Investigation into eradication of African swine fever in domestic pigs from a previous outbreak (2016/17) area of South Africa

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Highlights

- ASF outbreaks in an area were controlled with selective culling in South Africa.
- Clinical surveillance and a sero-survey suggests ASF was eradicated in area.
- Basic biosecurity measures are needed for smallholder pig keepers to prevent ASF.
- Investigation into possible wildlife ASF sylvatic cycle in the area is recommended.

Abstract

A serological survey was conducted to evaluate the eradication of African swine fever (ASF) infection eighteen months after clinical surveillance and selective culling had been completed during domestic cycle outbreaks in parts of South Africa in 2016/17. Three hundred and twenty-two serum samples from 85 pig keepers were collected in the study area and tested for the presence of antibodies against the ASF virus (ASFV). None of the samples contained detectable levels of antibodies against ASFV. These results together with the findings from clinical surveillance following culling activities suggest that the disease had been eradicated from the domestic pig population in this area following the outbreaks. Questionnaire responses from the pig keepers in this area highlighted the need to implement basic biosecurity measures in smallholder pig keepers to prevent outbreaks of ASF in South Africa.

Keywords: African swine fever; Domestic cycle; Survey; Biosecurity; Smallholder

1. Introduction

Some countries or zones are historically endemic for African swine fever (ASF), mostly due to the presence of the virus in wild suids and soft tick vectors (Jori et al., 2013; Penrith et al., 2004). Recently an increased number of countries have been affected by this transboundary animal disease, especially in domestic pigs (Penrith et al., 2019). Four epidemiological cycles have been described for ASF, including the sylvatic cycle between warthogs and soft ticks, the domestic pig-tick cycle, the domestic pig cycle and recently a wild boar-habitat cycle (Chenais et al., 2018; Haresnape and Wilkinson, 1989; Plowright et al., 1969). The sylvatic cycle between warthogs and soft ticks is present in eastern and southern Africa (Plowright et al., 1969). This sylvatic cycle of ASF, with occasional spill over infections in domestic pigs has been described in South Africa for several decades (De Kock et al., 1940; Magadla et al., 2016; Steyn, 1932).

A domestic pig cycle of ASF has only recently been reported to occur in South Africa (DAFF, 2018; Geertsma et al., 2012; Janse van Rensburg et al., 2020). In areas such as West Africa, the domestic pig cycle amongst free-ranging pigs has been described (Brown et al., 2018; Costard et al., 2009; Etter et al., 2011). In high-contact pig populations, for example where there are free-ranging pigs, the rapid reproduction rate of pigs provides a constant supply of susceptible pigs to maintain the circulation of ASF virus (ASFV), (Penrith et al., 2007). Circulation amongst confined domestic pigs also occurs under conditions of low biosecurity that may include feeding of catering waste (Penrith et al., 2013). This continued cycle of ASF spread amongst domestic pigs suggests that ASF mortalities may not in all cases be as high as previously thought, which could be attributed to ASF strain differences in virulence or resistance on the part of the pig to the circulating strains (Etter et al., 2011; Haresnape et al., 1985). Where clinically healthy pigs demonstrate antibodies to ASF, this could indicate resistance to the ASFV, although the basis of this resistance is unknown (Haresnape and Wilkinson, 1989; Penrith, 2013; Penrith et al., 2004).

Sero-prevalence can show how many pigs have become infected and survived, but does not necessarily indicate current infection with ASFV (Etter et al., 2011). The role of pigs surviving ASF outbreaks as healthy carriers of the ASFV has recently been challenged, with Petrov et al. (2018) showing that the viral genome can only be detected for about three months post infection and sentinel animals do not become infected when commingled after this period. Pigs can, however, shed the virus for up to a month following recovery but remain the highest risk source for spread of the disease during the incubation period (4–19 days – OIE, 2019), before clinical signs appear. Nevertheless, no lasting carrier state has been proven in pigs that have recovered from the disease (Penrith, 2009; Penrith et al., 2004; Penrith and Vosloo, 2009; Petrov et al., 2018; Ståhl et al., 2019).

