Investigation into the Epidemiology of African Swine Fever Virus at the Wildlife – Domestic Interface of the Gorongosa National Park, Central Mozambique

C. J. Quembo[1,2,3], F. Jori[4,5,6], L. Heath[1], R. Pérez-Sánchez[7] and W. Vosloo[1,2,8]

[1] Transboundary Animal Disease Program, Onderstepoort Veterinary Institute, Pretoria, South Africa
[2] Department of Veterinary Tropical Diseases, Faculty of Veterinary Sciences, University of Pretoria, Pretoria, South Africa
[3] Laboratório Regional de Veterinária em Chimoio, Centro Zonal de Investigação Agrária da Zona Centro, Instituto de Investigação Agrária de Moçambique, Chimoio, Mozambique
[4] UPR AGIRs, CIRAD, Montpellier, France
[5] Department of Zoology & Entomology, Mammal Research Institute, University of Pretoria, Pretoria, South Africa
[6] Department of Animal Science and Production, Botswana College of Agriculture, Gaborone, Botswana
[7] Instituto de Recursos Naturales y Agrobiologia de Salamanca (IRNASA), CSIC, Salamanca, Spain
[8] Australian Animal Health Laboratory, Geelong, Vic., Australia

Correspondence:
L. Heath. Department of Veterinary Tropical Diseases, Onderstepoort Veterinary Institute, PO Box X05, Onderstepoort, 0110 Pretoria, South Africa.
Tel.: +27 12 529 9501;
Fax: +27 12 529 9543;
E-mail: heathl@arc.agric.za

Summary

An epidemiological study of African swine fever (ASF) was conducted between March 2006 and September 2007 in a rural area adjacent to the Gorongosa National park (GNP) located in the Central Mozambique. Domestic pigs and warthogs were sampled to determine the prevalence of antibodies against ASF virus and the salivary antigens of *Ornithodoros spp*. ticks, while ticks collected from pig pens were tested for the presence of ASFV. In addition, 310 framers were interviewed to gain a better understanding of the pig value chain and potential practices that could impact on the spread of the virus. The sero-prevalence to ASFV was 12.6% on farms and 9.1% in pigs, while it reached 75% in warthogs. Approximately 33% of pigs and 78% of warthogs showed antibodies against salivary antigens of ticks. The differences in sero-prevalence between farms close to the GNP, where there is greater chance for the sylvatic cycle to cause outbreaks, and farms located in the rest of the district, where pig to pig transmission is more likely to occur, were marginally significant. *Ornithodoros spp.* ticks were found in only 2 of 20 pig pens outside the GNP, and both pens had ticks testing positive for ASFV DNA. Interviews carried out among farmers indicated that biosecurity measures were mostly absent. Herd sizes were small with pigs kept in a free-ranging husbandry system (65%). Only 1.6% of farmers slaughtered on their premises, but 51% acknowledged allowing visitors into their farms to purchase pigs. ASF outbreaks seemed to have a severe economic impact with nearly 36% of farmers ceasing pig farming for at least 1 year after a suspected ASF outbreak. This study provides the first evidence of the existence of a sylvatic cycle in Mozambique and confirms the presence of a permanent source of virus for the domestic pig value chain.

Keywords:
ticks; veterinary epidemiology; wildlife; virus

Introduction

African swine fever (ASF) is a highly contagious viral disease affecting domestic and wild pigs (*Sus scrofa*). It is characterized by fever and extensive haemorrhages throughout different organs and often leads 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*. African swine fever is endemic in most sub-Saharan African countries where it causes major

economic losses, threatens food security and limits pig production (Penrith et al., 2004b, 2007; Costard et al., 2009). In 2007, ASF spread to the Caucasus region and Russia, increasing the risk of introduction to Europe and Asia (Rowlands et al., 2008; 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 disease impacts negatively on the sustainability of these practices. Since the mid-1990s, ASF has caused severe losses in southern Mozambique (Penrith et al., 2013), Madagascar (Roger et al., 2001; Ravaomanana et al., 2010) and in several countries in West Africa (Etter et al., 2011), dramatically reducing pig numbers in these countries.

