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A study of domestic pigs, wild suids and ticks as reservoirs for African swine fever virus in Uganda

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ABSTRACT

As the world's population increases the need for food and good protein sources grows, especially in development countries like Uganda. The pig production is growing rapidly in Uganda and is not only a source of food but also an important income for many people living in the rural areas.

African Swine Fever (ASF) is a contagious viral disease, which can cause up to 100% mortality among domestic pigs. This disease is endemic in Uganda and causes major economical and personal losses for farmers whom it affects directly or indirectly. Outbreaks occur every now and then, and in most cases the source of the infection is unknown.

To better understand the dynamics of the disease, and sources of new infections, the aims of this study were to study different possible reservoirs for ASF (domestic pigs, wild suids and *Ornithodoros* ticks), and also to estimate the prevalence in one district, Rakai, in Southern Uganda, where pig production is an important source of income for the rural community. Another aim was to investigate the spatial behavior of a small number of bushpigs, to understand the interaction between these wild suids and their domestic relatives. Do they ever get in contact and thereby have the possibility to transfer ASFV from one another?

DNA from African swine fever virus (ASFV) was found by PCR in nine of 239 domestic pigs with no clinical signs and in three out of four sampled soft ticks (*Ornithodoros porcinus).* Antibodies against ASFV were found in two of four sampled bushpigs, the only warthog sampled in the study and in five of the 239 domestic pigs, using a commercially available ELISA. Three of the PCR positive pigs were borderline cases on the ELISA. GPS readings of the movement of the bushpigs indicated possible interface with the domestic pigs.

Conclusions made from this study are that domestic pigs, bushpigs, warthogs and soft ticks can act as reservoirs for ASFV. Domestic pigs and bushpigs can interact. The prevalence of ASFV in Rakai was 3.3% while the seroprevalence was 2.1%. The most important finding was the subclinical, ASFV positive pigs.

BACKGROUND

This project was part of a bigger, ongoing project on African swine fever (ASF), with scientists from the Swedish University of Agricultural Sciences (SLU), the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Makerere University, Uganda Wildlife Authority (UWA) and International Livestock Research Institute (ILRI). This was a Minor Field Study (MFS) financed by the Swedish International Development Cooperation Agency (Sida).

In the past few decades the meat consumption has increased enormously, especially in developing countries, with pork and poultry responsible for 73% of the increase. This phenomenon is referred to as "the livestock revolution". The increased demand for animal products might make a good opportunity for many farmers in the rural areas if they can expand their production and thereby rise from the most extreme poverty. On the other hand this may result in a higher density of animals, in the farms and also within the countries, and also an increased trade and consequently live animal movements. This necessitates better biosecurity to protect against transmissible animal diseases. Unfortunately this level can sometimes be hard to reach because of lack of knowledge, none or poor diagnostic methods and low qualityveterinary service in some areas. This makes farms more vulnerable to infectious diseases such as ASF (World Bank 2005).

ASF is an infectious disease of great importance. It is a highly contagious disease, described as a hemorrhagic fever, which can cause up to 100% mortality among domestic pigs during an outbreak. ASF is considered one of the most important transboundary swine diseases because of its rapid spread, the lack of vaccines or treatment and its socioeconomic consequences (Penrith et al 2009).

AFRICAN SWINE FEVER

Distribution and history

ASF was first described in the 1920's in Kenya and has been found in most sub-Saharan countries in Africa. In the 50's the disease spread to Portugal in Europe, probably by swill feeding from airports. More outbreaks occurred in Europe, for example in Spain, France and Belgium, during the 60's, 70's and 80's. The suspected sources of introduction were airports, harbors and imported pig products. By stamping out it was managed to eradicate the disease everywhere in Europe except for Sardinia, where it is now endemic. In the 1970's ASF also spread to some islands in the Caribbean (Cuba, Haiti and the Dominican Republic) and Brazil, but the eradication was successful and there have been no reports of the disease for several years (Costard 2009).

Recently, in 2007, an outbreak again was reported in Europe. The ASF started in Georgia and spread within the Caucasus region to Armenia, Azerbaijan and Russia. In January 2011 ASF was diagnosed among sick pigs in St. Petersburg in Russia (OIE 2011). In the rest of the world the disease has never been reported (Costard 2009).

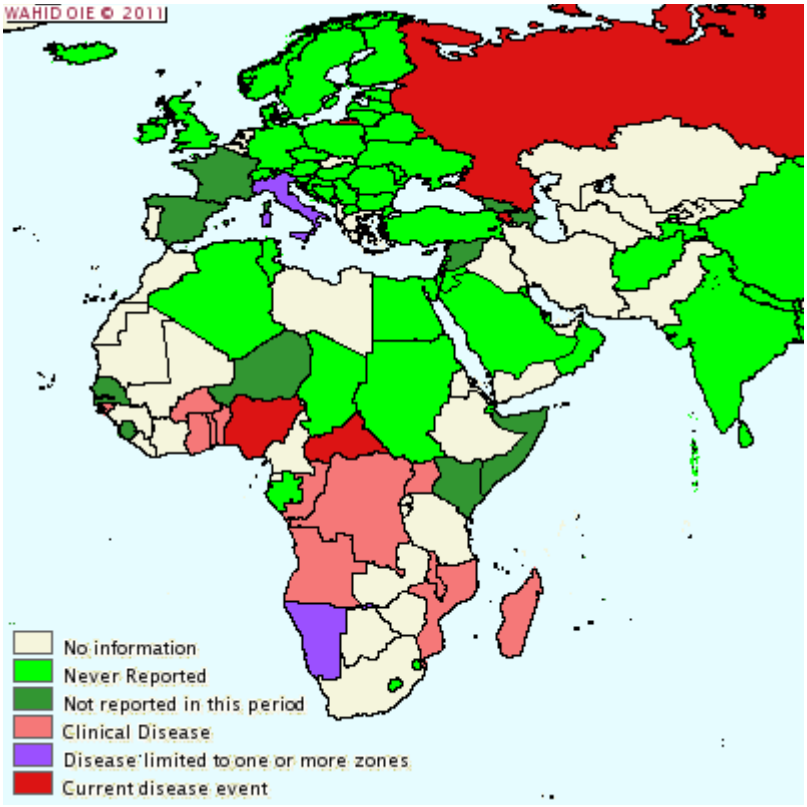

Fig. 1. Spread of ASF first 6 months of 2010 (OIE, 2011).

