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African swine fever in Uganda- description of a recent outbreak and studies of possible differential diagnoses

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ABSTRACT

This study had two different aims. The main aim was to investigate the dynamics and impact of African swine fever (ASF) on a farm in Uganda during a recent outbreak through a case study. The second aim was to estimate the presence of two important differential diagnoses of ASF: Classical swine fever (CSF) and Porcine Reproduction and Respiratory syndrome (PRRS).

The field and laboratory based case study of the farm level dynamics of ASF virus during a recent outbreak (October-December 2010) on a farm in the district of Mityana, Uganda, was conducted, using interviews, ELISA and RT-PCR. The financial impact on the farm was also estimated. The impact of the outbreak was profound. The farmer lost approximately over half of the population of pigs; mainly adults and newborn piglets were affected. Weaners and older piglets survived to a relatively larger extent. The outbreak spread between pens and units probably via direct and indirect contact. The source of the infection was difficult to identify since there were several suspected sources.

A pilot study of presence CSF and PRRS in Uganda was conducted using ELISA and RT-PCR in a cross-sectional study on 239 samples from the district of Rakai in southern Uganda and 80 samples from reported outbreaks of mortality in pigs where ASF virus had not been confirmed as the cause. All samples were negative for CSF and only one sample was seropositive for PRRS. The one positive sample for PRRS was suspected to be a false positive.

SAMMANFATTNING

Studien hade två olika syften. Det huvudsakliga syftet var att undersöka dynamiken och effekterna av Afrikansk svinpest (ASF) på en gård i Uganda under ett utbrott genom en fallstudie. Det andra målet var att uppskatta förekomsten av två viktiga differentialdiagnoser av ASF: Klassisk svinpest (CSF) och Porcine Reproduction and Respiratory syndrome (PRRS).

Den fält och laboratoriebaserade fallstudien av dynamiken på gårdsnivå av ASF virus genomfördes med hjälp av intervjuer, ELISA och RT-PCR, under ett pågående utbrott (Oktober-December 2010) på en gård i distriktet Mityana, Uganda. Den ekonomiska effekten av utbrottet på gården uppskattades. Effekterna av utbrottet var djupgående. Gårdsägaren förlorade ungefär hälften av populationen av grisar, främst vuxna och nyfödda grisar. Avvanda grisar och äldre smågrisar överlevde i en relativt sett större utsträckning. Utbrottet spreds mellan boxar och enheter via direkt och indirekt kontakt. Källan till infektionen var svår att identifiera eftersom det fanns flera misstänkta källor av introduktion av smittan till gården.

En pilotstudie av förekomst av CSF och PRRS i Uganda utfördes med ELISA och RT-PCR i en cross-sectional studie med 239 prover från distriktet Rakai i södra Uganda och 80 prover från rapporterade utbrott av dödlighet hos svin där ASF virus inte bekräftats som orsaken. Alla prover var negativa för CSF och endast ett prov var seropositivt för PRRS. Det enda positiva provet för PRRS misstänktes dock vara falskt positivt.

INTRODUCTION

Aims

The main aim of this study was to investigate the dynamics and impact of ASF on a farm in Uganda during a recent outbreak through a case study.

The secondary aim was to investigate the presence of CSF and PRRS in Uganda. These diseases had never been reported or studied in the country. In the study samples from the district of Rakai and samples from suspected ASF outbreaks in Uganda from 2010 where ASF had not been confirmed were analysed for antibodies and virus of CSF and PRRS.

Background

The project was a Minor Field Study (MFS) and part of a larger project on ASF epidemiology in Uganda. I was one of three students from the veterinary programme of the Swedish University of Agricultural Sciences (SLU) who conducted a MFS as my master thesis. The three of us focused on different parts in the epidemiology of ASF. The larger project, that this study was a small part of, was a collaboration between SLU, Makerere University in Kampala, Uganda, the Ministry of Agriculture Animal Industries and Fisheries of Uganda (MAAIF), Uganda Wildlife Authority (UWA) and International Livestock Research Institute (ILRI). The study was financed by the Faculty of Veterinary medicine and animal science and the Swedish International Development Cooperation Agency (Sida) and took place mainly in Uganda except for some preparations in Sweden.

Livestock in developing countries

Over the last twenty years the World Bank has spent over US$400 million dollars on agricultural education and training in developing countries to increase the income of farmers, where smallholder farms are the most common way of sustaining the household (The World Bank, 2010). "The Livestock Revolution" is the term used to describe the increased consumption of meat and other livestock products during the last decades. This has occurred especially in developing countries like Uganda. Because of the increased demand for meat and meat products there is an opportunity for poor farmers to raise themselves from poverty (The Livestock revolution, FAO, 2010). Livestock contributes to the livelihoods of an estimated 70% of the world's rural poor by providing a small but steady stream of food and income (PPLI project, FAO, 2010).

Uganda

Uganda is located in East Africa and is crossed by the equator. The country borders to Sudan, Kenya, Rwanda, the Democratic Republic of Congo, and Tanzania (Briggs, Roberts 2010). The population is 33,7 million persons (UNDP, 2010). The major exports are coffee, fish, tea and tobacco. Uganda was a colony of Great Britain before the country became independent in 1962. The ruling party is the NRM (National resistance movement) with President Yoweri Museveni. NRM and Museveni took charge of Uganda in 1986. Uganda has been coloured of several internal conflicts and wars, the most infamous ruler was Idi Amin (Briggs, Roberts 2010). The most recent conflict was between the Lord's Resistance Army (LRA) and the government. LRA was active mainly in northern parts of the country and affected the civilians' financial and security status negatively during

the two last decades. In 2005 LRA and Museveni came to a peace agreement and since then LRA has drawn back their main forces to the Democratic Republic of Congo. The northern Uganda that was part of LRA territory is still slowly recovering from the guerrilla wars (IRIN, 2010).

In the Global Human Development Report 2010 Uganda is rated as number 143 of 177 countries, with 51,3 % of the population living below $1,25 per day (UNDP, 2010).

Pig production in Uganda

As most other developing countries the civilian economy of Uganda depends on smaller farms. Smallholder pig production is a good way to raise money quickly due to the fast rate at which pigs can be produced and the good return of the investment. Pig production is increasing rapidly in Uganda, between 2000 and 2008 with approximately 600,000 pigs, an increase of 39% (FAO statistics division, 2010; see table 1). The pig producers of Uganda vary in scale of production and range in knowledge of diseases and biosecurity. During this study both large, modern pig producing farms with hundreds of pigs, and the more common rural pig producers that own only one to a few free-ranging or tethered pigs for their own consumption, were visited.

Table 1. Number of pigs (in thousands) in Uganda 2000-2008 (FAOSTAT, 2010)

Year	*2000*	*2001*	*2002*	*2003*	*2004*	*2005*	*2006*	*2007*	*2008*
Nr of pigs	*1573*	*1644*	*1709,8*	*1778*	*1940*	*2000*	*2060*	*2122*	*2186*

Legislation on epizootic diseases in Uganda

Uganda closely follows the OIE guidelines for disease reporting and control, albeit with many challenges. Despite these challenges, there are some laws that are supposed to prevent the spread of epidemic diseases. For example, any person who have an animal in his or her care or possession and/or suspect an animal being sick with an epizootic disease like ASF should prevent further spread of disease, isolate the animal and contact the local veterinary officer. The veterinary officer has the right to examine and sample any animal he or she suspects to be infected with an epizootic disease. When the existence of the epizootic disease is confirmed, the veterinary officer has the obligation to report this to the commissioner of livestock health and entomology. When the outbreak is confirmed, the commissioner will inform the farmers in the neighbourhood of the outbreak. The veterinary officers have the right to slaughter any affected animals, animals suspected of carrying the disease or have been in contact with other infected animals (Animal Diseases Act, Cp 38, 1918).

AFRICAN SWINE FEVER

ASF is a highly infectious and lethal disease of swine caused by a large DNA virus of the *Asfarviridae* family, genus *Asfivirus*. The ASF virus has only one serotype, but there are more than 20 genotypes and numerous subtypes of ASF virus of varied virulence (Penrith et al, 2009).

The ASF virus is highly resistant in a protein rich and moist environment. It can survive in chilled meat or carcasses for up to six months, and at 4 ºC for two years. It remains infective in smoked and salted pork. It is also highly resistant to putrefaction; it can remain in faeces for at least 11 days and in decomposed serum for 15 weeks (Penrith et al, 2009; Radostitis et al, 2007; Epiwebb 2010). The virus is inactivated at 60 ºC for 20 minutes, and can survive in pH ranging from 3,9-11,5 (OIE ASF, 2010). The virus is sensitive in the environment and is rapidly inactivated by sunlight and desiccation. In pig sties in tropical countries, even in absence of cleaning and disinfection, the virus do not remain infective for more than three to four days (Penrith et al, 2009).

