POTENTIAL ROLE OF *ORNITHODOROS MOUBATA* IN AFRICAN SWINE FEVER EPIDEMIOLOGY ALONG KENYA UGANDA BORDER

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Declaration

This thesis is my original work and has not been presented for degree in any other University

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Dedication

First, I dedicate this thesis to God for granting me sufficient grace to carry out the study and life in abundance to see the output of my research. Secondly, I dedicate the thesis to my family, who offered me unconditional love and support throughout the course of the study.

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LIST OF ABBREVIATIONS

ASF	African Swine Fever
ASFV	African Swine Fever Virus
BecA	Biosciences eastern and central Africa
CI	Confidence Interval
CSIRO	Commonwealth Scientific and Industrial Research Organization, Australia
DVS	Department of Veterinary Services
ELISA	Enzyme-Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FFS	Farmer Field School
FPR	Farmer Participatory Research
HAD	Haemadsorbing
ILRI	International Livestock Research Institute
MAAIF	Ministry of Agriculture, Animal Industries and Fisheries
OD	Optic Density
O.I.E	Office of International des Epizooties (World Organization for Animal Health)
OPD	Orthophenylendiamine
PBS	Phosphate Buffered Saline
SGE	Salivary Gland Extract
SI	Serological Index
SPSS	Statistical Package for Social Sciences
TMB	Tetramethylbenzidine

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ABSTRACT

Development of strategies for control of African swine fever (ASF) requires an in depth investigation of risk factors for the disease transmission. Such factors include exposure of pigs to *Ornithodoros moubata (O. moubata),* the known vector for ASF. The cross sectional study used a random sampling approach where 640 pig keeping households were randomly selected. Data collection done using a questionnaire followed by blood sample collection from domestic pigs. The study used tick specific enzyme-linked immunosorbent assay (ELISA) test (rtTSGP1 ELISA) to quantify the prevalence of pig exposure to the ASF tick vector and assessed risk factor for exposure. The test involved the use of a recombinant form of omTSGP1 to screen for anti-tick antibodies in exposed pigs. The results showed that of 181 out of 1085 pigs sampled (17%) were exposed to tick bites. Kenyan side of Busia had the highest prevalence of exposed pigs (22%) compared to Ugandan side (10%). The odds of exposure in farms that did not control ticks was about twice compared to those that controlled ticks in Busia county but in Uganda tick control had no influence. The result further show that acaricide control and farmer education interpreted as level of awareness can lead to reduction in the risk of pig exposure to ticks. There was significant association between tick exposure and previous ASF like outbreaks on farms, with Chi-square ($P<0.05$) and (Cramer's V value of 0.079) interpreted as ticks could play a role in the ASF outbreaks in Uganda. This study established that the pigs in the study region are exposed to tick bites from *O. moubata* and therefore ticks present a risk for ASF maintenance and transmission. The study recommends a further sampling of ticks and analysis of ASF virus

infection rate in the ticks followed by virus characterization to determine the extent of risk that the ticks portend.

Dr. Peter Gathura, (BVM, Msc, PhD), Prof. William Ogara, (BVM, Msc, PhD), Dr. Edward Okoth Abworo (BVM, Mvee, PhD) Were the Supervisors

CHAPTER ONE

1. GENERAL INTRODUCTION

1.1 Pig production

Pig farming is an important undertaking which provides opportunity as an income generating activity for small-scale farmers, especially in developing countries. It is most popular in Africa, Latin America and South East Asia (Costales *et al*., 2007; Huynh *et al*., 2007). Pig production and productivity in Kenya has been analysed in the recent past. The pig population is estimated at 334,689 (Kenya National Bureau of Statistics, 2009). Central, Western and Rift valley Provinces holding the highest number of pigs compared to other provinces (Kagira *et al.* 2010b). Small-scale pig production constitutes about 70% of the total pig farmers in Kenya (Government of Kenya, 2007)

The reasons for keeping pigs included provision of protein/meat, dowry, school fees payment and manure for fertilizing the soil, particularly for farmers that practice mixed farming. This implies that majority of small scale farmers depend on pig farming to improve their livelihood. Pig production has the potential to play a crucial role in poverty alleviation, because of the ability to convert low quality feed into high quality protein together with high reproductive potential (Penrith and Vosloo, 2009).

