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Emerging Infectious Diseases: a model of disease transmission dynamics at the wildlife-livestock interface in Uganda

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Sammanfattning

Nya infektiösa sjukdomar är ett återkommande hot mot hälsan hos både djur och människor. Förståelse för hur nya sjukdomar uppkommer är viktigt för att bättre förutse källor till nya utbrott. Listan på bidragande faktorer är lång, en blandning av antropogeniska effekter, miljöförändringar, molekylära och klimatiska förändringar är alla involverade i uppkomsten och spridningen av dessa sjukdomar. Två av de underliggande faktorerna får särskild uppmärksamhet i den här studien. Spridning av sjukdomar från vilda djur till tamdjur och zoonoser som sprids från djur till människa är centrala fenomen i uppkomsten av nya infektiösa sjukdomar bland både tamdjur och människor. Den här studien analyserar hur afrikansk svinpest sprids från vilda till tamsvin, och agerar därmed som modell för hur nya sjukdomar uppkommer. Fältarbetet sker i norra delarna av Uganda, ett land som är en ’hot spot’ för nya virussjukdomar.

Afrikansk svinpest spreds nyligen till Georgien, Ryssland och Ukraina, och har därmed hamnat i fokus som ett hot mot djurhälsan i hela Europa. Det är en mycket smittsam hemorrhagisk virussjukdom som tillhör familjen *Asfaviridae*. Sjukdomen kan smitta en rad olika svindjur, däribland vårtsvin (*Phacochoerus aethiopicus* och *Phacochoerus africanus*), busksvin (*Potamochoerus larvatus)*, skogssvin (*Hylochoerus meinertzhageni)*, samt tamsvin och vildsvin (båda *Sus scrofa)*. Mjuka fästingar av arten *Ornithodoros* agerar som vektorer för sjukdomen och sprider den till svinddjur i en sylvatisk cykel. Blodprover togs från 91 tamsvin, och fästingar samlades in från 6 vårtsvinsgryt i Murchinson Falls national park. Dessa analyserades med real-time PCR för att hitta ASF virus. Alla blodprover från fästingar var PCR-negativa för ASF. Den här studien fann ett tamsvin som var infekterat med afrikansk svinpest i PCR analys, men den var negativ för antikroppar i ELISA. Detta är en intressant fynd, eftersom det möjliggör spridning från en asymptomatisk bärare till andra tamsvin, och kan vara en källa till nya utbrott. Sekvensering och nucleotide Blast analys fann att viruset var 100% identiskt med genotyp IX som tidigare har hittats i Uganda.

Abstract

Emerging infectious diseases are a recurring threat to both human and animal health. Understanding the multiple causes behind the emergence of new diseases is key to the prevention of new and potentially devastating outbreaks. The list of underlying causes is long, including a variety of anthropogenic, environmental, molecular and climatic changes that promote the emergence and spread of disease. Two of these factors are central to the emergence of new diseases and receive special attention in this study. The spread of disease from wildlife to livestock and diseases that spread from animals to humans (zoonoses) are of importance as they implicated in the majority of EID events. This study aims to analyse how one emerging infectious disease (ASF) spreads between different wild and domestic hosts, and thereby provide a model for how disease emergence may occur. The field study is located in the northern parts of Uganda, a hotspot new and emerging viral diseases.

The recent spread of African swine fever to parts of Georgia, Russia and most recently to the Ukraine, has put this disease in the spotlight as a new and emerging threat to animal health in all of Europe. African swine fever is a highly contagious viral hemorrhagic disease of pigs, the causal agent of which is a double stranded DNA virus belonging to the family *Asfaviridae*. The disease is capable of infecting a number of suids, including warthogs (*Phacochoerus aethiopicus* and *Phacochoerus africanus*), bushpigs (*Potamochoerus larvatus)*, the giant forest hog (*Hylochoerus meinertzhageni)*, as well as the European wild boar and domestic pigs (both *Sus scrofa)*. Soft ticks (genus *Ornithodoros*) act as vectors for the disease, spreading it to suid hosts in a sylvatic cycle. 91 blood samples were collected from domestic pigs. Engorged ticks were collected from 6 warthog dens. These were analysed using real-time PCR for the presence of ASFV. All blood samples extracted from ticks were PCR negative for the virus. This study found one domestic pig that was PCR positive for ASFV, but negative for ASFV antibodies when run on an ELISA. This is an interesting find as it suggests the presence of asymptomatic carriers among domestic pigs, a possible source of new outbreaks. Sequencing of the viral DNA and nucleotide Blast analysis found the virus to be 100% identical to genotype IX which has previously been found to circulate in Uganda.

Introduction

Infectious diseases are caused by pathogenic microorganisms such as viruses, bacteria or parasites, which are capable of being transmitted from one individual to another. Non-infectious diseases comprise all other diseases, such as cancer, auto-immune disease, or genetic disease. An emerging infectious disease (EID) is a disease that has recently been discovered (for example HIV, SARS), has crossed the species barrier (Ebola, Nipah virus), or is a well-known disease that has recently increased in incidence or geographical range (West Nile virus, African swine fever) (Morse 1995, Daszak et. al 2001). There are also *re-emerging* infectious diseases, such as antibiotic-resistant strains of tuberculosis, or chloroquine-resistant malaria, that have existed for a long time but recently evolved to become more virulent and less treatable with current medicines.

For most EIDs there are no effective therapies, vaccines, or preventative strategies to combat the disease. In addition, there is a poor understanding of the factors underlying their emergence (Wolfe, 2005). In order to better predict when and where a new EID event will occur, research is required to understand the driving mechanisms behind disease emergence. This study uses the interface between bushpigs, warthogs, ticks and domestic pigs in northern Uganda to analyse the complex interactions between human behaviour, agricultural practices, ecology and viral epidemiology to determine the causes of disease emergence.

Factors in the emergence of infectious disease

In recent years much attention has been paid to EIDs that have the potential to cause global pandemics. These include the SARS outbreak in 2002, the H5N1 “bird flu” epidemic, the “swine flu” pandemic in 2009, and most recently the West Nile virus epidemic in the United States. EIDs that cause panzootics in animals or wildlife tend to be less publicised but are of equal importance. They impact animal health, have economic repercussions, and affect biodiversity through the endangerment and extinction of species (Smith et. al, 2006). The outbreak of foot-and-mouth disease in the United Kingdom in 2001 is a good example. Although not directly harmful to human health, it led to the culling of millions of head of cattle, sheep and goats, and cost the British economy several billion pounds in agriculture and lost tourism (Barclay, 2001). EIDs have also been responsible for 4% of all species extinctions and 8% of species endangerment (Smith et. al, 2006). This is well exemplified by the endangered African wild dog, currently under threat from rabies and canine distemper virus in east Africa (Cleaveland et. al, 2000).

The trend in infectious diseases shows a steady increase from the 1940’s to the present day (Jones et. al, 2008). There was a peak incidence in the 1980’s that coincided with the start of the global HIV pandemic. The estimated rise in EID events during recent decades is not the result of increased reporting due to better surveillance, as the trend is increasing even when reporting bias has been accounted for (Jones et. al, 2008).

Research into the mechanisms driving disease emergence has highlighted several contributing factors. As would be expected, the underlying causes are numerous and complex, reflecting the variety of individual EID cases. One aspect they all appear to have in common is that

anthropogenic change is usually involved. Many EIDs have their origins in wildlife, from where they spread to domestic animals and on to humans. Examples include Ebola virus, which emerged in Uganda in the 1930's from a suspected bat reservoir in the wild. Factors contributing to EIDs from wildlife can be traced to habitat fragmentation, human encroachment on wildlife reservoirs, increased population density of wildlife, pathogens, and host, as well as land clearance and changes in agricultural practices. Each of these contributes to creating an interface over which infection with new diseases may occur (Morse, 1995).

In other cases, it may be the susceptibility to disease in the recipient that allows pathogens to spread beyond their usual range. Factors such as breakdown of public health, poverty, war and famine can contribute to creating conditions within the human population that allows pathogens to thrive. For example, the peak in EIDs in the 1980's coincided with the HIV pandemic, as the large proportion of immunosuppressed individuals worldwide allowed other diseases, such as tuberculosis, to increase in prevalence (Jones et. al, 2008). Other factors may facilitate the spread of disease on a local and global scale, once it has become established in a population. An example is the SARS outbreak in Hong Kong in 2003, which was close to becoming a global pandemic as international travel allowed the disease to spread to more than two dozen countries worldwide (CDC, 2012). Factors such as increased population density, urbanization, globalisation, modern transport and tourism, and local and global trade all allow an EID to spread at a faster pace than ever before. Climate change may also play role in increasing the spread of a disease, although the effects of changing temperature and rainfall is difficult to predict. It is possible that increased temperature will spread vectors such as ticks and mosquitoes over a larger geographical range, thereby contributing to the spread of vector-borne pathogens (Morse, 1995).