Global distribution of African swine fever

ASF was first described in 1921 in Kenya. The disease is widespread in most countries of sub-Saharan Africa (Penrith et al, 2009). In Uganda the disease is enzootic (OIE, 2010). In 1959 ASF spread to Portugal, and from there to several other European countries: France, Italy, Malta, Belgium and the Netherlands in the following decades. The European countries were successful in their eradication, except for Portugal and Spain where eradication was possible first 30 years later. In Europe today ASF still exists on Sardinia where the infection is endemic (Penrith et al, 2009).

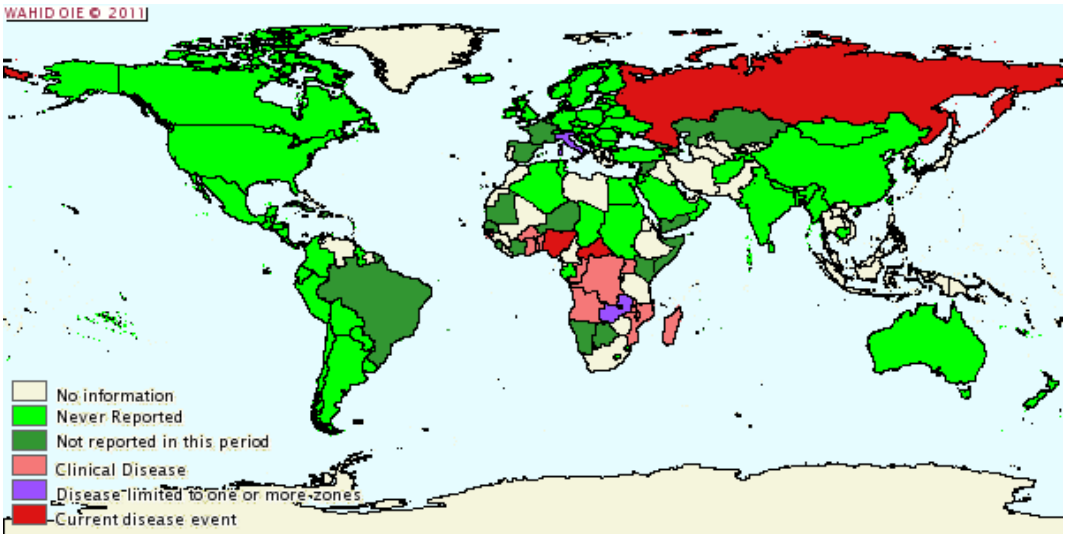

Figure 1. Global Distribution on African swine fever (OIE, 2011)

In the 1970s and 1980s severe outbreaks of ASF were reported in several countries in Central and South America. Eradication was achieved through stamping out and South and North America are now considered free of ASF (OIE, 2010).

In 2007 ASF was reported in Georgia. After analysing the genotype of the virus strain in Georgia the suspected origin of infection was from illegal import of meat from Madagascar. The disease spread from Georgia to neighbouring countries e.g. Armenia and Russia (Rowlands et Al, 2008). Outbreaks have been reported as far north as in Saint Petersburg and the latest one was reported in January 2011 (OIE, 2011).

The risk of spreading ASF to the rest of the European countries is considered to be a big threat to the large pig producing countries within the EU. The risk factors to be considered are a large population of wild boars in these parts of Europe and the rather unstable political situation in some of the affected countries, which complicate efficient control measures and reporting (Costard et al, 2009).

Hosts

ASF virus infects only species from the pig family (*Suidae*). Both wild and domestic pigs can be infected but domestic pigs are the most sensitive of infection, regardless of gender and age (Penrith et al, 2009).

Clinical symptoms

The course of the diseases is often acute or peracute with a mortality rate up to 100 %. The incubation time is 5-15 days, acute cases have a much faster course and incubation is often 3-4 days. Chronic and subacute cases may occur. These cases have a longer duration but inevitable end in death. The chronic cases may have been infected with a less virulent virus subtype then the acute and peracute cases (Penrith et al, 2009).

Peracute
The peracutely infected pigs are usually found dead without premonitory signs. Common signs before death, but not always seen because of the rapid course of the disease are: lethargy, high fever, reddened skin of the abdomen and legs (can be seen in white pigs), shade seeking, huddling together and shallow breathing (Penrith et al, 2009).

Acute
The acute course is slower than the peracute, and death normally occurs after two to seven days. Clinical signs that are often seen include high fever, huddling together, lethargy, anorexia, seeking shade and water, reluctance to move, reddened or cyanotic skin particularly on the ears, lower legs and ventral abdomen (seen in white pigs), abdominal pain and mucopurulent ocular and nasal discharges. Vomiting is common as well as constipation or bloody diarrhoea. As a result of the high fever, pregnant sows abort in any stage of the pregnancy. In the final stages lung oedema evolves resulting in clinical signs such as difficulty of breathing, bloody froth from mouth and nostrils. Usually the lung oedema is the primary cause of death but if the pigs survive longer they may evolve nervous signs because of viral encephalitis/vasculitis or of terminal nature. If the pig recovers from the acute course it is usually asymptomatic (Penrith et al, 2009).

Subacute
Pigs with subacute ASF can survive from weeks to several months, since they are often infected with a less virulent strain of the virus. Depending on if they develop a chronic form of ASF or not, they die or recover. The subacute form occurs mainly in enzootic areas (Epiwebb, 2010). Clinical signs consist of fluctuant fever, the pigs usually grow thin with swollen and painful joints, cardiac damage and a moist cough and difficulty of breathing. Secondary bacterial infections such as interstitial pneumonia are common (Penrith et al, 2009).

Chronic
Common signs of a chronic infected pig are emancipation, inhibition of growth and a long, dull hair coat. Respiratory signs, lameness, sores and ulcers over bony parts of the pigs can also be seen. Chronically infected pigs are susceptible to secondary bacterial infections. A chronically infected pig may survive for many months but recovery is unlikely (Penrith et al, 2009).

Epidemiology

The mortality rate of acute ASF may reach 100 %. In central Africa and in certain local breed populations of pigs higher survival rates has been observed, even in outbreaks with virulent strains of ASF virus. The theory behind this observation is that either some kind of inherent resistance may be evolving through natural selection in the exposed pig population (Penrith et al, 2009) or a decrease of the virulence of the virus occurs with time in enzootic areas (Radostitis et al, 2007). During the decades when ASF existed in Spain and Portugal chronic and persistent infected pigs were observed. The affected pigs had decreased mortality rates and a wide range of clinical symptoms, this made it harder to diagnose the disease. The spreading of the infection through introduction of infected pigs, either during the incubation period or by persistently infected pigs was identified as one of most important transmission routes (Wilkinson, 1984).

The virus is spread through two different cycles: a sylvatic and a domestic. The sylvatic cycle involves wild species of swine spreading the virus by soft ticks of the family *Ornithodoros* (Penrith et al, 2009). In Africa the major host for the ASF virus is the warthog but all wild species of swine in Africa can be silent carriers. The ticks live in the burrows of the warthogs (Penrith et al, 2009). The *Ornithodoros* ticks can survive for a long time and can harbour the virus for several years with only a gradual decrease of infectivity (Radostitis et al, 2007). The bushpig and giant forest hog can become infected by ASF virus but their roles in the epidemiology has not been fully determined (Jori and Bastos, 2009). In commercial farms it is unlikely for the domestic pigs to come in contact with wild pigs and their ticks, but this is considered more common in traditional free-ranging systems (Wilkinson, 1984).

The domestic cycle involves domestic pigs spreading the virus to other domestic pigs through direct or indirect contact. The virus infects the pig through the oronasal route. In the infected domestic pig the virus is shed in enormous amounts in all bodily secretions and excretions, tissues and blood 24 to 48 hours before clinical symptoms are shown. If the pig survives the acute disease it will remain infected for a couple of months but only shed virus for approximately one month. The virus has been found in lymphoid tissues in domestic pigs for up to three to four months after infection (Penrith et al, 2009). When the ASF virus existed in Spain in the 1960s reactivation of virus by stress factors like transportation, in recovered pigs was suspected to be a source of transmission. A study where recovered pigs which carried the ASF virus was administrated corticosteroids during 9 to 31 weeks showed that corticosteroids can reactive the virus replication up to six months after infection. The reactivated pigs did not produce sufficient levels of viremia to transmit the disease by direct contact to other susceptible pigs nor did the pigs show any symptoms of the disease. The theory was that virus levels might have been too low to be transmittable through direct contact but

might be high enough to transmit the disease through shedding in blood and through ingestion of tissues (Wilkinson, 1984).

Transmission of ASF virus through indirect contact are via fomites (equipment, vehicles, people, clothing) or swill feeding with infected meat (Penrith et al, 2009). Undercooked swill feeding has been identified as the major source of spread of ASF to former free areas and the source if infection can almost always be traced back to airports or harbours (Epiwebb, 2010). Domestic pigs can also sustain the infection in the population through ticks but without involvement of the wild pig species (Penrith et al, 2009; OIE ASF, 2010).

