Do wild suids from Ndumo Game Reserve, South Africa, play a role in the maintenance and transmission of African swine fever to domestic pigs?

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ABSTRACT

Warthogs (*Phacochoerus africanus)* and bushpigs (*Potamochoerus larvatus*) are considered as the wild reservoirs of ASF. They are both present in Ndumo Game Reserve (NGR), located in the Northern South African Province of KwaZulu on the border with Mozambique. In that area, the occurrence of tick-warthog sylvatic cycle of ASF has been suspected for years. To assess if wild suids represent a risk of ASF virus spillover to domestic pigs, wild suid abundance and incursions outside NGR boundaries were estimated using transect counts, fence patrols and camera traps. Also, the presence of *Ornithodoros* ticks was explored in 35 warthog burrows within NGR. In addition, blood samples were taken from 67 domestic pig farms located outside NGR to be tested for ASF antibodies. Information on interactions between domestic and wild suids and ASF occurrence was gathered using interviews with pig farmers (n = 254) in the study area. In conclusion, the bushpigs and warthog's population estimates in NGR are 5 and 3–5 individuals/km^2, respectively. Both species move out of the reserve regularly (15.4 warthogs/day and 6.35 bushpigs/day), with movements significantly increasing in the dry season. Some farmers observed warthogs and bushpigs as far as 8 and 19 km from NGR, respectively, but no reports of direct wild-domestic suids interactions or ASF outbreaks. Also, no soft ticks were detected in all warthog burrows and all the pig blood samples were negative for ASF antibodies. The absence of ticks in warthog burrows, the absence of antibodies in pigs sampled, the absence of reported outbreaks, and no familiarity with ASF in the study area, suggest that a sylvatic cycle of ASF is, at present, unlikely in NGR. This conclusion must be confirmed by a larger survey of warthog burrows and monitoring potential antibodies in warthogs from NGR.

Keywords: African swine fever, bushpigs, domestic pigs, South Africa, sylvatic cycle, warthogs

1 INTRODUCTION

In Southern Africa, warthogs (*Phacochoerus africanus*) and bushpigs (*Potamochoerus larvatus)* are considered as potential wild reservoirs of African swine fever (ASF). Warthog is found in open savannah habitats in most of Sub-Saharan Africa and their densities range from 1 to 10 individuals/km^2 in protected areas (Cumming, 1975). The bushpig is mainly distributed in forested areas of eastern-, southern- and western-central Africa with densities ranging from 1 to 10 individuals/km^2 in protected areas (Venter et al., 2016). ASF is a highly infectious and haemorrhagic disease affecting exclusively domestic and wild suids, a significant threat to the pig industry worldwide (Costard et al., 2013). In Africa, the virus is maintained in two epidemiological cycles: the sylvatic cycle, involving warthogs and bushpigs, and the domestic cycle involving domestic pigs. The disease can be transmitted by direct contact with an infected animal, its body parts or its secretions, or indirectly through fomites or contaminated food (Chenais et al., 2018). Warthogs do not become viraemic, apart from a brief period as young warthog piglets, and thus do not transmit the disease directly (Plowright, 1981; Thomson, 1985). Warthogs transmit ASF through soft tick bites, with *Ornithodoros moubata* acting as a vector of the disease among warthogs, but also between warthogs and other suids species, particularly domestic pigs. These ticks are the natural reservoir maintaining the disease (Pereira de Oliveira et al., 2019). In the absence of *Ornithodoros* ticks, warthogs do not excrete sufficient amount of virus to transmit the disease horizontally to domestic pigs, therefore, the presence of warthogs is not enough to maintain a permanent source of virus in the environment (Jori & Bastos, 2009). The bushpig has been proven to be naturally resistant by experimental infection (Oura et al., 1998). Previous studies suggest that bushpigs could have a potential role in the transmission of ASF (Okoth et al., 2013) because they have been occasionally found carrying the virus in different parts of Africa. Transmission of the virus through direct contact to susceptible domestic pigs has been proven in captivity (Anderson et al., 1998). However, their potential to maintain ASFV in its natural habitat and its transmission to domestic pigs has not been proven (Ravaomanana et al., 2011; Ståhl et al., 2014).

In Southern and East Africa, the maintenance of ASF in a sylvatic cycle linked to the presence and maintenance of ASF virus in *Ornithodoros* ticks in warthog burrows represents a challenge for the development of pig farming in rural areas due to a constant risk of ASF spill over to domestic pigs (Quembo et al., 2016). This can occur through direct physical or indirect contact between wild and domestic suids through the sharing of environmental resources such as soil, forage and water, facilitating disease transmission (Kock, 2005). ASF is a disease of global concern as it has the capacity to spread worldwide and can lead to severe socioeconomic impact, both in areas where it is newly introduced and where it is endemic (Chenais et al., 2019). In South Africa, ASF is a notifiable disease with a disease-free area and a control area defined in 1935 where ASF has been reported as endemic. The spread of ASF out of the control area should be notified internationally to the OIE and could result in an international ban of pigs and pork trade involving the disease-free area (OIE, 2011).

ASF is endemic in Mozambique, adjacent to areas with extensive subsistence pig farming in South Africa. The first laboratory-confirmed outbreak of ASF in Mozambique was reported in 1960. A number of outbreaks have since been observed around the country, including in the region south of Maputo, within 30 km of our study site. Though movements of domestic pigs from one province to the other played a role in disease transmission and spread, the sylvatic cycle was considered equally important particularly in the vicinity of conservation areas where warthogs and bushpigs are common (Penrith et al., 2007).