Aetiology

The African swine fever virus (ASFV) is an *Afsivirus* and the only member of the family of *Asfarviridae* (African swine fever related viridae). It is a large double stranded DNA virus, which can infect species of the pig family (*Suidae*). It is also the only known DNA virus that is an arbovirus (arthropod borne virus). There are

22-23 known genotypes and several subtypes of the virus, with different virulence (Sánchez-Vizcaíno et al 2009).

ASFV is a resistant virus in different pH-values (most stable between pH 4 to 10 but experiments has shown infectivity from organic materials in pH values ranging from 3.1 – 13.4) and in low temperatures. It remains stable at -70^{o}C but gets inactivated in 60^{o}C for 30 minutes. The virus can remain infective in raw, smoked or frozen meat for several months. In pig feces ASFV can persist for more than 60 days (Muller 1973, cited by Sánchez-Vizcaíno et al 2009). If the virus loses the protection from surrounding tissue/proteins it is easily inactivated by sunlight or disinfections. (Penrith et al 2009, Epiwebb 2010, Sánchez-Vizcaíno et al 2009)

Pathogenesis

The most common infection route is per orally and via the tonsils or the pharyngeal mucosa, to the closest lymph nodes. ASFV foremost replicates in the cytoplasm of monocytes and macrophages but can also infect megakaryocytes, platelets, neutrophils and hepatocytes etc. Virus replication has however not been demonstrated in all these different cell types. After the first replication a viremia quickly starts. Experiments have shown the first viremia 8 hours after infection and a second one as early as 15 hours after infection. The second replication can occur in the spleen, liver, lungs and body lymph nodes. ASFV causes hemorrhages, but the mechanism behind this are still discussed, and later in the course of the disease, disseminated intravascular coagulation (DIC). Lymphocytes usually undergo apoptosis and also here the reasons why are not completely understood (Sánchez-Vizcaíno et al 2009).

Clinical signs

The signs of ASF can vary, mostly depending on the virulence of the strain. Subacute or chronic ASF, which are primly caused by less virulent strains, have been seen in Europe and the Caribbean while the strains in Africa seem to cause more aggressive forms of the disease with peracute or acute course. The incubation period is between three to 15 days (Sánchez-Vizcaíno et al 2009).

Peracute ASF

Peracute ASF is one of the most common forms of ASF, the other one being acute ASF. Pigs die rapidly without any or with only a few clinical signs. If the pigs get any symptoms previous of death, they can be high fever, cyanotic–red parts on the abdomen, ears and legs, and decumbency (Penrith et al 2009, Epiwebb 2010).

Acute ASF

Pigs with acute ASF get high fever, become anorexic and lethargic. They may suffer from either diarrhea or constipation, and nausea. The same redness as in the peracute form can be seen, with cyanotic parts particular on the legs, the ears and the ventral abdomen (Fig. 2). Pregnant sows usually abort at all stages of pregnancy. Ataxia is common but other neurological signs, like convulsions, can also appear in the later course of the disease. Death usually occurs within two – seven days. Pigs can recover even though it is rare (Penrith et al 2009, Epiwebb 2010).

Fig. 2. A piglet with acute ASF with typical redness of the ears, snout and legs. This pig also suffered from neurological signs and died within a couple of hours, Mityana, Uganda, 2010 (personal photo).

Subacute ASF

Subacute ASF is usually seen when pigs get infected by less virulent strains. Signs of disease last for about three – four weeks and can be fluctuant fever, emaciation and cough, due to pneumonia. Pregnant sows usually abort. A pig with subacute ASF can die, recover or develop a chronic state of ASF (Penrith et al 2009, Epiwebb 2010).

Chronic ASF

Pigs with the chronic form of ASF get more diffuse symptoms like emaciation, arthritis, pneumonia and dermatitis. Viremia can reoccur with periods of fever. Secondary bacterial infections are common. The pigs usually die within a couple of months (Penrith et al 2009, Epiwebb 2010).

Pigs that recover from acute or subacute ASF can become subclinical persistent carriers of ASFV. The virus levels in recovered pigs are considered to be too low for the pig to transmit the virus by direct contact to other pigs, unless the virus becomes reactivated by stress. Experiments have been done where administrating corticosteroids to carrier pigs have caused new viremia. No major studies have been performed to investigate if persistently infected sows can transmit ASFV to their piglets but so far the literature indicates that so is not the case (Wilkinson 1984).

Pathology

The pathology varies depending on the form of the disease. Peracute and acute deaths in ASF does not cause major pathological findings. Fluids in the body cavities, cyanotic parts on extremities and the ventral abdomen are common findings. Since ASFV can cause intravascular coagulopathy and thrombocytopenia, bleedings in different organs like the kidneys, spleen, heart

and the gastrointestinal serosa can be present. Enlarged and hemorrhagic lymph nodes are also common (Sánchez-Vizcaíno 2009).

Pathological findings in pigs that die of subacute or chronic ASF varies more and can consist of pneumonia, emaciation, enlarged lymph nodes, arthritis, pleuritis and pericarditis (Sánchez-Vizcaíno 2009).

Immunity

Antibodies are formed after about 7–12 days after an acute infection. The antibodies do not protect completely against a new infection but some immunity against the same strain may develop. Virus replication can continue in the presence of antibodies. The antibodies persist for a long time and can be transmitted from the sow to the piglets trough the colostrum. Since there is no vaccine available and no known serological cross-reactions to other viruses findings of antibodies correlates to natural infection (Sánchez-Vizcaíno 2009).

Epidemiology

Domestic pigs are highly susceptible to ASF and the mortality rate can be as high as 100%. However, in enzootic areas the mortality can range from 10–90%, one reason might be a lower virulence among some strains (Epiwebb 2011). Some resistance among certain local breeds has also been discussed as a reason since a lower mortality rate sometimes can be seen in outbreaks with highly virulent strains (Penrith et al 2009).

