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Understanding African swine fever outbreaks in domestic pigs in a sylvatic endemic area: The case of the South African controlled area between 1977–2017

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Abstract

South Africa declared a controlled area for African swine fever (ASF) in 1935, consisting of the northern parts of Limpopo, Mpumalanga, North West and Kwa-Zulu Natal Provinces. The area was delineated based on the endemic presence of the sylvatic cycle of ASF, involving warthogs and argasid ticks. Occasionally, spillover occurs from the sylvatic cycle to domestic pigs, causing ASF outbreaks. In the period 1977 to 2017, 59 outbreaks of ASF were reported in domestic pigs within the ASF controlled area of South Africa. During these outbreaks, at least 4,031 domestic pigs either died or were culled. Season did not affect the number of reported ASF outbreaks, but the number of reported outbreaks in this area per year was thought to be slowly increasing, although not statistically significant. Outbreaks occurred predominantly in Limpopo province (93%) and were mostly due to contact (or suspected contact) with warthog or warthog carcasses. Clustering analysis of outbreaks found that the local municipalities of Ramotshere Moiloa, Lephalale and Thabazimbi had the highest relative risk for outbreaks. In 32 of the 59 outbreaks, the genotype of the ASF virus (ASFV) involved could be determined. Phylogenetic analysis of ASFVs detected in domestic pigs during the study period revealed that p72 genotypes I, III, IV, VII, VIII, XIX, XX, XXI and XXII had been involved in causing outbreaks within the ASF controlled area. No outbreaks were reported in the Kwa-Zulu Natal part of the controlled area during this period. South Africa is unlikely to eradicate all sources of ASFV as spillover from the sylvatic cycle in the controlled area continued to occur, but with the implementation of appropriate biosecurity measures pigs can be successfully farmed despite the presence of ASFV in African wild suids and soft ticks.

KEYWORDS

African swine fever, biosecurity, compartmentalization, pigs, sylvatic cycle

1 | INTRODUCTION

African swine fever (ASF) constitutes a global threat to food security, particularly in areas where pigs are predominantly kept as a protein source due to limited economic resources (Costard et al., 2009). Pigs in these areas are often kept free-range and left to scavenge for food, as their owners cannot afford commercial feed (Edelsten & Chinombo, 1995; Kebkiba, Antipas, &

Youssouf, 2015). The lack of confinement and basic biosecurity practices increases the risk of these pigs contracting the disease, resulting in subsistence and small-scale pig farmers being disproportionately affected by ASF.

African swine fever is caused by a double-stranded DNA virus, classified as the only member of the family *Asfarviridae*, in the genus *Asfivirus* (Alonso et al., 2018). To date, twenty-four genotypes of the virus have been described that are used to determine the genetic relationship between outbreaks (Achenbach et al., 2016; Penrith, 2009, 2013; Quembo, Jori, Vosloo, & Heath, 2018; Van Heerden, Malan, Gadaga, & Spargo, 2017). Genotypes are defined based on differences across the C-terminal region of the gene that encodes the p72 major capsid protein (Bastos et al., 2003). The genotyping and molecular characterization of the ASF viruses can give significant insights into the spread of the disease, since it can be used to determine whether outbreaks were caused by a single introduction or were the result of multiple independent introductions (Boshoff, Bastos, Gerber, & Vosloo, 2007; Rowlands et al., 2008).

In South Africa, ASF has historically been associated with the presence of the sylvatic epidemiological cycle, involving warthogs and soft ticks (Jori et al., 2013; Penrith, Bastos, Etter, & Beltrán-Alcrudo, 2019). The sylvatic cycle is characterized by the transmission of the African swine fever virus (ASFV) between the common warthog (*Phacochoerus africanus*) and soft ticks of the family Argasidae (*Ornithodoros* spp.; Chenais, Ståhl, Guberti, & Depner, 2018; Jori et al., 2013; Penrith, Vosloo, Jori, & Bastos, 2013; Sánchez-Vizcaíno, Mur, Bastos, & Penrith, 2015; Wilkinson, 1986). The disease is generally thought to have evolved in this cycle, with the ticks inhabiting the warthog burrows and transmitting the virus to warthogs during feeding (Plowright, Parker, & Peirce, 1969a; Thomson, 1985). Warthog piglets develop viraemia for a short period following infection, without showing clinical signs of disease. The virus can be transmitted to naive ticks during the viraemic phase of infection, thereby cycling the virus between the invertebrate and vertebrate host (Thomson, 1985). Transmission of ASFV amongst ticks of the *Ornithodoros moubata* complex occurs sexually, transovarially and transstadially (Plowright, Perry, & Greig, 1974; Plowright, Perry, Peirce, & Parker, 1970; Thomson, 1985). The efficiency of these ticks as biological vectors of ASF, however, differs according to the strain of ASFV (Plowright et al., 1970).

It has been reported that a Palaearctic species of *Ornithodoros* tick, *O. erraticus*, may remain infected with ASFV for up to eight years (Boinas, Wilson, Hutchings, Martins, & Dixon, 2011). Some ticks may even remain infected for the duration of their lifespan, depending on the level of virus–host adaptation and the viral strain involved (Kleiboeker & Scoles, 2001). *Ornithodoros* ticks are able to survive without feeding for at least five years, which suggests that infected ticks could be responsible for the periodic re-emergence of ASF (Boinas et al., 2011). Experimental infection of different *Ornithodoros* species has shown that the maintenance of ASFV is species-dependent, and even though the virus can replicate in most members of the genus, it does not establish persistent infections in all species (Plowright et al., 1970). The ASFV remains genetically stable in ticks and does not become attenuated, despite long-term persistence in host ticks (Plowright et al., 1970).

It has been shown that warthogs, even when sero-positive, did not transmit ASFV to other warthogs or to domestic pigs by direct contact. Transmission is accomplished by an intermediate tick vector (De Kock, Robinson, & Keppel, 1940; DeTray, 1957; Montgomery, 1921; Plowright, Parker, & Peirce, 1969b; Sánchez-Botija, 1963). Plowright et al. (1969b) showed that ASFV was not easily transmitted to pigs by feeding of warthog tissues, as the virus was mainly localized in the lymph nodes of warthogs. Thomson, Gainaru, and Van Dellen (1980) found that when lymph nodes from experimentally infected warthogs were minced and fed to pigs, they could infect domestic pigs. However, due to the consistency, size and encapsulation of lymph nodes, it is most likely that should they be ingested by pigs, they would be swallowed whole, which would not be favourable for the absorption of the virus. This implies that adult warthogs most likely spread ASFV by carrying infectious ticks on their hides (Costard et al., 2009; Plowright et al., 1969b).

Ornithodoros spp. ticks are not usually found on warthogs, as they tend to drop off in the burrows after their blood meal. However, tick nymphs (*Ornithodoros* spp.) have been found on warthogs outside of burrows in Namibia and South Africa (Boomker, Horak, Booyse, & Meyer, 1991; Horak, Biggs, Hanssen, & Hanssen, 1983; Horak, Boomker, De Vos, & Potgieter, 1988). This suggests that tick nymphs could play a role in the spread of ASF from warthogs to domestic pigs (Horak et al., 1983, 1988). Other blood-sucking invertebrates, such as lice, mites and ixodid ticks, do not transmit ASFV, with the exception of *Stomoxys calcitrans*, which could possibly mechanically transmit the virus for up to 24 hr after feeding on viraemic pigs (Mellor, Kitching, & Wilkinson, 1987).

Reports of ASF in South Africa date back to 1928. It was first reported in the north-eastern part of South Africa (now Limpopo province) that was part of the former Northern Transvaal (De Kock et al., 1940; Magadla, Vosloo, Heath, & Gummow, 2016; Steyn, 1928). Measures to control ASF were legislated in 1935, when South Africa declared a controlled area for ASF. This designated area consists of the northern parts of the Limpopo, Mpumalanga, North West and Kwa-Zulu Natal provinces (Figure 1) and was defined based on the presence of the sylvatic cycle in these areas. In accordance with the Animal Diseases Act, 1984 (Act 35 of 1984), pigs raised in this area need to be kept in pig-proof enclosures and the movements of pigs, warthogs and their products from these areas are subject to obtaining permits and complying with conditions stated in the permits (Magadla et al., 2016; Penrith, 2013).