The complexity in the eco-epidemiology of this multi-host pathogen disease makes it hard to implement disease mitigation strategies. Among other factors, farming practices in neighbouring communities, the distance from the neighbouring community to a protected area (PA), the availability of resources in PAs, and the nature of the fence (surrounding PAs) stimulate wildlife-livestock interactions that promote the introduction and spread of diseases (Kukielka et al., 2016). Cowled and Garner (2008) argued that behaviour, animal density, distribution, contact rate and habitat connectivity of both the vector and susceptible species are important for understanding how diseases are transmitted between wildlife and livestock. For ASF, the higher the wild suid density within a PA the greater the chances that some of the suids will get into contact, directly or indirectly, with domestic pigs. Also, the further wild suids move from the PA into farmland, the greater the risk of disease transmission. Pech and McIlroy (1990) argued that the movements of pigs increase the likelihood of contact between infected and uninfected pigs, and thus the spatial extent, and velocity of the spread of a disease.

Fencing has a long history in wildlife conservation and in many cases it has proven to be an effective tool for keeping wildlife out of specific areas, controlling animal movements and disease outbreaks (Durant et al., 2015). Fences are often deleterious to wildlife, preventing access to food and water as well as natural migration, but also protecting wildlife from human threats (Pirie et al., 2017). However, given that they undergo changes, fences probably do not work in the same way and with the same efficiency in all cases. Floods, breaks due to wildlife movement and damage due to theft are different factors increasing fence permeability. Therefore, a fence requires regular inspection and maintenance (Jori et al., 2011).

This study aims to provide quantitative information about the potential presence of a sylvatic cycle at the wildlife livestock interface around Ndumo Game Reserve (NGR), which is a part of the Lebombo Transfrontier Conservation Area, a transboundary conservation initiative including protected areas from South Africa, Swaziland and Mozambique. NGR is home to warthogs and bushpigs which play a role in maintaining and transmitting livestock diseases (Costard et al., 2009). Also, it is adjacent to a communal area with a substantial amount of farmed pigs, generating a wildlife-livestock interface. In addition, the reserve is within the boundaries of the ASF control zone in South Africa. That means the area is suspected to have a sylvatic cycle in which *Ornithodoro*s tick populations colonize warthog burrows, representing a permanent source of ASF. The reserve shares its borders with Mozambique where ASF is considered to be endemic. Understanding the extent to which wild suids from NGR interact with surrounding domestic pigs close to NGR is critical for the surveillance and control of ASF. Therefore, the specific objectives of our study were: i) to estimate the population size of warthog and bushpigs in NGR; ii) to assess the presence of ticks in warthog burrows from NGR; iii) to study the movements of wild suids between NGR and the adjoining farming areas and their potential interactions with domestic pigs.

2 METHODOLOGY

2.1 Study Area

NGR is a 10 117 ha wildlife reserve bordering both the Mathenjwa community of Northern KwaZulu Natal province, South Africa and also Mozambique (Figure 1). The boundary of the reserve is fenced except on its northern side, where the seasonal Usuthu River separates South Africa and Mozambique. There are two seasons: wet (October–March) and dry (April–September) with an average annual rainfall of 638 mm. The mean annual temperature is 21.9°C with dry season temperatures often reaching well above 40°C. Villages around the eastern, western, and southern boundaries of the reserve are typical of South African communal areas where subsistence farming is the major economic activity. The villagers grow mainly maize and keep livestock which includes cattle, goats, pigs and chickens. Dipping, the driving of cattle through a specially constructed concrete tank with water and an acaricide to control ticks on cattle, is a regular activity in these villages.

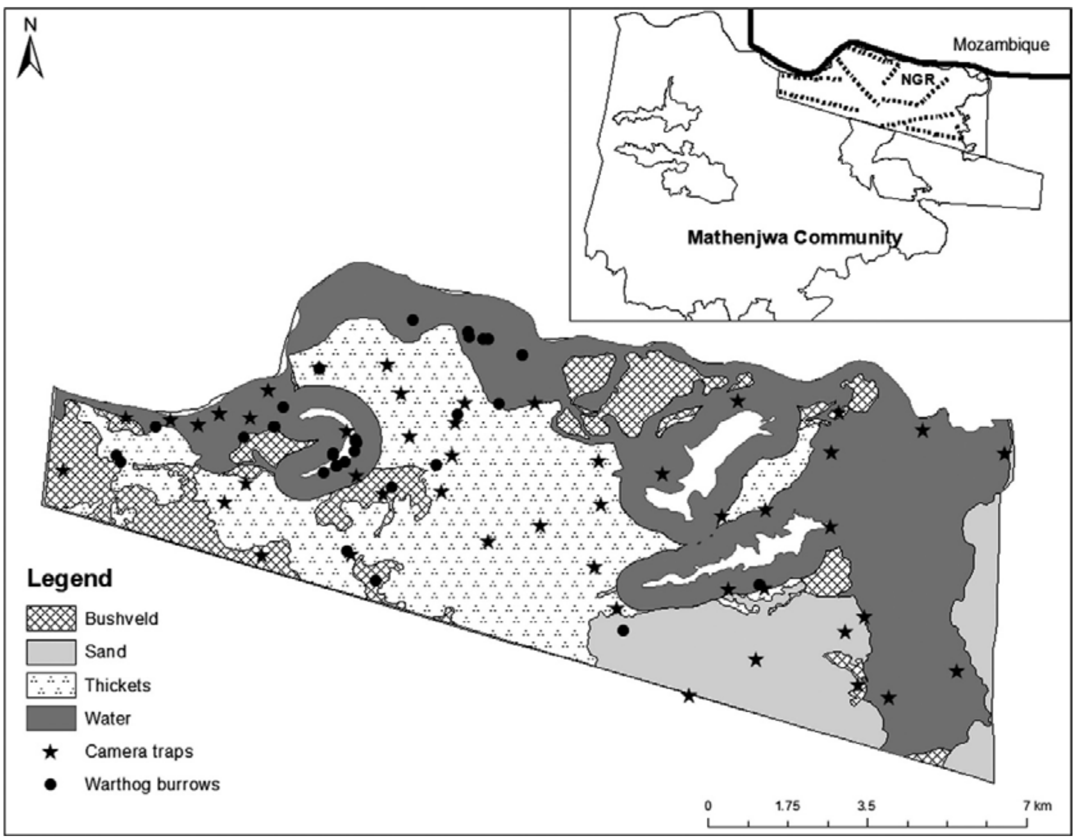

FIGURE 1. The distribution of four vegetation types present in Ndumo Game Reserve (NGR) as well as surveyed warthog burrows (black dots) and the location of the static camera traps (black stars). The transects walked (dashed lines on the top right map) during our study are presented as dotted lines within NGR in the upper figure

