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Outbreaks of African swine fever in domestic pigs in Gulu district, Uganda

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SAMMANFATTNING

Efterfrågan på animalieprodukter har ökat kraftigt i världen de senaste decennierna. Särskilt gäller detta griskött och fjäderfä. Grisproduktionen har ökat drastiskt även i Uganda, ett land där trettioen procent av befolkningen lever i fattigdom. Grisproduktion anses vara en viktig faktor i fattigdomsbekämpning, då det tillför viktigt inkomst såväl som tillskott på fullvärdigt protein.

Afrikansk svinpest (ASF) är en dödlig sjukdom bland domesticerade grisar som ofta karaktäriseras av hög feber, blödningar och dödlighet upp till 100%. Sjukdomen är endemisk i de flesta afrikanska länder söder om Sahara och sedan 2007 i Ryssland och Kaukasusområdet. ASF leder till allvarliga sanitära och socioekonomiska konsekvenser i länder där sjukdomen är endemisk såväl som i länder där den nyss introducerats. Epidemiologin som innefattar både tamgrisar och en sylvatisk cykel är komplex, och det tillsammans med avsaknaden av vaccin och virusets höga motståndskraft i kontaminerade svinprodukter gör ASF till en av de mest fruktade och därför viktigaste grissjukdomarna i världen. ASF utgör ett allvarligt hinder för grisproduktionen i Afrika, där kontroll svårgörs av ett flertal faktorer såsom avsaknad av grundläggande biosäkerhet, lösgående grisar och förflyttning av grisar.

Syftet med studien var att samla information från det gångna årets utbrott av ASF i distriktet Gulu i Uganda, för att öka förståelsen för den spatiotemporala dynamiken av sjukdomen och att klargöra de huvudsakliga smittvägarna. Sexton byar där ASF konfirmerats besöktes och 135 intervjuer utfördes. Mortaliteten under dessa utbrott uppskattades till 84,5 %. Nitton blodprov togs för analys. Tretton blodprover togs från grisar som misstänktes vara infekterade med ASF och fem av dessa var PCR-positiva. Utav de sex som togs från grisar som överlevt utbrott av ASF var tre ELISA-positiva.

De huvudsakliga smittvägarna var handel med levande grisar och grisprodukter. Detta var särskilt markant i början av ett utbrott (ofta på detta sätt som viruset introducerats i en by). Efter introduktion i en by så spreds viruset troligen via slaktavfall och direktkontakt mellan grisar, vilka båda underlättas genom bristande biosäkerhet och att grisar ofta går lösa. Inverkan av vilda reservoarer och subkliniska smittbärare undersöktes inte i denna studie men förmodas vara av mindre betydelse.

ABSTRACT

The demand for animal products has increased in the last decades, especially for poultry and pork. Pork production has increased drastically globally, and also in Uganda. Thirty-one percent of Uganda´s population lives in poverty. Pig production is believed to play a crucial role in poverty alleviation, because of the ability to convert low quality feed into high quality protein together with high reproductive potential.

African swine fever (ASF) is a lethal disease of domestic pigs often characterized by haemorrhagic fever and with mortality rates up to 100%. The disease is endemic in most sub-Saharan countries and since 2007 in the Russian Federation and Caucasus area. ASF is associated with severe sanitary and socio-economic consequences, in countries where it is endemic as well as in areas where it has recently been introduced. The epidemiology, involving a domestic and a sylvatic cycle, is complex. This, together with the absence of vaccine and the marked virus resistance in contaminated animal products, make ASF one of the most feared and therefore important diseases in swine. ASF poses a serious constraint on pig production in Africa, where control is made difficult by several factors, including lack of basic biosecurity measures, traditional free-ranging husbandry system and movements of pigs.

The aim of this study was to collect information from the past year´s outbreaks of ASF in Gulu district to better understand the spatial and temporal dynamics of the disease and to clarify modes of transmission.
Sixteen villages with history of confirmed outbreaks of ASF were visited and 135 interviews were performed. The average mortality during these outbreaks was estimated at 84.5%. Thirteen blood samples were collected from clinically affected pigs. Out of these, five were PCR-positive. Out of the six blood samples from pigs that survived outbreaks, three were ELISA-positive.

The main transmission routes were trade of live pigs and pig products, especially at onset of an outbreak (introduction into a village). When the virus had been introduced into a village, it was probably spread through swill/waste and direct contact between domestic pigs, both of which are facilitated through the traditional free range pig husbandry system and lack of biosecurity. The involvement of wild reservoirs and subclinical carriers was not investigated but is assumed to be less important.

BACKGROUND

During the last decades, the demand for meat and milk has increased in the world, particularly in developing countries. Consumption of meat increased almost three times more in developing countries than in developed countries from early 1970 to mid 1990s (Delgado & Narrod, 2002). The trend is expected to continue. Pigs and poultry are increasing the most. Global pork production increased more than threefold (from 25 to 87 million tons) between 1961 and 2002 (den Hartog, 2004). North America, Europe and East Asia are the main producers of pork, with China alone having almost 50% of the world´s pig population. The trend is visible also in Africa.

Infectious diseases pose a serious threat to livestock production as it leads to reduced production, higher mortality and greater environmental impact of the larger number of animals. Furthermore, there are the obvious risks of food poisoning and possible zoonotic aspects.

African swine fever (ASF) is one of the most important diseases in swine with high mortalities in domestic pigs and severe socio-economic consequences wherever it is introduced (Sánchez-Vizcaíno et al., 2012). The disease is endemic in many parts of Africa.

This project was conducted as part of ASFUganda, an ongoing collaborative research project financed by various Swedish research institutions (Sida, SLU, Formas), coordinated by the Swedish University of Agricultural Sciences (SLU) and scientists from Makerere University, the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Uganda Wildlife Authority (UWA) and International Livestock Research Institute (ILRI) as partners.

The study was carried out as a Minor Field Study, financed by Sida (Swedish International Development Cooperation Agency) with additional support from SLU.

AFRICAN SWINE FEVER

Aetiology

ASF virus, ASFV, is a large DNA-virus that replicates cytoplasmatically in infected cells (Chapman et al., 2008). It is the only member of the genus *Asfivirus* in the family *Asfarviridae*. Based on the structural protein p72, 22 different genotypes have been described, of which 14 are present in southern Africa (Boshoff et al., 2007). ASFV is also the only known DNA arbovirus (arthropod borne virus) (Penrith, 2009).

Warthogs (*Phacochoerus* spp.), bushpigs (*Potamochoerus porcus)* and soft ticks (*Ornithodorous* spp.) are natural hosts for the virus and are only asymptomatically infected (Chapman et al., 2008; Rowlands et al., 2008). By contrast, the European wild boar (*Sus scrofa*) develops clinical disease similar to domestic pigs and is not considered a long term reservoir (Costard et al., 2009).

The virus is very resistant and can, for example, persist in tissues for several months (Costard et al., 2009) and 1000 days in frozen meat (Sánchez-Vizcaíno et al., 2012). ASFV can also persist two months in pig faeces (Sánchez-Vizcaíno et al., 2009). Moreover, the virus is resistant to changes in temperature and pH, meaning that smoked, salted or dry pork products are as big risk as raw or frozen ones (Penrith & Vosloo, 2009). However, temperatures greater than 60°C for more than 20 minutes will inactivate the virus.