Earlier studies described the epidemiology of ASF in the ASF controlled area of South Africa up to 1974. Ten ASF outbreaks were reported between 1935 and 1938 in various districts of the Northern Transvaal (now Limpopo province) and were either associated with warthog contact or swill feeding (DAFF Annual Reports, 2018a, 2018b; Pini & Hurter, 1975). From 1939, no ASF outbreaks were reported until 1951 when three outbreaks were reported in the Pietersburg, Soutpansberg and Letaba districts. Another 17 outbreaks were reported in the former Northern and Eastern Transvaal

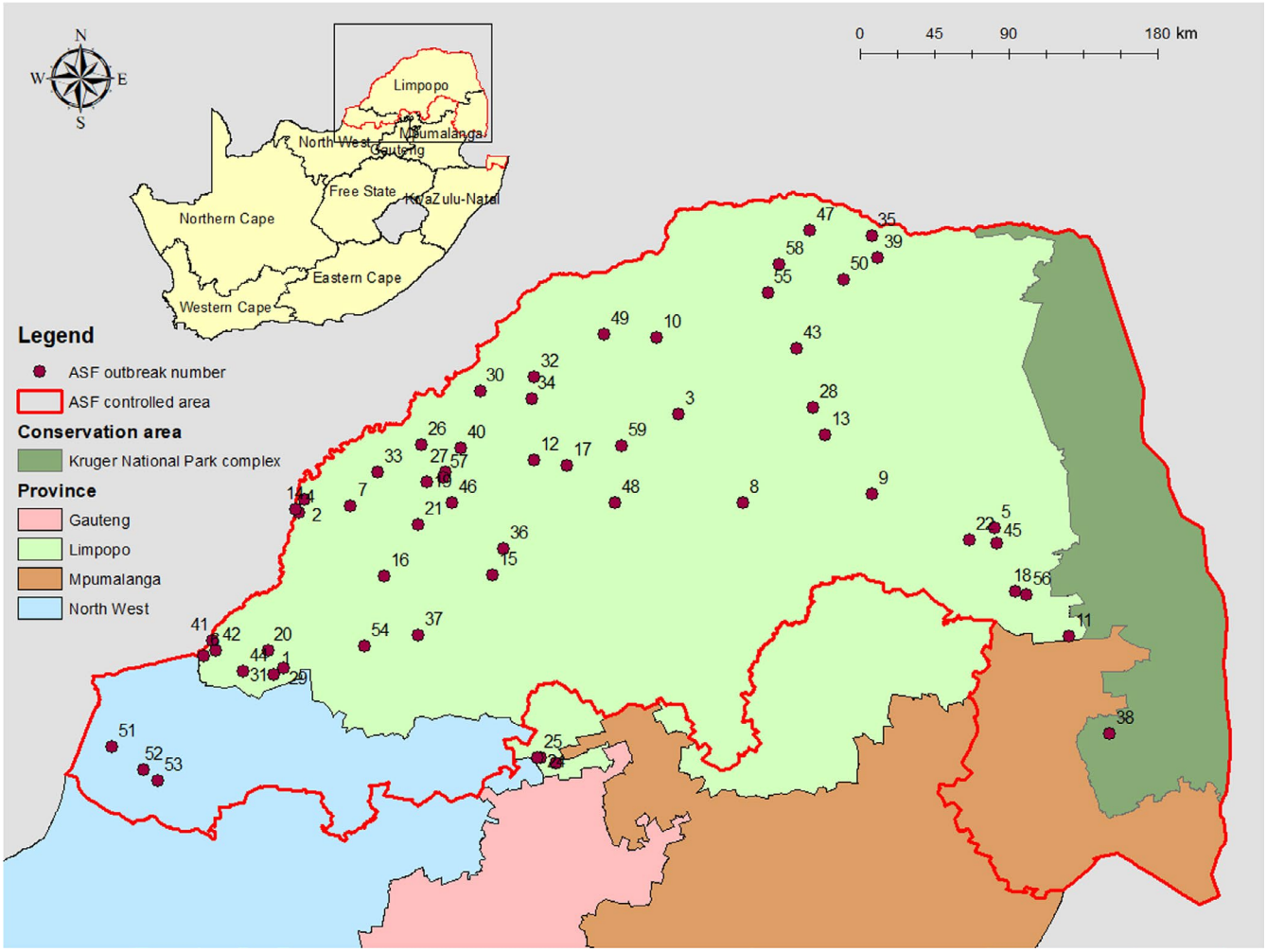

FIGURE 1 Spatial distribution of ASF outbreaks in domestic pigs from 1977 to 2017 in the South African controlled area

(now parts of Limpopo and Mpumalanga provinces) between 1953 and 1962, after which there were no further ASF reports until 1973 (DAFF Annual Reports, 2018a, 2018b; Pini & Hurter, 1975). Between 1973 and 1974, 18 outbreaks were reported with six focus areas. The first was in the Letaba District, which was ascribed to a warthog carcass that was brought to the farm. The second was on a farm about 35 km from the first focus, with no known origin. The third outbreak focus was in the Pietersburg District, with no known origin of infection, which spread to various farms in the vicinity. The fourth focus was in the Letaba District and was attributed to the movement of infectious pig products. The fifth focus was in the White River District, attributed to contact with warthog, and the last focus was in the Thabazimbi District, where ASF-infected ticks were discovered. During these outbreaks, almost 4,000 pigs either died or were culled due to ASF (DAFF Annual Reports, 2018a, 2018b; Pini & Hurter, 1975).

This study examines the occurrence of ASF in domestic pigs in the controlled area of South Africa for the period 1977 to 2017. For this specific period, there is little published information available. From 1977, the Department of Agriculture, Forestry and Fisheries started to file individual detailed records on outbreaks, including GPS coordinates. These records were analysed in this study to determine the frequency of spillover of ASF from the sylvatic cycle to domestic pigs and the pattern of spread once an outbreak occurred in domestic pigs, whether there was clustering of outbreaks as well as the spatial relative risk of infected local municipalities in this ASF controlled area where ASFV remains endemic in African wildlife. Factors that could possibly have influenced the occurrence of transmission from wildlife to domestic pigs were noted as well as what factors assisted farms in preventing outbreaks. The molecular epidemiology of the outbreaks is described and demonstrates the genetic diversity of ASFV recovered from domestic pigs in the ASF controlled area of South Africa. Biosecurity measures that may assist in disease prevention and control are discussed.

2 | MATERIALS AND METHODS

2.1 | Data collection

The ASF controlled area, as described in the Regulations of the Animal Diseases Act, 1984 (Act 35 of 1984), was used as reference

for the study area (Figure 1). Primary epidemiological information was collated from official veterinary disease reports submitted for each of the outbreaks by the provincial state officials responsible for the areas in which the events occurred. This was supplemented by utilizing the Department of Agriculture, Forestry and Fisheries (DAFF) annual reports, the DAFF disease database (DAFF Disease Database, 2018), OIE reports and personal communication with officials who had been involved in the outbreak control and eradication.

2.2 | Temporal analysis

For the temporal analysis, a linear regression model was used with the formula:

$$\hat{y} = a + bx$$

where $\hat{y}$ = expected outcome (no. of outbreaks); a = y-intercept; b = slope and x = independent variable (year).

This equation was used with the data collected on the number of ASF outbreaks reported in domestic pigs in the area per year, for the time period 1977–2017. The slope is calculated to determine the trend in frequency of ASF occurrence in domestic pigs in this area. A positive slope would indicate that there is a positive trend (the number of ASF outbreaks in domestic pigs in this area per year is increasing), while a negative slope would indicate a decreasing number of outbreaks over time. Microsoft Excel 2010® was used in the performance of the calculations. The weather data were summarized into four seasonal groups: summer (December to February), autumn (March to May), spring (September to November) and winter (June to August). The data were tested using a general linear regression model with a Poisson distribution on R software (R Core Team, 2013).

2.3 | Spatial analysis

The geographical distribution of outbreaks was mapped using ArcGIS® software by Esri.[1] The distribution was analysed for hierarchical clustering using a single linkage method and adopting a 'friends of friends' clustering strategy using R software. The cluster tree was cut using a height (threshold distance) of 60 km. This represented the maximal distance between pairs of elements from different clusters to agglomerate these clusters. We assumed a constant circulation in the sylvatic cycle over time based on the absence of major landscape restructuring in the area. A spatial relative risk (RR) of dead pigs linked to the ASF outbreak was calculated for each local municipality that was affected by outbreaks. It was decided not to include local municipalities where no outbreak was reported despite the presence of domestic pigs in these municipalities. The objective was to measure the risk relative to the population of pigs of the infected local municipalities rather than to produce a definitive risk map for ASF outbreaks. This relative risk was defined as the ratio between the observed number of dead pigs recorded over the period studied and the expected number of dead pigs calculated related to the density of pigs, assuming that the density of pigs would have been homogeneously distributed over the whole area affected by ASF outbreaks (all the local municipalities affected).

The following formula was used to calculate this RR:

$$RR_{lm=i} = \frac{\sum_{lm=i} DP_{lm} \times \sum_{lm=1}^{n} PC_{lm}}{\sum_{lm=1}^{n} DP_{lm} \times PC_{lm=i}}$$

where $\sum_{lm=i} DP_{lm}$ represents the total number of dead pigs for the local municipality i; $\sum_{lm=1}^{n} DP_{lm} \times PC_{lm=i}$ represents the sum of the total number of dead pigs within all the local municipalities that experienced ASF outbreaks; $PC_{lm=i}$ is the pig census for the local municipality i (StatSA, 2016); $\sum_{lm=1}^{n} PC_{lm}$ is the total population of pigs within the local municipalities (StatSA, 2016).

The clusters and relative risk were spatially represented using QGIS 3.4 software.[2]

2.4 | Virus propagation, DNA amplification and phylogenetic analysis

For confirmation of ASF, state veterinarians had submitted samples on suspicion of ASF to the Agricultural Research Council-Onderstepoort Veterinary Research (ARC-OVR) Transboundary Animal Diseases (TAD) Laboratory for testing. Testing performed prior to 1995 involved various techniques including inoculation of live pigs, histopathology, serology, fluorescent antibody test, immunoelectroosmophoresis test and virus isolation using primary pig macrophage cell lines. Since 1995, all samples were tested using conventional PCR based on Bastos et al. (2003), (DAFF Annual reports, 2018a, 2018b; Original laboratory reports (TAD)—DAFF archives).