The case of re-emerging diseases is directly the result of human behaviour, as the unrestricted use of medicines and antibiotics has lead to microbial adaptation to resist treatment (Davis et. al, 2001). Of all the factors involved in driving disease emergence, the most important appears to be anthropogenically induced change. In this light, EIDs can be seen as an unwanted side-effect of human economic development (Jones et. al, 2008).

Zoonotic EIDs

Zoonoses are diseases that can be transmitted from animals to humans and vice versa. Of all the diseases that affect humans, around half are zoonotic and have historically originated in animals (868 pathogenic agents, 61% of the total) (Taylor et. al, 2001). Zoonoses also make up a majority of EIDs, as two thirds of all EIDs can be traced to an animal origin. These include SARS, HIV, Nipah virus, Ebola, and Hantaviruses (Taylor et. al, 2001). Evidence suggests that viral transfer from animal to human is fairly common, but onward transmission from human to human is relatively rare. This is evident in the bushmeat trade, where hunters in Cameroon are regularly infected with simian foamy viruses, without further human-to-human transmission occurring (Wolfe, 2004). The very close interface with wild animals provides a platform where the disease can be transmitted to humans, but as the viruses do not yet have the means for onward transmission and therefore no outbreak occurs. Several viruses, including avian influenza and hendra viruses, appear to follow a similar pattern, transmitting to humans sporadically without further human-to-human transmission occurring. This process has been

called “viral chatter”, a mechanism that may be fundamental to viral EIDs (Wolfe, 2005). High rates of viral chatter increases the pathogens diversity and variation, and thereby increases the probability that one version of the virus will finally be able to replicate in humans. Monkeypox and Nipah viruses have reached the second stage, acquiring a minor ability to spread between humans, with small epidemics of limited human-to-human transmission (Wolfe, 2005).

Of the viruses that do manage to transmit between humans, most tend to do so at rates that are too low to allow the pathogen to become permanently established in the human population. Research into DNA sequences of HIV suggests that as many as 10 transmission events into the human population occurred before the virus attained rates of transmission that allowed it to become permanently established (Hahn et. al, 2000). On a molecular level, there are numerous barriers hindering animal diseases from infecting a human host. In order for a pathogen to cross the species barrier, it must first enter the host cell, replicate with the assistance of the foreign cell, evade the host immune response, exit the cell and then be able to infect other organisms. For each of these steps, a mutation is required in the pathogen to allow it to adapt to a foreign host. Most zoonotic viruses are RNA viruses, as these have a greater tendency to mutate during the replication process through point mutations, genetic drift or antigenic shift. DNA viruses, such as African swine fever, have better proof-reading mechanisms that remove unwanted mutations and therefore are less likely to mutate. Of all the RNA viruses that have achieved the feat of infecting humans, only HIV, H3N2 and H1N1 influenza A viruses achieved human-to-human transmission and are now permanently circulating in the human population (Webby et. al, 2004).

EIDs from Wildlife

The majority (71.8%) of zoonotic EIDs have an origin in wildlife (Jones et. al, 2008). Research shows that wildlife diversity and species richness is a determining factor for the appearance of zoonotic EIDs (Jones et. al. 2008, Wolfe 2005). Species diversity and richness can be plotted along a gradient that increases towards the equator. It is as yet unclear whether it is the diversity of pathogens or their wildlife hosts that is the driving force in the appearance of wildlife EIDs; perhaps it is a combination of the two. As species richness is a key in the designation of EID “hot-spots”, there should be an increase in surveillance and preparedness for EID events in lower latitudes (fig. 1). However, current research shows that EID research and surveillance is focused on higher latitudes, where EIDs are least likely to emerge (Jones et. al, 2008).

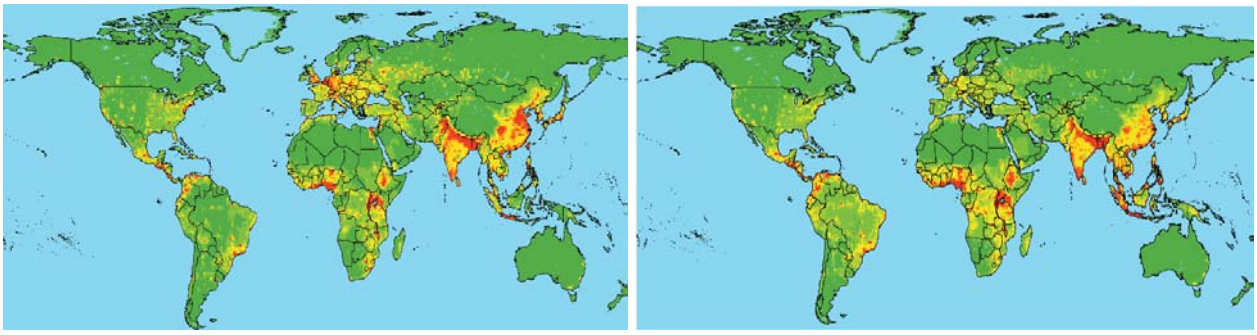

Figure 1. Global distribution of the relative risk of a zoonotic EID event from wildlife (left) and vector borne pathogens (right) (Jones et. al, 2008, image reprinted with the consent of *Nature Publishing Group*).

Along with species diversity, anthropogenic factors can contribute to diseases spreading from wildlife and causing outbreaks in domestic animals and humans. Nipah and Hendra viruses are two paramyxoviruses that followed this path. They emerged from pteropid fruit bat reservoirs in Malaysia in 1999 and Australia in 1994. The spread to humans occurred after amplification in domestic animal hosts, Nipah virus first spread to domestic pigs, and Hendra virus spread to horses. The emergence of Nipah virus can be explained through deforestation and agricultural expansion. The growth of intensive pig farming and the practice of having fruit orchards surrounding pig farms brought bats urine, faeces and partly eaten fruit in contact with domestic pigs (Daszak et. al, 2006). Nipah-virus infected bats have always been present in Malaysia, but the human-induced change in their environment caused it to emerge in domestic animals and thereafter spreading to humans. The outbreak led to the culling of 1 million pigs in Malaysia. To avoid future outbreaks, increase biosecurity on pig farms and controls over trade in live pigs is required, along with increased surveillance in high-risk areas, and buffer zones around farms.

Hendra virus followed a similar path, spreading from pteropid fruit bats to horses and then humans. It is believed that land clearance of lowland eucalyptus woodland brought fruit bats to fruiting trees in suburban gardens. The increased bat population density in these areas made contact between bat urine and horses more likely (Daszak et. al, 2006) SARS coronavirus had a similar human-wildlife interface. The outbreak in 2002 was traced to the farming of wild palm civets, and selling and live, subclinically infected civets at local markets. This brought humans and civets into close contact, facilitating disease transmission. Phylogenetic analysis confirmed that the virus originated in bats, then spread to humans via palm civets as an amplifier host. The outbreak lead to 10,000 palm civets being destroyed in Guangdong province, China.

Another wildlife-human interface that has lead to the emergence of disease is the bushmeat trade in Africa. The bushmeat trade has been responsible for the emergence of Ebola, HIV-1 and -2, and possibly anthrax and simian foamy viruses (Wolfe, 2005). The butchering, transportation, sale, purchase, and eating of wild animals creates ideal conditions for pathogenic transfer. There is a high demand for bushmeat in west and central Africa, an estimated 300 grams of bushmeat per person per day is consumed in the Congo basin. This amounts to 4.5 million tons of bushmeat being taken out of the forest annually (Wolfe, 2005). In Uganda, and specifically in the study area for this project, there is anecdotal evidence that illegal hunting takes place in Murchinson falls national park. Studies have also shown that bushmeat hunting occurs in the southern parts of Murchinson falls national park, and although the amount of poaching taking place is relatively low, both bushpigs and warthogs are included in the species hunted for food (Oluput, 2009). This creates the conditions necessary for the spread of African swine fever from the confines of the reserve to domesticated pigs living in the periphery. In the areas surrounding the park bushpigs may also be hunted legally, and taken to local communities to be sold or eaten. Thereby interface is created between wildlife and domestic animals as the latter come into contact with meat, skins or offal from carcasses infected with the virus. As meat products remain infective for several months after slaughter, there is a high risk of infection at this potential interface.

Human demographics may put pressure on the nature reserves and bring people into contact with wildlife. A recent study on the effect of migration on protected areas in Uganda, found

that 72% of those who engage in hunting in protected areas (in this case Budongo Forest Reserve, Masindi district, Uganda) were regional migrants. Results of the study also showed that regional migrants were more likely to be associated with deforestation than local inhabitants (Zommers et. al, 2011). The conflict that has affected northern Uganda between 1987 to 2006 has displaced up to two million people (WHO, 2005). As these people have begun to return home there is a risk that lack of jobs will induce people to turn to hunting and deforestation to earn a living, which could exacerbate the problem. Examples of this in past include arboviruses such as dengue and yellow fever, as well as cutaneous leishmaniasis, that have all emerged as a direct result of encroachment on wildlife habitats (Wolfe, 2005).