History and distribution

African swine fever was first described by Montgomery in Kenya 1921 (Costard et al., 2009). The disease is probably indigenous to the African continent south of Sahara and was revealed as a problem first when domestic pigs were introduced by colonists from Europe (Sánchez-Vizcaíno et al., 2009). From its origin, in the eastern and southern Africa, ASF spread slowly to central and western Africa. However, since 1994 a rapid increase in ASF has been observed in Africa, with many new countries affected, and ASF is now endemic in more than 20 countries south of Sahara (Figure 1) (Gallardo et al., 2011; Penrith, 2009).

Portugal was the first country outside of Africa to experience outbreak of ASF in 1957, and another in 1960, after which the disease remained endemic in the Iberian peninsula until 1995 (Costard et al., 2009; Penrith & Vosloo, 2009). Outbreaks of ASF were reported from several countries in western Europe between 1960 and 1986, but the disease was eradicated from all affected regions except Sardinia in Italy, where it still remains endemic. Cuba was the first transatlantic country to report outbreak of ASF in 1971, with subsequent reports from several Caribbean island countries as well as Brazil (1978) (Costard et al., 2009). The disease was eradicated from these countries with great effort and cost (Penrith & Vosloo, 2009).

ASF gained new global interest in 2007 when ASF was confirmed in the Republic of Georgia (Penrith & Vosloo, 2009; Costard et al., 2011), probably introduced through the port of Poti, where free-ranging pigs are thought to have come across food scraps from ships (Rowlands et al., 2008; Penrith, 2009). Delays in recognizing ASF resulted not only in great numbers of dead pigs in Georgia, but also swift subsequent spread to neighboring countries in the Caucasus region (Rowlands et al., 2008; Costard et al., 2009; Penrith, 2009). ASF was confirmed in wild boars in Chechnya (Russian federation) and spread further in wild boars as well as domestic swine in the area (Penrith & Vosloo, 2009). The rapid spread and uncontrolled situation in Russian federation (with outbreaks as far northwest as St Petersburg and Murmansk) is considered a real threat, particularly to the European Union (Sánchez-Vizcaíno et al., 2012) (see figure 2 for outbreaks during 2011). Luckily, ticks do not appear to be involved so far. However, there are some *Ornithodoros*-species in the region and it is believed that all species are susceptible to infection with ASFV (Sánchez-Vizcaíno et al., 2012).

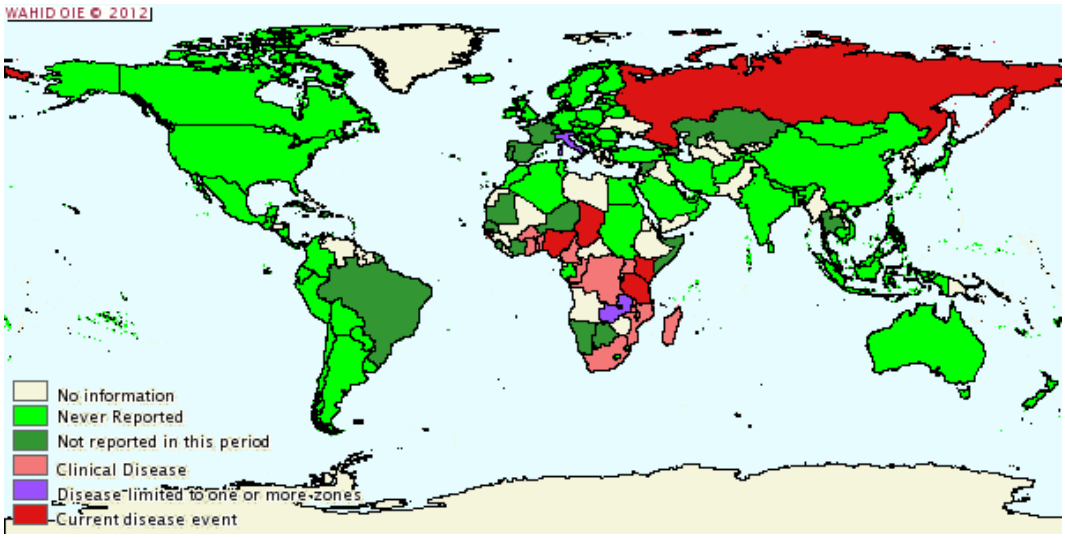

Figure 1. World distribution of ASF 2011 (WAHID OIE, 2012: http://web.oie.int/wahis/maps/temp/133009177230081.png).

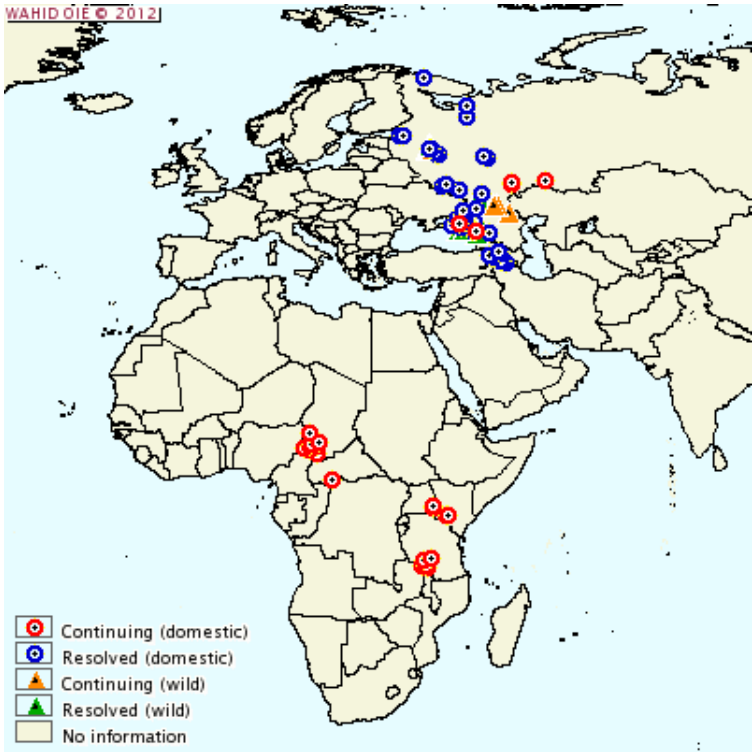

Figure 2. Outbreaks of ASF during 2011 (WAHID OIE, 2012).

Pathogenesis

The virus most commonly enters the body via the tonsils or pharynx to the regional lymph nodes (Sánchez-Vizcaíno et al., 2009). Viraemia is observed from three days after infection (Costard et al., 2009). The virus replicates primarily in cells of the mononuclear phagocytocis system (Sánchez-Vizcaíno et al., 2009). Injuries on endothelium lead to haemorrhages and dissiminated intravascular coagulation (DIC). Common pathological lesions of acute forms of the disease include widespread haemorrhage in organs and on parietal surfaces, blood fluid in

body cavities, petechiae, congestions of lungs, spleen, lymph nodes and gastrointestinal tracts (Sánchez-Vizcaíno et al., 2009).

