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Emerging Infectious Diseases: using PCV2 as a model of disease transmission dynamics at the livestock-wildlife interface in Uganda

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Uppsala

2013

Examensarbete inom veterinärprogrammet

ISSN 1652-8697
Examensarbete 2013:28

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Examensarbete inom veterinärprogrammet, Uppsala 2013
Fakulteten för veterinärmedicin och husdjursvetenskap
Institutionen för Biomedicin och veterinär folkhälsovetenskap
Kurskod: EX0751, Nivå A2E, 30hp

Key words: PCV2, disease transmission, livestock-wildlife, emerging infectious diseases (EID)
Nyckelord: PCV2, sjukdomstranmission, vilda djur – tamdjur, EID

Online publication of this work: http://epsilon.slu.se
ISSN 1652-8697
Examensarbete 2013:28

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SAMMANFATTNING

Uganda anses vara ett högriskland för utveckling av nya plötsligt uppdykande sjukdomar (emerging infectious diseases, EID). Grisproduktionen i Uganda har ökat drastiskt de senaste åren och en stor del av Ugandas grisar är frigående. De kan därför lätt komma i kontakt med vilda djur i deras närområde. Porcint circovirus 2 (PCV2) är ett ubikvitärt virus och etiologiskt agens för bland annat porcine multisystemic wasting syndrome (PMWS). Förutom att infektera tamgrisar över hela världen så har PCV2 också visats kunna infektera vildsvin. PCV2 används i den här studien som ett modellvirus för transmissionsdynamik mellan vilda och tama djur då det är spritt bland grisar över hela världen, har viss genetisk variation och har hittats på både tama och vilda grisar.

Målen för den här studien var att undersöka sjukdomstranmissionsdynamiken mellan vilda och tama djur i Uganda med PCV2 som modell, som ett led i EID-övervakningen, samt att uppskatta prevalensen av PCV2 bland domesticerade grisar i Uganda.

Nittioen tamgrisar runt Murchison Falls nationalpark provtogs och analyserades med SybrGreen RT-PCR med avseende på tre olika PCV2 genogrupper, SG1, SG2 och SG3. Försök att fånga vårtsvin gjordes men tyvärr utan framgång. Därför kunde inte heller några slutsatser angående sjukdomstransmission mellan vilda och tama grisar dras. Alla tamgrisprover var negativa på PCR för SG1 och SG2 men för SG3 fanns en punktprevalens på 77%. SG3 är även känd som PCV2b och är den vanligaste genotypen av PCV2 i de flesta andra länder. PCV2b är även den genogrupp som mest associerats med PMWS.

För framtida studier kan både Uganda och tama och vilda grisar vara viktiga områden att studera. Dels för att öka förståelsen för sjukdomstranmissionsdynamiken mellan tama och vilda djur och dels för EID-övervakning.

ABSTRACT

Uganda is considered a “hotspot” for emerging infectious diseases (EIDs), meaning that it is a high-risk country for new infectious diseases to originate. The pig production is increasing drastically in Uganda and many of the pigs are free ranging and can come in contact with wildlife. Porcine circovirus 2 (PCV2) is a ubiquitous virus and causal agent of porcine multisystemic wasting syndrome (PMWS). Not only is PCV2 found in domestic pigs all around the world but it has also been shown to infect wild boar. PCV2 is, in this study, used as a model for disease transmission dynamics because it is spread among pigs throughout the world, has some genetic variation and has been found in both domestic and wild pigs.

The aims of this study were to investigate the disease transmission dynamics in the livestock-wildlife interface in Uganda with PCV2 as a model, as a part of the EID surveillance, and to estimate the prevalence of PCV2 in domestic pigs in Uganda.

Ninety-one domestic pigs around Murchison Falls national park were sampled and analyzed with SybrGreen RT-PCR for three different PCV2 genogroups, SG1, SG2 and SG3. Attempts to catch warthogs were made but unfortunately without success. Consequently no conclusions regarding disease transmission between wild and domestic pigs could be made. The domestic pig samples were all negative for SG1 and SG2 but for SG3 a point prevalence of 77% was found. SG3 is also known as PCV2b and is the most common PCV2 type in most other countries as well. PCV2b is also the genogroup most associated with PMWS.

For future projects both Uganda and suids could be important areas of study both for increasing the understanding of disease transmission dynamics in the livestock-wildlife interface and also for EID surveillance.

INTRODUCTION

Background

This study was carried out in collaboration with, and as a part of, an ongoing project on African swine fever (ASF). This ongoing ASF-project involves the Swedish University of Agricultural Sciences (SLU), the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF), Makerere University, Uganda Wildlife Authority (UWA) and International Livestock Research Institute (ILRI).

Emerging infectious diseases

Emerging infectious diseases (EID) are diseases of infectious origin that have appeared new in a population or have existed for a period of time but are increasing in incidence or geographic range (Morse, 1995). EID is not a new phenomenon; in fact there are several examples throughout history. These include the plague, caused by *Yersina Pestis* during the 14th century, the Spanish flu pandemic in the beginning of the 20th century and HIV/AIDS during the 1980s (Bale Jr, 2012). Although EIDs is an old phenomenon, the incidence seems to have increased since the 1940s and is therefore a bigger threat now than it has been before (Jones et al, 2008). Many EIDs are zoonotic or potential zoonotic diseases, i.e. infectious agents in livestock or wildlife that can also infect the human population (Siembieda et al, 2011). The definition of an emerging zoonotic disease is according to World Health Organization (WHO): "…zoonoses that have newly appeared or have occurred previously but are increasing in incidence or geographical range" (WHO, FAO & OIE, 2004).

There are many factors that contribute to the emergence of new infectious diseases (Bale Jr, 2012). Examples of such factors are climate change, increased urbanization, increased international trade and travel, intensification of agriculture and change of land use (Daszak et al, 2012). Other examples include natural disasters, use of antimicrobial agents and mutation of pathogens (Bale Jr, 2012).

