

Comparison of African swine fever virus prevalence and risk in two contrasting pig-farming systems in South-west and Central Kenya

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ABSTRACT

We describe a horizontal survey of African swine fever virus (ASFV) prevalence and risk factors associated with virus infection in domestic pigs in two contrasting production systems in Kenya. A free range/tethering, low input production system in Ndhwa District of South-western Kenya is compared with a medium input stall fed production system in Kiambu District of Central Kenya. Analysis of variance (ANOVA) of data derived from cluster analysis showed that number of animals, number of breeding sows and number of weaner pigs were a significant factor in classifying farms in Ndhwa and Kiambu. Analysis of blood and serum samples using a PCR assay demonstrated an average animal level positivity to ASFV of 28% in two independent samplings in South-western Kenya and 0% PCR positivity in Central Kenya. No animals were sero-positive in either study site using the OIE indirect-ELISA and none of the animals sampled exhibited clinical symptoms of ASF. The farms that contained ASFV positive pigs in Ndhwa District were located in divisions bordering the Ruma National Park from which bushpig (*Potamochoerus larvatus*) incursions into farms had been reported. ASFV prevalence ($P < 0.05$) was significantly higher at distances between 6 and 16 km from the National Park than at distances closer or further away. One of the 8 bushpigs sampled from the park, from which tissues were obtained was PCR positive for ASFV. The data therefore indicated a potential role for the bushpig in virus transmission in South-western Kenya, but there was no evidence of a direct sylvatic virus transmission cycle in Central Kenya. ASF control strategies implemented in these areas will need to take these epidemiological findings into consideration.

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1. Introduction

African swine fever (ASF) is a rapidly lethal epidemic disease of domestic swine that constrains the development of the smallholder pig industry in Sub-Saharan Africa. The ASF outbreaks result in significant economic losses in

developing countries (reviewed by Penrith et al., 2004). The effects of the disease for small-scale pig keepers include the loss of a major source of income for farmers, and a major source of high quality and cheap protein for poor communities (el Hicheri et al., 1998; Nana-Nukechap and Gibbs, 1985). Infection with virulent strains of African swine fever virus (ASFV) causes a rapidly lethal disease in naive domestic pigs and slaughter with quarantine of infected areas is the only currently available method of disease control (Costard et al., 2009).

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The African swine fever virus (ASFV), the causative agent of ASF, is a large double-stranded DNA virus, which is the only member of *Asfviridae* family (Dixon Linda et al., 2006). The ASFV is the only known DNA *arbovirus*. The arthropod host for ASFV is *Ornithodoros* spp. This was initially discovered in *Ornithodoros erraticus* ticks from the Iberian Peninsula (Penrith et al., 2004; Penrith and Vosloo, 2009) and subsequently confirmed in Africa in *Ornithodoros moubata* ticks present in warthog (*Phacochoerus aethiopicus*) burrows (Plowright et al., 1969). ASFV can infect hosts through either a sylvatic cycle or a domestic cycle. In the sylvatic cycle, ASFV infects warthogs (*P. aethiopicus*) in which infection is asymptomatic and ticks in the genus *Ornithodoros* (De Kock et al., 1940; Detray, 1957, 1963; Detray et al., 1961; Montgomery, 1921; Steyn, 1932) and there is a well described transmission between ticks and neonatal warthogs in the burrows. Outbreaks can occur when domestic pigs are bitten by ticks that have fed on infected warthogs (Anderson et al., 1998). In the case of another African wild suid, the nocturnal bushpig (*Potamochoerus larvatus*), which is also asymptomatic following experimental infection (Anderson et al., 1998), the role of the species in the transmission of ASF to domestic pigs in natural agro-ecosystems is currently not established (Jori and Bastos, 2009). Direct transmission between bushpigs and domestic pigs has however been demonstrated experimentally (Penrith et al., 2004). The virus can also be transmitted between domestic pigs through either direct or indirect contact (Costard et al., 2009; Mebus, 1988). This can occur between acutely infected, recovered or carrier pigs and naïve animals. Fomites and contaminated feed are also important sources of infection.