African swine fever

African swine fever is an EID that has its origin in wildlife. It is a highly contagious disease among domestic pigs, and has a very high mortality rate (approaching 100%) (OIE, 2009). It is endemic in much of Sub-Saharan Africa, Russia and Sardinia, with sporadic outbreaks occurring in the rest of the world (fig. 2).

African swine fever is caused by a double-stranded DNA virus that is the only member of the *Asfarviridae* family. The virus infects warthogs (*Phacochoerus aethiopicus* and *Phacochoerus africanus*), bushpigs (*Potamochoerus larvatus)*, wild boar (*Sus scrofa)*, the giant forest hog (*Hylochoerus meinertzhageni)*, domestic pigs, and soft ticks (genus *Ornithodoros*). The ticks act as vectors, spreading the disease within and between different species in a sylvatic cycle.

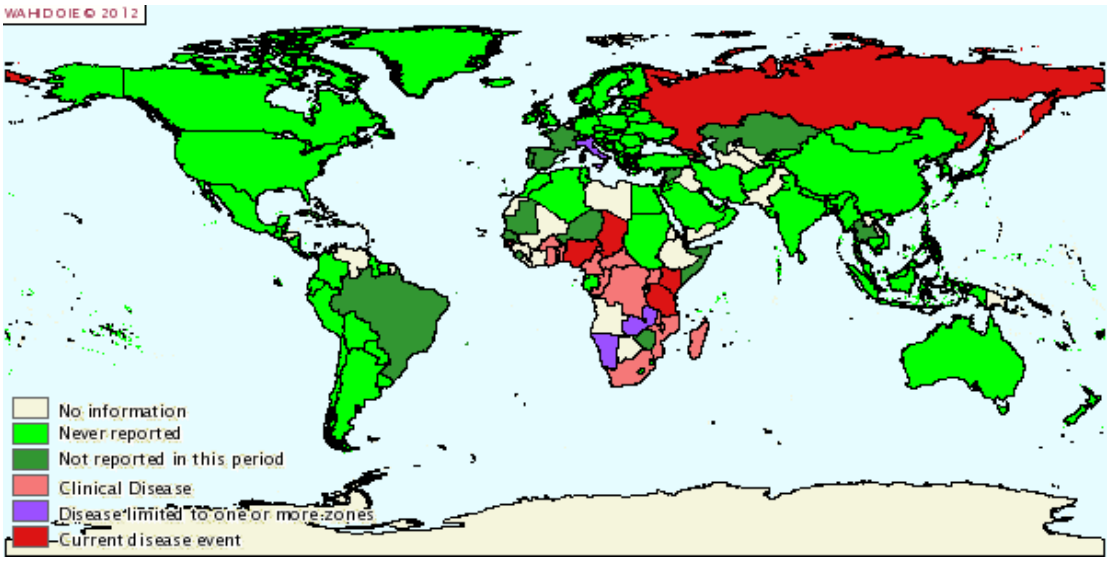

Figure 2. World distribution map of African swine fever 2010 (OIE WAHID).

When infected with the most virulent form of the virus, domestic pigs can rapidly deteriorate with death occurring within two weeks of contracting the disease (OIE, 2009). It is one of the most serious diseases of domestic pigs, with very high mortality rates and potential for international spread, which puts it on the list of notifiable diseases by the OIE. Symptoms in infected pigs include high fever (40-42˚C), anorexia, cyanotic skin on ears and extremities, bleeding from bodily orifices and eventually coma and death. The route of viral infection is usually via the tonsils or the dorsal pharyngeal mucosa to the mandibular or retropharyngeal lymph nodes, thereafter the virus spreads to other body organs via a viraemia. A primary

viraemia occurs within a few hours of infection, followed by a second viraemia within 24 hours. The liver, spleen, lungs and lymph nodes are the main sites of secondary viral infection, but all tissues of the body normally become infected within a few days. The haemorrhage and disseminated intravascular coagulation (DIC) that lead to death and typical post-mortem findings is the result of viral replication in endothelial cells of the capillaries. The virus also replicates in monocytes and macrophages, thereby affecting the pig's immune system and impairs the coagulation of blood through release of cytokines and complement factors (Penrith et al, 2004).

A subacute, chronic form of the virus exists in endemic areas, and with less virulent strains of the virus. The subacute form has lower mortality rates, with symptoms of undulating fever and sporadic abortions. Pigs that survive can develop a chronic subclinical infection, with the potential of infecting others (OIE, 2009). Pathogenesis of the disease and pig immune responses are still poorly understood. Antibodies do not protect against infection, but can confer some degree of protection against identical viral strains in individuals that have survived the original infection (Sánchez-Vizcaíno et. al, 2009).

Warthogs and bushpigs do not show any signs of clinical disease when infected, and mortalities are rare (Bastos et. al, 2009). Warthogs constitute the main mammalian reservoir of the disease in the wild, consisting of asymptomatic carriers among young warthogs, who spread the disease onwards via the ticks that feed on them.

Wild boar in Europe and parts of Africa can also become infected. They show clinical symptoms identical with those of domestic pigs (severe, unspecific clinical signs with fever, depression, anorexia, dyspnea, ataxia), and succumb to the disease with similar mortality rates (100%)(Blome et. al, 2012). This is especially true with the highly virulent strains circulating in the Caucasus, where studies find no chronic ASF carriers among the wild boar population (Blome et. al, 2012). Wild boar are likely to become infected through spill-over from domestic pigs, exposure to pig carcasses or contact with fomites, or consumption of contaminated feed.

The spread of ASF

ASF first appeared in Kenya in the 1920's as domestic pigs were brought to the country by foreign explorers. It was identified as a virus originating in subclinically infected warthogs in 1921, when it was noted that pigs became infected when they came in close contact with wildlife (Costard et. al, 2009). The disease spread through domestic pig populations in Central and West Africa and reached Madagascar in 1998 and Mauritius in 2007.

ASF first spread outside Africa in 1957 when it appeared in Portugal. A further outbreak appeared in Portugal in 1960, and although efforts were made to eradicate the disease, it remained endemic on the Iberian peninsula until the late 1990's. It was found that European ticks of the *Ornithodoros* genus were capable of acting as vectors for the disease, thus complicating efforts at eradication. After its introduction to the European continent in 1957, the disease appeared in numerous European countries in the following years. Cases appeared in Malta, Italy, France, Belgium and the Netherlands during the 1970's and 80's (Costard et. al, 2009). Europe is now free from ASF following a concerted eradication program, but it is still endemic in Sardinia. Spread of ASF has also occurred beyond the continent of Europe. In

1970's, outbreaks appeared in Cuba, Dominican Republic, Haiti, and Brazil (Costard et. al, 2009). Worldwide eradication efforts have largely been successful in countries where ASF outbreaks have occurred. This has been achieved through effective surveillance measures and stamping out programs to contain the disease where outbreaks occurred.

The most recent outbreak in Europe occurred in 2007 when cases were found in Georgia, which eventually spread to neighbouring countries including Armenia, Azerbaijan, Russia and the Caucasus (Beltrán-Alcrudo et. al, 2008)(fig. 3). ASF is now endemic in Russia, and 300,000 pigs have been culled as a result of ASF outbreaks at a cost of an estimated $240 million (FAO, 2012). In July of 2012 the virus spread to the Ukraine, probably from imported meat products from neighbouring Russia. The first case in the Ukraine was 170km from the Russian border, increasing the likelihood that the virus spread via live infected pigs or contaminated goods that were transported into the country, and not by direct contact between swine or wild boar (Roberts, 2012). The arrival of the disease to the Ukraine has increased the risk to neighbouring countries such as Moldova, Belarus, Romania and the Baltic states to the west. To the east, cases have appeared along the border with Kazakhstan, a country that shares a long border with China. Should the disease spread eastward, it could threaten China's 1 billion strong pig population. China is already at risk from its growing trade with African nations, and the virus may spread directly to the country via this route (Callaway, 2012).

