Suiform Soundings



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Understanding the dynamics and spread of African swine fever virus at the wildlife-livestock interface: insights into the potential role of the bushpig, *Potamochoerus larvatus*

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Abstract
African swine fever virus (ASFV) is a serious animal disease of pigs, causing high mortality in domestic swine. In Africa, the presence of a sylvatic cycle involving wild pigs and soft ticks means that the risk of introduction of ASFV into domestic swine is always present. Although warthogs are considered the main wild vertebrate host of the virus in the endemic African setting, they are not the only wild African suids with a potential role in ASF epidemiology. The bushpig, *Potamochoerus larvatus,* is an elusive, nocturnal pig known to be susceptible to ASF, and with a natural interface with both warthogs and domestic pigs. The bushpig therefore may play a significant role in ASF epidemiology and serve as a link between the sylvatic and nonsylvatic cycle. This paper presents initial results from an ongoing study investigating the role of the bushpig in the epidemiology of ASF at the wildlife-livestock interface. African swine fever (ASF) is a fatal, haemorrhagic disease of domestic pigs,

Keywords: African swine fever (ASF), bushpig, *Potamochoerus larvatus*, epidemiology

Introduction

African swine fever (ASF) is considered one of the most devastating diseases of pigs. It is a highly contagious disease, caused by a DNA virus (ASFV) resulting in up to 100 % mortality in naïve domestic pigs. There is currently no vaccine or treatment and ASF control is carried out by diagnosis and elimination of infected animals. ASFV is currently endemic in large parts of sub-Saharan Africa (Penrith et al., 2013), where it has a severe impact on livelihoods, food security and trade (FAO, 2010). The epidemiology of ASF is complex with a sylvatic cycle involving the natural asymptomatic reservoirs of the virus, specifically the common warthog (*Phacocheorus africanus*) and soft ticks of the genus *Ornithodoros*. There is also a more recently evolved nonsylvatic cycle in which the virus is transmitted directly or indirectly from pig to pig and appears capable of persisting in domestic pigs in the absence of sylvatic hosts (Jori and Bastos, 2009; Jori et al., 2013). Historically the sylvatic cycle predominated and ASF outbreaks occurred as a result of spill over to domestic pigs at the wildlife-livestock interface. This corresponded well with the distribution of warthogs in Eastern and Southern

Africa (de Glanville et al., 2014). In recent decades, however, the importance of the traditional sylvatic cycle has diminished, and pig to pig transmission is now considered the most important modality of ASF spread and persistence in endemic areas (Penrith et al., 2013). This is attributable to increasing numbers and densities of domestic pigs, and the extent of live pig movements and trade, combined with a reduced contact between warthogs and domestic pigs, as warthogs become restricted to national parks and other protected areas.

Although warthogs are considered the main wild vertebrate host of the virus in African agrosystems, they are not the only wild African suids with a potential role in ASF epidemiology. The bushpig, *Potamochoerus larvatus,* is an elusive, nocturnal, medium sized wild pig with a relatively wide range in Eastern, Central and Southern Africa, known to be susceptible to ASFV. Naturally infected animals, detected by PCR have been reported from several countries, and like warthogs, experimentally infected bushpigs exhibit no acute clinical reactions. Infected bushpigs can transmit ASFV to susceptible domestic pigs directly, unlike the warthog, which has only been demonstrated to transmit indirectly through the tick vector (Anderson et al., 1998). The overlap between bushpig and domestic pig habitat is likely to be significant in many regions, since bushpigs unlike warthogs are known to move into communal lands to feed on crops. Anecdotal reports even describe free ranging female pigs being mounted by male bushpigs, suggesting the possibility of inter-generic hybridization and the possible introgression of *Potamochoerus spp*. genetic material into domestic pigs, although scientific confirmation is currently lacking. Close interaction in areas where ASFV is circulating may result in virus exchange between the two genera. Overlap between warthog and bushpig habitats in the national parks and reserves may also result in virus transmission in one or both directions, perhaps mediated by soft ticks. The bushpig may play a significant role in ASF epidemiology and serve as a link between the sylvatic and non-sylvatic cycle. The aim of the ongoing study is to investigate the role of the bushpig in the epidemiology of ASF at the wildlife-livestock interface. This short report presents initial data.

Materials and Methods

Capture and sample collection

Sampling sites were identified at the interface between farmland and national parks (Murchison Falls National Park and Lake Mburo National Park, respectively), in Uganda, with assistance of local hunters and the responsible District Veterinary Officer (DVO), and inside the park borders with assistance of Uganda Wildlife Authority (UWA) staff. Capture was performed using game capture nets (50x3 mts, 150mm square mesh, 3.5mm nylon braid khaki, ALNET Ltd, SouthAfrica; see fig 1), and bushpigs were sedated with tiletamine and zolazepam (Zoletil 100, Virbac Laboratories, France) at a dose of 300-350 mg/ adult pig (Kock et al., 2006). Serum and blood was collected from captured bushpigs and stored on ice in cool boxes, and one bushpig from each location was equipped with a GPS/GSM tracking collar of the harness type (Savannah Tracking Ltd, Kenya). To increase the sample size, local hunters were encouraged by the DVO to report bushpigs killed during hunts, to enable sampling. From these bushpigs samples of spleen, heart, lung and kidney were collected.