The wild pigs in Africa, including the warthog (*Phacochoerus africanus* and *Phacochoerus aetiopicus)*, the bushpig (*Potamochoerus larvatus)* and the giant forest hog (*Hylochoerus meinertzhangeni)*, are also susceptible to the virus but do not seem to develop any clinical disease (Jori & Bastos 2009). Wild boars (*Sus scrofa*) in Europe are susceptible to the virus and develop clinical disease similar to what seen in domestic pigs (Penrith et al 2009).

The domestic cycle/new epizootic cycle

ASF exists in three different cycles (Sánchez-Vizcaíno et al 2009). In the domestic cycle, the direct contact between domestic pigs is the most important route of transmission and the infection route is dominantly oronasal. Transmission also occurs through aerosols, feces, urine and other body fluids. A pig with acute ASF shed high amounts of virus and if it survives it can continue to shed virus for about 30 days even if the infection may persist longer. Other routes of transmission, especially important for spreading to new areas, is through swill feeding with uncooked pork and through contaminated fomites (Penrith et al 2009, Jori & Bastos 2009). The opinion about how long a domestic pig can stay infected varies between different scientists. According to Penrith (2009) the virus can persist for maximum three to four months while Costard et al (2009) believe that it can persist for at least six months. Not much research is done on the possibility of vertical transmission of ASFV but available studies indicate that this does not happen (Wilkinson 1984).

The intermediate cycle

In this cycle the soft tick, *Ornithodoros porcinus,* plays a major role as a reservoir and transmits ASFV between domestic pigs. Soft ticks are long lived and can survive for over five years without eating. They can carry infectious amounts of ASFV for several years, without any remarkable decrease. In the soft ticks the virus is maintained through transmission sexually, trans-ovarially and trans-stadially, this makes it possible to maintain ASFV is an area without infecting any suids (Sánchez-Vizcaíno et al 2009). This can cause outbreaks of ASF when new pigs are introduced to an old pig pen that contains ticks and where the disease has previously occurred. ASFV has been found in ticks collected in pig sties that has been empty of pigs for four years (Ravaomanana et al. 2010)

The sylvatic cycle/old epizootic cycle

The third cycle is the sylvatic cycle, where the virus cycles between wild suids, especially warthogs, and the soft tick. The virus is endemic in the warthog population in East and South Africa without causing any clinical disease in the hogs. The virus is believed to transmit between neonatal warthogs and soft ticks in the warthog burrows. A viral replication occurs in the young warthogs and the blood virus levels become high enough to transmit to soft ticks that can infect new warthogs. In older warthogs the blood virus levels never become high enough to infect ticks or other suids. No horizontal or vertical transmission is believed to occur between warthogs (Jori & Bastos 2009, Costard et al 2009).

The spread of AFSV from warthogs to domestic pigs is thought to take place when warthogs and domestic pigs share the same feeding area and tick from the warthogs bites the domestic pigs. Carcasses from warthogs, brought by humans, are also believed to play a crucial role in meaning of carrying ticks. Feeding on carcasses from infected warthogs, on the other hand, has not lead to infection in the domestic pigs in experiments. Direct contact between infected warthogs and domestic pigs has not been shown to cause transmission. It is believed that infected wild suids only have an infective virus level in other tissue than the lymph nodes for about two months after infection but stay infected for life (Jori & Bastos 2009).

ASF in other wild suids

The role of other wild suids has not been investigated thoroughly. It is known that the bushpig and the giant forest hog can become infected with the ASFV and that they do not seem to get any disease. The giant forest hog very seldom exists in the same habitats as the domestic pigs and are not likely to play an important role in the transmission of ASFV. It is also worth to be mentioned that ASFV has only been found once in this species (Jori & Bastos 2009).

Fig. 3. A bushpig in Lake Mburo National Park, Uganda, 2010 (Ganowiak, J.).

The bushpig (Fig. 3) has been considered to be of less importance than the warthog, at least in South Africa, but since the bushpig is a nocturnal animal it is harder to catch and fewer studies have been carried out. Bushpigs also have a reduced immune response against ASFV and it could therefore sometimes be difficult to find antibodies, which might lead to an estimated prevalence that is lower than in reality (Jori & Bastos 2009). The bushpig is also suspected to be a reservoir of ASFV in areas where there are no warthogs, but where the virus is endemic. The blood virus levels in an infected bushpig are high enough to infect both the soft ticks and domestic pigs. Bushpigs do not however live in burrows and therefore do not get in contact with the soft ticks naturally (Costard et al 2009). It is reported that interbreeding has occurred between bushpigs and free ranging domestic pigs but no scientific confirmation has been done. It has been speculated that hybrids, if they exist, may become asymptomatic carriers among domestic pigs and thereby maintain the spread of the virus, this since pure breed bushpigs do not get any clinical signs, (Jori & Bastos 2009). Bushpigs are hunted for theirmeat (Fig. 4), and leftovers fed to domestic pigs could lead to infection if the virus amount in the tissues is high enough (Sánchez-Vizcaíno et al 2009).

Fig. 4. A bushpig carcass brought to a village by local hunters in Masaka, Uganda, 2010 (Andersson, M.).

Bushpigs have, in contrary to the warthog, been shown experimentally to transmit ASFV to domestic pigs by direct contact. Contact with feces from infected bushpigs is also considered to be a possible transmission route from wild to domestic suids (Costard 2009).

Uganda

Uganda is a sub-Saharan country lying on the equator in Eastern Africa. It was a British colony from early 1900's to 1962 when it won its independence. Since then Uganda has had a hard time struggling civil strife and also international conflicts (Briggs, 2010). Things are improving steadily but still the country is very poor and is rated as number 143 of 169 in the Human Development Report 2010, with 31.1% living in poverty.

Agriculture is of big importance in Uganda, accounting for about 60% of GNP and approximately 90% of Ugandans get some of their livelihood directly from agriculture. About 85% of Ugandans live in the rural areas. Because of the lack of proper infrastructure it is hard for the people living in the rural areas to keep up with and get advantages of the progresses in more central parts of the country (Rural poverty portal 2011).

