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THE ROLE OF THE BUSHPIGS IN THE EPIDEMIOLOGY OF AFRICAN SWINE FEVER AT THE WILDLIFE-LIVESTOCK INTERFACES IN UGANDA

BY

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REG. NO. 2010/HD17/877U

DISSERTATION SUBMITTED TO THE DIRECTORATE OF RESEARCH AND GRADUATE TRAINING IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN MOLECULARBIOLOGY AND BIOTECHNOLOGY

OF MAKERERE UNIVERSITY

JANUARY 2017

DECLARATION

I, **Ogweng Peter** do hereby declare that this dissertation is my original work and has not been published or presented for any award in any Institution.

Signed……Date…19TH JAN. 2017

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DEDICATION

To my late parents Mr Olal Bosco (RIP) and Mrs Helen Awel Olal (RIP), my wife Harriet and our lovely children, Awel Claire Pirwot and Ongom Benjamin Pirwot.

ACKNOWLEDGEMENT

I greatly appreciate my supervisors Dr. Karl Stahl and Associate Professor Charles Masembe for their tireless efforts in supporting, guiding and encouraging me throughout the research. I also appreciate ASF-Uganda research team supported by the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM), APHIS-USDA, and the Swedish Research Links Programme through the Swedish Research Council for providing all the finances and logistics during the study. I also thank the Wellcome Trust that provided additional financial support to Charles Masembe in the final stages of my MSc is highly appreciated. Appreciations also go to the UWA Officials in Murchison Falls and L. Mburo National Parks, the District Veterinary Departments of Gulu, Nwoya, Amuru, Nebbi, Masaka and Kalungu districts. I appreciate Dr. Tonny Aliro for his technical assistance especially in bleeding domestic pigs and bushpigs, Dr. Denis Muhangi, Mayega Johnson and Susan Ndyanabo for their guidance during the Laboratory work. I also appreciate Dr. Henrik Rasmussen for his time and work in making collars for tracking bushpigs, assistance in bushpig capture and data analysis. My heartfelt appreciations also go to my former Headmaster Mr. Simon Oscar Okumu who permitted me to go for further studies. Finally I thank God the Almighty for having given me the gift of life and seen me through this work.

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LIST OF ABBREVIATIONS

ASF	African swine fever
ASFV	African swine fever virus
C-DNA	Chromosomal DNA
CSF	Classical Swine fever
C_t-VALUE	Cycle threshold
CSV	Comma separated values
DNA	Deoxy ribonucleic acid
Ds-DNA	Double stranded DNA
DVOs	District Veterinary Officers
EDTA	Ethylenediamine Tetra-Acetic acid
FAO	Food and agricultural Organisation
GPS	Global Positioning System
GSM	Global System for Mobile Communication
KMZ	Keyhole mark-up language zipped
MAAIF	Ministry of Agriculture, Animal Industries and Fisheries
MCP	Minimum Convex Polygon
OIE	World Organisation for animal Health (Office Internationale des Épizooties)
PCR	Polymerase Chain Reaction
RNA	Ribonucleic acid
RT-PCR	Realtime- Polymerase Chain Reaction
TAD	Trans-boundary Animal Disease
UBOS	Uganda Bureau of Statistics
UWA	Uganda Wildlife Authority

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ABSTRACT

African swine fever is a devastating hemorrhagic fever of pigs, caused by the African swine fever virus (ASFV). In Africa, the presence of a sylvatic cycle involving warthogs and soft ticks means that the risk of introduction of ASFV into domestic swine is always present. Bushpigs (*Potamochoerus larvatus)* are nocturnal, susceptible to ASFV and share a natural interface with both warthogs and domestic pigs. The role of the bushpig in the epidemiology of ASFV if any is not understood, yet control strategies of the disease in areas with probable contact between bushpigs and domestic pigs would depend on such information. This study used a molecular and ecological approach to investigate the role of the bushpig in the epidemiology of African swine fever at the wildlife-livestock interface in Uganda. The GPS/GSM tracking collars were used to monitor the movement patterns of free-ranging domestic pigs and bushpigs. Four free-ranging domestic pigs and five bushpigs were collared and their movements monitored for two weeks and two months respectively. Domestic pigs had a peak daily distance of 2,521m; mean home range of 43,239m^2 (ranging from 3,929–143,822m^2) with a mean core utilization area of 13,328m^2 (ranging from 744–47,416m^2). The domestic pigs mainly roamed around homesteads, gardens, water points and rubbish pits during both day and night. The bushpigs were active from the evening and throughout the night wandering between game reserves and domestic farmlands with minimal activities during daytime. This study describes a new bushpig peak daily distance of 15.3Km, home range of 8.5Km^2 in Uganda. There was some overlap between free-ranging domestic pigs and bushpig activity times, indicating the possibility of interaction between the two species. The ASFV detection was done in domestic pig and bushpig samples. Thirteen (8.9 %) domestic pig blood samples were positive for ASFV. One (7.1 %) bushpig sample tested positive for ASFV with C_t value of 35.8. There was probable evidence of interaction between the potentially ASFV positive bushpig and free ranging pigs indicating that the bushpig could be playing a role as an ASF epidemiological link at the wildlife-livestock interface between the sylvatic and non-sylvatic cycles. However this observation requires deeper and full genome sequencing to compare the ASFV genetic characteristics for a robust conclusion.

Key words: African swine fever, bushpig, epidemiology, wildlife-livestock interface

1.0 INTRODUCTION

1.1 Background to the study

African swine fever (ASF) is a devastating haemorrhagic fever of pigs that causes up to 100% mortality (Arias and Sanchez-Vizcaino, 2002; Costard *et al.*, 2013; Penrith *et al.*, 2004). This typically lethal disease of domestic pigs is caused by a double stranded DNA (ds-DNA) virus, the African swine fever virus (ASFV) that is classified within the *Asfarviridae* family; with genus *Asfivirus*, as the only member (Dixon *et al.*, 2005; Takamatsu *et al.*, 2011). The disease is contagious and there is neither vaccine nor cure for the disease (Penrith *et al.,* 2004). The ASF disease is one of the most serious diseases of pigs in the sub-Saharan African countries that are still threatening the pig population and the rural economy of other continents (Arias and Sanchez-Vizcaino, 2002; Costard *et al.*, 2009).

Uganda is a country where 85% of the population live in rural areas with majority of the people depending on agriculture (UBOS, 2010). The livestock sectors, including pig farming are steadily increasing in response to the demand for livestock products. The country has the second largest and most rapidly growing pig production system in Africa (Phiri *et al.*, 2003), and the population of pigs in Uganda by the year 2010 stood at over 3.2 million (UBOS, 2010).

However, the pig sector in Uganda is affected by several constraints with farmers facing a continuous threat from infectious diseases, of which ASF is the most feared. African swine fever is endemic in Uganda's domestic pig population (Gallardo *et al.,* 2011c; Aliro *et al.,* 2012; Muhangi *et al.,* 2012; Muwonge *et al.,* 2012; Atuhaire *et al.,* 2013) with outbreaks occurring at regular intervals, with over 20 outbreaks confirmed in 2009 alone (Aliro *et al.,* 2012), and there have been several reported outbreaks annually throughout the country. More than 75% of Ugandan pigs are found in smallholder farms in the rural areas (UBOS, 2010) and some of these farms are within short distances from wildlife reserves, which poses challenges in disease control in case of disease spill over from the wild.

In Uganda, control of the disease has been and is still a big challenge for most farmers although the farmers are aware to a large extent of the signs and symptoms of the disease in addition to some control measures (Chenais *et al.,* 2015) but there seem to be other factors that prevent farmers from implementing the necessary actions. In some studies, it is suggested that the most

feasible approach would be confining the pigs to reduce contact between pigs from different farms during ASF outbreaks (Thomas *et al.,* 2013).

The epidemiology of ASF is complex with three different cycles. The traditional sylvatic cycle involves the natural non-affected reservoirs of the virus, the common warthog (*Phacochoerus africanus*) and the soft ticks in the genus *Ornithodoros*. The second is the tick-domestic pig (non-sylvatic) cycle, where the soft tick infests the pig farms. Thirdly, a domestic pig cycle in which the virus is transmitted directly or indirectly from pig to pig and appears capable of persisting in domestic pigs without either traditional vertebrate or invertebrate sylvatic host (Jori and Bastos, 2009).

The sylvatic cycle has been a dominant cycle, and ASF outbreaks occurred more or less frequently as a result of spill overs to domestic pigs at the wildlife-livestock interface (Penrith *et al.*, 2013). Today, however, the importance of the traditional sylvatic cycle is reduced. The man-made non-sylvatic domestic cycle in which the virus is transmitted directly or indirectly from pig to pig is considered as the only means of ASF transboundary spread across long distances and is today the main cause of persistence in endemic areas (Penrith *et al.,* 2013).

The reasons for the significance of this cycle are threefold. Firstly, the increasing number and densities of domestic pigs since many people are now venturing into pig farming. Secondly, uncontrolled pig movements due to trade in pig and pig products, which seems to escalate the rate of disease spread in case of an outbreak (Aliro *et al.,* 2012, Muhangi *et al.,* 2015). Thirdly, the interface between warthogs/ticks and domestic pigs is limited in many countries today preventing spill over from the sylvatic to the domestic cycle. However, the bushpig often has been reported to raid crops in domestic areas and it could be playing a role in the transmission of ASF from the wild to domestic pigs at the wildlife-livestock interface.

The ASFV infects the wild suids (warthogs, bushpigs and giant forest hogs) but no clinical signs are developed. Warthogs, however, are known for being ASFV reservoirs once infected (Plowright *et al.,* 1969; Pini and Hurter, 1975; Thomson, 1985). Soft tick vectors for ASFV have also been identified as ticks of the genus *Ornithodoros*. *Ornithodoros erraticus* is found in Spain, Portugal and western North Africa, while another species, *Ornithodoros sonrai* has been reported in Senegal in rodent burrows that may sometimes be found in or close to pigsties (Etter

et al., 2011 and Vial *et al.,* 2007).

The *Ornithodoros moubata* complex is found in East and Southern Africa where they are commonly found inhabiting human dwellings but also warthog burrows and pigsties, the latter demonstrated particularly in south- western Malawi and Mozambique (Haresnape and Mamu., 1986; Haresnape and Wilkinson., 1989; Haresnape *et al.,* 1985, 1987, 1988). *Ornithodoros moubata* ticks have, however been found very widely in warthog burrows in southern and eastern Africa in Kenya, Tanzania, Uganda, Namibia, south Africa, Zambia, Zimbabwe and most recently in Mozambique (Quembo *et al.,* 2015) and in Swaziland were the ticks were not infected with ASFV (Boshoff *et al.,* 2014).

Warthogs are considered as the main wild vertebrate host of the ASFV in the endemic African setting (Costard *et al.,* 2013). Warthogs unlike bushpigs live in burrows, which are also *Ornithodoros moubata* tick habitats. This provides opportunity for warthogs to get into contact with *Ornithodoros moubata* ticks, which if infected with ASFV, can transmit the virus to warthogs through bites. Baby warthogs bitten by ASFV infected ticks in warthog burrows, develop a high enough viraemia under natural conditions able to infect other ticks and keep the cycle going (Thomson, 1985). The infected warthogs remain asymptomatic for life (Jori and Bastos, 2009).

The bushpigs, which are found in many Ugandan wildlife reserves, are also often mentioned as possible ASFV reservoirs (Plowright *et al.,* 1969; Pini and Hurter, 1975; Thomson, 1985). In the absence of a natural interface between the warthog and domestic pig, the bushpig may therefore serve as an epidemiological link between the sylvatic and non-sylvatic cycle. Bushpigs are known to be susceptible to ASFV from experimental reports by Montgomery (1921) and Anderson *et al.* (1998). However, bushpigs do not inhabit burrows; this reduces the chances of contact between them and soft ticks (Costard *et al.*, 2009), and if and how these wild pigs fit into the sylvatic cycle is still unknown .

A significant overlap between bushpigs and domestic pig habitats around the national parks is also likely, possibly resulting in virus transmission to domestic pigs, with or without soft tick involvement (Ståhl *et al.,* 2014). This study therefore investigated the possible role of the

bushpig in the epidemiology of ASFV at the wildlife-livestock interface in selected regions of Uganda.

1.2 Statement of the problem

The bushpig has often been reported to interact with domestic pigs in many areas of their distribution range and are likely to share many pathogens (Jori and Bastos, 2009). Although it is known that bushpigs are susceptible to ASFV infection (Anderson *et al.,* 1998), estimates of ASFV prevalence in diverse natural populations of the bushpigs are lacking. To date, adequate information is lacking on the role of the bushpig in the epidemiology of ASF. The objective of this study was therefore to improve the understanding of the role of the bushpig in the epidemiology of ASF at the wildlife-livestock interface in selected regions of Uganda.

1.2.1 General objective

To investigate the influence of the bushpigs in the epidemiology of ASF at the wildlife-livestock interface in selected regions of Uganda.

1.2.2 Specific objectives

I. To determine the movement patterns and home range of free ranging domestic pigs at the wildlife-livestock interface in the study area.
II. To determine the bushpig movement patterns and home range at the wildlife-livestock interface in Uganda in the study area.
III. To investigate the level of interaction and presence of ASFV among bushpigs and free ranging domestic pigs at the wildlife-livestock interface in the study area.

1.3 Significance of the study

Pigs have a rapid rate of growth and reproduction with a gestation period of about four months. Sows produce 4-12 piglets, which makes them have a high multiplication rate and hence provide high economic returns to farmers. Pig farming requires a relatively small piece of land that is easily affordable for small-scale farmers who are the majority in Uganda. This has made pig farming an activity most ventured in by women and children making it a great activity for poverty alleviation in the poor and post conflict areas of the country, such as the previously war ravaged Northern Uganda.

However, diseases such as ASF constitute a major constraint to the pig farming industry. The respective DVOs of Gulu, Amuru, Lira, Wakiso, Mukono, and other districts in Uganda reported outbreaks annually since 2011. Although the high rate of uncontrolled trade in domestic pig and pig products across the districts of Uganda is mainly responsible for the spread of ASF from one district to the other, it is important to investigate the sylvatic cycle as well.

