ASFV replicates in both mammalian and arthropod hosts (Dixon et al 2004). The virus replicates in the cytoplasm of the host cell (Costard et al 2009) and is found in all fluids and tissues of acutely infected pigs (Clarence & Fraser 1991). It replicates in vitro in both macrophages and in the aortic endothelial cells (Dixon et al 2004).

ASF virus is highly stable at room and lower temperatures (temp), where it can survive for 18 months. It can be destabilised by heat treatment at 60 degrees Celsius (°C) for 30 minutes (Plowright & Parker 1967). The virus had been recovered in chilled and processed meat products up to six months after processing.

1.2 **Epidemiology**

1.2.1 **Affected species**

ASF affects domestic and wild pigs of all breeds and ages. In Africa there are three members of the wild Suidae, warthog (*Phacochoroerus spp*); bushpig (*Potamochoerus porcus)* and the giant forest hog (*Hylochoerus mienertzhageni*) (Anderson *et al* 1998).

*Phacochoroerus africanus (*common warthog*),* is the species of warthog mostly associated with the soft tick vector and ASF (Jori & Bastos 2009). They are asymptomatic carriers of ASFV (Costard *et al* 2013) with minimal or no shedding of virus by seropositive warthogs (Kleiboeker & Scoles 2001) and could as such act as reservoir of the disease. Common warthogs are naturally adapted to open woodlands savannah (Randi *et al* 2002; Muwanika *et al* 2006), where the virus is maintained and transmitted between wild and domestic suids (*Sus scrofa domestica)* by soft-shelled ticks of the *Ornithodoros* genus (Hurter & Pini 1975), most notably in the South African context, *Ornithodoros moubata* (colloquially known as tampans*).*

Common warthogs mainly inhabit burrows, which are excavated by aardvark (*Orycteropus afer*), for purposes of shelter, farrowing and rearing their young (White & Cameron 2009). In southern Africa warthogs are known to be seasonal breeders (Arnot *et al* 2009) farrowing in late spring to early summer. This is the time when the viraemia is known to drastically increase for up to 11 days in the young warthogs with higher possibilities of infecting naïve tampans occupying the burrows and feeding on them (Jori *et al* 2013).

Bushpigs, although widely distributed, are believed to be of less importance than warthogs in the epidemiology of the ASF (Costard *et al* 2013) although this needs to be confirmed (Jori *et al* 2013). Infected bushpigs do not usually exhibit clinical signs of the disease and are able to transmit the disease directly to domestic pigs (Costard *et al* 2009; Sánchez-Vizcaíno *et al 2012*), but it is not so for warthogs, where transmission into the domestic cycle occurs only via soft ticks (Bastos *et al* 2009). The Giant Forest hogs, whose role in the epidemiology of ASF is regarded as negligible (Costard *et al* 2013), are restricted to areas of dense forests where domestic pigs are not common, hence it is unlikely that they play a significant role in the transmission or dissemination of ASFV (Jori & Bastos 2009).

Infected African wild pigs, especially warthogs, are normally in-apparent carriers of the virus and act as reservoir hosts. European wild boars and feral pigs, both of genus *Sus* and species *Sus scrofa,* are fully susceptible to ASF and show a similar clinical syndrome to domestic pigs (OIE manual 2008; FAO, Arnot *et al* 2009). Although wild boars are fully susceptible, Jori & Bastos (2009) suggested that wild boars cannot maintain the virus for long periods of time in the absence of other factors such as infected domestic pigs. This was confirmed in a study done in Spain monitoring the wild boar population (Mur *et al* 2012).

ASFV has been isolated from the soft ticks of the genus *O. moubata* in Africa and *O. erraticus* in the Iberian Peninsula and it is believed that most ticks of the *Ornithodoros spp.* can carry the virus (Stärk 1998). Soft ticks are clearly involved in the maintenance of ASFV in Africa and have been regularly found in warthog burrows in eastern and southern Africa (Nesser *et al* 1994). ASFV can persist and is

amplified in *O. moubata* ticks (Costard *et al* 2013); the virus can also cause mortality in ticks (Kleiboeker & Scoles 2001).

1.2.2 **History and geographical distribution**

ASF was first observed and reported by Montgomery in Kenya in 1921 (Montgomery 1921). It was noted that disease outbreaks occurred when domestic pigs came into close contact with wildlife species (Costard *et al* 2009).

Before 1957 the disease was limited to the African continent south of the Sahara. ASFV was exported outside the African continent, possibly through Angola to Lisbon, Portugal in 1957 and 1960, by infected pork products (Dixon *et al* 2004), and in the same year spread to Spain. For decades the disease remained endemic in these two European countries before Spain's coordinated eradication programme was set up in March 1985. Portugal and Spain gained their freedom from the disease in December 1994 and December 1995 (Arias & Sánchez-Vizcaíno 2002) respectively. Portugal experienced another outbreak in Alentejo in 1999 before the disease was ultimately eradicated (Boinas *et al* 2001a referenced by Bonias *et al 2004)*.

Additional European outbreaks were reported in France in 1964, Italy in 1967, 1969 and 1993, Belgium in 1985 and the Netherlands in 1986 (Arias & Sánchez-Vizcaíno 1992). The disease was successfully eradicated in Europe, except in the Italian island of Sardinia (Costard *et al* 2013), where the disease has remained endemic in feral pigs since 1982 (Costard *et al.* 2009; Lubisi *et al*. 2009; Rowlands *et al* 2009). Here cases still occur despite attempts to eradicate the disease (Wieland *et al* 2011), with reported outbreaks as late as 2014 (OIE 2014).

ASFV entered South America and the Caribbean islands through Cuba in 1971 and again in 1980. It was successfully eradicated after a highly intensive programme with a total expenditure in excess of nine million dollars (Simeón-Negrín & Frías-Lepoureau 2002). The disease has also appeared in Brazil and part of the Caribbean islands in 1978-1979. The resolution of the Food and Agricultural Organisation (FAO) to coordinate control programs in Latin America and the Caribbean was taken in August 1978 (Alexander 1992) and the Brazilian Ministry of Agriculture implemented eradication and compensation programmes. Brazil was declared ASF free in December 1984 (De Paula Lyra *et al* 1982). The Dominican Republic and Haiti successfully eradicated ASF by stamping out all pigs in 1981 and 1984 respectively (McCauley 1982; Wilkinson 1989 cited by Costard *et al* 2009).

In 2007 ASF was confirmed in the Caucasus region of the former Soviet Republic of Georgia and the outbreak spread to 56 of 61 districts in Georgia (Rowlands *et al* 2008, Wieland *et al* 2011). Outbreaks of ASF were also reported in the neighbouring regions, including the Republic of Abkhazia. On August 29, 2007, ASF was confirmed in Armenia and on November 4, 2007, in Nagorno-Karabakh bordering Armenia. On November 5, 2007, an infection in a wild boar was confirmed in the Russian Republic of Chechnya bordering Georgia and further outbreaks of ASF were reported in Nagorno-Karabakh in April 2008 (Rowlands *et al* 2008). In January 2008 ASF was confirmed in Azerbaijan, East of Georgia and in December 2008 and January 2009 ASFV was diagnosed in wild boars in north-western Iran (Rahimi *et al* 2010). In January 2011 ASF was reported in St Petersburg, Russia (OIE 2011). ASF is now widely spread in the Caucasus region and Russia (Gogin *et al* 2013). The

disease has now also spread into Poland and Lithuania (European Union 2014) and poses a real threat to Europe and Asia.

ASF has spread through many West African countries with major economic loses (Dixon *et al* 2004). The disease was first identified in Senegal in 1959 (Etter *et al* 2011). Outbreaks were first reported from Senegal in 1978 (Plowright *et al* 1994). In 1996 West Africa experienced an outbreak in Côte d'Ivoire which spread to Benin, Togo, Nigeria, Ghana and Burkina Faso between the years 1997 to 2003 (Penrith *et al* 2013). Senegal, Gambia and Cape Verde also reported an increase in ASF outbreaks (Penrith *et al* 2004a).

Kenya confirmed an outbreak in 1994 (Penrith *et al* 2004a) after more than 30 years of a break from the disease. In Kenya, Tanzania and Mozambique the warthog/tick cycle had been confirmed. ASFV is endemic in domestic pigs in Malawi and Mozambique (Penrith *et al* 2013). The disease had been persistent in the continent over the years, reaching the Indian Ocean islands of Madagascar in 1998 and Mauritius in 2007 (Costard *et al* 2009, Rowlands *et al 2008*). The Mauritian outbreak was eradicated in 2008 (Lubisi *et al* 2009). The spread of the disease outside the African continent is thought to be mainly through the introduction of infected pigs or carriers or through infected processed meat products.

1.2.3 **History of outbreaks of African swine fever in South Africa**

In South Africa, the presence of ASF dates back to as early as 1926 when it was first recorded in the Northern parts of the country, formerly known as Transvaal (Boshoff *et al* 2007); the area which was later proclaimed the ASF control zone in 1935. In

this area ASF is maintained in the sylvatic cycle between warthogs and tampans (Penrith *et al* 2004a) with occasional spill over into domestic pigs. Two outbreaks were reported in the former Cape Province between 1933 and 1939 and since then no outbreaks have been reported there (Boshoff *et al* 2007).

Because of the presence of the sylvatic cycle, South Africa is not exempt from sporadic outbreaks. Most of the outbreaks in the ASF controlled areas occur within free ranging herds in the northern parts of the country because of contact between improperly confined pigs and warthogs, which results in infected tampans feeding on the pigs and transmitting the virus (Penrith & Vosloo 2009).

With the exception of the outbreaks which occurred in 1951 in Mpumalanga (former southern Transvaal), and just outside the control area in Bela-Bela, Limpopo Province in 1996 (Penrith *et al* 2004a), no other outbreaks had been reported outside the ASF control zone up to the end of 2011. During this period a number of cases/outbreaks occurred within the control zone in the Limpopo province and were reported by the Department of Agriculture, Forestry and Fisheries (DAFF) to the Office international des Epizooties (OIE) during the period of January 1993 to December 2011.

Figure 1 depicts the spatial distribution of ASF outbreaks in South Africa between 1993 and 2006 (DAFF 2007).

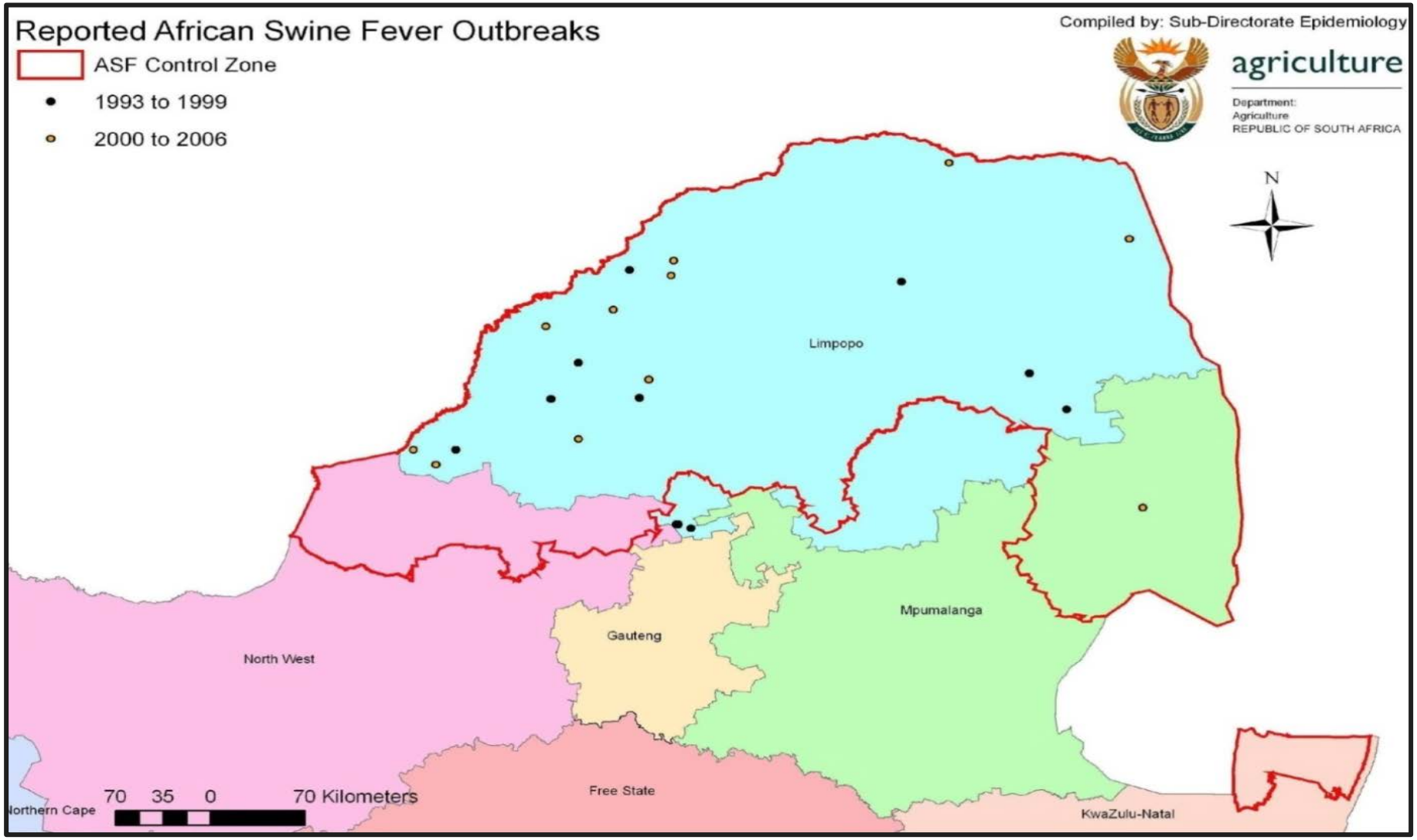

Figure 1: Reported African swine fever outbreaks in South Africa 1993 - 2006 (map provided by DAFF)

In January 2012 Gauteng Veterinary Services (GVS) reported a suspected case of ASF to DAFF in a group of pigs which demonstrated clinical signs at a Gauteng abattoir. The suspected pigs were from a small scale piggery farm in Delmas, Mpumalanga, outside the control zone that operated as a fattener for pigs before they were taken for slaughter. Pigs had been sourced from a local auction pen. No pigs were left on the farm and that was the only consignment destined for slaughter at the abattoir. Both the abattoir and the farm were placed under disease control quarantine and diagnostic samples tested positive. This was the first outbreak of ASF outside the ASF control zone since 1996 (Gauteng Veterinary Services 2012).

Subsequent to the immediate notification of the disease to DAFF, within a period of two months from the confirmation of the index case, South Africa reported an additional sixteen (16) outbreaks of ASF to the OIE. The reported ASF outbreaks were all diagnosed and confirmed outside the ASF controlled area in Gauteng and Mpumalanga Provinces (OIE 2012).

1.2.4 **Maintenance and transmission of ASFV**

ASFV is maintained in three different epidemiological cycles. The first and typical is the sylvatic cycle, where the virus is maintained in warthog-associated argasid ticks by transtadial, transovarial and sexual transmission (Thomson 1985, Costard *et al* 2009), i.e. the life cycle of the virus is adapted to the arthropod vector, Ornithodoros spp ticks and the wild suids, with occasional spill over to domestic pigs (Fasino *et al* 2012). Figure 2 shows the maintenance and transmission cycle of ASFV.

In the savannah regions the ticks can act as both reservoir host and sources of infection. Infected argasid ticks can be passively transported by adult warthogs and are mainly found in the burrows; habitats where warthogs are found at night.

The second epidemiological cycle, the endemic cycle (domestic pig/tick cycle) mainly occurs in West and Central/East Africa (Bastos *et al* 2004) and involves maintenance of ASFV in domesticated pigs (Sánchez-Vizcaíno *et al* 2012) and the ticks of *O. moubata* that has established themselves in the pig houses (thatched timber houses) commonly found in this part of Africa (FAO 2002; Lubisi 2005; Vial *et al* 2007).

In a third epidemiological cycle, once ASFV is established in the domestic pig population, it can be transmitted rapidly by direct contact between infected and susceptible domestic pigs (Costard *et al* 2009). The spread of the ASFV is mainly due to movement of infected or carrier pigs and pork products (Sánchez-Vizcaíno *et al* 2012). There is also a risk of indirect contact through transportation, contaminated farm workers, iatrogenic factors and lastly the virus can survive in pig tissues and meat products (Costard *et al* 2009).