One EID that has been studied extensively with regards to its reason for emergence is Nipah virus, a zoonotic disease that first emerged in 1998 in Malaysia (Daszak et al, 2012). One hypothesis regarding the emerging of this disease is that fruit bats, that naturally carry the virus, were forced to change habitat due to extensive deforestation. The bats therefore colonized fruit trees in the plantations that replaced the former rainforest and the former habitat of the bats. As a consequence of this, pigs on farms, where the bats colonized the fruit trees, were exposed to Nipah virus and later spread it to humans in close contact with the pigs (Bale Jr, 2012). Another hypothesis is that increased commercial pig production and a trend towards dual-use agriculture (fruit tree plantations) were the driving factors behind the emergence of Nipah virus (Daszak et al, 2012). To predict and understand the underlying causes of EIDs is a difficult task and interdisciplinary efforts are required (Daszak et al 2012).

Not only do EIDs pose a threat to human and domestic animal health, it can also have severe biological implications to wildlife species (Daszak et al, 2000). One threat that is especially

important to endangered species with small populations is the spill-over. This refers to when diseases spread from reservoir domestic animals to sympatric wildlife. Small naive populations can become locally extinct, as happened to the Serengeti wild dog population 1991 after a spill-over of canine distemper virus from the adjacent domestic dog population (Daszak et al, 2000). Even with the goal to maintain biodiversity and work for conservation of endangered species there are possible threats. When reintroducing animals into the wild you risk transferring new pathogens to the already threatened population (Daszak et al, 2000).

One reason that studies of the wildlife-livestock interface are important to predict EIDs is that more than two thirds of the zoonotic EIDs originate from wild species (Jones et al, 2008). Also diseases caused by pathogens that have the ability to infect several different species are more likely to become EIDs and are therefore of special concern, especially if they also are zoonotic (Siembieda et al, 2011). But it is not only zoonotic EIDs that affect humans, the economic cost for other EIDs, for example foot and mouth disease, can be substantial (Daszak et al, 2000).

Jones et al (2008) discuss EID "hotspots" as a phenomenon and define them as places were EIDs are more likely to occur. Developing countries in tropical Africa, Uganda included, are regarded as EID "hotspots" due to factors such as high human population density, wildlife biodiversity, increasing human population growth and low latitude (Jones et al, 2008). They also conclude that the surveillance and the efforts made controlling emerging diseases are not proportionate to where EIDs are most likely to occur.

Porcine Circo Virus 2

Porcine circovirus (PCV) belongs to the family *Circoviridae* and is a small, circular, single-stranded DNA virus, which is non-enveloped (Segalés et al, 2005). It was first discovered by Tischer et al (1974) as a viral contaminant in porcine kidney-15 (PK-15) cell line. During the eighties, when the initial studies on the virus were performed, results indicated that the virus was non-pathogenic (Tischer et al, 1986). When postweaning multisystemic wasting syndrome (PMWS) emerged in the nineties, however, it was associated with circovirus. A previously uncharacterized strain of PCV was isolated and sequenced from pigs with clinical signs of PMWS (Morozov et al, 1998). This strain was later named porcine circovirus type 2 (PCV2) and the original nonpathogenic PCV found in PK-15 cells was renamed PCV1 (Patterson & Opriessnig, 2010). PCV1 and PCV2 have been shown to have 69-76% sequence homology (Morozov et al, 1998 and Hamel et al, 1998).

PCV2 is nowadays recognized as a ubiquitous virus worldwide and can be divided into three subtypes, PCV2a-c of which PCV2a and PCV2b has been found all around the world (Segalés et al, 2008). The virus has been studied in many different countries in Asia, Europe, North America, Oceania and South America (Patterson and Opriessnig, 2010).

Although PCV2 was associated with clinical disease when it was first discovered in the late 1990s, retrospective studies have shown that PCV2 was common in the pig population long

before the first outbreak of porcine circovirus diseases (Rose et al, 2011). There also seem to have occurred a major shift in which PCV2 subtype is most common from PCV2a to PCV2b during or prior to 2003 (Rose et al, 2011). In a study performed in Sweden on PCV2 by Timmusk et al (2008) the genogroups are named Swedish Genogroup 1, 2 and 3 (SG1, SG2 and SG3) where SG1 and SG2 corresponds to PCV2a and SG3 corresponds to PCV2b (Grau-Roma et al, 2011).

PCV2 is highly persistent in the environment and stable even under high temperatures and under many different pH-values. The virus is also resistant against many common disinfectants such as iodine, alcohol, phenol and formaldehyde (Patterson and Opriessnig, 2010).

Viral transmission

Direct horizontal transmission of PCV2 is the most important route for infecting susceptible animals and spreading the virus in between pigs (Rose et al, 2011). A less efficient route of transmission of PCV2 to naïve animals is indirect via oral ingestion of uncooked infected meat (Opriessnig et al, 2009). Although vertical transmission occurs, the clinical importance is difficult to evaluate, but is believed to play a role in virus spread both within populations and between populations (Rose et al, 2011). PCV2 is able to infect fetuses through the transplacental route (Castor et al, 2012) and young piglets through infected milk (Rose et al, 2011).

Not only does PCV2 infect suids but it has also been shown to replicate in, and spread between mice. Therefore the presence of rodents is a potential risk factor for indirect transmission and could be important in the epidemiology of PCV2 (Patterson and Opriessnig, 2010). In a recent study by Pinheiro et al (2012) the authors verified that both rats (*Rattus rattus*) and mice (*Mus musculus*) in proximity to pigs can become naturally infected with PCV2. They found that the PCV2-positive rodents were infected with PCV2b and that these samples had a 98-99% sequence identity with previously known PCV2 isolates from pigs. The prevalence found was 14.8% in rats and 53.8% in mice (Pinheiro et al, 2012).

Pigs can shed the virus for a long time and it can be detected in a variety of different excretions (Rose et al, 2011). These include nasal and fecal excretions, serum and lymphoid tissue (Grau-Roma et al, 2009).

Because PCV2 is highly resistant in the environment, can infect susceptible animals through many different routes and it can be excreted for a long time, the virus can be maintained in a herd for an extensive period of time without further reintroduction (Rose et al, 2011).

Porcine circovirus associated diseases

PCV2 has been associated with several different diseases, which are collectively termed porcine circovirus associated diseases (PCVAD), a concept introduced by the American Association of Swine Veterinarians in 2006 (Opriessnig et al, 2007).