The role of the bushpig in the epidemiology of ASF at the wildlife-livestock interface is unknown, yet effective disease control strategies can only be designed based on an accurate understanding of the epidemiology of the disease. The findings of this study therefore will be important to domestic pig farmers and all other stakeholders in addressing the knowledge gap currently existing in understanding the epidemiology of ASF.

1.4 Research Questions

I. What is the movement patterns and home range of free ranging domestic pigs at the wildlife-livestock interface?
II. What is the bushpig movement patterns and home range at the wildlife-livestock interface?
III. What is the level of interaction between bushpigs and free-ranging domestic pigs at the wildlife-livestock interface?
IV. Are there ASFV infected bushpigs and free ranging domestic pigs at the wildlife-livestock interface in the study area?

2.0 LITERATURE REVIEW

2.1 African swine fever virus

African swine fever is one of the most serious diseases of pigs in the sub-Saharan African countries that are still threatening the pig population and the rural economy of the other continents (Arias and Sanchez-Vizcaino, 2002; Costard *et al.,* 2009). The ASF disease is a devastating haemorrhagic fever of pigs that causes up to 100% mortality (Arias and Sanchez-Vizcaino, 2002; Penrith *et al.,* 2004), and the disease has no cure (Penrith *et al.,* 2004).

African swine fever is caused by a ds-DNA virus of the family *Asfarviridae* and it is the sole member of the genus *Asfivirus*, in addition to being the only known DNA arbovirus, with a genome of about 170 Kbp (Dixon, 2005; Takamatsu *et al.,* 2011). The virus has been classified into several genotypes with the help of molecular epidemiological studies. These studies uncovered substantial field heterogeneity with at least 23 genotypes being identified to date on the basis of C-terminus p72 gene sequencing (Bastos *et al.*, 2003; Boshoff *et al.*, 2007; Lubisi *et al.*, 2005). The 23rd genotype was recently identified in Ethiopia, where ASF was reported for the first time in 2011 (Achenbach *et al.,* 2016)

The 23 genotypes are distributed differently in various countries where the disease is endemic. The genotypes that have been circulating in Uganda in the last decade were genotypes IX and X (Lubisi *et al.,* 2005). Gallardo *et al*. (2011c) also reported that sequence analysis of the C-terminal end of p72-gene and p-54-gene placed the Ugandan ASFV isolates from 1995 to 2003 ASF outbreaks within genotype IX Uganda.

The ASFV is a complex virus and has been reported to have more than 30 structural proteins (capsid proteins). Some of these proteins have long been considered as important immunodominant antigens for serological diagnosis, especially the p72 and p54 proteins that are encoded by the p72-gene (size is 72 K Da) and p54-gene (size is 54 K Da) respectively (Gallardo *et al.*, 2011a). These proteins are highly conserved, for example the p72 which has been proved to have 97.8%-100% amino acid identity in strains isolated from different parts of the world (Gallardo *et al.,* 2011a).

2.2 The distribution of ASF

The disease is believed to have evolved in Southern and Eastern Africa, where a sylvatic cycle of

maintenance and transmission exists. This involves its natural hosts and vectors that are the warthog and argasid tick respectively (Penrith *et al.,* 2004). The first outbreak of ASF was reported in Kenya by Montgomery in 1921 among the white settlers' pigs. This was followed by reports from South Africa (Steyn, 1932) and Angola (Gago da Câmara, 1933), although the disease in Angola was only confirmed as being ASF in 1943 (Mendes, 1994). ASF then spread from Angola to Portugal, with outbreaks occurring in Portugal in 1957 and 1960 (Wilkinson, 1989), from where the disease later on spread to the rest of the Iberian Peninsula (Arias and Sanchez-Vizcaino, 2002) and eventually to other European countries, the Caribbean and Brazil. ASF was also reported in Malawi (Matson, 1960) and Mozambique (Abreu *et al.,* 1962; Mendes, 1971).

In West Africa, ASF was reported from Senegal in 1978 (Plowright *et al.,* 1994) and in Cameroon there was two early separate outbreaks 1982 and 1985 (Awa *et al.,* 1999; Ekue and Wilkinson, 1990; Nana-Nukechap and Gibbs, 1985). ASF was first reported in Mozambique in 1993 and again in Kenya in 1994 after 30 years (Penrith *et al.,* 2004). In 1996 an outbreak of ASF occurred in Côte d'Ivoire and spread to the neighbouring countries of Benin, Togo and Nigeria (1997), Ghana (1999) and Burkina Faso (2002) and more outbreaks were reported in Senegal, Gambia and Cape Verde during the same period (Penrith et al., 2004).

In 1998 ASF was reported for the first time from the island of Madagascar, where it has since become endemic (Costard *et al.,* 2009; Roger *et al.,* 2001). ASF was reported from Mauritius in 2007 (Lubisi *et al.*, 2009) and more recently ASF has spread into the Caucasus, Russia, Ukraine, Belarus, Estonia, Latvia, Lithuania, and Poland (Costard *et al.*, 2015).

Gallardo *et al.* (2011c) stated that the disease has been reported in many African countries, including Uganda where outbreaks have been recorded in various parts of the country. The disease has been reported in Busia, central Uganda districts of Mukono, Nakasongola and Wakiso in October, 2007 (Gallardo *et al.,* 2011c). Atuhaire *et al.* (2013) reported 30 ASF outbreaks in Uganda between 2010 and 2013 alone. It is also reported in the OIE Animal Health Information System (OIE reporting history) that ASF outbreaks have been annually present in different districts across Uganda from January 2005 until December 2015 (http://www.oie.int, 14-9-2016). Between January to June 2016 alone, the ASF-Uganda Research Team investigated

and collected whole blood and tissue samples from ASF outbreaks in eleven districts (Gulu, Isingiro, Jinja, Kalungu, Kampala, Luwero, Mbarara, Masaka, Moyo, Mukono and Nwoya) in Uganda

2.3 Host range of African swine fever virus

The African wild suids such as the warthog (*Phacochoerus africanus*), bushpigs (*Potamochoerus larvatus*), Red River hog (*Potamochoerus porcus*), giant forest hog (*Hylochoerus meinertzhageni*) are immune to the pathogenic effects of the ASFV but wild boars (*Sus scrofa scrofa*) have the same susceptibility as domestic pigs (*Sus scrofa domestica*) (Jori and Bastos, 2009).

The warthog is considered the original vertebrate host of ASF virus, which together with *Ornithodoros moubata*, the soft tick vector, constitutes the ancient sylvatic virus cycle (Penrith *et al.,* 2004). Two additional virus cycles are recognized to occur in the endemic African setting, namely a domestic pig–tick cycle in which warthogs play no apparent role; and then the domestic pig-domestic pig cycle in which the virus appears capable of persisting in domestic pigs without the agency of either traditional vertebrate or invertebrate sylvatic host (Penrith *et al.,* 2004).

The bushpig has been reported to interact with domestic pigs in many areas of their distribution range (Haresnape *et al.*, 1985; Vercammen *et al.*, 1993; Jori and Bastos., 2009). Montgomery (1921) demonstrated that bushpigs are susceptible to ASFV, followed by later studies including those of DeTray (1963) who reported several isolates of the virus from free ranging bushpigs in Kenya. Other reports confirmed that the Red River hog (*P. porcus*) can become infected with ASFV (Luther *et al.*, 2007). The bushpig (*P. larvatus*) was experimentally infected in Zimbabwe and it became viraemic for 35 to 91 days without showing symptoms (Anderson *et al.*, 1998).

2.4 Transmission of African swine fever virus

The disease being contagious is transmitted through the pig value chain as well as natural means that involve the soft tick vector (*Ornithodoros moubata*) (Costard *et al.,* 2013). The value chain transmission risks include: feeding on garbage containing ASFV infected meat; naïve pigs mixing with pigs from other herds; and contact with fomites such as, vehicles, implements (spades, hoes, etc.), clothes and sampling tools that have been exposed to infected, blood or pork

(Mur *et al.*, 2012). In the tick vector, transmission is reported to occur in the following three ways; trans-stadially, trans-ovarially and sexually (Rennie *et al.,* 2001).

2.5 Bushpig taxonomy and ecology

The bushpig, *Potamochoerus larvatus*, and Red River hog, *P. porcus*, are the two representatives of the African genus *Potamochoerus*. Both species are of medium body size, with an elongated snout and a long, often brightly coloured coat. However, *P. porcus* is brighter in colour than the bushpig, with a distinct white dorsal stripe and crest, long white whiskers and ear tufts, occurring only in West and Central Africa (Vercammen *et al.,* 1993).

The bushpig (*Potamochoerus larvatus*) is darker in coat colour, often without the distinct white masks and long ear tufts characteristic of *P. porcus*. It has a relatively wide range covering most of the sub-Saharan Africa right from Ethiopia to South Africa. Although two morphological variants are suspected (Vercammen *et al.,* 1993), data on distribution are sketchy and imprecise. Genetic studies on *Potamochoerus* are lacking and would be useful for clarifying the systematic and distributional limits of all members and variants of this species.

The distribution of the bushpig appears to be limited by the continuous availability of food, water, and forest cover. Bushpigs are rarely reported in open woodland, savannah, or other more arid and open habitats (Seydack, 1990). Bushpigs are found more frequently in areas of intensive agriculture, probably due to their nocturnal habit of crop raiding which allows them to survive and flourish in such places. They are also found in swamps, thickly wooded areas, and forests, along water sources (Skinner and Smithers, 1990). Therefore, due to their use of habitat and resources, there is thus a greater chance of contact between bushpigs and domestic pigs than between warthogs and domestic pigs in Uganda, especially in communal areas with agricultural activities.

Bushpigs live in small family groups with an average of 2.4 and usually ranging between 1–10 individuals (Seydack, 1990). The density of *Potamochoerus spp.* tends to vary in different parts of the world. In South Africa where they have been studied extensively, their density varies from one region to another. This was found to be ranging from 0.35 to 0.5 individuals per square kilometer but can go up to 3 individuals per square kilometer in tropical forest regions of the country (Skinner and Smithers, 1990).

Bushpigs are found to have a pattern of life in which they are usually sedentary, territorial, and predominantly nocturnal, although diurnal activity occurs in cooler months (Seydack, 1990). During daytime periods they are mostly inactive, they take shelter in dense vegetation. Bushpigs are found to construct bad weather nests during cold and wet spells. The report of these early studies in South Africa revealed that average daily movement distances for bushpigs were found to be 3 kilometers, ranging between 0.5 and 5.8 kilometers in western South Africa (Seydack, 1990).

2.6 Laboratory diagnosis of ASFV

The ASFV can be diagnosed by a number of laboratory techniques, which include conventional Polymerase Chain Reaction (PCR), Enzyme-Linked Immunosorbent Assay (ELISA) and Real Time-PCR (RT-PCR). However, for the purpose of this study, only RT-PCR was used since it detects ASFV in pigs at a viraemic stage when transmission from one pig to another is most likely. The ELISA on the other hand, detects antibodies that are developed by the pig at a later stage, yet early detection of the disease is important in surveillance and effective control.

2.6.1 The RT-PCR detection of ASFV

Real-time PCR analysis detects specific nucleic acid amplification products as they accumulate in real-time. As the RT-PCR progresses, small amounts of PCR products, which can be DNA, cDNA or RNA are quantified. The RT-PCR is based on the detection of the fluorescence produced by a reporter molecule, which increases as the reaction proceeds. The fluorescence occurs due to the accumulation of the PCR product with each cycle of amplification.

During polymerization, a reporter fluorescence dye and a quencher dye are attached to a TaqMan® probe. Fluorescence from the reporter dye's emission is observed once both dyes are attached to the probe. Once PCR amplification begins, DNA polymerase cleaves the probe, and the reporter dye is released from the probe. The reporter dye, which is separated from the quencher dye during every amplification cycle, generates a sequence-specific fluorescent signal. The signal increases in real time as PCR cycles continue; the fluorescence intensity increases proportionally.

The RT-PCRs are quantitative or semi-quantitative with a cycle threshold (C_t) value that depends on the number of amplification cycles required to attain a threshold value (C_t value). A positive

sample results in a sigmoid curve, which clearly shows the C_t value of a particular sample. Lower C_t values correspond to higher concentration of viral DNA in the sample. The RT- PCR assays are advantageous because they are easy to perform, highly sensitive, specific and provide scope for automation.

3.0 METHODOLOGY

3.1 Study Area

The main part of the study was conducted in the districts of Amuru, Gulu, Nebbi and Nwoya, including Murchison Falls National Park (Figure 1). Part of the study, however, also included Lake Mburo National Park and Katonga river basin.