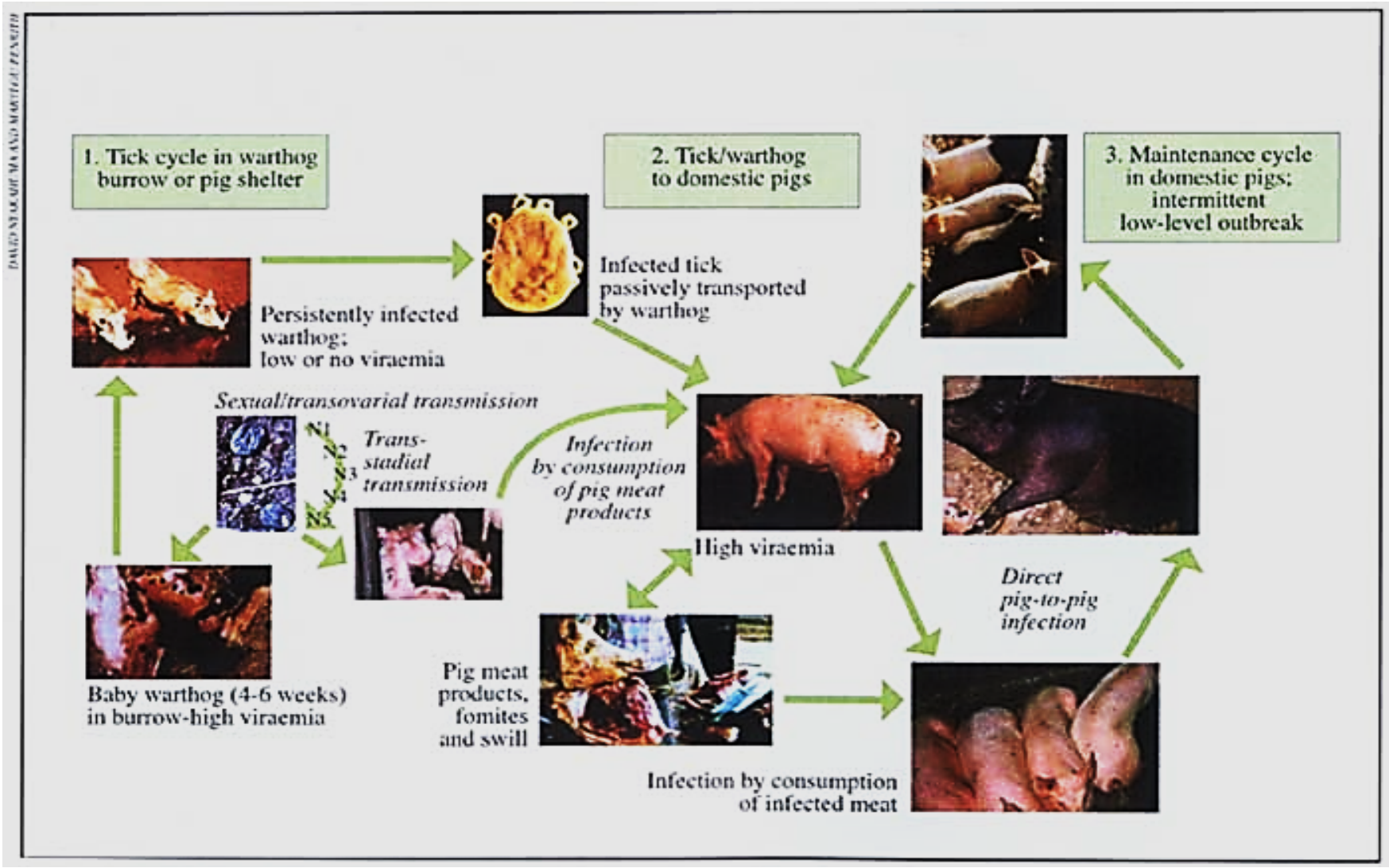

Figure 2: Maintenance and transmission cycle of ASFV (adapted from FAO website)

1.3 **Clinical signs and lesions**

ASF is both clinically and gross pathologically indistinguishable from other porcine diseases like classical swine fever and erysipelas (Sánchez-Vizcaíno 2006). This presents a need for confirmation of the disease through laboratory diagnostics. The incubation period varies from five to 15 days (OIE 2009), with clinical signs ranging from peracute to subclinical or in-apparent.

Peracute signs are characterised by sudden death with minimal clinical signs, which could be limited to elevated rectal temperature or no obvious clinical signs (Penrith *et al* 2004a).

Acute infection in domestic pigs is characterised by high morbidity, high fever of between 40°C and 42°C (FAO 2000), congestion and cyanosis of the skin of the extremities and abdomen, generalised visceral haemorrhages, abortions in pregnant sows and mortality rates of up to 100 % (Penrith 2009; Penrith & Vosloo 2009). During the early stages of infection the virus is mainly excreted via the naso-pharyngeal route. It is present in all secretions and excretions including oral, conjunctival, genital, urinary and faecal.

Subacute disease is caused by less virulent strains of ASFV resulting in fluctuating fever and loss of condition (Penrith *et al* 2004a). Interstitial pneumonia observed during this phase may result in respiratory distress. Infected animals may progress to the chronic stage of the disease.

Chronic disease and inapparent infections are characterised by diverse clinical signs and mortality of up to 10% (Arias & Sánchez-Vizcaíno 1992). Pigs in both subacute and chronic stages of the disease may experience secondary bacterial infections.

The gross pathological changes resemble those seen in classical swine fever (Penrith *et al* 2004a), with more severe lesions in acute cases and absence of lesions in some cases (OIE 2009). The lesions include haemorrhages in the spleen, lymph nodes and kidneys, and sometimes cardiac haemorrhages, pulmonary congestion and interstitial pneumonia (Arias & Sánchez-Vizcaíno 1992).

1.4 **Socio-economic impact of the disease**

Small scale pig production forms part of the socio-economic food clusters and poverty alleviation programmes within the Comprehensive Agricultural Support Programmes (CASP) in South Africa. This is because of the species' high reproductive potential, short production cycle and high feed conversion rate. Pigs have the ability to convert low quality protein into high quality protein (Penrith 2009).

ASF can have devastating effects on the commercial pig industry and almost inevitably leads to loss of international trade (Vial *et al* 2007) due to restriction or outright banning of the importation of any live pigs or processed pork from infected countries. The implementation of drastic and costly strategies to either eradicate or limit the spread of the disease can see severe socio-economic losses inflicted on poorer or small scale pig producers who are less likely to implement effective prevention and control strategies (Rowlands *et al* 2008; Costard *et al* 2009). The

small scale pig producers are also mainly affected by direct loses of animals dying in acute and subacute disease as pigs are often used as savings by some of them.

Unavailability of veterinary officials and lack of compensation for culled animals can pose further socio-economic problems (Sánchez-Vizcaíno *et al* 2012). The application of control measures in areas where ASF occurs, results in a forced change from traditional extensive low input husbandry to a more intensive system which places a higher demand for capital infrastructure investment on the producer (Penrith 2009).

1.5 **ASF control measures**

1.5.1 **General control measures**

The control of ASF is complicated by the absence of both treatment and an effective vaccine as well as the presence of the arthropod vectors (Penrith *et al* 2004b). The presence of the sylvatic cycle can obscure and / or delay the successful eradication of the disease, whilst countries with sporadic outbreaks and no arthropod vectors have a higher likelihood of eradicating the disease.

The control of ASF is based on rapid laboratory diagnosis, stamping out procedures, strict movement control of both live pigs and pig products and the enforcement of strict sanitary measures (Agűero *et al* 2004, Lubisi *et al* 2009). The rapid detection of infected animals reduces the potential transmission of the virus to uninfected herds and avoids the spread of the disease (Agűero *et al* 2004). Prevention of contact between the warthogs, their burrows and domestic pigs can limit the spread of the disease to domestic pigs and has proven to be successful (OIE). Sporadic outbreaks

may occur in endemic areas when the virus spreads from infected ticks or warthogs to domestic pigs (Blood & Rodastitis 1989). These could be controlled by quarantine, culling of infected and in contact pigs and proper destruction of carcasses.

1.5.2 **Control measures in South Africa**

Based on the presence of epidemiologically significant factors, warthogs and tampans, and the occurrence of outbreaks, South Africa has designated an ASF control area that mainly encompasses the Limpopo province, the northern parts of North West, Mpumalanga and KwaZulu-Natal Provinces as gazetted in 1935 (Penrith *et al* 2004b) (Figure 3 - DAFF, 2007). The disease control area and control measures are prescribed by the Minister of Agriculture according to section 31 of the Animal Diseases Act (Act 35 of 1984) and Animal Disease Regulations.

ASF in South Africa has, for more than thirty years, been well controlled by applying the existing legislation in the ASF control area (Penrith & Vosloo 2009). In these areas, commercial pig farming is discouraged and where it occurs, movement control; which includes control of movement of animals and animal products within the control zone and from the control zone to free areas, strict infrastructure requirements and husbandry practices are prescribed which have to be adhered to. Pig proof enclosures within a double perimeter fence or concrete wall with concrete floors have to be strictly effected, to ensure that pigs do not come into contact with wild pigs or ticks. All sickness and mortalities are to be reported immediately to the responsible veterinary authority and feeding of swill is forbidden.

Veterinary approved pig compartments with proven high bio-security levels of domestic pig farm production practices are found within the ASF control area. The

sale of pigs from the compartments to abattoirs outside the control area is only allowed under a special red-cross permit. These compartments are audited on an annual basis by DAFF. Accredited and approved piggeries within the ASF controlled area must be registered by the Provincial Director of Veterinary Services (PDVS) under the delegation of the National Director and registration is renewed on an annual basis. The State Veterinarian (SV), after inspection of these and any new facilities' compliance with minimum standards for a veterinary approved pig compartment, makes a recommendation to the PVDS for their registration or re-registration.

Scheduled monthly inspections of accredited farms are carried out to ensure compliance with prescribed farm bio-security requirements. All accredited piggeries and approved piggeries are to fully comply with the requirements as prescribed in the ASF protocol and Veterinary Procedural Notice 39/2011/1. Movement of pigs and pig products from these areas is also controlled by movement (Red Cross) permits.

In the event of an ASF disease report or outbreak, the affected farm is put under immediate quarantine. On confirmation of the ASFV infection all in-contact and affected animals are culled, without compensation, and carcases destroyed under veterinary supervision. If outbreaks are outside the control zone, movement protocols for suid and suid products are instituted and enforced for the entire country. The infected premises and establishments are disinfected, quarantined and restocked/reopened for use after 30 days. Active surveillance is implemented as determined by the Chief Veterinary Officer. In cases where ASF control measures

are not adhered to by the farmer and an outbreak is experienced, there is no compensation paid by the government alongside stamping out.

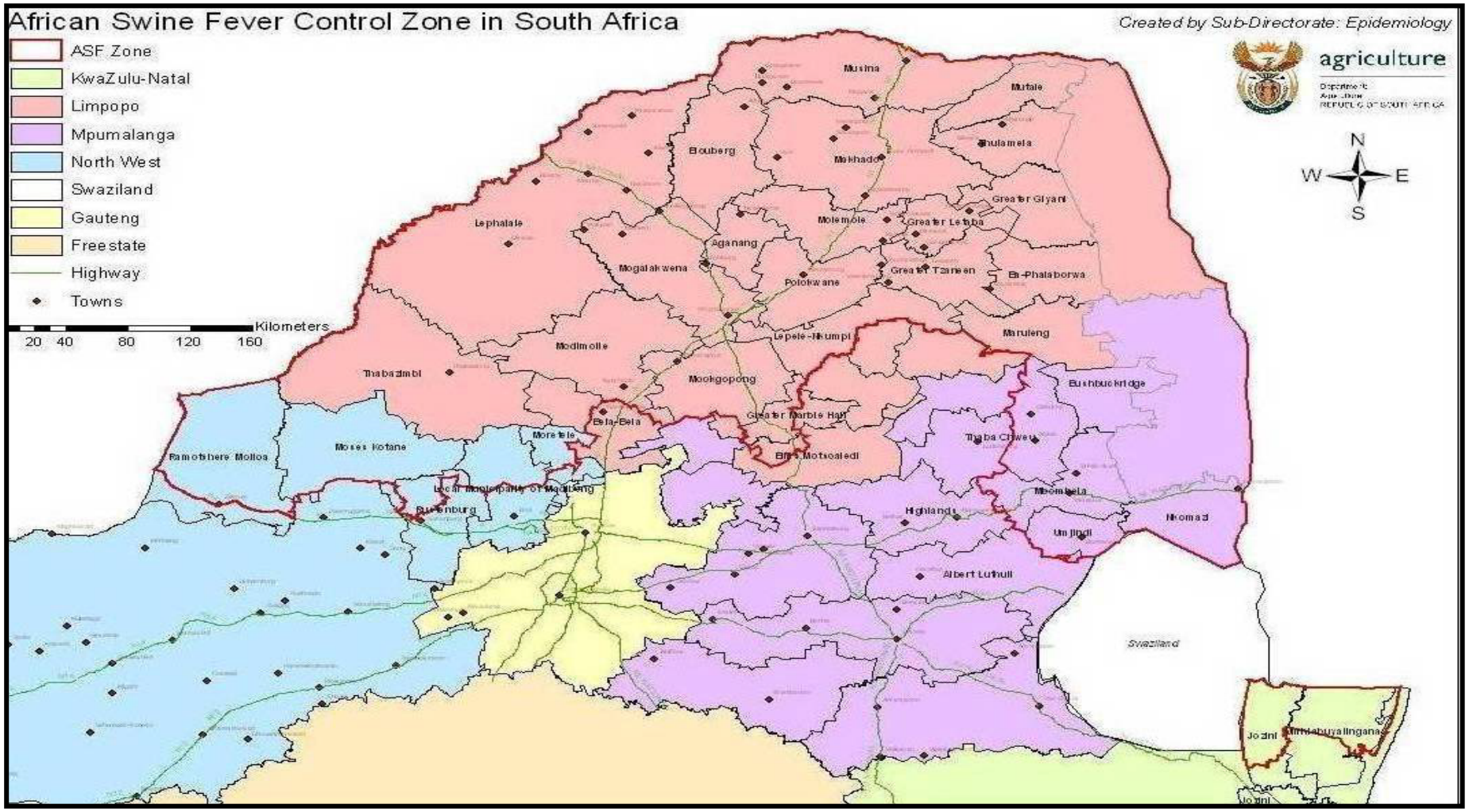

Figure 3: African swine fever control zone in South Africa

1.6 Problem statement

1.6.1 First problem statement

Sporadic outbreaks of ASF have recently been experienced outside the ASF control zone of the country. The control line has never been surveyed since its inception in 1935 and may no longer be correctly placed; hence the need to review its validity.

1.6.2 Second problem statement

Changes in farming practices and systems as well as changes in climate could directly modify the geographical distribution of hosts, vectors and pathogens along the control line; hence the need to survey the distribution of the relevant host, vector and pathogen.

1.7 Objectives of the study

1.7.1 First study objective

The first objective was to analyse the distributions and trends of ASF outbreaks that have occurred previously along the control line to see if the latter has been effective in controlling the spread of disease.

1.7.2 Second study objective

In South Africa, warthogs and warthog burrows are important secondary determinants of ASF, without them the disease is unlikely to flourish. The second

objective was to determine the location of farms with warthogs and warthog burrows within 20 km north and 20 km south of ASF control line.

1.7.3 **Third study objective**

A primary determinant in South Africa for ASF along the control line is the presence of the tick *O. moubata* on which the maintenance of infection is dependent. Thus the third objective was to determine the proportion of surveyed farms that have *O. moubata* ticks present in the warthog burrows.

1.7.4 **Fourth study objective**

The primary determinant is the presence of the ASFV itself, hence the fourth objective of the study was to survey an area 20 km north and 20 km south of the ASF redline with the aim of detecting and isolating ASFV from *O. moubata* in this area and determining the proportion of farms that had *O. moubata* ticks infected with ASFV.