Pig production in Uganda

The pig production in Uganda is growing rapidly with about 1.8 million pigs in 2003 and 3.2 million in 2009 (UBOS 2010). Uganda has the largest population of domestic pigs in East Africa. Most of the pigs are held by small scale farmers in the rural areas, either free-ranging or in small housing systems. There are also

bigger, commercial pig farms, often in the urban or periurban areas (Phiri 2003, Sánchez-Vizcaíno et al 2009).

African swine fever in Uganda

ASF is endemic in Uganda (OIE 2010) but there has not been much research done on the epidemiology of the disease and to assess the magnitude of the problem. In the last six months of 2010 at least nine outbreaks of ASF were confirmed in Uganda (Ståhl, ASFUganda, personal communication). The disease is reoccurring among domestic pigs but there is only minor knowledge of its origin. Further investigations about which epidemiological cycle (the domestic or the sylvatic) that is the main concern for introducing ASFV to domestic pigs is essential (Jori & Bastos 2009).

An outbreak of ASF can strike hard on the larger farms with intense pig production in Uganda but it also to a great extent affects the poorer farmer who cannot afford proper biosecurity or often do not have enough money to start over after a considerable financial loss (Costard et al 2009). Free-ranging pig can get in contact with ASFV by meeting wild suids or ticks while an important source of infection for pigs in commercial farming systems are newly introduced, recently infected (or possibly subclinically infected) pigs from the rural areas (Sánchez-Vizcaíno et al 2009). Many smallholder farmers lack knowledge about how diseases are transmitted, and mixing of live pigs from different farms in markets and on transports are common. Another essential factor is that poor farmers often cannot afford to lose pigs, therefore they often sell or slaughter their animals as soon as they start getting symptoms of a severe disease among their pigs (Costard et al 2009).

Among the wild suids in Uganda ASFV has been detected in warthogs but not in the bushpig or the giant forest hog. The warthog has been considered to be a main source of infection from wildlife to domestic pigs and the prevalence of infected warthogs is high (>80%) in most of the examined populations in East Africa. Since no direct transmission from warthogs to domestic pigs occur, in contrast to case of bushpigs, the importance of bushpigs in the ASF epidemiology needs to be further investigated and maybe more focused on (Jori & Bastos 2009).

OBJECTIVE

The main objective of this project was to find different reservoirs for ASFV in Uganda. A sub objective was to estimate the prevalence of the disease.

Aims

- Investigate if healthy domestic pigs can act as reservoirs for ASFV in Uganda
- Estimate the prevalence of ASFV and the seroprevalence in domestic pigs in the Rakai district in southern Uganda
- Investigate if wild suids (warthogs and bushpigs) and soft ticks can act as reservoirs for ASFV in Uganda and also if bushpigs and domestic pigs can interact

METHODS AND MATERIAL

Sampling of domestic pigs and interviewing local pig farmers

Blood samples were collected from eight different sub counties in the district Rakai in southern Uganda. In each sub county samples were collected from five different villages and from each village, from two farms. In each farm three pigs, with no clinical signs of disease were sampled, with some exceptions, ending up with a total of 239 samples from domestic pigs. The selection of the farms was done in collaboration with the local veterinary officers (VO), with the ambition to achieve a wide geographical distribution (see map, Fig. 5).

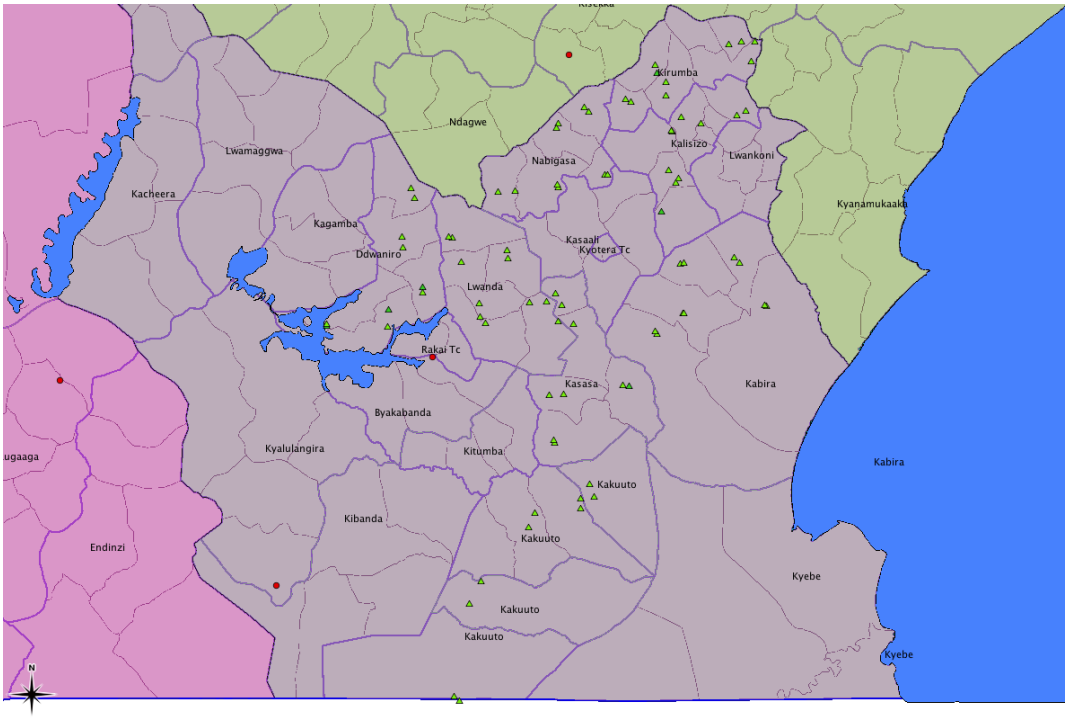

Fig. 5. Map of the sub counties and the sampled farms in Rakai, Uganda, 2010.

The blood was collected from the jugular vein in medium to large sized pigs (Fig. 7), while the cranial vena cava was used for smaller pigs. Serum and EDTA tubes where collected from each pig. Sex, breed (local, mixed or improved) and an estimated age were noted. The samples were named after a system with three letters representing the sub county, one number representing the farm and one number representing the individual pig. For example, pig number two at farm number 8 in the sub county Kasasa would be named KAS 8:2. The serum tubes were centrifuged either the same day as the sampling or the day after and the serum was separated from the blood. The samples were stored in duplicates, at -80^{o}C for long term storage, and at -20^{o}C for diagnostic analysis, respectively.