African swine fever virus is maintained in three epidemiological cycles (Penrith et al., 2004b). The sylvatic cycle, involving warthogs *(Phacochoerus africanus*) and argasid ticks belonging to the *Ornithodoros moubata* complex, is restricted to regions where the two species coexist, predominantly central, east and southern Africa (Jori and Bastos, 2009). Warthog piglets are born free from the virus (Thomson, 1985; Kleiboeker et al., 1998) and are infected by ticks during the first 6–7 weeks spent inside the burrow (Thomson, 1985; Jori and Bastos, 2009). As the natural arthropod host of ASFV, infected soft ticks also represent the link between wild suids and domestic pigs. In areas where domestic pigs are kept within the home range of wild suids, the spread of ASFV to pigs is often facilitated by soft ticks (Plowright et al., 1994; Kleiboeker et al., 1998; Penrith et al., 2004b). When pig shelters are infested with ASFV-infected *Ornithodoros moubata* ticks, the disease can be maintained between domestic pigs and the arthropod host in the absence of warthogs. Once established in the domestic pig populations, the virus can be maintained independently of the wild suids and ticks.

The first description of a disease resembling ASF in Mozambique appeared in the mid-1950s. However, it was not 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 this time, Mozambique has experienced regular outbreaks of the disease resulting in ASF being considered endemic in that country (Penrith et al., 2007). A study conducted in the Angonia district, close to the border with Malawi, found that the disease is exclusively maintained in domestic pigs (Penrith et al., 2004a). In contrast, the frequent occurrence of ASF in the areas surrounding the Gorongosa National Park (GNP) 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 ASFV in warthogs and domestic pigs found at the domestic/wildlife interface of the GNP and highlight the potential risk factors contributing to the dissemination of ASF in the region.

Materials and Methods

The study was approved by the joint ARC-Onderstepoort Veterinary Institute (OVI) and Faculty of Veterinary Science, University of Pretoria Animal Ethics Committees (Ref. 21/2006) prior to execution.

Study area

The field study was conducted in the Gorongosa district, located in the central province of Sofala at 18°45′/19°15′ latitude South and 33°30′/34°45′ longitude East (Fig. 1). The district was selected as a study area based on the high number of small-scale domestic pig farms and its proximity to the GNP. The GNP is an unfenced wildlife conservation area located within the district. It covers a total area of 3770 km^2, and in 2006, the warthog population was estimated to be approximately 4000 (C.L. Pereira, personal communication).

According to the Ministry of Agriculture, there were no commercial pig farms in the area and the subsistence farmer held, on average, a herd composed of a maximum of eight pigs (often a sow and offspring) per farm (District Agricultural Directorate, Unpublished report). Pigs are predominantly reared under an extensive production system in which the animals are left roaming free, except during the rainy season (between November and March) when crops are still in the field and the pigs are kept indoors to protect the season's production. The typical pig pens, also called *Tanga* in the local language, are constructed from mud and wooden poles and are covered by grass or corrugated iron. Each farm generally maintains only one pen without internal divisions, located in the backyard.

Sampling protocol

Pigs

The protocol used to determine the sampling frame was based on the information provided by the District Agricultural Directorate, Mozambique. 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. As the pig population size in each of the different villages was not available, it was decided to distribute the number of farmers equally across the villages, and farmers were selected for the survey following a multistage sampling approach. Of the 19 villages, one was excluded from the study as the pig farms