CHAPTER 2. MATERIALS AND METHODS

2.1 Study area

The study area comprised the area along the ASF control line, as determined by the Animal Diseases Act (Act 35 of 1984). The infected area is defined as the area north of the ASF control line whilst the ASF free zone is defined as the area south of the ASF control line. The provinces included in the study were North West, Limpopo, Mpumalanga and Gauteng. A map of the ASF control zone (Figure 3) was obtained from DAFF, Food and Veterinary services, epidemiology unit. The study area maps were obtained from government printers and Department of Water Affairs. The ASF control line was superimposed on area maps and the area 20km north and 20 km south of the control line was measured using the maps' grid scale. The different provinces were visited to conduct training on the sampling method and discuss the sampling area and technical support.

2.2 Survey, sample size calculations and sampling strategy

A sampling frame consisting of the exhaustive lists of farms and their locations in the survey area was compiled from the area maps obtained from government printers. The list and location of farms for Gauteng and Limpopo Provinces were obtained from the Gauteng and Limpopo Provincial Veterinary Services offices respectively. The list of farms to be sampled was randomly selected using Survey Toolbox, Random Village sampling (Cameron 1999). Area maps and the list of farms were confirmed with the officials from different PDVS.

To obtain a cross section of the potential presence of tampans along the ASF red line, a northern and southern area were designated and an attempt was made to include in the sample size at least one farm with warthog burrows every 20 km north and 20 km south of the red line.

Because very little is known about the prevalence of warthog burrows, a number of assumptions were made to begin the survey:

First assumption

Using the formula $n = 1.96^2 \frac{P(1-P)}{d^2}$ to calculate the sample size, where n is the sample size, P is prevalence of warthog burrows with infected tampans and d is the margin of error (Thrusfield 2005), it was assumed that 20% of warthog burrows had infected tampans i.e. prevalence of burrows with infected tampans = 20%, error margin = 10% and confidence interval (CI) = 95%, then the sample size needs to be 61 randomly selected burrows to confirm this prevalence.

Second assumption

On the assumption that 20 in every 100 farms had warthog burrows, using the formula N = n + Negative Binomial (n + 1, p), where n is the number of farms needed to be found and p is the proportion with warthog burrows with infected tampans (Vose 2001), a median (i.e. at 50%) of 244 farms needed to be randomly sampled to find 61 warthog burrows. To be 95% sure of finding 61 burrows, 304 farms needed to be sampled. As information was collected, these assumptions were adjusted and the sample size changed accordingly.

To obtain the number of farms to be sampled by province, the sample (n = 304) size was proportionally weighted by the number of farms in each province using the formula $n_K = nN_K/N$ (Cameron 1999), where n_K is the number of farms selected per province, n is the total number of farms to be selected, N_K number of farms in the province and N is the total number of farms in the study area. There were fewer farms along the ASF control line in Gauteng than for example in Mpumalanga and Table 2.1 reflects the density of farms along the control line.

Table 2.1: *Number of farms selected from sampling frame*

Province	**% of Total farms**	**Number of farms selected (n_k)**	**Minimum number of burrows to be sampled per province**
Gauteng	8.2	25	5
Limpopo	24.7	75	15
Mpumalanga	43.4	132	26
North West	23.7	72	15
Total	100	n=304	61

2.3 **Data on previous disease outbreaks**

Data on the previous cases and outbreaks of ASF were acquired from the DAFF database of controlled diseases with the permission of respective senior managers from PDVS. The collection of such data was based on the disease reports submitted to DAFF by PDVS and subsequently reported to the OIE.

2.4 Sampling procedures

2.4.1 Sampling of farms

Provinces were visited for consultation and sampling demonstration/training between 2007 - 2008. Survey manuals (Annex 2) were distributed to the provincial contact officials.

The sampling unit was a farm where warthog dwellings are found. The criterion used for farms to be visited and sampled, considering the vast area to be sampled, was the presence of warthog burrows or any similar warthog dwellings as well as the presence of warthogs. Farms were selected from the initial list of 304 farms. If the burrows could not be found on the selected farm, the officials were allowed to select the next closest farm as an alternative, and if up to four neighbouring farms did not meet the criteria, the sampling official had to move to the next sampling point on the list. For each farm visited specific predefined data were collected (Annex 1). The GPS coordinates were taken using a Garmin hand held GPS.

The farm information collected included estimated number of warthog burrows on the farm, main farming activities, use of acaricides, presence of warthogs and other suid spp. on the farm, contact of warthogs and other domestic pigs and estimated number of warthogs on the farm.

Information was collected on the name of the province, state veterinary area, assisting sampling official, farm name and GPS coordinates of the farm location and details of alternate farms where applicable.

2.4.2 **Sampling of warthog burrows**

Farms were visited during May - November, 2008 - 2012, and the location of the warthog burrows was identified with the assistance of farmers. Where farmers couldn't assist with location, burrows were searched for by the sampling team. Direct sampling technique using the manual collection method (*Jori et al* 2013) was used. Tampans were collected from the warthog burrows (Figure 4A) using a specially designed spade (Figure 4B) for scraping the walls of the burrows hence determining the presence or absence of tampans.

The average number of burrows sampled per sampling site in the previous studies ranged from 2.8 – 4.8 (Vial et al 2007) and considering that a maximum of three burrows had to be sampled in each farm. In farms where burrows were in close proximity, this was regarded as a cluster and three burrows were sampled within the cluster. The burrows, which were interconnected, were regarded as individual burrows.

For homogeneity of the sampling procedure the number of scrapings and time spent in each burrow was standardised. Each burrow was scraped ten times using a spade specially modified for this purpose, spending a minimum of 30 minutes and maximum 45 minutes per burrow. Scraping followed a set pattern of two times in the proximal (entrance) area and two times in the deep areas, two times on each of the sides and two times on the bottom. A black plastic sheet was spread next to the burrow. The collected soil scrapings were spread on the sheet (Figure 4C) under direct sunlight to facilitate the ease of detection of the movement of the tampans. The tampans were also found through sieving of the soil from the soil scrapings.

Figure 4: Procedure used for collection of tampans (A) Warthog burrow (B) Specially designed spade for scraping of WB (C) Sampled soil spread on plastic sheet for allowing movement of tampans (D) Collected tampans

Individual warthog burrow information (Annex 1) included GPS coordinates for location of the burrow. The habitat where the burrow was found was rated as open veld, bushveld, riverine / wetlands, cultivated lands, mountains and other. Other variables were rated as follows: the soil type, where the burrow was found was graded as sandy, rocky, muddy or clay; whether the burrow appears to be in use was classified as active or inactive; presence of soft ticks and the number of ticks present was represented by three categories namely many (>20), few (5 - 20) and very few (>5).

2.5 **Weather data**

The weather data for the period 1993 - 2012 were requested from the South African Weather Services (SAWS). The requested data included monthly averages of minimum (min) and maximum (max) temperatures, average monthly rainfall in millimetres (mm) and average humidity. The weather data were required for the purposes of assessing whether changes likely to have a bearing on the preferred habitat for warthogs and the geographical distribution of host, vector and pathogen had occurred.

The weather data were received from SAWS in Microsoft Excel 2010. The data were summarised into daily maximum (max) and minimum (min) temperatures for the period 1993 - 2012. The rainfall and humidity data were summarised into monthly averages. The weather data were further summarised into four seasonal averages, summer (December - February), autumn (March - May) winter (June - August) and spring (September - November). Winter was omitted from the rainfall analysis as the study area is a summer rainfall area and very little rain is measured during winter which would have skewed the analysis. The moving average of four time periods was

calculated using the formula $MA_j = 1/n\sum_{j=1}^{n-1} at$ where MA_t (forecasted value) is the moving average at time (j), n is the number of prior periods to include in the moving average and a_t is the actual value at time (t). The centred moving average of two time periods was calculated using the same formula. The linear regression analysis and time series graphs from Microsoft Excel 2010 data analysis tool was used to prove the statistical significance of values

2.6 **Data management**

The central submission point for all the data and samples was the ARC-Onderstepoort Veterinary Institute, Transboundary Animal Diseases Programme (TADP). The tampans were collected and submitted to TADP for detection of ASFV and were further used for virus isolation and sequencing. The sequencing results are not reported as part of this study.

The information collected through sample forms (Annex.1) during sampling of burrows was captured and sorted using a spread sheet programme, Microsoft Access 2010, and was converted to Microsoft Excel 2010 for purposes of analysis. Epi Info™ 7 was also used for basic statistical functions. The data for disease outbreaks, obtained from DAFF, were captured in Microsoft Excel 2010. All Microsoft Excel data were exported to CSV (Comma delimited) for purposes of mapping.

2.7 Distribution patterns of ASF outbreaks and disease determinants

2.7.1 Temporal distribution of outbreaks

Data collected on the past occurrence of the outbreaks, as reported by DAFF to the OIE, were analysed to discern the trends, dissemination rate and effectiveness of control of the disease. As part of analysis disease data were clustered into four seasons of the year, autumn (March - May), winter (June - August), spring (September - November) and summer (December - February) for evaluation of the possible links of outbreaks to peak warthog production activities using statistics analysis command of Epi Info™.

2.7.2 Geographic distribution of outbreaks and disease determinants

A map showing the distribution of previous outbreaks was created together with maps of the distribution of sampled warthog burrows, with and without tampans. These were produced using GIS mapping software through the assistance and facilities of ARC - Institute of Soil, Climate and Water, together with the maps showing the distribution of sampled farms in both the ASF controlled area and ASF free zone.

The geographical distribution of warthogs and warthog burrows with uninfected and infected tampans was mapped using the Geographical Information System software ArcGIS 10.1 for desktop (ESRI 2012) and DIVA-GIS 7.5.0.0 (Hijmans *et al* 2012). A distribution map to compare the current ASF control zone and the study findings was also produced.

2.8 **Detection of ASF virus DNA in tick samples**

All ticks were sent to TADP for analysis. Total DNA was extracted from a sample of ticks crushed in a 1.5 ml Eppendorf tube containing 1 ml of phosphate buffered saline (PBS) supplemented with 1% foetal calf serum (FCS) and 1% of a combination of antibiotics and an antimycotic. Homogenates were centrifuged at 10 000 x g for 1 min and the supernatant frozen at -70° C.

DNA was extracted from 200 µl of each tick homogenate and recovered in a final volume of 50 µl DNA solution using the Qiamp kit (Qiagen GmbH, Hilden), according to the manufacturer's instructions. A nested PCR that targets the C terminal end of the *p72* gene of ASFV was used to screen soft tick samples for the presence of ASFV DNA (Basto et al., 2006).

CHAPTER 3. RESULTS

3.1 Details of previous ASF disease outbreaks

3.1.1 Distribution of outbreaks within the ASF control zone

The first objective of the study was to review the ASF outbreaks that have been reported in South Africa from 1993 to 2011. All the outbreaks from the ASF control zone were confirmed by laboratory testing. Table 3.1 shows the dates, location, case morbidity and mortality data of the thirty one (31) outbreaks reported by DAFF to the OIE between the years 1993 and 2011. There were no major outbreaks affecting the commercial pig production industry. Outbreaks occurred sporadically over the period and were all reported in the Limpopo Province with the exception of two from the Kruger National Park, which constitutes the central disease reporting region in Limpopo and Mpumalanga provinces.

Table 3.1: *ASF outbreaks reported to the OIE 1993-2011*

Date	District name	Local municipality	No of outbreaks	No of cases	No of dead	No culled
10/1993	Waterberg	Modimolle	1	41	8	33
11/1993	Waterberg	Lephalale	1	9	3	6
01/1995	Mopani	Maruleng	1	10	10	0
11/1995	Waterberg	Thabazimbi	1	22	20	0
11/1995	Waterberg	Lephalale	1	1	1	0
12/1995	Waterberg	Lephalale	1	12	0	12
01/1996	Mopani	Ba-Phalaborwa	1	11	8	3
02/1996	Waterberg	Bela-Bela	1	3	2	0
02/1996	Waterberg	Bela-Bela	1	23	23	36
02/1996	Waterberg	Bela-Bela	1	3	3	0

07/1997	Vhembe	Makhado	1	2	2	15
12/1998	Waterberg	Lephalale	1	27	20	7
07/2001	Waterberg	Thabazimbi	1	27	27	3
07/2001	Waterberg	Thabazimbi	1	27	27	3
08/2001	Waterberg	Lephalale	1	24	24	0
08/2001	Waterberg	Lephalale	1	24	24	0
04/2002	Waterberg	Lephalale	1	2	1	0
04/2002	Waterberg	Lephalale	1	10	7	0
12/2002	Vhembe	Musina	1	55	20	35
05/2003	Waterberg	Lephalale	1	40	36	4
10/2003	Waterberg	Thabazimbi	1	2	2	4
10/2004	Kruger National Park	Kruger National Park	1	2	2	0
10/2004	Kruger National Park	Kruger National Park	1	2	2	0
07/2005	Waterberg	Lephalale	1	6	5	1
09/2006	Waterberg	Thabazimbi	1	16	16	0
07/2007	Waterberg	Thabazimbi	1	27	27	3
02/2008	Mopani	Ba-Phalaborwa	1	12	6	22
02/2008	Mopani	Ba-Phalaborwa	1	5	5	30
01/2009	Waterberg	Lephalale	1	9	9	0
01/2009	Vhembe	Musina	1	19	4	15
01/2011	Waterberg	Lephalale	1	14	10	3
Total			**31**	**487**	**354**	**235**

Outbreaks in the Limpopo Province occurred in the districts of Waterberg, Mopani and Vhembe (Table 3.1). The frequency of outbreaks in these three affected Limpopo districts and the central region, Kruger National park, over the time period 1993 - 2011 is summarised in Table 3.2. More than 70% of the outbreaks were reported in the far western Waterberg District of Limpopo Province, which consists of the local

municipalities of Lephalale (38.71% of outbreaks) and Thabazimbi (19.35%). Mopani and Vhembe districts, which are both bordering the Kruger National Park, reported 13% and 10% of total outbreaks respectively. The outbreaks were limited to low biosecurity pig farming practices.

Table 3.2: *Summary of ASF 1993 – 2011 outbreaks in the ASF control zone per district*

Province	District Name	Local Municipality	Frequency	Percentage of total outbreaks
Central	**Kruger National Park**	**Kruger National Park**	2	6.45%
Limpopo	**Waterberg**	**Bela-Bela**	3	9.68%
Limpopo	**Waterberg**	**Lephalale**	12	38.71%
Limpopo	**Waterberg**	**Modimolle**	1	3.23%
Limpopo	**Waterberg**	**Thabazimbi**	6	19.35%
Limpopo	**Vhembe**	**Makhado**	1	3.23%
Limpopo	**Vhembe**	**Musina**	2	6.45%
Limpopo	**Mopani**	**Ba-Phalaborwa**	3	9.68%
Limpopo	**Mopani**	**Maruleng**	1	3.23%
		Total	31	100.00%

The seasonal distribution of the total number of outbreaks from 1993 - 2011 indicated a noticeable increase in outbreaks during the summer period (December - February) (Figure 5). The number of outbreaks in spring (September - November) and summer combined was 21 compared to 10 in autumn (March - May) and winter (June - August).