Positive samples received by ARC-OVR TAD were passaged several times on pig bone marrow or blood macrophages as described by Malmquist and Hay (1960), from which the viruses were then isolated where possible. DNA was extracted from 200 µl of cell culture sample using the High Pure PCR Template preparation kit (Roche) and used as a template for the amplification of the B646L gene region. Epidemiological primers p72-D and p72-U designed to amplify the 3′ end of the B646L gene were used for p72 genotyping according to the methodology described by Bastos et al. (2003). The nucleotide sequences were determined by automated cycle sequencing at Inqaba Biotec. The sequences (±400 bp in length) were analysed using Sequencer 5.2.4 sequence analysis software (Gene Codes Corporation), where after they were aligned using Bioedit.

Phylogenetic analyses were conducted, and neighbour-joining (NJ) p72 trees were constructed using Mega version 7 (Kumar, Stecher, & Tamura, 2016) employing the p-distance nucleotide substitution model. Bootstrap confidence values were calculated on 10,000 replicates. Bayesian inference was performed using BEAST 1.0.4 software (Suchard et al., 2018) with default settings, and the first 1,000 trees discarded as "burn-in."

3 | RESULTS

In total, there were 59 reported outbreaks of ASF in pigs (including farmed European wild boar, which are susceptible to ASF, and excluding African wild suids, which are resistant to the pathogenic effects of ASF) in the controlled area of South Africa for the period 1977–2017, with at least 4,031 pigs affected. The details of these outbreaks are compiled in Table 1, and information per year on these outbreaks is provided. The reported outbreaks are displayed in Figure 1 with the chronological number of the outbreak according to this study indicated.

Out of the 59 outbreaks reported 1977–2017 in the ASF controlled area, 55 were in Limpopo (93%), three linked outbreaks in North West (5%) and only one outbreak in Mpumalanga (2%). Fifteen local municipalities within the ASF controlled area reported outbreaks in domestic pigs in the study period (Table 2 and Figure S1). Most of these outbreaks occurred in the north-western part of the controlled area, with the local municipalities of Thabazimbi, Lephalale and Musina most affected, with more than half of the outbreaks occurring within these municipalities. This could, however, be influenced by the fact that a relatively large part of the eastern section of the controlled area is occupied by the Kruger National Park and surrounding game parks, in which no domestic pigs are kept.

The analysis of the geographical distribution revealed five main clusters of outbreaks (Figure 2). Cluster 1 consisted of 36 outbreaks from 1977 to 2017 distributed over five local municipalities. Cluster 2 grouped seven outbreaks in three local municipalities in the east of Limpopo from 1979 to 2017. Cluster 3 grouped ten outbreaks in four local municipalities in the north of the Limpopo from 1985 to 2017. Cluster 4 grouped three outbreaks (represented by a larger dot in Figure 2) in 1996 in the south of Limpopo, close to the border of Gauteng, and these three outbreaks were included in this study even though they were technically just outside of the ASF controlled area, but of a similar epidemiology. Similarly, Cluster 5 grouped three outbreaks that occurred in the North West province in 2014. The temporal distribution of the clusters is shown in Table 3. The relative risk values calculated for each of the municipalities varied from 0.002 to 10 (median = 0.78), with more than 50% of the 15 local municipalities (Figure 2) affected by outbreaks associated with relative risk values lower than 1. Three local municipalities (Ramotshere Moiloa, Lephalale and Thabazimbi) had relative risk values higher than 2 (RR = 10.6, 4.0 and 2.9 respectively). The RR of Ramotshere Moiloa resulted from the fact that despite being the location of only three outbreaks this local municipality had a low pig density.

Analysis of the temporal distribution of outbreaks using the linear regression model suggested a positive trend (b = 0.024), indicating a possible increase in the frequency of outbreaks. This trend was, however, found not to be statistically significant (p-value = .1622). The general linear distribution model showed no statistically significant association with the month during which outbreaks occurred using both the likelihood ratio test and the general linear distribution model (p = .6252). Sixteen outbreaks occurred in summer, 11 in autumn, 18 in winter and 14 in spring. Analysis of the seasonal distribution of the outbreaks using the chi-square method confirmed that there was no seasonality of outbreaks (p = .2).

ASF viruses were isolated for 32 of the 59 outbreaks reported from 1977 to 2017. These viruses were genetically characterized and classified into corresponding genotypes based on partial sequencing of the p72 gene, and their origin locations are shown in Figure 3. Phylogenetic trees with similar topologies and support values were recovered with NJ and Bayesian inference (Figure 4). The p72 gene sequences generated in this study were aligned with sequences from previous studies describing the phylogeny of ASFV in South Africa resulting in a final dataset of 62 taxa. Viruses from domestic pigs clustered within nine genotypes, all of which were previously described (Bastos et al., 2003; Boshoff et al., 2007; Lubisi, Bastos, Dwarka, & Vosloo, 2005).

Genotype I included a single isolate (Spec/43) recovered from domestic pigs in 1985. Four genetically similar viruses were isolated from ticks collected in Kruger National Park two years prior to the outbreak in Lephalale. A fifth virus (MK Mkuzi) isolated from ticks collected within the Mkuze National Park also clustered within Genotype I. Viruses clustering within Genotype III were isolated from domestic pigs in Lephalale in 1993, 1995, 2002 and 2011, and Makhado in 2017. The sequences of these viruses were identical despite having caused temporally unrelated outbreaks over a 14-year period. Several viruses with the same sequence were also found in ticks collected from various locations in Limpopo province. Viruses isolated from ticks with C-terminal portions of the p72 gene identical to viruses from domestic pigs were also identified in Genotypes VII, VIII, XIX, XX, XXI and XXII (Table 4).

Genetic similarities between viruses recovered from outbreaks in domestic pigs and the sylvatic cycle were not only restricted to ticks. Viruses isolated from warthogs in Genotype VI and VII shared 100% identity with outbreak viruses. Genotype XXI included four genetically diverse viruses from domestic pigs isolated between 1985 and 2008. This genotype also included a single virus isolated from a warthog in 2003, which shared 100% identity with a virus from a European wild boar that was translocated to the Kruger National Park in 2008, as well as a virus isolated from a tick collected in the Kruger National Park in 1993 (Table 4). The genetic similarity between viruses from domestic pigs, warthogs and tick provides strong evidence that the sylvatic cycle continues to be the predominant source of outbreak of the disease in domestic pigs raised within the ASF control area of South Africa.

4 | DISCUSSION

This study describes the frequency and distribution of ASF outbreak within the control area of South Africa from 1977 to 2017. The data suggest that the number of outbreaks may be slowly increasing, but the increase was not found to be statistically significant. They further suggest that the likelihood of outbreaks is not influenced by seasons.

TABLE 1 Details on the year, location, number of pigs affected, control measures applied, suspected origin and phylogeny of reported ASF outbreaks in domestic pigs within the ASF controlled area of South Africa 1977–2017

Year	Outbreaks/ year	Outbreak no. in study	Local municipality	State veterinary area	Total no. of pigs affected	Pigs affected	Control measures[a]	Suspected origin	Phylogeny	Genbank accession number
1977	1	1	Thabazimbi	Thabazimbi	12	9 dead 3 culled	Pigs were culled with compensation and the pens disinfected by burning with peanut husks (as pens had ground floors). Property was quarantined for 3 months	Piglets escaped pens and came into contact with warthog carcasses	N/S[b]	
1978	2	2	Thabazimbi	Lephalale	4	All died	Property disinfected and quarantined for 6 months	Contact with warthog carcasses	N/S	
		3	Blouberg	Aganang	34	4 died	N/A[c]	N/A	N/S	
1979	2	4	Thabazimbi	Lephalale	9	8 dead 1 culled	Culling with compensation and destruction of carcasses	Reported as unknown	N/S	
		5	Ba-Phalaborwa	Phalaborwa	26	23 died 3 culled	Culling and carcasses destroyed	Piglets escaped pens. Warthogs were found on farm as well as warthog carcasses	N/S	
1981	1	6	Thabazimbi	Thabazimbi	15	8 dead 7 culled	Culling with compensation, carcasses disposed of by deep burial, disinfection and quarantine of property for 2 months	Piglets escaped pens and had contact with warthog carcasses	N/S	
1985	3	7	Lephalale	Lephalale	14	10 died 4 culled	Culling with compensation and carcasses destroyed	Suspected contact with ticks	Genotype I (Spec/43/1985) (This paper)	KC519625
		8	Polokwane	Polokwane	42	28 dead 14 culled	Culling and carcasses destroyed.	Pigs were kept free-roaming	Genotype XXI (Spec53) (Boshoff et al., 2007)	DQ250111
		9	Greater Tzaneen	Tzaneen	47	28 died 19 culled	Culling	N/A	N/S	
1987	1	10	Blouberg	Blouberg	20	16 died 4 culled	Culling with compensation, carcasses burnt	Contact with warthog carcass	Genotype XIX (Spec 120 & Spec 125) (Boshoff et al., 2007)	AF302812; DQ250112
1988	1	11	Maruleng	Maruleng	5	4 died 1 culled	Culling with compensation	N/A	N/S	
1989	1	12	Mogalakwena	Mokopane	7	5 died 2 culled	Culling with compensation, carcasses burnt and property quarantined for 3 months	Contact with warthog carcasses	N/S	
1992	2	13	Molemole	Polokwane	30	16 died 14 culled	Culling with compensation, carcasses burnt and property quarantined for 3 months	Contact with warthog carcass	Genotype XXII (Spec245) (Boshoff et al., 2007)	DQ250117
		14	Thabazimbi	Lephalale	24	20 died 4 culled	Culled pigs with carcasses burnt	Contact with warthog carcasses	Genotype XIX (Spec251) (Boshoff et al., 2007)	DQ250118