Postweaning multisystemic wasting syndrome (PMWS) is a disease that causes considerable economic losses worldwide (Ge et al, 2011; Alarcon, 2011). PMWS usually affects pigs during 2-3 weeks post weaning and the clinical signs include wasting, pallor of skin, enlargement of lymph nodes, diarrhea and dyspnea (Harding and Clark, 1997). In China the estimated morbidity of PMWS is around 10-20% in affected herds and the mortality is estimated to less than 10% (Ge et al, 2012). On an affected farm in Spain the mortality was appromimately 25% (Quintana et al, 2001). In a study by López-Saria et al (2011) the PMWS associated mortality varied between 1,8% to 12,4% between different groups.

The criteria for diagnosing PMWS, as proposed by Sorden (2000), in individual animals include clinical signs consistent with the disease, presence of typical histopathological lesions in lymphoid tissues and detection of high amounts of PCV2 in lymphoid tissues. Although PCV2 is necessary for development of PMWS, all pigs infected with the virus do not develop the disease (Segalés et al, 2005). This indicates that other factors, such as co-infections (Allan et al, 1999; Ellis et al, 2000; Ha et al, 2010) or immunostimulation (Kyriakis et al, 2002; Ha et al, 2010), are essential for development of the clinical disease.

Porcine dermatitis and nephropathy syndrome (PDNS) is another disease associated with PCV2 (Rosell et al, 2000). It can be observed in nursery, growing and even adult pigs (Drolet et al, 1999). Hemorrhagic, necrotizing skin lesions on the caudal part of the pig's body are the most striking feature of PDNS in affected pigs (Thibualt et al, 1998). Pigs can also develop anorexia, depression, stiff gait and mild fever (Drolet et al, 1999). Cause of death in affected animals is acute kidney failure (Segalés et al, 1998).

Reproductive failure such as abortion has been linked to the presence of PCV2 (Segalés et al, 2005), but although PCV2 has been found in aborted fetuses the correlation between PCV2 and abortion is not fully understood. In many cases there are co-infections with other bacteria or viruses (Castro et al, 2012). In the study by Castro et al (2012) PCV2 was found in 10.7% of the investigated 168 aborted fetuses. In the same study they found co-infections with brucella or porcine parvovirus (PPV) in 7.2% of the investigated fetuses and only 3.6% was found to be mono-infections.

Factors contributing to the development of PCVAD

Factors that seem to affect whether or not infected pigs develop PCVAD include vaccination schedules, housing, husbandry practices and biosecurity measures. Rose et al (2012) suggest that these factors can function as triggers for massive PCV2-replication and thereby cause clinical disease.

When infected with PCV2 many co-pathogens can enhance or induce PCVAD lesions and symptoms and contribute to triggering clinical disease (Rose et al, 2011). Examples of these co-infectious agents are porcine parvovirus (PPV) (Castor et al, 2012; Allan et al, 1999; Ellis et al, 2000; Ha et al, 2010), brucella (Castor et al, 2012), porcine reproductive and respiratory syndrome virus (PRRSV) (Ge et al, 2011) and mycoplasma (Opressnig et al, 2004).

Breed predisposition in terms of susceptibility to PCVAD has been suspected based on clinical experience and it has been shown that there are differences between breeds but whether these differences is breed related or linked to specific genes is yet to be decided (Rose et al, 2011; Segalés et al, 2005). López-Saria et al (2011) performed a study where they inseminated sows with different breed boars and found a difference in clinical expression of PMWS in the different litters.

Vaccines

There are currently several commercial vaccines available on the market in China (Ge et al, 2012), some European countries (Segalés et al, 2005) and USA (Shen et al, 2012). The vaccination of growing animals is a good measure of controlling PCVAD (Ge et al, 2012). In the USA it is estimated that 99% of the pig farmers vaccinate their growing animals against PCV2 (Shen et al, 2012). All the available vaccines today are based on PCV2a isolates, and in a study by Shen et al (2012) they found that all the serum samples from healthy confirmed vaccinated pigs were negative for PCV2a, suggesting a highly efficient protection against PCV2a viraemia. They also found that PCV2b is more common in both vaccinated pigs and unvaccinated pigs. This indicates that the vaccines are not as efficient in preventing PCV2b viraemia as they are in preventing PCV2a viraemia (Shen et al, 2012).

Prevalence

Out of 1675 serum samples from healthy pigs in the USA 7.7% were positive for PCV2a DNA and 8.4% of the samples were positive for PCV2b DNA. In the same study they found that the prevalence of PCV2a in PCVAD cases was 13% and for PCV2b the prevalence was 65% (Shen et al, 2012). In China the seroprevalence varies between 10-90% depending mainly on area and age group (Ge et al, 2012). In a study performed in Slovakia on 198 pigs, both healthy and with suspected PMWS, viral DNA was found in 35.5% of the pigs and antibodies against PCV2 in 54% of the pigs (Csank et al, 2011).

Aside from infecting domestic pigs and rodents PCV2 can also infect other suids such as wild boars (Toplak et al, 2012; Cadar et al, 2010; Cadar et al, 2012). In a study by Cadar et al (2010) they found PCV2 DNA in 13.5% of the wild boars in Transylvania. Another study in Transylvania has shown a phylogenetic difference between circulating PCV2 in wild boar compared to the domestic pig´s PCV2 in the same area (Cadar et al, 2012). In the same study evidence suggesting a potential spread of PCV2 from wild boars to domestic pigs was found (Cadar et al, 2012).

Pig production in Uganda

Uganda is an east-African country where 78% of the population is involved in agriculture, and livestock production contributes to more than 5% of the GDP. The majority of Ugandan farmers are subsistence farmers, who produce mainly for household usage with traditional methods. Pig production accounts for 29% of Uganda’s 292 800 tons annual meat production and has been the fastest growing livestock sector in Uganda since 1980 (FAO & AGAL,

2005). Approximately 45% of the rural farmers are estimated to live in poverty. Therefore improvements in the livestock sector are important in improving people's quality of life (FAO & AGAL, 2005). Uganda is said to have the largest population of pigs in East Africa (Phiri et al, 2003). According to the livestock census in 2008 Uganda has a pig population of around 3.2 million (MAAIF & UBOS, 2009). The main management system for keeping pigs in East Africa is extensive, free-ranging and on a small scale (Phiri et al, 2003).

OBJECTIVE

The purpose of this project was to study the disease transmission in the livestock-wildlife interface in Uganda with PCV2 as a model virus. Through investigating the presence of PCV2 and its different genogroups, as well as comparing the genetic material, in domestic pigs and wart hogs in the livestock-wildlife interface, a better understanding of the viral transmission dynamics can be provided. Since many EIDs originate in wildlife populations and spread to domestic animals in their proximity this could function as a model for the origination of EIDs.

