**Investigation into the epidemiology of African
swine fever virus at the wildlife –domestic
interface of the Gorongosa National Park,
Central Mozambique**

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Abstract

An epidemiological study of African swine fever (ASF)
was conducted between March 2006 and September 2007
in the Gorongosa District (Central Mozambique) at a study

1 site spanning the wildlife interface and Gorongosa
2 National park (GNP). Interviews held with farmers
3 indicated that domestic pigs are kept in free-ranging
4 husbandry system, are not fed commercial supplements
5 and generally not treated for any disease. Biosecurity
6 measures are mostly absent and pigs share the same space
7 with other domestic animals such as ruminants, dogs/cats
8 and poultry. Sera from 629 domestic pigs from 310 small
9 scale farmers and 12 warthogs from GNP were tested for
10 antibodies to ASF virus (ASFV) and salivary antigens of
11 *Ornithodoros* spp. ticks. The overall sero-prevalence to
12 ASFV in pigs was 9.1% and that on farms 12.6%,
13 compared to 75% in free-ranging warthogs.
14 Approximately 33% of pigs tested sero-positive to salivary
15 antigens of *Ornithodoros* spp. ticks, whereas in warthogs
16 the sero-prevalence was 77.8%. There were marginally
17 significant differences between farms in the buffer zone,
18 close to the GNP where there is greater chance for the
19 sylvatic cycle to cause outbreaks, and those considered to
20 be in the rest of the district, where pig to pig transmission
21 more likely occurs. *Ornithodoros* ticks were found in 10%
22 of inspected pig pens in the GNP buffer zone and in the
23 rest of the district. ASFV DNA was present on both soft
24 tick pools and virus was isolated from one. This study
25 provides the first evidence of the presence of a sylvatic
26 cycle in Mozambique and confirms the availability of a
27 permanent source of virus for the domestic pig value
28 chain.

1. INTRODUCTION

African swine fever (ASF) is a highly contagious viral disease characterised by fever and extensive haemorrhages throughout different organs of affected pigs (*Sus scrofa*) often leading to high mortalities in domestic pigs. The disease is caused by the ASF virus (ASFV) which is the only member of the *Asfarviridae* family, genus *Asfivirus*. ASF is endemic in most sub-Saharan African countries where it causes major economic losses, threatens food security and limits pig production in the affected countries (Costard et al., 2009, Penrith et al., 2007, Penrith et al., 2004b). In 2007 ASF spread to the Caucasus region and Russia, increasing the risks of further spread to Europe and Asia (Rowlands et al., 2009, Rahimi et al., 2010).

The high mortality rate associated with the disease, coupled with the highly infectious nature of the virus, makes it one of the most serious threats to the swine industry worldwide. In large parts of sub-Saharan Africa, subsistence livestock farming involves raising small herds of pigs. However, in areas where ASF is endemic, the

1 disease impacts negatively on the sustainability of these
2 practices. Since the mid-1990s, ASF has caused severe
3 losses in southern Mozambique, Zambia, Madagascar and
4 in several countries in West Africa, dramatically reducing
5 the pig herds in these countries.

6
7 ASFV is maintained in three epidemiological cycles
8 (Plowright et al., 1994). The sylvatic cycle involving
9 warthogs (*Phacochoerus africanus*) and argasid ticks
10 belonging to the *Ornithodoros moubata* complex, is
11 restricted to regions where the two species coexist and
12 predominantly occur in central, east and southern Africa
13 (Jori & Bastos, 2009). Warthog piglets are born free from
14 the virus (Thomson, 1985, Kleiboeker et al., 1998) and are
15 infected by ticks feeding on young warthogs during the
16 first 6 - 7 weeks spent inside the burrow (Thomson, 1985,
17 Jori and Bastos, 2009). As the natural arthropod host of
18 ASFV, soft ticks also represent the link between wild suids
19 and domestic pigs. In areas where domestic pigs are kept
20 within the home range of wild suids, the spread of ASFV
21 to pigs is often facilitated by soft ticks, representing the
22 second cycle (Plowright et al., 1994, Kleiboeker et al.,
23 1998, Penrith et al., 2004b). Once established in these
24 populations the virus can be maintained independently of
25 the wild suids and ticks.