(Continues)

TABLE 1 (Continued)

Year	Outbreaks/ year	Outbreak no. in study	Local municipality	State veterinary area	Total no. of pigs affected	Pigs affected	Control measures[a]	Suspected origin	Phylogeny	Genbank accession number
1993	2	15	Modimolle	Waterberg	41	8 died 33 culled	Culling with compensation, carcasses burnt	Contact with warthog	Genotype III (Spec257) (Boshoff et al., 2007)	DQ250120
		16	Thabazimbi	Lephalale	9	3 died 6 culled	Culling with compensation, disinfection and quarantine of property	Free-roaming, contact with warthog, possible tick contact	Genotype VII (Spec260) (Boshoff et al., 2007)	DQ250121
1994	1	17	Mogalakwena	Mokopane	5	5 died	Quarantine of property	Contact with warthog carcasses	N/S	
1995	4	18	Maruleng	Maruleng	10	10 died	Property disinfected and quarantined for 3 months	Contact with warthog	Genotype XX (RSA 1/95) (Boshoff et al., 2007)	DQ250123
		19	Lephalale	Lephalale	45	22 died 23 culled	Culling with carcasses destroyed and property quarantined for 3 months	Contact with warthog	Genotype III (RSA 5/1995) (Boshoff et al., 2007)	DQ250124
		20	Thabazimbi	Thabazimbi	236	20 died 216 culled	Culling with carcasses destroyed and property disinfected and quarantined	Contact with warthog	Genotype VIII (RSA 2/1995) (this paper)	KC519627
		21	Lephalale	Lephalale	5	1 died 4 culled	Culling with carcasses burnt and property disinfected and quarantined	Contact with warthog	Genotype VII (RSA 4/1995) (This paper)	KC662374
1996	4	22	Ba-Phalaborwa	Phalaborwa	10	7 died 3 culled	Culling with carcasses destroyed and property quarantined	Pigs free-roaming with possible contact with warthog	Genotype XXI (RSA 1/96) (Boshoff et al., 2007)	DQ250125
		23	Bela-Bela	Waterberg	89	46 died 43 culled	Three adjacent properties affected with pigs culled, carcasses destroyed and property quarantined[d]	Properties borrow boar for mating	Genotype XIX (RSA 2/1996 & RSA 3/1996) (Boshoff et al., 2007)	DQ250126; DQ250127
		24	Bela-Bela	Waterberg						
		25	Bela-Bela	Waterberg						
1997	3	26	Lephalale	Lephalale	32	8 died 24 culled	Culling with carcasses destroyed and property quarantined	Contact with warthog	N/S	
		27	Lephalale	Lephalale	4	2 died 2 culled	Culling, carcasses burnt and property quarantined	Possible contact with warthog	N/S	
		28	Makhado	Makhado	17	2 died 15 culled	Culling with compensation, carcasses destroyed and property quarantined	Possible contact with warthog	N/S	
1998	2	29	Thabazimbi	Thabazimbi	20	9 died 11 removed	Property depopulated and quarantined for 3 months	Possible contact with warthog	N/S	
		30	Lephalale	Lephalale	27	20 died 7 culled	Culling and quarantine	N/A	Genotype VII (RSA/1/1998) (Bastos et al., 2003)	AF302818
2001	2	31	Thabazimbi	Thabazimbi	30	27 died 3 culled	Culling with carcasses destroyed and property quarantined	Possible contact with warthog	Genotype VII (RSA 2/2001) (This paper)	JX403671
		32	Lephalale	Lephalale	24	All died	Property quarantined	Pigs were free-roaming with contact with warthog	N/S	

(Continues)

TABLE 1 (Continued)

Year	Outbreaks/ year	Outbreak no. in study	Local municipality	State veterinary area	Total no. of pigs affected	Pigs affected	Control measures[a]	Suspected origin	Phylogeny	Genbank accession number
2002	3	33	Lephalale	Lephalale	2	1 died 1 culled	Culling and property quarantined for 3 months	Contact with warthog	Genotype XXII (RSA 1/2002) (This paper)	JX403678
		34	Lephalale	Lephalale	10	7 died 3 culled	Culling with carcasses destroyed and property quarantined for 3 months	Contact with warthog possible	Genotype III (RSA 2/2002) (This paper)	JX403679
		35	Musina	Musina	55	20 died 35 culled	Culling with carcasses destroyed and property quarantined for 3 months	N/A	Genotype XXI (RSA 4/2002) (This paper)	JX403683
2003	2	36	Lephalale	Lephalale	40	36 died 4 culled	Culling with carcasses destroyed and property quarantined for 3 months	Pigs were European wild boar that were free-roaming as well as fed swill and had contact with warthog	Genotype VII (RSA 4/2003) (This paper)	JX403657
		37	Thabazimbi	Thabazimbi	6	2 died 4 culled	Culling with carcasses destroyed	N/A	N/S	
2004	2	38	Mbombela	Skukuza	2	1 died 1 culled	Culling with carcasses destroyed	Piglets were confiscated—European wild boar thought to be bush pigs and moved into Mpumalanga game park	Genotype XX (RSA 2/2004) (This paper)	JX403653
		39	Musina	Musina	123	65 died 58 culled	Culling with carcasses burnt and property quarantined	Pigs were free ranging with contact with warthogs	Genotype IV (RSA 3/2004) (This paper)	JX403645
2005	1	40	Lephalale	Lephalale	6	5 died 1 culled	Culling with carcasses destroyed and property quarantined for 3 months	Possible contact with warthog	Genotype XIX (RSA 1/2005) (This paper)	JX403636
2006	2	41	Thabazimbi	Thabazimbi	22	All 22 died	N/A	N/A	N/S	
		42	Thabazimbi	Thabazimbi	16	All 16 died	N/A	N/A	N/S	
2007	2	43	Makhado	Makhado	N/A (at least 1)	All died	N/A	N/A	Genotype III (RSA 1/2007) (This paper)	JX310053
		44	Thabazimbi	Thabazimbi	30	27 died 3 culled	Culling with carcasses destroyed	N/A	N/S	
2008	1	45	Ba-Phalaborwa	Phalaborwa	35	5 died 30 culled	Culling with carcasses destroyed and property quarantined	Pigs were European wild boar that were kept with other wildlife	Genotype XXI (RSA 1/2008) (This paper)	JX310055
2009	2	46	Lephalale	Lephalale	9	All 9 died	N/A	N/A	Genotype VII (RSA 1/2009) (This paper)	JX310045
		47	Musina	Musina	19	4 died 15 culled	Culling with carcasses destroyed	N/A	Genotype VII (RSA 2/2009) (This paper)	JX310044
2010	1	48	Mogalakwena	Mokopane	N/A (at least 1)	N/A	N/A	N/A	Genotype XIX (RSA 2/2010) (This paper)	JX310037

(Continues)

TABLE 1 (Continued)

Year	Outbreaks/ year	Outbreak no. in study	Local municipality	State veterinary area	Total no. of pigs affected	Pigs affected	Control measures[a]	Suspected origin	Phylogeny	Genbank accession number
2011	1	49	Blouberg	Lephalale	13	10 died 3 culled	Culling with carcasses destroyed and property quarantined	Contact with warthog possible	Genotype III (RSA 1/2011) (This paper)	JX294722
2012	1	50	Musina	Musina	15	12 died 3 culled	Culling with carcasses destroyed	N/A	N/S	
2014	3	51	Ramotshere Moiloa	Ramotshere Moiloa	1788	326 died 1,462 culled	Culling with carcasses destroyed and property quarantined	Possible contact with warthog (situated on game farm)	N/S	
		52			50	50 died	N/A	N/A	N/S	
		53			3	3 cases	N/A	N/A	N/S	
2016	1	54	Thabazimbi	Thabazimbi	9	5 died	Farm was quarantined and remaining 4 pigs slaughtered for own consumption	N/A	N/S	
2017	5	55	Makhado	Makhado	577	470 died 107 culled	Culling with carcasses destroyed and property quarantined	Pigs situated on a game farm with warthog contact	Genotype III (RSA 7/2017) (This paper)	MN537825
		56	Maruleng	Maruleng	72	57 died 15 culled	Culling with carcasses destroyed and property quarantined	Possible contact with warthog as the pigs were fed swill and warthogs were shot for workers	N/S	
		57	Lephalale	Lephalale	200	77 dead 123 culled	Culling with carcasses destroyed and property quarantined	Free-roaming pigs, with possible contact with warthog	Genotype XIX (RSA 10/2017) (This paper)	MN537828
		58	Musina	Musina	18	11 died 7 culled	Culling with carcasses destroyed and property quarantined	N/A	Genotype VII (RSA 09/2017) (This paper)	MN537827
		59	Mogalakwena	Mokopane	16	All 16 died	Property quarantined	Slaughtered warthogs on property and hung skins on walls of piggery	Genotype XX (RSA 08/2017) (This paper)	MN537826

[a]Where compensation is not specifically mentioned, compensation was not paid; where quarantine length not specified, the information was not included in the report.