Fig. 6. A local VO interviewing a farmer (Ganowiak, J.) Fig. 7. Blood sampling a mixed breed pig from the jugular vein (Andersson, M.). Both pictures from Rakai, Uganda, 2010.

The local VOs accompanied us to all farms and assisted in data collection using a questionnaire (Fig. 6). Since this study is a part of a bigger project only some of the data is used in the thesis.

Collecting wildlife data

Four bushpigs were caught and sampled for this study. Three of them were caught at the same time in Lake Mburo National Park and one was caught in a swamp in the greater Masaka district. Blood samples were collected in serum and EDTA tubes for analyses, from all four of them. One of the bushpigs in Lake Mburo and the one in Masaka were equipped with harnesses with GPS tracking devices (Fig. 8).

Fig. 8. Providing a bushpig with a GPS equipped harness, Masaka, Uganda, 2010 (Ståhl, K, ASFUganda.).

The GPSs provided information about the bushpigs' positions every third hour. The data was used to study the movement of the bushpigs to see if they came in contact with farms with domestic pigs. Data was collected for about three months from the Lake Mburo bushpig and for about two month for the Masaka bushpig.

Soft ticks were collected from warthog burrows in Lake Mburo National Park. Ticks from two different burrows were used in this study. One warthog was also sampled from Lake Mburo Narional Park. Blood was collected in EDTA and serum tubes.

Laboratory analyses

Some of the blood samples collected in serum tubes from Rakai, were centrifuged and the serum separated from the blood, in a small laboratory in Rakai. The rest of the samples in serum tubes were centrifuged at the Faculty of Veterinary Medicine, Makerere University, Kampala, Uganda. All laboratory analyses were run in the Molecular Biology Laboratory at the Makerere University Institute of Environment and Natural Resources (MUIENR), , in Uganda. The diagnostic techniques used were among the once recommended, for diagnosing ASF, by the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (2008) for detecting antibodies against and virus DNA.

PCR

For DNA extraction, a commercial kit from Qiagen, DNeasy Blood & Tissue Kit, was used. The test procedure that came with the kit was followed and 100µl of anticoagulated blood was used for every purification. The samples from Rakai were pooled farmwise before purification, mixing 100µl of blood from every individual from a farm in a collective tube. 100 µl from this tube was then used for the purification. The ticks were first grounded with sterile sand, as described by Bastos (2009), and then the same procedure as for the blood was followed. The extracted DNA was either used immediately or stored in a -20^{o}C freezer.

For the detection of ASFV DNA a realtime polymerase chain reaction (RT-PCR), targeting a gene for a structural protein, p72, which is highly conserved in ASFV, was used with reagents from Tetracore. The protocol used was, as described by Zsak el al (2004), 45 amplification cycles (95^{o}C for 2 s and 60^{o}C for 30 s) and run on a portable instrument, connected to a laptop, called SmartCycler (Cepheid, Inc., Sunnyvale, California). Two positive controls were used, one included in the kit from Tetracore and also one consisting of diluted, purified ASFV DNA (kindly provided by Dr J. Fernandez, from the international reference laboratory for ASF, CISA-INIA, Spain). As a negative control phosphate buffered saline (PBS) was used. The assay has recently been evaluated in a ring trial organized within the EC Network of Excellence EPIZONE, showing a diagnostic performance in line with or superior to the OIE recommended RT-PCR (unpublished). This method is quantitative and with a cycle threshold (Ct) value that depends on how many amplification cycles needs to be run before a threshold value is reached. Lower Ct values correspond to higher concentration of viral DNA in the sample. Positive samples results in a sigmoid curve (Fig. 9).

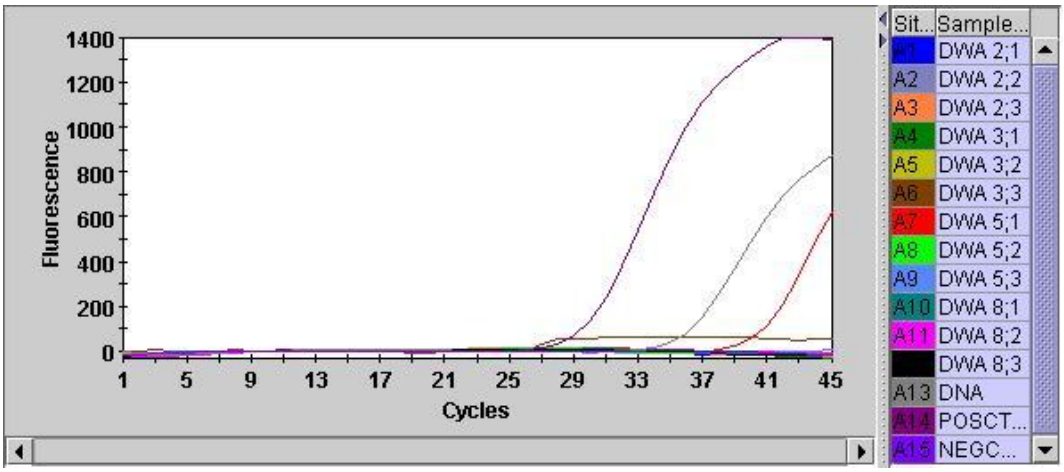

Fig 9. Sigmoid curves from one RT-PCR run on individual samples from the sub county Ddwarino, Rakai, Uganda. The two positive controls (POSCT and DNA) and DWA 5:1 shows sigmoid curves corresponding to positive samples. DWA 3:3 shows a doubtful curve but a rerun showed a negative result.

From the farms with positive PCR results, DNA purification and RT-PCR were rerun on an individual level, using 100 µl of anticoagulated blood. If no positive individual sample was found two more reruns were made, one on the same purificated DNA used on the first run and one from purificated DNA from serum. RT-PCR was also run on whole blood from the bushpigs and the warthog.