26
27 The first description of a disease resembling ASF in
28 Mozambique appeared in the mid-1950s. However, it

wasn't until 1960 that the disease was confirmed by laboratory diagnosis following an outbreak in Tete Province located in the Central West region of the country. Since then, Mozambique has experienced regular outbreaks of the disease resulting in ASF being considered endemic to Mozambique (Penrith et al., 2007, Abreu et al., 1962). A study conducted in the Angonia district, close to the border with Malawi (Penrith et al., 2004a), found that the disease is exclusively maintained in domestic pigs. In contrast, the frequent occurrence of ASF in the areas surrounding the Gorongosa National Park, suggests that a sylvatic cycle may also contribute to the maintenance of the disease in Mozambique (Penrith et al., 2007). Here we report on the prevalence of antibodies against ASF in warthogs and domestic pigs found at the domestic/wildlife interface and highlight the potential risk factors contributing to the dissemination of ASF in the region.

2. MATERIALS AND METHODS

2.1. Study area

The field study was conducted in the Gorongosa District (GD), located in the central province of Sofala at 18° 45'/19° 15' latitude South and 33° 30'/34° 45' longitude East (Figure 1). The district was selected as a study area based on the high number of small scale domestic pig

1 farms and its proximity to the Gorongosa National Park
2 (GNP). The GNP is an unfenced wildlife conservation area
3 located within the district. It covers a total area of 3770
4 km² and in 2006 the warthog population was estimated to
5 be approximately 4,000 (Pereira, C.L. personal
6 communication).

7
8 According to the Ministry of Agriculture, there were no
9 commercial pig farms in the area and the subsistence
10 farmer hold, on average, a herd of maximum 8 pigs (often
11 a sow and offspring) per farm (District Agricultural
12 Directorate, 2005). Pigs are predominantly reared using
13 extensive production systems with pigs left roaming free
14 except during the rainy season (between November and
15 March) when crops are still in the field. The typical pig
16 pens, also called *Tanga* in the local language, are
17 constructed from mud and wooden poles, covered by grass
18 or corrugated iron. Each farm generally maintains only one
19 pen without internal divisions, located in the backyard.

20 21 **2.2. Sampling protocol**

22 23 *Pigs*

24 The study was approved by the joint Onderstepoort
25 Veterinary Institute and Faculty of Veterinary Science,
26 University of Pretoria Animal Ethics Committees (Ref.
27 21/2006) prior to execution. The sampling protocol used
28 as a sampling frame was based on the information

provided by the District Agricultural Directorate. The pig population in 2005 in the area surrounding the GNP was estimated to be 17,348 animals owned by approximately 10,990 small scale farmers distributed in a total of 19 villages. Since the pig population size of the different villages was not available, it was decided to distribute the sample size equally over the villages, and farmers were selected for the survey following a multistage sampling approach. In one village the pig farms were depopulated and was therefore not included in the study. As a result, a total of 18 villages were selected according to their proximity to the GNP boundary. Six villages were located at the edge of the GNP (less than 10 km), termed the Gorongosa National Park Buffer Zone (GNPBZ). An additional 12 villages at an average of 15 km from the GNP boundaries were selected, referred to as Rest of District (ROD) herein (Figure 1). The GNPBZ is characteristically rural with a lack of basic infrastructure such as electricity and poor roads, low human population density and crops interspersed with bushy vegetation. In contrast, the ROD is mostly a peri-urban area with a high human population density, basic facilities (electricity) close to a tarmac road and less cropland. We hypothesized that an environment more influenced by human activities such as the one found in ROD, would influence the abundance of warthogs in the area, and potential contacts between warthogs and domestic pigs would be more frequent in the GNPBZ compared to the ROD.

To estimate the required sample size for detecting at least one diseased pig, a prevalence of 50% for ASFV antibodies was assumed with an accepted error of 5% and a level of confidence (CI) of 95%. Villages were considered as clusters of pig farms. In each village, pig farms were chosen randomly following a multistage sampling approach, and up to 5 pigs per farm were sampled. The minimum sample size required was 385 pigs as calculated using the free software Win Episcopo 2.0 (www.clive.ed.ac.uk/winepiscopo).

2.3. Sample and data collection

Warthogs

Twelve warthogs from 4 different locations with approximate ages of less than 1 year (n=3) and older than 1 year (n=9) were sampled opportunistically inside the GNP during 2006. Capture locations were chosen based on the accessibility of the site and abundance of animals. Warthogs were captured by darting them from the vehicle with a compressed air dart gun and plastic darts (Daninject[®]) at a distance of 5 to 8 m. A dose of 250 mg of Zoletil[®] was used to induce anaesthesia, topped up with a combination of 100 mg of Ketamine and 20 mg of Azaperone to allow a light level of anaesthesia, good immobilisation and reduction of recovery time (Kock and Burroughs, 2012). Approximately 5 ml of blood was

1 obtained from the anterior *vena cava* in each animal using
2 10 ml plain Vacutainer® tubes.