[b]N/S—not sequenced; either no virus could be isolated or sequencing was not possible.

[c]N/A—not available; Information was not included in the report.

[d]Included although technically just outside of the ASF controlled area.

TABLE 2 Details on the number of ASF outbreaks per local municipality and the year of occurrence

Local municipality	Province	No. of outbreaks	%	Year (s)
Thabazimbi	Limpopo	14	23.7	1977, 1978, 1979, 1981, 1992, 1993, 1995, 1998, 2001, 2003, 2006, 2006, 2007, 2016
Lephalale	Limpopo	13	22.0	1985, 1995, 1995, 1997, 1997, 1998, 2001, 2002, 2002, 2003, 2005, 2009, 2017
Musina	Limpopo	5	8.5	2002, 2004, 2009, 2012, 2017
Mogalakwena	Limpopo	4	6.8	1989, 1994, 2010, 2017
Blouberg	Limpopo	3	5.1	1978, 1987, 2011
Ba-Phalaborwa	Limpopo	3	5.1	1979, 1996, 2008
Maruleng	Limpopo	3	5.1	1988, 1995, 2017
Bela-Bela	Limpopo	3	5.1	1996, 1996, 1996
Makhado	Limpopo	3	5.1	1997, 2007, 2017
Ramotshere Moiloa	North West	3	5.1	2014, 2014, 2014
Polokwane	Limpopo	1	1.7	1985
Greater Tzaneen	Limpopo	1	1.7	1985
Molemole	Limpopo	1	1.7	1992
Modimolle/Mookgophong	Limpopo	1	1.7	1993
Mbombela	Mpumalanga	1	1.7	2004

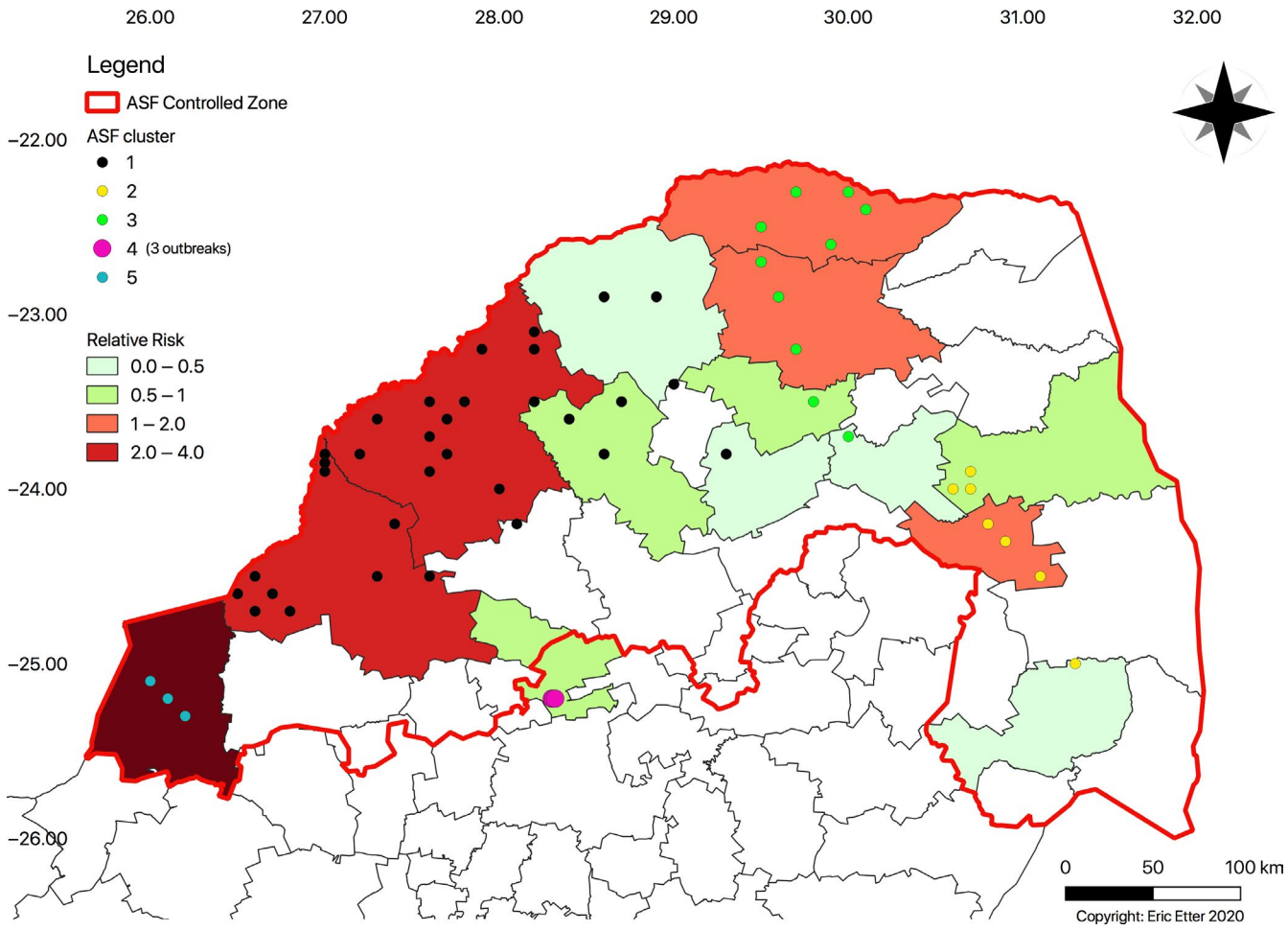

FIGURE 2 Map of municipal relative risk with clustering of ASF outbreaks in domestic pigs from 1977 to 2017 in the South African controlled area

TABLE 3 Temporal distribution of outbreaks amongst clusters

Cluster (number of outbreaks)	Year (number of outbreaks)
Cluster 1 (36)	1977 (1), 1978 (2), 1979 (1), 1981 (1), 1985 (2), 1987 (1), 1989 (1), 1992 (1), 1993 (2), 1994 (1), 1995 (3), 1997(2), 1998 (2), 2001 (2), 2002 (2), 2003 (2), 2005 (1), 2006 (2), 2007 (1), 2008 (1), 2009 (1), 2010 (1), 2011 (1), 2016 (1), 2017 (2)
Cluster 2 (7)	1979 (1), 1988 (1), 1995 (1), 1996 (1), 2004 (1), 2008 (1), 2017 (1)
Cluster 3 (10)	1985 (1), 1992 (1), 1997 (1), 2002 (1), 2004 (1), 2007 (1), 2009 (1), 2012 (1), 2017 (2)
Cluster 4 (3)	1996 (3)
Cluster 5 (3)	2014 (3)