ELISA

To detect antibodies from serum from the domestic pigs, the bushpigs and the warthog, a commercial blocking enzymatic immunoassay (Blocking ELISA) kit from Ingenasa was used. The test has a high sensitivity and specificity but the sensitivity decreases if the samples are poorly preserved (OIE 2008). The wells are coated with a purified protein extract from ASFV (p72) as antigen. This protein is considered the most antigenic one in the virus. All reagents used were provided in the kit. The samples were diluted 1:1 (50µl + 50µl) with diluents, directly in the 96 well microtitration coated plates, which came with the kit. Two wells were used for negative control and two for positive control, the controls came with the kit and were also diluted 1:1 (50µl + 50µl). The recommended test procedure was followed and then the Optical density (OD) values were read at 450 nm. The negative cut off (NC) was, as recommended, set to the mean OD values of the negative controls and the positive cut off (PC) was set to the mean value of the positive controls. The test was validated if $NC/PC \geq 4$.

RESULTS

Blood samples

Five out of 239 pigs were ELISA positive resulting in a seroprevalence of 2.1%. For samples with OD values between the PC and the NC a rerun was made but there were still 15 samples with doubtful results. Eight pigs were positive on the PCR, 3.3 %, and three of these pigs were also doubtful on the ELISA test (DWA 3:3, DWA 5:1 and DWA 8:3). All the PCR positive pigs had Ct values over 38.2 (table 1), which are considered to be high and correspond to a low concentration

of virus DNA. This relates good to the fact that no pigs had any clinical signs of disease.

Table 1. The cycle threshold (Ct) values on the PCR positive pigs from Rakai, Uganda. The PCR is run on anticoagulated blood or serum

Pig id	KAS 6:3	KLZ 10:1	KIR 10:2	DWA 2:2	DWA 3:2	DWA 5:1	DWA 5:3	DWA 8:3
Ct value	40,2	39,1	38, 2	41,1	41,0	39,9	39,5	40,2

Five of the PCR positive pigs came from the same sub county, Ddwaniro (DWA) and one farm had two PCR positive pigs (DWA 5). The last three PCR positive pigs came from different sub counties; Kasasa (KAS), Kalisizo (KLZ) and Kirumba (KIR) (see map, Fig. 5). Also the ELISA came out with most positive (two) and doubtful (11) results from the Ddwaniro sub county. One sub county, Lwanda, had no pigs that tested positive or doubtful on neither the ELISA nor the PCR. In total 24 pigs were positive or doubtful on the lab analyses made.

*Table 2. Distribution of the pigs testing positive or doubtful on ELISA or positive on the PCR, in the different sub counties. Values between the negativt and the positive cut off is considered to be doubtful on the ELISA. *Three pigs were both positive on the PCR and doubtful on the ELISA*

	Number of pigs			
Sub county	ELISA positive	Doubtful ELISA results	PCR positive	Total
Ddwaniro	2	11	5	14*
Kakuuto	0	1	0	1
Kasasa	1	0	1	2
Kabira	0	1	0	1
Kirumba	1	1	1	3
Kalisizo	0	0	1	1
Lwanda	0	0	0	0
Nabigasa	1	1	0	2
Total	5	15	8	24*

The distribution of breed, sex and age among all the sampled domestic pigs in the study and also among the once tested positive on the PCR is shown below in Fig 10-12. The breed distribution varied significantly between the two groups with 87% of local breed among the PCR positive compared to only 45% of local breed in the whole population (χ^2=4.346, p=0.037 with Yates' correction). Also the age distribution varied between the two groups with only adult pigs and pigs in the estimated age of three-five months that were PCR positive. Among all the tested domestic pigs 21% were in the age of three-five months but among the PCR positive pigs 50% were in the same age group.

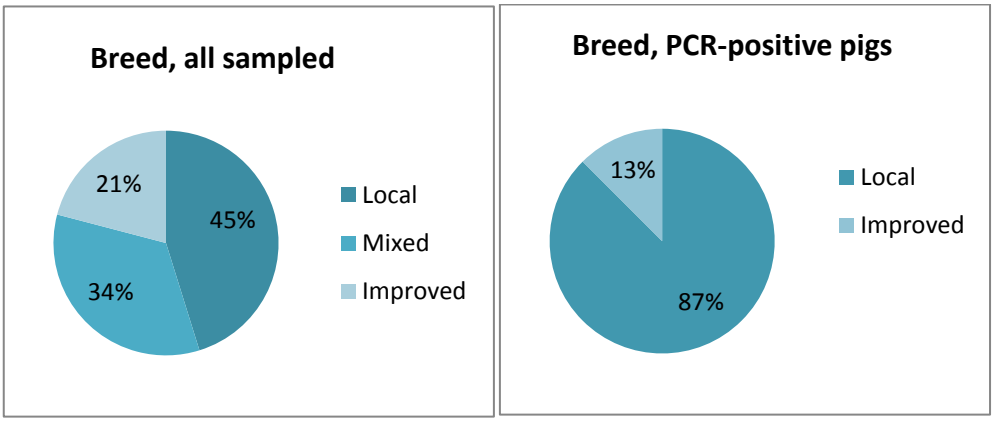

Fig 10. The breed distribution among all sampled pigs from Rakai and among the PCR positive pigs from Rakai. Local = black pig, mixed = black and white, improved = white.

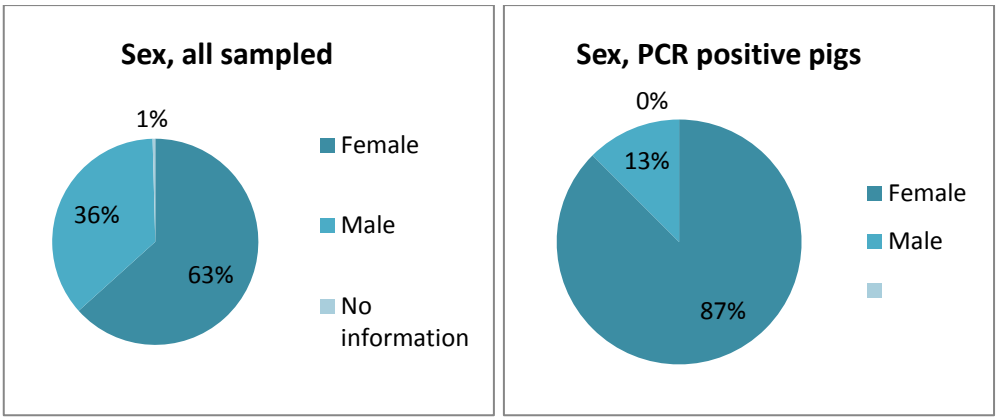

Fig. 11. The sex distribution among all the sampled pigs from Rakai and from the PCR positive pigs from Rakai.