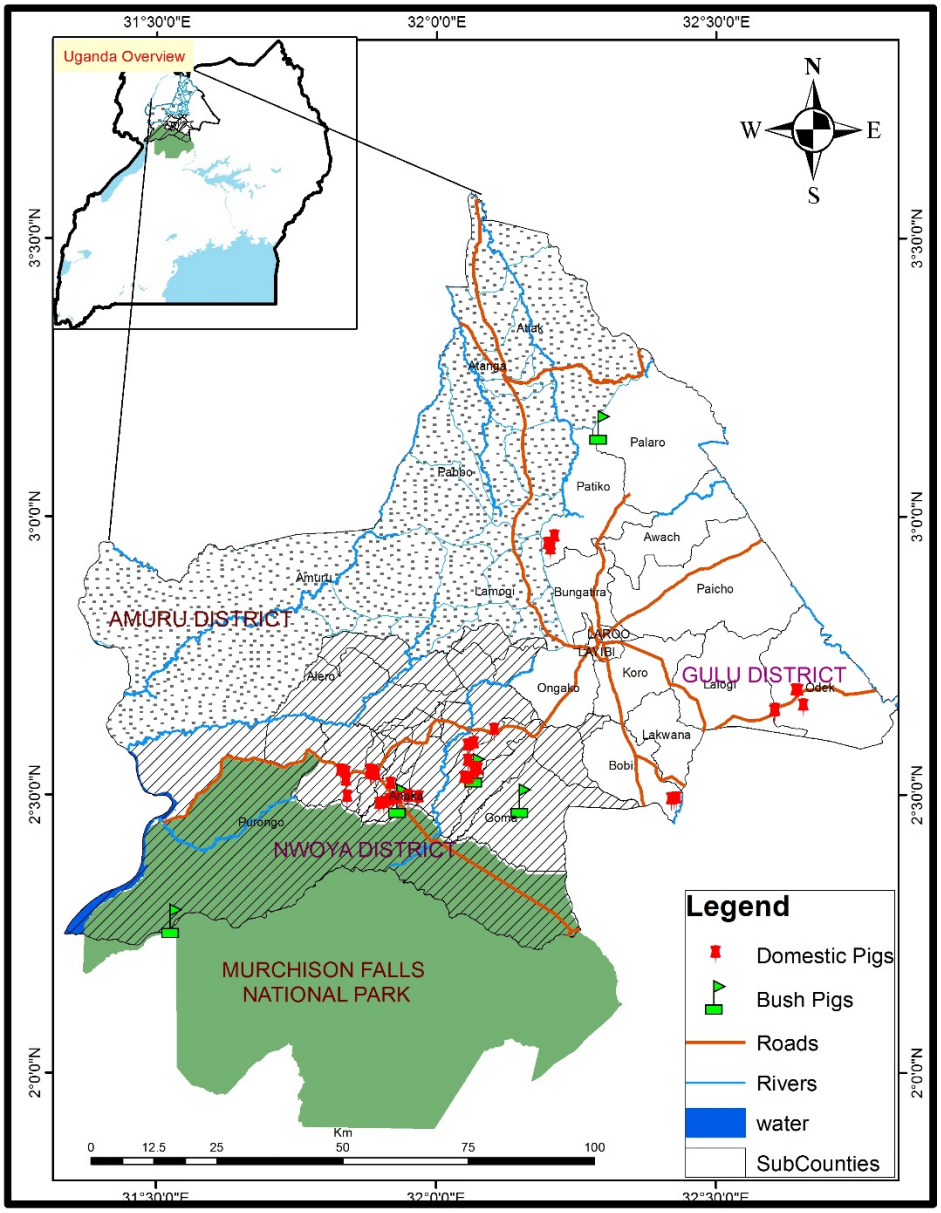

Figure 1: Locations of domestic pig farms sampled and bushpig capture sites in the Districts of Amuru, Gulu, Nwoya and Murchison Falls National Park

Map generated using ArcGIS v10.3.

3.1.1 Amuru District

The Ugandan Parliament established Amuru District in 2006. Prior to that, the district was part of Gulu District. Amuru District is bordered by Adjumani District to the north, South Sudan and Lamwo District to the northeast, Gulu District to the east, Nwoya District to the south, Nebbi District to the southwest and Arua District to the west. The administrative headquarters of the district at Amuru are located approximately 60 kilometers, by road, west of Gulu, (*Amuru District Information Portal [Online]*, 2014).

The district lies at the coordinates of $02^0 50'$ N and $33^0 05'$ E, and it is well known for its fertile soils. About 98% of the people in Amuru District earn a living through subsistence farming. The extreme eastern part of the district shares a boundary with the River Nile, whose banks are characterised by thick forests and swamps.

3.1.2 Gulu District

Gulu District lies 332 kilometers from the national capital Kampala and, covers an area of 11,732 square kilometers, comprised of open waters and swamps (180 square kilometers), arable land (10,301 square kilometers), national parks and Games Reserves (982 square kilometers) and forest coverage (371square kilometers).

The main economic activity in the district is subsistence agriculture, in which over 90% of the population are engaged in livestock rearing of which domestic pigs form part. The district is located at the coordinates: 02° 45′N 32° 00′E and is bordered by Lamwo District to the north, Pader District to the east, Oyam District to the south, Nwoya District to the southwest and Amuru District to the west (*Gulu District Information Portal [Online]*, 2014).

3.1.3 Katonga river basin

The Katonga river basin (KRB) is a vast wetland with thick marshes formed along River Katonga. This area follows the borderline between Kalungu and Gomba Districts and drains into Lake Victoria. The wetland is located between latitude $32^o 02'$ to $30^o 29'$ E and longitude $0^o 08'$ S to $0^o 10'$ N and covers an area of about 2,478 km^2. The wetland can be classified as a permanent peat forming freshwater swamp.

The predominant vegetation of the wetlands consisted of *Cyperus papyrus* mixed with other vegetation characteristic of wetlands. Most of the areas in KRB were under intensive agriculture, providing gardens for bushpig crop raiding which further supported their existence in this area. Besides practicing intensive crop agriculture, the farmers in this area are involved in domestic pig rearing.

3.1.4 Lake Mburo National Park

Lake Mburo National Park (LMNP) is located in Kiruhura District in Western Uganda. The park is located at the co-ordinates of 00° 36ˈS and 30° 57ˈE. It is situated about 30 kilometers east of Mbarara Town by road, and approximately 240 kilometers from Kampala. LMNP is the smallest park of the ten National parks in Uganda; the park occupies an area of 370 square kilometers (*www.ugandawildlife.org*). The park harbours over 350 bird species and 68 different mammal species including the warthogs and bushpigs.

3.1.5 Murchison Falls National Park

The Murchison Falls National Park lies at the northern end of the Albertine Rift Valley, where the bulky Bunyoro escarpment merges into the vast plains of Acholi land. The Acholi land constitutes seven districts to date, but only the districts of Nwoya and Amuru and Gulu lie within the study area.

The park covers 3,893 square kilometers and is Uganda's largest protected area. Today it is part of the even larger Murchison Falls Protected Area (5,072 square kilometers) that includes the adjoining Karuma and Bugungu Wildlife Reserves. The park is dominated by mammals such as warthog and bushpigs among others (*www.ugandawildlife.org*).

3.1.6 Nebbi District

Nebbi District lies to the west of Nwoya District which is located approximately 44 kilometers, by road, southwest of the Northern Town of Gulu, the largest metropolitan area in the sub-region. This location is approximately 330 kilometers, by road, north of the city of Kampala, Uganda's capital and largest metropolitan area. The coordinates of the district are: 02^0 38ˈN, 32^0 00ˈE.

3.1.6.1 Locations of sampled domestic pig farms in Nebbi District

Nebbi District lies along the western side of River Nile and also borders MFNP from the Northwestern side (*Nebbi District Information Portal [Online]*, 2014). The communities in Nebbi, especially those along the Nile are involved in both fishing and agriculture (including domestic pig rearing) for economic gain. The swamps along river Nile in the district together with MFNP are homes to bushpigs and warthogs in this area (Figure 2).

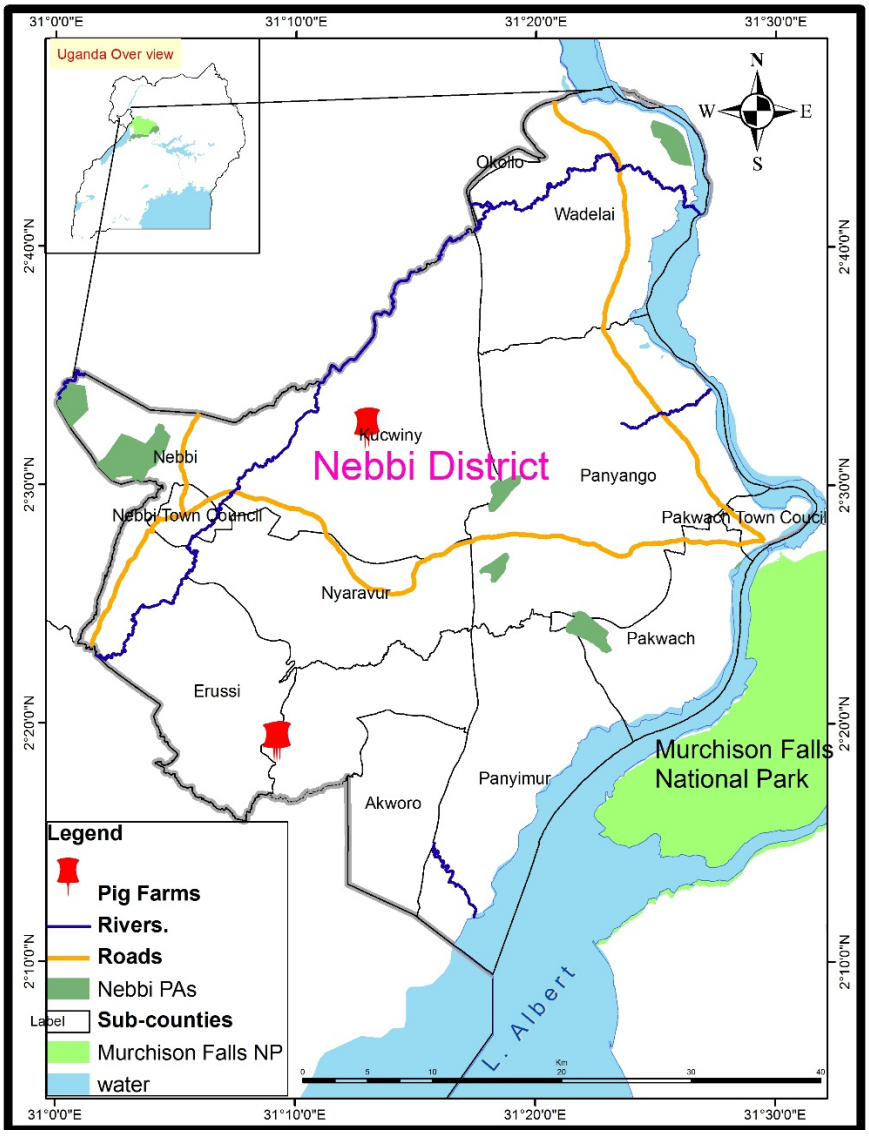

Figure 2: Locations of sampled domestic pig farms in Nebbi District
Map generated using ArcGIS v10.3.

3.1.7 Nwoya District

Nwoya District is one of the newest districts in Uganda. It was established by the Act of Parliament and began functioning on 01 July 2010. Prior to that date, it was part of Amuru District. The district is bordered by Amuru District to the north, Gulu District to the northeast, Oyam District and MFNP to the east, Kiryandongo District, Masindi District and Buliisa District to the south.

The major economic activities of the people of Nwoya are intensive agriculture and livestock keeping of which domestic pig rearing forms a sizeable percentage. The proximity of MFNP to Nwoya District provided easy movement of wild pigs from the park to raid crops in gardens and possibly interact with domestic pigs (*Nwoya District Information Portal [Online],* 2014).

3.2 Study Design

The suitable study locations were identified with the assistance of local hunters, DVOs of the respective districts and UWA staff. Bushpigs were captured and sampled from the wildlife-livestock interfaces of Murchison Falls National Park, Katonga River Basin and Lake Mburo National Park, while domestic pigs were sample from farms within 10 Kilometers from the boundaries of the national parks. Twenty-nine sampling sites were selected purposively to get ASFV positive samples, provided they were within 10 kilometers from the park boundary at the wildlife-livestock interface or had reported cases of bushpig activities or ASF outbreaks in the study areas (Table 1).

Table 1: Sites that were selected for domestic pig and bushpig sampling

District	Sub-county	Parish	Village
Amuru	Alero	Panok-Rach	Lakang
Gulu	Palaro	Mede	Got-Oywec
	Odek	Binya	Rom-kituku
	Patiko	Pugwinyo	Adak
	Lalogi	Jaka	Wanglobo
	Bobi	Lukwo	Bar-Olam
		Patek	Bar-dyel
KRB	Lwabenge	Kiraga	Mabaale
			Kasabwera
		Bugomola	Serinya
		Bukulula	
Nebbi	Kucwiny	Vurr	Vur-waradi
			Jupasonga
	Parombo	Pagwata	Jupukok
Nwoya	Purongo	Latoro	Latoro
		Patira-Kiba E	Job
			Pabit
			Olwiyo
			Patira E
			Lagaji
			Wipolo
	Anaka	Todora	Laliya
			Agung pabali
		Orum	Alwaka
	Koch-Goma	Agonga B	Lamin-latoo
		Koch-li	Kamcoo
		Kal	Lutuk
Nebbi/Nwoya	MFNP	MFNP	MFNP
Kiruhura	LMNP	LMNP	LMNP

Key: MFNP Murchison Falls National Park

LMNP Lake Mburo National Park

3.2.1 Domestic pig restraining and collaring

Four free ranging domestic pigs were collared from different farms at the wildlife-livestock interface in the main study area to determine their home range. A mature (three month old and above) free ranging domestic pig was purposively selected from each of the selected farms with consent of the farmers. The selected domestic pig was then captured and restrained using a pig catcher after which a GPS/GSM tracking collar (Savannah Tracking Ltd, Kenya) was then tied and fastened on it. The collared domestic pigs' movements were monitored for two weeks (Figure 3).

Figure 3: A collared free-ranging domestic pig in Agung, Nwoya District

3.2.2 Bushpig capture and collaring

Bushpig capture was performed using game capture nets (50x3 m, 150mm square mesh, 3.5mm nylon braid khaki, ALNET Ltd, South Africa). The research assistants, local hunters and park rangers assisted in tracking the bushpigs during early mornings. Once their locations were identified and their presence ascertained by presence of tracks moving into a bush or swamp and absence of track moving out, then the nets were quietly and quickly set on one side of the bush or swamp and the bushpigs were chased into the nets.

The hunting sessions involved walking in the parks and other identified bushpig hideouts in the communities for six hours every day, which on many occasions resulted in no capture success. The team had to endure months of hectic hunting sometimes without capture; these difficulties that were experienced during the study limited the sample size, and this prompted the study to get tissue samples from hunted and killed bushpigs as well.

Figure 4: A research assistant carrying a game capture net (ALNET Ltd, South Africa), as the rest of the team set the other net

The captured bushpigs were immobilized with zoletil 100, at a dose of 300-350 mg/ adult pig (Kock *et al,* 2006). Bushpigs are known to be susceptible to overheating and stress especially after capture, which causes subsequent death. To overcome this problem, immediately after immobilization, the captured bushpigs were then quickly moved under tree shades in some to protect them from direct sunlight and their eyes covered with a cloth. Cold water was poured on the captured bushpigs to reduce their body temperature. Five bushpigs were captured and fitted with GPS/GSM tracking collars (Figure 5) of harness type (*Savannah Tracking Ltd, Kenya*).