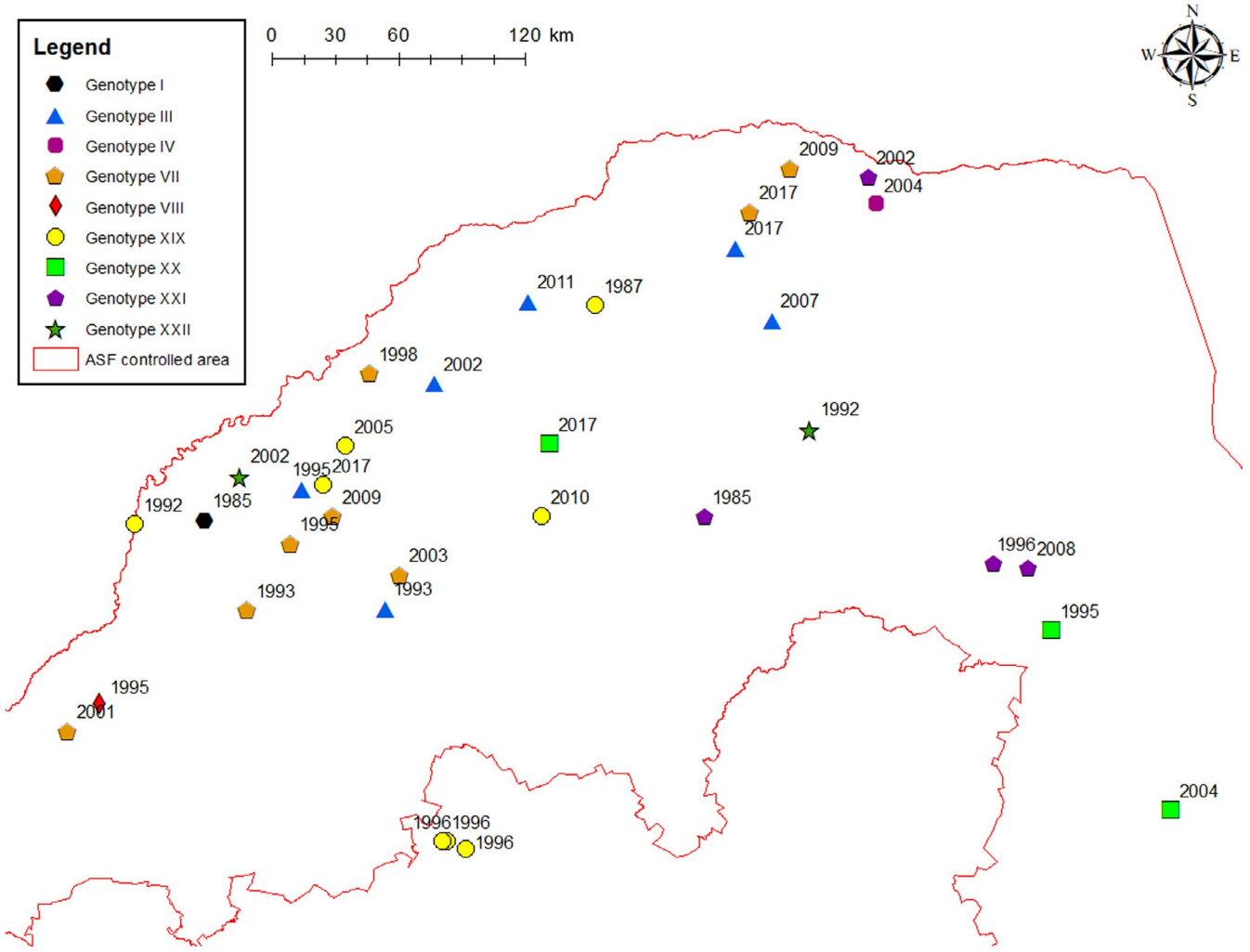

FIGURE 3 Map of ASF genotypes sequenced from outbreaks in domestic pigs (1977–2017)

Rather than producing a risk map based only on the spatial concentration and possible diffusion of outbreaks, the approach used to calculate the relative risk for the affected local municipalities enabled calculation of the risk of dead pigs due to the ASF outbreaks in a specific local municipality, relative to the expected number of dead pigs. A homogeneous distribution of all dead pigs relative to the population of pigs of all affected local municipalities was assumed. The limitation of this weighting approach was that the population of pigs of the local municipalities at the date of each outbreak could not be taken into consideration since the available census data were limited to data produced in 2016 by StatsSA. The results of this approach allowed consideration of some local municipalities as high-risk locations even if the number of outbreaks was not as high as for other municipalities, as is the case for Ramotshere Moiloa. Therefore, these high-risk

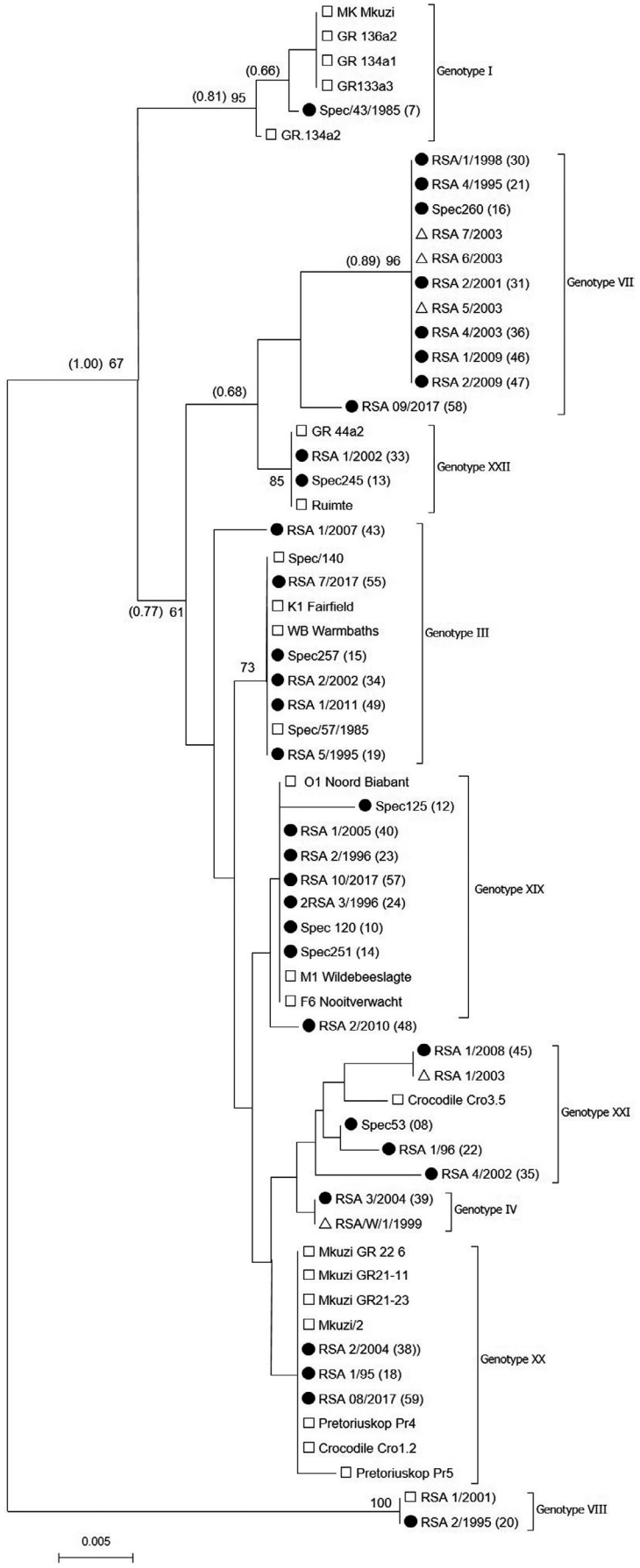

FIGURE 4 Neighbour-joining tree depicting p72 gene relationships of African swine fever viruses from outbreaks in domestic pigs (solid circles) in the South African controlled area (1977–2017), as well as other viruses isolated from warthog (open triangles) and ticks (open squares) during the same period. Bootstrap values > 60% obtained following 10,000 replications and are indicated next to the node together with the posterior probability value obtained from the Bayesian inference. Nine genotypes were designated based on previous studies (I–X, Bastos et al., 2003 and II–XVI, Lubisi et al., 2005, Boshoff et al., 2007 XVII-XXII)

TABLE 4 Summary of ASF viruses isolated from sylvatic hosts in the South African ASF controlled area for the period 1977–2017

Genotype	Number of domestic outbreaks	Sylvatic isolate	Sylvatic host specimen	Year	Area	Reference	Other countries genotype isolated from
I	1	MK Mkuzi	Tick	1978	Mkuze, KZN	Zsak et al. (2005)	Angola, Belgium, Benin, Brazil, Cameroon, Côte d'Ivoire, Dominican Republic, DRC, Gambia, Ghana, Holland, Kenya, Namibia, Nigeria, Malta, Portugal, Sardinia, Senegal, Spain, Togo, Zambia, Zimbabwe (Bastos et al., 2003; Boshoff et al., 2007; Lubisi et al., 2005)
		GR 133a3	Tick	1981	Kruger National Park (KNP)	This paper, KC519623	
		GR 134a1	Tick	1981	KNP	This paper, KC489774	
		GR 134a2	Tick	1981	KNP	This paper, KC519624	
		GR 136a2	Tick	1981	KNP	This paper, KC489776	
III	6	Spec/57/1985	Tick	1985	Thabazimbi, LP	Arnot et al. (2009)	Botswana (Bastos et al., 2003)
		Spec/140	Tick	1987	Waterberg, LP	Arnot et al. (2009)	
		WB Warmbaths	Tick	1987	Waterberg, LP	Zsak et al. (2005)	
		K1 Fairfield	Tick	1996	Unknown	Zsak et al. (2005)	
IV	1	RSA/W/1/1999	Warthog	1999	Makhado, LP	Bastos et al. (2003)	–
VII	8	RSA 5/2003	Warthog	2003	Lephalale, LP	This paper, JX403658	Botswana (Boshoff et al., 2007)
		RSA 6/2003	Warthog	2003	Lephalale, LP	This paper, JX403659	
		RSA 7/2003	Warthog	2003	Lephalale, LP	This paper, JX403660	
VIII	1	RSA/1/2001	Tick	2001	Mkuze, KZN	This paper, JX403670	Malawi, Mozambique, Zambia, Zimbabwe (Bastos et al., 2003; Bastos, Penrith, Macome, Pinto, & Thomson, 2004; Lubisi et al., 2005)
XIX	6	O1 Noord Brabant	Tick	1996	Thabazimbi, LP	Zsak et al. (2005)	–
		M1 Wildebeeslagte	Tick	1996	Polokwane, LP	Zsak et al. (2005)	
		F6 Nooitverwacht	Tick	1996	Lephalale, LP	Zsak et al. (2005)	

(Continues)

TABLE 4 (Continued)

Genotype	Number of domestic outbreaks	Sylvatic isolate	Sylvatic host specimen	Year	Area	Reference	Other countries genotype isolated from
XX	3	Mkuzi GR21-11	Tick	1978	Mkuze, KZN	Arnot et al. (2009)	-
		Mkuzi GR21-23	Tick	1978	Mkuze, KZN	Arnot et al. (2009)	
		Mkuzi GR22 6	Tick	1978	Mkuze, KZN	Arnot et al. (2009)	
		Mkuzi/2	Tick	1978	Mkuze, KZN	This paper, KC519621	
		Pretoriuskop Pr4	Tick	1996	KNP	Zsak et al. (2005)	
		Crocodile Cro1.2	Tick	1996	KNP	Zsak et al. (2005)	
		Pretoriuskop Pr5	Tick	1996	KNP	Zsak et al. (2005)	
XXI	4	Crocodile Cro3.5	Tick	1996	KNP	Zsak et al. (2005)	-
		RSA 1/2003	Warthog	2003	Unknown	This paper, JX403664	
XXII	2	GR 44a2	Tick	1979	KNP	This paper, KC489777	-
		Ruimte	Tick	1998	Waterberg, LP	This paper, KC835274	

municipalities should be explored more closely in order to find the specific risk factors that could explain why they experienced a high number of outbreaks relative to their small pig population size.