NGR is home to a variety of mammals including different species of antelope, black and white rhinos, hippos, crocodiles and both species of wild suids (bushpigs and warthogs). The

Usuthu and Pongola Rivers feed the pans (water-logged areas), namely Nyamithi, Banzi, Shokwe, Usuthu and Pongola pans. Within the game reserve, fence and reserve patrols are a daily activity for field rangers to combat poaching.

The reserve has seven major vegetation types: Western Maputaland Clay Bushveld, Makatini Clay Thicket, Lowveld Riverine Forest; Western Maputaland Sandy Bushveld, Sand Forest, Subtropical Alluvial Vegetation; and Subtropical salt pans (Mucina et al., 2006). In order to facilitate interpretation and analysis of the results, only four vegetation types were used after grouping vegetation types with similar physical vegetation structure and plant species: Bushveld, Thicket, Sandveld and Water (Figure 1).

2.2 Abundance estimation

2.2.1 Warthog numbers derived from annual transect counts

NGR has eight permanent line transects used for annual game counts, ranging from 1.5 km to 8 km with a combined length of 50.915 km. The transects cut across the four vegetation types. ArcGIS 10.6 (ESRI, Redmont, California, USA) was used to measure lengths of transects based on their position within different vegetation types (Table S1). These transects were used to conduct annual warthog counts and bushpig track counts. To maximize the accuracy of estimates, the eight transects in NGR were walked 16 times annually, during the dry seasons (April–September) of 2017 and 2018 and using distance sampling methodology. The researcher, together with NGR game scouts and students from Tshwane University of Technology (TUT), conducted the counts in the morning (5–8 a.m.) to maximize chances of detecting warthogs because they are difficult to detect later in the day when they rest in the shade. Two observers, each focusing on one side of the transect, counted warthogs observed and, for each encounter, recorded the coordinates of the observer, distance from observer to animal (r), group size (n) and angle of the animal from the transect (ϴ). In addition, transect count data from 2013 to 2016 were made available for analysis by Ezemvelo KwaZulu Natal Wildlife (EKZNW). The number of warthogs in the reserve was estimated using the Distance sampling software (Distance V8) using the negative exponential cosine model as the detection function. This function computes the likelihood contributions for off-transect sightings distances, scaled appropriately, for use as a distance likelihood. Only those years with consistent and reliable count data that fitted statistical models were used. Warthog transect sightings were mapped according to vegetation types using ArcGIS V10 and recorded in Excel (Microsoft Corporation, 2018) as follows: (a) year of transect counts (b) transect number (c) vegetation type in which that observation was made (d) length of the transect within that specific habitat (e) the number of warthogs seen and (f) the number of warthogs per km of transect. Descriptive analyses and linear mixed effect regression (Pinheiro & Bates, 2006) were performed. Summary statistics, including mean and standard error of the mean (*SEM*), were computed.

2.2.2 Bushpig relative abundance using the transects

Bushpigs are crepuscular and nocturnal and seldom observed. Therefore, the only realistic method of assessing bushpig abundance is the use of indirect evidence provided by tracks, as well as photographs obtained by static camera traps. Bushpig track transect counts, using the eight transects used for warthogs, were conducted on a separate occasion (June 2018–December 2018). In order to maximize statistical reliability, each transect was walked 21 times recording bushpig tracks encountered. The researcher was assisted by game scouts with experience in animal tracking. Bushpigs tracks, when compared to those of warthog, have broader hoofs and their claw mark show clearly on the tracks. To avoid the risk of double-counting, tracks were erased using branches. For each observation the following data were entered in MS Excel: date, repetition, transect number, vegetation type, length of that particular vegetation type in a transect, GPS coordinates of where the observation was made, number of tracks and, number of events (an event is a discrete cluster of tracks on a particular transect). Tracks indicated the total count of tracks recorded per event. A linear mixed-effects regression model was performed to predict the mean number of events and tracks based upon habitat (Pinheiro & Bates, 2006), allowing an assessment of relative bushpig abundance indicators (tracks and events) in each of the four habitat categories in NGR.

2.2.3 Camera trap surveys

A pilot study was conducted from May to July 2017 to fine-tune the camera trap data collection method. Twenty-four static camera traps were deployed based on field rangers' perception of areas where bushpigs could be found. During the pilot study, the duration of each camera trap placement was variable because NGR has a history of extensive theft of cameras by poachers. Camera traps were removed immediately after signs of human activity were observed near a camera. After the pilot study, 48 camera trap stations were randomly positioned within the four main vegetation types (Figure 1). Camera trap surveys were conducted from February to December 2018. At each camera trap station, a single camera was tied to a tree or stump at bushpig height (30–50 cm above the ground) or higher (150–200 cm) with a downward pointing inclination, depending on the vegetation. Surrounding vegetation that would promote triggering by wind in front of the camera was cleared. Despite the nocturnal behaviour of the target species, camera traps were set to record photographs 24 hr a day. Trophy Cam® (Bushnell Outdoor Products, USA) cameras had continuous triggering of a one-second interval between consecutive images while ScoutGuard® cameras were set to record 10 s video footage each time a movement was detected within the distance range (15 m). The date and time were shown on each photograph/video. The objective was 60 consecutive days periods of observation, but the period was sometimes shortened in the case of poacher activity around the camera traps. All images were downloaded from the cameras after which the date and duration of each observation of suids were entered into MS Excel, each record reflecting an event per specific camera trap station (an event is an observation of at least one suid within a single 30-min time interval). The following were recorded (a) camera trap station ID (b) GPS coordinates of the camera, (c) habitat in which the camera trap was installed (d) bushpig count (the total number of animals seen for all the pictures taken during one event on a camera trap station) (e) duration of each event (f) time duration since the previous event. Wild suid rate is the number of wild suid individuals (bushpig or warthog) photographed per camera day. Likewise, events are the number of events per camera day. Due to the small sample size of suid observations in the sand and bushveld habitats, these two vegetation types were merged into a single category. Log transformation of raw data was performed to obtain a normal-like statistical distribution of abundance values. A one-way ANOVA was performed for detecting differences in wild suid rate between habitats.