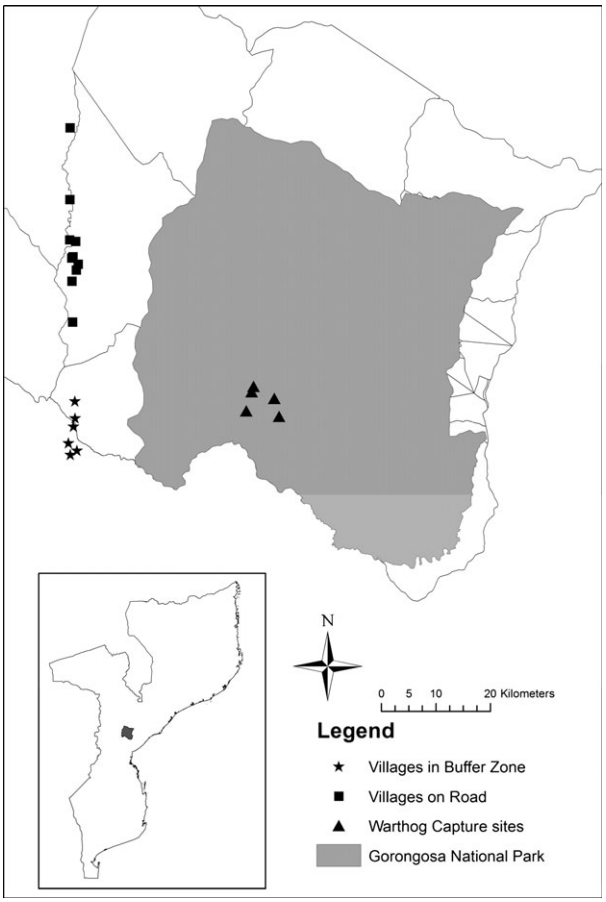

Fig. 1. Map of the Gorongosa district, including the area of the Gorongosa National Park, and the locations where warthogs and pig farms were sampled.

had been depopulated. The remaining 18 villages were grouped according to their proximity to the GNP boundary. Six villages located at the edge of the GNP (<10 km) are referred to as the Gorongosa National Park Buffer Zone (GNPBZ). The additional 12 villages, at an average of 15 km from the GNP boundaries, are referred to as rest of district (ROD) (Fig. 1). The GNPBZ is characteristically rural with a lack of basic infrastructure such as electricity and 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 95% level of confidence. Villages were considered as clusters of pig farms. In each village, pig farms were chosen randomly following a multistage sampling approach, and up to five pigs per farm were sampled. The minimum sample size required was 385 pigs as calculated using the free software WIN EPISCOPE 2.0 (www.clive.ed.ac.uk/winepiscope).

Sample and data collection

Warthogs

Twelve warthogs from four different locations aged <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®, DanWild LCC, Austin, TX, USA) at a distance of 5–8 m. A dose of 250 mg of Zoletil® (Virbac, Centurion, South Africa) 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 immobilization and reduction of recovery time (Kock and Burroughs, 2012). Approximately 5 ml of blood was obtained from the anterior *vena cava* in each animal using 10-ml plain Vacutainer® tubes (Becton, Dickinson and Company, Franklin Falls, NJ, USA).

Soft ticks in pig pens

Twenty farms from the study area (GNPBZ and ROD) were assessed for the presence of soft ticks using the vacuum aspiration method (Butler and Gibbs, 1984; Vial, 2009; Ravaomanana et al., 2010; Jori et al., 2013). A petrol-powered mulching blower/vacuum was used to collect material from different parts of pig pens and small mammal burrows in close proximity to the pen. Spades were used to collect bigger volumes of material where appropriate. Soft ticks were collected by spreading litter on black plastic sheets exposed to the sun. Specimens were placed in a plastic sample bottle with sand at the bottom and kept in cool, dark conditions until they were dispatched to the Transboundary Animal Disease Programme (TADP), OVI in South Africa for further analysis. The specimens were transported under a permit issued by the South African Department of Agriculture, Forestry and Fisheries.

Pigs

A total of 629 pigs of local breed ranging from 6 months to 3 years old were sampled from 310 small-scale farmers clustered in 18 villages. Sera were obtained by standard methods, transferred to labelled cryotubes and stored at −20°C until sent to the TADP to be tested for antibodies

against ASFV. Aliquots of these sera were also sent to the *Instituto de Recursos Naturales y Agrobiologia de Salamanca* (CSIC) in Spain to be tested for antibodies to the salivary proteins of *Ornithodoros* spp. ticks.

Questionnaire

At the time of blood collection, 314 pig owners were interviewed using an extensive questionnaire. The GPS coordinates of the farms were recorded, and the presence of soft ticks or other ecto-parasites was also noted. Information about the sampled animals, including age, sex, health status, clinical signs of haemorrhage, herd size, breed and the suspicion of ASF or recent fatalities was gathered. In addition, information on the farm type, husbandry and feeding practices was obtained to identify possible risk factors for the transmission of ASFV. A presumptive diagnosis for ASF was assessed according to clinical details given by the pig owner. Soft ticks were shown to farmers to assess whether similar parasites have been observed in their premises or village.