This increase in number of outbreaks and number of pigs affected by ASF as well as the clustering in this area could be due to various factors. One factor may be an increase in the number of households keeping pigs in South Africa. StatsSA (2016) found in their Community Survey on Agricultural Households that the number of households keeping pigs in South Africa increased from 112 678 in 2011 to 210,504 in 2016. Of these households, 192,257 (91%) kept between 1 and 10 pigs and are less likely to invest in biosecurity measures to protect their pigs from disease, as they are usually kept informally and free-roaming to allow for food scavenging. Considering the relative risk, which eliminates the pig density factor, other factors to be considered are whether there was a higher concentration of sylvatic hosts in the areas most affected, whether there was a higher number of circulating viruses in these sylvatic hosts or whether there were perhaps differences in reporting, which may not necessarily reflect the true incidence. Farm factors could also play a role, such as fencing and other biosecurity measures to prevent contact with wildlife. The absence of strong significance in the temporal trend favoured an absence of variation of risk factors over the time and was more in line with purely geographical differences.

Contact with warthogs or warthog carcasses was noted in the reports (DAFF Annual Reports, 2018a, 2018b; Original laboratory reports (TAD)—DAFF archives) for 41% of the ASF outbreaks in domestic pigs in the controlled area (Table 1). A further 24% of outbreaks were suspected to have been caused by contact with warthogs and no information on the possible source of the outbreaks was available for 35% of the outbreaks (Table 1). Taking these factors into consideration, together with the elapsed time between ASF outbreaks in domestic pigs and genotype diversity of viruses associated with outbreaks, it can be surmised that the maintenance of ASFV in the sylvatic cycle remains the predominant epidemiological cycle in the controlled area with spillover to domestic pigs and that the disease is not maintained in a domestic cycle within the domestic pig population.

Each of the outbreaks in this study (with the exception of the outbreaks in 1996 in Bela-Bela, Limpopo and 2014 in Ramotshere Moiloa, North West) was contained to one property, which indicates that there were 55 separate introductions of ASFV into domestic pig herds in the controlled area of South Africa from 1977 to 2017. This is supported by the finding that nine different genotypes of ASFV were isolated from these outbreaks, all of which were also found either in ticks or warthogs (Table 4). The control measures prescribed for pigs kept in the controlled area in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) requires pigs to be kept in pig-proof housing and should an outbreak occur the property is quarantined, and no pigs or pig products are allowed to leave the property. Due to this area's long history of ASF in wildlife, veterinary services are sensitized to the risk of ASF, which may account for the success in containing outbreaks to single properties.

The study area in the Mpumalanga, Limpopo and North West provinces of South Africa thus remains an area where the ASFV is maintained in the sylvatic cycle and can serve as a source of virus for domestic outbreaks. Although Magadla et al. (2016) found the current South African ASF controlled area still relevant, the southern border will need continuous evaluation to ascertain whether wildlife reservoirs are migrating south.

This study shows that no outbreaks had been reported in domestic pigs during 1977–2017 in the ASF controlled area of Kwa-Zulu Natal. However, during a study in 1978 three isolates of ASFV (Genotype XX, Figure 4) were obtained from 5,018 ticks collected in the Mkuze Game Reserve in the north of Kwa-Zulu Natal but south of the controlled area (Arnot, du Toit, & Bastos, 2009; Thomson et al., 1983). These ASF isolates were found to be of lower virulence, and the sero-prevalence in warthogs in the area was found to be very low at 2% (Thomson et al., 1983). A later study in the same area found that the tick and warthog population had increased in the area, but none of the 348 ticks collected yielded positive results (Arnot et al., 2009). This raises the question whether ASFV is still present in this area. Future studies should focus on investigating larger areas in the Kwa-Zulu Natal area of the control zone for ASFV presence in hosts.

As early as the 1950s, it was found that certain biosecurity measures would protect pigs from ASF in the northern parts of South Africa. The Veterinary Services, together with the pig farming sector, started developing a system of compartmentalization, which could allow commercial pig farmers to farm pigs in this area without contracting the disease. Basic biosecurity measures included that pigs should be kept in pig-proof pens. These pig pens should be secure with cement flooring, surrounded by pig-proof fencing that was at least 1.3 m high and anchored in the ground with concrete, to prevent digging by warthogs, effectively providing a double barrier, with a "pig-free" area in between the fence and the houses. Swill feeding was prohibited, and record keeping was required (DAFF, 2011). These compartment piggeries would be officially approved after inspection. Since the implementation of this system, no ASF outbreaks have occurred in these approved pig compartments, based on monthly clinical inspection and six-monthly serological testing (DAFF, 2011). This confirms that these biosecurity measures are protective in areas where the sylvatic cycle of ASF is present.

In conclusion, South Africa is unlikely to eradicate all sources of ASFV as the virus was found to still be circulating in the sylvatic cycle in this area, but with the implementation of relevant biosecurity measures pigs can be successfully farmed despite the presence of ASFV in African wild suids and soft ticks. When breaks in biosecurity occur transmission to domestic pigs can ensue, but if quarantine is quickly and effectively implemented, domestic spread can be prevented.

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CONFLICT OF INTEREST

The authors declare that they have no actual or potential financial or personal conflict of interest regarding this publication.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to, and the appropriate ethical review committee approval has been received. Research ethics approval was obtained from the University of Pretoria, Faculty of Veterinary Science and Section 20 approval in terms of the Animal Diseases Act, 1984 (Act 35 of 1984) was obtained from the South African Director of Animal Health.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ENDNOTES

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REFERENCES

Achenbach, J. E., Gallardo, C., Nieto-Pelegrín, E., Rivera-Arroyo, B., Degefa-Negi, T., Arias, M., … Sánchez-Vizcaíno, J. M. (2016). Identification of a new genotype of African swine fever virus in domestic pigs from Ethiopia. *Transboundary and Emerging Diseases*, *64*, 1393–1404. https://doi.org/10.1111/tbed.12511

Alonso, C., Borca, M., Dixon, L., Revilla, Y., Rodriguez, F., Escribano, J. M., & ICTV Report Consortium (2018). ICTV virus taxonomy profile: *Asfarviridae*. *Journal of General Virology*, *99*(5), 613–614. https://doi.org/10.1099/jgv.0.001049

Arnot, L. F., Du Toit, J. T., & Bastos, A. D. S. (2009). Molecular monitoring of African swine fever virus using surveys targeted at adult *Ornithodoros* ticks: A re-evaluation of Mkuze Game Reserve, South Africa. *Onderstepoort Journal of Veterinary Research*, *76*, 385–392. https://doi.org/10.4102/ojvr.v76i4.22

Bastos, A. D. S., Penrith, M.-L., Crucière, C., Edrich, J. L., Hutchings, G., Roger, F., … Thomson, G. R. (2003). Genotyping field strains

of African swine fever virus by partial p72 gene characterisation. *Archives of Virology*, *148*, 693–706. https://doi.org/10.1007/s00705-002-0946-8

Bastos, A. D. S., Penrith, M.-L., Macome, F., Pinto, F., & Thomson, G. R. (2004). Co-circulation of two genetically distinct viruses in an outbreak of African swine fever in Mozambique: No evidence for individual co-infection. *Veterinary Microbiology*, *103*, 169–182. https://doi.org/10.1016/j.vetmic.2004.09.003

Boinas, F. S., Wilson, A. J., Hutchings, G. H., Martins, C., & Dixon, L. J. (2011). The persistence of African swine fever virus in field-infected *Ornithodoros erraticus* during the ASF endemic period in Portugal. *PLoS One*, *6*(5), e20383. https://doi.org/10.1371/journal.pone.0020383

Boomker, J., Horak, I. G., Booyse, D. G., & Meyer, S. (1991). Parasites of South African wildlife. VIII. Helminths and arthropod parasites of warthogs, *Phacochoerus aethiopicus*, in the eastern Transvaal. *Onderstepoort Journal of Veterinary Research*, *58*, 195–202.

Boshoff, C. I., Bastos, A. D., Gerber, L. J., & Vosloo, W. (2007). Genetic characterisation of African swine fever viruses from outbreaks in southern Africa (1973–1999). *Veterinary Microbiology*, *21*, 45–55. https://doi.org/10.1016/j.vetmic.2006.11.007

Chenais, E., Ståhl, K., Guberti, V., & Depner, K. (2018). Identification of wild boar-habitat epidemiologic cycle in African swine fever epizootic. *Emerging Infectious Diseases*, *24*(4), 810–812. https://doi.org/10.3201/eid2404.172127

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., … Dixon, L. (2009). African swine fever: How can global spread be prevented? *Philosophical Transactions of the Royal Society B*, *364*, 2683–2696. https://doi.org/10.1098/rstb.2009.0098

DAFF (Department of Agriculture, Forestry and Fisheries of South Africa) (2011). VPN 39: Standards for the registration of a veterinary approved pig compartment. Retrieved from https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food-Safety/Animal-Health/importexport/vpnson

DAFF (Department of Agriculture, Forestry and Fisheries of South Africa) (2018a). Annual Reports. Retrieved from https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food-Safety/Animal-Health/information/host

DAFF (Department of Agriculture, Forestry and Fisheries of South Africa) (2018b). Online disease database. Retrieved from https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-Food-Safety/Animal-Health/Epidemiology/diseasedatabase

De Kock, G., Robinson, E. M., & Keppel, J. J. G. (1940). Swine fever in South Africa. *Onderstepoort Journal of Veterinary Science and Animal Industry*, *14*, 31–93.