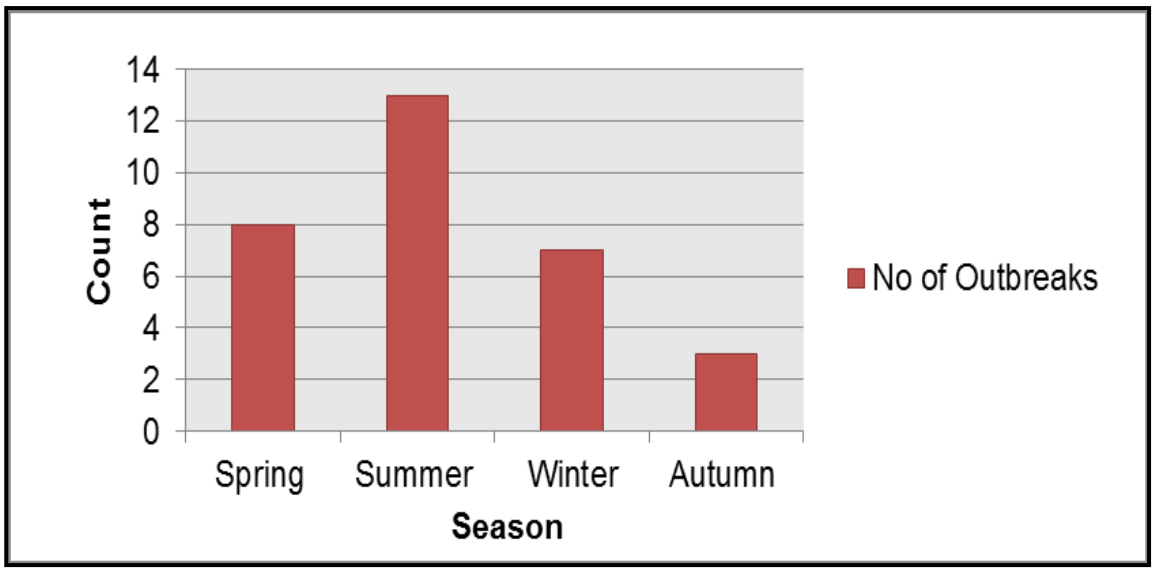

Figure 5: Seasonal distribution of outbreaks in ASF control zone 1993 – 2011

3.1.2 Distribution of outbreaks outside the ASF control zone

Between January and March 2012 several outbreaks were confirmed and reported to DAFF, and subsequently to the OIE (Table 3.3). These all occurred outside South Africa's ASF control zone, in Mpumalanga and Gauteng Provinces. The disease was first diagnosed and confirmed in pigs sent for slaughter to an abattoir in Gauteng. Subsequent to that, 16 more outbreaks were reported in five different districts (Table 3.3). The infected properties belonged to speculators and the source of the outbreak was linked to one property and illegal movement of pigs from the infected area in Limpopo Province to an auction outside the ASF control area.

Table 3.3: *Recent South African ASF outbreaks reported to the OIE 2012*

Date	Province	SV Area	District	No. of Outbreaks	No. of cases	No. dead	No. culled
01/2012	Mpumalanga	Delmas	Delmas	1	44	37	52
01/2012	Mpumalanga	Victor Khanye	Victor Khanye	9	152	131	276
01/2012	Mpumalanga	Govan Mbeki	Govan Mbeki	1	196	196	603
02/2012	Gauteng	Germiston	Lesedi	5	152	151	165
02/2012	Gauteng	Pretoria	City of Tshwane	1	10	10	0
		TOTAL		**17**	**554**	**525**	**1096**

The geographical distribution of the ASF outbreaks from 1993 - 2012 is illustrated in Figure 6. Outbreaks indicated in blue cycles were reported from 1993 - 2006 and the outbreaks reported from 2007 - 2011 are shown with yellow circles. The 2012 outbreaks which occurred outside the control zone are shown using red circles.

A total of 31 outbreaks in the ASF control zone were reported between 1993 and 2011 (Table 3.1) compared to 17 outbreaks reported over two months outside the ASF control zone in 2012. The intensive coordinated stamping out, quarantine and movement restrictions implemented by the joint operations of the State Veterinary Service, pig industry, non-governmental organisations and affected pig farmers contributed to bringing the 2012 outbreaks under control. A serological survey performed during the outbreak and extended to two months after the last case, confirmed that ASFV had been eliminated from outside the control area.

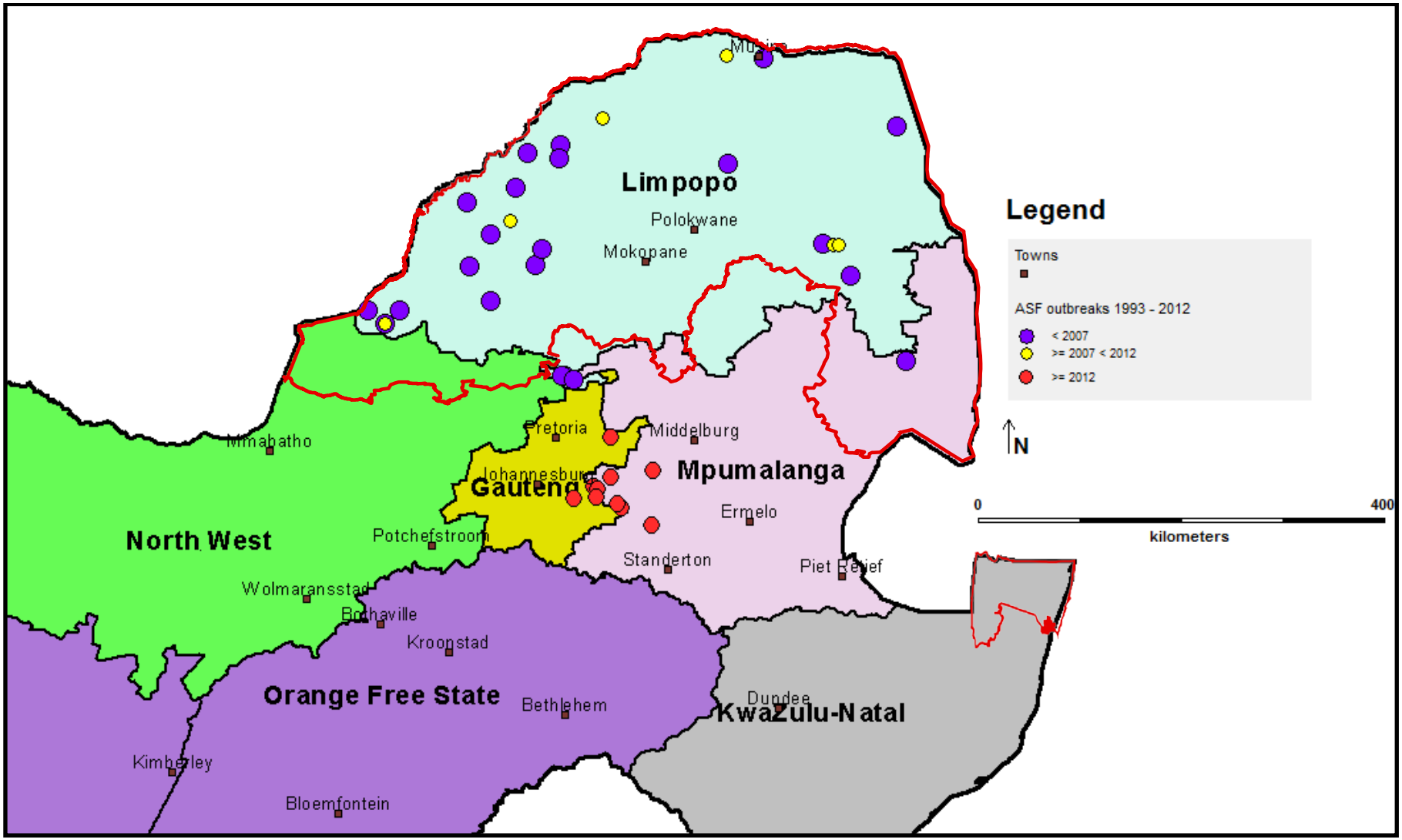

Figure 6: Spatial distribution of ASF outbreaks 1993 - 2012

3.2 Sample collection

3.2.1 Distribution of farms visited

The second study objective was to determine the location and number of farms with warthogs and warthog burrows in the pre-set radius of the ASF control line. Based on the assumption that 20 out of every 100 farms visited would have warthog burrows, 304 farms were selected for sampling. From the original 304 randomly selected farms, 73 farms were visited for sampling during the study period and a total of 157 warthog burrows were found (Figure 7). The farmers assisted in providing information on farms where warthogs and warthog burrows were known to exist and some farms were targeted based in on this information. This was necessary given the size of the area to be covered and the fact that the objective of the survey was to find burrows and look for ticks.

The actual proportion of farms sampled by the end of the study differed from the initial proportional weighted intended numbers (Table 3.4). This could have introduced some bias into the study as some provinces were better represented than others.

Table 3.4*: Final proportion of farms sampled for tampans along the ASF control zone*

Province	Initial proportion of farms to be sampled	Final proportion of farms sampled	Final number of farms sampled
Gauteng	8.2	13.70	10
Limpopo	24.7	32.88	24
Mpumalanga	43.4	36.99	27
North West	23.7	16.44	12
Total	100		73

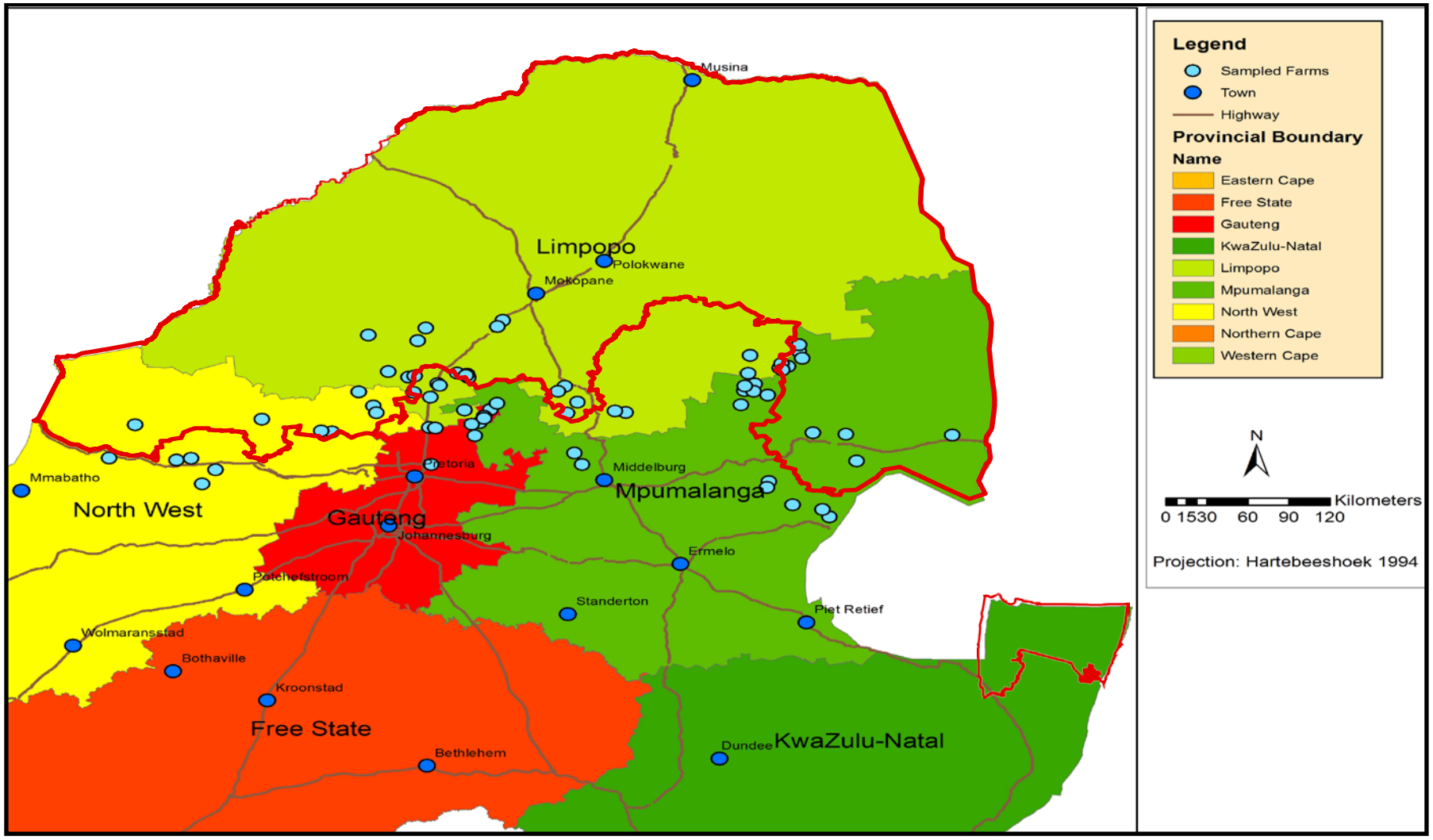

Figure 7: Geographical distribution of farms sampled for presence of warthog burrows and tampans

On a number of farms assistance was provided by the farmer, which made it easier to locate warthog burrows and receive warnings about avoiding burrows occupied by dangerous animals. A limited number of farmers could not provide information and the sampling team had to search for warthog burrows in camps identified by the farmer.

The main findings obtained from the questionnaire regarding warthog distribution are summarised in Table 3.5. Almost half of the farms were wildlife/game farms (50.68%), in 30.14% of the farms the main farming activity was livestock other than pigs, in 10.96% the main farming activity was crops and 8.22% was residential areas and mixed farming. On approximately 63% of these farms warthogs were seen during the visit. A total of 71.43% of farmers stated to have warthogs on the farm (Table 3.5) whilst 57.53% of farmers had noticed warthogs on neighbouring farms. On farms where a form of tick control was practised, this was directed to either livestock or other wildlife animals and not wild pigs.

The proportion of farms with warthog burrows was 86.30% (Table 3.5). The latter constituted the farms where warthogs were occupying storm drains, nests and farms where no burrows could be located. None of the farmers had actual counts of warthogs and 23.29% estimated they had 1 - 20 on their farms, 17.81% had 21 - 40, 17.81% had 41 - 60 and 30.14% had more than 60 warthogs. Just over 24% of farmers claimed to have an increase in the number of warthogs and these increases were attributed to conservation practices. On one farm, a nature reserve, the sampling team observed warthogs in direct contact with domestic pigs.

Table 3.5: *Summary of farm information gathered through questionnaire survey*

Questionnaire variable	No. of incidents	Proportion of farms (%)
No. of Provinces sampled	4	
No. of farms sampled	73	
No. of farms with warthog burrows	63	86.3
Main farming activity, Wildlife	37	50.68
Main farming activity, livestock (other than pigs)	22	30.14
Main farming activity, crops	8	10.96
Mixed farming	3	4.11
Residential	3	4.11
Farms where a form of tick control was practised	28	38.36
Farms where there was no tick control	45	57.53
Farms where warthogs were seen during visit	48	65.75
Farms where neighbouring properties were reported to have warthogs	38	52.05
Farms with estimated number of warthogs 1 - 20	17	23.29
Farms with estimated number of warthogs 21 - 40	13	17.81
Farms with estimated number of warthogs 41 - 60	13	17.81
Farms with estimated number of warthogs more than 60	22	30.14
Farms which claimed to have an increase in number of warthogs	18	24.66
Farms where there was contact of domestic pigs with warthogs	1	1.37

3.2.2 **Distribution of warthog burrows and tampans collected (study objective no. 3 and 4)**

The GPS coordinates of all the warthog burrows located and sampled were recorded and plotted, a total of 157 warthog burrows (Table 3.6) were found across the study area on 63 farms (Figure 8).

Table 3.6: *Summary of warthog burrows (WB) where tampans were found*

Province and number of farms with burrows	%. of farms with WB	No of farms where tampans were found	No. of WB where tampans were found	% of WB with tampans	Total WB sampled	% of WB sampled
Gauteng (10)	40	4	6	21.50	28	17.9
Limpopo (20)	27	7	10	17.54	57	36.54
Mpumalanga (24)	19	2	3	6.67	45	28.85
North West (9)	13	1	1	3.7	27	16.67
TOTAL (63)	**21**	**14**	**20**	**12.74**	**157**	
95 % CI	**Lower limit** **Upper limit**			7.96% 18.99%		

This exceeded the required sample size of 61 warthog burrows in order to find 20% of burrows with infected tampans with a desired absolute precision of 10%.

Tampans were found and collected in 12.74% of the total burrows (Table 3.6), which was 19.10% of the farms sampled. On 45% of the farms many (>20) tampans were collected, 35% had few (5 - 20) tampans and 20% of the farms had very few (<5). On two of the farms visited, all three sampled warthog burrows were infested with tampans, on two farms two of the three sampled warthog burrows were infested with

tampans and on the rest of the farms (10 farms) tampans were recovered in one of the three sampled burrows per farm.

Gauteng Province, located outside the control zone, had the highest proportion of warthog burrows with tampans (21.5%). In Limpopo Province, 17.54% of warthog burrows were infested with tampans. In Mpumalanga Province we found only three warthog burrows with tampans; we could recover a significant number of tampans (>20) in a burrow located inside the ASF control zone, the other burrow located in a farm at the border of Gauteng and Limpopo Provinces had few (5 - 20) tampans

Pools of tampans per burrow were tested for the presence of ASFV DNA and one(1) out of the 14 farms, with one positive warthog burrows, situated along the control line and infested by tampans (7.14% of the farms), located in Limpopo Province in close proximity to the ASF control line, tested positive. No live virus was isolated from these PCR positive tampan samples.