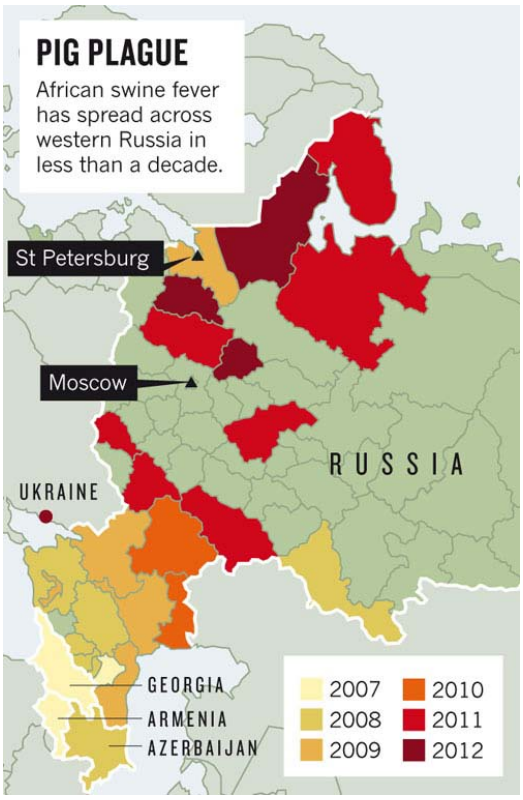

Figure 3. The recent outbreak and spread of African Swine Fever across Russia and Eastern Europe (Callaway, 2012, image reprinted with the consent of *Nature Publishing Group*)

Contingency plans have been put in place to hinder the spread of the disease in Eastern Europe and the European Union. This includes banning all imports of live pigs and pork products to the EU from affected countries. In 2011, a real-time simulation exercise on African swine fever

took place jointly with the Nordic and Baltic countries including Denmark, Estonia, Finland, Iceland, Latvia, Lithuania, Norway and Sweden (OIE, 2011). In 2012, a further simulation exercise took place in Denmark, Spain, the Czech Republic, Belarus and Croatia to plan for an eventual outbreak.

Studies by the FAO in Russia indicate that swill feeding is responsible for 97% of all new infections in domestic pigs. It tends to be small-holder farms with few or non-existent biosecurity measures that are typically infected first before subsequently passing the virus on to small commercial farms and eventually industrial farms (FAO, 2012). Upholding bans on swill-feeding to pigs (which is already banned in the EU), could prevent disease transmission in Russia and Eastern Europe, but may be difficult to enforce to poor small-holder farmers. The main risk factors in the spread of ASF include high proportion of backyard pig farms, wild boar contact, illegal trade, and the practice of swill feeding. The EU member states mainly have high biosecurity commercial pig farms, bans on swill feeding as well as import restrictions and contingency plans, all of which will hopefully safeguard against a spread to the EU.

Epidemiology and disease transmission

Warthogs

The main reservoir of ASF in the wild is the warthog (*Phacochoerus africanus*). Warthogs live over a wide range in most of Sub-Saharan Africa, and are more widespread and numerous than bushpigs, who also carry the disease. The transmission of ASF between warthogs follows a sylvatic cycle where the ticks of the genus *O. moubata* act as a vector. Young warthogs become infected in their burrows when ticks feed on them. After a short period of viraemia lasting two or three weeks, the warthogs recover and show no further clinical symptoms (Costard et. al, 2009). Animals remain infected for life, with low levels of virus latent in the lymph nodes of the head. Adults do not tend to contract the disease from either ticks or direct contact, and experimentally transmission through horizontal or vertical contact between individuals has not been found. For the disease to spread from warthog to domestic pig, there must therefore be a tick vector acting as a go-between. The disease can spread to domestic pigs if they come into contact with the vector either by frequenting warthog burrows, or from ticks being carried to areas inhabited by domestic pigs (Bastos et. al, 2009).

Warthogs show a seroprevalence that is consistently around 80% in areas where tick vectors are present (Plowright et. al, 1994). There can be a significant variation in prevalence of ASF antibodies between different regions, which is largely unexplained. For example, in a study the seroprevalence of ASF among warthogs in the Serengeti was found to be 100%, whereas the seroprevalence in the neighbouring Magadi region was only 50% (Heuschele and Coggins 1969). In South Africa even greater variation has been found, with a difference between a seroprevalence of 90% and 4% in very close geographical locations (Penrith et. al, 2004). The presence of antibodies in adult warthogs is the result of infection as a juvenile, as antibodies persist through the entire life of the warthog. The transmission dynamics are complicated and incompletely understood, as high seroprevalences have been found in areas of Kenya where no Argasid ticks are found.

Ticks

The soft ticks (*Argasidae*) that are responsible for spreading ASF have a different life cycle from hard ticks (*Ixodidae*) in that they inhabit burrows, pig sties or other areas that are frequently visited by their mammalian hosts, but are restricted to one location. This is in contrast with hard ticks, that are widely distributed in the environment. As soft ticks have a limited distance over which they travel, the range over which they may spread disease is likewise limited. Yet if carried on the bodies of their warthog hosts, the ticks may be transported from wild to domestic areas and thereby initiate outbreaks in domestic pigs (Bastos et. al, 2009). This is dependent on co-habitation with warthogs in areas inhabited by people and their animals.

Infected ticks play an important role in the long-term maintenance of disease in a geographical area. They may survive in a burrow for several months and up to several years after feeding on an infected host (Oleaga-Pérez et. al, 1990). The virus may even be transmitted through transstadial and sexual transmission between ticks (Endris et. al, 1994). This allows the virus to persist for a long period of time even without a mammalian host on which to feed. For these reasons, it is necessary to eradicate all disease-carrying ticks if in an endemically infected area is to become disease-free. Efforts to achieve tick control on a large scale have been fruitless, due to their long life, resistance to fasting, the possibility of alternative hosts to pigs, and their ability to hide in fissures in buildings to avoid insecticides. This has lead to a great difficulty in eradicating ASF from endemic areas where a sylvatic cycle is present (Sánchez-Vizcaíno et. al, 2009). An alternative has been proposed through inoculating pigs with avermectins to protect against ticks, but as yet no vaccine against ticks exists.

Bushpigs

Bushpigs may also infect soft ticks, but unlike warthogs, are even capable of infecting domestic pigs through direct contact (Bastos et. al, 2009). After infection, bushpigs remain viraemic for a period of 35 to 91 days, during which they are capable of spreading the disease to the tick vectors (Anderson et. al, 1998). The virus also persists in lymphatic tissue for up to 34 weeks, during which time they may infect pigs through direct contact (Anderson et. al, 1998). Similar studies have shown that reverse infection of bushpigs from domestic pigs does not occur. As bushpigs live predominantly nocturnal lives, they are able to frequent agricultural areas and are therefore more likely than warthogs to come into contact with domestic pigs. The frequency of infection in bushpigs may be lower than warthogs however, as they do not live in burrows and therefore are less likely to come into contact with disease-carrying ticks. This may reduce the frequency with which bushpigs become infected, and thereby reduce their importance as a wildlife reservoir (Anderson et. al, 1998). However, outbreaks of ASF have occurred in areas of Malawi were warthogs and soft ticks are absent, thereby implicating bushpigs in the spread of the disease to domestic pigs.

A suspected hybridization between bushpigs and domestic pigs has been reported, with sightings of male bushpigs mounting free-ranging female pigs (Vercammen et al. 1993). This may add to the complexity of disease transmission dynamics, as the susceptibility of these hybrids to infection and their roles as disease carriers are yet to be elucidated. As bushpigs are

a known to be asymptomatic carries, potential hybrids could become asymptomatic carrier pigs that spread the disease to other individuals.

Giant forest hog and wild boar

There is little data about infection with ASF in the giant forest hog, but it is most likely a rarity and has a negligible effect on the spread of the disease (Penrith et al., 2004). Since the distribution of this species is restricted to areas of dense forest, contact with domestic pigs is unlikely.

Wild boar are susceptible to ASF and can become infected through direct contact or ingestion of infected meat products (Costard et. al, 2009). They exhibit similar clinical signs and have very high mortality levels, on a similar scale to domestic pigs. This probably limits their role as disease carriers, as the animals die before they have a chance to spread the disease onwards. Wild boar are also sedentary animals that do not migrate over large distances. This also limits the risk of cross-boundary spread. In areas where large wild boar populations have been present during ASF outbreaks, such as in Spain and Sardinia, only low seroprevalence in wild boar populations have been found. Their roles as reservoirs in the wild depend on re-infection from infected domestic pigs, without which the disease in wild boar will eventually die out (Mur et. al, 2012). This suggests that wild boar may only be of limited importance in maintaining the disease in the long-term (Mur et. al. 2012, Blome et. al. 2012), which should limit their role in the current outbreak in Russia and Eastern Europe.

Domestic pigs

ASF can spread in an independent cycle within pig populations, without coming into contact with ticks or wild suids. Domestic pigs can transmit the disease through direct contact with other pigs for up to one month after infection (Costard et. al, 2009). Transmission via direct contact is the most common form of transmission in much of West Africa, where the sylvatic cycle has not been demonstrated (Costard et. al, 2009). Even in endemic areas, such as Uganda, there is evidence that the most common source of infection is from other domestic pigs and not directly from wildlife. The initial source of an outbreak in an area otherwise free of ASF will most usually originate from wildlife, and the subsequent spread within the domestic pig population will be between infected individuals. However, studies show that the overall seroprevalence of the disease in periods between outbreaks is low. In a study in Tanzania, the seroprevalence in the domestic pig population was only 7% (Swai et al., 2004).