The incubation period is generally between 4-6 days (6-8 days in subacute cases) (Sánchez Botija, 1982). Clinical signs vary from peracute to subacute, much depending on the virulence of the virus (OIE, 2009; Sánchez-Vizcaíno et al., 2009). In peracute ASF, pigs can die without previous clinical signs. In the acute form, high fever (more than 40°C), reddening of the skin (ears, tail distal limbs, ventral areas of the trunk), anorexia, incoordination, vomiting and diarrhea (sometimes bloody) are common symptoms, and almost all animals die within 6-13 days (8-12 days, Costard et al., 2009). The clinical signs of peracute and acute ASF are very similar to other haemorrhagic diseases such as CSF (classical swine fever), salmonellosis and erysipelas (Sánchez-Vizcaíno et al., 2012). The clinical signs of subacute ASF are milder, with undulant fever for several weeks, anorexia, dyspnea and abortion in pregnant sows (Sánchez-Vizcaíno et al., 2009). Mortality varies (30-70% according to Sánchez-Vizcaíno et al., 2009), and some pigs recover or stay chronically infected. Recovered pigs gain immunity towards related virulent viruses (King et al., 2011). Chronic ASF is connected to various signs such as respiratory signs, growth retardation, arthritis and increased susceptibility to secondary infections (Sánchez-Vizcaíno et al., 2009). There are some disagreement concerning the existence and importance of chronical carrier animals. Penrith & Vosloo (2009) claim that there is no evidence that recovered pigs can become long-term virus carriers, whereas Sánchez-Vizcaíno et al. (2012) mean that carriers have been demonstrated in both wild and domestic animals, and are important for persistence and dissemination in endemic areas. A review article by Costard et al. (2009) summarizes that pigs might remain persistently infected for 6 months and during this time act as a source of transmission to susceptible pigs.

Despite many attempts, there is so far no effective and safe vaccine available against ASFV, and the current control methods are therefore limited to prevention, e.g. quarantine/movement restrictions, and stamping out (Penrith, 2009; King et al., 2011). Immunity against ASFV is not fully understood; however, it has been shown that protective immunity can be accomplished after infection with low virulent isolates or attenuated viruses (Costard et al., 2009; King et al., 2011), as well as by passively transferred ASFV-antibodies (Onisk et al., 1994). Live attenuated vaccines have been tested, for example in Spain and Portugal during the 1960´s, but with unsatisfactory results (King et al., 2011; Sánchez-Vizcaíno et al., 2012). Besides deaths, caused by the insufficiently inactivated vaccine virus, there were also a large number of carrier animals, which made eradication more difficult (King et al., 2011).

However, development is progressing, much thanks to progress in identifying ASFV genes involved in virulence and immune invasion (King et al., 2011). A recent study demonstrated that domestic pigs experimentally immunized with non-virulent ASFV of genotype I were protected up to 100% against challenge from two virulent African ASFV isolates of different genotypes. Since cross-protection between different genotypes has been demonstrated, it is possible that one vaccine will be effective against multiple circulating genotypes (Costard et al., 2009: King et al., 2011).

Some domestic pig populations have shown a higher level of natural resistance against ASFV, for example in Malawi and north-western Mozambique (Penrith et al., 2004). Unfortunately, resistance appears not to be inherited. Offspring to resistant pigs that were challenged with ASFV developed clinical disease and almost all died (Penrith et al., 2004). Penrith & Vosloo (2009) believe that these more resistant pig populations are fairly widespread.

Epidemiology

Domestic cycle

ASFV is transmitted by direct contact between pigs, as well as indirect by contact with contaminated equipment, clothes etc. (Penrith & Vosloo, 2009). Virus is excreted in blood, faeces, urine and saliva from infected pigs. When the virus is introduced into a naïve herd, there is a rapid horizontal transmission between pigs (Lubizi et al., 2005). Transmission through direct contact can occur up to 30 days after infection, whereas blood is infective for eight weeks (Penrith & Vosloo, 2009) and in putrefied blood as long as 15 weeks (Sánchez-Vizcaíno et al., 2009).

Meat from infected pigs or contaminated pork products is another important source of infection, due to the virus´ long persistence in tissues (Costard et al., 2009). Carcasses from diseased pigs can be a great risk to in-contact pigs. Pig products are most likely responsible for the outbreaks on other continents, when food scraps from airplanes and boats have been fed to pigs (e.g. Portugal in 1957, Brazil in 1978 and Georgia 2007) (Costard et al., 2009; Rowlands et al., 2008).

Movements of pigs and pig products are also responsible for a vast majority of recent outbreaks in African countries (Penrith & Vosloo, 2009). Tracing pig-related outbreaks is extremely difficult, because movements are often illegal. However, advances in molecular genetic characterization of the virus have facilitated our understanding of origin and subsequent spread of outbreak viruses. It is clear that humans play a major role in spreading ASF. Gulenkin et al. (2011) identified the major significant risk factors for spread of ASF as density of road networks, water bodies and density of domestic swine production.

The role of subclinical carriers has been discussed in several studies. Costard et al. (2009) reviewed current knowledge and reached the conclusion that recovered pigs might remain persistently infected for 6 months and during this time act as a source of transmission to susceptible pigs.
Björnheden (2011) found eight PCR-positive pigs, with no known clinical disease, whereby five were ELISA-negative and three were ELISA-doubtful. Virus isolation would be needed to determine if they were carrying live virus. According to Sánchez-Vizcaíno et al. (2012), symptomatic carrier animals play an important role in the persistence and dissemination of the disease in endemic areas.

The information on the epidemiology of ASF in Uganda is limited, and so is the knowledge about prevalence. One study from Rakai district in southern Uganda estimated the prevalence of ASFV in healthy domestic pigs, from areas where ASF had not been reported, to 3.3% (Björnheden, 2011). The seroprevalence was

2.1%. Another recent study from central Kenya demonstrated that 49% of the domestic pigs (n=83) were PCR-positive for ASFV but none were serologically positive (Gallardo et al., 2011). These were abbatoir samples from pigs that had no clinical or pathological signs of ASF.

Sylvatic cycle

The ancient sylvatic cycle is well described from eastern and southern Africa (Costard et al., 2009). It involves soft ticks and wild suids, especially warthogs, who are natural hosts for the virus.
As direct, or vertical, transmission between warthogs is not thought to occur, soft ticks of the *Ornithodoros* spp. are necessary for maintaining ASFV. Studies have shown that about 80% of free living warthogs are infected with ASF virus in areas where ticks are present and can act as vectors. Infected ticks feed on warthogs, and young warthogs develop sufficient viremia to infect new ticks feeding on them. The levels of ASFV in adult suids are not sufficient for transmission to domestic animals, neither through direct contact nor through ticks (Sánchez-Vizcaíno et al., 2012). Ticks can survive up to five years with occasional feeding, maintain the virus for four months after their last blood meal, and transmit it to other ticks through transstadial, sexual and transovarial transmission (Costard et al., 2009). Persistent infection in ticks has been observed up to several years (Sánchez-Vizcaíno et al., 2012). Taken together, the role of the *Ornithodoros* tick in the epidemiology of ASF is mainly as long-term reservoir.
The sylvatic cycle has never been demonstrated in West Africa (Costard et al., 2009).

Interaction between sylvatic and domestic cycle

Transmission of ASFV from warthogs to domestic pigs is believed to be dependent on *Ornothodorus* ticks (Costard et al., 2009), although it has also been suggested that carcasses from infected warthogs could be a source of transmission. Nevertheless, the involvement of the sylvatic cycle appears not important for maintaining the disease among domestic pigs. A recent study from an area of Spain where ASF was endemic until 1995 confirms that ASFV cannot persist long time in isolated wild boar populations without the involvement of factors that promote re-infection, like feeding on contaminated swill or contact with domestic pigs (Mur et al., 2012). As mentioned before, ticks are important mainly to maintain ASFV in an area for a long time (Costard et al., 2009). For example, ASF re-emerged in Portugal in 1999 on a farm that had been affected in the past (Costard et al., 2009). Infected ticks were found on the farm, suggesting that they had maintained the virus.