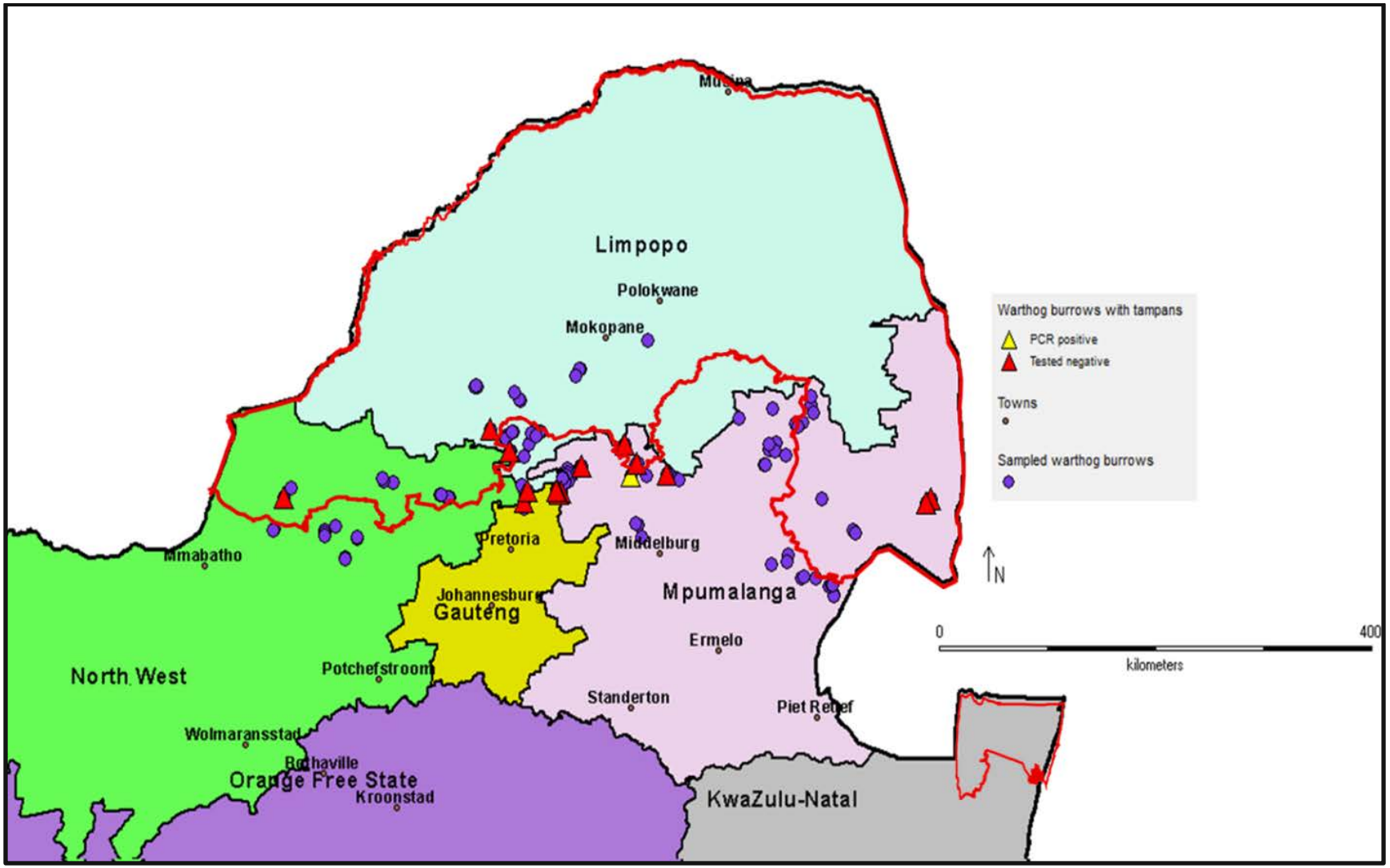

Figure 8: Spatial distribution of warthog burrows sampled for presence of tampans

3.2.2.1 Warthog burrow information

Individual warthog burrow information including the habitat, soil type and the state of activity was gathered by the sampling official (Table 3.7). The warthog burrows were predominantly located in the Bushveld area (73.25%). Sixteen percent of the warthog burrows were in the Savannah grassland / open veld areas. About 3% of the sampling points were storm water drains, which warthogs are known to inhabit. The sampling teams identified 61.78% of burrows to be in sandy soil with 60% of tampans recovered in the type of soil identified to be sandy, 17.20% of burrows were in muddy soil and contained 30% of tampans and 10 % of tampans were found in burrows in rocky areas. One out of 20 infested burrows was inactive although infested with a considerable number of tampans (>20).

Table 3.7: *Summary of warthog burrow information gathered through questionnaire survey*

Questionnaire variable	No. of incidents	% of total burrows
No. of warthog burrows sampled	157	
1. Habitat of warthog burrow		
No. of warthog burrows in the bushveld	115	73.25
No. of warthog burrows in the cultivated lands	8	5
No. of warthog burrows in the grasslands	13	8.28
No. of warthog burrows in the mountains	4	2.55
No. of warthog burrows in the open veld	12	7.64
No. of other areas sampled (storm drains)	5	3.18
2. Nature of soil where warthog burrow was found		
Clay	20	12.74
Muddy	27	17.20

Rocky	13	8.28
Sandy	97	61.78
3. State of warthog burrow activity		
No. of active warthog burrows sampled	145	92
No. of farms where tampans were found	14	22.2
No. of warthog burrows where tampans were found	20	12.74
		% of infested burrows
No. of tampans found (very few < 5)	4	20
No. of tampans found (few 5 – 20)	7	35
No. of tampans found(Many >20)	9	45

3.3 Changes in climate along the ASF control line 1993 - 2012

3.3.1 Maximum and minimum temperature

The average daily maximum and minimum temperatures, in degree Celsius by months, was summarised by obtaining the monthly averages for the period 1993 - 2012. The city of Carolina showed the lowest average daily minimum and maximum temperatures whilst the highest averages were recorded for Lephalale. Temperatures tended to be closely uniform in the interconnected local municipal areas, Lephalale and Thabazimbi and Marble Hall and Belabela (Warmbaths). Analysis of the preliminary data supplied by SAWS indicated that the temperature deviations during the study period were within the expected range. There were no noticeable changes in the average daily maximum and minimum temperatures (Table 3.8 and Table 3.9) in the areas along the ASF control line from 1993 - 2012.

The higher proportion (85%) of tampan infested warthog burrows was situated in areas located closer to weather stations that recorded warmer temperatures: Marble Hall and Warmbaths (Bela-Bela). No tampans were recovered in areas corresponding to Carolina, Lydenburg and Rustenburg weather stations.

Table 3.8*: Average monthly max temp (°C) along the ASF control line: monthly 1993 - 2012*

Town\Month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Carolina	24.90	24.95	23.89	21.93	19.88	17.87	17.72	20.64	23.60	23.76	23.83	24.63
Lydenburg	26.34	25.93	25.36	23.32	21.22	19.28	18.98	21.53	24.39	24.81	24.85	25.99
Marble Hall	31.45	31.91	30.56	28.34	25.67	23.19	23.14	25.61	29.14	30.97	30.91	31.32
Warmbaths	30.2	30.5	29.4	27.1	24.6	22.3	22.1	25.5	29.4	30.6	30.2	30.3
Rustenburg	29.62	29.76	28.49	25.99	23.76	21.29	20.95	24.01	28.09	29.33	29.48	29.59
Lephalale	32.57	32.95	31.51	29.01	26.67	24.25	23.88	27.06	30.54	31.96	32.24	32.39
Thabazimbi	31.88	31.91	30.88	28.45	25.78	23.39	23.11	26.56	30.44	32.14	31.99	32.03

The data for maximum average seasonal temperature showed an increasing trend (Figure 9). On linear regression analysis using Microsoft Excel 2010 the increase was statistically significant ($p = 0.00018$ $r^2 = 0.165$).

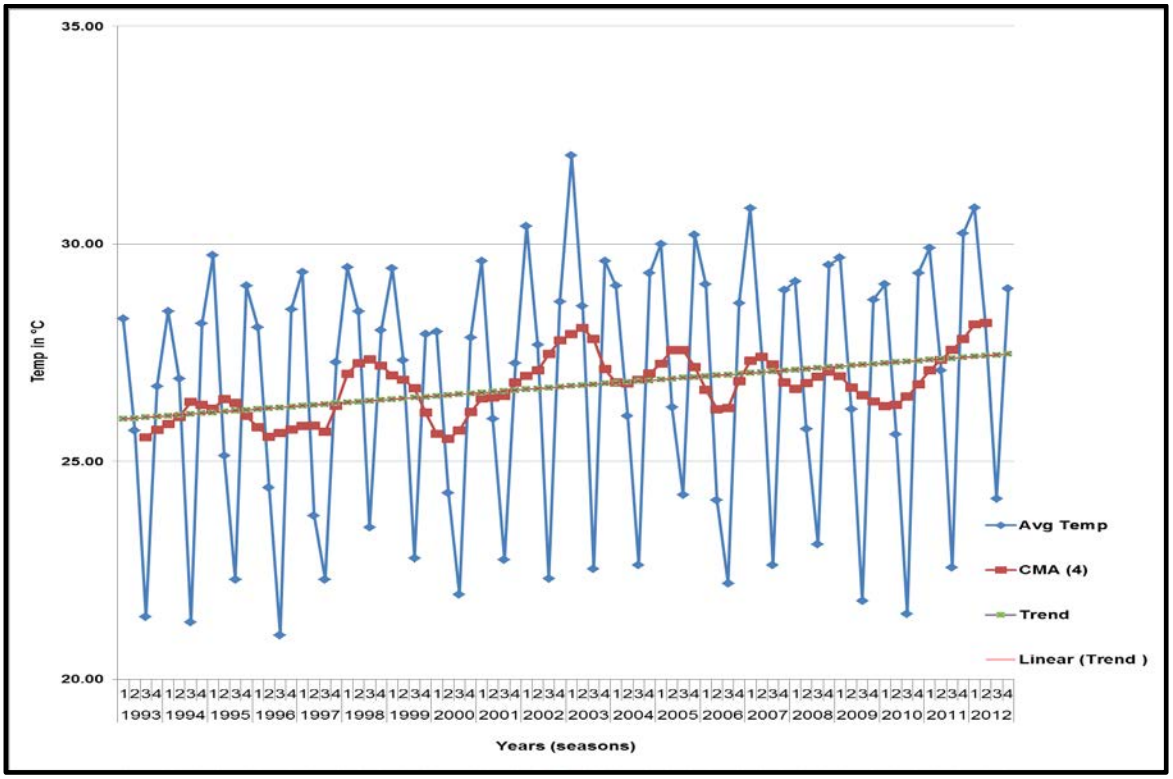

Figure 9: Average seasonal maximum temp along the ASF control line: 1993 - 2012

(CMA (4) = centred moving average (four seasons))

Table 3.9: *Average daily monthly min temp (°C) along the ASF control line: monthly 1993 – 2012*

Town\Month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Carolina	13.74	13.55	12.10	8.84	4.77	1.78	1.20	3.91	7.43	10.25	12.02	13.03
Lydenburg	15.29	14.96	13.59	10.67	6.94	4.14	3.54	6.01	9.09	11.76	13.61	14.59
Marble Hall	19.80	19.69	18.00	14.34	9.44	5.83	5.53	8.63	13.21	16.93	18.23	18.90
Warmbaths	17.4	17.0	15.4	11.7	6.9	3.6	2.6	5.9	10.2	14.0	15.8	16.7
Rustenburg	17.61	17.42	15.75	11.86	7.37	3.94	3.29	6.09	10.48	14.23	15.79	16.90
Lephalale	20.55	20.31	18.86	14.77	9.85	6.31	5.18	8.78	13.32	17.12	18.97	19.90
Thabazimbi	19.91	19.41	17.65	13.17	7.05	3.48	2.45	6.62	12.75	17.03	18.47	19.24

The average minimum seasonal temperature (Figure 10) was not statistically significant ($p = 0.6$; $r^2 = 0.0017$).

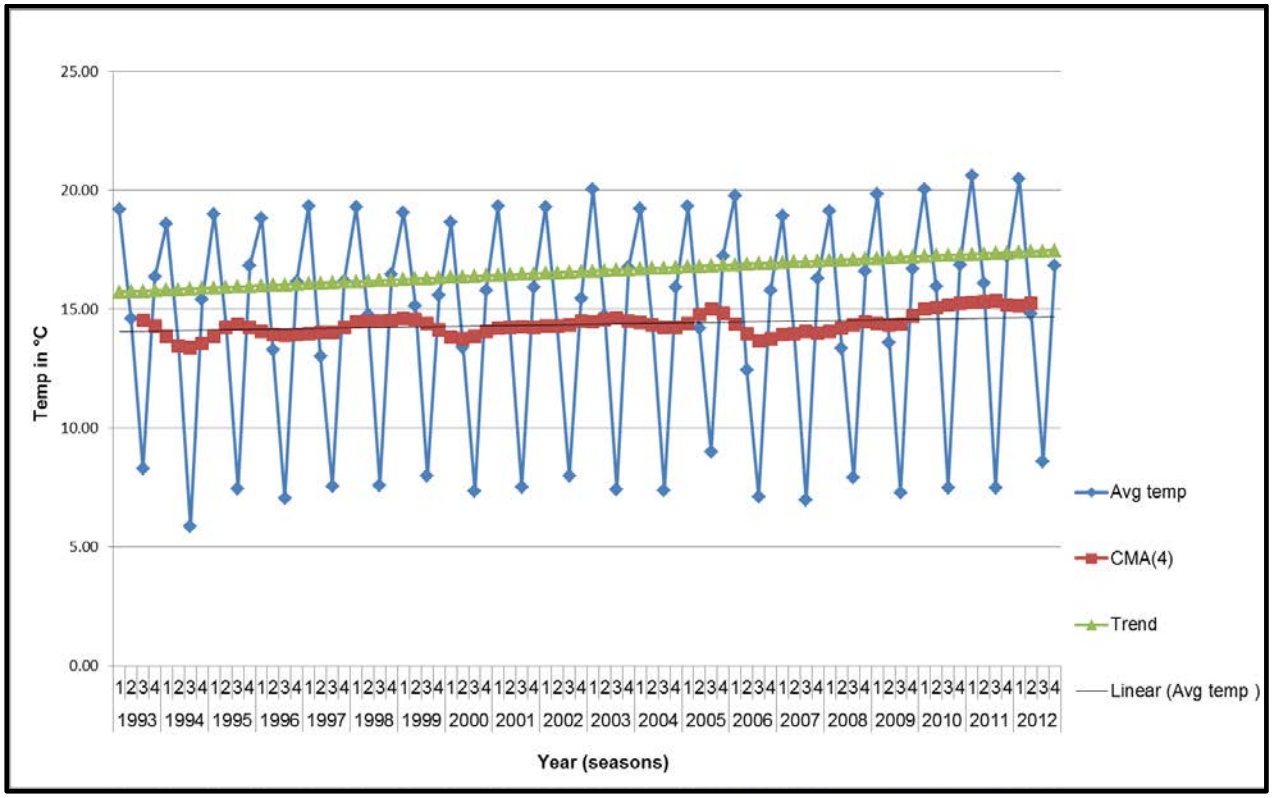

Figure 10: Average seasonal minimum temp along the ASF control line: 1993 – 2012

(CMA = Centred Moving Average)

3.3.2 **Rainfall**

The average monthly rainfall measured in mm was summarised by obtaining the average monthly rainfall for each month during the period 1993 – 2012 for main town areas (Table 3.10). The highest (722.3mm) average annual rainfall was recorded for Carolina and the lowest for the Lydenburg and Lephalale areas. Both Lydenburg and Lephalale received average annual rainfall below the country's average (450mm). It should be noted that there were variations in total recorded mm of rainfall (average monthly rainfall) and seasonal fluctuations with recorded dry and flooding seasons during the period analysed.