In countries such as Senegal, where the role of ticks in disease transmission to domestic pigs is limited (Vial et al., 2007), it is likely that trade in animals and animal products, or chronically infected pigs are main sources of new outbreaks. Pork products are often implicated, as the virus can persist in carcasses for several months. Another line of infection may be through pigs feeding on remnants of warthog or bushpig carcasses, as the trade in bushmeat brings meat from wild animals to inhabited areas. However, there exists no experimental data to support that pigs can become infected by ingestion of warthog tissues (Penrith et. al, 2004). As infected bushpigs have higher levels of circulating virus, it is more likely that these could spread the disease onwards if consumed. Another hypothesis is that pigs may consume infected ticks on the bodies of warthogs, and thereby ingest enough viral particles to become infected.

ASF in Uganda

African swine fever is endemic in Uganda, with regular outbreaks occurring in different parts of the country every year. Pig production in Uganda is predominantly conducted by rural small-holder farmers, who own on average five pigs per farm. Death rates amongst pigs are high, with between 2 and 12 pigs dying per household in 2002 (Doble, 2007). Of these the chief cause of death was found to be African swine fever followed by accidents and other diseases. Large scale commercial farming exists in some parts of Uganda, but was not the focus of this study. In a 2010 census, there were 2.3 million pigs in the country and the population is steadily growing, representing an important source of income for a large proportion of rural people (FAO, 2010).

In most cases the pigs on smallholder farms are kept free-ranging or tethered to trees. In some cases the pigs are permanently kept in stalls, or kept in stalls overnight and free-ranging during the day. It is the extensive nature of pig farming in Uganda that allows pigs to come into contact with wildlife and thereby contract ASF. The absence of any biosecurity measures on the farms, or proper control programs once a disease outbreak has occurred, is a continuing problem and allows the disease to spread uncontrollably once an outbreak has begun. The loss of one or all of a farmer's pigs has serious implications to a farmer, as they are commonly poor and live on subsistence farming.

It is common practice that once an outbreak occurs, farmers try to sell or slaughter their sick animals. This increases the likelihood that the disease will spread further, as both live animals and meat products brought to market are contagious. Knowledge about the disease and the routes of infection are lacking, and better education programs at the local level are required. For example, a recent survey of 181 pig farmers in Kampala found that 29% of farmers vaccinated their pigs. Of these 25% did not know what disease they vaccinated them against and the remaining 75% believed they were vaccinating against ASF (Doble, 2007). This despite the fact that there is no current vaccine available against ASF. The same study found that the most common course of action if a pig contracted ASF was to send it to the butcher for human consumption. Other measures included injecting it with oxytetracycline (an anti-parasitic that has no effect on the disease), feeding it plants, local herbs, fish or tobacco, or if possible selling it to another farmer. The least common course of action was to slaughter the animal and disposing of the carcass in a sanitary way (Doble, 2007).

Control and Prevention of ASF

To combat ASF control and surveillance policies need to act on the locally where outbreaks occur, at the regional level where the disease is endemic, and internationally through regulations in trade of animals and animal products. The World Organization for Animal Health (OIE) has set out international standards and regulations for the trade in livestock. Given the ability for the disease to persist for long period in animal by-products, strict regulations in trade in pork products need to be followed. Many of the past outbreaks of the disease (Portugal 1957, Georgia 2007) have been traced to international movement and careless handling of pig meat.

Risk assessment is required for all countries currently free of the virus, to establish which pathways are high-risk for bringing the disease into the country. This facilitates more targeted control and surveillance strategies at the national level. Once spread of ASF has been confirmed, the method of eradication is through stamping out of all pigs within the affected area and the establishment of a security zone. In endemic areas eradication is more difficult to achieve, as the disease has become rooted in a wildlife reservoir and within the domestic pig population via chronic carriers. In 2005, the European Commission set out an eradication plan for Sardinia to rid the island of the disease. The plan included increased surveillance, improved biosecurity measures, a strategy to contain the wild boar population, and stricter regulations against export of pig meat products (European Commission, 2005). The plan has had positive results, with a declining prevalence in both wild boar and domestic population. Yet total eradication has not been achieved. In 2012 a total of 37 outbreaks were confirmed in domestic pigs and 23 outbreaks among wild boar (Standing Committee, 2012). This is an increase from 30 outbreaks in 2011, which points to a failure of the current eradication plans.

In South Africa, successful control measures have been implemented in the form of ASF free zones. Transport of pigs, warthogs and animal products between infected and non-infected zones is controlled by permits. In infected areas, commercial pig production is only allowed in special enclosures behind double-fence barriers and other biosecurity as set out by the OIE (Costard et. al, 2009). The program appears to be successful, with very few outbreaks of ASF in commercial farms. Free-ranging pig production does occur, but is discouraged in endemic areas because of the high risk of spreading the disease. The success of the program has to be seen in light of the large financial cost it requires, making it the reserve of large-scale commercial farming and not a feasible option for small-holder subsistence farmers in Africa.

Objectives of this study

Investigating the presence of ASF in wildlife and domestic hosts in Uganda as well as comparing the genotypes of the virus will indicate whether ASF spreads between wildlife and domestic pigs. Research into the spatial distribution of the diseases will indicate whether the proximity to wildlife increases the incidence of virus transmission. A better understanding of the variety of human and animal interfaces and wildlife-livestock interactions also will give a better understanding of how to reduce the spread of the virus. Thereby the study of disease transmission dynamics between wild and domestic pigs will act as a model of emerging infectious diseases originating in wildlife populations.

In summary, the study aims to:

1. Gain insights into the epidemiology and transmission dynamics of viruses between wild and domestic animals, with the transmission of ASF between warthog, bushpig, ticks and domestic pigs acting as a model for this process.
2. Study the prevalence of ASF in domestic pigs in Gulu and Nwoya Districts in northern Uganda.
3. Analyse the husbandry techniques of smallholder farmers and how it affects susceptibility to disease.

The Study Area

The study area is within Gulu and Nwoya districts in northern Uganda (fig. 4). This area has traditionally suffered from poverty and underdevelopment, with a prevalence of HIV that is higher than the rest of the country (WHO 2005). The area has also been affected by the intermittent conflict between the Lord's Resistance Army (LRA) and Ugandan government forces that took place between 1987 and 2005. The region has only begun to be re-populated in the last 3 to 5 years, as internal refugees have begun returning to their homes. This brings people back into areas that had been reclaimed by wildlife during the years of war, and creates a new interface betweeen domestic and wild animals that may facilitate disease transmission.

Blood samples from domestic pigs were collected from homesteads along the border with Murchinson fall national park. The study location was chosen based on its proximity to the park as this presents a potential interface zone where free-ranging pigs can come into contact with warthogs, bushpigs and infected ticks. In addition to domestic pig samples, the intention was to collect blood samples from warthogs and bushpigs inside Murchinson falls national park. Due to problems with the seasonally high grass, no warthogs or bushpigs were captured. Ticks were collected from warthog burrows inside the park. GPS coordinates were registered for each of the sampling locations for domestic pigs, tick and bushpigs (fig. 4).