Immunity

The pigs that survive the peracute and acute phase of ASF have detectable levels of antibodies in serum against the ASF virus after 7-12 days after the first clinical symptoms. Both in warthogs and domestic pigs the antibodies persist for long time, probably for life. In domestic pigs the antibodies do not protect fully against further infection with ASF virus, but a certain degree of protection against infection with homologous strains of the virus has been reported. Sows that are serologically positive transmit antibodies to their piglets through the colostrums. In subacutely and chronically infected cases the virus replication continues regardless the presence of antibodies. There is no vaccine available against ASF virus (Penrith et al, 2009).

Diagnostic methods

ASF cannot be distinguished from Classical swine fever (CSF) by symptoms or macroscopic lesions. Therefore CSF is the most important differential diagnosis of ASF. Diagnosis can be confirmed by virus isolation, antigen detection e.g. ELISA, direct immunofluorescence, histopathology with immunohistochemistry, virus nucleic acids detection with PCR, or antibody detection with ELISA (antibodies can be analysed after seven to twelve days after infection and are lifelong). Blood or infected tissues for example lung, spleen, liver, kidney, tonsils and lymphnodes can be used in diagnostic methods (Penrith et al, 2009; Radostits et al, 2007).

To learn more of the histopathological lesions and immunohistochemistry of ASF see the thesis written by Justine Ganowiak.

Differential diagnosis

CSF and ASF cannot, as mentioned earlier, be separated only on symptoms and macroscopic findings and therefore CSF is considered to be one of the most important differential diagnoses to ASF. Other diseases to consider are Porcine Reproductive and Respiratory Syndrome (PRRS), Porcine dermatitis/nephropathy syndrome (PDNS), Pasteurellosis, Salmonellosis and Erypesipelasis (Epiwebb, 2010; Penrith et al, 2009).

Classical Swine Fever

CSF or hog cholera is a contagious, febrile and lethal disease of pigs (Merck Vet Manual, 2008). The disease is caused by a small, enveloped RNA virus of the family *Flaviviridae*, genus *Pestivirus*. Only one serotype has been found but antigenic differences have been defined between different viral strains. CSF virus

is related to other Pestiviruses for example Bovine virus diarrhoea virus (CFSPH, 2009; Radostits 2007). Hog cholera was first described in the early 19th century in the USA. A condition in Europe called swine fever was later recognized to be the same disease. Today the disease is called CSF to distinguish it from ASF (Merck Vet Manual, 2008).

CSF is widespread in Asia, some Caribbean islands, Madagascar, Mauritius and South and Central America (CFSPH, 2009). CSF is endemic in wild boar in parts of Europe. Hungary, Serbia and Russia have had outbreaks in 2010. South Africa had one outbreak in august 2007 but since then there have not been any reported cases. Uganda has never reported a case (OIE, 2010). In 1997-98 the Netherlands had an outbreak that involved 429 herds and over 12 million pigs were stamped out to eradicate the disease (Merck Vet Manual, 2008).

The incubation time of CSF is between 2-15 days depending on the dose and virulence of the virus strain and the age and susceptibility of the pig. Less virulent strains often cause subclinical, subacute and chronic cases and more virulent strains cause acute and peracute cases. Younger pigs are more likely to show more severe symptoms than older pigs (CFSPH, 2009). In the most severe form of CSF, acute CSF, pigs often die within 1-3 weeks. The incubation time is often shorter because of more aggressive course of the disease, 2-6 days (Merck Vet Manual, 2008). Symptoms of acute CSF include high fever, lethargy, anorexia, constipation followed by diarrhoea, conjunctivitis, nervous symptoms like unsteadiness, staggering gate, convulsions which progress to posterior paresis, vomiting, respiratory signs, reddened to cyanotic skin on ears, ventral abdomen, legs (seen in white pigs). The course of the subacute form is similar to the acute form but the symptoms are less severe, since it is caused by moderate virulent strains of CSF virus. Survival rates differ, some will survive others die within a month (CFSPH, 2009).

Chronic cases are caused by less virulent strains of CSF virus and infected pigs often survive over 30 days but the course of the disease is almost always fatal. Symptoms resemble the acute and subacute course in the initial stages with the difference that the affected pigs often improve after a couple of weeks. However, chronically pigs develop recurrent symptoms like intermittent fever, anorexia, periods of constipation or diarrhoea, wasting or slowed growth, alopecia and skin lesions. They often develop secondary bacterial infections (CFSPH, 2009).

In subclinical cases the only symptoms can be poor reproductive performance and this makes the disease difficult to diagnose on clinical symptoms only. Subclinical infected pigs are infected with low virulence strains of the CSF virus. Other symptoms of subclinical infected sows are abortion or birth of stillborn, mummified, malformed or weak piglets. Newborn piglets can have congenital tremors or congenital malformations of visceral organs and the central nervous system. The unborn piglets can become persistently infected in utero if they survive. These animals are persistently viremic and can spread the virus before they become clinically ill themselves. The persistently infected pigs often stay asymptomatic for several months before they get symptoms like lethargy, depression, slowed growth, dermatitis, diarrhoea, conjunctivitis, ataxia and

posterior paresis. Affected pigs often survive six months or longer but die within a year (CFSPH, 2009).

Transmission route of CSF is usually direct or indirect contact between pigs or with infected material, like pork, from pigs by oral or oronasal route. Transmission can also occur through mucus membranes, conjunctiva and skin abrasion. Infected pigs can spread the virus through blood, all body secretions and excretions and tissues. Virus shedding can begin before clinical signs are visible and continues throughout the acute and subclinical course. Chronically infected pigs can shed the virus continuously or intermittently for a long time (months) (CFSPH, 2009). Meat that has not been properly cooked can also be a way of transmission since the virus is partially resistant to heat (inactivated at 56 ºC) and a wide range of pH values (OIE, Classical Swine Fever, 2010). Furthermore the virus can remain infectious up to three months in refrigerated meat and more than four years in frozen meat. The CSF virus does not become inactivated by smoking or salt curing; survival range from 17 to more than 180 days. The virus can be spread through mating and artificial insemination. Other routes of transmission are through fomites, mechanically by insects, birds and other animals. Airborne transmission seems to be possible but the maximum distance of spread is unclear (CFSPH, 2009).

Diagnostic methods are similar to the ones used to diagnose ASF: virus isolation, antigen detection with ELISA, direct immunofluorenscense, serological analysis with ELISA (antibodies are produced two to three weeks after infection and persist for life) and virus' nucleic acids detection with PCR. Congenitally infected pigs are immunotolerant and therefore negative on antibody detection tests (CFSPH, 2009).

Porcine reproduction and respiratory syndrome

PRRS is caused by a small enveloped virus from the family *Arteriviridae*, genus *Arterivirus*. PRRS is also known under the names: Mystery Swine Disease, Blue Ear Disease, Porcine Endemic Abortion and Respiratory Syndrome (PEARS) and Swine Infertility Respiratory Syndrome (SIRS) (Beltran-Alcrudo et al, 2007). The virus is very stable under cold/freezing conditions. It can retain infectivity for 4 months at -70 ºC but is inactivated at temperatures above 56 ºC (15-20 min) (Merck Vet Manual, 2008).

PRRS is an important contagious swine disease and was first identified in USA in 1987 and later found the Netherlands in 1990. Today the virus is spread worldwide and is found in all larger pig producing areas of the world. It is endemic in parts of Asia e.g. China and Vietnam. In Africa the disease situation is unknown, and South Africa is the only country that have reported outbreaks, in January 2005 and in April 2008 (OIE 2010; Beltran-Alcrudo et al, 2007). In 1992 two genotypes of PRRS virus were identified: type I representing a European strain and type II a Northern American strain. The different strains of PRRS virus differ greatly in their pathogenicities. Vaccination has been used as an appropriate strategy for prevention of PRRS but it has been suggested that several newly identified virulent PRRS virus isolates have their source in PRRS virus-derived inactivated vaccines (Tian et al, 2007).

The pig is the only species known to be susceptible to the PRRS virus. The incubation period has experimentally been four to eight days but it can vary from 3-37 days (Beltran-Alcruid et al, 2007). In outbreaks of PRRS in China, 2006, the course of the disease varied between 5-20 days and the infected pigs spread the disease to the entire herd in three to five days. The virus can remain in lymphoid tissues for up to 150 days after exposure, even if it is cleared from the blood (Tian et al, 2007).

The clinical symptoms can vary between different herds but in general PRRS is characterized by reproductive failure of sows and respiratory distress of piglets and growing pigs. Reproductive symptoms can be: infertility, foetal mummification, abortions, agalactia, stillbirths and weak piglets that usually die shortly after birth due to secondary bacterial respiratory infection. Young piglets have the highest mortality and losses can reach 60-70 % in the peak of an outbreak, but 30-50 % is more common. The respiratory symptoms are mostly seen in weaners and porkers: anorexia, depression, cutaneous hyperaemia, dyspnea, rough hair coat, slowed growth and increased susceptibility and mortality to secondary bacterial infections. Finishing pigs, sows and boars have more often a subclinical course of disease (Beltran-Alcrudo et al, 2007).