Table 3.10*: Summary, average monthly rainfall (mm) along the ASF control line: 1993 – 2012*

Town\Month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	Average annual rainfall
Carolina	116.2	79.9	89.4	49.8	16.4	8.2	5.6	9.8	14.4	89.2	113.6	129.8	722.3
Lydenburg	44.1	46.1	39.8	37.0	12.1	2.0	3.5	2.9	13.8	61.9	71.4	75.5	410.1
Marble Hall	91.8	68.8	76.6	23.9	10.7	2.0	1.8	2.0	5.5	64.0	104.3	112.1	563.5
Warmbaths	109.7	76.5	79.5	37.9	16.1	4.3	1.6	2.8	8.6	55.8	87.7	114.3	594.8
Rustenburg	100.9	83.9	69.1	35.5	19.1	5.0	1.0	3.5	10.5	53.2	74.9	99.1	555.7
Lephalale	80.9	47.7	45.1	26.2	13.0	3.5	1.1	0.1	2.8	31.5	69.7	89.2	410.8
Thabazimbi	121.4	97.8	77.0	31.5	15.9	7.9	1.3	0.9	9.5	51.6	74.4	122.8	612

The average seasonal rainfall in the area along ASF control line showed no observable trend (Figure 11). This was however not statistically significant at a 95% confidence level ($p > 0.34$; $r^2 = 0.015$).

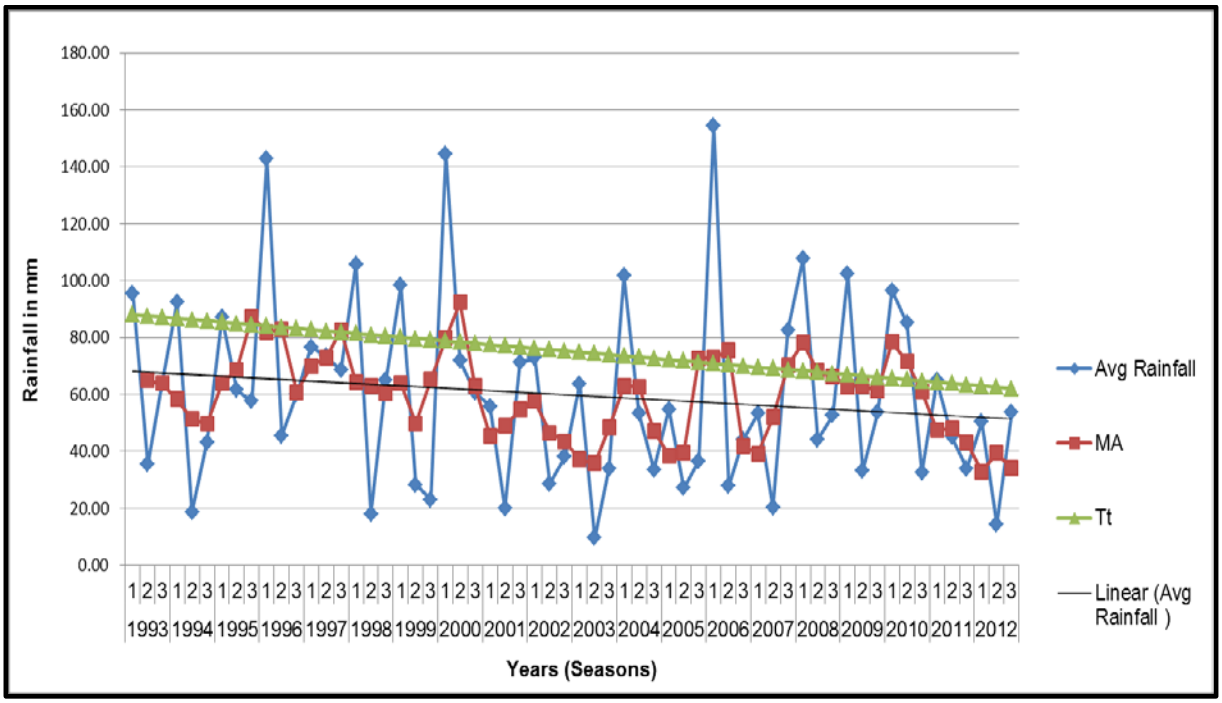

Figure 11: Average seasonal rainfall along the ASF control line: 1993 – 2012

(MA = Moving Average, T_t = Trend)

3.3.3 **Humidity**

The humidity data for Carolina and Marble Hall were not considered due to missing information. The average monthly humidity along the ASF control line for the study period ranged between 45 and 85%.

Table 3.11*: Summary, average monthly humidity (%) along the ASF control line: 1993 – 2012*

Town\Month	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Lydenburg	80	82	83	83	75	72	72	68	63	71	75	79
Warmbaths	71	72	74	75	70	71	68	58	50	58	63	68
Rustenburg	73	71	74	76	76	77	72	61	52	60	65	71
Lephalale	73	72	74	75	75	76	71	60	51	54	59	69
Thabazimbi	70	71	74	77	76	76	70	57	46	51	58	66

A decreasing trend was observed in the humidity (Figure 12) which was statistically significant ($p = 0.003$; $r^2 = 0.106$).

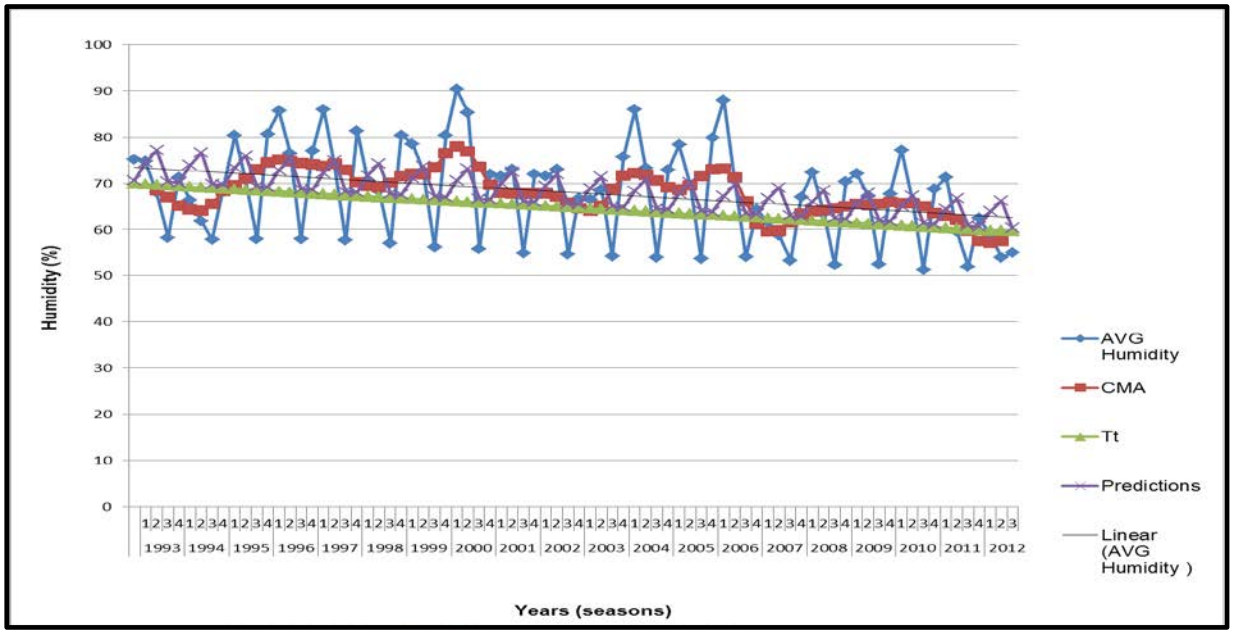

Figure 12: Average seasonal humidity along the ASF control line: 1993 – 2012

(CMA = Centred Moving Average, T_t = Trend)

CHAPTER 4. DISCUSSION

4.1 Introduction

According to DAFF 2012 reports, Limpopo, North West, Mpumalanga and Gauteng Provinces contribute up to 63% of pork production in South Africa (Anonymous 2014). In addition, the contribution of pig production to the primary agricultural sector is approximately 2.15%, with a gross value of pigs slaughtered increasing more than 200 fold from 2001-2011. The country exports some of its pork to the South African Development Community countries and to eastern, western and central Africa. The marketing directorate of DAFF identifies some of the strengths of the pig industry as being short turnaround production time and increased demand for pork in the light of high prices and unavailability of red meat. The weaknesses include susceptibility to diseases where health, safety and phyto-sanitary issues can inhibit growth of the industry. The challenges include outbreaks of diseases such as swine fevers. Animal disease outbreaks are a limiting factor in any production system as they affect production levels and have the potential to prohibit international trade agreements.

ASF has previously been reported in South Africa from the gazetted and proclaimed ASF control area. The aim of this study was to survey the determinants of ASF disease along the ASF control line to assess the current validity and effectiveness of the control line. An analysis of the spatial and temporal distribution of the outbreaks that occurred between 1993 - 2012, using the data from national disease reports, provided insight into the regions that are most at risk.

4.2 Significance of outbreaks within the ASF control zone

Sporadic outbreaks, confined to Limpopo Province, were reported from 1993 - 2011 with no reported outbreaks in the ASF control zones of both the Mpumalanga and North West Provinces. An outbreak, which is not part of this study, was reported from North West Province in January 2013 inside the ASF control zone. The outbreaks were in areas where pigs were not kept in full confinement and were most probably due to a spill-over from the sylvatic life cycle of the disease. The presence of a sylvatic cycle has been suggested as the limiting factor to the eradication of ASF (Penrith 2013) whilst it has played a major role in the sporadic occurrence of ASF in domestic pigs (Costard *et al* 2013). The reporting pattern and number of pigs affected in the outbreaks in the ASF control zone point to the low input and lack of biosecurity as the main contributory factors. Although three outbreaks from Bela-Bela occurred adjacent to the control line and were confirmed and reported to the OIE, the disease had not spread beyond the borders of the gazetted control zone prior to 2012. This suggests the DAFF policy applied within the control zone has been effective in containing the disease north of the control line.

The spatial distribution of the disease indicated that the outbreaks of 1993-2011 were mainly skewed to the western municipalities of the Limpopo Province which is in agreement with the distribution of tampans recorded between 1970 and 1990 (Penrith *et al* 2004a) and where ASFV had been isolated from tampans in the past. Limpopo hosts more than 50% of the game farms in South Africa and a considerable number of these are located in the Waterberg district, situated in the west of the Province. It is likely therefore that game farms have contributed to the sporadic reports of the disease. This is further supported by the fact that there have been few

reports of ASF in the south central districts of Limpopo, where there are fewer game farms. A number of accredited piggeries are situated in this area and there is strict movement control of pigs from the accredited facilities directly for slaughter.

Although the outbreaks from 1993 - 2011 were sporadic, the analysis performed in this study showed that there were more outbreaks in summer compared to winter, with autumn being the least affected. Late spring to early summer is a recognised farrowing season for warthogs (Arnot *et al* 2009) with increased viraemia in neonatal warthogs inhabiting the burrows. During this period it is expected that the majority of ticks will be infected with ASFV with an increased risk of infection to domestic pigs that come into contact with these ticks. The only three outbreaks reported during the autumn period, in 2002 and 2003, were from Lephalale, the region which reported the highest number of outbreaks during the period 1993 - 2011. This is also amongst the regions with the highest distribution of tampans (Penrith *et al* 2004a). The high prevalence of virus and tampans in Lephalale may contribute to the spill-over of disease into autumn.

Another reason why there has been a clustered contribution of outbreaks could be the variable demographics of small scale pig farms and related socio-cultural and religious practices that influence pig movement and contact with warthogs and infected tampans. This is supported by the events that took place in 2012.

4.3 Significance of outbreaks outside the ASF control zone

Prior to 2012, the only two outbreaks reported outside the ASF control area date back to 1996 and 1951, and occurred outside Bela-Bela (Warmbaths in Gauteng Province) and Mpumalanga Province respectively. After 15 years of successfully containing ASF within the ASF control zone, the first outbreak was confirmed outside the ASF control zone and reported to the OIE in January 2012; subsequent to that sixteen other outbreaks were confirmed within two months in the Gauteng and Mpumalanga Provinces.

The infected farms were placed under immediate veterinary quarantine when they were identified. South Africa established and imposed a movement protocol for the movement of pigs and pig products from the infected areas in Gauteng and Mpumalanga Provinces and a surveillance programme to determine the magnitude of the outbreaks, and control measures were instituted. Movement was only permitted for direct slaughter under strict monitoring of the veterinary services and was accompanied by red-cross permit. The institution of the Joint Operations and Veterinary Operations Committees, which dealt with interprovincial monitoring and coordination of the disease, played a pivotal role in the curbing and controlling the outbreak before overwhelming the pig industry.

Investigations into the first identified outbreak in Gauteng revealed that pigs were brought from an auction in the ASF free area into the farm for fattening and slaughter in early December 2011 (Gauteng Veterinary reports). Pigs started showing clinical signs and first deaths were recorded towards the end of December 2011 and were presented for slaughter to an abattoir, where suspected diagnosis of ASF was

subsequently made and laboratory confirmed by TADP. The collaboration between Gauteng Province, Mpumalanga Province and DAFF in backward and forward tracing of the movement of pigs from the auctions assisted in the identification of further related outbreaks in the Lesedi and City of Tshwane municipalities of Gauteng Province, and Delmas, Victor Khanye and Govan Mbeki districts of Mpumalanga Province. Retrospective investigation of all outbreaks outside the ASF control zone linked them to the same source property inside the ASF control zone and was attributed to the illegal movement of infected pigs from the identified property to an auction outside the control zone (Penrith 2013). The 2012 outbreaks of ASF were successfully controlled and the country's free status reinstated. Collaborations of stakeholders, urgency of response and the availability of diagnostic resources all contributed in the control of these outbreaks. Since then no further outbreaks were reported in the ASF free zone.

The 2012 outbreaks highlight the importance of other methods of ASF transmission, which could result in devastating effects in the commercial pig production industry. ASFV has demonstrated its potential to spread and establish itself in new areas through the uncontrolled outbreaks in the Caucasus and Eastern Europe. The movement of infected animals and animal materials has been found to play a critical role in the spread of highly infectious diseases like ASF, FMD and CSF and could result in devastating effects on the pig production industry. The introduction of ASFV into either the free ranging - low biosecurity farms, or the highly concentrated and intensive pig farming areas could have devastating effects on the pig industry in the free zone. It is thus the responsibility of the state to safeguard this industry from such

occurrences by constant monitoring, active surveillance and ensuring that the regulations are enforced.

4.4 **Distribution of farms with warthog burrows**

The study of the epidemiology of the disease constitutes the core in implementing, monitoring and assessing disease control interventions. Under South African conditions, a very important determinant of the epidemiology of ASF is the presence of warthogs and warthog burrows, which, if they are colonised with tampans, could remain permanently so and if subsequently infected with ASFV, serve to perpetuate the sylvatic cycle of the disease.

The final number (157) of warthog burrows sampled was higher than the initial targeted number (61) resulting in an increased proportion of sampled warthog burrows per province, thus we sampled enough warthog burrows to prove the presence of infected tampans. One of the contributing factors to the final number of warthog burrows sampled was the inconsistencies in assistance offered by farmers as well as the willingness of provincial officials to assist with sampling that varied from province to province. The manual method of sampling tampans in warthog burrows is laborious and time consuming (Jori *et al* 2013); it is the least expensive and most practical but it proved not to be acceptable with some sampling teams because of the perceived risks associated with potential encounters with predators whilst in the process of scraping or entering the burrows.

Gauteng Province has conducted on-going surveillance for ASFV and sampling for tampans is conducted in warthog burrows in areas bordering the ASF control line. Since 2002, infected tampans have been found on five different farms outside the control zone (Dwarka *et al* 2004). During our study, ten farms with 28 warthog burrows were sampled and tampans were found in 21.5% of these burrows, all located on game farms south of the red line. This constituted the highest percentage of infested burrows found during the survey and could partly be attributed to the fact that the location of some infested warthog burrows was known. ASF DNA was not detected in any of these tampans. The area in which these game farms are located has been gazetted as a 'Big Five' game reserve – the Dinokeng Game Reserve located south of the ASF control line. Since both the vertebrate and arthropod hosts were identified in the study area of the province, it indicates that the conditions continue to be favourable for maintenance of tampans in the Northern Gauteng area. This poses a question on whether the control line needs to be moved further south in this part of the country.