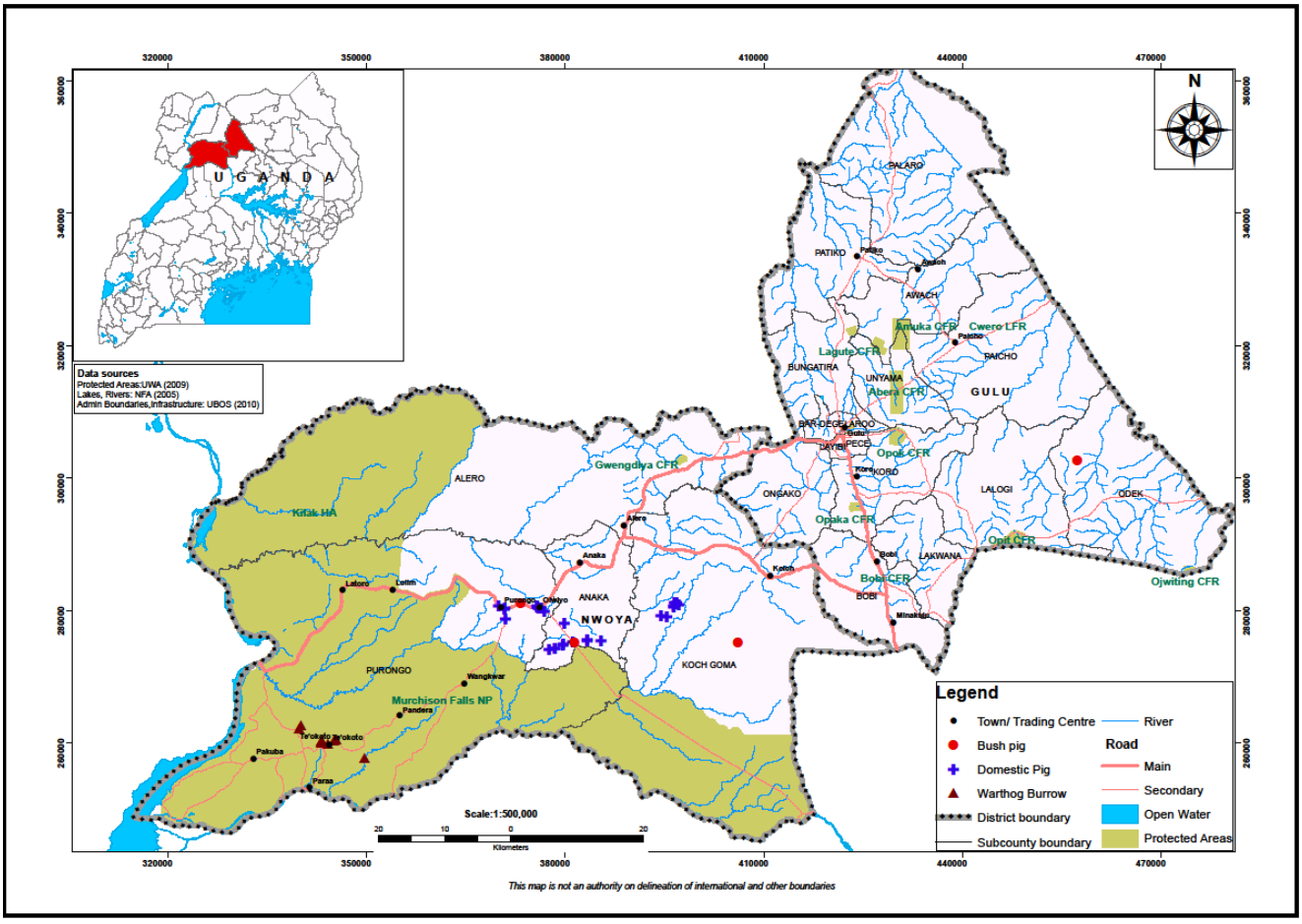

Figure 4. Map of Study Area: Gulu and Nwoya Districts. Marked are GPS-coordinates of each of the domestic pig, bushpig, and tick samples. The area marked in beige is Murchinson Falls NP.

METHOD AND MATERIALS

Interviews

At each of the homesteads visited, a short questionnaire was used to answer basic questions about the health status, feeding practices and husbandry of the pigs, as well as the presence of warthogs and bushpigs in the area. The farmers were also asked if there were any recent outbreaks of ASF in or near their village. The interviews were conducted with the aid of the district veterinary officer (DVO) (fig. 5).

Sampling domestic pigs

Blood samples were collected from domestic pigs in smallholder farms along the border with Murchinson Falls National Park (fig. 6). The farms were selected for their proximity to the park and a total of 30 homesteads were visited. An average of 3 pigs were sampled per location, with a variation from just one pig for the smaller farms up to 11 pigs for the larger farms. Samples were collected during a period of 7 days, with a total of 91 blood samples taken. In order to gain access to the farms we worked with the local DVO who introduced us to farmers willing to take part in the study. As a courtesy to the farmers taking part in the study, the pigs that were sampled were dewormed free of charge.

Blood was sampled from the jugular vein in serum and EDTA vacutainers. Sex, breed (local, mixed, improved) and an estimated age were taken for each of the sampled pigs. In addition, the pigs were given ear notches in order that they may be identified if one were to be found positive for ASF. The serum samples were allowed to settle, and the supernatant transferred to a collecting tube, after which it was transported to the Molecular Biology laboratory in Makerere University, Kampala. As recommended, the samples were stored at a temperature of -20°C while used for analysis in the lab, and transferred to -80°C for long-term storage.

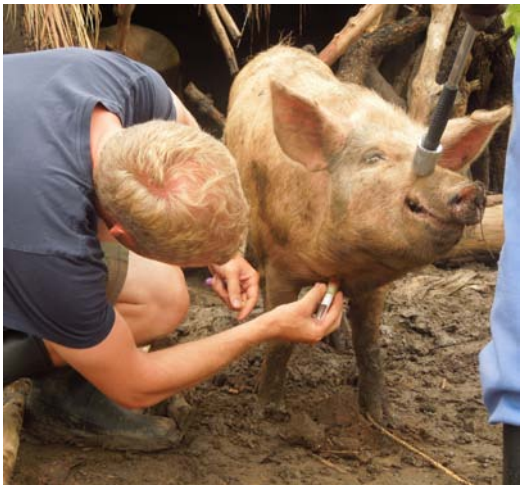

Figure 6. Taking a blood sample with a serum vacutainer, Nwoya district, Uganda 2012.
Figure 5. Interviewing a local pig farmer with the assistance of the DVO, Nwoya district, Uganda 2012.

Sampling bushpigs, warthogs, and ticks

Attempts were made to capture warthogs in the northern sections of Murchinson Falls national park. This area was close to where the domestic pigs had been sampled, and was therefore of interest as a wildlife interface. When warthogs were sighted, game capture nets measuring 100 meters in length were erected and the warthogs herded toward the nets. The efforts were hampered by the seasonally high grass, as the field research was carried out during the rainy season. For this reason as well as logistical constraints on the ground, no warthogs were captured. Bushpigs were captured as part of a different study between the Swedish University of Agricultural Studies (SLU) and Makerere University. The bushpigs were captured using the same technique during the dry season of July and August, when the grass is lower and the bushpigs more easily sighted. Ticks were collected from a total of six warthog burrows in the study area. Only engorged ticks were collected, as these were most likely to contain blood from warthogs living in the burrows (fig. 7).

Figure 7. Collecting ticks from warthog burrows, Murchinson Falls National Park, Uganda 2012.

Laboratory analyses

The samples from domestic pigs and ticks were analysed at the Molecular Biology laboratory at Makerere University Institute of Natural Resources. DNA was extracted using GeneJet Whole Blood Genomic DNA Purification Mini Kit (Thermo Scientific, Waltham, USA), using the Whole Blood Main Protocol for extraction of blood samples from both domestic pigs and the blood retrieved from engorged ticks. In order to achieve a higher concentration of viral DNA, the samples were eluted to 60µl and not 200µl as recommended by the protocol.

PCR

Blood samples

The samples were pooled farm-wise with no more than 3 samples from each farm and run in a real-time PCR using assay from Tetracore (ASFV PCR Assay, Tetracore). The viral assay is a real-time PCR method that utilizes a specific set of forward and reverse primers for the detection of ASF viral DNA. The PCR amplifies a section of the gene coding for a structural viral protein 72 (VP72) in the ASF virus. The p72 protein (B646L gene) is the main component of the viral capsid that surrounds the inner virus envelope. It makes up about 32% of the total protein mass and has a high antigenicity (Bastos et. al, 2003).

Each Smartcycler tube was loaded with 22.5µl Rehydration Buffer and 2.5µl of extracted material (sample) or 2.5µl ASF positive control (provided by Tetracore), or 2.5µl distilled water as a negative control. Each tube was then briefly centrifuged in a microfuge before being loaded into the PCR Smartcycler (Cepheid Inc., Sunnyvale, Calif). The cycling conditions, as set out by Tetracore, were 1 denaturation cycle at 95°C for 120s, followed by an amplification of the DNA strands with 1 cycle at 95°C for 5s, and one cycle at 60°C for 60s. Steps two and three were repeated 45 times. A positive sample results in a sigmoid curve, with a lower Ct (cycle threshold) corresponding to higher concentrations of viral DNA in the sample (fig. 8).