Figure 5: The GPS/GSM tracking collars used in collaring bushpigs and domestic pigs

Figure 6: A captured bushpig being fitted with a GPS/GSM tracking collar

The captured and collared bushpigs' movements were monitored in real time for a period of two months provided the collars remained on them. The bushpig movement data were uploaded at Savannah Tracking Data Manager Website (www.savannahtracking.com) every six hours.

3.2.3 Collar recovery from bushpigs and domestic pigs included in the study

The last six-hour GPS position of the bushpig was downloaded from the main server as Keyhole mark-up language zipped (kmz) files. The kmz files were imported to Google Earth to generate an aerial view of the pig movement pattern in real time. The recorded movement pattern and last GPS co-ordinates were then used to track the bushpig.

The GPS co-ordinates were then plotted on the hand held GPS available in the research area. This particular position was then located in the field and that formed the starting point of tracking the bushpig although it was very challenging. The challenges were caused by collars falling off the bushpigs in areas with no GSM network coverage or bushpigs being killed by local hunters and throwing the collar in areas without GSM network coverage.

In free ranging domestic pigs, the collar recovery was easier because most of the time these pigs came back to their homesteads. The farmers were notified a day before the collar recovery date to capture the pig in advance. There were some instances that the farmer failed to locate and capture the collared domestic pigs. In such situations, the last co-ordinates of the domestic pigs were downloaded from the main server *(www.savannahtracking.com)* and plotted on a hand held

GPS. The domestic pig was then tracked using the plotted co-ordinates until the collar was recovered.

3.2.4 Pig movement monitoring using GPS/GSM collars

The movements of bushpigs and domestic pigs were monitored using data from deployed GPS/GSM tracking collars. A GPS/GSM tracking collar acquires GPS points using GPS at user defined interval and for this study it was every one-hour (1loc/hr.) except for the bushpig in Lake Mburo, which was one location every fifteen minutes (4loc/hr.). The GPS data were then downloaded via internet to the savannah data manager (*www.savnnahtracking.com*) for further analysis.

All data were stored in memory on the collar as backup and transmitted only once the collar was within GSM coverage of a mobile phone network. The recorded bushpig and domestic pig locations were visualized in real time through Google Earth (*www.google.com/earth*) using kmz files.

3.2.5 Pig movement GPS/GSM data analysis

The GPS/GSM pig movement data from the Savannah Tracking server were downloaded as Comma separated value (csv) files. The csv files were then imported into ArcGISv10.3 and projected into UTM WGS 36 N to give latitude and longitude co-ordinates. The latitude and longitude co-ordinates were converted to distances in meters and then imported into Microsoft Excel. Imported Microsoft Excel data was then used to calculate daily distances and average hourly activity pattern of each collared pig. These figures were used to generate graphs of daily distances and average hourly activity patterns of pigs.

Ground truthing of the movements of each collared pig was done to ascertain what were present at the most utilised locations of the pigs. These locations were identified from the Google Earth print out of each pig's movement data, where the most utilised locations were defined as those locations that were frequently visited by the pig and therefore had many recorded GPS points crowded together. This was vital in the identification of water points and rubbish disposal points in each pig's core utilisation area.

The estimated home range (HR, 90% Utilization Distribution, UD) and core area (50% UD) (Borger *et al.,* 2006) for each collared bushpig and domestic pig was done using the fixed-kernel method function from the ADEHABITAT package (Calenge, 2006) in R version 3.1.1. Kernels were estimated using the reference bandwidth method because the least-squares cross-validation method failed to converge. Each home range was generated from all the available locations except the 2 first days of tracking to avoid biased movement due to abnormal behavior after the capture for each animal to maximize the precision of the estimate (de Solla *et al.,* 1999).

The number of locations available for each animal was above 50, sufficient for estimating the surface of the home range (Seaman *et al.,* 1999).The home range was also estimated using the minimum convex polygon, taking into account 100% of locations (100% MCP), in order to compare our results with other studies and to determine the maximum area covered by the animals.

3.2.6 Bushpig blood and tissue sample collection

Blood samples were collected from 11 captured bushpigs and three tissue (spleen and kidney) samples from bushpigs were opportunistically obtained from local bushpig hunters in the study area. Whole blood was collected from the jugular vein of captured bushpigs using EDTA coated vacutainer tubes.

3.2.7 Domestic pig blood sample collection

Whole blood samples from 146 free ranging domestic pigs from different farms within 10 kilometers from the National park boundary with the exception of the farms from Gulu, in the study area, were collected during ASF disease outbreaks. The sampling targeted sickly pigs, if available, as well as contact pigs in affected villages. The samples were collected from Gulu district (Lalogi S/C, 11 and Patiko S/C, 10), KRB (Bukulula, 4), Nwoya district (Anaka S/C, 41; Koch-Goma S/C, 38 and Purongo S/C, 27) and lastly Nebbi district (Kucwiny S/C, 8 and Parombo S/C, 7). Pigs were restrained using the pig catcher and whole blood (5 ml) was collected from the jugular veins into EDTA-coated sterile vacutainer tubes (Figure 7). All bushpig and domestic pig samples in the field were kept in a cool box and then transferred to -20^0C and -80^0C in the Molecular Biology Laboratory located in the Department of

Environmental Management, College of Agriculture and Environmental Sciences, Makerere University, for short and long-term storage respectively.

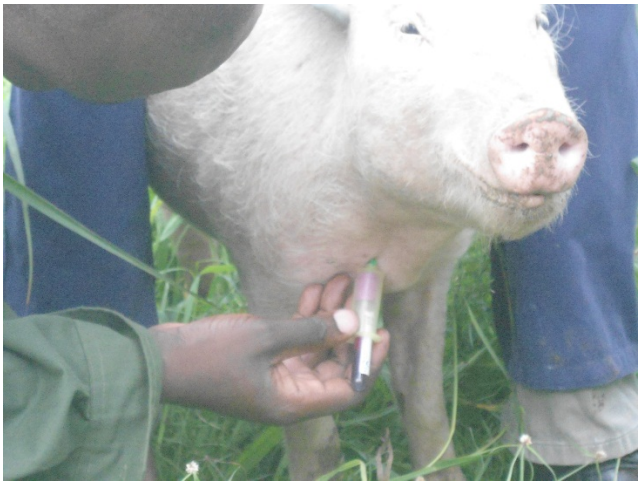

Figure 7: Jugular vein blood sampling of a domestic pig

3.2.8 Laboratory detection of AFSV from pig whole blood and tissue samples

The molecular analysis of samples was done at the Molecular Biology Laboratory in the Department of Environmental Management, College of Agriculture and Environmental Sciences, Makerere University.

3.2.8.1 Whole genome DNA extraction and RT-PCR detection of ASFV

Whole genome DNA was extracted from 100µl of whole blood and 10mg of tissue using a nucleic acid extraction kit (QIAGEN/DNeasy Blood and Tissue kit) following the manufacturer's procedures. The extracted DNA products were analysed by electrophoresis through 2% agarose gel visualised under ultra violet light.

A commercially available RT-PCR kit (Tetracore Inc., Rockville, MD, US), based on the method described by Zsak *et al.,* (2005) was used for the detection of ASFV DNA in the blood and tissue samples in accordance with the instructions from the manufacturer. To each ASFV PCR Assay Smart Cycler tube, 22.5 µl of rehydration buffer was added. 2.5 µl of each extracted sample DNA was then added to the different tubes marked for the particular samples, two tubes were marked for the controls (positive and negative controls) and for the positive control 2.5 µl of rehydrated ASFV DNA positive control sample was added, and 2.5 µl of 1 x Tris-EDTA was

added to the negative control tube, making a final PCR reaction volume of 25 µl for all the tubes. The Smart Cycler tubes were then incubated at room temperature for three minutes and then run on a Smart Cycler Real Time PCR machine (Cepheid, Inc., Sunnyvale, California) with 45 amplification cycles (95°C for 2 s and 60°C for 30 s). This method is quantitative, with a cycle threshold (Ct) value that depends on the number of amplification cycles required to attain a threshold. Lower Ct values correspond to higher concentration of viral DNA in the sample.

4.0 RESULTS

4.1 Free ranging domestic pigs movement and home range at the wildlife-livestock interface

4.1.1 Activity patterns of collard free ranging domestic pigs

The GPS collars stayed on all the four domestic pigs for the required duration of two weeks (Table 2). However, the collar harness caused injuries on one of the domestic pigs in Gony-cogo, which was properly treated after collar removal and the farmer reported full recovery. In another instance a collar fell off from one of the domestic pigs in Olwiyo after two weeks before collar recovery. This collar was never recovered probably because it could have fallen in an area with very limited or no mobile phone network.

Table 2: Sites where domestic pigs were collared and the duration of collaring

Date	Duration of collaring	Location
18/7/2012	2 weeks	Nwoya, Agung
29/3/2013	2 weeks	Nwoya, Gony-cogo
29/3/2013	2 weeks	Nwoya, Lutuk
03/11/2012	2 weeks	Nwoya, Olwiyo

The recorded free ranging domestic pigs movement pattern showed that the domestic pigs were generally active during both day and night hours, with high activity patterns during certain hours although this varied from one pig to the other.

The Gony-cogo free ranging domestic pig was more active between 06:00 to 23:00 hours with a peak daily distance of 1502.4 meters (Figure 8) and a peak average hourly activity of 53.6 meters at 14:00 hours (Figure 12). This pig had the third highest peak average hourly activity pattern, compared to the activity patterns of all the collared free ranging domestic pigs.

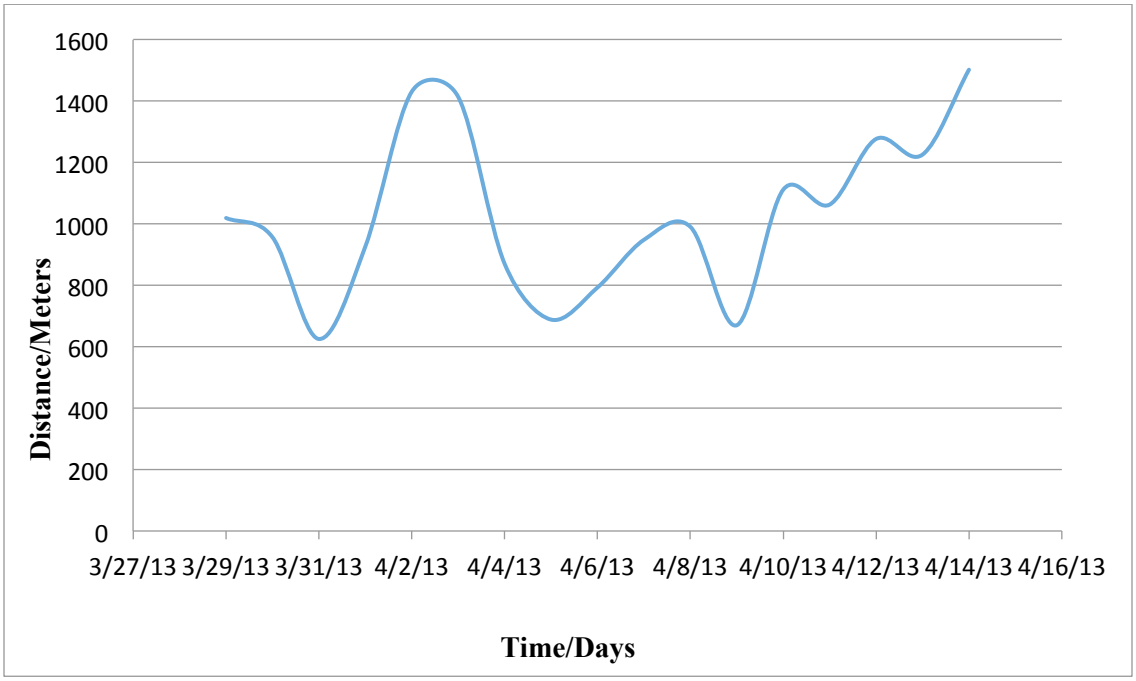

Figure 8: Daily domestic pig movement in Gony-cogo, Koch-Goma sub-county

The Lutuk free ranging domestic pig was more active between 18:00 to 20:00 hours with a daily peak distance of 1070.3 meters (Figure 9) and a peak average hourly activity of 56.2 meters at 19:00 hours (Figure 12). This pig had the shortest peak average hourly activity pattern, compared to the activity patterns of all the collared free ranging domestic pigs.

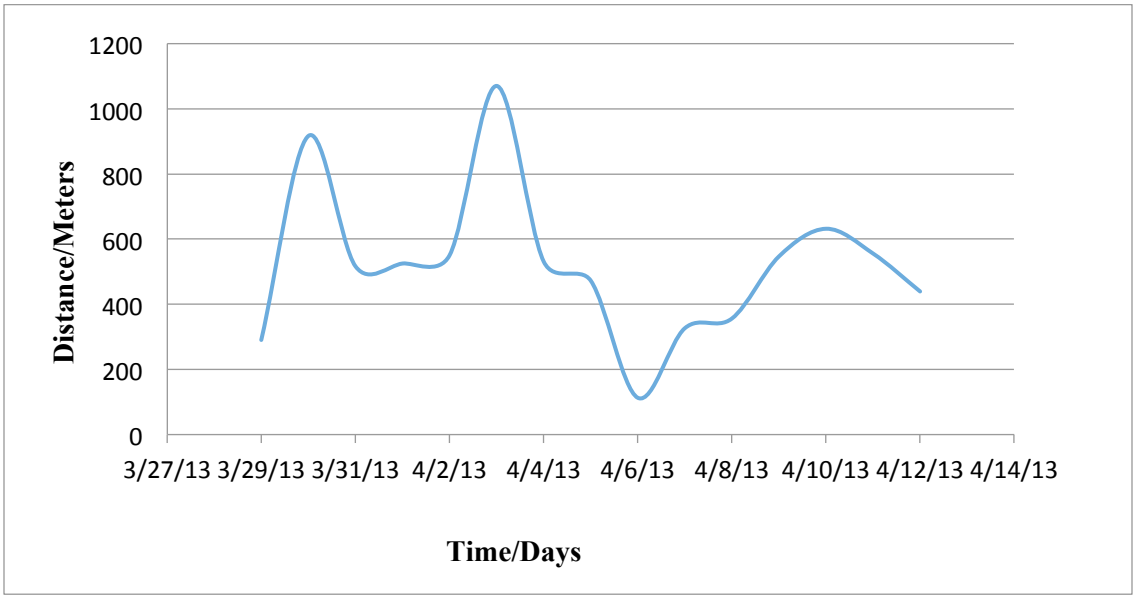

Figure 9: Daily domestic pig movement in Lutuk, Koch-Goma sub-county

The Olwiyo free ranging domestic pig was more active between 06:00 to 19:00 hours with a daily peak distance of 958 meters (Figure 10) and a peak average hourly activity of 228.7 meters at 13:00 hours (Figure 12). This pig had the second highest peak average hourly activity pattern, compared to the activity patterns of all the collared free ranging domestic pigs.