DeTray, D. E. (1957). African swine fever in Wart Hogs (*Phacochoerus Aethiopicus*). *Journal of the American Veterinary Medical Association*, *130*, 537–540.

Edelsten, R. M., & Chinombo, D. O. (1995). An outbreak of African swine fever in the southern region of Malawi. *Revue Scientifique Et Technique De l'OIE*, *14*(3), 655–666. https://doi.org/10.20506/rst.14.3.865

Horak, I. G., Biggs, H. C., Hanssen, T. S., & Hanssen, R. E. (1983). The prevalence of helminth and arthropod parasites of warthog, *Phacochoerus aethiopicus*, in South West Africa/Namibia. *Onderstepoort Journal of Veterinary Research*, *50*, 145–148.

Horak, I. G., Boomker, J., De Vos, V., & Potgieter, F. T. (1988). Parasites of domestic and wild animals in South Africa. XXII. Helminth and arthropod parasites of warthogs, *Phacochoerus aethiopicus*, in the eastern Transvaal Lowveld. *Onderstepoort Journal of Veterinary Research*, *55*, 145–152.

Jori, F., Vial, L., Penrith, M.-L., Pérez-Sánchez, R., Etter, E., Albina, E., … Roger, F. (2013). Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian Ocean. *Virus Research*, *173*(1), 212–227. https://doi.org/10.1016/j.virusres.2012.10.005

Kebkiba, B., Antipas, B. B., & Youssouf, M. L. (2015). Factors contributing to the introduction and the spread of African swine fever virus in Chad. *International Journal of Current Microbiology and Applied Sciences*, *4*(8), 607–613.

Kleiboeker, S. B., & Scoles, G. A. (2001). Pathogenesis of African swine fever virus in *Ornithodoros* ticks. *Animal Health Reviews*, *2*(2), 121–128. https://doi.org/10.1079/AHRR200133

Kumar, S., Stecher, G., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, *33*(7), 1870–1874. https://doi.org/10.1093/molbev/msw054

Lubisi, B. A., Bastos, A. D., Dwarka, R. M., & Vosloo, W. (2005). Molecular epidemiology of African swine fever in East Africa. *Archives of Virology*, *50*, 2439–2452. https://doi.org/10.1007/s00705-005-0602-1

Magadla, N. R., Vosloo, W., Heath, L., & Gummow, B. (2016). The African swine fever control zone in South Africa and its current relevance. *Onderstepoort Journal of Veterinary Research*, *83*(1), a1034. https://doi.org/10.4102/ojvr.v83i1.1034

Malmquist, W. A., & Hay, D. (1960). Hemadsorption and cytopathic effect produced by African swine fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research*, *21*, 104–108.

Mellor, P. S., Kitching, R. P., & Wilkinson, P. J. (1987). Mechanical transmission of capripox virus and African swine fever virus by *Stomoxys calcitrans*. *Research in Veterinary Science*, *43*, 109–112. https://doi.org/10.1016/S0034-5288(18)30753-7

Montgomery, R. E. (1921). On a form of swine fever occurring in British East Africa (Kenya Colony). *Journal of Comparative Pathology and Therapeutics*, *34*, 159–191. https://doi.org/10.1016/S0368-1742(21)80031-4

Penrith, M.-L. (2009). African swine fever. *Onderstepoort Journal of Veterinary Research*, *76*, 91–95.

Penrith, M.-L. (2013). History of "swine fever" in Southern Africa. *Journal of the South African Veterinary Association*, *84*(1) Art. #1106. https://doi.org/10.4102/jsava.v84i1.1106

Penrith, M.-L., Bastos, A. D. S., Etter, E. M. C., & Beltrán-Alcrudo, D. (2019). Epidemiology of African swine fever in Africa today: Sylvatic cycle versus socio-economic imperatives. *Transboundary and Emerging Diseases*, *66*, 672–686. https://doi.org/10.1111/tbed.13117

Penrith, M.-L., Vosloo, W., Jori, F., & Bastos, A. D. S. (2013). African swine fever virus eradication in Africa. *Virus Research*, *173*, 228–246. https://doi.org/10.1016/j.virusres.2012.10.011

Pini, A., & Hurter, L. R. (1975). African swine fever: An epizootiological review with special reference to the South African Situation. *Journal of South African Veterinary Association*, *46*(3), 227–232.

Plowright, W., Parker, J. A., & Peirce, M. (1969a). African swine fever virus in ticks (*Ornithodoros moubata* Murray) collected from animal burrows in Tanzania. *Nature*, *221*, 1071–1073. https://doi.org/10.1038/2211071a0

Plowright, W., Parker, J., & Peirce, M. A. (1969b). The epizootiology of African swine fever in Africa. *The Veterinary Record*, *85*, 668–674.

Plowright, W., Perry, C. T., & Greig, A. (1974). Sexual transmission of African swine fever virus in the tick, *Ornithodoros moubata porcinus*, Walton. *Research in Veterinary Science*, *17*, 106–113.

Plowright, W., Perry, C. T., Peirce, M., & Parker, J. (1970). Experimental infection of the argasid tick, *Ornithodoros moubata porcinus*, with African swine fever virus. *Archiv Für Die Gesamte Virusforschung*, *31*, 33–50. https://doi.org/10.1007/BF01241664

Quembo, C. J., Jori, F., Vosloo, W., & Heath, L. (2018). Genetic characterization of African swine fever virus isolates from soft ticks at the wildlife/domestic interface in Mozambique and identification of a novel genotype. *Transboundary and Emerging Diseases*, *65*, 420–431. https://doi.org/10.1111/tbed.12700

R Core Team (2013). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from http://www.R-project.org/

Rowlands, R. J., Michaud, V., Heath, L., Hutchings, G., Oura, C., Vosloo, W., … Dixon, L. (2008). African swine fever virus isolate, Georgia, 2007. *Emerging Infectious Diseases*, *14*(12), 1870–1874. https://doi.org/10.3201/eid1412.080591

Sánchez-Botija, C. (1963). Reservoirs of ASFV: A study of the ASFV in arthropods by means of the haemadsorption test. *Bulletin, Office International Des Épizooties*, *60*, 895–899.

Sánchez-Vizcaíno, J. M., Mur, L., Bastos, A. D. S., & Penrith, M.-L. (2015). New insights into the role of ticks in African swine fever epidemiology. *Revue Scientifique Et Technique De l'OIE*, *34*(2), 503–511. https://doi.org/10.20506/rst.34.2.2375

Statistics South Africa (2016). Community Survey 2016: Agricultural households, Report no. 03-01-05. Retrieved from www.statssa.gov.za

Steyn, D. G. (1928). Preliminary report on a South African virus disease amongst pigs. In *13th and 14th Reports of the Director of Veterinary Education and Research, Union of South Africa* (pp. 415–428). Pretoria, South Africa: Government Printer.

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., & Rambaut, A. (2018). Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evolution*, *4*(1), vey016.

Thomson, G. R. (1985). The epidemiology of African swine fever: The role of free-living hosts in Africa. *Onderstepoort Journal of Veterinary Research*, *52*, 201–209.

Thomson, G. R., Gainaru, M. D., Lewis, A., Biggs, H., Nevill, E., Van der Pypekamp, H., … Condy, J. (1983). The relationship between African swine fever virus, the warthog and *Ornithodoros* species in southern Africa. In P. J. Wilkinson (Ed.), *African swine fever* (pp. 85–100). EUR 8466 EN. Luxembourg, Luxembourg: Office for Official Publications of the European Communities.

Thomson, G. R., Gainaru, M. D., & Van Dellen, A. F. (1980). Experimental infection of warthog (*Phacochoerus aethiopicus*) with African swine fever virus. *Onderstepoort Journal of Veterinary Research*, *47*, 19–22.

Van Heerden, J., Malan, K., Gadaga, B. M., & Spargo, R. M. (2017). Reemergence of African swine fever in Zimbabwe, 2015. *Emerging Infectious Diseases*, *23*(5), 860–861. https://doi.org/10.3201/eid2305.161195

Wilkinson, P. J. (1986). Epidemiology of African swine fever. *Revue Scientifique Et Technique De l'OIE*, *5*(2), 487–493. https://doi.org/10.20506/rst.5.2.243

Zsak, L., Borca, M. V., Risatti, G. R., Zsak, A., French, R. A., Lu, Z., … Rock, D. L. (2005). Preclinical diagnosis of African swine fever in contact-exposed swine by a real-time PCR assay. *Journal of Clinical Microbiology*, *43*, 112–119. https://doi.org/10.1128/JCM.43.1.112-119.2005

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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