As mentioned before, China had outbreaks of PRRS in the summer of 2006. The infected pigs had the following symptoms: reddened skin, petechiae, erythematous blanching raches and pimples often observed in ears, mouth, noses, back and inner thigh. Furthermore high fever, lethargy, anorexia, cough, lameness, shivering, diarrhoea was also observed. Many adult pigs died during this epidemic period, which is uncharacteristic for PRRS. ASF or CSF was suspected in the beginning but investigators found that the cause of the epidemic was a highly virulent strain of PRRS (Tian et al, 2007).

The diagnosis of PRRS can be difficult, mainly because virus isolation is made from the alveolar macrophages which need to be harvested from specific pathogen free pigs under 6-8 weeks of age. Otherwise serological test like ELISA and virus detection by PCR can be used for diagnose (OIE, PRRS, 2010).

MATERIAL AND METHODS

Study region and population

The main part of the study was conducted on a farm in Mityana district, Uganda during three months (October-December) 2010. During three visits to the farm and close phone contact with the manager of the farm the development of the outbreak could be monitored. No contact was ever made with the owner of the farm; all communication was made through the manager.

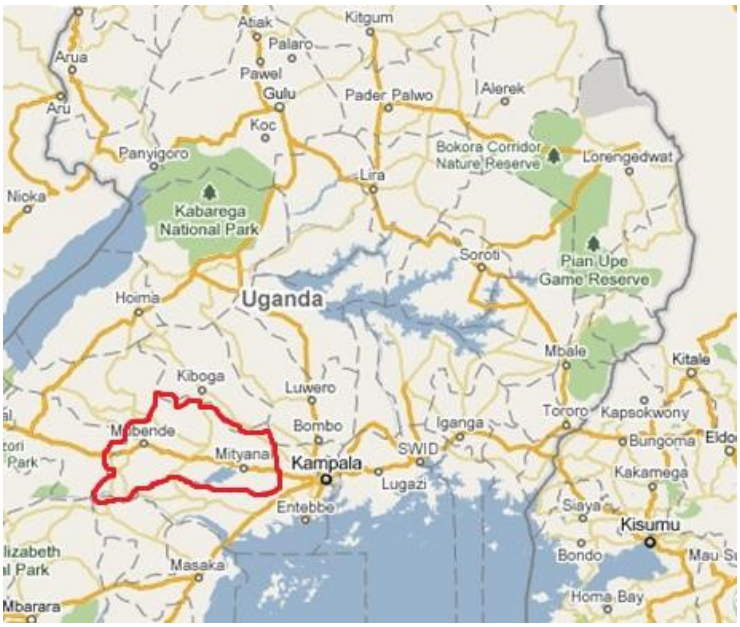

Figure 2. Map of Mityana district, Uganda (Google Earth, 2011)

The farm

The farm was founded in 1995 and has an area of approximately 200 acres. The manager had been working at the farm since 2008, and during his two years of work no outbreaks of enzootic diseases like ASF or other diseases with high mortality rate had been reported. The farm had an integrated pig production and also kept cattle. The farm consisted of five units A, B, C, D and E. Unit A and B held pregnant sows, sows with piglets and, weaners (the piglets were weaned at around eight weeks of age) and three boars. Unit C held gilts and porkers and Unit D had two pens with porkers (for schematic drawing see Appendix 1). The fifth unit, unit E the isolation unit, a hut approximately two kilometres away from the rest of the farm, up on a hill.

The farm had a contract with a meat product company to whom they sold their porkers. The porkers were slaughtered on the farm at about nine to twelve months of age and the meat was then transported with the farms' own transport vehicle to the meat company. Old sows and other pigs in the production were sold to smaller slaughter houses in Kampala. The biosecurity measures of the farm consisted of two footbaths, one in front of unit A and one in front of unit D and C. To limit the access to the farm there was a gate in the entrance of the farm and there were also plans to fence in the farm.

The farm had eight workers employed. Three of the workers were responsible only for the pig production units. One worker was responsible for unit C and D and the other two for unit A respective unit B. The workers were not supposed to go between the units. The isolation unit was handled by the worker handling the tractor, he did not come in contact with the rest of the units. Three of the remaining workers were diggers. One of the diggers also mixed the feed for all the pigs. The remaining worker handled the cattle. The workers lived in the neighbourhood of the farm area and some of the workers kept pigs of local type. One of the workers who took care of the pig production had had pigs at home but there was no record if he still kept pigs. Additional animals, apart from the pigs

and cattle, which were kept on the farm were some dogs and a cat. The cattle, dogs and cat were roaming freely over the farm area.

Feed for the pigs were bought from a local market in Kisenyi. The feed consisted of maize brand, wheat brans, fish and snails shells, no swill feeding occurred on the farm. The vehicle for transport of the feed was hired but otherwise the entry to the farm was minimized.

Waste from the pig production was put away in a waste ditch. The waste was used in the garden as manure, but only on the farm.

There was also a well on the farm, which people from the nearby villages used. The villagers had to pass through the farm to access this well.

There were wild pigs, bushpigs, in the forest around the farm but they did as far as it is known not come in contact with the domestic pigs. There were other domestic free-ranging pigs in the neighbourhood, which might have been sick. Some of the workers' pigs had died but there were no information on whose since all pigs were free-ranging. No new pigs had been introduced to the farm during the last six months, and the last time any animals were sold was in August 2010 when nine sows were sold for slaughter because of lack of reproductive out-put.

The outbreak
The manager suspected that the source of infection originated from somewhere outside the farm. A possible source of infection was pig carcasses from a neighbouring lady who had pigs that had died before the outbreak started on the farm. The carcasses were dropped on the farm area and the dogs belonging to the farm had eaten from the remains. The lady had nine pigs from the beginning, then a few were sold and the rest died.

The clinical symptoms that had been observed were firstly loss of appetite then, one to two days later, the affected pigs started to show reddened areas on the ears and the abdomen and an increased rate of breath. Later the pigs started shivering and shortly after they died. The manager tried to report the outbreak to the local district veterinary officer (DVO) but had not been able to get in contact with him.

Rakai
The other part of the study consisted of a screening for CSF and PRRS in the district of Rakai in south Uganda and in 80 samples from reported outbreaks of mortality in pigs where ASFV was not confirmed as the cause. In Rakai, eight sub-counties were included, and in each, five villages were selected. In each village two pig farmers were chosen and asked if they wanted to be part of the study. On each farm three pigs were chosen for sampling. The DVOs chose the farms for the sampling. As compensation for participation in study to the farmers, each of the sampled pigs was dewormed with Ivermectin.

Sample and data collection

Blood samples were taken from the pigs in the study, one serum and one EDTA tube from each pig. The pigs were captured with a pig catcher, or if they were piglets laid on their back, and blood was collected using vacutainer from the jugular vein.

Data on sex, age and breed of the pig was collected and each sample from the pig was labelled. The breeds were classified as: improved (white pig), local (black pig) or mixed (black and white pig). The age was estimated and the different categories were defined as: 0-2 months, 3-5 months, 6-9 months, 9-12 months, and adults (>12 months).

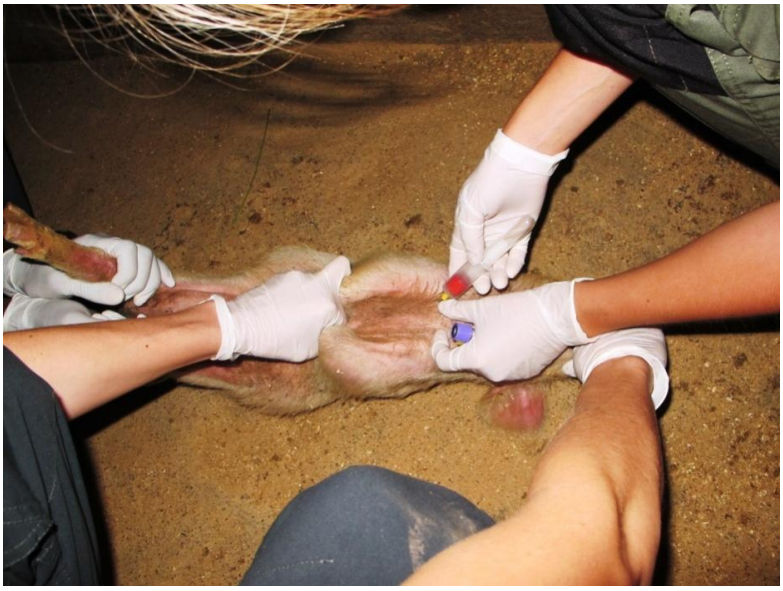

Figure 4. Sampling of piglet with acute ASF in the farm, Mityana district (picture J. Ganowiak)

Laboratory analyses

All laboratory analyses were performed at the molecular biology laboratory of the Makerere University Institute of Environment and Natural Resources at Makerere University, Microbiology lab, Kampala.