Impact of ASF

In areas where ASF is endemic, as well as where it has been recently introduced, it has serious socio-economic consequences (Costard et al., 2009). Countries with well developed commercial pig industry face the highest impact but the greatest losses are usually inflicted on the poorer smallholder producers who are less likely to implement prevention and biosecurity measures, and often lack financial support to restart production in case of an outbreak (Costard et al., 2009). It is estimated that the introduction of ASF into Madagascar caused a loss of about half the pig population (due to mortality or pre-emptive slaughter) (Roger et al.,

2001). Countries with confirmed ASF suffer not only from high mortalities, but also from trade restrictions and costly control strategies for eradication. Introduction of ASF into countries outside Africa have all been eradicated at high costs. For example, the costs of the last five years eradication program in Spain alone were estimated at USD 92 million (Costard et al., 2009), and since 2010, the economic losses in the Russian Federation have been estimated at about one billion USD (Sánchez-Vizcaíno et al., 2012).

UGANDA

Uganda is situated on the equator in eastern Africa, north of Lake Victoria and bordering Kenya, Tanzania, Sudan, Democratic Republic of Congo and Rwanda (Briggs & Roberts, 2010). The former British colony (from the end of 1800 until 1962) is about half the size of Sweden, with a population almost four times bigger (32.7 million) (World Bank, 2011). Thirty-one percent of the population lives in poverty. Agriculture contributes more than 20% to GDP (Uganda Bureau of Statistics (UBOS), 2008). Poverty is most apparent in rural areas, where people live out of smallholder agriculture cut off from major market trade, financial services and technology to improve production (Rural Poverty Portal, 2012). Social issues and climate contribute to the poverty. The poorest areas of Uganda are located in the northern and northeastern parts, where the climate is drier than the rest of the lush country, and where civil strife has disrupted smallholder agriculture for two centuries. Pig production is becoming increasingly popular as a source of food, income and employment, especially in the rural areas, and with 3.3 million pigs (UBOS, 2010), Uganda is said to have the largest and fastest growing pig production in eastern Africa (International Livestock Research Institute (ILRI), 2009). Pig production has the potential to play a crucial role in poverty alleviation, because of the ability to convert low quality feed into high quality protein together with high reproductive potential (Penrith, 2009). Pigs are kept as “walking banks”, mostly on free-range, and are slaughtered or sold at certain occasions when money is needed, for example to pay school fees (Ståhl and Aliro, ASFUganda, personal communication).

ASF in Uganda

Control of ASF in endemic areas like Uganda is made difficult by several factors, including lack of basic biosecurity measures, traditional free-ranging husbandry system and movements of pigs (Costard et al., 2009). In most rural areas, local slaughter places are small and poorly equipped, and waste (and sewage) is directly accessible to other animals such as dogs or roaming pigs (see fig 3 and 5). Also, many pig owners sell their animals as soon as they suspect ASF among them.
Quarantine and movements restrictions are set up officially when ASF is confirmed in an area, but the implementation is very poor (Ståhl and Aliro, ASFUganda, personal communication).

Figure 3. Slaughter place in one of the villages in Gulu, DVO interviewing. Gulu, Uganda, 2011 (personal photo)

Gulu district

Uganda is divided into more than 110 districts (see fig 4), which are subdivided into counties, subcounties, parishes and finally into villages. Northern Uganda, Gulu district included, has long been considered dislocated from the country, geographically as well as economically and culturally (Briggs & Roberts, 2010). Internal conflicts have stricken hard, the latest being 20 years of terror from the rebel group Lord´s Resistance Army (LRA). Most of the rural population was forced to seek shelter in towns, or move to so called Internally Displaced Camps (IDPs). There is a great number of Non-Governmental Organisations (NGOs) working in and around Gulu town, some working with distribution of pigs (e.g. World Vision, IOM, Laroo CFP) (Aliro, ASFUganda, personal communication).

Figure 4. The districts of Uganda; Gulu district marked in red.

ASF is a big problem in Gulu, as in other parts of Uganda. During the past 1.5 years, outbreaks of ASF has been confirmed in 14 out of Gulu´s 16 subcounties (Ståhl and Aliro, ASFUganda, personal communication)

OBJECTIVE

The main objective of this study was to better understand the spatial and temporal dynamics of the disease and to clarify major modes of transmission between domestic pigs, by collecting information from the outbreaks of ASF in Gulu district.

Aims

The aims were to:

- ✓ Estimate the mortality for the outbreaks in Gulu district
- ✓ Identify the main risk factors for, and routes of, transmission of ASF, between and within villages
- ✓ Gain understanding of the impact of ASF in Gulu district

Figure 5. Pigs tethered close to a slaughter place. Note the closeness to the intestines from a recently slaughtered pig in the foreground. Gulu, Uganda 2011 (personal photo).

MATERIALS AND METHODS

Study population

The study area was confined to Gulu district in northern Uganda (figure 4), where ASF had been confirmed in 14 of the 16 subcounties during the last 1.5 years. The aim was to visit a number of villages from several of these subcounties, and to interview all people in the village that had suffered loss of pigs within that time.

Data collection and sampling

All data was collected using a questionnaire that was developed together with the District Veterinary Officer (DVO) (see Appendix A). GPS-locations were captured for all interviews.

Figure 6. The DVO interviewing a farmer family (Lindström, L.)

Figure 7. Bleeding a young pig from the cranial vena cava (Aliro, T.). Gulu district, Uganda, 2011.

The interviews were conducted together with the DVO and a key informant (fig 6). Blood samples were collected from the jugular vein or the cranial vena cava from domestic pigs that survived outbreak of ASF or that presented with clinical signs in villages with ongoing outbreaks (fig 7). A pig was registered as survivor if it survived the acute phase of ASF among pigs within the same or neighboring households. Serum and EDTA tubes were collected from each pig and stored in cold boxes until transported to the lab. Sex was recorded and age was estimated. Body temperature was recorded if pigs had been in contact with sick pigs or appeared dull.

Laboratory analysis

All laboratory analyses were carried out at the molecular biology laboratory at Makerere University, Institute of Environment and Natural Resources (MUIENR), Kampala, Uganda. All serum tubes were centrifuged within a maximum of three days after collection, and the serum was separated from the blood. All samples were stored in duplicates at -20°C, one for diagnostic analysis and one for future long term storage at -80°C.

For extraction of viral DNA, a commercial kit was used (DNeasy Blood & Tissue, QIAGEN, Hilden, Germany). The protocol for purification of total DNA from animal blood (Spin-Column Protocol) was used, in accordance with

manufacturer´s instructions. In brief, 100 µl anticoagulated blood was used for each sample. PBS, ethanol and lysis buffer were added to all samples. The solution was transferred to the spin columns and washed twice. Elution was repeated as recommended for maximum DNA yield. The extracted DNA was used immediately or in some cases stored at -20°C.

For amplification and detection of ASFV DNA, a commercial realtime PCR was used (ASFV PCR Assay, Tetracore, Rockville, USA).
The PCR targets a gene for the structural protein p72, which is highly conserved. The protocol was run according to the instructions of the manufacturer, that is, 45 amplification cycles (5 seconds at 95°C and 60 seconds a 60°C) and run on SmartCycler (Cepheid Inc., Sunnyvale, California). A positive control included in the kit was used. The negative control was prepared during the extraction and contained all reagents from the protocol. The RT-PCR is a quantitative method where positive samples result in a sigmoid curve (figure 8). Lower Ct (cycle threshold) values correspond to higher concentrations of viral DNA.