2.3 Fence survey

In February 2018, a pilot fence survey was conducted to identify portions of the fence with holes used by wild suids. These holes are termed sites. The fence was divided into four main sections to relate fence crossings by pigs to the localities of farms. Thirty-two sites were identified on the western part of the reserve, 57 on the South-western side, 46 on the south-eastern and 6 on the eastern side. Two bouts of fence surveys were conducted to identify wild suid activities at the respective sites: the wet season survey was conducted on 27 consecutive days of February 2018 and the dry season survey was conducted in August 2018 for 30 consecutive days. The number and location of all the sites were the same for both surveys. On each day, each site was inspected for the presence of bushpig and/or warthog tracks. Once tracks were observed, the researcher, with the help of experienced tracker game scouts, took note of the species responsible, identity of the site, and the number of tracks counted. For each observed set of tracks, the species was identified, based on the footprints (on a few occasions droppings). Three items were recorded for each site: (a) whether tracks indicated wild suids crossing the fence (= a crossing event); (b) the species of suid; (c) an estimate of the number of wild suids that had crossed at that point, based on the tracks leading to and from the site. A crossing event refers to an occasion when one or more warthogs/bushpigs crossed the fence at a specific site. The mean number of crossing events/site/day during a survey (f_c) is the mean daily number of crossing events/site for a specific section of the fence during the survey (Table 1). Similarly, the mean number of wild suids/site/day during a survey (f_p) represents the mean daily number of wild suids/site for a specific section of the fence during the survey (Table 1). Since the statistical distribution of the number of crossings was similar to that described by a negative exponential function, analyses were performed on the natural log-transformed values of f_c and f_p. For detecting differences among the four sections of the fence, a one-way ANOVA was separately performed on f_c as well as on f_p classified by fence section. For detecting differences between the dry and wet seasons, a repeated-measures ANOVA was separately performed on f_c as well as on f_p categorized by season. Standard errors of the estimates (*SEM*) for the number of wild suids crossing the whole fence each day were generated by performing 1,000 bootstrap samples of the observations at each fence site and finding the *SEM* of the 1,000 estimates of the wild suid crossing rate. R V3.4.4 (R Core Team, 2020) was used for all statistical analysis and statistical significance was set for *p*-value lower or equal to .05.

TABLE 1. Fence survey results for bushpigs and warthogs in NGR for the dry and wet season with two variables: The number of crossings per site per day and the number of pigs per day

Season	Fence section	West	South West	South East	East	Whole fence Bootstrap	1-way ANOVA *df* = 137,3
Wet Season	No sites	32	57	46	6	141	
	No.surveys†	27	27	27	27	27	
	No.site-days†	864	1539	1,242	162	3,807	
	Crossing events/site/day	*0.0046*	*0.0012*	*0.0008*	*0*	*0.0066*	*F = 1.7414* *p = .1614*
		0.0335	**0.0292**	**0.0418**	**0.0062**	**0.1107**	***F* = 1.1436** ***p* = .3338**
	Mean no. pigs /day	*0.4074*	*0.0741*	*0.0741*	*0*	*0.56 ‡*	*F = 3.0318** *p = .0315*
		2.5185	**3.7037**	**3.9629**	**0.0741**	**10.26 ‡**	***F* = 0.9285*** ***p* = .4289**
Dry season	No. surveys‡ No. site-days‡	30 960	30 1,710	30 1,380	30 180	30 4,230	
	Crossing events/site/day	*0.0343*	*0.0187*	*0.0007*	*0.0055*	*0.0592*	*F = 8.6126* *p < .0001*
		0.0542	**0.0392**	**0.0246**	**0.0277**	**0.1457**	***F* = 2.2483** ***p* = .0855**
	No. pigs /site/day*	*3.0333*	*3.1333*	*0.1000*	*0.1000*	*6.367 ‡*	*F = 9.0643** *p < .0001*

		5.0000	**6.3666**	**3.6666**	**0.4000**	**15.4** ‡	**$F = 1.4405$*** **$p = .2337$**
Effect of season: **Repeated-measures ANOVA**	Crossing events/site/day						*$F = 39.100$* *$p < .0001$*
							$F = 2.0090$ **$p = .157$**
***df* = 278,1**	No. pigs/site/day						*$F = 46.83$* *$p < .0001$*
							$F = 34.665$ **$p \leq 0.0316$**

Note: Outcomes for 1-way and repeated measures ANOVA are indicated. The numbers in bold indicate warthogs results while the italicised ones are for bushpigs.

* Data are represented as the number of pigs crossing each day. However, since fence sections each had a different number of sites, statistics are performed on the number of pigs per site per day.

[†] Figures for no. surveys and no. site-days apply to both bushpig and warthog.

[‡] Bootstrap estimates of the standard error of the mean no. pigs crossing the whole fence each day.