The first description of the disease was from Kenya (Montgomery, 1921). A number of outbreaks have subsequently been reported in the country. These include 1954 in Laikipia District; 1958 in Kiambu District; and 1964 on a farm in Trans-Nzoia District in Kenya (Kenya Department of Veterinary Services). In 1994 further outbreaks of ASF occurred after an absence of 30 years. Eleven farms in Kiambu, Thika and Nairobi districts comprising a total of 9000 pigs were affected. Further outbreaks were reported to OIE in May 2006 (Busia District) and November 2006 to early 2007 in Rift Valley and central provinces (OIE disease report, February, 2007). The five outbreaks affected 1011 out of the total population of 13,601 domestic pigs present in these areas resulting in 630 pig deaths. The affected pigs were housed in different sties but ranged freely in search of food. The EU-OIE reference laboratory at CISA-INIA, Spain tested and confirmed samples from the affected areas as positive for ASFV by indirect enzyme-linked immunosorbent assay (OIE-ELISA), immunoblotting and PCR (Gallardo et al., 2009a).

Preliminary studies of pig production systems among rural smallholders in Western and Central Kenya have recently been initiated. These have focused primarily on farmer perceptions relating to pig keeping (Mutua et al., 2010), market value chain analyses (Kagira et al., 2010) and pig farming system characterization (Wabacha et al., 2001, 2004). However, the prevalence and risk of ASF and other porcine diseases has not yet been assessed in these production systems.

The data reported herein was collected as part of wider study on the epidemiology of African swine fever in Kenya and was designed for the purposes of assessing the risk factors associated with African swine fever (ASF) virus prevalence, including the potential role of the bushpig in ASFV transmission in a free range system in Ndhiwa District, Homabay County, Kenya. ASFV prevalence in a stall feeding pig production system in the central region of Kenya, where the sylvatic cycle was not thought to be important was also assessed.

2. Materials and methods

2.1. Study areas

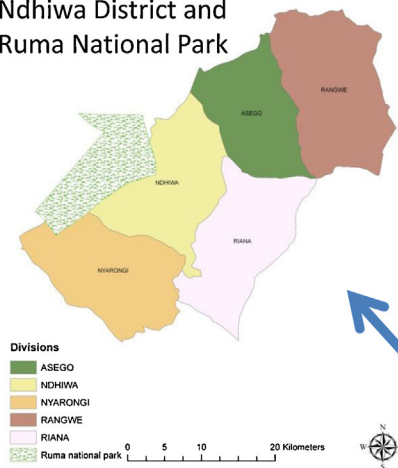
The study was conducted in Homabay and Kiambu Counties (Fig. 1). In addition Ruma National Park was selected for bushpig sampling. Homabay County is located in South-west Kenya close to Lake Victoria and is one of the 6 counties within Nyanza Province. The Country is divided into six administrative districts namely: Homabay, Ndhiwa, Gwasi, Rachuonyo South, Rachuonyo North and Mbita. Ndhiwa District, where the study was located, is divided into four administrative divisions namely: Ndhiwa, Nyarongi, Riana and Kobama. The specific study area, Ndhiwa District, was selected because it represents a predominantly free-range smallholder pig production system and lies in close proximity to the national park, factors increasing the risk of ASF occurrence. Ruma National park is situated along the western border of Ndhiwa District adjacent to Ndhiwa, Nyarongi and Kobama divisions. The park contains a population of bushpigs (*P. larvatus*). The exact number of wild suids in the park is difficult to quantify, but estimated at 300 (Ruma National Park KWS Warden, personal communication).

Kiambu County (Fig. 1) is located in central Kenya; it borders Muranga County to the North and North East, Machakos County to the East, Nairobi and Kajiado counties to the South, Nakuru County to the West, and Nyan-darua County to the North West. The study was located in Kiambaa District.

2.2. Selection of pig farming households and animals

With the help of Department of Veterinary Services of the Ministry of Livestock Development, a list of pig farming households was obtained. Pig-farming households within each administrative division (a subunit of a district) were selected using a systematic random sampling approach. This sampling approach selected the households at a fixed interval throughout the sampling transect from a random starting point. Using a list of 800 and 600 pig-farming households every 20th and 15th household was selected in Homabay and Kiambu, respectively. The final number of households selected was limited by logistics and available financial resources. In total 44 farms were selected in the first period in April and May 2008 and 39 in the second sampling period in March 2009 in Homabay District. A total of 40 farms were selected in Kiambu County in the period January to April 2010. All pigs (all sexes and ages) in each of the selected farms were sampled. A total of 143 and 103