3 4 *Soft ticks in pig pens*

5 Twenty farms from the study area (GNPBZ and ROD)
6 were assessed for the presence of soft ticks using the
7 vacuum aspiration method (Butler and Gibbs, 1984,
8 Ravaomanana et al., 2010, Jori et al., 2013, Vial et al.,
9 2007). A petrol-powered mulching blower/vacuum was
10 used to collect material from different parts of pig pens,
11 small mammal burrows in close proximity to the pen
12 Spades were used to collect bigger volumes of material
13 where appropriate. Soft ticks were collected by spreading
14 litter on black plastic sheets exposed to the sun. Specimens
15 were placed in a plastic sample bottle with some sand
16 added and kept in cool, dark conditions until they were
17 dispatched to the Transboundary Animal Disease
18 Programme (TADP), ARC-Onderstepoort Veterinary
19 Institute (OVI) in South Africa for further analysis. The
20 specimens were transported under a permit issued by the
21 South African Department of Agriculture Forestry and
22 Fisheries.

23 24 *Pigs*

25 A total of 634 pigs of local breed ranging from 6 months to
26 3 years old were sampled from 314 small scale farmers
27 clustered in 18 villages. Sera from blood samples were
28 obtained by standard methods, transferred to labelled

1 cryotubes and stored at -20°C until sent to the TADP to be
2 tested for antibodies against ASFV. Aliquots of these sera
3 were also sent to the *Instituto de Recursos Naturales y*
4 *Agrobiología de Salamanca* (CSIC) in Spain to be tested
5 for antibodies to the salivary proteins of *Ornithodoros* spp.
6 ticks.

7 *Questionnaire*

8 At the time of blood collection, 314 pig owners were
9 interviewed using a short questionnaire. The GPS
10 coordinates of the farm were recorded and the presence of
11 soft ticks or other ecto-parasites was also noted.
12 Information of sampled animals in terms of age, sex,
13 health status, haemorrhagic symptoms, husbandry practice,
14 herd size, breed and the suspicion of ASF or recent
15 fatalities was gathered. In addition, information on the
16 farm type, husbandry and feeding practices was obtained
17 to identify possible risk factors for the transmission of
18 ASFV. A presumptive diagnosis for ASF was assessed
19 according to clinical details given by the pig owner. Soft
20 ticks were shown to farmers to assess whether similar
21 parasites had been seen in their premises or village.

22 **2.4. Sample and data processing**

2.4.1. ASFV Antibody detection using Enzyme-linked Immunosorbent Assay (ELISA)

Serologic analysis was performed using an indirect ELISA following the protocol described in the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2012). The antigen used in the assay consisted of purified ASFV isolate Zaire collected in 1977 and subsequently adapted on Vero cells. The results were considered positive when sera had an absorbance value of more than twice the mean absorbance value of the control negative sera on the same plate.

2.4.2. ASFV and DNA detection in soft ticks

For pig pens where ticks were found, a sample of ticks was 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 antimycotic. The soft tick homogenates were centrifuged at 10 000 x g for 1 min and the supernatant frozen at -70° C until further use.

ASFV was isolated from soft ticks using a haemadsorption assay previously described in the literature (Malmquist and Hay, 1960). One hundred microliters of supernatant from tick homogenates were inoculated into peripheral blood mononuclear cells (PBMC) cultured in 96 flat bottom well plates according to standard procedures. The plates were

1 observed for up to seven days, virus harvested when
2 haemadsorption was observed and stored at -80°C. All
3 samples were submitted to three serial passages on PBMC,
4 where every passage was performed on a weekly basis.
5 Samples were considered negative for ASF virus if no
6 haemadsorption was observed.

7 DNA was extracted from 200 µl of each tick homogenate
8 and recovered in a final volume of 50 µl DNA solution
9 using the Qiagen kit (Qiagen GmbH, Hilden) according to
10 the manufacturer's instructions. A nested PCR that targets
11 the C terminal end of the *p72* gene was used to screen soft
12 tick samples for the presence of ASFV DNA (Basto et al.,
13 2006). All DNA samples were tested for tick
14 mitochondrial 16S rDNA according to previously
15 published methodology (Black and Piesman, 1994, Vial et
16 al., 2007) to exclude the occurrence of false-negative
17 results due to inhibitors in the tick supernatant.