2.4 Farmers interviews

A structured questionnaire was used to collect data from all smallholder pig farmers within the Mathenjwa community (*n* = 254) from April 2017 to December 2017. The purpose of the interview was to gather information on potential wild-domestic pigs interactions observed by the local rural communities and to gather information on prevalent pig diseases in the area. Pig farmers were identified at dip tanks and interviews were conducted on their farms. The 45 min interview comprised of 22 questions administered in the Zulu language by the first author. To ensure that no pig farm was missed, the exhaustive snowball method was used (Etikan et al., 2016). Farmers were asked if they observed wild suids near their farm (Table S2). Observations were clustered into two groups of distances from their farms to the observed suids: near (0–20 km) and far (>20 km). This clustering represented farmer's opinions on contact rate of wild and domestic pigs from those that are either very close or very far away from the reserve. If any observation was made, they were asked to respond to whether they had seen the suids (a) in direct contact with domestic pigs (physical contact) (b) on their farms (close to domestic pigs) or (c) close to their farms or (d) elsewhere. They were also asked to comment on diseases of their domestic pigs and about potential outbreaks of ASF occurring in the area. Farmers were asked if their pigs got sick or died from any disease. If affirmative they provided symptoms. They also were asked to name diseases that were prevalent in the study area.

2.5 Soft ticks survey

Warthog burrows within NGR were identified with the assistance of game scouts during their regular patrols. When a warthog burrow was encountered, the researcher and the game scouts looked for signs of activity (tracks and droppings) then GPS coordinates were recorded. A 20-litre bucket was used to collect sand from each burrow which was spread in a thin layer across a large, black plastic sheet in the sun. Due to photophobic nature of soft ticks, the sunlight and warm temperatures encourage *Ornithodoros* tick movement and facilitates tick detection as well as collection. A minimum period of 30 min per burrow was allowed, to ensure that tick movement would be elicited and that all visible ticks were collected (Jori et al., 2013).

2.6 Serological survey

2.6.1 Sampling

A serological survey was conducted to investigate the potential exposure of domestic pigs in the study area to ASF virus. A total of 67 animals (one individual per farm) were sampled within a 15 km range of the NGR boundary. This sample size was calculated in order to detect a seroprevalence of 4.4% with 95% confidence. The sampling was done risk-based in order to increase the chances of finding positive pigs, if any. Samples were preferably collected from households with a recent history of clinical signs such as anorexia, weight loss or mention of previous pig's death. However, none of the sampled pigs showed any clinical symptoms of ASF during the time of the study. Whenever possible, animals that were 6-months-old and above were preferred for antibodies testing as it was assumed that the young ones were less likely to have been exposed to the diseases in their short lives and this could show cross-reactions due to their developing immune system.

At the farm, domestic pigs were held while blood samples were obtained from the anterior vena cava and collected into BD Vacutainer™ Serum Tubes with the assistance of qualified veterinary technicians. Sera were aliquoted and stored at 4°C and transported to the ASF Reference Laboratory of Transboundary Animal Diseases (TAD) at the Onderstepoort Veterinary Research, Agricultural Research Council (ARC-OVR), South Africa.

2.6.2 Laboratory analysis

Domestic pig blood samples were analysed for the presence of ASF antibodies. Antibodies against ASF virus were detected using the commercial competitive ASFV antibody Blocking p72 Enzyme-linked Immunosorbent Assay (ELISA) (manufactured by Ingenasa®, Madrid, Spain), based on purified p72 protein. The Ingezim PPA Compac kit was previously validated by the OIE/FAO/EU Reference Laboratories using 1,069 porcine samples. The relative measured sensitivity was 99.36% while the specificity was 98.6% (Gallardo et al., 2013). Using the classic epidemiological formula (Dohoo et al., 2003) we recalculated the real threshold prevalence according to our results.

$$P_r = \frac{P_t + Sp - 1}{Se + Sp - 1} \tag{1}$$

2.7 Ethics

The methods used for collecting data from pig farmers were assessed and approved by the University of Pretoria's human ethics committee (EC 161129-084). Permission to conduct the study within the NGR was also obtained from Ezemvelo Kwazulu Natal Wildlife authorities. Verbal consent from the local chief was obtained before project inception. For the interviewees, participation was voluntary, private and confidential and there was no penalty if they decided not to participate. Written informed consent was obtained from each participant.

3 RESULTS

3.1 Wild suid counts derived from transect counts

3.1.1 Warthog numbers from distance sampling and habitat preference

Using the Distance software, data for the years, 2013–2018 were analysed. The years 2013 and 2018 produced interpretable results. The estimation of the number of warthogs in NGR were n = 632, 95% confidence interval = $[490, 815]_{95\%}$ and n = 383, CI = $[271, 541]_{95\%}$ respectively. This suggests a population ranging between 400 and 500 warthogs, that is, 3–5 individuals/km^2 in NGR. Table 2 shows the raw counts of warthogs along the transects for each year. Warthogs had a significant preference for water areas compared to other vegetation types (one-way ANOVA p = .002).

TABLE 2. Annual warthog counts along transects in the four vegetation types of Ndumo Game Reserve

Year		Habitat				*n*
		Bushveld	Sand	Thicket	Water	
2013	Length (km)	8.34	9.7	17.5	15.36	236
	No. warthog	44	33	95	64	
	Mean density	**5.276**	**3.402**	**5.429**	**4.167**	
2015	No. warthog	15	5	46	184	250
	Mean density	**1.799**	**0.515**	**2.629**	**11.979**	
2016	No. warthog	36	25	20	154	235
	Mean density	**4.317**	**2.577**	**1.143**	**10.026**	
2017	No. warthog	12	28	25	104	169
	Mean density	**1.439**	**2.887**	**1.429**	**6.771**	
2018	No. warthog	13	1	67	60	141
	Mean density	**1.559**	**0.103**	**3.829**	**3.906**	
Overall	No. warthog	120	92	253	566	1,031
	Mean density/year	**2.88**	**1.9**	**2.89**	**7.37**	
	SEM	0.7999	0.6646	0.7932	1.5956	

Note: Habitat length is the length of the respective habitats in different transects. Mean density indicates number of warthogs/km. There is a significant preference for thicket and water (1-way ANOVA $p < .002$).