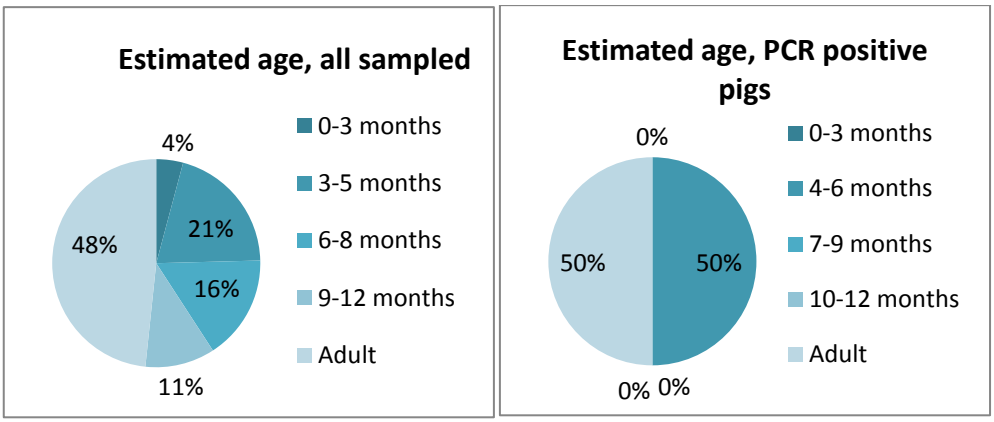

Fig 12. The estimated age distribution among all the sampled pigs from Rakai and among the PCR positive pigs from Rakai.

Two of the three bushpigs from Lake Mburo were seropositive but none were PCR positive. The Masaka bushpig was negative both on ELISA and PCR. Three out of four sampled soft ticks were PCR positive with Ct values between 38.4 and 41.4. The warthog was seropositive but PCR negative.

Questionnaire

Two out of seven farms (29%) with PCR positive pigs had experienced disease among their pigs within the last year, the clinical signs being fever in one case and fever, cough and red skin in one case. The same number among all farms visited was 31%. None of the farms with PCR positive and ELISA borderline cases had experienced any disease in the last year.

One of the farms with the PCR positive pigs had knowledge about wild suids in the surroundings (14%, table 3). Six (7.5%) of all the visited farms had knowledge about wild suids in the area and six (7.5%) of the farms did not know if there were wild suids in the surroundings or not.

Table 3. Answers on the questionnaire among the farms with PCR positive pigs

Farm id	Wild suids in the area	Disease among the pigs in the last year	Specified disease
KAS 6	Yes	Yes	Fever
KAL 10	No	No	-
KIR 10	No	Yes	Fever, red skin, cough
DWA 2	No	No	-
DWA 3	No	No	-
DWA 5	No	No	-
DWA 8	No	No	-

Bushpig movement

Information from Lake Mburo showed that the bushpig did not move outside the national park and consequently did not come in contact with human habitat. The Masaka bushpig, on the other hand, repeatedly visited areas around farms and could theoretically interact with domestic pigs and thereby represent a possible risk for introducing infections to domestic pigs (Fig. 13).

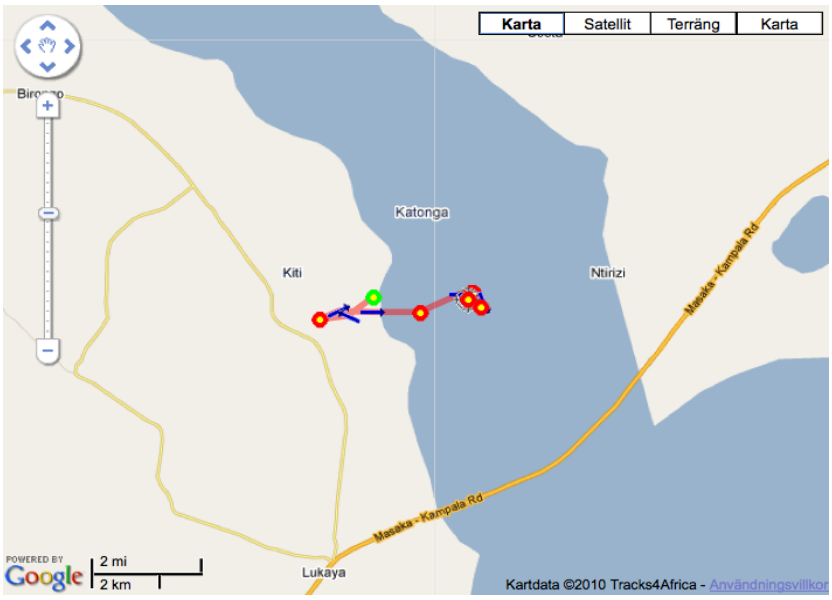

Fig. 13. GPS readings from the Masaka bushpig showing how the bushpig leaves the swamp for an area with human habitation. (ASFUganda, Savannahtracking).

DISCUSSION

The finding of either antibodies or viral DNA in healthy domestic pigs, bushpigs, warthogs and soft ticks means that all these species must be seen as potential reservoirs for ASFV in Uganda. The bushpigs involvement in the maintenance of ASF in Uganda has not been proven before (Jori & Bastos 2009) but this needs to be reevaluated. To know what reservoirs are the main sources of new outbreaks among domestic pigs, deeper studies like virus isolation and phylogenetic studies, are necessary. By learning more about the different reservoirs it is easier to develop better biosecurity recommendations for the individual farmers but also on a national level. If all the reservoirs listed above show to be important, the strategy to prevent outbreaks need to be broad. Different measurements like better control during trade with domestic pigs, preventing from possible interaction with wild suids, eradication of ticks in sties before introducing new pigs etc., needs to be considered apart from the basic biosecurity (footbaths, restricted visitation to the farm and so on).