18 19 2.4.3. Detection of antibodies against tick salivary protein 20

21 The presence of antibodies against the salivary proteins of
22 *Ornithodoros* spp. ticks was assessed in domestic pig and
23 warthog sera using the ELISA test developed by Díaz-
24 Martín et al (2011), which is based on a recombinant
25 salivary lipocalin protein (rOmTSGP1) of *O. moubata*
26 complex. This assay demonstrated 99.4% specificity and
27 100% sensitivity in detecting antibodies 3 months after
28 exposure to tick bites (Díaz-Martín et al., 2011).

2.4.4. Statistical analysis

In order to detect the influence of age on the detection of ASFV antibodies in the study area (GNPBZ and ROD), animals were classified into two groups: younger than 1 year (n=522) and older than 1 year (n=112). We calculated the number of months between time of sampling and the last reported outbreak of ASF on farms to determine any effect of the time elapsed after the outbreak on the prevalence results. Farms were subsequently divided into those with pigs sampled less than 3 months after a reported outbreak (n=132) and those sampled more than 3 months after a reported outbreak (n=84). The remaining sampled animals (n=418) were from areas with unknown suspicions or reports of ASF outbreaks. A farm was considered positive for ASFV or rOmTSGP1 antigen when at least one pig was found positive to one of the two serological tests. Associations between sero-positivity and the different variables such as pig age, location and the time between sampling and reported outbreaks were tested with the chi square test and Odds ratio calculations for homogeneity of two populations (Fischer exact test). Values of $p < 0.05$ were considered significant.

Prevalence of tick infestation in warthog burrows and pig pens and tick infection with ASFV with 95% confidence intervals were calculated with Epi-Info v.3.5.3, 2011 (CDC, Atlanta, US).

3. RESULTS

3.1. Questionnaire

We considered a herd the total number of pig heads belonging to the same owner. The average herd size per farm was calculated to be 8 pigs (Median 6, IQR [2;10]). Some variations in herd size and composition (sexes and age groups) were identified between farms ($p < 0.05$). Approximately 20.2% (128 / 634) of the sampled populations of pigs were adults with 85.9 % female pigs (110 / 128) and 14.1 % boars (18 / 128). Juveniles (less than one year) represented 79.8% (506 / 634) of total sampled population with 55.7 % (282 / 506) fatteners (i.e. after weaning and before being used for reproduction) and 44.3 % (224 / 506) suckling piglets. All respondents mentioned that the pig reproduction was by natural mating using own or borrowed boars. Pigs were not fed commercial feed supplements, but 100% of questioned pig-owners mentioned giving maize bran, approximately 48% gave their stock post harvest by-products and between 29 to 35% of respondents fed kitchen left-over and wild legumes respectively. Most of the 314 interviewed pig farmers kept other animals on their premises with poultry accounting for 49% of the species, small ruminants (goats and sheep) for 19.7%, beef cattle 2.3% and 29.6% kept dogs and cats.

1 In many cases (76.4%), pigs were reported to share the
2 same space with other animals. In addition, farmers
3 acknowledged that their pigs were left free ranging (65%)
4 and free mating (47%) and 72.3% declared the common
5 practise of lending/borrowing boars. A few farmers (7.0%)
6 shared materials and equipment and 1.6% slaughtered pigs
7 on their premises. Concerning bio security measures and
8 risk factors in favour of ASFV transmission, farmers
9 reported that visitors entered the premises in 51.3% of the
10 cases to purchase pigs.

11 Pigs were not treated for any diseases and 100% of
12 respondents indicated that they do not administer any
13 prophylactic treatment such as vaccination, de-worming or
14 iron supplement. None of the pig-owners in the villages
15 acknowledged having seen soft ticks. However, 100% of
16 the respondents confirmed to have seen hard ticks (*Ixodid*
17 group). Pigs were also often affected by lice, fleas and
18 mange.