In 2012, South Africa for the first time experienced an ASF epidemic in several areas outside the ASF controlled area, without a direct link with warthogs. The initiation of this epidemic was linked to illegal movement of pigs from the ASF controlled area to an auction, but the subsequent spread was via a domestic cycle (Geertsma et al., 2012; Janse van Rensburg et al., 2020). Following a national pig disease serological survey in 2013, throughout South Africa (including the ASF controlled area), in which all pigs sampled were negative for antibodies to ASFV, it was concluded that the epidemic had been eradicated and the South African domestic pig population was free from circulating ASFV at the time of the report (De Klerk and Pienaar, 2014).

Subsequently, outbreaks of ASF were again reported from May 2016. This 2016/17 ASF epidemic in South Africa comprised of two isolated outbreaks in North West Province and more extensive (11 reported) outbreaks in Free State Province followed by three outbreaks in Northern Cape Province during 2017. The map of the outbreaks of the 2016/17 epidemic showed a marked concentration of outbreaks around the Bloemfontein area in the Free State and adjacent Kimberley area in the Northern Cape (Janse van Rensburg et al., 2020).

A selective culling policy was followed for the control of the ASF outbreaks of the 2016/17 epidemic. All the pigs on enclosed properties where an outbreak occurred were culled. In communal areas where it was difficult to clearly define epidemiological units, only pigs that were infected or confirmed to be in contact with infected pigs were culled. Other groups of pigs in the area for which there was no compelling evidence of contact with infected animals were first monitored for any signs of disease and/or tested to determine whether to proceed with culling. The last reported outbreak for this epidemic was in July 2017 and the epidemic was declared over by the South African Department of Agriculture, Forestry and Fisheries (DAFF) in December 2017 (Janse van Rensburg et al., 2020).

Following the conclusion of the epidemic, based on the clinical surveillance as well as the culling that had been implemented, there remained a question as to whether some exposed pigs may have survived due to subclinical or chronic infections in the area of the 2016/17 outbreaks. A serological survey designed to detect any possible antibodies to ASFV was proposed to assist in evaluating whether the domestic pig cycle had been eradicated in this area by the control measures that were carried out. A questionnaire regarding the presence of potential risk factors for ASF was compiled to administer to pig keepers in this area.

2. Materials and methods

2. 1. Study area

The area sampled was determined by the DAFF and provincial officials as the highest risk area implicated in the 2016/17 ASF outbreaks in the previous free area, due to the high proportion of the outbreaks (15/17) occurring in this area (Fig. 1). This area is comprised of four state veterinary areas, the Kimberley state veterinary area in the Northern Cape Province and the Fauresmith, Bloemfontein and Thaba ‘Nchu state veterinary areas in the Free State Province. The outbreak areas in the North West Province were excluded as these had been limited to single properties with no evidence of spread in the surrounding area. Maps for this study were created using ArcGIS® software by Esri. [1]