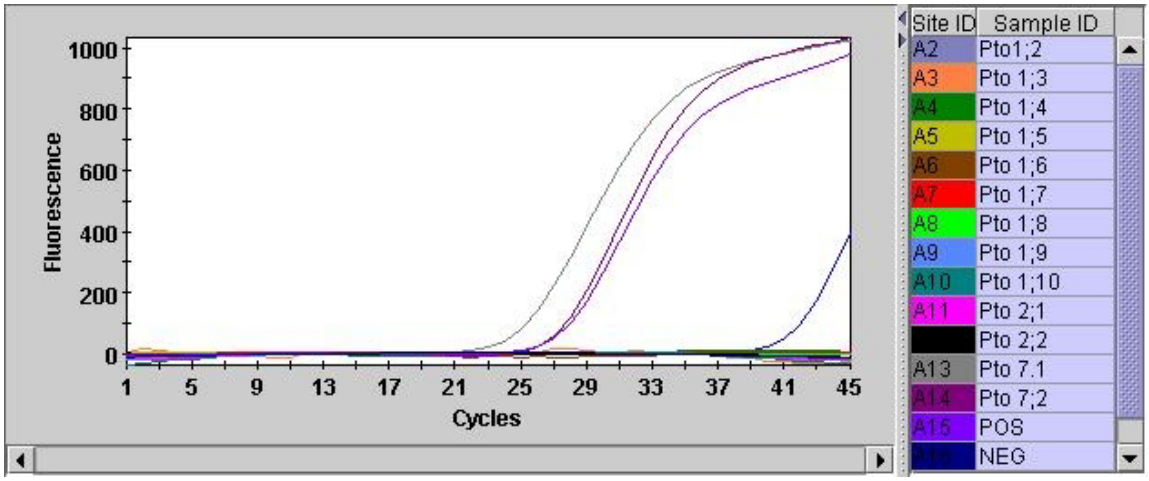

Figure 8. Results from one RT-PCR run on individual samples from Gulu, Uganda. Samples Pto1;1 (not shown), Pto7;1, Pto7;2 and the positive control (POS) show sigmoid curves corresponding to positive samples.

To detect specific antibodies against ASFV, a commercial blocking ELISA kit (INGEZIM PPA COMPAC, Ingenasa, Madrid, Spain) was used. All reagents used were provided with the kit, including positive and negative controls, and the recommended test procedure was followed. In brief, the samples were diluted 1:1 (50µl diluents and 50µl serum) directly into the wells. Two wells were used for positive as well as negative controls, these were diluted in the same manner as the samples. The wells of the plate are coated with a purified protein extract from the VP73, the major structural and most antigenic protein from ASFV. If a sample contains specific ASFV antibodies, they will bind to the antigen coating in the wells. The specific monoclonal antibodies (Mab) added will only bind to the antigen coat if the serum sample does not contain specific antibodies, e.g. negative samples. The presence of labeled Mab is demonstrated with a specific substrate, and the optical density (OD) values measured at 450 nm.

RESULTS

Interviews

A total of 135 interviews were performed during six field trips in October and November 2011. Sixteen villages in seven subcounties were visited (figure 9). The number of interviews in each village varied from two to nineteen (two interviews were made in two villages, in all other villages a minimum of five interviews were performed). The outbreaks of ASF in Gulu district had been going on from August 2010, so the interviews covered time aspects from August 2010 to November 2011. With few exceptions, all pigs we saw were kept on free range.

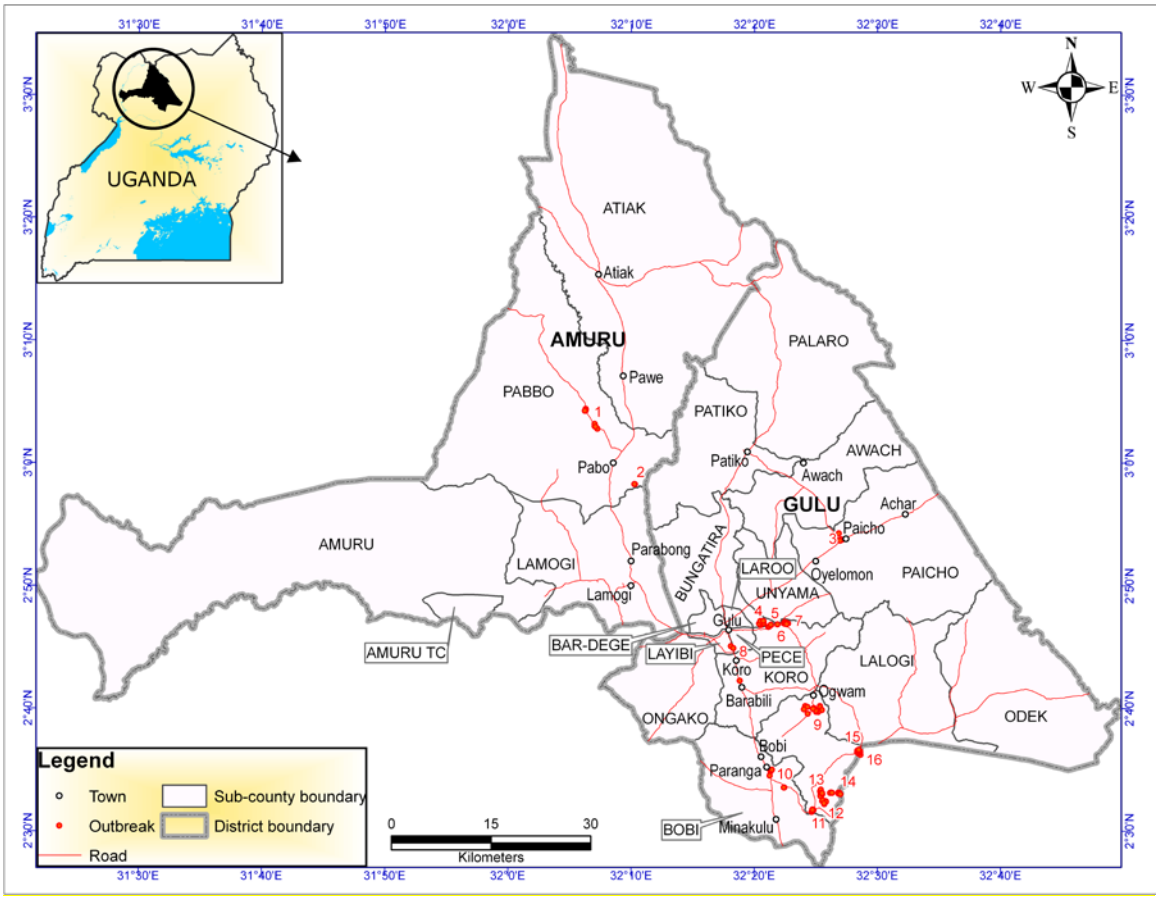

Figure 9. Map of the interviews performed in Amuru (1-2) and Gulu (3-16) districts, Uganda, 2011. The numbers represents the 16 villages.

Mortality

Based on the interviews, a total of 610 pigs died and 111 survived the outbreaks of ASF (see table 1), giving an estimated mortality of 84.5%. The gap between number of survivors and number remaining, as seen in Table 1, has its explanation in the time gap between outbreak and interview. Some pigs survived outbreaks but had been sold or slaughtered since. Furthermore, the inconsistency between "Number remaining" and "Number of pigs now" reflects the impact of ASF; some people had no remaining pigs and still have no pigs while others have acquired (bought or received) new pigs.