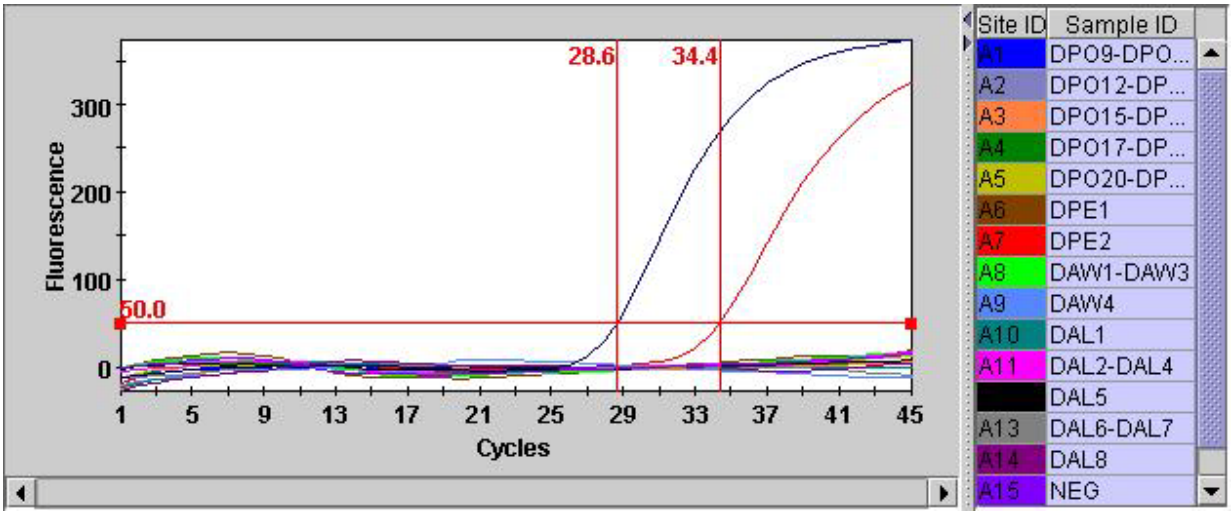

Figure 8. RT-PCR results from domestic pigs in Nwoya district. The sigmoid curves represent positive control (blue) and positive DPE2 sample (red).

Samples that were positive in the real-time PCR were run on a classic PCR using two oligonucleotide primers termed P72-U (5' GGCACAAGTTCGGACATGT 3') and P72-D (5' GTACTGTAACGCAGCACAG 3') amplifying a region of 478 bp at the C-terminal end of the p72 protein (Bastos et. al, 2003). Genomic amplification was performed in a 50µl volume in the presence of 0.2mM dNTP, 2.5µl of each primer, 1µl Pfu polymerase and 2µl of DNA extract. The eluted material was then run in a Mastercycler PCR. Amplification products of the expected 478 bp size run on a 2% agarose gel electrophoresis and identified against a molecular weight marker. When the electrophoresis was performed, no viral DNA was detected. Therefore a Touchdown PCR was used to avoid amplifying nonspecific DNA sequences. This is achieved by using higher annealing temperatures, thereby increasing the specificity of the primers (table 1). Higher specificity avoids unwanted binding by the primers, which leads to amplification of the wrong segments of DNA, thereby obscuring the amplification of the desired segments. The earliest steps of the PCR reaction are run at the highest possible temperature below the melting point of the primers. At this temperature the primers will bind with the highest specificity. At each of the following rounds the temperature is decreased, and the desired fragments are amplified.

PCR Conditions					
Classic PCR Pfu protocol			Touchdown PCR		
Step 1	95°C 7min		Step 1	95°C 7 min	
Step 2	95°C 30 sec	x35	Step 2	95°C 30 sec	x10
Step 3	55°C 30 sec		Step 3	65°C 15 sec	
Step 4	72°C 30 sec		Step 4	72°C 30 sec	
Step 5	72°C 10 min		Step 5	95°C 30 sec	x35
			Step 6	56°C 30 sec	
			Step 7	72°C 30 sec	
			Step 8	72°C 10 min	

Table 1. Mastercycler PCR temperature conditions for each step of the classic PCR Pfu protocol and Toudown PCR with higher annealing temperatures.

Blood extracted from Ornithodoros ticks

Blood was removed from the ticks using a scalpel and pipette. Some ticks had already digested the blood, and could therefore not be used. The viral DNA was extracted using the Thermo Scientific Whole Blood Protocol as above. The samples were then run in the same real-time PCR Smartcycler from Cepheid using the kit and cycling conditions from Tetracore.

Tick tissue (whole ticks)

ASFV can be found both in the blood the tick has consumed, but also in the salivary glands, coxal glands, reproductive tissue and midgut epithelium of the ticks themselves (Kleiboker, 1998). For this reason, it is useful to analyze the tick tissue for presence of ASFV. The whole ticks were ground down using a sterile mortar and pestle. First a dry homogenization was carried out, after which 600µl PBS was added and the homogenization continued until the ticks were well separated. The solution was then centrifuged in a low speed centrifugation at 4000rpm for 10 minutes to allow the debris to settle and the supernatant to surface. The supernatant was then siphoned off for use in DNA extraction using the Thermo Scientific Whole Blood Protocol.

When the extracted material was run in a gel electrophoresis, no bands of extracted DNA were found. Efforts were made to adjust the extraction method to retrieve the ASFV DNA. The Thermo Scientific Tissue Protocol was used and the incubation period, which was 10 min at 56°C according to the Tissue Protocol, was extended to 30 min, 60 min, and 12h to give the Digestion Solution and Proteinase K longer time to take effect. Neither the Blood Protocol, Tissue Protocol or extended incubation times had any effect, and none of the subsequent gels showed any evidence of extracted DNA. Therefore the attempts at analyzing tick tissue were abandoned.

Purification and sequencing

Samples positive for ASFV were run in a Touchdown PCR as described above, and the DNA products purified using the QIAquick PCR purification kit from QIAGEN. The buffers provided in the kit recover the desired DNA and removes contaminants such as impurities,

salts, enzymes, and unincorporated nucleotides. The DNA binds to the silica membrane in the spin column while contaminants pass through the column. As the impurities are washed away, the pure DNA is refined and eluted with 50µl Buffer EB. The purified DNA products were then sent to Macrogen in Amsterdam for sequencing.

The sequences were assembled and edited using Seqman Lasergene9 from DNAstar. The sequences where aligned using BioEdit Sequence alignment software. A phylogenetic neighbour-joining tree was constructed, using MEGA5 with a bootstrap value of 1000 (fig. 9).

ELISA

To detect antibodies against ASFV, the positive sample (DPE2) and samples from nearby farms were run in a commercial blocking ELISA kit from Ingenasa (INGEZIM PPA COMPAC, Ingenasa, Spain). All reagents as well as positive and negative controls were provided in the kit. The wells are coated with VP73 protein extract, which is a protein antigen of the ASF virus. If the sample contains ASF antibodies, it will bind to the antigen coating in the wells.

The sample was diluted 1:1 with 50µl serum sample and 50µl diluent. The same procedure was used for the positive and negative control. The samples were incubated for one hour, and then washed four times using 300µl washing solution. After this 100µl conjugate was added and the samples incubated for 30 min at 37°C. The samples were then washed five times, after which 100µl substrate solution was added to each well. Finally 100µl stop solution was added to each well, and the optical density (OD) values measured at 450nm. The viability was determined if the relationship between negative control (NC) and positive control (PC) was $NC/PC \geq 4$. According to this formula all tests were determined to be viable. The formulas for the positive and negative cut-offs were:

Positive cut off: NC – ((NC-PC) x 0.5)

Negative cut off: NC – ((NC-PC) x 0.4)

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.

RESULTS

Blood samples

Of the 91 domestic pigs sampled in Gulu and Nwoya districts, one was found to be PCR positive for ASFV (fig. 8). The Ct value was 34.4, indicating a positive reaction with moderate amounts of target nucleic acid in the sample. When the sample was run through an ELISA, no antibodies for ASFV were found.

All the blood samples extracted from ticks were PCR negative for ASFV.

Sequencing

Nucleotide Blast analysis confirmed 100% relationship with ASFV p72 nucleotide sequences from Uganda. In the phylogenetic tree the positive ASFV sample from this study (id:DPE2)

was used along with strains of ASFV from other parts of Africa and the outbreak in Georgia in 2007 (fig. 9).