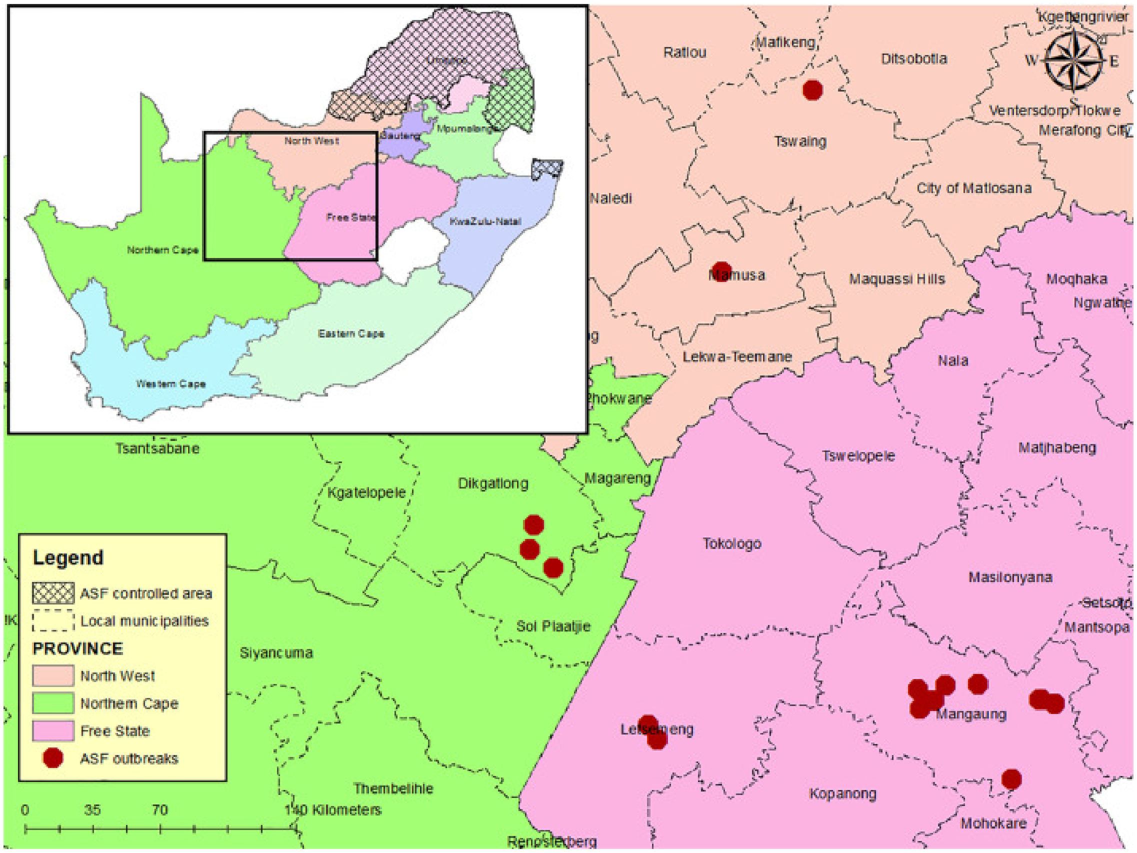

Fig. 1. Location of ASF outbreaks in South Africa, March 2016 to August 2017.

2. 2. Sample size determination

At a 95% confidence level, assuming a 5% farm level sero-prevalence if present, for an "infinite" population size (larger than tenfold sample size which was a very conservative approach), the following equation can be used to calculate the sample size to detect the presence of disease assuming random sampling (Dohoo et al., 2003):

$$n = \ln \alpha / \ln q \tag{1}$$

Where n = required sample size; α = 1 – confidence level and *q* = 1 – prevalence.

For the number of pigs to be sampled per pig keeper in order to detect the disease within the farm, a minimum estimated within-herd prevalence of 55% was used, with a 95% confidence level and an estimated average herd size of 25 (finite population), the following equation from Dohoo et al. (2003) was utilized:

$$n = \left(1 - (\alpha)^{1/D}\right)\left(N - \frac{D-1}{2}\right) \tag{2}$$

Where: *n* = required sample size; *α* = 1 – confidence level set at 95%; *D* = estimated minimum number of diseased animals in the group (population size * minimum expected prevalence) and *N* = population size.

2. 3. Sample collection

A two-stage sampling strategy was used in the survey, in which pig keepers (sampling points) were first selected, followed by animals, as described by Cameron and Baldock (1998). Serum samples were collected in May 2019 by provincial veterinary officials and/or business development managers from the South African Pork Producers' Organisation (SAPPO). As the outbreaks had only occurred in the smallholder pig sector, they were targeted for this study, whereas commercial pig farms maintain a certain level of biosecurity and are regularly visited by veterinarians to support and confirm maintenance of ASF freedom. As no precise recent information on the number of pigs and pig farms was available for the target area, a modified snowball sampling method (Naderifar et al., 2017) was used to select pig keepers. Smallholder pig keepers that the veterinary officials were aware of were included for pig sampling, and if these pig keepers were aware of others, those pig keepers were also included. This process was followed by the sample collectors to sample as many as they could in the time given for sampling (2 days). Information pertaining to biosecurity, herd management, marketing and ASF knowledge was recorded using a questionnaire for each pig keeper whose pigs were sampled.

2. 4. Serological test

ELISA was performed with the commercially available blocking ELISA, which uses a monoclonal antibody (Mab) specific for VP72 ASFV protein, manufactured by Ingenasa (Ingezim PPA Compac K3, Ingenasa, Madrid, Spain). The tests were performed according to the manufacturer's instructions at the Serology section of the Transboundary Animal Diseases Laboratory of Onderstepoort Veterinary Research – Agricultural Research Council.