Table 1. Data from the 16 villages where interviews were performed. Collected data regarded number of dead pigs, numbers of pigs that survived, number of pigs still remaining with the owner and total number of pigs at the time of the interview. The mortality was estimated from these data, as well as duration of the outbreak in each village. Village no. 1 had two separate outbreaks and therefore is mentioned twice

	Number of pigs					
Village	Dead	Survive	Remain	Now	Mortality (%)	Estimated duration (days)
1	26	15	14	25	63.4	25
1´2	26	0	1	10	100	10
2	11	4	4	4	73.3	20
3	73	4	1	1	94.8	25
4	21	4	2	12	84.0	70
5	22	1	0	0	95.7	25
6	41	7	0	2	85.4	30
7	28	0	0	5	100	10
8	63	12	6	6	84.0	30
9	20	2	2	2	90.9	20
10	23	10	1	5	69.7	15
11	63	11	1	28	85.1	15
12	26	2	0	0	92.9	40
13	34	15	3	14	69.4	25
14	17	5	0	2	77.3	15
15	68	10	0	41	87.2	>200*
16	48	9	0	22	84.2	60
Total	**610**	**111**	**35**	**179**	**84.5%**	

*Duration about 7 months, probably multiple outbreaks.

The duration of the outbreaks varied between a couple of weeks to a couple of months in different villages (see table 1). Some villages had, or are likely to have had multiple outbreaks. There was also some variation in the estimated duration between households within the same village, from one day to one month.

Transmission

In most cases it was not possible to confirm the source of introduction of ASFV into a village. However in many villages it was possible to make a qualified guess and in some villages the source of introduction was clear (see table 2).

Table 2. Time of outbreaks of ASF in the 16 villages, and assumed sources of virus introduction into the individual villages

Village	Outbreak	Introduction
1	July 2011	New pigs, died within 2 weeks
1´2	Oct-Nov 2011	Pork roasted at home, left overs fed to pigs
2	Oct-Nov 2011	Dead pig brought in October
3	May 2011	Infected pork suspected
4	Sep-Nov 2010	New pig (according to neighbors)
5	Oct-Nov 2010	
6	Oct-Nov 2010	Skin and swill from Gulu town (pork eating joint)
7	Feb 2011	Sick pig brought (say neighbors)
8	Sep-Oct 2011	On main route to Gulu town
9	Oct-Nov 2011	Infected pork sold on market 8 Oct
10	Nov-Dec 2010	On main route to Gulu town, major slaughter centre
11	Oct 2010	Pork from other village brought 9 Oct
12	Oct-Nov 2010	Pork from another village
13	Nov 2010	Pork from another village
14	Sep 2010	Infected pork suspected
15	Apr-Dec 2010*	Major slaughter centre
16	Aug-Oct 2010	Major slaughter centre

*Probably multiple outbreaks

The outbreak in village 11 in October 2010 started after Independence day (9th October), when a diseased pig was sold on the local market. One year later, the same thing happened in another village. Pigs started dying one week after the diseased pigs were sold on the market. In another village, a majority of the people claimed they knew that the butcher used to have brought sick or dead pigs for slaughter. Some more or less known transmission events are shown in figure 10.

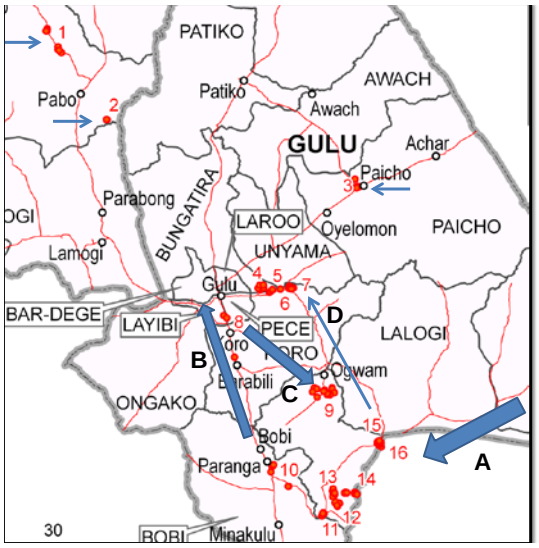

Figure 10. Arrows demonstrating some transmission routes. The thin arrows at 1, 2 and 3 indicate pigs and pork introduced from distant areas not known or shown in the map.

- *A. Aug 2010. ASF spreads from eastern to southern Gulu, probably through pork. The virus circulates between the villages 11-14, with at least one known transmission on Oct 9th (Independence Day)*
- *B. Arrow demonstrating the main road to Gulu town. The many transports and slaughter places/trading centers along this road should provide plenty of transmission possibilities (live animals, infected pork products, vehicles)*
- *C. Outbreak in Aug-Sept 2011, source unknown but situated by the main road. Diseased pigs taken to village 9 and sold on the market for Independence Day*
- *D. Outbreak probably originating from infected pork (15-16 major slaughter centre)*

When it comes to the question about how their own pigs could have become infected, 46 people blamed free roaming pigs (neighbors) and 46 blamed pork or swill from slaughter slabs (fig 11a). Out of the 62 people that clearly answered the question about how it might have spread further from their premises, 33 stated they sold sick or dead pigs and 21 said they threw away or buried the carcass (fig 11b). Most people affected by an outbreak sold pigs or pork at a lower price than normal, or even on credit, and many people also told us that they had bought pork cheaply from neighboring villages, knowing or not knowing that there was an outbreak of ASF (fig 12).

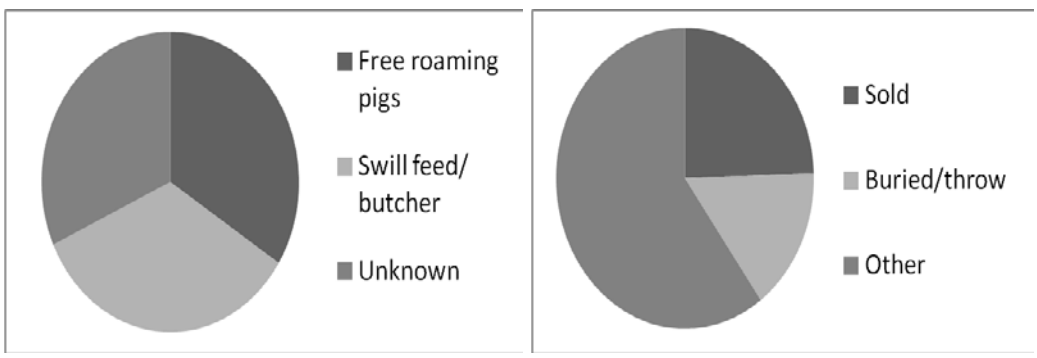

Figure 11a. Estimated introduction of ASF. Figure 11b. Estimated spread of ASF.

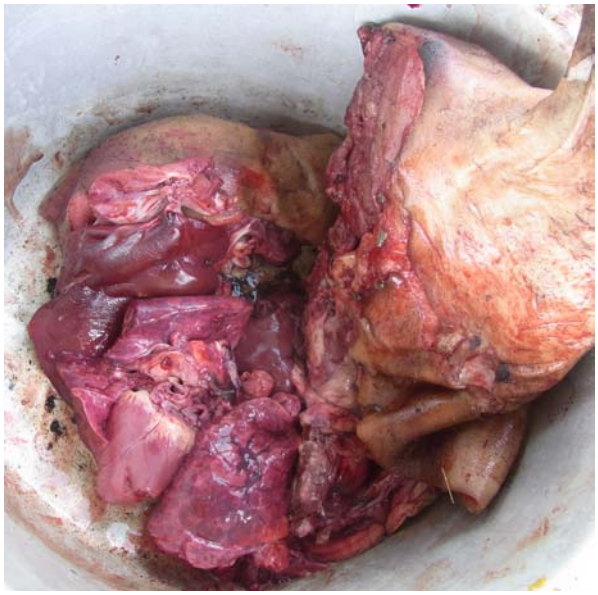

Figure 12. Parts of a pig that died the same morning. Note the dark discoloration of the organs, due to haemmorhage and congestion. This meat was later eaten. Gulu,Uganda, 2011 (personal photo).