3.1.2 Bushpig relative abundance and habitat preference using line-transect counts

Linear mixed-effects ANOVA indicates that thickets (15.601 tracks/km, Table 3) had by far highest density of bushpig tracks when compared to Sandveld (2.566 tracks/km) and water areas (5.374 tracks/km). The trend was similar for both events data as well as for the tracks data. Bushveld areas had no bushpig tracks.

TABLE 3. Bushpig tracks encounter rates in four vegetation types of the NGR

	Mean	Standard error	*t*	*p*
Events				
Sand	1.311	0.457	2.868	<.05
Thicket	3.024	0.357	8.458	<.001
Water	0.982	0.366	2.681	<.05
Bushveld	0	0.429	0	n/a
Tracks				
Sand	2.566	2.608	0.984	NS
Thicket	15.601	2.039	7.649	<.001
Water	5.374	2.09	2.571	<.05
Bushveld	0	2.696	0	n/a

Note: An event indicates a cluster of tracks on a particular transect. Tracks indicates number of bushpigs inferred from tracks for each event. The *t* and *p*-values reflect the outcomes of a repeated-measures linear mixed model using REML, comparing the bushpig encounter rate in the Bushveld with each of the other three habitats. These differences are mostly statistically significant with Thicket having the highest encounter rates.

TABLE 4. Warthog and bushpig abundance in the four vegetation types in NGR, inferred from camera traps

Vegetation types	Number of cameras	Camera days	Number of wild suids		Events		Mean number of wild suids		*SEM* (wild suids)		Mean number of events		*SEM* (events)		*p*	
Sand (S)	3	97	**52**	*7*	**26**	*2*	**0.536**	*0.072*	**0.418**	*2.330*	**0.268**	*0.021*	**0.241**	*0.660*	**0.903**	*0.634*
Bushveld (B)	5	170	**4**	*6*	**3**	*5*	**0.024**	*0.035*	**0.015**	*0.063*	**0.018**	*0.029*	**0.011**	*0.064*		
Combined S + B	8	267	**56**	*13*	**29**	*7*	**0.210**	*0.049*	**0.179**	*0.869*	**0.109**	*0.026*	**0.090**	*0.246*		
Thicket	17	514	**77**	*174*	**20**	*27*	**0.150**	*0.338*	**0.249**	*1.007*	**0.039**	*0.052*	**0.069**	*0.087*		
Water	23	602	**168**	*307*	**36**	*51*	**0.279**	*0.515*	**0.249**	*1.323*	**0.060**	*0.086*	**0.069**	*0.182*		
Overall	48	1,383	**301**	*491*	**85**	*85*	**0.218**	*0.355*	**n/a**	*n/a*	**0.061**	*0.061*	**n/a**	*n/a*		

Note: The total number of wild suid species and events are shown. Mean number of wild suids/events indicate the number of observations per camera day. The numbers in bold indicate warthogs results while the italicised ones are for bushpigs. *p* = statistical significance of a 1-way ANOVA on the log-transformed number of images with wild suids, comparing different habitats. Since the total number of camera days is relatively small, differences among habitats are not significant.

3.2 Wild suid abundance estimation using camera traps

Forty-eight camera traps were installed in 4 different vegetation types for a total of 1,383 days. Bushpigs (n = 483) were observed more frequently than warthogs (n = 304). Similarly, the mean observation rate was higher for bushpigs (0.3518 animal/day, Table 1) than for warthogs (0.221 animal/day, Table 4). This suggests that bushpigs are at least as common as warthogs in NGR.

3.2.1 Warthogs

Although the camera-derived results suggested that warthogs were more common in the sand vegetation (0.536 animals/camera day; 0.268 events/camera day, Table 4), the effect was not statistically significant due to low numbers of warthog recorded.

3.2.2 Bushpigs

Even though camera data suggested that bushpigs preferred water areas to other habitats, this was not statistically significant due to a relatively small sample size of bushpigs observed. The findings were consistent with events (Table 4).

3.3 Tick presence in warthog burrows

Despite intensive sampling, no soft ticks were recovered from any of the 35 warthog burrows in NGR (Figure 1).

3.4 Pig serology

The serum was obtained from animals of 10 months old on average ranging from 6 to 16 months. They were collected in small farms with a median herd size of 2 pigs. All the 67 serum samples tested were negative for ASF antibody presence with the competitive blocking ELISA test. According to the sampling procedure and after applying the formula in Equation 1, this absence of positive results amongst 67 animals tested allowed us to consider that the prevalence of ASF, if any, should be below 3%.

3.5 Movements of wild suids across NGR boundaries

Pig count data: The mean number of bushpigs crossing the fence in wet season (0.56 pigs/day, Table 1) was 11 times lower than the mean number observed in dry season (6.348 pigs/day, Table 1) and this difference was statistically significant ($p < .0001$). Similarly, the mean number of warthogs crossing the fence was significantly lower during the wet season (10.26 pigs/day, Table 1) compared with the dry season (15.4 pigs/day, Table 1; $p = .03$).

Crossing event data: Bushpigs crossed the reserve fence at a significantly higher rate (0.0592 events/site/day, Table 1; $p < .0001$) during the dry season survey than during the wet season survey (0.0066 events/site/day). Warthogs had a similar trend as bushpigs. However, there was no seasonal effect in the number of warthog crossing events (0.036 events/site/day) during the dry season survey and (0.028 events/site/day) in wet season survey (Table 1). Bushpigs crossed the western section at a significantly higher rate than the south-eastern section in both dry (1.592 pigs/day) ($p < .0001$) and wet (0.1389 pigs/day) ($p = .0315$) seasons. In the wet season, no bushpigs crossed through the eastern fence. Warthogs showed a similar trend although the differences are not statistically significant (Table 1).