The presence of tampans had been previously recorded in the Mpumalanga Province in the area along the Kruger National Park (Penrith *et al* 2004b). During this study, 2008 - 2012, 27 farms with 45 warthog burrows were sampled inside and outside of the ASF control area of Mpumalanga. Although the proportion of sampled farms (37%) was lower than the study design required (43.4%), the required number of warthog burrows (n = 26) was easily found since areas with known presence of warthogs were targeted. Tampans were found in only 6.67% of warthog burrows on both sides of the control line but no viral DNA was detected by PCR. The presence of only a few burrows with tampans in an area outside the control zone in

Mpumalanga could mean that there is probably no need to shift the control line in these areas. However, further monitoring is required because the area where tampans were found is bordered by communal residential areas and the probability of warthogs coming into contact with domestic pigs cannot be disregarded. Fortunately, Mpumalanga PDVS has detailed information on the areas where warthogs and warthog burrows can be found (Figure 13) and are proactive in monitoring the situation.

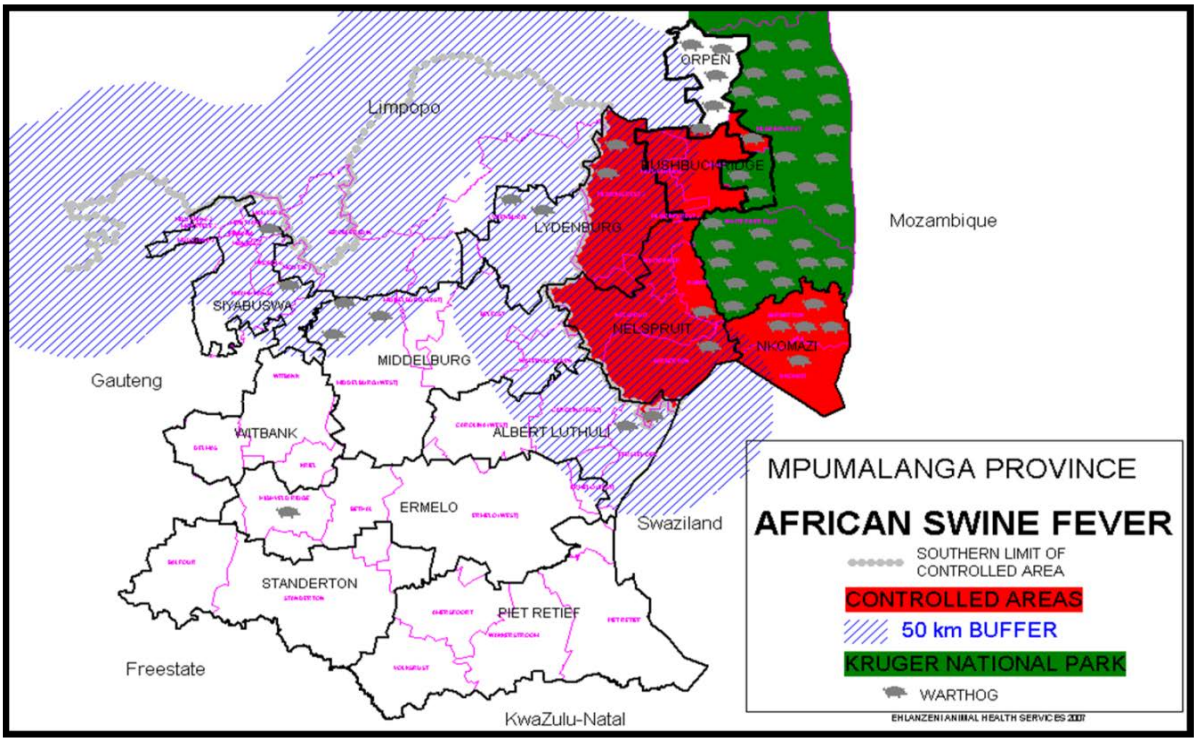

Figure 13: Distribution of warthogs along the ASF control line in Mpumalanga Province – 2008 (provided by Mpumalanga Veterinary Services)

As part of a monitoring programme, the Lydenburg (Thaba Chweu) State Veterinarian was given a surveillance project (1997 - 2010) in which serum samples from warthogs, bush pigs and domestic pigs along the ASF control zone (Lydenburg and Pilgrim's Rest) were collected and tested (Mpumalanga PDVS reports). All of the

138 serum samples collected were sero-negative for ASFV except samples collected at two farms, Mooiplaas and Vlakfontein, in 2004. The warthog serum samples from these two farms tested positive for ASF viral DNA but no virus could be isolated. In the subsequent serum collections performed by the province between 2004 and 2010 including repeat serum collections from Mooiplaas and Vlakfontein farms, all warthog serum samples tested negative for ASFV DNA. This further supports the argument that the control line does not need changing in this region. It should however be taken into consideration that these were conveniently collected suid serum samples and are not necessarily representative of the warthog population in the area. The interaction of the different suid species deserves an intensive study.

Limpopo Province is the largest and leading producer of pork in South Africa (Anonymous 2011). The province has a number of accredited piggeries, which are high biosecurity compartments approved by DAFF, from where pigs can be moved directly for slaughter at approved and registered abattoirs outside the ASF control area. The province constitutes the largest section of the ASF control area where outbreaks of ASF occur and tampans are known to occur (Penrith *et al* 2004b). Tampan infested warthog burrows were distributed across the different farming activities both north and south of the ASF control line with 60% on wildlife farms, 20% on livestock and 20% on crop farms. Tampans which tested positive for ASFV during PCR screening were from within a crop farming area situated south but in close proximity to the ASF control line. The relatively high proportion of burrows with tampans, both north and south of the control line, and the presence of virus south of the control line suggest that the control line should be realigned in this area of the country. The movement towards mixed farming could also be contributing to the risk

of widespread distribution of warthogs. Warthogs are known to scavenge crop fields in many areas of Africa and they have been recorded as top contributors in destruction of maize and bean fields (FOA 2010). Hence they are likely to gravitate towards cultivated areas of the province.

The northern portion of the North West Province is part of the ASF controlled area. A number of the farms in the sampling frame in this province couldn't be visited because of prevailing service delivery community uprisings and labour strikes which resulted to limited access to some farming areas. The farms in the Moses Kotane local municipality were not sampled because it consisted mainly of rural residential areas but there were no known warthog burrows in this municipality. The area that could not be surveyed in North West Province was relatively extensive and hence the results may not be a true reflection of the situation along this part of the control line. The province borders south-western Limpopo Province which is considered as a high risk area that has previously been identified as a locality where tampans of *O. moubata* complex are found (Penrith *et al* 2004b) and this should potentially pose a high risk to the neighbouring farming communities. Nevertheless, a total of 12 farms were visited where 27 warthog burrows were sampled; which is above the minimum number of 15 burrows calculated in the original sample size for North West Province. An insignificant number of tampans (less than 5) was found in one warthog burrow situated north of the ASF control line and the PCR screening test was negative. However, considering the poor representativeness of sampling in this province, it is recommended that the area be revisited when the social community conditions are back to normal to get the more representative sample.

In summary the total number of farms visited in this study contributed to more than double the number of warthog burrows that were targeted for an assumed prevalence of 20% of burrows being infested with infected tampans. The infestation rate of the warthog burrows was found to be 12.74% (20/157) (CI95% 7.96% - 18.99%). In 5% (1/20) of the total infested warthog burrows, tampans tested positive for ASF viral DNA but no virus could be isolated. PCR detects viral genome and can be positive even when no infectious virus is detected by virus isolation. The test is used as a screening method because of its sensitivity and specificity with the ability to detect even non-hemadsorbing and low virulent virus isolates (Agüero *et al* 2004). The epidemiological significance and risk posed by these positive PCR tests are currently not known.

The overall infestation rate of the warthog burrows varied from province to province and was consistent with what other researchers have found (Parker *et al* 1969; Bastos *et al* 2009). In previous studies the infection rate of tampans with ASFV was in the range of 0.3% - 1.7% (Penrith *et al* 2004b) whilst Kleiboeker & Scoles (2001) cited a rate of 0 - 3.8%. In a study done in the Mkuze Game Reserve (MGR) in 2007, 60.2% of warthog burrows contained tampans but PCR failed to detect the presence of ASFV DNA (Arnot *et al* 2009). The highest infection rate of tampans with ASFV has been found in Livingstone Game Park where it was recorded to be 5.1% (Wilkinson *et al* 1988). Wilkinson *et al* (1988) emphasised that the overall infection rate of tampans depends on the relative proportions of different stages of ticks with a higher rate where the populations have a higher proportion of adults. This is however not so in the MGR study where 75% of ticks were adults (Arnot *et al* 2009). The tampans collected for this study had approximately equal proportion of adults and

nymphal stages. The status of the ASFV in the arthropod vector, *O. moubata*, needs to be constantly monitored. Any change in ASFV status, with the spread of infected *O. moubata* outside the control zone, could have considerable impact on the South African pig production industry.

4.5 **Questionnaire survey**

The main objective of the questionnaire survey was to collect data related to warthog burrow habitat and soil type. The habitat can influence the ability of warthogs to feed and escape predators while soil can have an impact on the ability of fossorial species and predators to excavate and modify burrows (White & Cameron 2009). Also of importance were the farm data which determined which farming activities could influence the presence of warthogs on the farm and the farmer's observed status of warthogs on the farm and neighbouring farms.

4.5.1 **Farm data**

Aspects of farm management activities can influence the spatial distribution of the agent and host and therefore the disease. The survey questionnaire focused on activities that could have an influence on the distribution and number of warthogs on farms. The farms that were sampled were predominantly wildlife (50.68%) and livestock (30.14%) farms. In 65.75% of the farms, warthogs were seen during the visit, whilst 52.05% of the farmers indicated that there were warthogs on neighbouring farms. Warthogs are herbivores which get most of their food from grazing and occasional browsing and digging for roots. Most farmers practising crop and mixed farming identified them as destructive to both planted crops and grazing fields. Farmers in the residential / communal farming areas, mixed farming and crop

farming areas claimed that they had observed a decreasing number of warthogs over the years. The main reasons for the decrease in number of warthogs were ascribed to hunting, changes in farming practises and changes in human population distribution.

Only 24.66% of the farmers claimed to have observed an increase in the warthog numbers. A farmer in Mbombela local municipality claimed to have reintroduced warthogs on his farm after they had been completely eliminated. The increase was mainly credited to nature conservation practices, although on some farms signs of poaching traps were identified. Common warthogs have been identified as having characteristics of an invasive species based on studies in the Eastern Cape where common warthogs have spread beyond the targeted introduction site (Nyafu 2012).

Our study therefore confirmed the presence of warthogs along the control line. This has been the case throughout the existence of the control line. What has changed in the recent times is an increase in game farming in South Africa, and in the areas along the ASF control line. A change from approximately 575 thousand to 18.6 million game animals has been documented between 1964 and 2007 (Carruthers 2008) with a threefold increase in the number of game farms between 1981 and 1992. The shift to wildlife-based production has been recognized as the most rapidly expanding agricultural activity (Snijders 2012). The increase in the number of game farms poses a potential risk for movement and increase in the number of warthogs and the arthropod vector, making continual surveillance of the control line even more important.

4.5.2 **Warthog burrow data**

Common warthogs are mostly associated with the savannah biome and can be found in grasslands, open woodlands and flood plains. The savannah biome is the largest of the biomes in the southern Africa, covering 46% of its area (Rutherford and Westfall 1994) and hosting the major game reserves in South Africa. *O. moubata* is also known to be widely distributed in the savannah regions where warthogs occur (Penrith *et al* 2004b). The grassland biome borders the savannah along the study area. A great portion of the grassland biome is used for agricultural activities, including livestock and crop farming. In our observation most of the burrows were located in the savannah and grassland biomes. The grassland and savannah biomes share the areas along the KwaZulu-Natal coast and the northern parts of the Eastern Cape coast to approximately the Sundays River, indicating that potential conditions exist away from the current control zone that are favourable for warthogs to survive.

It is also reported that the soil type would influence the vegetation surrounding the burrow and the burrows' humidity (White & Cameron 2009). The study showed that tampans were most commonly recovered (60% of warthog burrows) from the soil which sampling teams identified as sandy. Warthogs inhabit pre-excavated aardvark burrows. As they do not dig their own burrows, soil type would not be expected to play a major role in their habitat. They were found to also occupy other areas like storm drains and nests. The distribution of the biomes where common warthogs can flourish and the availability of the places suitable for protection from predators can contribute to the dispersal of warthogs southwards. With warthogs occupying pre-excavated burrows, there could be a risk of the arthropod vectors moving southwards as demonstrated in the Gauteng Province survey.

In summary the study confirmed that there is a high distribution of farms with warthogs and warthog burrows both south and north of the ASF control line. A representative sample of warthog burrows (157) to test the assumption that there is 20% prevalence of ASFV along the line was attained however; the assumed 20% prevalence of ASFV could not be verified. An even distribution of warthog burrows infested with tampans was found with most concentrated in the central parts of the study area, which is Bela-Bela, Northern Gauteng, Ephraim Mogale and Elias Motsoaledi municipalities. The study did not show any evidence of active virus infection, as ASFV could not be isolated from the tampans that tested PCR positive.

4.6 **Changes in climate along the ASF control line 1993 – 2012**

Both warthogs and tampans are required for long term maintenance of ASFV in the sylvatic cycle (Kleiboeker and Scoles, 2001). It was therefore essential to consider aspects of climate as a determinant of environmental factors influencing the spatial / geographical distribution of the disease thus any changes to the climatic conditions which impact on the distribution of vegetation type and farming practices would be expected to have significant impact on the distribution of the vectors (Van den Bossche & Coetzer 2008), which in turn will have a bearing on the incidence and prevalence of the disease. Soft ticks are very specific vectors not comparable with hard ticks and their biology is not directly linked with climatic conditions but more on condition of their microenvironment (Vial 2009).

The ASF control line area is subjected to warm to hot months from October - March accompanied by mainly summer rainfall (Table 3.11). Warthogs are herbivores and

rainfall would be one climatic factor that highly contributes to the ecological dynamics involved in their survival. For most of the weather stations in this study, the average total annual rainfall recorded from 1993 - 2012 had surpassed the country's annual average (450mm) with the exception of Lydenburg and Lephalale which were below the average. Ogutu *et al* (2008) recorded that seasonal abundance of warthogs is related to both current and cumulative rainfall. This would mean that both can result in a potential abundance of warthogs in areas where habitat allow their existence. On the other hand, warthogs are known to occur in areas of Somalia where the annual rainfall is between 250mm and 450mm (d' Huart & Grubb 2001) thus showing they can survive in arid habitats.

Tampans in our study were recovered in areas where the average daily maximum temperature was above 20°C. This concurs with an experimental study by Vial (2009) where conditions for thriving of tampan species have been proven to be temperatures ranging from 22 °C - 32°C with 25°C being the optimal temperature inside the microhabitat for *O. moubata* (Jori *et al* 2013).

In the same experimental study, tampans have been quoted to survive at relative humidity of 50% - 95% with 50% being the optimal humidity for *O. moubata.* In Egypt, the experimental optimum temperature and humidity conditions for the life cycle of *O. erraticus* were found to be 28°C and 75% respectively (El Shoura 1987). Ogutu *et al* (2008) similarly found that the population of ungulates, which includes warthogs, was influenced by cumulative past rainfall and seasonal rainfall fluctuations in Mara- Serengeti, Kenya.

The results showed an increasing trend for maximum temperatures and it can be assumed that it is getting warmer in areas along the ASF control line. This has been accompanied by a decreasing trend in humidity and insignificant change in seasonal rainfalls. Although there were observed changes in the weather trends between 1993 and 2012, these are gradual and the impact may not have been felt yet. There is no definite trend to suggest that the area along the ASF control line is becoming dryer and thus could lead to warthogs moving southwards in search of better conditions for survival. The data in this study serve as a reference for future studies.

CHAPTER 5. CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This is the first scientifically designed study of ASFV determinants along the control line since its inception in 1935. It is also the first study of weather patterns along the control line which could have an impact on ASFV determinants in the long term. The study therefore provides, for the first time, important baseline information on the current status of ASFV determinants and weather variables along the control line. The study confirms that the control line is an important mechanism for controlling the spread of ASF in South Africa and it can now be used for comparative purposes in future studies to examine whether risk factors may be changing or whether the control line needs moving.