Impact

Since we performed 135 interviews, we can assume that this number of people used to have pigs. Eighty-three smallholders, 61%, no longer kept pigs (fig 13a). In numbers of pigs, the total number before the outbreaks was estimated at 721. As described earlier, 610 out of these died and 111 survived. The estimated number of pigs at the time of the interviews was 179, which is 25% of the total number of pigs before the outbreak (fig 13b). These numbers, however, are only rough estimates but do give a picture of the importance of the disease.

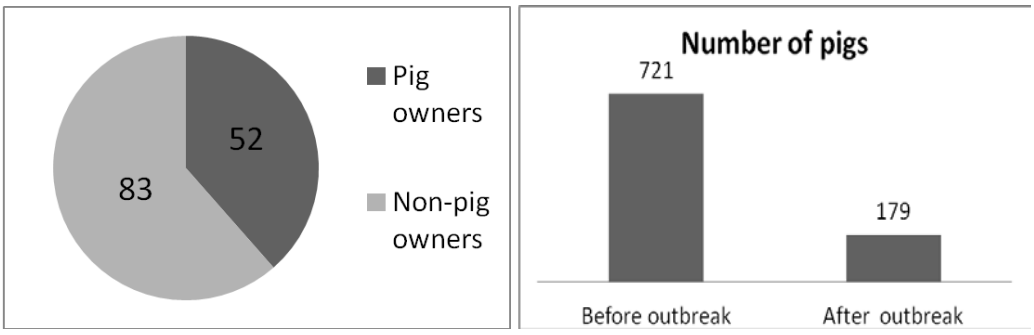

Figure 13a. Number of pig owners and pigs before and after outbreaks of ASF. Out of the 135 that were interviewed, 52 still kept pigs and 83 did not keep pigs.

Figure 13b. The estimated number of pigs in the villages at the time of interviews were 179, 25% of the 721 before the outbreaks.

Blood samples

Nineteen blood samples relevant to this study were collected. Out of these, 5 were PCR-positive and 3 ELISA-positive (shown in table 3).

Table 3. 8 out of the 19 samples were positive on either PCR or ELISA. The sex and age of all pigs were noted, and the rectal temperature was measured from pigs that presented with clinical signs

Pig ID	PCR	ELISA	Sex	Age (estimated)	rtemp
Bar4:1	N	POS	Female	2 months*	
Alo1:1	N	POS	Female	15 months	
Tee1:1	N	POS	Female	8 months**	
Pto7:1	POS	N	Female	4 months	41,8°C
Pto7:2	POS	N	Male	4 months	41°C
Pto14:1	POS	N	Female	10 months	
Lac2:1	POS	N	Female	12 months	41°C
Lac2:2	POS	N	Female	12 months	41,4°C

*born after outbreak, offspring to survivors
** PCR pos twice before (May and July)

Figure 14. Pigs showing clinical signs of acute ASF (fever, huddling together, with reddening of the ears). The two adults were bled and ASF was confirmed. They both died within two days. Gulu, Uganda, 2011 (personal photo).

PCR – detection of ASFV

Thirteen pigs were bled in villages with ongoing outbreaks; these were bled to confirm ASFV. 5 out of these were PCR-positive (table 3 and 4). All presented with fever (up to 41,8°C) and some had pinkish-red ears and abdomen (see fig 14).

Table 4. The cycle threshold (Ct) values of the five PCR positive pigs and their positive controls

Pig ID	Pto7:1	Pto7:2	Pto14:1	Lac2:1	Lac2:2
Ct value	24,2	26,7	35,0	27,8	35,5

ELISA – detection of antibodies against ASFV

Six pigs were bled in villages without ongoing outbreak of ASF (5-12 months after outbreak). Out of these, 4 were survivors (Awo1:1, Bar7:1, Alo1:1, Tee1:1), and 2 were offspring of survivors (Bar4:1 and Bar7:2). Three of these six were ELISA-positive (table 5).

Table 5. Optical density (OD) values for the three ELISA positive pigs (read at 450 nm)

Pig ID	Tee 1:1	Bar4:1	Alo1:1
OD value	0,192	0,119	0,168

The ELISA test was considered valid because NC / PC $\geq$ 4.
The cut off calculations (NC and PC) were set to
NC = NC-[(NC-PC)*0,4] = 0,9438.
PC = NC-[(NC-PC)*0,5]) = 0,825.
OD values lower than the positive cut off (0,825) are positive and values higher than the negative cut off (0,9438) are negative to ASFV antibodies.

DISCUSSION

The average mortality during the studied outbreaks was estimated at 84.5%. Some kind of immunity/increased tolerance could have been expected to develop, considering that ASF is endemic in the area. Nevertheless, the form of ASF seen in Gulu appears to be the acute form where mortality normally is high. One problem when calculating this number is the increased activity of trade at onset of an outbreak. Many pigs are sold or slaughtered when it is expected that they are or might be sick. That means it is sometimes not clear whether they would have survived the disease if they had not been removed. Also, we only interviewed people whose pigs had died. There is therefore a risk that we missed out some data, either because the key informants lacked information from some people or because some pigs that we neglected as not affected were actually infected by ASF but survived. Considering the high mortality, it is not likely that this happened to a big extent.

Movements of pigs and pig products are known to be a major transmission route, which was confirmed in this study. Although it is difficult to point out the exact source of infection, it is clear that outbreaks in most villages were connected to introduction of new pigs or pork (see table 2). Some villages slaughter greater quantities than the average village. These major slaughter centers are particularly vulnerable because of the higher inflow of pigs from different origins. For example, in one village, two pigs were slaughtered during the three hours we performed interviews. It is a well known trading centre and many people declared that they usually buy pork from that village. The constant passage of new pigs could explain the long duration of the outbreak, alternatively the occurrence of multiple outbreaks, in this centre. Consequently, it is likely that it acted like a constant source of infection to other villages. Many people also suspected pork from that area to have caused disease outbreak in their village.

Density of road networks was identified as one of the major significant risk factors for spread of ASF by Gulenkin et al. (2011). In some villages, no evident source of introduction could be demonstrated. Some of them were situated along the main road to Gulu town and it is likely that the virus was carried with vehicles or infected pork transported to or through these villages. The outbreaks in these villages appear to have occurred more or less simultaneously (September to December 2010). One interesting finding is the two outbreaks connected to Independence Day in two different villages in 2010 and 2011. On both occasions, dead pigs were taken from another village to be sold on the local market for Independence Day. The consumption of meat increases for this celebration and presumably it is easier to sell pork at Independence Day because of this increased demand.

We did not have a good response frequency on the questions about thoughts of transmission (fig 4). We had more specific answers towards the end of the study. One reason could be that these interviews were closer to the time of outbreak, but also that we asked more specific questions.

The main interest of sampling blood in this study was to capture survivors. However, since some outbreaks took place over a year before the visit, there were

few survivors up to date. One interesting finding was the pig Tee1:1 which had tested positive for ASFV twice (at the time for outbreak and two months after) but in this study was PCR-negative but now ELISA–positive (5 months after outbreak). Did she carry live virus up to five months after the outbreak and did she during this time constitute a potential source of infection to other pigs? The other interesting finding was the ELISA-positive 2 months old pig Bar4:1 whose parents had survived an outbreak 8 months earlier. Unfortunately, the parents had recently been slaughtered. Antibodies from the mother had probably been transferred to the young through colostrum. The other offspring tested was 6 months old (born 4 months after outbreak) and was not positive. It is likely that he also had maternal antibodies but that these had now waned. Penrith et al. (2004) demonstrated that maternal antibodies persist three months before waning, but in some instances longer, up to seven months.