Ndhiwa District and Ruma National Park



Kiambu County Districts

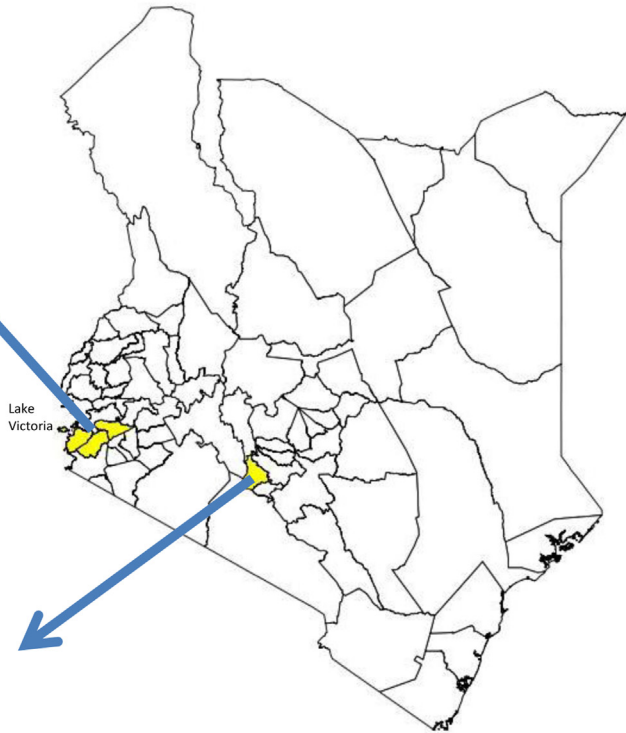


Fig. 1. Map showing location of Homabay and Kiambu Counties in Kenya. Homabay County is located in the South-western region and runs along the southern part of Lake Victoria and Kiambu County is located in central region of Kenya.

domestic pigs were sampled in the two locations. A total of 8 bushpigs were sampled in Ruma National Park in 2008.

2.3. Farm description

All the farms in Homabay used both free-range foraging and tethering as feeding strategies. In the free-range foraging system the pigs were left to roam freely in search of food whereas in the tethering system the animals were secured adjacent to the home or in nearby pastures. A combination of both management systems was used in all the farms studied. In addition, the animals were fed food remains from the homestead and other feeds from the farm. The types of feed utilised were diverse and included tubers (potatoes, cassava or peelings), fruits (guavas, mangoes and pawpaw), vegetables (kale, sweet potato vines, local vegetable (*machicha*)), cane and cereals (maize or maize meal, porridge).

All the farms in Kiambu were stall fed pigs with either commercial feeds or swill or both combined.

2.4. Household spatial information

Pig farming households were geo-referenced using Garmin Global Positioning System (GPS) 12XL unit (Garmin Olathe, KS, USA). GPS data was downloaded using Ozi Explorer and exported into ArcGIS® 9.3 (ESRI, CA, USA) for analysis. Distances between the pig farming households and the Ruma National Park were calculated in ArcGIS 9.3. The distances were included in statistical modelling of ASF risk.

2.5. Rapid rural appraisal

The study employed the rapid rural appraisal technique (Chambers, 1980; Kumar, 1993; McCracken et al., 1988). This approach used a range of investigation procedures. Their chief characteristics are that they take only a short time to complete, are relatively cheap to perform and make use of 'informal' data collection procedures. The techniques primarily utilised expert observation coupled

with semi-structured interviews of pig farmers, local leaders and veterinary professionals. Data was collected using: (1) existing written records from the District Veterinary Officer; (2) direct observations made on the farming households; (3) a triangulation method, where information from one of the data sources was validated using information from the other two independent data sources to increase the scientific rigour of the study. The primary aims of the rapid appraisal were to rapidly characterize farms based on perceived potential risk of ASF infection.

2.6. Questionnaire administration

A questionnaire was administered through interviews of pig farmers and animal health service providers. Information was collected on three aspects of the farming system; household information (size of household, source of labour and land size), pig production data (herd size, feed and feeding strategies, breeding, restocking and inter-farrowing intervals) and animal health constraints (number of sick animals, diseases, disease symptoms and disease control).