Another purpose of the study is to investigate the prevalence of PCV2 in Ugandan pigs since, to the author's knowledge, no published prevalence studies on PCV2 in Uganda have been made.

Aims

- To gain knowledge of the transmission dynamics of viruses between wildlife and livestock with PCV2 in domestic and wild suids as a model.

- To investigate the prevalence of PCV2 in domestic pigs and warthogs in Murchison Falls and its adjacent area.

MATERIAL AND METHODS

Part of this project, mainly field work but also to some extent laboratory work, was conducted in cooperation with another veterinary student writing his master's thesis.

Study region

All the fieldwork of this study took place in Uganda in the two neighboring districts Gulu and Nwoya during September and October, 2012. The samples were collected in proximity to the border, or in, Murchison Falls national park in northern Uganda (figure 1). The area is in this case representative for the livestock-wildlife interface since both domestic free-ranging pigs and wild suids are present in the area. Ninety-one domestic pigs were sampled from 31 different farms in nine separate villages belonging to one of the three subcounties Koch goma, Anaka or Purongo. The farms were selected based on proximity to the park and availability based on farm owners´ willingness to let their pigs participate in the study. To maintain a good relationship between the farmers and researchers, all the pigs that were sampled for the study were dewormed.

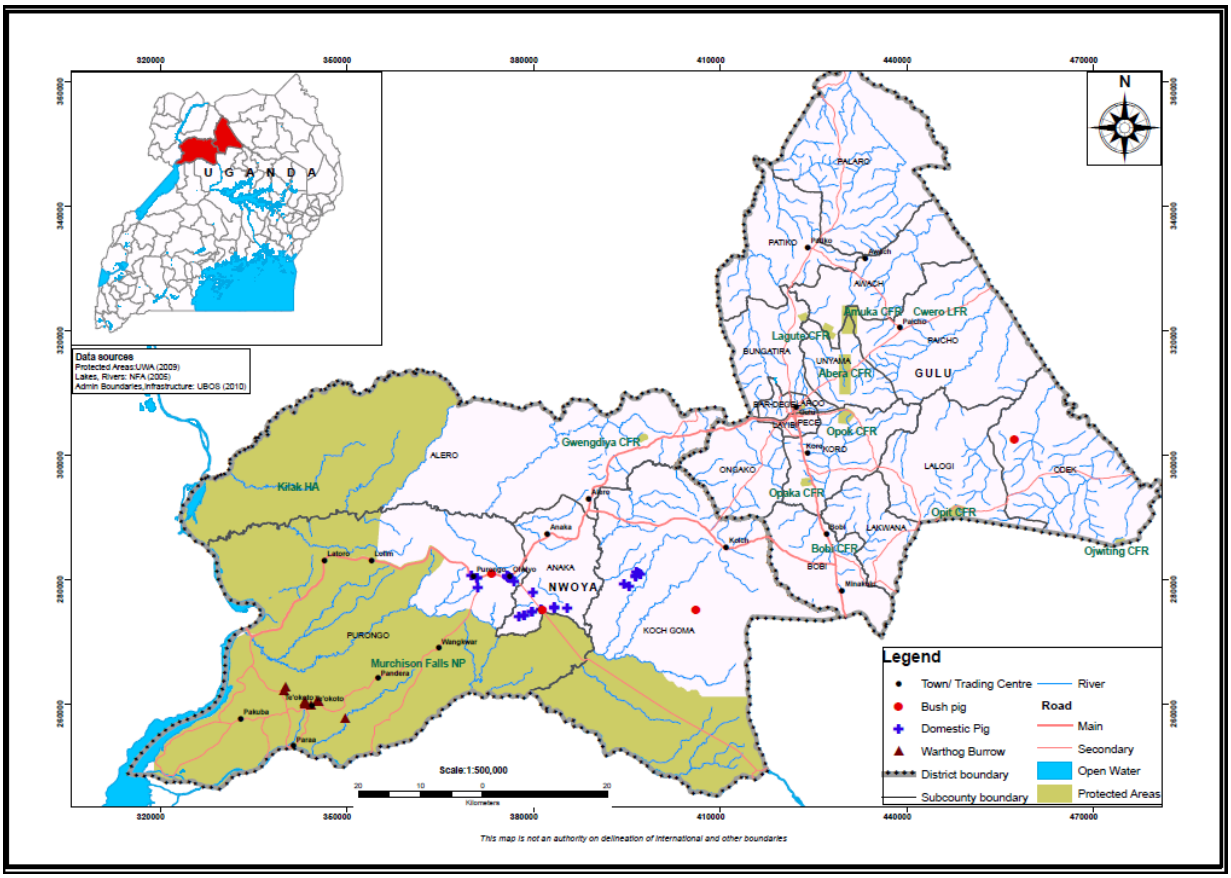

Fig 1. Map showing the sampling area and indicating the GPS-points were domestic pigs were sampled.

Sampling and data collection

From every individual pig serum and EDTA samples were collected. Serum samples were left standing over night and then separated from the blood into two cryo tubes the day after sampling. The EDTA samples were kept in a cooler directly after sampling and until they could be stored in a fridge. The EDTA also was separated into two cryo tubes, one for long-term storage in -80°C and one for short-term storage and diagnostic analysis in -20°C.

The pigs were restrained using a pig-catcher and bleed from the jugular vein if they were large or medium sized (figure 2). The smaller pigs were restrained manually and bleed from the cranial vena cava. The district veterinary officer (DVO) of Gulu-district accompanied us and assisted us in our sampling process. We also had local hunters assisting us as guides and helping in restraining the pigs. For each pig, estimated age category (0-3 months, 3-6 months, 6-12 months or 12+ months), breed (local, mixed or improved) and gender were noted at the time of the sampling.