19 Over 39% (123/ 314) of respondents mentioned having
20 experienced what they suspected to be ASF outbreaks in
21 the past and reported pig deaths. An outbreak reportedly
22 occurred prior to 2005 and was subsequently followed by
23 two outbreaks in November 2006 (more than 3 months
24 prior to sampling) and February 2007 (less than 3 months
25 prior to sampling). Amongst farmers suspected to have
26 experienced ASF outbreaks, 40.7% (50 / 123) mentioned
27 that not all animals died during the outbreaks. Almost
28 35.8% (44 / 123) of affected farmers stopped rearing pigs

for at least one year after an outbreak due to high mortality and losses. The remaining 60.8% (191/314) of pig farmers answered that they had never experienced mortalities which they suspected to have been due to ASF. These farmers were mostly from the ROD (80.6% (154/191)) while 19.4% (37/191) were from the GNPBZ.

3.2. Detection of antibodies against ASFV

Warthogs

Prevalence of antibodies directed against ASFV in warthogs (n=12) was 75%, 95% CI (42.8-94.5) in adult animals (n=9) showing 66.7%, 95% CI [29.9-92.5] seropositivity and 100% in individuals less than 1 year old (n=3).

Domestic pigs

The mean number of pigs sampled per village was 35 (Median 33, IQR [16;55]) and the mean number of pigs bled per farm was 2 (median:1, IQR [1;3]). During 2006, 50 pigs were sampled in the GNPBZ and 74 in the ROD compared to 120 sampled in the GNPBZ and 390 in the ROD in 2007.

In total, 634 serum samples of pigs were collected from 314 farms in 18 villages: 170 pigs from 79 farms were sampled in 6 villages from the GNPBZ and 464 pigs from

235 farms were sampled in 12 villages in the ROD. However, 5 pig sera from 4 different farms from ROD (all in Tambarara Village) were of poor quality and had to be discarded. Therefore, only 629 sera from 310 farms were considered for the analysis (Table 1).

The overall antibody prevalence against ASFV in 629 tested domestic pig sera (table 1) was 9.1%, 95% CI [7-11.7]. The ASF sero-prevalence in 170 tested pig sera from the GNPBZ was 12.5%, 95% CI [7.4-17.6] whereas from 459 tested pig samples from the ROD, 7.8%, 95% CI [5.4-10.3] were positive for ASFV antibodies (Table 2). However, these differences were only marginally significant (OR=0.59, p=0.05).

The overall farm sero-prevalence (310 tested farms) in the GD was 12.6%, 95% CI [8.9-16.3]. The farm sero-prevalence (Table 2) in 79 tested pig farms from GNPBZ was 12.8%, 95% CI [5.4-20.2] compared to 12.5%, 95% CI [8.2-16.8] in the 231 tested farms from the ROD which was not significantly different (Table 2; OR=0.97, p=0.53).

When the sero-prevalence of different age groups was compared, 91.2%, 95% CI [80.7-97.1] of the sero-positive animals were younger than one year with the remaining 8.8%, 95%, CI [2.9-19.3] older than a year, a statistically significant difference (OR=0.42, p=0.043).

There was a strong association between the reported occurrence of recent outbreaks (from 1-3 months) and sero-positivity against ASFV in pig sera. Indeed, 40.4%,

95% CI [27.6-54.2] of the positive animals corresponded to pigs having been exposed to a recent outbreak between 1 and 3 months prior to sampling (OR=5.7, p=0.004). Equally, there was a significant association (OR=1.93, p=0.0276) between those farms having experienced recent outbreaks and the presence of piglets (younger than 6 months).

3.3. Soft tick collection and ASFV and DNA detection

Two of the 20 inspected pig pens in the GNPBZ and ROD were found infested with *Ornithodoros* spp. ticks, suggesting an infestation prevalence of 10%, 95% CI [3.1 - 23.1]. In both cases the ticks were found in the GNPBZ. ASFV DNA was detected in homogenates of ticks from both infested pig pens and live virus was isolated from one pool (results not shown). All reactions, with the exception of the negative controls, amplified the expected 414 bp 16S of soft tick mitochondrial gene used as internal control, thereby confirming template DNA and the reaction integrity and precluding the possibility of false negative results (results not shown).

3.4. Detection of antibodies against tick salivary protein

Warthogs

The prevalence of antibodies against tick salivary proteins in sampled warthog population was 77.8%, 95% CI [40.0-

97.2]. When different age groups of warthogs were compared, all 100% of warthogs less than 1 year (n=3) and 66.7%, 95% CI [22.3-95.7] of older animals (n=9) showed sero-positivity against the rOmTSGP1 antigen.