2.7. Animal sampling

Bushpigs in Ruma National Park were captured using specially designed trapping nets and restrained chemically using Azaperon 40 mg/ml (Stresnil®-P/M; Mallinckrodt) at a dose of 0.5 mg/Kg body weight. A two-point sampling strategy was used for domestic pigs in Homabay, the first in April and May 2008 and the second in March 2009. Domestic pigs from the selected farms were physically restrained prior to sampling. Blood was collected from the jugular veins using BD Vacutainer® needles (gauge × length: 21 × 1-1/2 in.) into 10 ml BD Vacutainer® glass serum tube and 4.5 ml 15% EDTA tubes (Becton, Dickinson and Company, United Kingdom). Non-EDTA blood was allowed to clot and serum separated. Both serum and EDTA blood aliquots were dispensed into 2 ml cryo-vials (Greiner bio-one, Germany) and stored at −20 °C. The samples were shipped to the European Union ASF Reference Laboratory CISA-INIA (Madrid, Spain) on dry ice according to standard protocols for shipping infectious biological materials.

2.8. African swine fever diagnosis

2.8.1. Antibody detection

Antibody detection of ASF was performed using the OIE-approved serological tests that consist of initial screening of sera by an indirect enzyme-linked immunosorbent assay (OIE-ELISA) followed by an immunoblotting assay to confirm ambiguous results. Briefly, both conventional ELISA and immunoblotting assays were performed using a lysate of stable monkey kidney cell line (ECACC, 91070510) infected with ASFV E70MS48 as the antigen and protein-A conjugated to the enzyme horseradish peroxidase (HRPO) as the reporter system. Both procedures were carried out following the protocols described in the OIE Diagnostic Manual. The OIE-ELISA has a sensitivity of 98.8% and a specificity of 87.8 (Perez-Filgueira et al., 2006).

2.8.2. Virus detection

2.8.2.1. PCR. DNA was extracted directly from serum, blood or 10% suspensions of ground tissues using a nucleic acid extraction kit (Nucleospin/Machery-Nagel – Cultek) following the manufacturers procedures. A PCR assay using the ASF diagnosis primers PPA1/PPA2 that generates an amplicon of 257 bp within the p72 protein (Aguero et al., 2003) was used to confirm the presence of ASFV DNA. The PCR products were analysed by electrophoresis through 2% agarose gels and the specificity of the amplicons obtained was confirmed using the NdeI restriction endonuclease (Gallardo et al., 2011).

2.8.2.2. Virus isolation. Macrophage cultures used for the isolation of ASFV were derived from naïve domestic pigs as previously described (Malmquist and Hay, 1960). Briefly, cells were seeded into 96-well tissue culture grade microtitre plates (volume 200 µl; 300,000 cells per well) in homologous swine serum, and incubated in a humidified atmosphere containing 5% CO₂ at 37 °C. Three-day cultures were infected at a multiplicity of infection (moi) of 1:10 with serum, blood or 10% suspensions of ground tissues supplemented with 5 µg/ml gentamycin sulphate (BioWhittaker) and incubated for 24 h at 37 °C. After inoculation, a preparation of 1% homologous red blood cells in buffered saline was added to each well. The plates were examined for haemadsorption over a 6 day period. The samples were blind passaged three times.

2.8.2.3. Virus characterization. Viruses isolated were characterized at CISA-INIA using molecular techniques as described (Boshoff et al., 2007). A two-step genetic characterization approach was used in which initially, P72 and P54 gene sequencing was used to delineate genotypes. This was followed by higher resolution dissection of viral relationships by central variable region characterization of the 9RL ORF (locus B602L).

2.8.2.4. Data entry and analysis. Field data, results of laboratory analysis and GPS distances derived from ArcGIS were recorded on prescribed forms in Microsoft Access 2000 (Microsoft Corporation). Statistical analyses were conducted using SPSS 12.0.1 2003 (SPSS Corporation, Chicago, USA) and GenStat® 10th edition (VSN International, United Kingdom). The analysis included pig farming system characterization and typing using summary statistics and K-mean cluster analysis within the SPSS package. Modelling of disease risk was performed using Poisson regression analysis in GenStat®. The response variable was a count of the number of ASFV positive animals per farm. Variables fitted to the data were an arbitrary constant combined with farm size, distances from Ruma National Park and the two sampling periods.

3. Results

3.1. Pig farm characterization

Boars used for servicing of sows were either kept on-farm, or borrowed from neighbours. In Homabay District only 30% of the farms had breeding boars, whereas in

Kiambu District 48% of farms had boars, with a mean of 2 breeding boars per farm. The main breeding approaches in Homabay and Kiambu districts were use of a boar on-farm or, more frequently, contractual-breeding where a farmer would borrow a boar from the neighbourhood to mate his sows, or alternatively a sow was taken to a boar when in heat. In both the districts re-stocking was through a combination of piglets born on the farm, animal loans from neighbours and purchases from local sources.