2. 5. Animal prevalence threshold calculation

By inverting the equation of the sample size adjustment to take into account a cluster size (Eq. (3)) (Dohoo et al., 2003) using a clustered sample size (n') we can recalculate the sample size for a sample without cluster (n) and from then reversing the Eq. (1) to obtain an estimation of the animal prevalence threshold if no sample tested positive.

$$n' = n \times [1 + \rho \times (m - 1)] \quad (3)$$

with ρ being the intra-cluster coefficient and m the cluster size.

2. 6. Questionnaires

The pig keepers were firstly approached with questions on whether there were any recent mortalities or clinical signs of disease in the herd in order to report any suspicions of further outbreaks. The questionnaire then requested the pig keepers' perception regarding the presence of certain risk factors on their properties (Dione et al., 2016). These included questions on the level of pig confinement, the pig keepers' knowledge of pig contact with African wild suids whether they felt that their pigs may have had contact with African wild suids, whether they fed potentially infectious swill, bought or sold via auctions, practiced home slaughter and allowed visitors unrestricted access to the pigs. The risk factors included in the questionnaire were identified primarily through information from previous ASF outbreaks in South Africa, in consultation with Veterinary Service officials (Janse van Rensburg et al., 2020). The pig keepers' level of knowledge of ASF was also assessed and they were asked whether they implement specific biosecurity measures to prevent ASF introduction.

3. Results

The number of pig keepers selected and the number of pigs sampled per state veterinary area are shown in Table 1. In total 85 pig keepers were selected, which exceeds the calculated required number of 59, if random sampling had been possible (cf Eq. (1)). As there were no herds with suspicions of ASF, no tissue samples were taken for PCR. Serum was collected from 86 pigs in Kimberley, 73 pigs in Fauresmith, 80 pigs in Bloemfontein and 83 in Thaba 'Nchu (Table 1). These serological sampling sites are shown in Fig. 2. A minimum of four pigs were needed per farm in accordance with Eq. (2) but due to the number of pigs available at the sampling point this number could have been reduced. The average number of pigs per farm was around 4 for Kimberley, Bloemfontein and Thaba 'Nchu state veterinary areas and 3 for Fauresmith. Information on the ages of the pigs sampled was not captured as the sampling was performed on pigs available at the time of the visit. However, since the sampling occurred eighteen months after the conclusion of the control measures, pigs born after the outbreak would have been included.