3.6 Reports of wild suid presence in surrounding pig farms

Most pig farms were concentrated adjacent to the western (78%) and south-western (13%) sections of the fence. While some farms were also located in proximity of the south-eastern fence section (9%), the eastern section had no adjacent farms. The highest number of domestic pigs ($n = 631$) was located close to the western section of the fence while a considerably smaller number ($n = 172$) was found on the south-western section of the fence (Figure 1).

Among the 254 pig farmers, eight reported bushpigs near their households (20km radius) while three reported to have seen warthogs. None of them reported any direct interaction between those wild suid species and their domestic pigs. Bushpig sightings were reported as far as 19 km from the fence while for warthogs the furthest was 8 km (Figure 2).

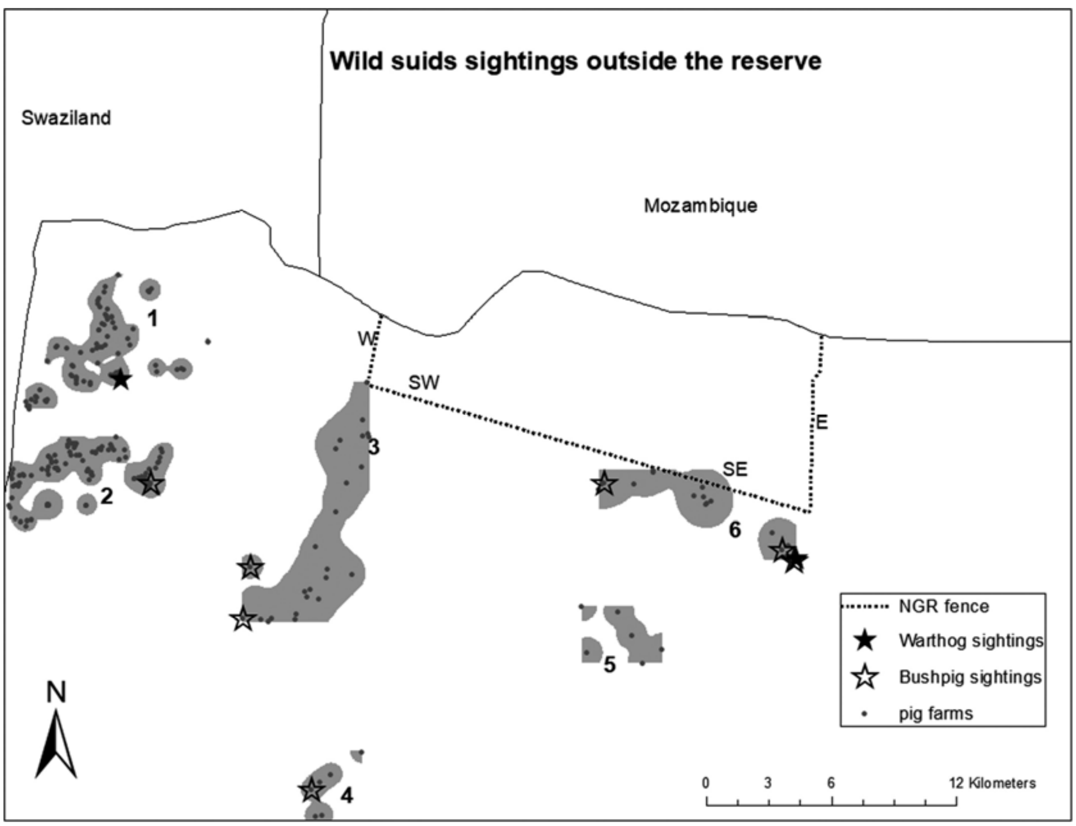

FIGURE 2. Clusters (in grey shade) of pig farmer households and wild suids sightings as reported by farmers in the Mathenjwa Community surrounding NGR. Numbers indicate cluster identity (i.e. 1 denotes Cluster 1)

Pig farmers who reported bushpigs near their households were located adjacent to the western, south-western and south-eastern section of the fence, whereas those who had seen warthogs tended to be more evenly distributed adjacent to the western and south-eastern section of the fence. (Figure 2). None of the farmers reported disease or disease symptoms compatible with ASF outbreaks.

4 DISCUSSION

Warthogs and bushpigs are common in the study area. Annual transects counts in NGR revealed a warthog density of 3–5 individuals/km^2, with a total population of 400–500 warthogs in the area. These estimates fall on the lower end of population densities found by Cumming (1975) who reported that in Africa warthog densities range from 1 to 15 individuals/km^2. This could be partly explained by their preference for open savannah (Deribe et al., 2008), instead of the bush thicket that is common at NGR. The camera trap survey indicated that NGR has a significant bushpig population, which may be similar to warthogs in numbers. This approach was useful in detecting the abundant bushpig population which had not been recorded using the diurnal line transects. Most of the bushpig recorded on camera traps (0.515 animals/ camera day) were found in habitats close to water, consistent with the observations of different authors who noted that bushpigs are water-dependent (Kingdon, 2014; Seydack, 2017). Warthogs (0.536 animals/camera day) on the other hand, were also found in sandy environments. Therefore, NGR provides a good habitat for both wild suids.

Movements of both wild and domestic suids can facilitate direct or indirect interactions with domestic pigs as well as ASF transmission at the interface of a protected area (Arias et al., 2018).

Both wild suid species regularly crossed the game fence, moving into adjacent farmland. Standard wire or wire-mesh fencing is not very efficient to contain suiform species and warthogs are often reported to escape from other protected areas by digging under fences (Jori et al., 2011; Swanepoel et al., 2016). Therefore, in cases where it is important to contain wild suids in conservation areas, using other kinds of barriers such as wild boar-proof fences are recommended (EFSA, 2014; Satheeshkumar et al., 2012).