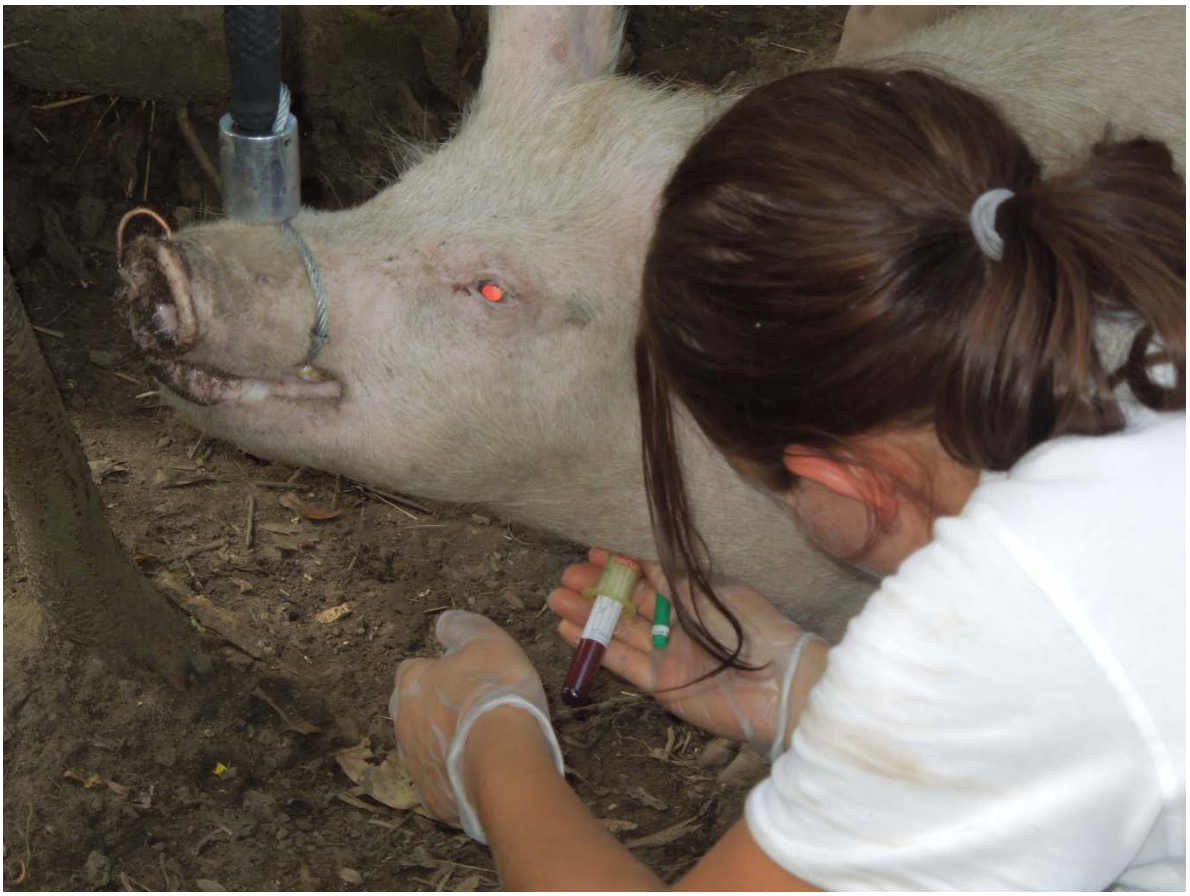

Fig. 2. Bleeding of a large pig from the jugular vein.

A questionnaire was filled out for each farmer (see appendix A) and GPS-coordinates were registered at every farm. Since many of the farmers did not speak English, either the DVO or one of the local guides helped with translation of the questionnaire to the farmers.

During three days in the Murchison Falls national park attempts to catch and sample warthogs were made. With the assistance of local hunters large game capture nets were put up and attempts to drive warthogs in to the nets were made. Unfortunately, due to tall grass and therefore difficulties keeping track of the warthogs, none were caught.

Laboratory analysis

The initial laboratory analyses, DNA extraction and PCR, were performed in Uganda at the Molecular Biology Laboratory, Makerere University, Institute of Environment and Natural Resources (MUIENR), Kampala. Agarose gel to confirm real positives as well as an additional PCR in order to extract PCR-product for sequencing was performed at the Swedish University of Agriculture (SLU). Some of the positive samples were sent to Macrogen in the Netherland for sequencing.

DNA extraction

The DNA extractions were performed with the commercial kit GenJET Genomic DNA Purification Kit (Thermo Scientific). The manufacturer's protocol for mammalian blood was followed except for the amount of elution buffer used. Two hundred µl EDTA-blood was used from each sample. Elution buffer was added, 30 µl repeated twice instead of 200 µl, in order to achieve a higher DNA concentration.

PCR

A SYBR Green real-time polymerase chain reaction (RT-PCR) was performed in order to amplify and detect viral DNA from the extracted samples. A commercial kit, Thermo Scientific Maxima SYBR Green qPCR Master Mix (Thermo Scientific), was used and the instructions that came with the kit were followed. The thermal cycling protocol used was the two-step protocol starting with 95°C for 10 minutes once, thereafter 95°C for 15 seconds and 60°C for 60 seconds repeated in 40 amplification cycles. Melting curve analysis was carried out from 60-95°C increasing the temperature with 0.2 degrees per second. For both forward and reverse primers a final concentration of 0.3 µM was used in the Master Mix. No ROX solution was used in the reaction set-up. For SG1, SG2 and SG3 three different forward and reverse primers was used in order to differentiate the three genogroups. The primer sequences used were the same as used in a prevalence study by Blomström et al (2010).

The reactions were carried out using the Cepheid SmartCycler RT-PCR machine. For the SG2 PCR the DNA samples were pooled three and three according to farm and the positive samples were then run individually. For the SG1 and SG3 PCR all the DNA samples were run individually from start. For all the PCR reactions the amount of template DNA was 2µl. As a negative control, nuclease free water, provided in the kit, was used.

SYBR Green RT-PCR is a quantitative method of analyzing samples for DNA. SYBR Green is a dye that binds to double-stranded DNA (dsDNA) with high affinity. When the dye binds to dsDNA it becomes highly fluorescent. The fluorescence is measured and a cycle threshold (CT-value), when the fluorescence rises above a certain level, is acquired. Since the amount of fluorescence generated is proportional to the amount of product present in the sample this value can be used as quantification (Simpson et al, 2000).

Since SYBR Green binds to all dsDNA it can also bind to primer-dimers and other nonspecific amplified products, therefore a melting curve analysis was performed in order to differentiate between the real positives, which have a higher melting point, and primer-dimers which have a lower melting point. Samples that even after melting curve analysis were inconclusive were run on a 1.5% agarose gel and visualized with ultraviolet light to confirm or dismiss as real positives.