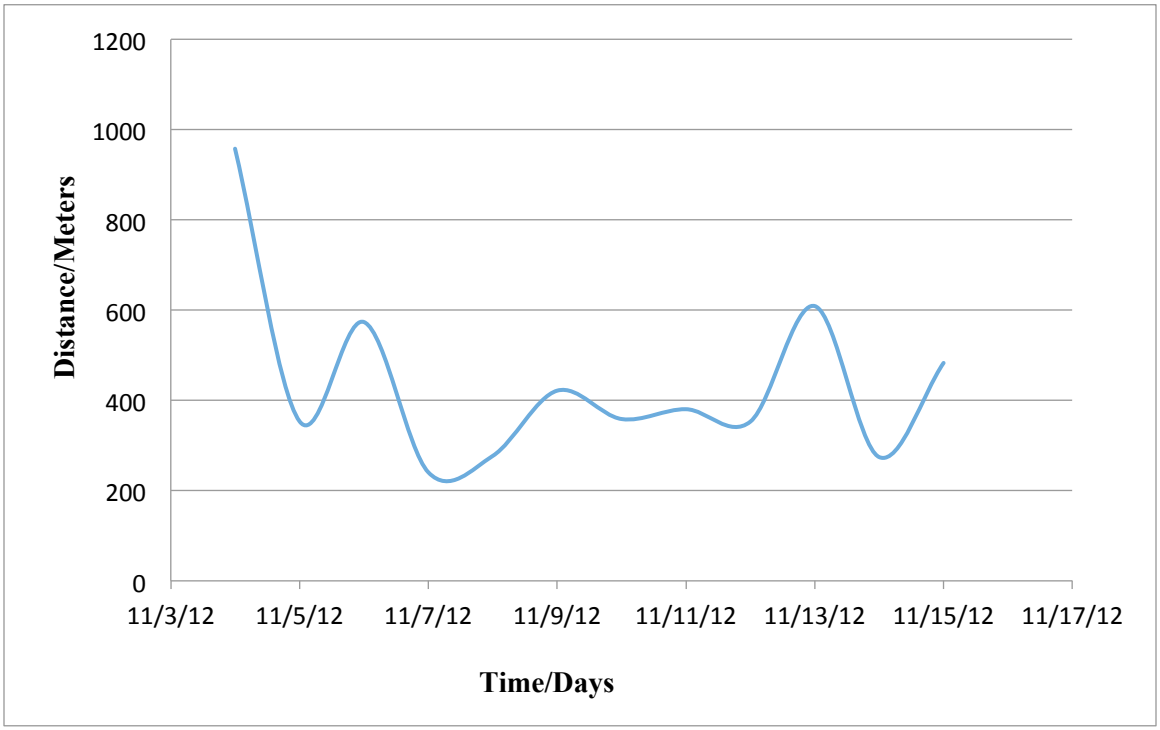

Figure 10: Daily domestic pig movement in Olwiyo, Purongo sub-county

The Agung free ranging domestic pig was more active between 18:00 to 08:00 hours with a daily peak distance of 2520.5 meters (Figure 11) and a peak average hourly activity of 352.9 meters at 03:00 hours (Figure 12). This pig had the highest peak average hourly activity pattern, compared to the activity patterns of all the collared free ranging domestic pigs.

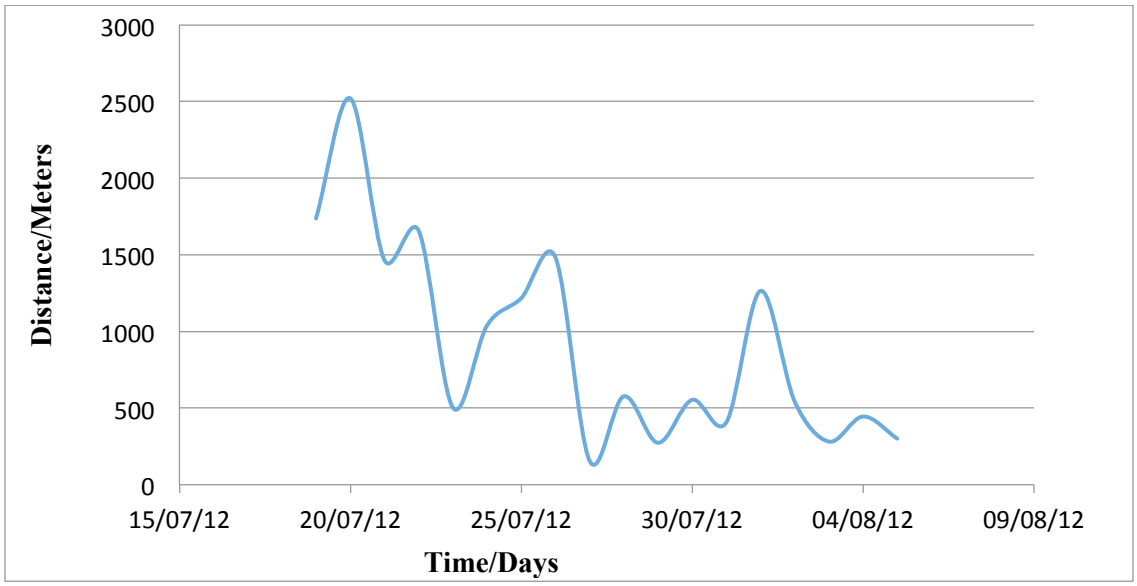

Figure 11: Daily domestic pig movement in Agung, Anaka sub-county

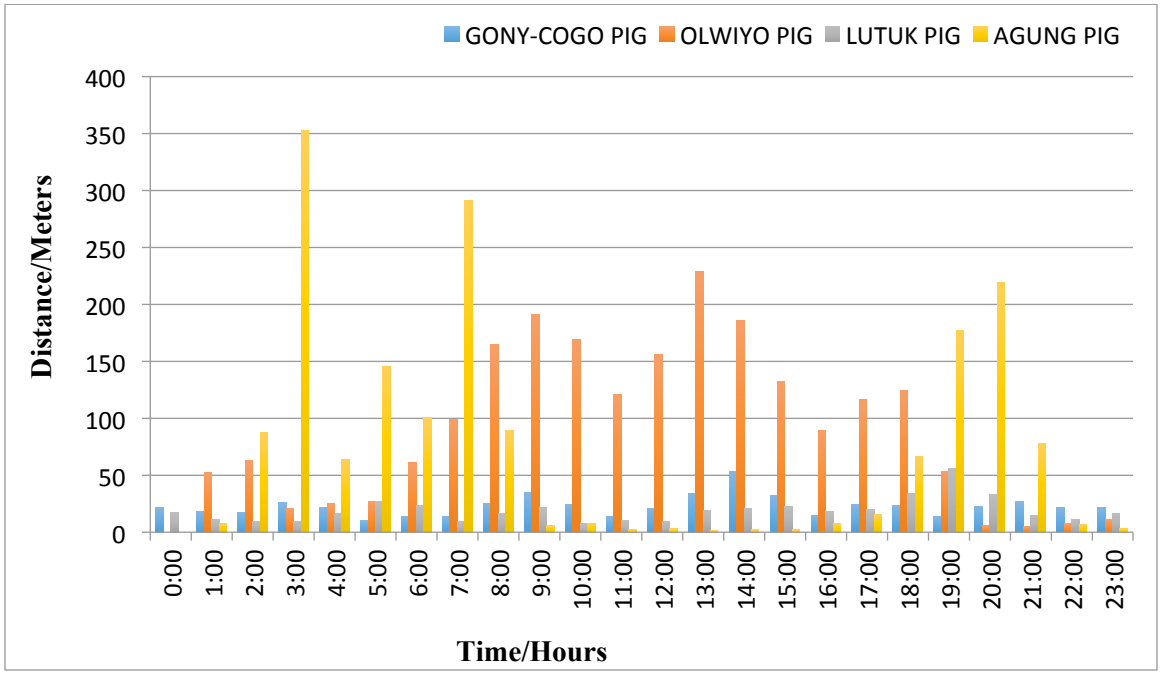

Figure 12: Average hourly domestic pig activity pattern in Gony-cogo, Olwiyo, Lutuk and Agung

The collared free ranging domestic pigs roamed between homes possibly in search of food (Figure 13). The pigs targeted rubbish disposal points and this enabled pigs from different farms

to share feeding grounds among them. Ground truthing of the domestic pig movement also showed that the free ranging domestic pig were some-times involved in crop raiding.

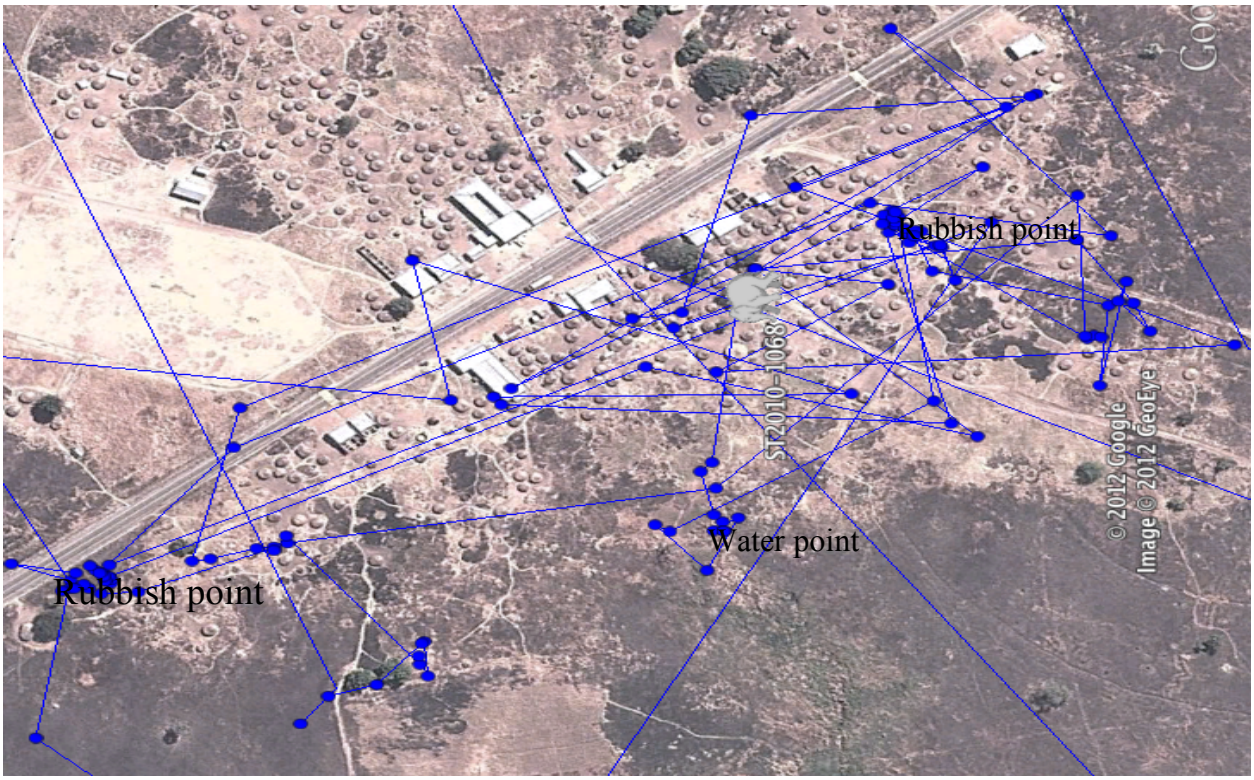

Figure 13: The recorded GPS/GSM domestic pig movement pattern

Key:

GPS/GSM recorded free ranging domestic pig locations

GPS/GSM recorded free ranging domestic pig movement pattern

4.1.1 Home ranges of collard free ranging domestic pigs

The Minimum convex polygon (MCP), Home range and Core area, were calculated for all the four collared domestic pigs (Table 3 and Figure 14). The domestic pigs had a mean home range of 43,239 m^2 (ranging from 3,929 – 143,822 m^2) and a mean core utilization area of 13,328 m^2 (ranging from 744 – 47,416 m^2). There was no collared domestic pig with a home range stretching up to the park boundaries. All the pigs had their core utilization areas at the homesteads, water points and rubbish disposal points.

Table 3: Domestic pig home range, core value and minimum convex polygon calculated values

DOMESTIC PIG ID (Village)	TRACKING DURATION (Days)	START & END OF TRACKING (Date)	FREQUENCY (Location/Hr.)	NUMBER OF LOCATION	MCP AREA (M^2)	HOME RANGE (M^2)	CORE AREA (M^2)
AGUNG	17	18/07/2012 – 05/08/2012	1 location	432	293,402	143,822	47,416
OLWIYO	13	03/11/2012 – 15/11/2012	1 location	294	83,042	9,748	2,027
LUTUK	15	29/03/2013 – 12/04/2013	1 location	340	34,113	3,929	744
GONY-COGO	17	29/03/2013 – 15/04/2013	1 location	395	72,139	15,455	3,126

MEANS: MCP (120,674 M^2), HOME RANGE (43,239 M^2) AND CA (13,328 M^2)

Minimum convex polygon (MCP): Smallest convex polygon enclosing all the recorded GPS locations of the animal.

Core area (CA): Most frequently used part of the home range.

Home range (HR): Area traversed by the animal during its normal activities such as foraging, mating and caring for the young.