The K-means cluster analysis procedure within SPSS was used to group farms based on their characteristics. The farm characteristics used in the analysis were farm size, number of animals per farm, number of breeding males, number of breeding females, number of piglets farrowed and number of piglets weaned. Four clusters were found to provide consistent differentiation according to the farming characteristics. Cluster 1 exhibited small farm size (cluster centres of 6.6 ha, herd size of 7 pigs with 1 breeding sow, 1 breeding boar, 3 weaners and 2 piglets). Most of the farms (95%) in both South-west Kenya and Central Kenya were found in this cluster. Analysis of variance (ANOVA) derived from the cluster analysis showed that all the variables used were significant ($P < 0.05$) but the number of animals, number of breeding sows and number of weaner pigs provided the widest separation between clusters with an F value of 574.982, 510.443 and 1375.484, respectively.

3.2. Serology and virus detection

In Homabay detection of virus in blood and serum samples using p72 PCR demonstrated an average of 28% (at 95% CI [21,36]) positivity to ASFV in two independent samplings at different times and involving different farms and pigs within the same general area. However, no sero-positive pigs were detected using the OIE-ELISA. The farms with ASFV positive pigs according to PCR analysis were collected from Ndhiwa, Kobama and Nyarongi divisions that either border, or are located close to, the park. Bushpig incursions into some of the PCR positive farms had been reported to KWS by pig farmers. Of the pigs sampled from Kiambu District (central region), no animals tested positive using the p72 PCR assay. As in South-west Kenya, none of the samples collected from pigs were positive for ASFV antibody using the OIE-ELISA.

Eight different bushpigs were sampled at three independent time points. In the second time point one bushpig was humanely sacrificed and sampled for tissues and the kidney sample was found to be positive by PCR. All the eight bushpig serum and blood samples were negative by both PCR and OIE-ELISA. No virus could be isolated after three passages in macrophages from the PCR positive sample.

3.3. Virus genotypes

Sequencing of the 3' end of the gene encoding the C-terminal end of the p72 protein and the full length p54-gene of Kenyan ASFV viruses from domestic pigs and bushpigs in Ndhiwa District indicated that the viruses clustered together within genotype p72 genotype X and also

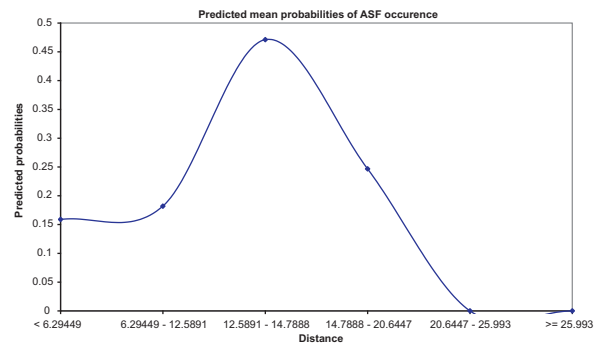


Fig. 2. Graph showing Poisson regression model predicted mean probability of ASF occurrence by distance from Ruma National Park. Y axis shows the predicted probabilities and X axis the distances categories from Ruma National Park in relation to location of pig farming households.

exhibited the same p54 genotype, indicating a close genetic relationship.

3.4. Farm level African swine fever risk

Regression analysis involved fitting the following variables into a Poisson model: sampling period (years 2008 and 2009), feeding sources (on-farm and off-farm), breeding (loaned boar or own boar), tick control (acaricide used or not used), distance/distance categories from Ruma National Park, and herd size as an offset, to explain the count of pigs infected with ASFV per farm. The Poisson model indicated that period of sampling and distance from Ruma National Park were statistically significant ($P < 0.05$). The prevalence of ASFV was higher in 2009 (31%) compared to 2008 (25%). The model estimates for period and distance categories is shown in Table 1. A higher disease probability was found in farm to park distance range between 6 km and 16 km (Fig. 2). There were 36 farms in this distance range. The risk of ASF was non-significant in farms less than 6 km (15 farms). The risk to ASF was also not significant at more than 16 km from the park (30 farms) and since there were more pigs at this distance range (>16 km), this prediction was interpreted as reduction of ASFV infection risk as a result of increased distance from the park.