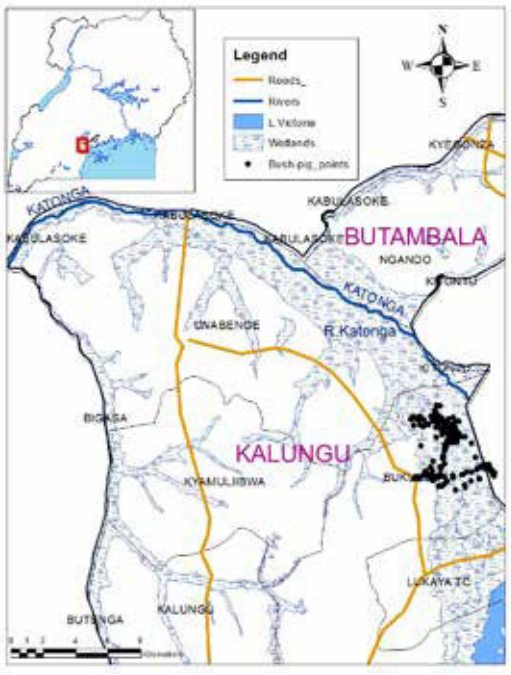


Figure 1. Map showing the location and home range of a bushpig that was captured and monitored during two months between late October and mid December 2010. Black dots represents collected GPS positions of the bushpig. For a more detailed map see figure 4.

Laboratory analyses

After each capture exercise, samples were transported on ice to the Molecular Genetics Laboratory in the College of Agriculture and Environmental Sciences, Makerere University, Kampala. Serum tubes were centrifuged at 2000 g for 10 minutes to separate serum from the clotted blood. Serum and whole blood were then aliquoted into duplicate 2 ml cryovials (Cryo.s, Greiner Bio-one, Wemmel). All samples were stored in duplicate at -20°C and -80°C as working and long-term storage sample aliquots, respectively. Serum samples were screened for presence of ASFV specific antibodies using a commercially available blocking ELISA (Ingezim PPA, INGENASA, Spain) in accordance with the instructions of the manufacturer. Positive samples were re-tested using the recently released SVANOVIR® ASFV-Ab (Boehringer Ingelheim Svanova, Uppsala, Sweden) indirect ELISA. The SVANOVIR® ASFV-Ab ELISA kit was used according to the instructions by the manufacturer.

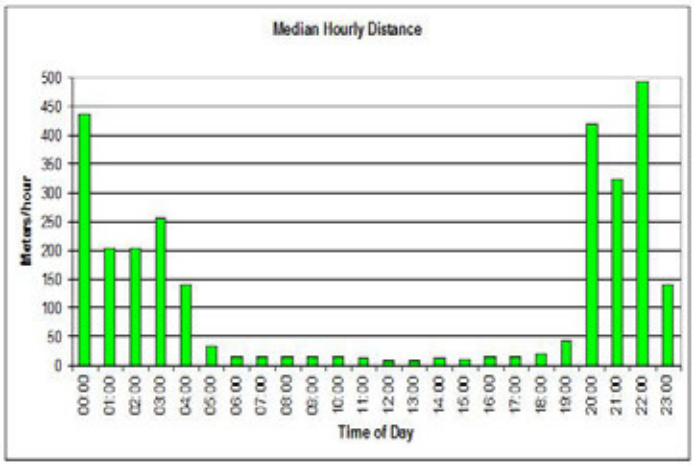


Figure 2. Average diurnal activity pattern: The bushpig showed as expected limited movements during the day and with peak distances travelled between sunset and midnight.

All blood and tissue samples were screened for presence of ASFV nucleic acids. In brief, genomic DNA was extracted using DNeasy Blood & Tissue kit (Qiagen, Duesseldorf, Germany) following the manufacturer's protocol. The extracted DNA was then used as template in a commercially available ASF real time PCR assay targeting the p72 gene (Tetracore Inc., Rockville, USA) according to the instructions of the manufacturer. The assay was optimized for use on a SmartCycler® (Cepheid Inc., Sunnyvale, California, USA).

Bushpig movement data

Hourly GPS position data from the deployed tracking collars was transmitted every 6 hrs to a database at Savannah Tracking Ltd (www.savannahtracking.com). Data was downloaded to the Savannah Data Manager software for further export and GIS analysis. Movement data could further be visualized in realtime through Google earth (www.google.com/earth)

Results and discussion

To date six bushpigs have been captured and sampled, and tracking collars have been deployed on four individuals in three different locations. In addition, samples have been collected from five bushpigs captured and killed by local hunters.

Blood and/or tissue samples from 11 bushpigs were tested for ASFV DNA, and so far one tested positive on PCR (CT 35.8). This bushpig was captured by local hunters in the farmland adjacent to the northern borders of Murchison Falls National Park, an area where several outbreaks of ASF have been reported and confirmed in domestic pigs during the last few years (Aliro et al., 2012). Serum samples from seven bushpigs were tested for presence of specific antibodies against ASFV, and two tested positive in both the blocking and indirekt ELISAs used. This is the first time seropositivity to ASFV has been demonstrated in bushpigs[1]. These bushpigs were captured within Lake Mburo National Park, an area with an abundant warthog population.