Sequencing

The positive PCR products that were selected for sequencing were purified using the GeneJET PCR Purification Kit (Thermo Scentific). The purification was performed according

to the manufacturer's instructions. The purified PCR-products were divided in to two different microcentrifuge tubes and a SG3 forward, and a SG3 reverse primer (Blomström et al, 2010), were added respectively. The microcentrifuge tubes were labeled and sent to Macrogen (The Netherlands) for sequencing.

A search tool at National Center for Biotechnology Information (NCBI), the Basic Local Alignment Search Tool (BLAST), was used to scan the acquired sequences and can be reached from http://blast.ncbi.nlm.nih.gov/Blast.cgi.

RESULTS

Prevalence of PCV2 in domestic pigs

Out of the 91 sampled domestic pigs in Uganda, Gulu and Nwoya districts, none were positive for PCV2 SG1 or PCV2 SG2. For PCV2 SG3, 70 out of the 91 pigs sampled were positive on RT-PCR. The positive pigs were found in all the villages. These results represent a point prevalence of 77% for SG3 and a point prevalence of 0% for SG1 and SG2 (figure 3).

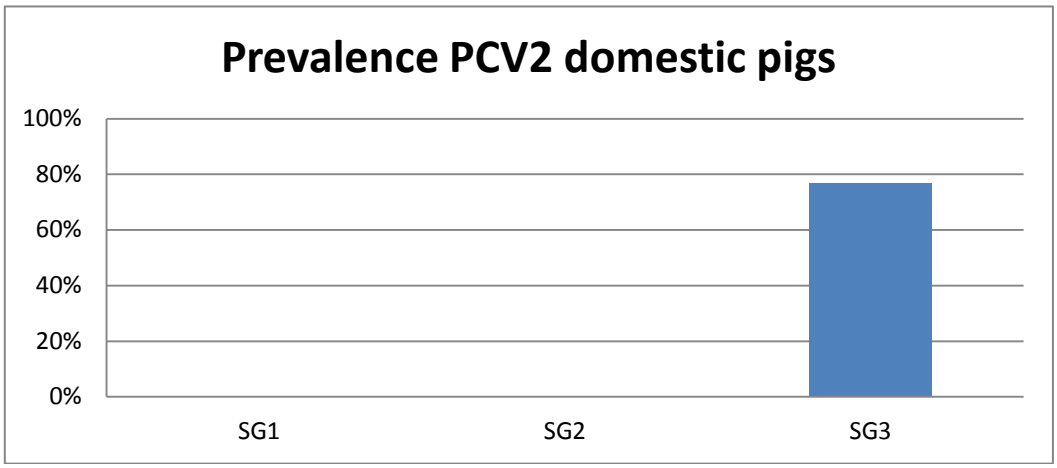

Fig. 3. Prevalence of PCV2 in domestic pigs sampled in Gulu and Nwoya districts for SG1, SG2 and SG3 respectively.

The CT-values for the positive samples were all relatively high, ranging from 34 to 38, indicating a low concentration of viral DNA.

Distribution of estimated age (p=0.88) sex (p=0.78) and breed (p=0.94) showed no statistical significant difference for the PCR-positive pigs compared to all the sampled pigs. Figures 4 to 6 shows the different distributions for breed, estimated age and sex for all the sampled pigs and for the SG3 PCR-positive pigs respectively.

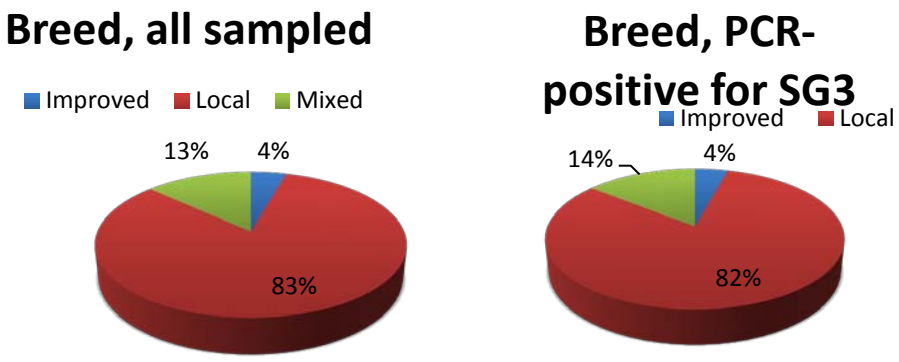

Fig. 4. Distribution of breed among all the sampled pigs and among the SG3 PCR-positive pigs.

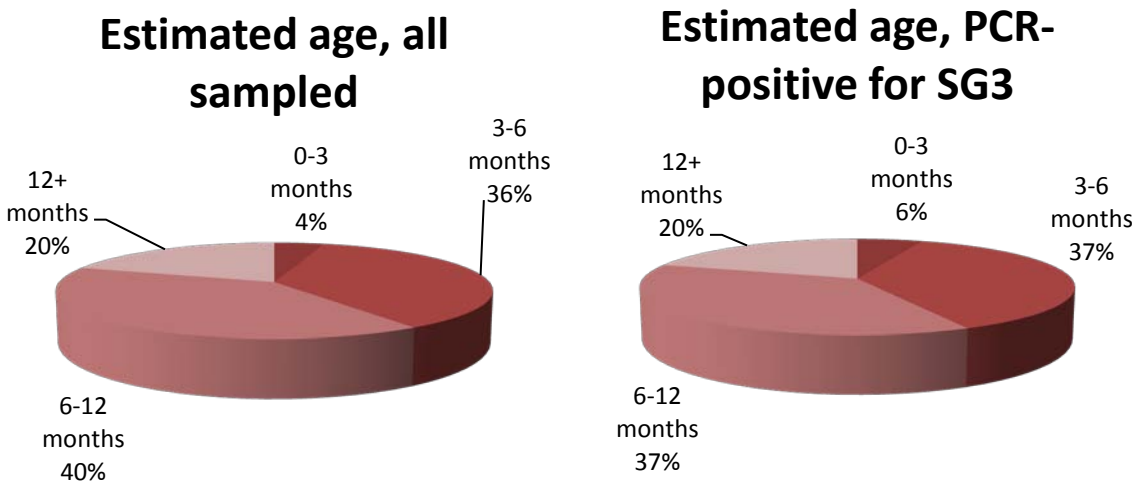

Fig. 5. Age distribution among all the sampled pigs and among the SG3 PCR-positive pigs.