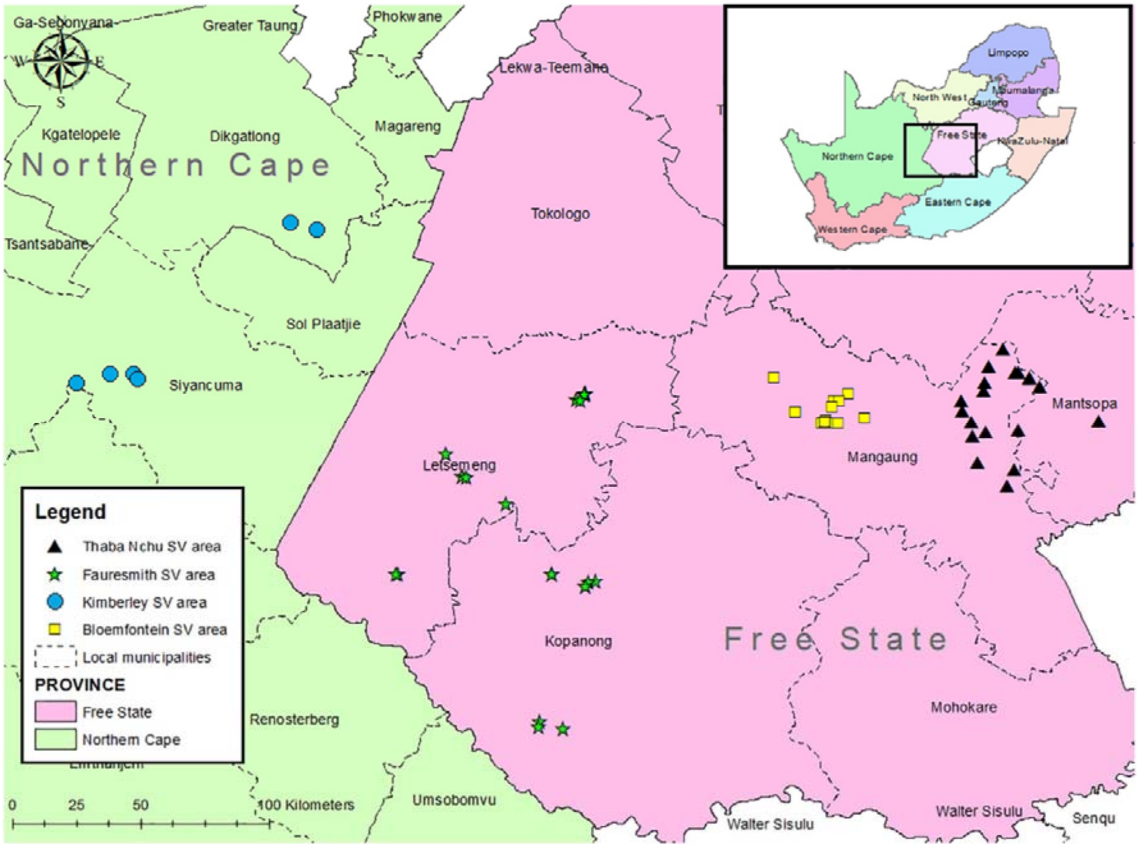

Fig. 2. Location of sampling sites from previous (2016/17) ASF outbreak area of South Africa.

Table 1. Number of pig holdings and pigs sampled per state veterinary area.

State Veterinary area	Number of pig keepers selected	Number of serum samples collected
Kimberley	19	86
Fauresmith	24	73
Bloemfontein	20	80
Thaba 'Nchu	22	83
Total	**85**	**322**

All of the serum samples tested negative for antibodies against ASFV on ELISA. With the two stage sampling approach used in this study, it shows that should ASFV still be circulating, the farm prevalence should be below 3. 5% at 95% confidence level if our sample had been randomly selected. The sample size of 322 sera from 85 farms would give the clusters' size of 3. 79, which is equivalent to a sample size without clustered sera of 207 using an intra-cluster coefficient of 0. 2 using Eq. (3) (Dohoo et al., 2003). This would indicate that the prevalence of infection (if present) was lower than 1. 44% with 95% confidence.

Of the 85 pig keepers selected, 84 answered the questionnaire although not all of the questions were necessarily completed. A herd was considered to be the total number of pigs belonging to the same pig keeper. In total the number of pigs kept by 83 of these pig keepers was 1701 with an average herd size of 21 pigs per keeper (range from 1 to 98). Some pig keepers had only kept pigs for a few months, while one pig keeper had been farming with pigs for 34 years. The median duration of pig keeping was, however, only 3 years and almost 50% of pig keepers had been farming with pigs for less than 3 years.

Table 2. The presence of some of the risk factors for ASF in the study area.

Risk factor	% respondents
Pigs free roaming some or all of the time	20% (16/81)
Pigs may have contact with African wild suids according to keeper	17% (12/72)
Pigs fed with swill potentially containing meat fed (kitchen/restaurant waste) without being heat treated	47% (36/77)
Buy pigs through auctions	14% (11/78)
Sell pigs through auctions	23% (16/71)
Pig keeper slaughters pigs at home	60% (49/81)
Visitors allowed where the pigs are kept	61% (44/72)
Little or no knowledge of ASF	73% (58/79)
No biosecurity implemented to prevent ASF	78% (45/58)

Pig keepers (20%) acknowledged that their pigs were, at least in part, free roaming. Pigs may have contact with African wild suids (warthog and/or bushpig) according to their keeper (17%). Pigs were not only fed commercial feed, but 47% of the owners fed their pigs with swill potentially containing meat (kitchen/restaurant waste) without being heat treated (Table 2). Regarding marketing of pigs, about 17% (12/71) respondents mentioned that they provide pigs to the abattoir, whilst 23% (16/71) marketed pigs via auctions and about 49% (35/71) indicated that they sold pigs live (either private sale or to 'resellers'). Only 14% of pig keepers bought their pigs at auctions, with most of the pig keepers indicating that they acquired pigs via private sales. These smallholder pig keepers mostly slaughter pigs at home

(60%). A large number of the pig keepers (61%) reported that visitors entered the premises where pigs were kept. Most of pig keepers (78%) reported no specific biosecurity measures implemented to prevent ASFV transmission, possibly due to a lack of knowledge of ASF (73%), (Table 2).