ELISA

The samples from the farm in Mityana were tested for antibodies against ASF virus using a commercially available ELISA kit (Ingenasa, Madrid, Spain) in accordance with the instructions from the manufacturer. The samples were diluted 1:2 by diluting 50 µl of sample with 50 µl of sample diluents. The samples were not tested in duplicates. The wells in the test were washed with approximately 300 µl washing solution and then emptied manually by turning the plate upside down and forcefully hit the plate against a towel. The samples were measured at 450 nm. The test was considered valid when the optic density (OD) of the negative control was, at least, four times higher than the OD of the positive control. The calculation for the positive and negative cut-offs were:

Positive cut off: CN – ((CN-CP) x 0.5)

Negative cut off: CN – ((CN-CP) x 0.4)

(CN= control negative, CP= control positive)

The sample was considered positive if the OD was smaller than the positive cut-off and negative if the OD was greater than the negative cut-off. If the sample had an OD in-between the two cut-offs it was considered to be in the grey zone.

Samples from Rakai and the 80 samples of undiagnosed mortality in pigs were tested for antibodies against CSF and PRRS (PRRS Antibody X3 Herdcheck kit and CSF Antibody test kit, IDEXX Laboratories Inc., Maine, USA). The test procedure followed the IDEXX Herdcheck manual that came with the test kit. The samples was diluted 1:40 for the PRRS test by diluting 5µl with 95 µl in a clean 96-well diluting plate and then taken 50 µl of that mix and dilute further another 50 µl of sample diluents. The PRRS x3 Herdcheck had a specificity of 99.9% and sensitivity of 98.8% (IDEXX, 2010). The CSF samples were diluted 1:2 by diluting 50 µl of sample with 50 µl of sample diluents. The samples were not tested in duplicates, with exception for the positive samples that were rerun in duplicates for confirmation. The wells in both the PRRS and CSF tests were washed with approximately 300 µl washing solution and then emptied manually by turning the plate upside down and forcefully hit the plate against a towel. The absorbance of the CSF samples were measured at 450 nm and the PRRS samples was measured at 630 nm instead of 650 nm. The positive cut-off in the PRRS test was OD 0.40.

PCR

The samples from Mityana were tested for presence of ASF virus nucleic acids. DNeasy Blood and tissue kit from Qiagen was used for extraction of viral DNA from EDTA blood samples. The samples from the district of Bundibugyo were tested for presence of CSF and PRRS nucleic acids. The PRRS kits detected both European and the American strains of the virus. The RNeasy Mini Kit from Qiagen was used to extract viral RNA. The extracted RNA samples were kept on freezer blocks when handled for the PCR analysis. The RT-PCR was performed with the Cepheid Smartcycler v3. For ASF, PRRS and CSF the Tetracore ASF resp. CSF kits were used and test procedure was performed according to the manual that came with the kit.

The result of the RT-PCR was considered positive when the samples accumulated enough fluorescent signal to cross a defined threshold. The unit used, CT (cycle threshold), was defined as the number of cycles required for the sample to accumulate enough fluorescence to cross the threshold. The threshold for a positive CT was programmed in the smartcycler according to the test manual. The positive control that came with the test kit had an average positive CT, for the ASF test normally around CT 28-30. The positive CT values of the samples were compared to the positive control. The sample was considered to be highly positive for virus if the positive CT was lower than the positive control. If the sample had a higher positive CT than the positive control it was interpreted as a positive result but with a lower amount of virus.

RESULTS

Chronological development of the outbreak in Mityana

7th of October 2010 one sow, mother of nine piglets approximately two months of age, was found dead by the workers in the morning in unit B, block 1, pen 5. The nine piglets and piglets from neighbouring unit B, block 1, pen B11 were able go

between the pens. The manger believed that the cause of death was that the sow had been over suckled and therefore exhausted. The nine piglets were moved to unit A, block 2, pen 2 together with some weaners.

8th of October one of the nine piglets died suddenly. The remaining eight piglets were moved back to unit B, block 1, pen 5 and the passage to pen 11 was blocked.

12th of October another three piglets of the litter of nine died. In unit B, block 1, pen 11 the sow with eleven piglets was getting sick and two of her piglets died. The sow and remaining piglets of pen 11 were put in the isolation unit.

13th of October one piglet of unit B, block 1, pen 5 and three piglets in the isolation unit died (former unit B, block 1, pen 11).

14th of October, the first investigation was performed. Three other piglets of unit B, block 1, pen 5 had died during the night/morning and the one remaining piglet was very sick with symptoms like shivering, reddened ears and legs. It died later during the night.

Figure 5. Piglet from pen B5 with acute ASF, note the reddened ears and legs (picture M. Andersson)

In the isolation hut, there was one dead piglet and the remaining piglets and the sow were feverish and weak. The sow died the next day and her piglets died one by one the following days.

Necropsies were performed on three pigs of unit B, block1, pen 5 that had died earlier that day. The sick piglet from pen 5 and three others from the isolation unit were sampled (org. pen 11). RT-PCR analysis 15th of October confirmed ASF as diagnosis. The piglet from pen 5 and two from the isolation unit tested strongly positive for ASF. The outbreak was reported to the commissioner of livestock and entomology and the DVO.

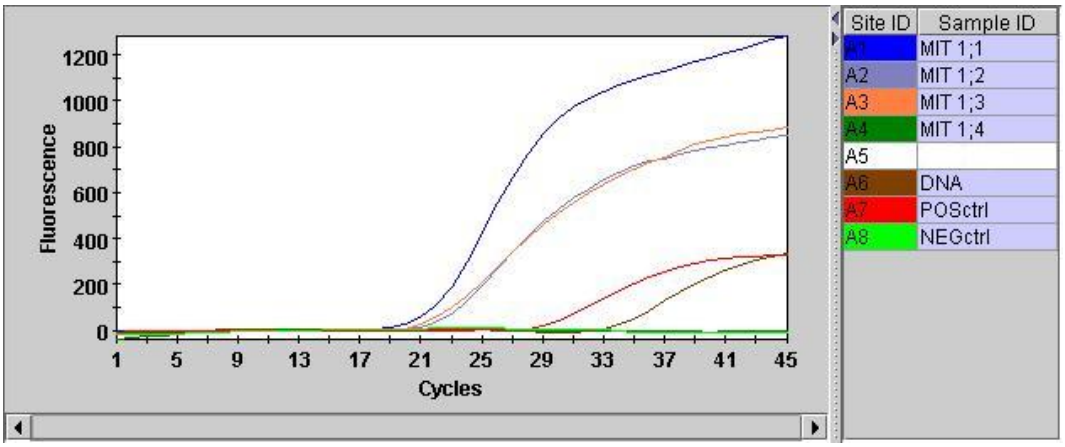

Figure 6. Results from real-time PCR assay. First visit at the farm, 14th of October.

18th of October a sow, mother of three, in unit B, block 1, pen 6 died. Her piglets were put in the isolation unit. The following days after the sows´ death her piglets started dying. On the second visit, the 23rd of October, only one of these piglets remained alive.

20th of October pregnant gilt in unit B, block 1, pen 7 died.

23rd of October, the second visit. The night before the visit on 23rd of October 2010 one of the sows in unit B had given birth to 5 mummified foetuses and eight live piglets, no other reproduction problems had been reported on the farm. Minor problems with lameness in piglets and weak born piglets had been observed, but similar problems had occurred before the outbreak as well. At the visit 18 pigs from five different pens were chosen for sampling (see Appendix 2). All pigs that were sampled were also earmarked so they could be identified later. The RT-PCR results of the samples showed that all pigs sampled in block A, unit 2, pen 2 were positive except for one. In unit B, block 1, pen 13 one pig was also positive but with a higher CT of 39,6. The rest of the samples were negative on the RT-PCR.