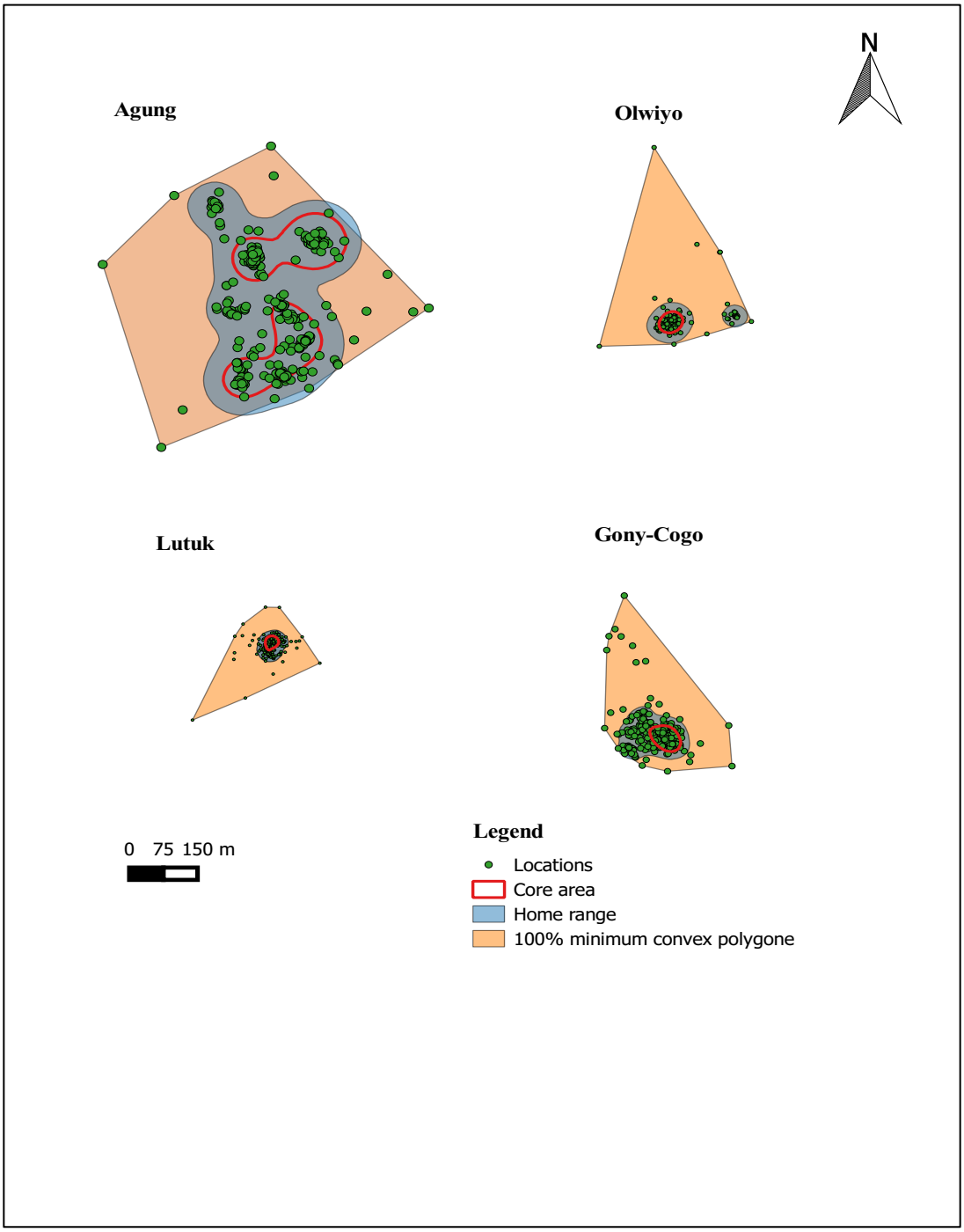

Figure 14: Locations, home range, core area and minimum convex polygon of the Agung, Olwiyo, Lutuk and Gony-cogo domestic pigs

Generated using R version 3.1.1.

4.2.The bushpig movement pattern and home range at the wildlife-livestock interface

4.2.1 Activity pattern collard bushpigs

Out of the five collared bushpigs, data were successfully collected from two bushpigs for a period of two months. The third collar stopped reporting after five days and the collar was never recovered. The fourth collared bushpig died after two weeks from the capture date possibly due to stress, the collar was recovered from the decomposing carcass, although the data were not usable. The fifth bushpig completely disappeared after two weeks of capture and collaring, and its collar was never recovered with no usable data recorded as well (Table 4).

Table 4: Locations where collared bushpigs were captured and the duration of collaring

Date	Duration of collaring	Location
March-May-2010	2 months	L. Mburo NP
Oct-Dec 2010	2 months	KRB
Apr 2012	2 weeks	MFNP
May 2013	5 days	MFNP
Mar 2013	2 days	MFNP

The recorded Katonga bushpig movement patterns showed that this bushpig, moved across the wildlife-livestock interface, from the swamps in the night into farm areas and then back to the swamp at daybreak. The bushpig rested during daytime under the swamp thickets, game reserves and game parks from where it emerged late in the evening to raid crops in the farmlands (Figure 16). The crop raiding behavior of bushpigs prompted farmers in Buliisa District to invent a bushpig control measure that prevented them from reaching the gardens (Figure 15).

Figure 15: A garden encircled by used mineral water bottles in Buliisa District as a control method against bushpig farm raiding

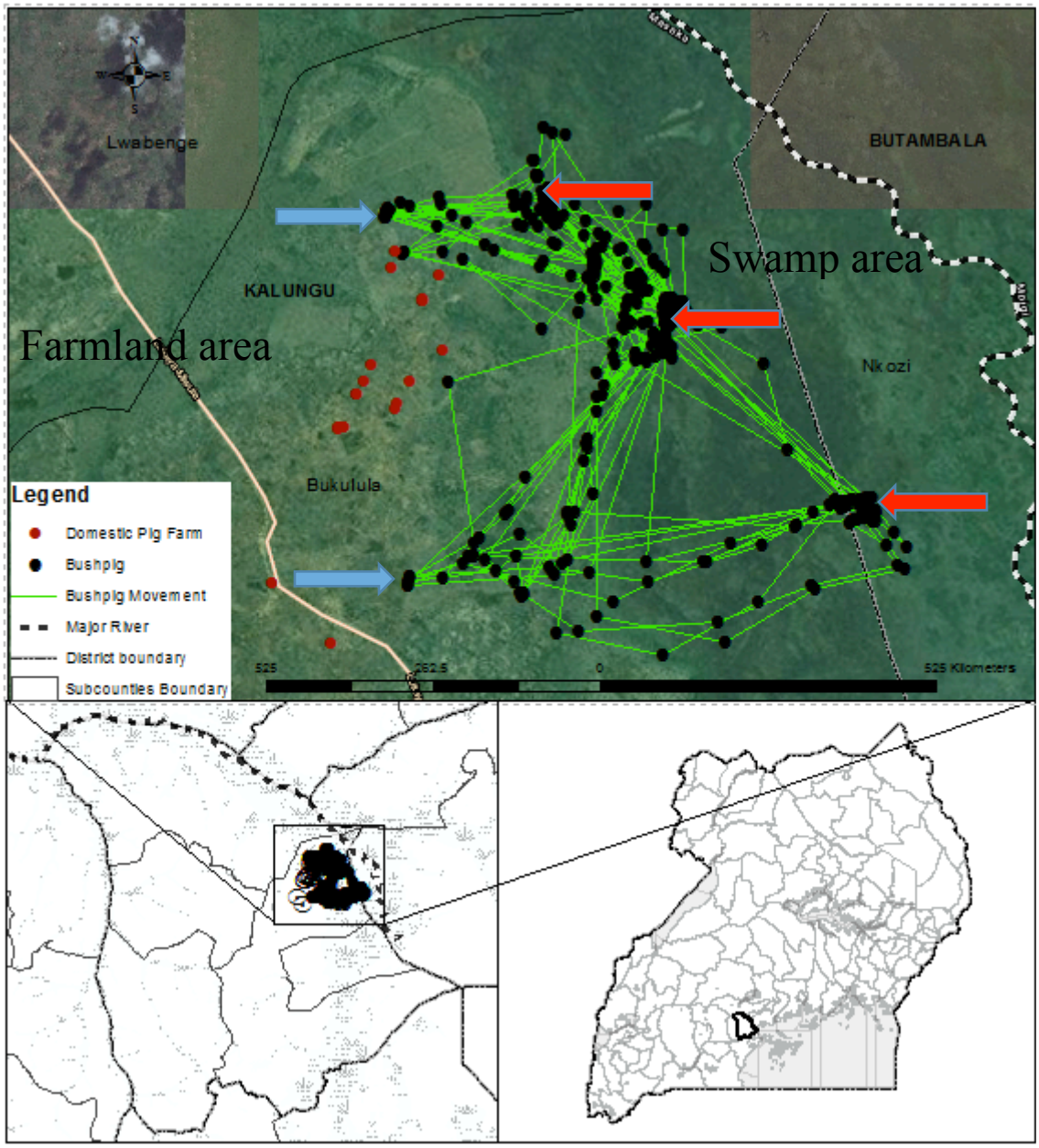

Figure 16: Katonga river basin bushpig movement pattern

Key: The red arrows show daytime resting sites in the swamps, while the blue arrows shows night time bushpig crop raiding of farms.

There was evidence of crop raiding by the Katonga bushpig in farms at the wildlife-livestock interface during nights when the bushpigs showed highest activity levels (Figure 16). This was

observed in farms within the home range of the Katonga bushpig that were visited during the study including other farms within the study area.

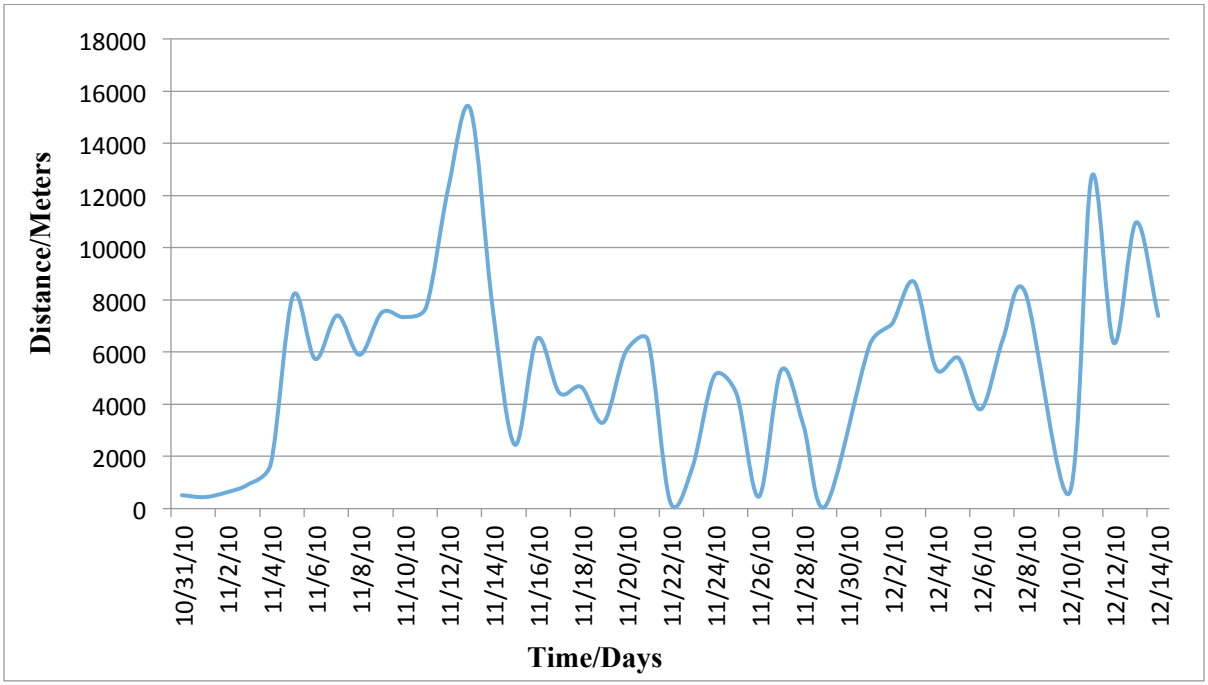

Figure 17: Daily Katonga river basin bushpig movements

There was an initial resting phase following capture of the bushpigs lasting about one week (Figure16: 29th Oct.-3rd Nov 2010) for the Katonga river basin and five days for Mburo (23-27 March 2010) bushpigs before resuming normal activity. The Katonga bushpig's peak daily movement was found to be 15.3 kilometers (Figure 17) while the Mburo bushpig's peak daily movement was 10.5 kilometers (Figure 18). However, there was a large variation in activities between days with what appeared to be 2-3 day cycles of high and low movement (Figure 17 and 18). The daily movements of the Uma bushpig was not calculated because the GPS data collected were for only 5 days after capture where it had very minimal activity presumably because it was in its initial resting phase like the other two bushpigs (Katonga and Mburo) and its collar stopped reporting before it had normalised its daily activities.