[1]Also reported as preliminary results in a student thesis by Björnheden, 2011, The Swedish University of Agricultural Sciences, Uppsala. http://stud.epsilon.slu.se/2355/4/Bjornheden_L_110314.pdf

Although tracking collars were deployed on four bushpigs, movement data was only successfully collected from two individuals. One bushpig was killed shortly after capture and the collar was left in a location from which it was subsequently recovered. The other bushpig was found dead at the site where it was initially captured, possibly due to stress and hyperthermia after capture and sedation (Jori et al., 2013). One of the remaining collared bushpigs was captured within Lake Mburo National Park, and all movements during the 6 weeks it was monitored were registered within the park borders and there was no possibility for interaction with domestic pigs. This was one of the seropositive bushpigs, and the absence of a natural interface with domestic pigs within the park borders thus indicates that the seropositivity was a result of exposure to ASFV within the sylvatic cycle, rather than through contact with domestic pigs. The fourth bushpig was captured in a swampy area bordering farmland where there was a high level of production and rearing of domestic pigs (see Figure 1). This pig was monitored for two months until it was killed by local hunters. Movement data showed limited movements during daytime, with most movement observed between sunset and midnight (Figure 2). The maximum daily travel distances were up to 12 km (Figure 3). During the day the bushpig rested in swamps or thick bush with nightly movements into farming areas where domestic free ranging 136 pigs are kept (Figure 4).

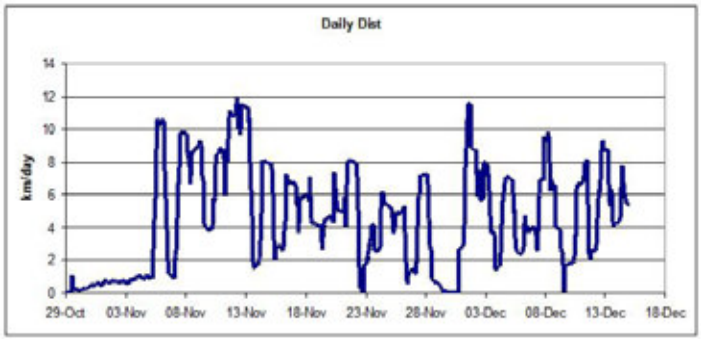


Figure 3. Daily travel distance: The bushpig moved up to 12 km per day but with very large variation between days with what could appear to be 2-3 day cycles of high and low movement. Note the initial resting phase following capture.

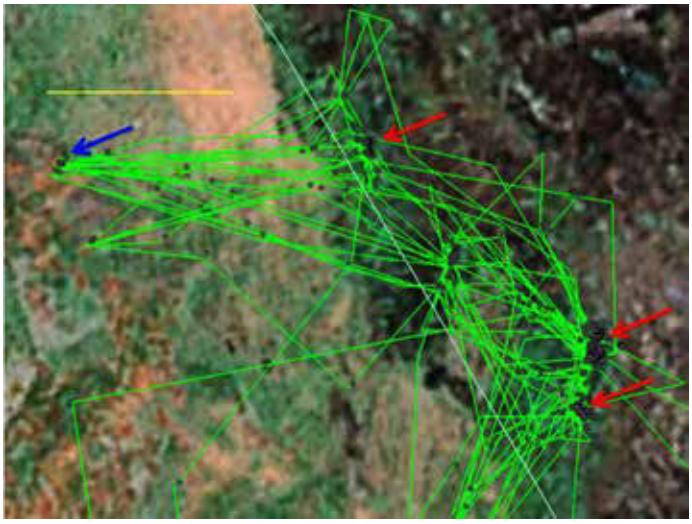


Figure 4. Bushpig movements monitored between 28th of October and 15th of December 2010. Yellow line = 1 km. sites in the swamps. Blue arrow: night time crop raiding of farms. Yellow line = 1 km.

These results confirm that bushpigs can be naturally infected by ASFV, and demonstrate for the first time that seroconversion occurs in some animals. This is also the first study in which tracking technology has been used to study the potential interaction of bushpigs and domestic pigs. The data clearly demonstrate a spatial overlap between bushpig and domestic pig home ranges. Additional GPS tracking data from bushpigs and from domestic pigs and from multiple seasons is required for in depth modelling of the spatial and temporal interactions between the two species, supported by molecular characterization of the virus genotypes present with in both species in order to determine the likelihood and direction of transmission between the two.

In conclusion, our findings are consistent with a role for the bushpig in the epidemiology of ASF, but additional data is required to confirm this.

Acknowledgements

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Mburo and Murchison Falls National Parks for invaluable assistance during bushpig capture and sampling. This work was financed through the USDA/FAS cooperative agreement 58-3148-1-252. Support was also obtained through the Swedish Research Links Programme (Swedish Research council).



Figure 5. Bushpig in Lake Mburo National Park, Uganda. Foto: Karl Ståhl

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