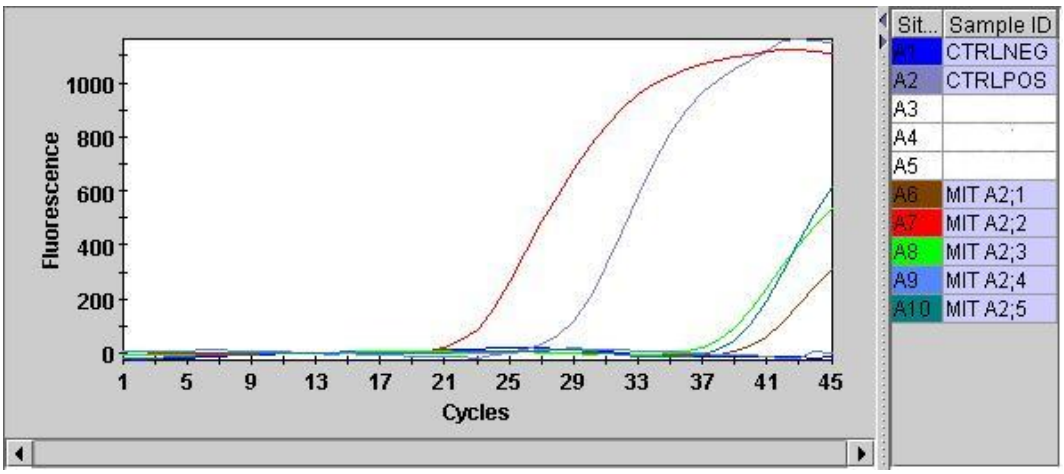

Figure 7. Results from RT-PCR assay. The farm unit A block 2 pen 2.

All samples were also tested for antibodies of ASF virus with ELISA. All samples except for two pigs in unit B, block 1, pen 12 were serologically negative.

25th of October, one of the gilts in unit C aborted. One day after the gilt was found dead. After that the manager decided to send all gilts in that pen to slaughter to prevent further transmission. The gilts had direct contact with the porkers in the

neighbouring pen through a gate, but none of porkers had shown any clinical signs around the time when the gilt died. The manager suspected that the infection had spread from unit A and B to unit C through the workers. According to him it was difficult to enforce the workers to follow the new directions of strict hygiene rules between units and pens. After the gilts were sent to slaughter fences were put up around every unit to minimize spreading between units. Separate rubber-boots was introduced for each unit. The workers were allowed only to use the unit specific rubber-boots in their unit.

The weaners in unit A, block 2, pen 2 started to die around this date. They were put in the isolation unit but all died during the following days.

Shortly after the visit on 23rd of October 2010 the two sows in unit B, block 1, pen 13 started to show clinical signs (loss of appetite) and therefore they were sold for slaughter. The sows were mothers of piglets in unit B, block 1, pen 6 and piglets in unit B, block 1, pen 12. Two of the piglets in unit B, block 1, pen 12 were serologically positive for ASF virus on the visit the 23rd of October but without any clinical signs.

27th of October seven sows from unit A, block 1, and 2 (Block 1; Pen 1, 3 and 4, Block 2; Pen 1 and 4) started to show clinical signs like loss of appetite. The sows were sold for slaughter to a small slaughterhouse in Kampala. One of the sows from unit A, block 1, pen 2 did not have any clinical signs like the others and because of that she was put in unit B, block 1, pen 7 separated from the rest of the pigs in that block. The manager did not want the sow to be placed in the isolation unit because the sow was pregnant, approximately 3 months. If she was to be placed in the isolation unit and she did not develop any symptoms she would not be able to go back to the rest of the pigs since the isolation unit was considered contaminated. The sow was supposed to be in "quarantine" until the sign of disease developed or she delivered. At the third visit on 12th of November, 2010 the sow had no symptoms of ASF.

A sow with three piglets in unit B, block 2, pen 3 was put in isolation unit after getting clinical signs, loss of appetite. In the isolation she got worse and was sold for slaughter. Her three piglets died in isolation during the following days.

12th of November, the third visit. Following pigs had clinical symptoms at the visit:

- Unit A, block 1, pen 2 one sow had loss of appetite. The sow had aborted seven foetuses and had delivered six live piglets four days ago.
- Unit A, block 2, pen 3 and 5, two sows in each pen had symptoms like loss of appetite and lethargy.
- Unit B, block 2, pen 5 a sow had aborted a few days ago but was still without any clinical symptoms at the visit, she was only having problems moving.
- One of the boars, in unit B, block 1, pen 8, had reddened skin on its hind legs and abdomen. The worker started the cleaning of the block in the pen of the boar and then continued cleaning all the rest of pens in that block. If the boar was infected the disease had spread to the whole block since the workers do not change rubber boots or cleaning equipment between the

pens, expect for the "quarantine" sow in unit B, block 1, pen 7 which had its own equipment.

The three weak born piglets that were in unit B, block 1, pen 10 at the last visit were now moved to unit B, block 2, pen 4 but they showed no clinical signs. No other pigs had been moved.

15th of November 2010, information was gathered through phone contact with the manager. The manager had sold the boar in unit B, block 1, pen 8 for slaughter as a preventive measurement. Two sows of the four sows with loss of appetite in unit A, block 2, pen 3 and 5 had died and the remaining two sows had been sold for slaughter. The sow with newly born piglets in unit A, block 1, pen 2 had died, both sow and piglets were buried on the farm. The sow in the "quarantine" in unit B, block 1, pen 7 had been sold to slaughter because it had developed clinical signs.

29th of November, information was gathered through phone contact with the manager. In unit A all sows were dead or slaughtered, there were only a few piglets left in that unit. In unit B there were remaining five sows and one boar. There had been no more spread of the infection since the boar from unit B, block 1, pen 8 was sold for slaughter.

13th of December, information was gathered through phone contact with the manager. On 3rd of December, four of the porkers in unit C started to show clinical signs and where sold for slaughter on the 3rd of December and the 5th of December. The remaining 16 porkers were sold as a preventive measure to the pork abattoir in Kampala on the 10th of December. No more pigs with clinical symptoms had been seen in unit B.

Impact of the outbreak on the farmer

Since ASF is endemic in Uganda the farmers do not get any compensation for their losses. The outbreak of ASF was very devastating for the economy of the farm. During the visits to the farm and the conversations with the manager a sense of hopelessness was felt. The manager was more and more concerned about the possibility of ever eradicate the infection and about how many pigs that would die.

When looking at the numbers of pigs lost either to clinical disease or to slaughter over half of the population disappeared because of the ASF outbreak (see table 2).

Table 2. Summary of the impact of the number of pigs on a commercial pig farm during an outbreak of ASF in Uganda 2010

Date	*23/10*	*13/12*	*Difference between 23/10 and 3/12*
Sows	*41*	*5*	*-39*
Boars	*3*	*1*	*-2*
Porkers	*87*	*-*	*-87*
Gilts	*11*	*-*	*-11*
Weaners	*89*	*70*	*-19*
Piglets	*109*	*75*	*-34**
Total	*340*	*151*	*-192*

**The loss of piglets was probably higher since several have been born during this time period.*

To estimate the losses the following numbers are presented:

- The porkers were sold for 4200 UGX/kg at dressed weight ~50 kg (live weight ~80-100 kg) in total 210 000 UGX/porker to the meat company the farm had a contract with.
- The price for buying a sow was 450-500 000 UGX.
- According to the manager, the biggest boar was bought for 500 000 UGX and the two smaller ones for 260 000 UGX each.
- The value of pregnant gilt was >500 000 UGX and a nonpregnant gilt was worth around 400 000 UGX.
- The mean value of piglets per litter was estimated to 6 piglets/litter from the number of piglets per litter at the first visit. Assuming that the production of porkers are equal to the amount the piglets/litter minus expenses for feed etc the profit of each litter was estimated to 600 000 UGX/litter (210 000 x 6 – 50%).
- The future production of litters of gilts and sows was estimated from numbers from Swedish pig production. In average a sow in Sweden produces three litters before she is slaughtered (Fellström, 2009). No statistics was found on the number of litters a gilt produces so this was estimated as higher than the sows and therefore the number six was used.

The estimated losses are presented in Table 3 and 4.

Table 3. Estimated losses because of deceased animals

Category of animals	*Number of deceased animals*	*Estimated loss (million UGX)*
Gilt	*1*	*0,5*
*Future litters of gilt**	*6 x 1*	*3,6*
Sows	*25*	*11,25-12,5*
*Future litters of sows***	*3 x25*	*45*
Piglets	*34*	*3,4*
Weaners	*19*	*1,9*
Total	***79***	***65,65-66,9***

**Estimated production of litters is six, lost profit of litter is estimated to 600 000 UGX/litter*
***Estimated remaining litters to produce is three, lost profit of litter is estimated to 600 000 UGX/litter*

Table 4. Estimated losses because of sanitary slaughter

Category of animals	*Numbers of sanitary slaughtered animals*	*Estimated loss (in million UGX)*
Gilts (pregnant) [1)]	*10*	*1,35*
*Future litters of gilts**	*6 x 10*	*36*
Sows [2)]	*14*	*0,7*
*Future litters of sows***	*3 x 14*	*25,2*
Total	***23***	***63,25***

1) Loss by slaughtering a pregnant gilt was estimated to 135,000UGX/gilt
2)Loss by slaughtering sows was estimated to 50,000 UGX/sow
** Estimated production of litters is six, lost profit of litter is estimated to 600,000 UGX/litter*
*** Estimated remaining litters to produce is three, lost profit of litter is estimated to 600,000 UGX/litter*

Total loss was estimated to: 128,9-130,15 million UGX (so far) = 362,000-365,500 SEK[1].