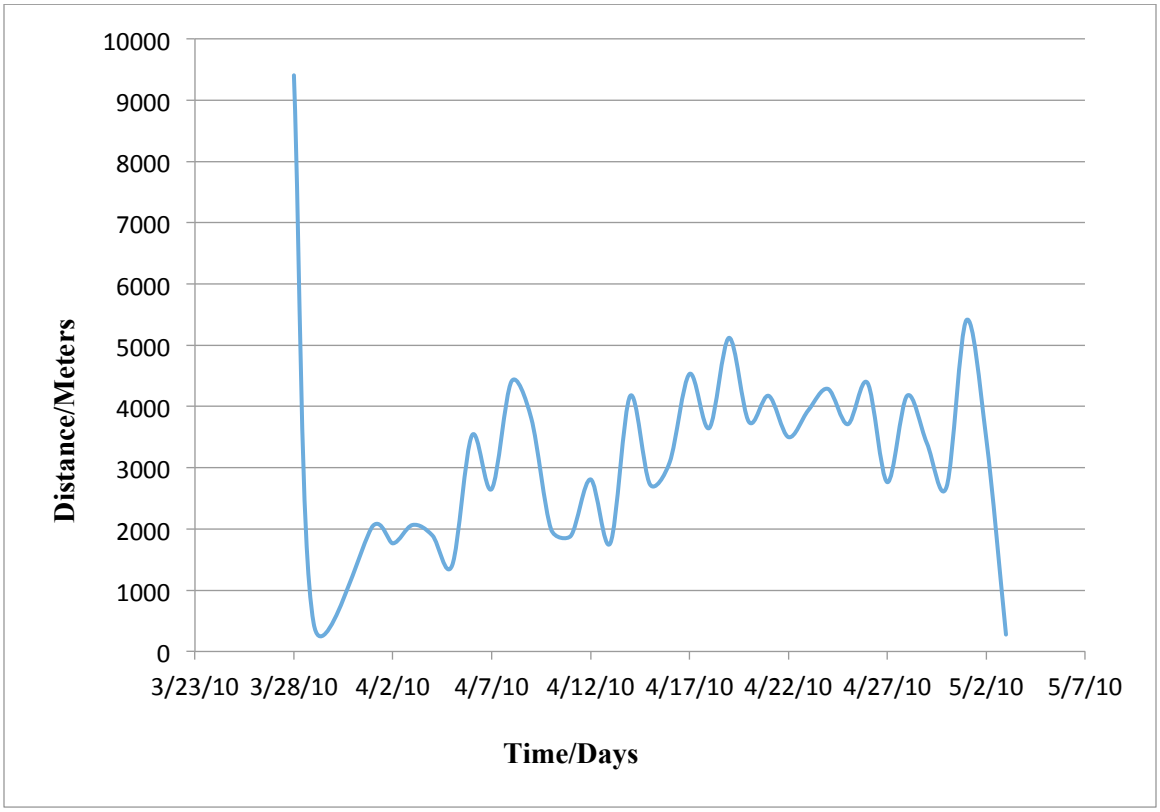

Figure 18: Daily Mburo bushpig movements

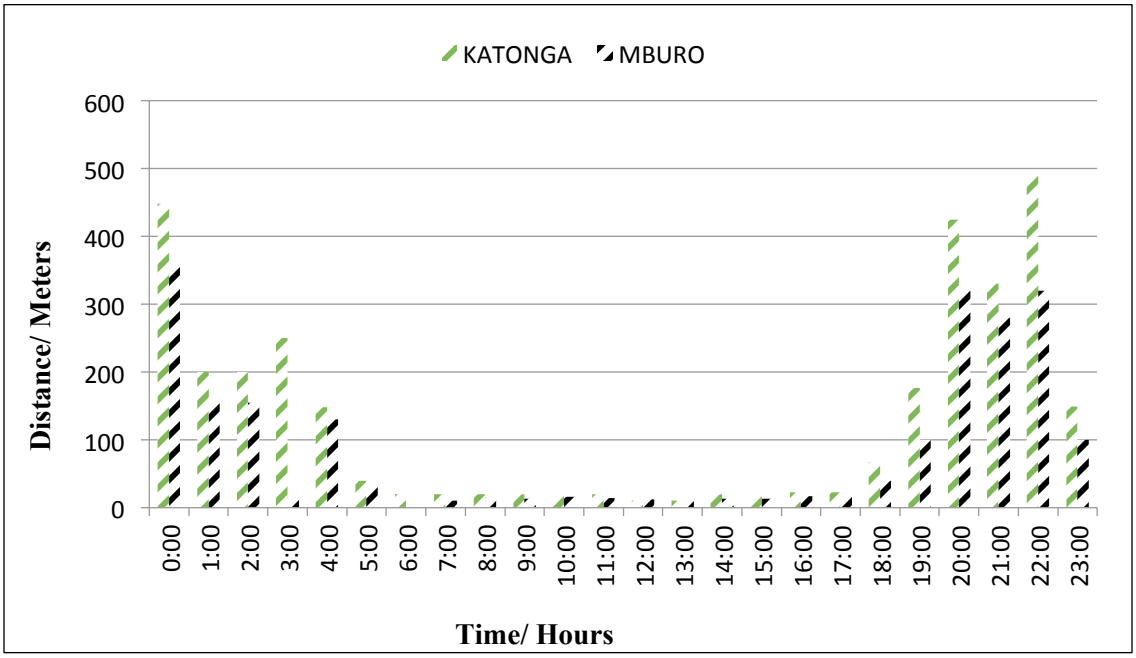

Figure 19: Average hourly bushpig activity pattern

The bushpigs (Katonga and Mburo) showed an activity pattern of limited movements during the day between 7:00 to 17:00 hours (Figure 19) but showed peak activity levels between sunset and mornings from 18:00 to 06:00 hours with peak activity at 20:00 – 0.00 hours (Figure 19).

4.2.2 Home ranges of collard bushpigs

The Minimum Convex polygon (MCP), home range and core area of the three collared bushpigs were calculated (Table 5 and Figure 20). The bushpigs' mean home range and mean core utilization area were not calculated on assumption that the sample size was only three and in addition to having only five days of the third Uma bushpig's data. The MCP of Puss was also drawn with a different scale from the rest of the collared bushpigs because of having only five days of GPS data collected. The Katonga bushpig had the largest home range of 8.5 square kilometers (Table 5 and Figure 20) stretching up to domestic pig and crop farms (Figure 16), followed by the Mburo one with 2.1 square kilometers and then lastly the Uma bushpig with only 0.002 square kilometers as its movements lasted just five days and were probably biased because of post-capture reaction.

Table 5: Bushpig home range, core area and minimum convex polygon calculated values

BUSHPIG ID (Village)	TRACKING DURATION (Days)	START & END OF TRACKING (Date)	FREQUENCY (Location/Hr.)	NUMBER OF LOCATIONS	MCP AREA (Km^2)	HOME RANGE (Km^2)	CORE AREA (Km^2)
Mburo	37	28/3/2010-3/5/2010	4 locations	1574	4.6	2.1	0.8
Katonga	45	31/10/2010-14/12/2010	1 location	902	17.8	8.5	1.4
Uma	5	03/05/2013-08/05/2013	1 location	124	0.009	0.002	0.0004

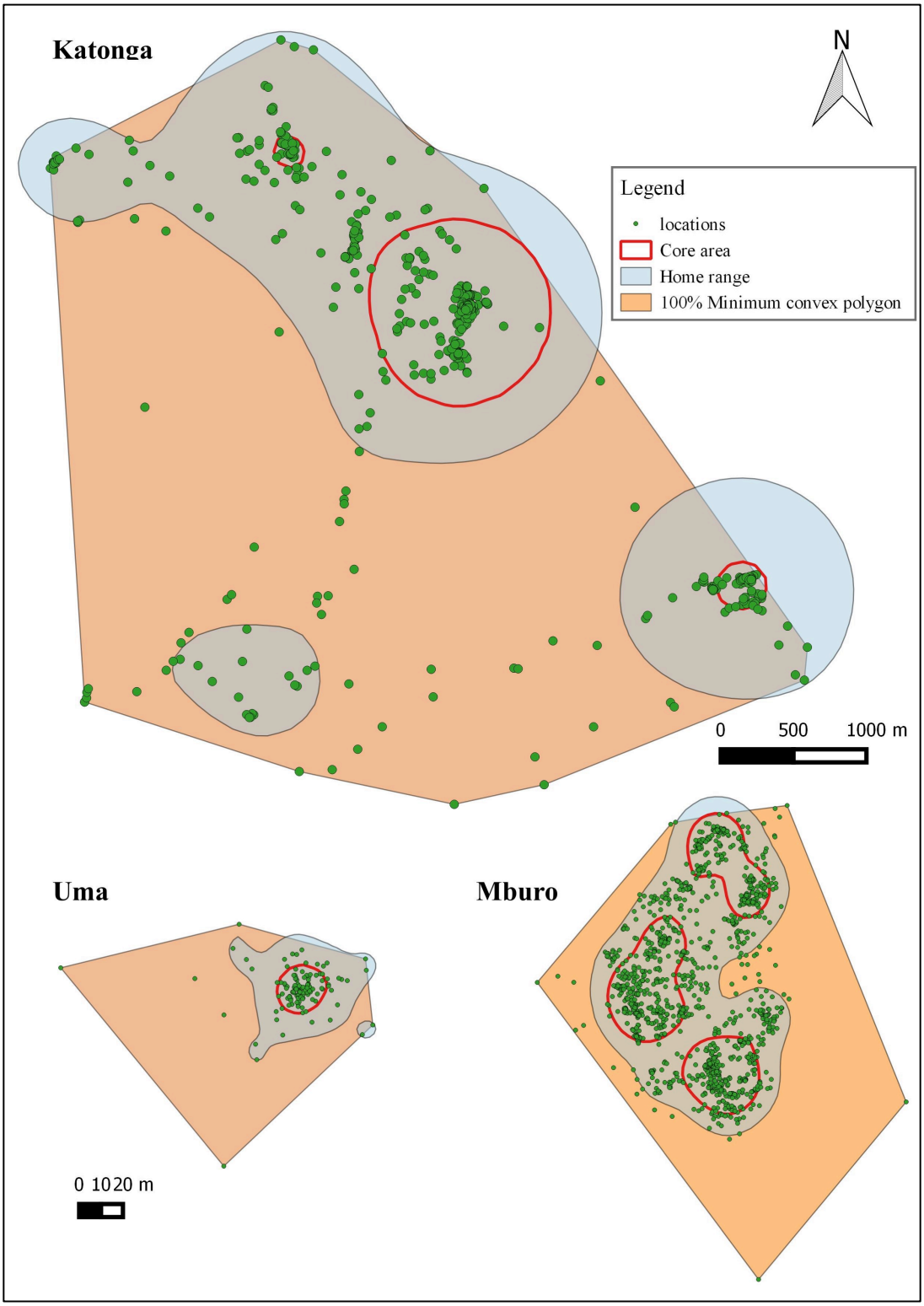

Figure 20: Home range, core area and minimum convex polygons of Shrek, Mburo, and Puss bushpigs Generated using R version 3.1.1 and note the different scale for the Uma bushpig

4.3 The ASFV infection in whole blood and tissue of sampled domestic pigs and bushpigs

Out of the 146 domestic pigs sampled from farms in the study area (Gulu district, 21; KRB, 4; Nwoya district, 106 and Nebbi district, 15), 14 samples, collected from pigs with clinical signs of ASF were positive for ASFV (Table 6 and Figure 21).

Table 6: Locations and Ct values of the positive domestic pig samples detected by real time-PCR

District	Sub-county	Parish	Village	Number of blood samples	ASFV Positive Sample ID	C_t value (Cycles)
Gulu	Lalogi	Jaka	Wanglobo	21	DPLW 1	28.6
					DPLW 2	**40.7?**
					DPLW 7	38.0
KRB			Bukulula	4	BKL2	24.2
Nwoya	Anaka		Laliya	41	DPL1	24.6
					DPL2	25.5
					DPL3	28.1
					DPL4	29.0
					DPL5	27.5
	Koch-Goma	Kal	Lutuk	38	DPK 1	29.1
					DPK 2	31.7
	Purongo	Patira-Kiba E	Patira E	27	DPE 2	34.4
Nebbi	Kucwiny	Vurr	Vur-waradi	8	DPKV 3	39.6
	Parombo	Pagwata	Jupukok	7	DPKJ1	24.6

The C_t values above were considered positive for ASFV, however C_t 40.7 was considered doubtful.

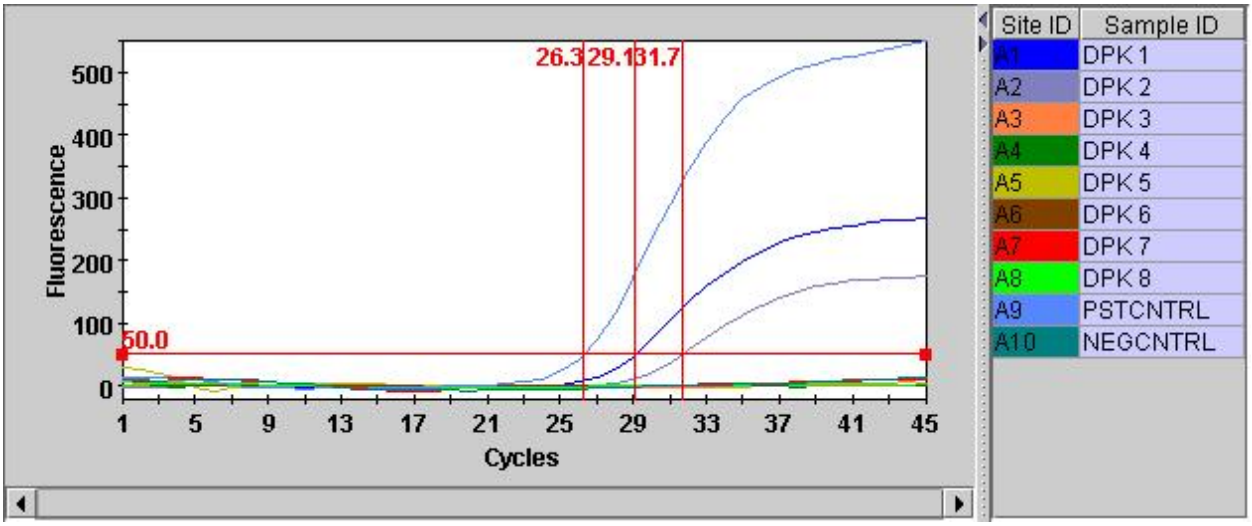

Figure 21: Real time-PCR results that were run on domestic pig blood samples

The RT-PCR results revealed that 1 bushpig sample was positive for ASFV, and one had a doubtful reaction (Table 7 and Figure 22).

Table 7: Results of real time-PCR diagnosis of ASFV in blood and tissue samples of bushpigs

Sample ID	Sample type	Sampling location	Bushpig Sex	Bushpig estimated age (Years)	ASFV RT-PCR Result	C_t Value (Cycles)
BP2	Blood	LMNP	M	1.5	Neg.	0
BP3	Blood	LMNP	F	2	Neg.	0
BP4	Blood	LMNP	F	1	?.	43.2
BP Gulu	Tissue	Lutuk	M	1	Neg.	0
Shrek	Blood	Bukulula	F	2	Neg.	0
BPNAA3	Blood	MFNP	M	2	Neg.	0
BPNAA4	Blood	MFNP	M	3	Neg.	0
BPNA1	Blood	Agung	M	1	Neg.	0
BPNA2	Tissue	Agung	M	2	Neg.	0
BPGME	Blood	Mede	F	1	Pos.	35.8
BPNK1	Blood	Koch	F	0.2	Neg.	0
BPNA3	Tissue	Agung	M	1.5	Neg.	0
BPMF2	Blood	MFNP	M	2	Neg.	0
BPMF3	Blood	MFNP	M	2	Neg.	0

Key: Pos. represent a positive result, Neg. represent a negative result and ?. represent a doubtful result.