Domestic pigs

Of the 629 pig sera collected in the field and tested, 44 from 9 farms (table 1) were not tested as there was insufficient serum available to perform the analysis. The overall prevalence of antibodies to tick salivary proteins among 590 tested domestic pigs from the study area was 32.7%, 95% CI [29.2-36.9] with 42.0%, 95% CI [36.4-47.7] of farms positive. More than 45% of pigs samples in the GNPBZ had antibodies to salivary proteins (95% CI [37.3-54.3]), compared to 28.9%, 95% CI [24.8-33.4] of pigs in the ROD. The on-farm prevalence between the GNPBZ and the ROD was 66.2%, 95% CI [54.3-76.8] and 34.2%, 95% CI [28.1-40.7] respectively (Table 2). Substantial significant differences were observed in the proportion of sero-positive domestic pigs (OR=0.48, p=0.0002) and sero-positive farms (OR=0.27, p=0.0001) between the two areas (Table 2).

When the sero-prevalence against the rOmTSGP1 antigen within different age groups was compared, 32.0%, 95% CI [27.9-36.3] of the sero-positive animals were younger than one year with the remaining 38.0%, 95%, CI [28.1-48.8] older than a year, but these differences were not statistically significant (OR=1.3, p=0.16).

3.5. Correlation between the sero-prevalence and ASFV and tick salivary proteins

Among the 585 sera tested for the presence of antibodies to ASFV and the rOmTSGP1 antigen, only 20.0%, 95% CI [10.4-33.0] contained antibodies to both antigens. The association between the tests was statistically significant (OR=0.48, $p=0.01$). Among the 301 farms tested 28.6%, 95% CI [14.6-46.31] farms had pigs that tested positive on both assays. However, this association was only marginally significant (OR=0.51, $p=0.056$). Sera collected from pigs in 4 villages in the ROD and 1 village in the GNPBZ only contained antibodies to tick antigens (table 1).

4. DISCUSSION

The socio-economic impact of ASF is due to high mortality and morbidity rates that can threaten food security, as pigs represent a good source of cheap protein and a source of income to many poor people (Costard et al., 2009). In African countries and other developing nations ASF impacts negatively on the sustainability of pork production. Farmers lack the resources to implement prevention or control measures and are often unable to restart production following an outbreak (Edelsten and

Chinombo, 1995), as indicated by the fact that 36% of farmers interviewed in this study ceased to farm with pigs for at least 1 year following of an ASF outbreak. The questionnaire also revealed that most villagers sold their stock alive or slaughtered without reporting the disease to local veterinary authorities. Similar behaviour by farmers following ASF outbreaks has previously been shown to contribute to the spread of ASF in other developing countries (Nana-Nukechap and Gibbs, 1985, Penrith et al., 2013, Costard et al., 2009, Fasina et al., 2010) and could account for the low number of adults pigs observed in our study.

The combination of serology and a questionnaire in this study provided a good indication that ASFV was circulating in the area a few months before blood samples were collected as the data showed > 40% sero-positive animals have been exposed to outbreaks less than three months prior to sampling. The overall sero-prevalence of ASFV (9.1%) observed in domestic pigs in the GD was slightly lower compared to the results obtained in the Angónia District (14.3%) of Mozambique (Matos et al., 2011, Penrith et al., 2007) and significantly lower when compared with the serological data of a survey carried out in the Mchinje district (48%) of Malawi (Haresnape et al., 1985). ASF is endemic in both areas and both experience regular outbreaks of the disease often characterized by lower than usual mortality rates in adult domestic pigs (Haresnape and Wilkinson, 1989, Penrith et al., 2004a).

1 The results of this study strongly suggest that the sylvatic
2 cycle is present in the GNP. The serological survey in
3 warthogs presented in this study, despite its limited size, is
4 the first described in Mozambique to date. The high sero-
5 prevalence to ASFV (75%) and tick salivary proteins
6 (78%) is consistent with previous observations in warthog
7 populations from other parts of East and southern Africa
8 (Jori and Bastos, 2009, Penrith et al., 2004a, Jori et al.,
9 2013). Although the GNP is not fenced, warthogs do not
10 venture too far away as they are hunted and killed once
11 they reach the GNPBZ and ROD. The prevalence of ASF
12 per farm was highly similar in both areas, however a
13 marginally significant ($p=0.05$) higher sero-prevalence
14 was detected in the GNPBZ as compared to ROD (12.5%
15 versus 7.8%). Furthermore, 80% of farmers who reported
16 not having experienced any outbreaks on their farm were
17 located in the ROD area. This suggests that ASFV
18 infections could be more common in the buffer zone
19 farms, which are located closer to the park and to a
20 permanent source of virus from wild host than the farms
21 in the ROD.