In our study area, crossings were more common during the dry season for both bushpigs (6.35 bushpigs/day) and warthogs (15.4 warthogs/day). The high number of fence crossings represents a challenge in the management of diseases in domestic pigs. This is because, in the farming area (as in many African rural areas) a considerable population of domestic pigs is left free-ranging, which increases the chances of direct and indirect interactions between wild and domestic suids and disease transmission (Jori et al., 2018). This risk is exacerbated by the fact that most crossings were observed on the western side of the reserve where the highest number of pig farms are located. A possible explanation for more crossings on the western section could be that this terrain is mountainous and it has a moister thicket vegetation, providing a more suitable habitat for a shy species such as the bushpig (Flamand et al., 1991; Jori & Bastos, 2009). This habitat also provides the suids with fruit and bulbs (Nyafu, 2009) particularly during the dry season when resources in the game reserve are scarce. The farms, therefore, have the potential for high levels of direct or indirect wild-domestic and domestic–domestic pig interactions, facilitating the transmission of shared pathogens, such as ASF. Despite limited veterinary research on the pathogenic burden of wild African pigs compared to the Eurasian wild boar (*Sus scrofa*), warthogs and bushpigs are known to be carriers of ASF as well as other pathogens such as trichinella, bovine tuberculosis and several porcine viruses that could be transmitted to domestic pigs sharing the same environment (Jori et al., 2018).

Similar to other studies, some farmers (*n* = 11) reported to have seen at least one of the wild suid species outside the reserve (Kukielka et al., 2016), but none of the suids was observed interacting with domestic pigs. While natural hybridization between domestic pigs and warthogs has not been reported, there are a number of reports of cross-breeding between bushpigs and domestic pigs (Jori & Bastos, 2009; Kingdon, 2015). It is, therefore, assumed that while interactions could occur, they are not necessarily observed due to the elusive and nocturnal behaviour of bushpigs (Payne et al., 2018). Incursions of bushpigs in farming areas are likely to occur at night and warthogs might have human avoidance behaviour (Kassilly et al., 2008). Therefore, questionnaires alone are not the best method for conclusions on potential nocturnal interactions and other methodologies such as radio-tracking and setting up camera traps near pigsties should be considered.

In many African rural areas, a considerable number of domestic pigs are free-ranging (Nantima et al., 2015; Penrith et al., 2013; Quembo et al., 2016), increasing the chances of direct and indirect interactions between wild and domestic suids interactions and potential pathogen transmission (Jori et al., 2018; Penrith et al., 2013). Even though the sharing of the same habitat and resources represents an ideal situation for the transmission of pathogens between wild suids and domestic pigs (Barth et al., 2018). In this study, no indication suggested that NGR currently harbours a sylvatic cycle that would allow wild suid species to act as carriers of ASF. No evidence of *Ornithodoros* tick infestation among our surveyed burrows (*n* = 35) was found, suggesting that the tick reservoir is currently unlikely to be present in NGR and a permanent source of ASF virus is not maintained in the reserve despite the presence of warthogs and bushpigs. These findings are similar to similar studies in Mkuze Game Reserve (approximately 100 km south of NGR) who inspected 98 warthog burrows and collected ticks (Arnot et al., 2009). Given the importance of the sylvatic cycle in Mozambique and the observations of some ASF outbreaks on the Mozambican side close to NGR (Penrith et al., 2013), further research should be undertaken on a larger number of burrows in order to confirm this suspicion.

The apparent absence of a sylvatic cycle in NGR is consistent with the fact that none of the farmers interviewed was concerned with severe disease outbreaks compatible with regular ASF. Furthermore, considering that ASF antibodies have a long life span of at least 3 years, the 67 serum samples from domestic pigs tested negative for ASF antibodies, supports the hypothesis that ASF has not been circulating recently. In addition, our sampling was risk-based in order to maximize the chances of detecting antibodies, based on the age of the animals (6 months or older) and the reports of anorexia, weight loss or some history of pig mortality. This absence of circulating antibodies in our sample is a good indicator that ASF virus was not present or at least below 3% in the study area at the time of our study and an additional indicator of the likely absence of ASF circulation in the area. Despite our sampling design was not robust enough to confirm freedom of disease, the combined absence of antibodies in our sample, mortality reports among pig households and ticks in our sample of warthog burrows strongly suggest a potential absence of a sylvatic cycle in the NGR.

However, considering the endemic ASF status in Mozambique (northern boundary of NGR), the presence of significant numbers of warthog and bushpig within NGR, the regular movements of both species at the interface with domestic pigs, we strongly recommend to maintain veterinary surveillance and management efforts in order to identify potential infectious disease transmission to the local pig population, and which can act as a sentinel species of potential pathogen circulation.

5 CONCLUSION

This study is an investigation of the interface between wild and domestic pigs and the potential presence of a sylvatic cycle in an African-protected area. Both warthogs and bushpigs are common in NGR and they often move out of the park, sharing home ranges and resources with domestic pigs, particularly in the dry season. Therefore, fencing should not be the method of choice to prevent transmission from potentially infected wild suids to neighbouring pig farming areas since they are prolific diggers. Despite many opportunities for wild-domestic interactions, it seems unlikely that the wild suids currently transmit ASF to domestic pigs as no ticks were found in warthog burrows and surrounding pig farmers were not familiar with ASF outbreaks in their area. Further research should explore a larger number of warthog burrows to confirm absence of ticks and potential antibodies against ASF and other diseases should be monitored in pigs and wild suids that are potentially exposed to ASF. An awareness programme among smallholder farmers is also encouraged particularly targeting the western and southern sections that could potentially have a high burden of contact between wild and domestic pigs. Confining pigs during periods of high potential interactions (at night and/or in the dry season) is also recommended since full-time penning is expensive and impractical. Considering the proximity of the Mozambican border, regular surveillance of wild and domestic suids is equally important for monitoring potential incursions of ASF in this high-risk area.

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CONFLICT OF INTEREST

There was no conflict of interest. None of the authors received any funding from Ezemvelo Kwazulu-Natal Wildlife to do the work.

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