The role of subclinically or chronically infected carrier animals in the epidemiology of ASF remains largely unclear, but is nevertheless highly interesting to investigate. Questions have been raised (for example by Björnheden, 2011) whether distribution of pigs could actually contribute to disease transmission. The importance of pig production in poverty alleviation is reflected in the many NGOs that distribute pigs. We already know that transport of pigs constitute a major risk factor for transmission. Pigs could be transported in incubation state, or perhaps be in a chronical carrier state where stress during transportation and entry into a new herd could reactivate the virus and cause a viremia, sufficient to infect other in-contact pigs. Screening these animals for antibodies before transport would not be sufficient to rule out carriers. ELISA-negative, PCR-positive domestic pigs have been demonstrated for example by Björnheden (2011) and Gallardo (2011). Gallardo et al. (2011) demonstrated 49% PCR-positive domestic pigs without clinical or pathological signs of ASF. This number is extremely high (as a comparison, Björnheden (2011) found the prevalence of ASFV in healthy domestic pigs from Rakai district in southern Uganda to be 3.3%). Important to note is that the samples in the former study were taken from an abattoir. It is possible that these pigs were sent for slaughter due to suspicion of sickness, meaning they could have been in incubation state, which could explain the high prevalence.

Wildlife gets a lot of blame in the debate of ASF, as well as many other diseases in livestock, in Uganda. The sylvatic cycle is sometimes described as an important part in the epidemiology of ASF in eastern Africa. Perhaps this is true if compared to e.g. western Africa, and the wildlife reservoirs are probably important in the persistence of the virus in this area, but not as source of infection. Compared to the apparent role of trade and movements of domestic pigs and pork, the sylvatic cycle cannot be considered to be of major concern.

ASF has been shown to be connected to great losses and big socio-economic impact, regardless where it hits. In this study, I have only included information from people who were affected by ASF, in one particular area of Uganda. I have no numbers from the population of pigs as a whole. Nevertheless, it remains clear that ASF with its high mortality has an enormous impact on affected people and areas, and leads to severe economic losses. Many of the people I interviewed lost

their pigs up to a year ago, and a great majority has not resumed pig production (fig 6).

Knowledge about the disease and different transmission routes varied considerably, but was poor in general. Most people were aware of the disease, but as we only visited villages that we knew have had outbreaks, this is not very surprising. One main source of information in the villages was the radio. The DVO talks for 15 minutes every Monday morning about for example animal husbandry, feed and diseases. When we started asking around how people found out about the disease, the answer was radio and to a lesser extent neighbors and veterinarians.

My impression is that most people answered the questions very sincerely. The DVO, who has a thorough knowledge about the people and the situation, was a good help in evaluating the credibility of the answers. Many people stated how they ate, sold or gave away dead pigs. I believe that many people did not see any other option. In many of the affected families, the pigs are the main source of income and live as "walking banks". It is slaughtered or sold first when money is needed, mainly to pay school fees for the children. As there is no compensation system, people are desperate to lose this income when pigs die unexpected.

It must not be forgotten that Gulu district is a special and sensitive area in many ways. It is located in northern Uganda which has been described as the poorest part of the country. More important, the history of civil strife has had a grim impact on people´s lives. People have suffered physically as well as psychologically, and the way back to a normal life is long. Many people have become dependent on aid, and some are still. Lots of valuable knowledge in agriculture was lost when people were forced to leave their premises and relocate, some to IDP-camps. Pigs are often kept on free range (as are most other livestock like goats and poultry) and are thought to "feed themselves". The consequences are that some pigs do not have enough food, which in turn can lead to reduced growth and increased susceptibility to diseases. Moreover, they are able to come in contact with waste from households or slaughter places and might possibly be more prone to eat such waste because of under feeding.

Current control and eradication strategies include quarantine, movement restrictions and stamping out. Obviously, there is a big difference in control strategies in countries where the disease is endemic compared to countries where it is newly introduced. Fact remains, ASF dies out in the absence of susceptible hosts. In Uganda, quarantine and trade restrictions are set up at outbreak of ASF but the implementation is very poor, and the means to reinforce the quarantine are lacking. Also, the lack of a compensatory mechanism makes people desperate to sell pigs or pork at onset of an outbreak. Stamping out is not an option. Penrith (2009) discusses the ethical and environmental dilemmas of stamping out, and come to the conclusion that it is impossible to implement successfully in countries with limited financial resources and veterinary services. She means that the only choice is farmer-based control, with focus on prevention for example through correct biosecurity measures. Sánchez-Vizcaíno et al. (2012) reached the same conclusions, stressing that improved biosecurity measures and pig husbandry systems would make disease control and disease-free areas possible. Since these

measures require drastic changes in traditional farming and market habits, it is crucial that all involved, from the individual farmer to the government, are united and well-informed about the advantages and consequences. The radio appears to reach out to many people. This channel for information should be used more in my opinion, to give an update on current disease situations and increase knowledge about transmission and suitable control measures. Pig farmers are probably more likely to comply if they feel that they are involved in the development and if they receive a meaningful benefit (as stated by Costard et al., 2009). In this case, it is important that all involved realize that it is not solely a question about winning, but also not to lose.

CONCLUSIONS

1. African swine fever caused a high mortality among domestic pigs in Gulu district in Uganda. In this study, the mortality was estimated at 84.5%.

2. Movements of pigs and pork are responsible for a vast majority of outbreaks of ASF, and are therefore the main challenges for control of ASF. (This is applicable not only for Gulu and Uganda.)

3. The lack of biosecurity, together with the traditional free range management, facilitates spread of disease(s). In other words, disease spread within villages could be limited with improved biosecurity and knowledge.

4. Outbreaks of African swine fever are associated with severe socio-economic consequences. In this study, this could be demonstrated in the big proportion of pigs that were lost and the big number of people that did not resume pig production.

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APPENDIX A

Survey No: ..... Date: ..... (DD/MM/YY)

Herd owner name……..... Phone.....

District.....County….....Subcounty.....

Parish.....Village……..

Gender of respondent M□ F□

Age group in years

0 – 18 □ 19 – 35 □ 36 – 55 □ Over 56 □

Breeds of the pigs

Local□ Crosses □ Exotic □

System of pig management

Free ranging □ Tethered □ Semi-intensive □ Intensive □

---

1. **Do you keep pigs?** Yes □ No□
2. **How many pigs do you keep?** .....
3. **Are you aware of massive death of pigs in this village during the last year?** Yes □; No□
4. **If yes, when?** .....
5. **Did you experience massive deaths?** Yes □ No□
6. **If yes, when did it start?** .....
7. **How many pigs died?** .....
8. **How many survived?** .....
9. **What was the duration of the outbreak?** .....
10. **What actions did you take to prevent losses?**

    No actions□ Slaughtered □ Sold□ Other……
11. **How many pigs remained in the herd after the outbreak?** .....
12. **Do you have any of the remainders in the herd now?** .....
13. **If yes, how many?** .....
14. **Did you introduce pigs into the herd within one month before disease outbreak?** Yes □ No□
15. **If yes, where did you bring the pig(s) from?** .....
16. **What do you think could have caused outbreak of disease in your herd?**

    i……

    ii……