The domestic pigs, with no recent history of clinical disease, that were PCR positive is an important finding. If these pigs carry live virus (needs to be confirmed by virus isolation) this means that it is possible to spread ASFV with trading of healthy pigs, acting as asymptomatic carriers. Since none of them were seropositive, even biosecurity measures like testing for antibodies before any

trade, is not sufficient to prevent the spread of ASFV. Transmission of ASFV from subclinically infected pigs to naïve pigs is though considered to be rare and probably the virus needs to be reactivated (Wilkinson 1984). Another conclusion is that a screening for antibodies is not enough to declare an area as free from ASFV. Additional PCR screening is necessary. If there were only adult pigs in this group, one reason for the lack of antibodies, could have been that antibodies had been formed earlier in life and disappeared. However, previous studies (Sánchez-Vizcaíno et al. 2009) indicate that antibodies persist for at very long time, maybe even for life. Given that some of these pigs were only three-five months old, it is more likely that no antibodies have been formed, either because of an early stage in the infection or because of lack of a proper immune response to the virus. Three of the PCR positive pigs where borderline cases on the ELISA and could maybe be explained with an early and ongoing but subclinical infection, a decrease in antibodies or a lower antibody response than usual. Another reason, not to be excluded, could be falsely high OD values (false negative) on the ELISA because of decreased sensitivity in the test when samples are not properly kept. Some samples were a bit haemolysed and this could represent a source of error. Both Ingenasa and OIE recommend running these samples with a different technique, like an indirect fluorescent antibody test, but this was not possible in this study.

Only two out of seven farms with PCR-positive pigs had had any disease among their pigs in the last year. The fact that some of these pigs were under six months of age may points both to subclinical carriers among domestic pigs and maybe even intrauterine infection, causing persistently infected pigs. Intrauterine infection of ASFV has nevertheless not been demonstrated (Wilkinson 1984), but could potentially be an explanation to the lack of immune response in some individuals. Other reasons for finding young PCR positive pigs could be infection by ticks or by interaction with wild suids. Since it is proved that soft ticks can survive, in for example pig sties, for a long time and carry infective amounts of ASFV without feeding on any suid for several years (Ravaomanana et al. 2010) this is a very possible source of infection.

In the study 45% of all sampled pigs where of local breed but among the PCR positive pigs 87% were of local breeds. This finding supports the theory that some local breeds have some sort of inherited resistance to ASFV (Penrith et al 2009). Since the age of the sampled pigs were only estimated it is impossible to make any conclusions from the fact that so many of the PCR positive pigs came from the same age group (three-five months), the second lowest age group. Possible reasons could be that a persistently infected animal could have a slowed growth rate and are therefore easily misjudged in the matter of age. Another reason could be that the pigs of local breed were generally smaller than the improved once and therefore maybe estimated as younger than they in fact were.

The finding of ASFV antibodies in bushpigs in Uganda, together with the fact that the bushpigs exists in the surroundings of villages and feed on the corps grown by farmers, shows that bushpigs must be considered a possible reservoir and source of transmission of ASFV. An important factor not being enlightened by the literature, but mentioned by Sánchez-Vizcaíno et al (2009) is the use of bushpig meat. Bringing possibly infected carcasses to the villages and feeding the domestic pigs with leftovers could definitely be a source of infection.

The tick/warthog cycle is, as already proven by Costard et al. (2009), Jori & Bastos (2009) among many others, also important for the maintenance of ASF in Uganda. From this study no conclusions about the importance of this cycle for the ASF outbreaks among domestic pigs, can be made. The seropositive warthog, the PCR positive soft ticks and the seropositive bushpigs all came from the same area, suggesting a possible transmission between warthogs and bushpigs, with or without tick involvement.

Irrespective of which the most important reservoir for ASFV is in Uganda, information about proper biosecurity must be one of the most important instruments in helping small scale farmers to prevent from infectious diseases among their pigs.

Future aspects

Further clinical experiments on persistently infected pigs could be helpful to see how long and if a pig carrying the virus could infect and cause disease to a naive pig, and such studies are in the pipeline. By inducing a physiological stress by administrating corticosteroids one can try to imitate a real stressful situation, like transportation, and then see if a new viremia is present and if it is high enough to cause transmission to naive pigs. Experiments like this have previously been performed (Wilkinson, 1984) but new studies could show more reliable results with new diagnostic methods being available. It would also be of interests to study the possibility of piglets being born as persistently infected due to intrauterine infection. If the piglets are infected before the immune system is fully development this could possible cause a lower immune response to ASFV than usual and therefore result in PCR positive and seronegative pigs.

By isolating and comparing virus from PCR positive pigs from outbreaks and from subclinical carriers one can get more knowledge about why some pigs do not develop a clinical disease. The information may give answers to if it mostly the virulence of different strains that differs or if it is the resistance among certain breeds and/or individuals of pigs. Since ASFV is known to have at many genotypes, with different virulence (Sánchez-Vizcaíno et al. 2009), a genotyping must be performed before taking any premature conclusions about resistance among certain pigs. To develop a resistance against a certain virus one may think that the virus needs to be stable and not, like ASFV, have a high level of variability.

As discussed by Jori & Bastor (2009) it would be of great interest to learn more about the importance of wild suids, especially bushpigs, in the epidemiology of ASF. To do this it would be interesting to perform virus isolation, sequence analysis and comparing the virus strains found in the domestic pigs and in the bushpigs. Since only antibodies were found in the bushpigs and the warthog in the study it would be interesting to run a PCR on tissue samples, preferable from lymph nodes, from these animals and see if they are PCR positive and if so, perform a genotyping to compare the virus DNA.

Another appealing, but maybe difficult, study would be to compare the genome of bushpigs and different domestic pigs of local breed to see if there are scientific evidence of some interbreeding. As mentioned by Sánchez-Vizcaíno et al (2009),

if hybrids exist they could hypothetically act as asymptomatic carrier pigs. A further aspect on this would be to see if the interbreeds were more immune to ASF than purebred domestic pigs.

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