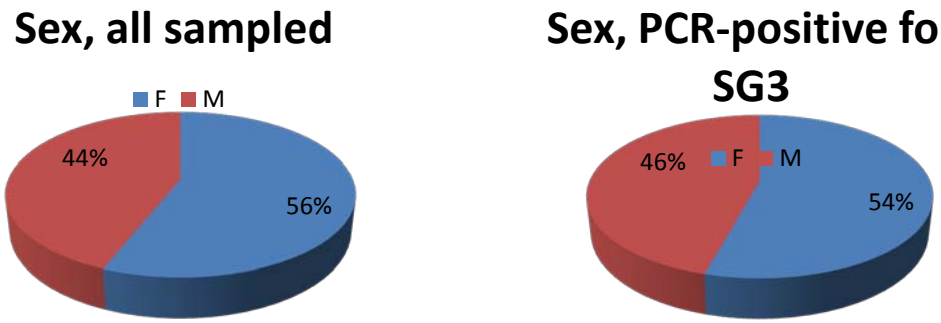

Fig. 6. The distribution of sex for all the sampled pigs and for the SG3 PCR-positive pigs.

Sequences

Twelve PCR-products, with inconclusive results on PCR, were run on gel electrophoresis to confirm or dismiss as positive. On the gel weak bands of the expected size could be visualized with the aid of ultraviolet light. Twelve of the confirmed positive samples were sent for sequence analysis and the nucleotide sequences acquired were screened in BLAST in order to see if the DNA sequence of the samples matched DNA sequences of PCV2. The sequenced material was found to only correlate to the PCV2-primers used and therefore no phylogenetic studies could be performed.

Questionnaire

A total of 31 pig farmers answered the questionnaire. Six of the respondents were female and 25 were male. Out of the 91 pigs 24 pigs (26%) were stated to live in areas where bush pigs and/or warthogs were present in the surroundings (figure 7). As perceived by their owners 74 out of the 91 pigs (81%) were considered healthy. In this study, 47% of the pigs were kept in stalls, 29% were free ranging, 11% were free ranging and kept in stalls and 13% were tethered (figure 8).

The number of pigs in each household in the study varied from 1-20 with a mean value of 5.1 pigs per household.

There was no statistical significant difference for the group “all the sampled pigs” versus the group “SG3 PCR-positive pigs” regarding the following factors: health status (p= 0.48), bush pigs or warthogs in the area (p= 0.38) or husbandry (p= 0.83).

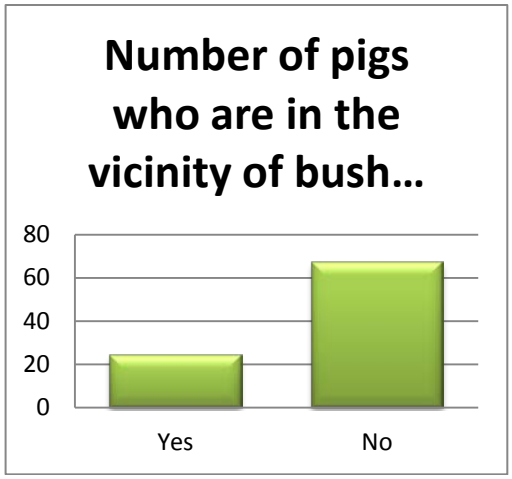

Fig. 7. The number of pigs that, as stated by their owners, were living in the vicinity of bush pigs and/or warthogs.

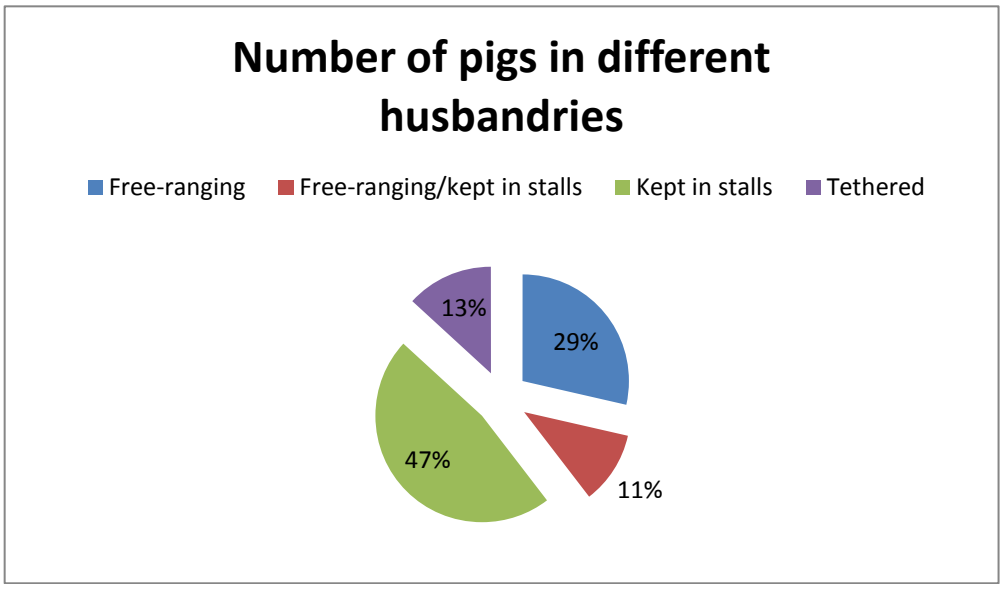

Fig. 8. The different husbandries of the pigs in the study were the highest percentage was kept in stalls.

DISCUSSION

In order to study the transmission dynamics between wild and domestic suids it is necessary to analyze samples from wild suids in the same area as the sampled domestic pigs. Unfortunately the efforts made to catch and sample wild suids, in this study represented by warthogs, was futile. Therefore no conclusions could be made regarding the transmission dynamics neither could a prevalence study of PCV2 be made in the warthog population of Murchison Falls.

Although it is not possible to draw any conclusions regarding the transmission dynamics in the wildlife-livestock interface from this study, the potential for pathogens to spread between wildlife and domestic animals and the risk for EIDs in developing countries cannot be ignored. The keeping of pigs in Uganda is a risk factor for domestic pigs to come into contact with wildlife since many pigs are free-ranging or partly free-ranging, 29% and 11% respectively in this study, and many pigs, 26%, as stated by their owner, were living close to bush pigs and/or warthogs. A possible consequence of this is increased risk of exposure to wildlife pathogen and thereby also increased risk for EID development. Uganda is considered a ”hotspot” for infectious diseases in general including EIDs and with the increasing pig population and human growth development currently taking place in Uganda the wild and domestic suid species and their viruses is an important area of study for the EID surveillance.