Sample and data processing

ASFV antibody detection using ELISA

Serologic analysis was performed using an indirect Enzyme-linked Immunosorbent Assay (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.

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 an anti-mycotic. The soft tick homogenates were centrifuged at 10 000 ***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 (Malmquist and Hay, 1960). One hundred µl of supernatant from tick homogenates were inoculated into peripheral blood mononuclear cells (PBMC) cultured in flat bottom 96-well plates, according to standard procedures. The plates were observed for up to 7 days, and virus was harvested when haemadsorption was observed and stored at −80°C. All samples were subjected to three serial passages on PBMCs, with each passage performed on a weekly basis. Samples were considered negative for ASF virus if no haemadsorption was observed.

DNA was extracted from 200 µl of each tick homogenate and recovered in a final volume of 50 µl DNA solution using the Qiamp kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's instructions. A nested PCR that targets the C terminal end of the *p72* gene was used to screen soft tick samples for the presence of ASFV DNA (Basto et al., 2006). All DNA samples were tested for tick mitochondrial 16S rDNA according to previously published methodology (Black and Piesman, 1994; Vial, 2009) to exclude the occurrence of false-negative results due to inhibitors in the tick supernatant.

Detection of antibodies against tick salivary protein

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

Statistical analysis

To determine 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 ($n = 112$). The number of months between time of sampling and the last reported outbreak of ASF on farms was calculated to determine any effect of the time elapsed after the outbreak on the prevalence results. Farms were subsequently divided into those with pigs sampled <8 months after a reported outbreak ($n = 132$) and those sampled more than 8 months after a reported outbreak ($n = 84$). The remaining sampled animals ($n = 418$) were from areas where suspicions or reports of ASF outbreaks had not been recorded. A farm was considered to have been at risk of the disease or exposure to the arthropod host when at least one pig was found positive to one of the two serological tests used. 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-squared test and odds ratio calculations for homogeneity of two populations (Fischer's 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 (CI) were calculated with EPI INFO v.3.5.3, 2011 (CDC, Atlanta, GA, USA).

Results

Questionnaire

A herd was considered as the total number of pigs belonging to the same owner. The average herd size per farm was calculated to be eight 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% (128/629) of the sampled populations of pigs were adults with 85.9% female pigs (110/128) and 14.1% boars (18/128). Juveniles (<1 year) represented 79.8% (506/629) of the 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% and 35% of respondents fed kitchen leftovers 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.

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

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

Over 39% (123/314) of respondents mentioned having experienced what they suspected to be ASF outbreaks in the past and reported pig deaths. An outbreak reportedly occurred prior to 2005 and was subsequently followed by two outbreaks in November 2006 and February 2007. Among farmers suspected to have experienced ASF outbreaks, 40.7% (50/123) mentioned that not all animals died during the outbreaks. Almost 35.8% (44/123) of affected farmers stopped rearing pigs for at least 1 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 191 farmers were mostly from the ROD (80.6%), while 19.4% were from the GNPBZ.

Detection of antibodies against ASFV

Warthogs

The prevalence of antibodies directed against ASFV in all warthogs tested ($n = 12$) was 75%, 95% CI [42.8–94.5]. The sero-positivity in adult animals ($n = 9$) was 66.7%, 95% CI [29.9–92.5], and in individuals <1 year old ($n = 3$), it was 100%.

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, 629 pig serum samples were collected from 310 farms in 18 villages (Table 1): 170 pigs from 79 farms were sampled in six villages from the GNPBZ, and 459 pigs from 235 farms were sampled in 12 villages in the ROD. The bias in sampling towards the ROD was due to the relatively low number of farms and pigs in the GNPBZ. The overall ASFV antibody prevalence in domestic pigs was 9.1% 95% CI [7–11.7]. The ASFV sero-prevalence in pigs from GNPBZ was 12.5%, 95% CI [7.4–17.6] and in pigs from the ROD was 7.8%, 95% CI [5.4–10.3] (Table 2). However, these differences were only marginally significant (OR = 0.59, $P = 0.05$).