There was no information on the price for the boars and the porkers were sold to the smaller slaughterhouses, but one can assume that they were also sold for a lower price than normal. Another factor that affects the farm was the boars which also were important for the future production and loss of genetic material.

Presence of CSF and PRRS

In total 319 samples were analysed for antibodies for both CSF virus and PRRS virus. All 239 samples from Rakai were negative. Of the 80 samples from outbreaks in which ASF had not been confirmed, one tested positive for PRRS, all the rest were negative for CSF and PRRS. The positive sample was rerun in duplicates to confirm the result and both duplicates were still positive. The positive sample measured OD 0.43 first time and OD 0.47 and 0.46 in the rerun. According to test manual the sample is positive if the OD measures over 0.40.

[1] 0,0028089 2011-02-03 http://omvandlare.com/valuta

The samples in Rakai were 239 instead of 240 because in one farm the owner did only have two suitable pigs for sampling instead of three.

DISCUSSION

ASF has existed and been enzootic in Uganda for a long time. There seem to be several factors that contribute to the presence of the disease in the country. The sylvatic cycle of ASF exists in Uganda (based on the results of this study) and this is one reason that can possibly explain why the disease is maintained in the area. Also in comparison to the other problems in Uganda, poverty and human disease like HIV and malaria, the control of ASF is probably not prioritized. In Uganda there are many other important animal diseases, like Foot-and-mouth disease and Contagious bovine pleuropneumonia. Diseases affecting cattle may be more prioritized since in comparison with pigs, cattle are considered more valuable. Another factor are the Veterinary Officers (VOs) who have an important role during an outbreak and also for the control and diagnostics of ASF. From my observations in Uganda it seemed like the VOs lacked resources in terms of diagnostic methods and transportation (running costs in general). The VOs sometimes lack access to cars and are forced to ride motorbikes, often lacking enough fuel. The farms were also generally difficult to reach because many of the roads were in poor condition. Another contributing factor is probably the structure of husbandry and the knowledge of ASF amongst the farmers in Uganda. During the field trips it was noted that in many of the smaller farms the pigs were often free-ranging or tethered. This may create a greater risk for the pigs of coming in contact with infected ticks and/or pigs, wild or domestic. The knowledge of the spreading of the ASF virus did not seem to be sufficient amongst several of the farmers that were visited. Out of my observations during the study it seemed like farmers rarely report an outbreak of disease with high mortality to their DVO, for example the neighbouring lady of the Mityana farm. If pigs in a smaller farm start to acutely die, the normal thing seemed to be either to sell the pigs that were still alive or slaughter them and sell the meat. This increases the risk of spreading ASF since movements of infected pigs and pork are the most important routes of spreading. The question is why are not all outbreaks reported? It may be that the farmers do not have enough tradition or knowledge of the importance of reporting an outbreak and/or do not gain anything on reporting. One example was the farm in Mityana where both the manager and personnel from the project reported the outbreak but no one from the authorities contacted the farm. Regardless, since most outbreaks are not reported it is difficult to have a good control of the spread and make preventive actions. More reported and monitored outbreaks would make it possible to get a better overlook of the spread of the disease, a better knowledge of which areas are more exposed and maybe aid the affected farmers in a better way.

On the farm level, the farm in Mityana, the impact of the ASF outbreak was profound. From the first visit and the continuous contact with the manager it was possible to follow how the infection spread from pen to pen and unit to unit. The source of infection probably originated from somewhere outside the farm. It is difficult to point out the exact route from where the infection entered the farm since in the interviews with the manager he pointed out several possible routes of infection. The most likely source of infection was the lady with the neighbouring

farm, but it is difficult to definitely say since no investigation was carried out to trace the infection. Another possible route was the workers, if they had pigs themselves that were infected and in turn brought the infection with them to the farm. The spread of disease in the farm between units and pens were more clearly visible with the direct and indirect contact between pigs. The infection seemed to have started in unit B block 1 and then spread through the movement of pigs, the workers and the equipment to unit A and then further to units C and D. The workers seemed to be an important route of spreading and are probably the reason for the spread to units C and D.

During the early phase of the outbreak the pigs with clinical disease seemed to be acutely or peracutely affected, indicating the possibility of a strain of virus with high virulence. The outbreak lasted three months and during the later phase the course of the disease went more slowly which indicate a more chronic state. One interesting observation of the infection during the outbreak was that mostly the older pigs showed clinical signs and died. The weaners and older piglets did not die in the same numbers as the adults and newborn piglets. One example of that was the weaners from unit B, block 1, pen 12 that are, presumably, still alive and did not shown any clinical signs at the visits of the farm. The results from the sampling showed that one of them was positive on the serology and one was in the gray zone. According to the literature domestic pigs are susceptible of the infection irrespective of gender and age but some pigs are more likely to develop a subclinical to chronic infection or they might inherit a bigger resistance to the infection (Penrith et al, 2009). Since the outbreak is still going on and not all pigs have been sampled it is not possible to evaluate the status of all pigs fully. The weaners of pen 12 seemed to have been exposed to the virus since at least one of them developed antibodies against ASF. Antibodies are detectable 7-12 days after the first clinical symptoms are shown and in infected pigs the virus can be shed for up to one month after being infected (Penrith et al, 2009). None of the weaners of pen 12 were positive on the RT-PCR. The infection might have come in to the population much earlier than suspected and the weaners have therefore stopped shedding the virus, which then is not detectable in the blood but might still be present in other tissues like lymphoid tissues. The weaners and older piglets can also be more likely to develop a chronic state than the older pigs. Another possible theory is that they have been able to eliminate the virus because they have a bigger resistance to the infection. These theories may explain the larger survival rate of the weaners and older piglets, but for more information about the status of the pigs it is needed to sample all remaining pigs and if possible take samples from lymphoid tissues.

As earlier discussed, there are several reasons for the disease existence in Uganda of the disease amongst the famers is a contributing factor. In the farm in Mityana the biosecurity measures were probably one reason why the infection was spread to the farm. At the first visit, the farm had a few footbaths and they seemed to not be changed regularly. The farm had a gate but since people from the villages nearby walked through the farm to get to a well the gate did not help to limit the access to the farm. There were also little knowledge of the workers and if they owned any sick pigs. During the outbreak the manager improved the level of biosecurity with better fencing, even around every unit, better footbaths and separate equipment for each unit. He tried to limit the workers' movements

between and in the units so they would not spread the disease further. He started to slaughter all pigs with clinical symptoms to prevent further spreading. The biosecurity measures taken to stop the spreading might have saved some pigs in the population but most likely all pigs have already encountered the disease but has different levels of resistance to the ASF virus and therefore a different rate of survival. In unit B block 2 almost no deaths or pigs with clinical infection had occurred and it is not known if the spreading of the disease was prevented there or those pigs have not shown any clinical signs yet. No pigs were sampled in that block so the virus and serology status are unknown. It would be interesting to sample all remaining pigs in the population in a couple of months, if some survive, to see if they have been able to eradicate the infection or if they are chronically infected but with no clinical symptoms. This will be done in February 2011.

In the screening for PRRS and CSF in Rakai and the undiagnosed samples there was only one positive sample for PRRS, from the district of Bundibugyo. It was the only positive sample from that region and outbreak. The result turned out to be a very weak positive result. Therefore the most likely interpretation of the result was that it was a false positive. To confirm the result the sample need to be analysed with an alternative test procedure and the best would be to take another sample from the animal and from the other pigs in that population. In the study there was no opportunity to test the sample in another procedure nor take another sample of the pig, but it is something that can be done in future studies. CSF was not found in the samples that were analysed. But since this is a smaller study one should consider that further studies are needed to clearly show that Uganda is free from CSF and PRRS.

Conclusions

ASF has existed a long time in Uganda and there are several reasons why it is maintained in the country. The lacking of reports and follow-ups of reported outbreak makes the controlling of the disease difficult.

ASF is an important disease for the individual farmer but also in a global view. For the individual farmer, as in the farm in Mityana, the impact of an outbreak of ASF can devastate the whole production. In a global view the disease causes economical losses in both developed and developing countries.

Lacking strong biosecurity, knowledge and resources to eradicate the infection is the main reason of the spreading to the farm in Mityana. Both the manager and the representatives from the lab have reported the outbreak to the authorities but no one has answered or given any directions to the manager.

The serologically positive sample for PRRS was probably a false positive. To evaluate it further the animal should be re-sampled and run in another test procedure. Since this was a small study the negative result of CSF and suspected negative result of PRRS cannot rule out that the diseases are present in Uganda.