Objective 1

The analysis of previous ASF outbreaks in South Africa shows that up until 2011 the control line was not being penetrated and was probably effective as a defined cut point between the endemic and disease free areas of ASF. The 2012 outbreaks were due to illegal movement of infected pigs from the control zone to the ASF free zone and were not as a result of the breakdown of the control line as a barrier established by environmental factors. Enforcement of movement control and stock auction inspections are still enforced to avert further unexpected ASF outbreaks.

Objective 2 and 3

The second and third objectives aimed to address the location of farms with warthogs and warthog burrows in them and the proportion of warthog burrows with

tampans. There is a high prevalence of warthog burrows on farms along the control line with 86.3% of farms testing positive for warthog burrows and warthogs were seen on 65.75% of farms during visits. Only 12.74% of warthog burrows had tampans. The environmental conditions, with the expansion of game farming practices and suitable climatic conditions continue to favour the maintenance of both the vertebrate and the arthropod hosts within the ASF control area. The study confirmed that warthogs, warthog burrows and tampans are found beyond the ASF control line. The causes for this spread are unclear and could be multi-factorial including their adaptability to Savannah grassland and movements towards game farming. The detected changes in the trends of weather conditions in line with the distribution of the disease determinants need further analysis as they are not comparable to any previous data.

Objective 4

The main determinant of ASF is the presence and isolation of ASFV in the ticks of *O. moubata complex.* Only one farm had tampans infected with ASFV, but no live virus was isolated. Therefore, our study found limited evidence of ASFV in tampans along the ASF control line. Further surveillance needs to be conducted to monitor the status of ASFV in tampans.

5.2 Recommendations

From the results of the study, it could be suggested that the position of the ASF control line would need to be realigned along the areas where there is a possible higher risk of the movement of the risk factors southwards. Emphasis should also be placed on regular surveillance of warthog burrows along the ASF control line with

skills development and skills transfer. Coupled with active and passive surveillance, the on-going intense awareness on the long-term incentives of livestock movement control, the preventative and control measures for ASF involving both provincial officials and farming communities would considerably minimise the unnecessary spread of ASF. Stringent enforcement of current control measures and animal disease regulations could limit unexpected outbreaks outside the ASF control zone.

There is a need for conducting more research using contemporary diagnostics methods. This would include research in the use of diagnostic methods for screening of porcine blood samples such as the use of an anti – tick ELISA test for detecting specific exposure of pigs to *O. moubata* (Diaz-Martin *et al,* 2011*).* A retrospective study of the disease using the current suid serum bank could be utilised for assessing the presence of virus along the control line.

The only incursion of ASF out of the control zone is due to illegal movement of animals. There is a need to study the socio-economic influences and demographics on pig farming practices in the ASF control area and an urgent need for exploring the commodity and value chain of pigs and pork in this area. This could ascertain the role of different suid sub-species in the maintenance of ASFV.

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Annexure 1

Warthog burrow / soft tick sampling form

1. Province: Mpumalanga ☐ Sampling date: ……
Limpopo ☐
North West ☐
Gauteng ☐

2. State vet area:……

3. Name of sampling official: ……..

4. Tel no. of official:……

5. Sampling frame reference number_____

6. GPS coordinates of property as provided on the list: _____

7. Farm name……

7.1 Could you find any burrows on this farm? Yes ☐
No ☐

If no, please state reasons (such as human settlement/no warthogs in the area/farmers destroy warthogs/simply no warthog activity on that farm, etc) and move to next closest farm (alternate farm 1). If yes continue with 12 below.

.....

****

8. Name of alternate farm 1 (if applicable) ……..

8.1 GPS coordinates of alternate farm 1: ……..

8.2 Date sampled:……

8.3 Could you find any burrows on the farm? Yes ☐
No ☐

If no, please state reasons and move to next closest farm (alternate farm 2). If yes continue with 12 below.

.....
****

9. Name of alternate farm 2 (if applicable) ……..

9.1GPS coordinates of alternate farm: ……..

9.2 Date sampled:…….

9.3 Could you find any burrows on the farm? Yes □
No □

If no, please state reasons and move to next closest farm (alternate farm 3). If yes continue with 12 below.

****

10. Name of alternate farm 3 (if applicable) ……..

10.1 GPS coordinates of alternate farm: ……..

10.2 Date sampled:…….

10.3 Could you find any burrows on the farm? Yes □
No □

If no, please state reasons and move to next closest farm (alternate farm 4). If yes continue with 12 below.

****

11. Name of alternate farm 4 (if applicable) ……..

11.1 GPS coordinates of alternate farm: ……..

11.2 Date sampled:…….

11.3 Could you find any burrows on the farm? Yes □
No □

If no, please state reasons and record this area as having no warthog activity and move onto the next sampling point on the list.

.....

FARM INFORMATION

12. Please give an estimate of the total number of burrows on the farm

0 ☐ 1 - 5 ☐ 6 - 10 ☐ 11 - 15 ☐ more than 15 ☐

13. Main Farming activities:

Crops ☐
Livestock (other than pigs) ☐
Wildlife ☐
Poultry
Intensive ☐
Extensive ☐
Pigs
Intensive ☐
Extensive ☐

Other (specify)……..

14. Tick control

Dipping ☐
Other ☐
None ☐

15. Have you seen warthogs/bushpigs on this farm Yes ☐
No ☐

16. Do they only live in warthog burrows? Yes ☐
No ☐

16.1 If no, specify where else they live?.....

……

17. Have you seen warthogs/bushpigs on the surrounding farms? Yes ☐
No ☐

18. Does the farmer believe there is increasing numbers of warthogs/bushpigs?
Yes ☐
No ☐

18.1 If yes, can he/she give reasons for the apparent increase?

……

……

19. How many warthogs does the farmer think he/she has on the farm?……

20. Do they come into contact with domestic pigs? Yes ☐
No ☐

INDIVIDUAL WARTHOG BURROW INFORMATION

21. GPS coordinates of **burrow 1**:……

21.1. Describe the habitat in which the burrow was found

Open veld ☐
Bushveld ☐
Riverine/wetlands ☐
Cultivated lands ☐
Mountains ☐
Other (please describe)

……..

21.2. Describe the nature of the soil where the burrow is found:

Sandy ☐
Rocky ☐
Muddy ☐
Clay ☐

21.3. Does the burrow appear to be in use?

Active ☐
Inactive ☐

21.4. Were any soft ticks present? Yes ☐
No ☐

21.5. How many ticks were present? Many (>20) ☐
Few (5 - 20) ☐
Very few (<5) ☐

22 GPS coordinates of **burrow 2**:……

22.1. Describe the habitat in which the burrow was found

Open veld ☐
Bushveld ☐
Riverine/wetlands ☐
Cultivated lands ☐
Mountains ☐
Other (please describe)

……..

22.2. Describe the nature of the soil where the burrow is found:

Sandy ☐
Rocky ☐
Muddy ☐
Clay ☐

22.3. Does the burrow appear to be in use?

Active ☐
Inactive ☐

22.4. Were any soft ticks present? Yes ☐
No ☐

22.5. How many ticks were present? Many (>20) ☐
Few (5 - 20) ☐
Very few (<5) ☐

23. GPS coordinates of **burrow 3:**……

23.1. Describe the habitat in which the burrow was found

Open veld	☐
Bushveld	☐
Riverine/wetlands	☐
Cultivated lands	☐
Mountains	☐
Other (please describe)	

……..

23.2. Describe the nature of the soil where the burrow is found:

Sandy	☐
Rocky	☐
Muddy	☐
Clay	☐

23.3. Does the burrow appear to be in use?

Active	☐
Inactive	☐

23.4. Were any soft ticks present?

Yes	☐
No	☐

23.5. How many ticks were present?

Many (>20)	☐
Few (5 - 20)	☐
Very few (<5)	☐

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24. Comments or other facts that may be important to the study:

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Annexure 2

ASFV Control Zone Surveillance

Summary of the objectives and benefits of the project

The primary objectives of the project are to:

(1) To determine the prevalence of warthogs within 20 km north and south of ASF control line.
(2) To determine the proportion of farms within the study area that have *Ornithodoros moubata* ticks present in the warthog burrows.
(3) To determine the prevalence of ASFV in *Ornithodoros moubata ticks* in this area.

Benefits

The research is aimed at evaluating the extent of presence of the warthogs and the *Ornithodoros moubata* ticks infected with ASFV north and south of current control boundaries.

The data generated will be used to evaluate the relevance of the current ASF control boundaries and could assist with establishing safer pig farming practices with specific reference to small scale farmers.

The epidemiological data collected on wild suid movements could assist in a better understanding of the epidemiology of ASF and also assist in determining the control boundaries.

The confirmation of the relevance of the boundaries could have an impact on the agricultural economy by convincing trade partners that our pork industry does not pose a significant threat to other parts of the world.

The rural pork keepers along the ASF control zone of Mpumalanga, Limpopo, Gauteng, North West and KwaZulu-Natal will benefit as the Provincial Directorate of Veterinary Services (PDVS) can advise them whether they should take extra precaution to ensure the survival of their pigs.

The PDVS will benefit from the knowledge that safe pig farming is ensured with a better understanding of the possible distribution of viral hosts that could potentially be the source of outbreaks.

The Department of Agriculture (DoA), Directorate of Animal Health (DAH), will benefit as ASF is a notifiable disease to the World Animal Health Organisation (OIE) and widespread outbreaks of the disease could have economic implications as far as control measures, such as destruction of animals and carcasses, compensation etc. are concerned.

The commercial pork industry will benefit as widespread outbreaks of ASF could lead to export bans and negatively affect the economy.

Contact Information

Project coordinator

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Principle Investigators and Project Supervisors

Dr Wilna Vosloo: Onderstepoort Veterinary Institute

Prof. Bruce Gummow: University of Pretoria

Sample Cataloging and Diagnostics

Dr Livio Heath: Onderstepoort Veterinary Institute

Tel. no: 012 529 9593, Fax no. 012 529 9595

Sample Collection

a. SAMPLING EQUIPMENT PROVIDED

A Sampling Kit will be supplied to each of the teams. The following equipment will include the following equipment:

- Specimen containers
- Small spade
- Forceps
- Scissors
- Black plastic sheets
- Tailor made long-handled shovels with spikes

b. Sampling Procedure

1. A list of farms selected in each study area is included in the sampling manual and have been sent to the regional PDVS offices.
2. It is the responsibility of the team leader to arrange farm visits with the owner/manager.
3. Complete the first page of the sample collection form for each of the farms visited by answering as many of the questions as possible.
4. Locate suitable warthog burrows within the farm boundaries. If possible note the GPS coordinates of the each borrow sampled.
5. Spread the black plastic sheet on reasonable levelled ground next to the burrow in direct sunlight.
6. Using the spade collect some soil from the burrow by scraping the roof, sides and bottom of the burrow.
7. Spread the soil you have collected onto the plastic sheet. If ticks are present they should start moving about after a few minutes.
8. Collect some soil from the burrow using the specimen containers and place the tick in the container.
9. Punch a few small holes in the lid of the specimen container to allow air to move freely between the inside and outside of the container.
10. Mark the specimen container clearly by including the following information:

    Province

PDVS district

Farm name or code

Burrow number

Date

11. At least three burrows should be sampled in each farm.
12. No more than 45 minutes should be spent searching for ticks in a single burrow. Do not spend more than the allocated time on burrows in which ticks are abundant.
13. If the necessary equipment is available to the sampling team, ticks can be collected by sieving. This should however be noted on the sampling form.
14. Please complete the relevant sections of the sampling form for each of the burrows sampled.
15. All sampled burrows should be recorded irrespective whether ticks were found or not.
16. The specimen containers should be stored in cool and dry environment at the State Veterinary office before forwarding it to the Onderstepoort Veterinary Institute.

c. DEVELOPED FARMING AREAS

Some of the farming areas have been developed into residential areas. In such cases farms adjacent to the intended farm can be selected as alternative study areas. Farms on either side of the intended farm may be used.

Farms that are not listed in the original sampling framework, but are known to have either warthogs and/or O. moubata tick present and fall within the grater study area can be added to the list.

Please include full details of alternative/additional farmers and specify on the sampling form whether the farm is replacing an original selected farm or is an additional farm sampled.

ADDRESS FOR DELIVERING OF SAMPLES

Please mark the shipments for attention Dr L Heath and send it to:

Trans-Boundary Animal Diseases Programme

Onderstepoort Veterinary Institute

Onderstepoort

0110

South Africa

Samples should be sent to the Onderstepoort Veterinary Institute within one (1) month of being collected.

LIST OF FARMS TO BE SAMPLED IN LIMPOPO

Bela-Bela District] (ASF controlled area)

FARM_NAME	PORTION	EAST	SOUTH
HANOVER	00001	28.59	-24.94
WITFONTEIN	00000	28.10	-24.74
RIETSPRUIT	00003	28.00	-24.84
BERLIN	00000	28.57	-24.89
ARNOT	00001	28.17	-24.78
ELANDSFONTEIN	00027	28.12	-24.68
BUFFELSPRUIT	00018	28.19	-24.81
LISBON	00002	28.56	-24.88
DROOGEKLOOF	00081	28.12	-24.83
KWARRIEHOEK	00000	27.86	-24.71
SCHRIKKLOOF	00004	28.17	-24.75
VAALWAL	00000	27.96	-24.76
CYFERFONTEIN	00009	28.04	-24.80
VINGERKRAAL	00002	27.96	-24.72

BUFFELSPRUIT	00003	28.20	-24.83
DROOGELAAGTE	00010	28.11	-24.89
MALMESBURY	00002	28.54	-24.88
DIEPDRIFT	00004	28.06	-24.69

Limpopo (ASF controlled area)

FARM NAME	PORTION	SOUTH	EAST
KROMDRAAI	00104	-24.9615	27.8391
VYGEBOOMSPOORT	00005	-24.7994	28.4116
BYZONDERHEID	00045	-24.5904	29.0852
STEILPOORT	00003	-24.8504	28.7207
RIETSPRUIT	00037	-24.6552	28.4191
RUIMTEPLAATS	00019	-24.5855	29.0477
GROENFONTEIN	00110	-24.3508	28.0495
DOORNSTOCK	00001	-24.6891	29.0561
DOORNKOP	00011	-24.4883	28.0123
RHENOSTERPOORT	00008	-24.6416	28.1565
RIETFONTEIN	0000R	-24.4676	28.6029
ROODE KOP	00003	-24.6339	29.1813
ELANDSPOORT	0000R	-24.6689	28.3709
BYZONDER	00009	-24.8456	28.7454
KROMDRAAI	00179	-24.9539	27.8747
TWEEFONTEIN	00032	-24.4377	28.0337
KROMDRAAI	00037	-24.9751	27.8542
VYGEBOOMSPOORT	00014	-24.7428	28.4080
MORGENZON	00005	-24.8688	27.8288
TOBIAS ZYN LOOP	00042	-24.4661	28.7701
KROMDRAAI	00151	-24.9474	27.8436
DOORNRAND	0000R	-24.4154	29.2215
WELTEVREDEN	00018	-24.5755	28.0155

DOORNFONTEIN	00046	-24.5525	28.2990
MIDDELFONTEIN	00021	-24.6010	28.2064

Limpopo (south of the ASF controlled zone)

FARM NAME	PORTION	EAST	SOUTH
TWEEFONTEIN	00062	28.39	-24.88
DOORNPUT	00009	28.48	-24.88
ZOETDOORNLAAGTE	00011	28.47	-25.06
ROODEPOORT	00116	28.25	-24.89
LANGKUIL	00030	28.25	-25.01
BUISKOP	00048	28.33	-24.85
ROODEKUIL	00110	28.35	-24.98
NOODHULP	00025	28.26	-24.91
HETBAD	00046	28.28	-24.87
THORNESS	00001	28.54	-24.93
BLAAUWBOSCHKUIL	00054	28.29	-25.07
WELGEGUND	00018	28.27	-25.04
VLAKLAAGTE	00012	28.39	-25.01
BOSPOORT	00012	28.29	-24.86
KALKBULT	00002	28.59	-25.04