4. Discussion

Potential risk factors for ASFV infection including feed sources, breeding strategies, sources for restocking of pigs, tick control, grazing management and proximity to the adjacent Ruma National Park were investigated. Domestic pigs, in this study, were fed cereals and cereal food waste, vegetables and fruits, in addition to free range foraging. Feeding of pork or other pig products was not cited by any farmer interviewed. Family food was sourced on-farm or locally, therefore feed was unlikely to represent a source of viral infection.

Free-grazing of pigs, as in Ndhiwa District creates the possibility of the pigs coming into contact with excreta deposited by wild pigs scavenging in the farms at night. Where farm sizes were large the farmers tethered the pigs in pastures or left them to roam freely. Free-range pigs,

Table 1
Poisson regression estimates of parameters.

Parameter	B	Std. error	95% Wald confidence interval		Wald chi-square	Hypothesis test	
			Lower	Upper		df	Sig.
(Intercept)	-8.271	1.2203	-10.663	-5.879	45.939	1	0
[Period = 1.00]	3.045	0.5482	1.971	4.12	30.858	1	0
[Period = 2.00]	0 ^a
[DistCateg = 1.00] < 6.29	2.083	1.2249	-0.318	4.484	2.892	1	0.089
[DistCateg = 2.00] 6.29–12.59	2.715	1.1009	0.558	4.873	6.084	1	0.014
[DistCateg = 3.00] 12.59–14.79	3.039	1.146	0.793	5.285	7.034	1	0.008
[DistCateg = 4.00] 14.79–20.64	2.151	1.1628	-0.128	4.43	3.421	1	0.064
[DistCateg = 5.00] 20.64–25.99	1.317	1.4477	-1.52	4.155	0.828	1	0.363
[DistCateg = 6.00] ≥ 25.99	0 ^a
Farm size	0.001	0.0386	-0.075	0.077	0.001	1	0.979
(Scale)	1 ^b

Dependent variable: ASF count. Model: (intercept), period, distance category (DistCateg), farm size, offset = number of animals.

^a Set to zero because this parameter is redundant.

^b Fixed at the displayed value.

if uncontrolled, caused damage in farms owned by neighbours resulting in conflict. For this reason, farmers with smaller land holdings more frequently confined the animals by tethering. Coincidentally, in this study, large farm sizes were also associated with close proximity to Ruma National park, probably due to both tsetse infestation in this area and also conflict with wildlife straying into farms. A sparse human population resulting from infestation with tsetse flies carrying human-infective trypanosomiasis in this area (Wilde, 1989) has been described. These factors can explain a lesser risk of domestic-wild pig interaction on farms closer to the park.

Both animal loans for breeding and purchases could be a factor contributing to transmission of the virus between farms, through direct pig to pig contact. No acaricide application was practised in any of the farms, even where cattle were also present, and we did not screen for antibodies against the soft tick that transmits the ASF virus (*Ornithodoros* spp.) in this study. The possible role of ticks in the epidemiology of ASFV in the two regions therefore cannot be entirely excluded and requires further investigation.

There appears to be a relatively high prevalence of ASFV in domestic pigs in the Ndhiwa District of South-western Kenya, adjacent to Lake Victoria, in the absence of frequent outbreaks of detectable disease. Although the average prevalence of viral infection in the domestic pigs and farms as confirmed through laboratory diagnosis is relatively high (28% in pigs and 35% farms, respectively, in the two sampling periods), it is also not accompanied by observable clinical signs in the positive animals. Additionally the animals were not sero-positive using the OIE indirect ELISA test. This suggests either the presence of a virulent or moderately virulent strains of the virus as has been observed in Europe (Costard et al., 2009), or a degree of tolerance of the virus in the mammalian host, or some combination of these two factors.