Of the 314 farms tested, 12.6%, 95% CI [8.9–16.3] had pigs that were sero-positive to ASFV. There was no significant difference in sero-prevalence based on region (Table 2; OR = 0.97, $P = 0.53$) with 12.8%, 95% CI [5.4–20.2] sero-prevalence in farms from GNPBZ ($n = 79$) and 12.5%, 95% CI [8.2–16.8] in farms from the ROD ($n = 231$).

When the sero-prevalence in different age groups was compared, 91.2%, 95% CI [80.7–97.1] of the sero-positive animals were younger than 1 year with the remaining 8.8%, 95%, CI [2.9–19.3] older than 1 year (OR = 0.42, $P = 0.043$).

There was a strong association between the reported occurrence of recent outbreaks resulting in 40.4%, 95% CI [27.6–54.2] of the positive animals being pigs that had been exposed to an outbreak between 1 and 8 months prior to sampling (OR = 5.7, $P = 0.004$). There was also a significant association (OR = 1.93, $P = 0.0276$) between those farms which experienced recent outbreaks and the presence of piglets (younger than 6 months).

Soft tick collection and ASFV and DNA detection

Two of the 20 inspected pig pens in the GNPBZ and ROD were found to be infested with *Ornithodoros* spp. ticks, cor-

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	Percentage of pigs with abs to ASFV	Percentage of farms with pigs sero-positive to ASFV	Percentage of pigs with abs to tick salivary proteins	Percentage of farms with pigs sero-positive 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 [7.0–11.7]	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.

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)

Sero-prevalence (%)	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

responding to an infestation prevalence of 10%, 95% CI [3.1–23.1]. In both cases, the pens were in the GNPBZ. African swine fever virus DNA was detected in homogenates of ticks from both of the infested pig pens, and live virus was isolated from one pool (results not shown). All samples, with the exception of the negative controls, were positive in the 16S soft tick mitochondrial gene PCR confirming the integrity of template DNA and precluding the possibility of false-negative results (results not shown).

Detection of antibodies against tick salivary protein

Warthogs

The prevalence of antibodies against tick salivary proteins in the sampled warthog population was 77.8%, 95% CI [40.0–97.2]. When different age groups of warthogs were compared, all warthogs <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, 44 from nine 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 the 585 domestic pigs tested was 32.7%, 95% CI [29.2–36.9], with 42.0%, 95% CI [36.4–47.7] of farms positive. Approximately 45% of pigs (64/140 sera) in the GNPBZ were sero-positive to tick salivary proteins (95% CI [37.3–54.3]), compared to 28.9%, 95% CI [24.8–33.4] of pigs (129/445) in the ROD. The on-farm prevalence in 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). Significant differences were observed in the proportion of sero-positive domestic pigs (OR = 0.48, $P = 0002$) and sero-positive farms (OR = 0.27, $P = 0.0001$) between the two areas (Table 2).

When the sero-prevalence of tick salivary proteins in pigs of different ages was compared, antibodies were found in 32.0%, 95% CI [27.9–36.3] of the animals younger than 1 year ($n = 493$) and 38.0%, 95%, CI [28.1–48.8] of the animals older than 1 year, respectively, but these differences were not significant ($P = 0.16$).

Correlation between prevalence of antibodies to 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, $P = 0.01$). Of the 301 farms, 28.6%, 95% CI [14.6–46.31] had pigs that tested positive in both assays. However, this association was only marginally significant (OR = 0.51, $P = 0.056$). Sera collected from pigs in four villages in the ROD, and one village in the GNPBZ only contained antibodies to tick antigens (Table 1).

Discussion

The socio-economic impact of ASF is due to high mortality and morbidity rates that can threaten food security, as pigs represent a source of cheap protein and 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). This is demonstrated by the fact that 36% of farmers interviewed in this study ceased to farm with pigs for at least 1 year following an ASF outbreak. The questionnaire used in this study 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; Costard et al., 2009; Fasina et al., 2010; Penrith et al., 2013) and could account for the low number of adult pigs observed in our study.