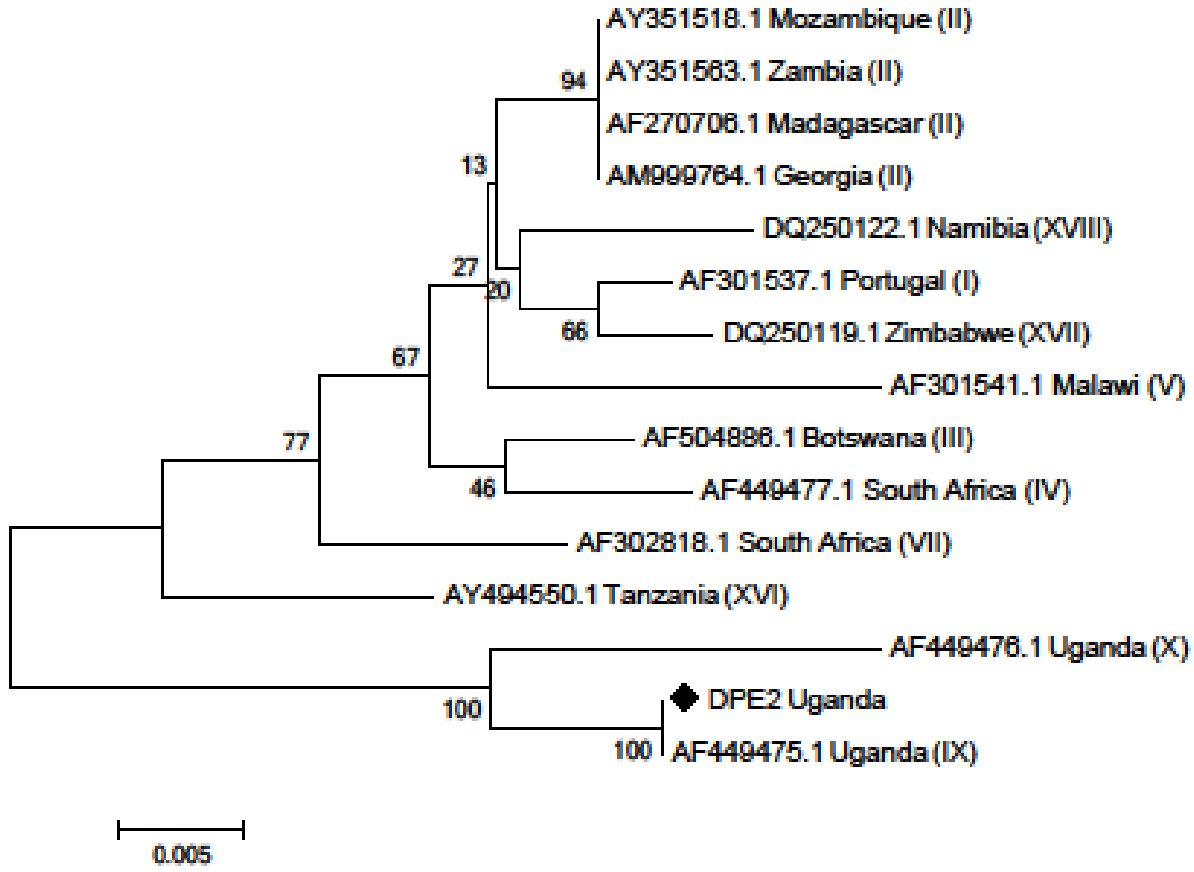

Figure 9. Phylogenetic tree of ASFV p72 nucleotide sequences from several African and European outbreaks. Accession numbers are displayed, followed by the country of origin and the genotype of each strain in roman numerals. The positive sample from this study, DPE2, is labelled.

Questionnaire

A total of 31 farmers were interviewed for this study. Of these, the majority owned between one and four pigs (65%). The husbandry was fairly evenly divided between free-ranging, keeping the pigs in stalls, and having them tethered outdoors. The breed of the pigs was in most cases (87%) of the local variety, with only a few having improved or mixed breeds. Two thirds of the farmers found their pigs to be generally healthy, with none specifying their pigs as being infected with ASF. Nine of the farmers were aware of there being warthogs and bushpigs in their local area, and eight farmers were aware of outbreaks of ASF having occurred in or near their village.

Discussion

This study did not find any infected wild suids or tick vectors. The blood extracted from the ornithodoros ticks that were collected from warthog burrows were all PCR negative for the virus. If the ticks had fed on warthogs, which is likely given their origin, these would by extension also be negative for the virus. The find of one PCR positive pig confirms that the virus is circulating among the domestic pig population in northern Uganda. The PCR positive

pig showed no adverse clinical signs when sampled and was negative for ASFV antibodies. This could be because it has very recently been infected and had not time to develop clinical symptoms, or had survived the disease and was in recovery. The lack of antibodies could be explained by an insufficient immune response, or a low-virulent strain of the virus inducing a weak reaction. Another possibility is that the sample was contaminated in the laboratory, but this was judged to be unlikely as all sanitary steps were followed. When the owner of the infected pig was interviewed, it was confirmed that a recent outbreak of ASF had occurred in the neighboring village, but the owner claimed that the sampled pig was healthy. The owner also had a few smaller piglets and claimed these were not in good health, but it was not clear what they were suffering from. These piglets were too small to be sampled for this study.

The presence of virus but lack of antibodies is an interesting find, and could suggest the presence of subclinically infected pigs that could potentially harbor the disease. The presence of asymptomatic carriers means that screening for the virus in an area would require both PCR analysis and ELISA, as a lack of antibodies does not rule out infection.

When genotyped, the virus was found to belong to genotype IX, which is one of many genotypes previously identified in Uganda. Historically, all ASFV p72 genotypes (22 in total) have been circulating in eastern and southern Africa, and genotype I has been circulating in Europe, South America, the Caribbean and West Africa. The outbreak in Georgia in 2007 was of genotype II (GenBank accession no. FR682468), which marks a spread of the disease from its natural boundaries on the African continent. This highly virulent strain clustered with ASFV isolates from Mozambique, Zambia, and Madagascar and was 100% identical to a strain of p72 genotype II that caused an outbreak in Tanzania between 2010-2012 (GenBank accession nr.JX391987 [TAN/10/Kyela]) (Misinzo et. al, 2012).

This study cannot confirm that ASF is spread from wildlife to domestic pigs in northern Uganda, as there is insufficient evidence to support such a hypothesis. To confirm such a theory, larger volumes of data and the sampling of bushpigs, warthogs, ticks and domestic pigs would be required. However, this study can confirm that the conditions are in place for wildlife to livestock transmission to occur, as several farmers kept free-ranging pigs and had seen warthogs and bushpigs near their farms. This is a clear indication of the possibility of a wildlife-domestic interface. The literature on the epidemiology of ASF supports the transfer of virus from wild to domestic pigs, but the exact nature of the transmission between vector and host in Uganda is yet to be established. The respective roles of bushpigs, warthogs, ticks and asymptomatic domestic carriers in causing outbreaks needs to be identified for a comprehensive understanding of how to combat the disease.

The role of the bushpig in maintaining and spreading the disease in Uganda is one of the main lines of research on this topic. Bushpigs can under experimental conditions infect domestic pigs, but to what extent this happens in the wild in not yet known. This study can not arrive at any conclusions about the potential roles of bushpigs in disease transmission, but from the locations where bushpigs were captured, it is clear that bushpigs do at least occasionally stray into inhabited areas. GPS tracking of bushpigs to confirm the extent they stray into inhabited areas would be useful, as would be tracking free ranging domestic pigs to see how far they stray into adjoining lands. It would also be interesting to perform virus isolation and sequence analysis on

positive samples from bushpigs and warthogs, and comparing these with virus strains in domestic pigs to see if the same virus appears in all species. If so, it would support the hypothesis that the virus spreads between them.

A key to future control of the virus is the development of a vaccine. There is currently no vaccine available against ASF, but research indicates that developing a vaccine should be feasible (Lewis et. al, 2000). The live-attenuated, inactivated or recombinant vaccines that have been developed so-far have been unsuccessful. This is thought to be the result of the lack of effective antibodies to ASFV and the great variability of the virus. An attenuated vaccine may be found using low-virulence strains of the virus or by deletion of disease-causing genes could weaken the virus. Another approach may be to study viral interactions in macrophages, and thereby stimulate a protective immune response (Sánchez-Vizcaíno et. al, 2009). The ineffectiveness of the antibody response despite high levels of antibodies produced during acute ASF infections points to a role for cellular immunity. The promotion of natural killer cells (NK) and cytotoxicity of leucocytes may have an effect on the progression of the disease (Sánchez-Vizcaíno et. al, 2009).

Until a vaccine has been developed, the best method for disease control is the physical separation of domestic pigs from wildlife. Practical experiences from other endemic areas show that this can have a great effect. In Uganda this would be difficult to implement, as the farmers tend to be poor and lack the means to build proper housing for their animals. There also appeared to be a lack of motivation among farmers to deal with the disease and similarly a lack of motivation among the DVO, who appeared resigned to a cycle of disease outbreaks in the area. In addition, the veterinary officers most probably lack the resources required to properly control an outbreak when it does occur. The roads in the villages are notoriously bad, especially in the rainy season, which creates practical difficulties in reaching areas where outbreaks occur.

Conclusion

The many potential modes of transmission in Uganda (trade, slaughter, asymptomatic carriers, bushmeat, wildlife-livestock interface) suggests that any control measures against the disease have to be broad to encompass all routes of infection. The most important step in this regard is education programs at the community level, to inform farmers of the disease and how it spreads. The farmers must also be given incentives not to try to slaughter and sell the meat once a pig becomes sick, nor selling the live animal once it contracts the disease. Farmers must also be taught the clinical symptoms of the disease, to allow for a quicker response to contain an eventual outbreak.

Of fundamental importance is government funding of proper disease surveillance and control programs, as well as proper compensation schemes to increase farmer compliance. This is a challenge in Uganda, where veterinary services lack resources and government compensation is non-existent. The feasibility of ASF-free zones has been demonstrated in South Africa, and may act as a model for other African nations where ASF is endemic.

Further research will be required to confirm the main mode of disease transmission from wildlife in northern Uganda, and thereby identify the source of new outbreaks.

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