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REFERENCES

Agriculture Education and training, The World Bank (online) Available: http://web.worldbank.org/WBSITE/EXTERNAL/TOPICS/EXTARD/0,,contentMDK:20445369~isCURL:Y~menuPK:1308455~pagePK:148956~piPK:216618~theSitePK:336682,00.html) 2010-11-08

Animal Diseases Act 1918, Chapter 38 (online) Available: http://www.ulii.org/ug/legis/consol_act/ada1918138/ (2010-11-21)

Beltran-Alcrudo et al, Focus on Porcine Reproductive and Respiratory Syndrome (regional awareness), FAO Empres, No2 2007

Briggs P, Roberts A. Brandt travel guides: Uganda (1994), 6th ed 2010, Brandt Travel guides, ISBN-13 978 184162309 2

the Centre for food security and public health (CFSPH), Institute for international cooperation in animal biologics, Iowa State University; OIE, Classical Swine Fever, Last updated 16 September 2009 Available: http://www.cfsph.iastate.edu/DiseaseInfo/disease.php?name=classical-swine-fever&lang=en (2011-02-03)

Costard et al, African swine fever: how can global spread be prevented? 2009,The Royal Society

Epiwebb, Afrikansk Svinpest (online) Available: http://epiwebb.se/02_afrikansk_svinpest/index.shtml (2010-12-04)

Fellström C, Griskompendiet, version 7, Instutionen för kliniska vetenskaper, SLU, 2009

FAO statistics division 2010 (online) Available: http://faostat.fao.org/site/573/DesktopDefault.aspx?PageID=573#ancor (2010-12-04)

Google Maps (online) Available: http://maps.google.se/maps?hl=sv&rlz=1T4SVEC_svSE380SE383&q=Mityana%20uganda&um=1&ie=UTF-8&sa=N&tab=wl (2011-02-03)

Human Development Report, UNDP (online) Available: http://hdrstats.undp.org/en/countries/profiles/UGA.html (2010-12-21)

Jori F. et Bastos A., Role of the wild suids in the epidemiology of African Swine Fever, International Association of ecology and health (Ecohealth), 2009

NAADS (online) Available: http://www.naads.or.ug/naads.php (2010-11-08)

Penrith et al, Preparation of African Swine fever contingency plan, FAO, 2009, ISBN 978-92-5-106426-9

Pro-poor livestock policy initiative, FAO (online) Available: http://www.fao.org/ag/againfo/programmes/en/pplpi/about.html (2010-11-08)

Radostitis O. et al, Veterinary Medicine, Elsevier Limitied 2007 10th ed, Chapter 21: Hog Cholera s 1157-1167, African Swine Fever s1167-1172 ISBN 10: 7020 2777 4 ISBN: 13: 978 0702 07772

Rowlands et Al, 2008, African swine fever virus isolate, Georgia, 2007, Emerging Infectious Diseases, 2008 December; 14(12): 1870–1874.

Söderling Liselotte, Personal Note, 2010-12-08

Tian et al, Emergence of Fatal PRRSV Variants: Unparalleled Outbreaks of Atypical PRRS in China and Molecular Dissection of the Unique Hallmark , PLos One 2007 June 13.

The Livestock revolution, FAO (online) Available: http://www.fao.org/WAIRDOCS/LEAD/X6115E/x6115e03.htm#TopOfPage (2010-11-08)

The Merck Veterinary Manual 2008,

Classical Swine Fever (online)Available: http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/53400.htm&word=classical%2cswine%2cfever (2010-12-21)

PRRS (online) Available:

http://www.merckvetmanual.com/mvm/index.jsp?cfile=htm/bc/54100.htm&word=PRRS (2010-12-21)

Uganda: government halts forced IDP repatriation, IRIN (online) Available: http://www.irinnews.org/Report.aspx?ReportId=86958 (2010-11-08)

WAHID Interface, World organisation of animal health, OIE

African Swine fever (online) Available: http://www.oie.int/wahis/public.php?page=disease_outbreak_map&disease_type=Terrestrial&disease_id=12 (2010-11-21)

Available: http://web.oie.int/wahis/public.php (2011-02-03)

African Swine fever distribution (online) Available: http://www.oie.int/wahis/public.php?page=disease_status_map (2011-01-25)

Classical Swine Fever, (online) Available: http://www.oie.int/wahis/public.php?page=disease_immediate_summary&disease_type=Terrestrial&disease_id=13 (2010-12-04)

PRRS (online) Available: http://www.oie.int/wahis/public.php (2010-11-21)

Wilkinson P.J, The persistence of African swine fever in Africa and the Mediterranean, Preventive Veterinary Medicine, 2 (1984) 71-82 Elsevier Science Publishers B.V Amsterdam.

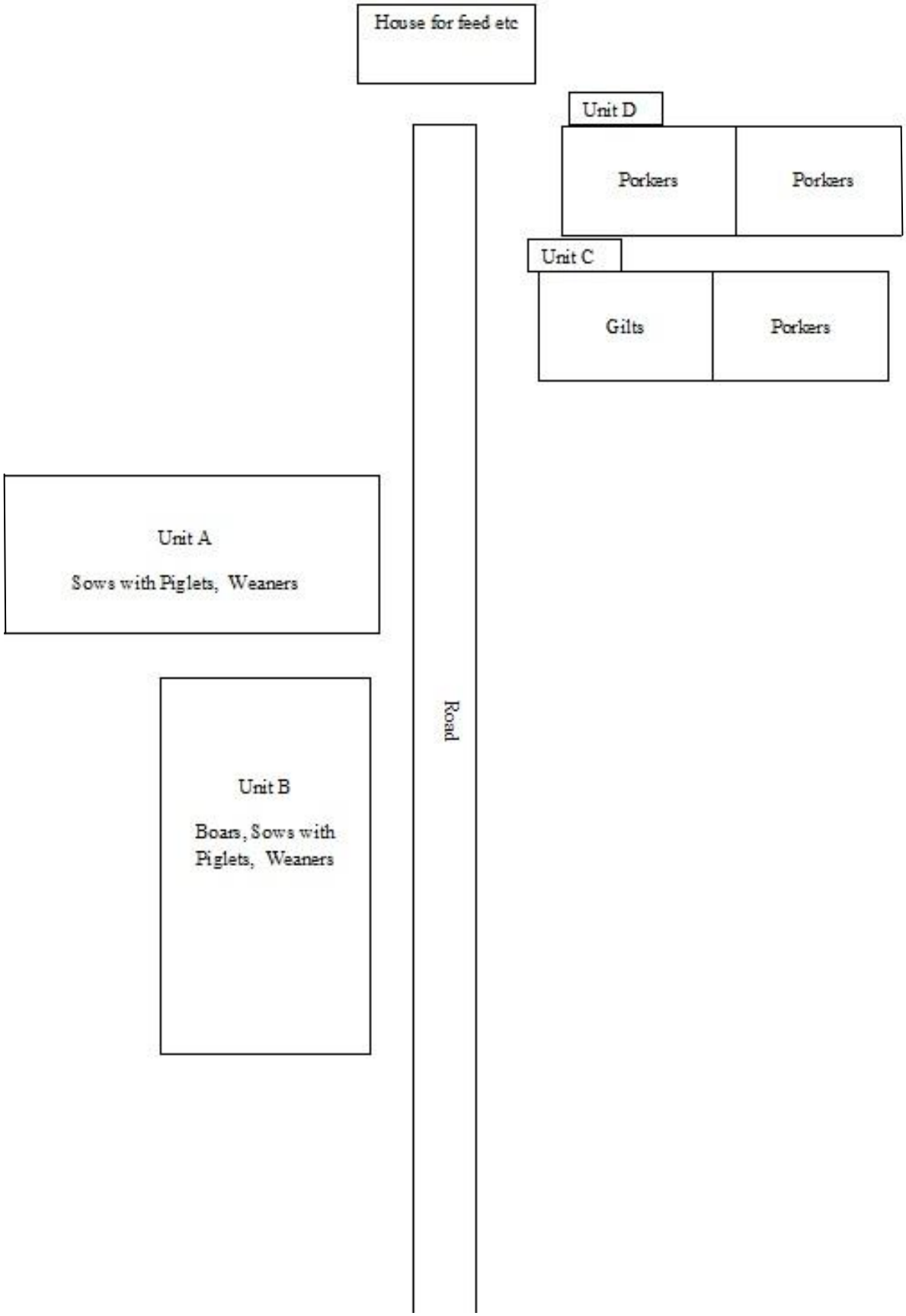
World Organisation of animal health, OIE, African Swine Fever updated 22/04/2002 (online) Available: http://www.oie.int/eng/maladies/fiches/a_a120.htm#5 (2010-12-04)

World organisation of animal health, OIE, Classical Swine Fever (online) Available: http://www.oie.int/eng/maladies/fiches/a_a130.htm (2010-12-04)

World organisation of animal health, OIE, PRRS (online) Available: http://www.oie.int/esp/normes/mmanual/a_00099.htm (2010-12-04)

APPENDICES

Appendix 1



Appendix 2

Naming of pens sampled and numbers of pigs in each pen that were sampled 23/10-10. Arrows indicate sewer draining.