22 The results of serology assessment of antibodies to tick
23 salivary protein provided evidence that 42% of the farms
24 had pigs that had been bitten by soft ticks recently and
25 support the premise that contact between soft ticks and
26 domestic pigs is widespread in the area. The presence soft
27 ticks at two farms outside GNP provides evidence of the
28 presence of the infected ASF vector in pigsties outside

wildlife areas. In addition, the significant differences between prevalence of antibodies to the tick antigen in the GNPBZ compared to the ROD suggest that the proximity of wildlife areas is a major contributing factor to the exposure of domestic pigs to soft ticks.

Pigs from a number of farms did not have antibodies to ASFV, but were positive for antibodies the rOmTSGP1 antigen. Previously some divergence has been observed between the presence of antibodies to tick salivary proteins and evidence of tick presence (Ravaomanana et al., 2010, Oleaga-Perez et al., 1994). Convergence between serological and tick collection data depends on several factors such as the sensitivity of the methods used to collect soft ticks, the time elapsed between the collection of sera and the tick collection as well as, the use of acaricides before sampling (Jori et al., 2013).

Conclusions

Results from this study showed that ASF virus is widespread in the pig population living in the region adjacent to the GNP. Results from the farmer questionnaire and serology suggested that outbreaks occur periodically and that new stock becomes re-infected on a regular basis. The presence of antibodies to salivary proteins of *Ornithodoros* spp. ticks and observation of ASF infected *Ornithodoros* spp. ticks in pigsties suggest that soft ticks are common in the region. The presence of

1 the vector and warthog population in the wildlife areas
2 contribute to maintenance of the disease in the district.

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5
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Figure 1: Map of the Gorongosa District including the

Table 1: Summary of the villages, number of farms and percentage sero-positivity for ASFV and tick salivary proteins in the Gorongosa District

Village	No. of pigs	No. of farms	% pigs with abs to ASFV	% farms with sero-positive pigs to ASFV	% pigs with abs to tick salivary proteins	% farms with sero-positive pigs to tick salivary proteins
Aerodromo	64	13	1,6	7,7	9,4	38,5
Canda	60	15	0	0	51,7	80,0
Gorongosa sede	74	34	2,7	5,9	33,8	38,2
Madibe	39	34	0	0	25,6	29,4
Missão Cristo Rei	11	1	18,2	100	N/D	N/D
Mukodza	42	18	0	0	54,8	72,2
Mutukuduri	27	17	33,3	41,2	14,8	23,5
Nhanguo	2	2	0	0	100	100
Nhataca	4	4	0	0	N/D	N/D
Tambarara	72	49	19,4	24,5	27,3	26,4
Twassicana	22	14	22,7	14,3	13,6	21,4

Vunduzi	42	30	7,3	10,0	9,5	10,0
Matchisso	14	10	0	0	28,6	40,0
Mbulawa	23	3	0	0	N/D	N/D
Mutiwambamba	45	16	13,3	37,5	40,0	56,3
Nhambita	6	1	0	0	N/D	N/D
Pungue	59	45	3,4	2,2	64,4	73,3
Tsiquiri	23	4	59,1	75,0	17,4	75,0
Total	629*	310*	9,1 95% CI [7-11,7]	12,6 95% CI [8.9-16.3]	32,7 95% CI [29.2-36.9]	42,0 95% CI [36,4-47.7]

In grey, villages located in the Gorongosa National Park Buffer Zone,

N/D=not done, CI=confidence interval, abs=antibodies

***The total sample tested for ASFV antibodies out of 634 sera collected in 314 farms**

Table 2: Comparison of sero-prevalence to ASFV and tick salivary proteins (rOmTSGP1) in the Gorongosa National Park Buffer Zone (GNPBZ) and the rest of the district (ROD)

Seroprevalence (%)	GNPBZ	ROD	OR	p value
ASFV (pigs)	12.5	7.8	0.59	0.05
ASFV (farms)	12.8	12.5	0.97	0.53
rOmTSGP1 (pigs)	45.7	28.9	0.48	0.0002
rOmTSGP1 (farms)	66.2	34.2	0.27	0.0001

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