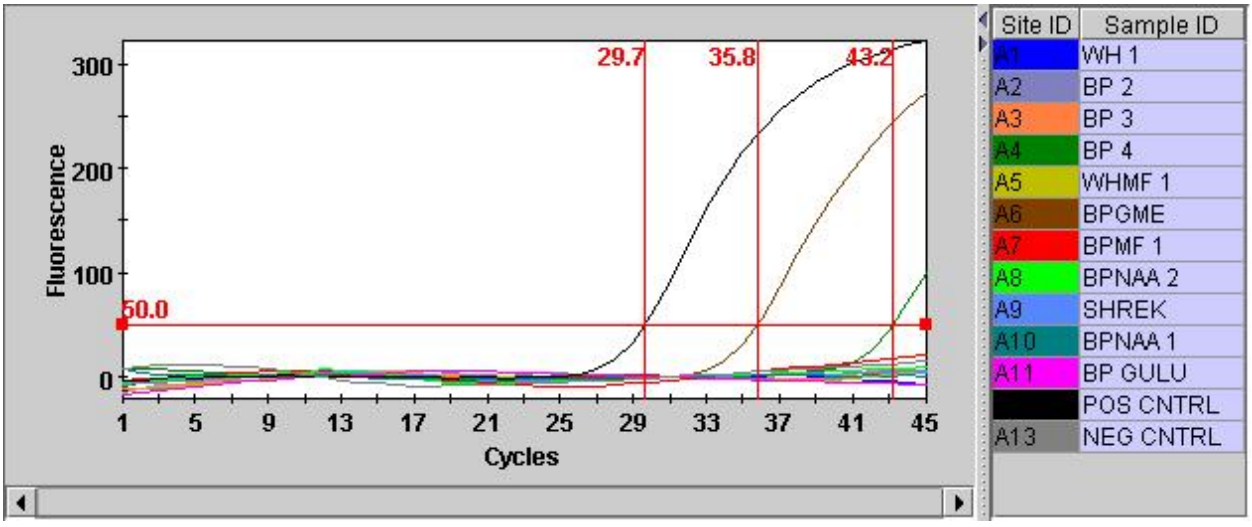

Figure 22: Real time-PCR results that were run on bushpig samples from different parts of Uganda

5.0 DISCUSSION, CONLUSIONS AND RECOMMENDATIONS

This section of the dissertation discusses, draws conclusions and makes recommendations on the findings of the study on the potential role of the bushpig in the epidemiology of AFS at the wildlife-livestock interface in Uganda.

5.1 Discussion

The bushpig capture process was quite difficult and a lot of hurdles were met during the study that limited the sample size and collaring of the bushpigs. In many occasions bushpig hunting sessions that lasted for six hours every day ended without capture. The use of game capture nets consumed a lot of time and had a low capture success rate, and therefore one of the alternatives to increase on the sample size was to collect tissue samples of slaughtered bushpigs from local hunters.

Hunting as a means of capturing bushpigs was also found to be inflicting a lot of stress on the animals, which was suspected to have caused the death of one of the captured and collared bushpigs. The difficulty to find the most proper fit of the GPS harnesses that were used during the study also affected the GPS data duration because on two occasions the collar dropped off before the required duration.

Increasing the sample size of bushpigs would be important in determining the prevalence of ASF in bushpigs. This has not been done yet; and also increasing the movement data, which would facilitate better investigation of the spatial pattern of the movement of bushpigs. This will aid to identify the sites where the interactions between them and free ranging domestic pigs are more likely to occur.

The pig farmers in the study area practiced the free-range system for the greater part of the year, with intervals of tethering especially during planting and harvest times (Chenais *et al.,* 2015). The pig movement pattern showed activities throughout a 24-hour period but with more activities during the mornings, afternoon and evenings although one pig (the Agung pig) showed an exception of peak activities at 3:00 hours (Figure 12). The difference in the activity pattern of the collared free ranging domestic pigs most likely can be explained by the variation of husbandry practices from the smallholder farms. The pigs that were periodically provided some food in their homesteads remained around the homesteads most of the time with minimal

movements to other areas. However, the pigs that were rarely given food in their homesteads were then forced to walk long distances in search for food.

The collared free ranging domestic pigs had a mean home range of 43,239 m^2 (ranging from 3,929 – 143,822 m^2) and a mean core utilization area of 13,328 m^2 (ranging from 744 – 47,416 m^2). The free ranging domestic pigs in this study had larger mean home range and mean core utilization area than the ones in the study in Kenya (Thomas *et al.,* 2013) although this difference may have been due to the high movements of the Agung pig whose home range and core utilization area were extremely higher (about ten times) than that of the other collard pigs in the study.

The free ranging domestic pigs in the study area mainly roamed around homesteads, water points and rubbish disposal points as their core utilization areas (Figures 13 and 14). This finding is consistent with that of a similar study in Kenya in which it is reported that free ranging domestic pigs spent approximately 50 % of their time around their homesteads and 47 % outside homesteads of their origin (Thomas *et al.,* 2013).

The roaming of free-range pigs on the other hand allowed interactions between pigs from different farms in rubbish disposal and water points, (Figures 13 and 14). An interaction of free ranging domestic pigs in such places could enhance pig-to-pig ASFV transmission during times of outbreaks. This finding is in agreement with that of Muhangi *et al* (2015), which reported the presence of higher potential ASF risk factors on small-scale pig farms that were most common in the study area, with multiple potential risk factors like sale and slaughter of infected pigs, rubbish disposal and water points.

The rubbish disposal points could potentially expose naïve free ranging domestic pigs to ASF infectious materials (Thomas *et al.,* 2013). It is well accepted that ASFV is highly resistant to inactivation in the environment when protected by organic material such as blood or meat. The virus persists for more than 15 weeks in putrefied blood, 11 days in faeces kept at room temperature or 1000 days in frozen meat. The ASFV also remained stable at pH 4.10 and was thus not affected by meat maturation, requiring constant heating at 60^{o} C for 20 minutes to be inactivated. Smoked sausages and air-dried hams required smoking at 32.49^{o} C for up to 12

hours and 25 to 30 days of drying respectively to be free of ASFV (Plowright *et al.,* 1994).

Due to the lengthy persistence of ASFV in tissues such as muscles, fat and bone marrow, and other pork products, access of naïve domestic pigs to such tissues presents a serious risk of infections (Plowright *et al.,* 1994). Therefore the free ranging pigs like the ones in the study area could be at risk of contracting and transmitting ASFV.

The collared bushpigs showed an activity pattern of limited movements during the day between 7:00 to 17:00 hours and peak activity levels between sunset and mornings from 18:00 to 06:00 hours with peak activity between 20:00 – 0.00 hours (Figure 18). One of the bushpigs (Katonga bushpig) in this study had a peak daily movement distance 15.3 kilometers from a swampy area into the farmland (Figure 17). Thus it was capable of reaching domestic pig farms and gardens within a range of 15.3 kilometers, confirming an overlap between bushpig foraging areas and pig farms at the wildlife-livestock interface (Figure 16).

The crop raiding behavior of bushpigs prompted farmers in Buliisa District to invent a bushpig control measure that prevented them from reaching the gardens. The method involved lining empty mineral water bottles (300 ml) with ropes around the garden (Figure 15). The farmers reported that bushpigs would knock the lined mineral water bottles, and the noise created by the bottles forced the bushpigs to turn back. However, in most areas in Nwoya district, farmers made bonfires in their gardens to keep watch throughout the nights to keep bushpigs out of their gardens until crop harvesting was done.

The home range calculation of the collared bushpigs showed that the Katonga bushpig had the largest home range, stretching up to domestic pig farms, and measured 8.5 square kilometers (Figures 16, 20 and Table 5), while the Mburo bushpig home range was 2.1 square kilometers. The Katonga bushpig inhabited the swamp at the wildlife-livestock interface while the Mburo bushpig had its territory deep inside the park. The difference in the home range of the two collared bushpigs could have come due to the variations in food availability and hunting pressure in their habitats.

The high hunting pressure and scarcity of food at the wildlife-livestock interface which is not a protected area may have forced the Katonga bushpig to move long distances in search of food and avoid hunters. The Mburo bushpig on the other hand had a small home range since its habitat

was located deep inside the park in a protected area where there was less hunting pressure with good and easy access to feed compared to the interface. The findings of the bushpig home range size in this study is not very different from that of an earlier study by Seydack (1990) in western South Africa that reported an average home range of 3 kilometers, ranging between 0.5 and 5.8 kilometers.

The positive bushpig and 14 positive domestic pig samples for ASFV confirmed the presence of naturally infected bushpigs with ASFV; and circulation of ASFV in bushpigs and free ranging domestic pigs in farms at the wildlife-livestock interface of MFNP in the study area (Tables 5 and 6). This finding concurs with that of a previous study in Kenya that reported the presence of ASFV in domestic pigs from farms bordering a National park (Okoth *et al.,* 2013).

Montgomery first reported ASF in East Africa in Kenya in 1921 among the white settlers' pig farms from where the disease then spread to other African countries. Bastos *et al* (2003) reported that East Africa is one of the richest regions in Africa in terms of ASFV p72 genotypes identified, with thirteen genotypes. Out of the thirteen genotypes in East Africa, eight genotypes (V, VI, IX, XI, XIII, XIV, XV and XVI) are apparently considered to be country specific while five genotypes (I, II, VIII, X and XII) do not seem to be restricted by national boundaries (Lubisi *et al.,* 2005).

In Uganda, an earlier study by Lubisi *et al* (2005) reported that until 1995, two ASFV genotypes; IX and X circulated in domestic pigs, wild pigs (warthogs) and the soft tick. Therefore, all the ASFV isolates which have been analyzed, which is a limited number, from outbreaks from 1954 to 1995 were caused by genotype X (Lubisi *et al*., 2005), while outbreaks from 1995 to 2012 have been caused by genotype IX in Uganda (Atuhaire *et al.,* 2013). The sequence results from one of the studies in our research team (Unpublished., 2014) also confirmed the circulation of ASFV genotype IX in domestic pigs in the study area.

During the study, one of the bushpigs moved into farmlands, potentially overlapping home ranges of domestic pigs in a range of up to 15.3 kilometers (Figures 16, 17 and 18). The collared domestic pigs did not move up to the protected areas but roamed around homes at the same time as bushpigs. However, the bushpig movement data recorded in the main study area during the study were not sufficient to draw comparison on the activity pattern of the bushpigs and free

ranging domestic pigs since no good bushpig movement data were recorded in the same area and period with domestic pigs.

The frequent movement of free ranging domestic pigs to rubbish disposal and water points could potentially enable direct or indirect interaction between the two species of pigs in the same area, providing suitable opportunities for ASF transmission among bushpigs and domestic pigs in the area. Indirect interaction among bushpigs and free ranging domestic pigs was also reported in the study area in one of the studies, indicating that it was frequent especially at water points during the dry season (Kukielka *et al.,* 2016). The direct interaction between bushpigs and free ranging domestic pigs has been reported to take place in many areas of their distribution range in previous publications (Haresnape *et al.*, 1985; Jori and Bastos., 2009; Vercammen *et al.*, 1993).

The findings of this study show that the husbandry of domestic pigs together with the behavior of the bushpig constitute suitable grounds for disease transmission at the interface, but transmission of the disease has only been evidenced from bushpigs to domestic pigs through direct contact by Anderson *et al.* (998); and as yet no direct contact between the two species has been confirmed in this study. There is a need to employ the use of motion sensor cameras (camera traps) in gardens and rubbish disposal points where bushpigs and free ranging domestic pigs are reported to interact so as to have evidence of natural direct contact between the two species.

5.2 Conclusions

The free-range domestic pigs in the study area spent more than 50% of their time around their own homesteads and about 47% around homesteads not belonging to them. The free-range domestic pigs that were not frequently given food in their own homesteads had wider home ranges than the pigs that were frequently fed in their homesteads.

There was direct or indirect interaction among free-range domestic pigs from different farms at rubbish disposal and water points, and these interactions provided suitable grounds for ASF transmission in three ways; from infected domestic pig to naïve domestic pigs or from infected bushpigs to naïve domestic pigs and finally from infected domestic pigs to naïve bushpigs.

The peak distance covered by a bushpig at the wildlife-livestock interface in the study area was 15.3 kilometers, from the park to farmlands were bushpigs possibly interacted with free-range domestic pigs paving way for ASFV spread to domestic pigs or even from domestic pigs to

bushpigs. There was an ASFV genotype circulating among bushpigs and free-range domestic pigs in the study area although its genotype was not confirmed.

The bushpigs that inhabited the wildlife-livestock interface and were consistently experiencing serious hunting pressure, had larger home ranges (8.6 Km^2) compared to their counterparts (2.1 Km^2) that lived deep inside the parks with less hunting pressure.

5.3 Recommendations

The farmers in the study area should be encouraged to use local materials to construct pig stys and reduce on the number of free-range pigs which increase on the rate of ASFV spread during out breaks.

Appropriate sedation drugs with a reversal antidote should be used other than zoletil 100 which has no antidote to reduce on the death toll of captured bushpigs due to long recovery time and stress, as bushpigs are known to be very susceptible to stress.

Efficient harness materials should be used for making GPS tracking collars to prevent collars from falling off the collared bushpigs and domestic pigs.

The satellite collars should be preferred to the GPS/GSM tracking collars in future to avoid loss of collars and missing data especially in areas where the GSM network coverage is weak or completely absent.

More efficient bushpig capture techniques such as electromagnetic drop nets and metallic drop door cages should be employed to increase the sample size and reduce the number of bushpig deaths due to stress that results from hunting.

There is need to sequence the virus from the positive bushpig and domestic pig samples for confirmation of its genotype.

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