PCV2 is a well-known virus and have been found in pig populations all over the world, including Asia, Europe, North America, Oceania and South America (Patterson and Opriessnig, 2010). However, no earlier studies on the prevalence of PCV2 have, to the author´s knowledge, been performed in sub-Saharan Africa except for South Africa (Grau-Roma, 2011). The results of this study suggest that PCV2 is present in Uganda and that it is

not an uncommon virus. The point prevalence for PCV2 SG3 in this study, 77%, cannot be assumed to be applicable to all of Uganda since the sample selection is too small to draw that conclusion. More extensive studies are necessary in order to achieve more applicable results when it comes to prevalence. Compared with study in the USA by Shen et al (2012) the prevalence of SG3 is very high in this area of Uganda, 77% compared to 8.4% for PCV2b in the USA. This comparison should not be made without consideration to the fact that the USA study was performed on mostly healthy pigs. Also, a large portion of the pigs in the USA study were vaccinated. In this current study, vaccination status was unknown, and no consideration to health status among the sampled pigs was made. Compared to the seroprevalence in China of 10-90% (Ge et al, 2012) and the seroprevalence of 54% in Slovakia (Csank et al, 2011) the prevalence of 77% is reasonable.

It is interesting that the point prevalence was quite high for SG3 and 0% for the other two genogroups tested for. In Sweden the genogroup SG1 is, compared to the other genogroups, quite rare (Timmusk et al, 2007). SG3, or PCV2b, is thought to be the most common genogroup in pigs naturally infected with PCV2 in the world (Grau-Roma, 2011) as it also was for the pigs in this study. A larger prevalence study would be interesting in order to see if there are any SG1 and SG2 (PCV2a) in Uganda at all or if there is only SG3 (PCV2b) and also to see if the true prevalence of SG3 is as high as in this study. To see if PCV2, and what genogroup, is present in the wild population of pigs would also be interesting to investigate.

Some authors suggest that there could be a correlation between pathogenicity and different genogroups of PCV2. Timmusk et al (2008) found that PCV2 SG1 only was present in healthy pigs in non diseased farms. PCV2 SG2 was mostly found in healthy pigs or on farms with recent outbreaks of PMWS and PCV2 SG3 was found to have a high association with PMWS-affected pigs. Timmusk et al (2008) conclude that there is increasing indications of there being a true correlation between in vivo pathogenicity and specific genogroups. Grau-Roma et al (2011) also concludes, in a review, that PCV2b (SG3) seems to be more virulent than PCV2a (SG1 and SG2).

None of the examined factors recorded for the pigs (estimated age, breed, sex, bush pigs/warthogs in the area, husbandry or health status) were risk factors for PCV2 SG3 PCR-positive pigs in this study. This could be because the sample selection was too small and therefore no significant conclusions could be made or because the factors studied truly does not pose an increased risk for PCV2. In order to investigate whether there is a breed predisposition for PCV2, a study with a larger sample quantity for the different breeds must be performed. In this study the group “improved” consisted of only 4 pigs out of 91 and therefore conclusions regarding breed predisposition is impossible.

The SG3 PCR-positive samples can be assumed to contain low concentrations of viral DNA since the main part of the CT-values were quite high and the bands on the gel electrophoresis were weak. Primer-dimer formation is a well known problem when running SybrGreen PCR, but the PCR protocol in this study was probably not completely optimized since a large

portion of the samples came out doubtful and/or with primer-dimer formation. The primer-dimer formation and the apparently low concentration of viral DNA in the positive samples makes the interpretation of the results more difficult and can pose as a source of error. There is a potential risk that the positive samples are non-specific amplifications, although when chosen samples were run on agarose gel for confirmation, bands of the expected size were visualized.

The sequencing generated no results of significance since none of the sequences matched PCV2 except for the primers. This is probably caused by the fact that the positive samples contained very low concentrations of viral DNA. In order to generate sequencing results in spite of the low DNA concentration attempts to amplify the DNA additionally could be attempted.

Conclusion

One of the aims of this project, to investigate the transmission dynamics in the livestock-wildlife interface, was unfortunately difficult to achieve because of technical difficulties in catching and sampling wild suids. Still the current situation in Uganda and its location on the equator makes it a “hotspot” for EID origination. This partly due to the large percentage of the pigs in Uganda that are free ranging and have a high risk of coming in contact with wild suids and thereby increasing the chance of viral transmission in the livestock-wildlife interface. Moreover the human growth and consequently the encroachment on wildlife make EID surveillance in Uganda an important task.

The finding that PCV2b (or SG3) is present in Uganda was expected since the virus has been found essentially all over the world. The prevalence in this study among domestic pigs was 77%.

ACKNOWLEDGEMENT

As a Minor Field Study this projects main sponsor is Swedish International Development Cooperation Agency (SIDA) with the additional sponsor being SLU. Without these sponsors I could not have conducted this project.

A special thanks to my supervisor Dr. Anne-Lie Blomström for guiding me through my laboratory work, both in Sweden and in Uganda through quick mail responses, and for help with the writing process. I would also like to thank Dr. Karl Ståhl and Dr. Charles Masembe for the help with planning and conducting fieldtrips and for their encouragement.

For great companionship and support I would like to thank all my travel companions; Fredrik Backlund, Ellen Jönsson, Linn Lernfelt, Camilla Eklundh and Sandra Björk.

Finally I would like to thank all my colleagues at the laboratory in Makerere, and all the people making the fieldtrips possible.

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APPENDIX A

Questionnaire Gulu District Date:

Owner's name:

Owner´s gender:

Subcounty/Village:

1. How many pigs do you keep?
2. Which breed or breeds are your pigs? Improved Local Mixed
3. Are they free-ranging or kept in stalls?
4. What kind of feed do you give to your pigs?
5. Are they healthy? YES NO
6. Do you know if there are bush pigs/warthogs in this area? YES NO
7. Do bush pigs and/or warthogs come in contact with your pigs? YES NO
8. Have you had an outbreak of African Swine Fever recently? YES NO