A similar observation of infection in domestic pigs in the absence of detectable clinical symptoms has been made in Northern Mozambique (Penrith et al., 2004). In the Mozambique study, neither host genetics nor lack of viral virulence provided a complete explanation of the lack of clinical symptoms observed. However, it has been shown that virus

populations with variable virulence, immunogenicity and infectivity to pigs can be generated by passaging African swine fever viral isolates in cell culture (Pan, 1992). Both naturally derived avirulent isolates from ticks (Chapman et al., 2008) and experimentally generated avirulent viruses (Burrage et al., 2004; Neilan et al., 2002) have been associated with the deletion of open reading frames (ORFs) within multicopy gene families located in the terminal regions of the ASF virus. It would be of interest to sequence a genotype X ASF virus from Ndhiwa District to see if sub-terminal ORFs are lacking in the genomes of these isolates, however we were unable to obtain virus isolates from either domestic pigs or bushpigs in this study. This was possibility due to a limited ability of ASF virus to replicate in the Landrace breed porcine macrophages used to attempt virus isolation. Since this exotic breed is genetically distinct from the indigenous African pigs found in Nhiwa District (Ramirez et al., 2009). It is interesting to note that the only Kenyan ASF virus for which a complete genome sequence is currently available (Chapman et al., 2008; de Villiers et al., 2010) was an isolate obtained in the 1950s that is also classified in p72 genotype X, but, unlike the viruses described in this study, is described as highly virulent in domestic pigs.

The failure of the 'indigenous' pigs to seroconvert observed in this study supports previous findings documenting a low incidence of detectable serological responses to ASFV in East Africa (Gallardo et al., 2009b, 2011; Perez-Filgueira et al., 2006). Recent unpublished studies have demonstrated the possibility that alternative serological methods may perform better than the OIE test, although this requires further validation (Gallardo C., personal communication). An alternative explanation for the low seropositivity may be related to the host phenotype/genotype. There is evidence that pigs from Western Kenya exhibit some introgression of genetic material from the Asian centres of wild boar domestication (Ramirez et al., 2009) and are therefore not identical to European and West African pig breeds. In concordance with the pattern observed in the indigenous pigs, previous findings indicate that the viraemia in bushpigs generally lasted longer than in warthogs in the absence of seroconversion, perhaps due to B lymphocyte apoptosis, indicating that antibody

mediated immunity may not be critically important for bushpig survival (Anderson et al., 1998; Oura et al., 1998).

Statistical analysis showed that distance from the park significantly explained the count of ASFV cases on-farm. The risk of ASF was non-significant in farms less than 6 km. This result is explained by the fact that the predicted probability was weighted by the number of animals at this range of distance from the park, since there were fewer pigs <6 km from the park. This range of distance from the park also lacked cultivated land that was attractive to bushpigs. There was thus limited interaction between bushpigs and domestic pigs at this distance from the park. The Lambwe Valley where the park is located also supports large numbers of tsetse fly (*Glossina pallidipes Austeni*) and infections of *Trypanosoma brucei rhodesiense* (Allsopp et al., 1972; Otieno and Darji, 1985; Turner, 1986; Wilde, 1989). This has led to fewer farms located close to the park and thus large farm sizes that are also associated with free-range production of pigs. The ASF risk was also not significant at more than 16 km from the park and since there were more pigs at this distance range, this finding was interpreted as reduction of ASFV infection risk as a result of increased distance from the park. Distances more than 16 km were not within the nightly foraging radius of the bushpigs. This data on the spatial distribution of virus infections in Ndhiwa District suggests the possible existence of a sylvatic cycle involving bushpigs, although this requires further genetic analysis of viral isolates. Reported farmer-bush pig conflict in the study area adjacent to Ruma National Park in South-west Kenya further supports the hypothesis of direct or indirect contact between the two pig species.

We conclude that our data are consistent either with the presence of an avirulent isolate of ASFV that may originate in bushpigs or that the pigs infected exhibit a degree of innate or acquired immunity to the specific virus that is currently circulating in South west region of Kenya. The contrasting absence of virus in Kiambu pigs detectable by PCR or serology despite recent disease outbreaks (Gallardo et al., 2009a; Department of Veterinary Services Kenya 2011 and 2012) suggests that the virus responsible for these outbreaks does not induce a long term carrier state. The data also provides evidence for the complexity of ASF epidemiology in East Africa, even within geographically restricted areas, since recent outbreaks involving highly virulent ASFV isolates classified within p72 genotype IX have recently been recorded in (Gallardo et al., 2009a) Busia County which is only approximately 100 km from Ndhiwa District. These results have implications for the design of targeted ASF control strategies in the study areas. Given that, in some instances infections do not result in clinical manifestation and are not detectable using conventional OIE-ELISA, some ASFV infections will escape serological screening and require the application of alternative nucleic acid-based approaches for virus detection.

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