4. Discussion

Although simple random sampling would have been preferred, this can only be performed in an area where the target population is known, whereas snowball sampling can be used to access a targeted population which is difficult to locate (Naderifar et al., 2017). This is an efficient and cost effective method to sample from a previously unknown population with the characteristics required for the study (in this case, keeping pigs). According to the 2016 national community survey on agricultural households (Statistics South Africa, 2016) our sample represents almost 4% of the households that have declared keeping pigs in the local municipalities covered by the different state veterinary areas. The concern associated with the representativeness of snowball sampling in this study was assuaged by the diversity of the pig keepers included in the survey as proved by the very large range of the number of years people were keeping pigs and of numbers of pigs within each household. This obtained diversity in our sample in addition to the fact that no pig keepers refused the sampling of their pigs decreased the bias of non-inclusion of reluctant participants, a frequent criticism when such methods are used (Kirchherr and Charles, 2018). In addition the information obtained from blood sampling was not subjective information that required diversity of opinion. Therefore we were confident that the results of this survey gave a good picture of the epidemiological situation in the area even if an additional random sampling would be needed to definitively confirm freedom of disease in this area. Regarding the collection of information about risk factors, the survey could have missed some but those gathered were present and need attention in the management of the control of ASF.

All the serum samples collected tested negative for antibodies against ASFV. Although the exact ages of the pigs were not captured, the samples were collected from pigs of varying ages. Given that antibodies due to exposure to ASFV should last for at least two years (Penrith et al., 2004), these results strengthened the assertion that the clinical surveillance together with the culling policy implemented was successful in eradicating domestic pigs exposed to the ASFV in this area following the 2016/17 epidemic.

Haresnape et al. (1985) performed a serological survey together with owner interviews to determine which areas of Malawi had become endemic for ASFV following outbreaks. It was found that in some areas that experienced outbreaks, all the sera tested negative (similar to the current study), indicating that all infected pigs should have succumbed to the disease. In other areas of Malawi, serological positives indicated that these had become endemic for ASFV, thus showing the usefulness of serology to determine whether an area has become endemic following ASF outbreaks (Haresnape et al., 1985). In Malawi, endemic maintenance was associated with the presence of *Ornithodoros moubata* complex ticks whose distribution in pig shelters coincided with the endemic area (Haresnape and Wilkinson, 1989). In South Africa serological surveys to detect the presence of antibodies to ASFV were undertaken each year from 2000 to 2005 in commercial pig herds, followed by a national pig survey in 2009 as well as in 2013, each of which yielded negative results (De Klerk, 2012; De Klerk and Pienaar, 2014).

A rising concern is the increase of introductions of ASFV into domestic pig populations outside the South African ASF controlled area. Historically, in South Africa, outbreaks in domestic pigs only occurred sporadically on single farms due to spill over from the sylvatic cycle in the ASF controlled area, while these recent outbreaks showed spread amongst domestic pigs and, with the exception of the two outbreaks in North West, were not contained to one location (Janse van Rensburg et al., 2020). Similar to previous studies, this study indicated certain risk factors for the introduction and spread of ASF, including free-roaming pigs, swill-feeding, auctions, informal pig slaughter, lack of ASF awareness and education, visitors allowed access to the pigs and ASF outbreaks at neighbouring properties (Bastos et al., 2014; Chenais et al., 2017; Costard et al., 2009; Etter et al., 2007; Fasina et al., 2015; Penrith et al., 2013; Penrith and Vosloo, 2009; Van Heerden et al., 2017). With these risk factors, the level of occurrence is not as important as the fact that they do occur, since only one introduction of the virus is required to result in an outbreak.

Another aspect that requires further investigation is possible spill over of ASFV into wildlife in the area due to the possible contact between infected domestic pigs during the outbreaks and African wild suids. As 17% of the pig keepers questioned indicated that their pigs may have contact with African wild suids, there is a risk that a sylvatic cycle of ASF may have been introduced in the area, which requires further investigation.