The combination of serology and a questionnaire provided a good indication that ASFV was circulating in the area a few months before blood samples were collected, as >40% of ASFV sero-positive animals had been exposed to an ASFV outbreak <3 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 (Penrith et al., 2007; Matos et al., 2011) 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). African swine fever is endemic in both these areas, and regular outbreaks of the disease occur, often characterized by lower-than-usual mortality rates in adult domestic pigs (Haresnape and Wilkinson, 1989; Penrith et al., 2004a).

The results of this study strongly suggest that the sylvatic cycle is present in the GNP. The serological survey in warthogs presented in this study, despite its limited size, is the first described in Mozambique to date. The high sero-prevalence to ASFV (75%) and tick salivary proteins (78%) is consistent with previous observations in warthog populations from other parts of East and southern Africa (Penrith et al., 2004a; Jori and Bastos, 2009; Jori et al., 2013). Although the GNP is not fenced, warthogs do not venture too far away as they are hunted for food by local rural communities once they reach the GNPBZ and ROD.

The prevalence of ASF per farm was highly similar in both areas; however, a marginally significant ($P = 0.05$) higher sero-prevalence was detected in the GNPBZ compared to ROD (12.5% versus 7.8%). Furthermore, 80% of farmers who reported not having experienced any outbreaks on their farm were located in the ROD area. This suggests that ASFV infections could be more common in the buffer zone farms, which are located closer to the park and to a permanent source of virus from wild host than the farms in the ROD.

The results of the serological assessment of antibodies to tick salivary protein provided evidence that 42% of the farms had pigs that had been bitten by soft ticks recently and support the premise that contact between soft ticks and domestic pigs is widespread in the area. In addition, the significant differences in 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. The detection of ASFV DNA and infectious virus in ticks collected from two farms outside GNP provide evidence of the presence of the infected ASFV vector in pigsties outside wildlife areas. Pigs from a number of farms did not have antibodies to ASFV, but were positive for antibodies to the rOmTSGP1 antigen, which could indicate that the ASF infection rate in ticks may be low and needs further study.

Conclusions

Results from this study showed that ASFV is maintained within the sylvatic cycle involving warthogs and *Ornithodoros spp.* ticks in the GNP as evidenced by the presence of ASFV-infected ticks in warthog burrows and the high percentage of warthogs with antibodies to the virus. The demonstration of antibodies to salivary proteins of *Ornithodoros* spp. ticks and finding ASFV-infected *Ornithodoros* spp. ticks in pig pens suggest that domestic pigs are periodically exposed to soft ticks which may lead to the dissemination of the virus beyond the sylvatic cycle. However, the contribution of the domestic pig/tick cycle in the epidemiology of the disease in our study area seems to be limited

considering the low level of infestation of pig pens with infected ticks. Conversely, the survival rate among infected pigs is higher than would be expected suggesting that domestic pigs in GD may have developed a degree of resistance to the pathogenic effect of ASFV, similar to what has previously been described for Angonia and the Mchinje district of Malawi.

It is likely that the presence of this sylvatic cycle at the GNP interface acts as a permanent source of ASFV for domestic pigs. Further studies are necessary in Mozambique to identify the role of other wildlife areas as sources of ASF to design management plans to control and prevent the transmission of the virus to the domestic pig value chain and its dissemination at regional, national or international level.

Acknowledgements

We would like to express our gratitude to The Wellcome Trust (Project number 210183. 183 AHDW03/04) for funding the entire research project. We thank the field staff at the Agricultural District Directorate in Gorongosa and The Carbon Sequestration Project in Nhambita at Gorongosa National Park Buffer Zone for their support during the field works. The Gorongosa National Park authorities and especially to Dr. Lopes Pereira who helped us in sampling warthogs must be thanked. Our grateful acknowledgement goes to the technicians of the Regional Veterinary Laboratory (Mr. Geraldo Cherene, Mr. Manuel Popinsky and Mr. Jacinto Chapala) and colleagues (Ms. Brenda Botha and Mr. Jan Esterhuysen) of the Transboundary Animal Disease Program of OVI in RSA for their valuable contribution during sampling pigs and helping during the laboratory testing. The former head of Regional Veterinary Laboratory (Dr. Elisabeth Specht) should be also thanked for her unfailing support and allowing the technicians their involvement in the field work.

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