With an increased emphasis on food security, combined with the fact that the capital investment needed in informal pig keeping is minimal, smallholder piggeries are on the increase in the southern African region (Penrith et al., 2013; Penrith et al., 2019). According to South Africa's Community Survey on Agricultural Households an increase of more than 90, 000 households keeping pigs in South Africa occurred from 2011 to 2016, most of these keeping less than 10 pigs (Statistics South Africa, 2016), making them less likely to invest in biosecurity measures. It follows that a larger or increasing pig population would have a greater risk for contact with ASFV due to the higher pig density and resultant increased movement and contact of pigs in a context of low biosecurity. The reported increase in households keeping pigs is supported by the finding in the study area that most of the pig keepers had only started keeping pigs in the last three years. With these smallholder piggeries, more factors that may lead or contribute to ASF outbreaks are added as indicated in similar studies, including the marketing systems used in this sector, such as the use of auctions and lack of access to abattoirs with proper meat inspection (Fasina et al., 2015; Penrith et al., 2013). The informal slaughter as indicated by pig keepers in this study (Table 2) lacks proper meat inspection to detect signs of the ASF, which could contribute to the transmission and maintenance of the disease in local pig populations (Penrith et al., 2013).

People play the major part in the spread of ASF in domestic pigs by moving pigs, pig products and other objects contaminated with infectious virus (Chenais et al., 2019; Penrith et al., 2019; Schulz et al., 2019). Outbreaks linked to pig and pig product movements are preventable by good biosecurity practices (Penrith, 2013). Due to the lack of knowledge on ASF and prevention of the disease (Table 2), the pig keepers may not have been aware of which measures would be effective in prevention of ASF.

A major concern is that almost half of the pig keepers (47%) fed swill to their pigs without a treatment to inactivate possible ASFV contamination (Table 2). Untreated kitchen or restaurant waste used by pig keepers in this area (Table 2) could contain meat products as they are generally obtained from the community without knowledge on what is included in this swill. This practice of swill feeding could be due to ignorance of the risks involved but is more likely due to the cost implications of obtaining commercial feed, especially when the costs in obtaining feed would most probably make the enterprise unprofitable with the available marketing options (Penrith et al., 2013). In South Africa, although swill feeding is not allowed unless it is properly cooked, for at least 60 min (Animal Diseases Act, 1984 (Act 35 of 1984) Regulations), it is very difficult to enforce. The treatment of swill can inactivate ASFV, but this has practical and cost implications that need to be taken into account.

Entry of ASFV via fomites (Etter et al., 2007; Mazur-Panasiuk et al., 2019) and other sources mentioned in Table 2 can be prevented by basic biosecurity. A pro-active programme of continuing education for pig keepers, community leaders and extension officers should be advocated to promote ASF prevention and control (Penrith et al., 2007). Biosecurity measures to prevent ASF will have the added benefit of protecting against other disease introductions concurrently (Fasina et al., 2012; Penrith and Vosloo, 2009). Biosecurity measures should be cost-effective for the farmer and practically implementable (Fasina et al., 2012). There is a need for such biosecurity measures to be developed and implemented in the smallholder pig farming sector in South Africa, as this was the sector impacted by the 2016/17 ASF outbreaks in South Africa, with no commercial pig farms affected (Janse van Rensburg et al., 2020).

Due to the presence of the sylvatic cycle in some areas of South Africa, all sources of ASFV cannot be eradicated, but as the transmission of ASFV from African wild suids to domestic pigs is less efficient, the domestic cycle results in more extensive spread (Penrith et al., 2019), and can be eradicated if biosecurity measures are successfully implemented. These biosecurity measures should focus on treated or safe feed as well as preventing contact between the keeper's pigs and pigs of an unknown health status, African wild suids, soft ticks and potentially ASFV etc.), (Penrith et al., 2013).

5. Conclusion

contaminated objects (such as equipment, vehicles, shoes, clothing

The results of this study suggest that ASFV was eradicated from domestic pigs in the study area following the 2016/17 epidemic in South Africa. However, from the responses of pig keepers in this area, risk factors for the reintroduction of ASFV remain, which needs to be addressed by affordable and practicable biosecurity measures as well as efficient surveillance of this pig sector.

Declaration of Competing Interest

The authors declare that no conflicting financial or personal interests exist.

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