African swine fever (ASF) is a highly contagious and fatal viral disease of domestic pigs. The farm-level risk of sero-positivity to African swine fever virus (ASFV) has been shown to be higher in free-range than in farms using partial or total-confinement (Mannelli *et al*., 1997). In developing countries, particularly in Africa, poverty conditions condemn pig owners to let their pigs in free ranging conditions to scavenge for food. This has the implications on transmission of

ASFV. In these circumstances, the disease is uncontrollable. This situation is aggravated by lack of infrastructure and resources from the veterinary services to confirm the diagnosis and react promptly, and lack of provisions from the governments to compensate pig owners for eventual implementation of stamping out operations. High mortalities in pig herds due to ASF outbreaks has devastating effects on the economies of rural people in developing countries and the potential spread of the disease to other geographic locations beyond the African continent. For example, the introduction of ASF disease in Madagascar (Roger *et al*., 2001) or the Caucasus (Penrith *et al*., 2004). Kenya and Uganda could also be facing the same calamity as pig production is composed of small holdings.

Pig production systems in Kenya range from large scale /commercial/ intensive production to backyard and free-range farming systems characterized by low input low output enterprises (Kagira *et al*., 2010a, 2010b). Uganda has the largest and fastest growing pig production in Eastern Africa. It has reported several outbreaks with the latest in Gulu District and neighboring areas since April, 2011 (ILRI, 2012) in a post conflict zones of Uganda, probably due to its potential to accelerate poor farmers out of poverty.

Specific pig farming systems may define how healthy pigs contract ASF (Okoth *et al*., 2013), either through direct contact or through bite by vectors (soft ticks). The transmission of African swine fever virus through vector bites needs to be comprehensively studied; in part this involves structured studies with the application of laboratory diagnostics.

African swine fever constitutes one of the major constraints to pig production and productivity in developing countries. Recent outbreaks in Kenya (Okoth *et al*., 2013) , for example, resulted in

about 100% morbidity and mortality in the affected herds. Similar cases have been reported in Uganda (Nantima, personal communication).

1.2 African swine fever

African swine fever is a disease caused by the African swine fever virus (ASFV), genus *Asfivirus* and that is the only member of the family *Asfarviridae*. The disease is characterized by high morbidity and high mortality and has been observed in all breeds and types of domestic pigs. All age groups of domestic pigs are equally susceptible. In Africa, the virus produces no apparent infection in 2 species of wild swine, (warthog (*Phacochoerus africanus*) and bush pig (*Potamochoerus larvatus*) and in the soft tick *O. moubata* (Jori and Bastos., 2009; Coetzer and Tustin., 2004)).Wild suids, specifically bush pigs, warthog and giant forest hog (*Hylochoerus meinertzhageni*) are all known to be carriers of the ASFV (Anderson *et al*., 1998). Domestic pigs are accidental hosts as they share the same environment with the vector. In the case of warthogs, there exists a complex cycle of infection involving argasid (soft) ticks (Plowright *et al*., 1969). Outbreaks of ASF can occur when domestic pigs come into contact with the ticks that have fed on warthogs or by direct contact between infected pigs. ASF causes severe threat to pig industry in sub-Saharan Africa and is a major risk hindering investment in pig production. Currently, there is no treatment or vaccine against ASFV (Chang *et al*., 2006; Dixon *et al*., 2004). Prevention of ASF relies entirely upon preventing contact between the virus and the susceptible host.

ASF was first reported in Kenya in 1910 and recorded in 1920 (Montgomery, 1921). Outbreaks in new areas have been associated with movement of domestic pigs and their products rather than contact with the wild pigs that are natural hosts of the virus. In endemic areas, ASFV spread

is associated with traditional free-range pig production system and lack of biosecurity measures on pig farms (Randriamparany *et al*., 2005).

The first outbreak of ASF disease outside Africa was in Portugal in 1957; the outbreak was successfully contained. The second introduction of ASF in Portugal was in 1960. The introduction was not contained resulting to rapid spread of ASF to several countries in Europe including Belgium and the Netherlands. The disease became established in the Iberian Peninsula and was only declared free of ASF in 1995 (Onisk *et al*., 1994)

Kenya has been experiencing continuous ASF outbreaks from 1994 after an apparent absence since 1963, (O.I.E, 2001). Other outbreaks in 2006, 2007, 2010 and 2011 in Busia County (current study site) have been associated with genotype IX of ASF virus (Gallardo *et al.*, 2011) and suspected but not confirmed to be caused by carrier pigs surviving between outbreaks, but also probably could have been caused by infection by tick vectors.

In most sub-Saharan Africa, where ASF is endemic, ASFV persists in nature by a sylvatic cycle of transmission between wild suids mainly the warthog, (*Phacochoerus aethiopicus*) and *Ornithodoros moubata* ticks, which infest their burrows (Wilkinson, 1984). Relevant for the maintenance of the virus in *O. moubata* ticks, previously showed that ASFV can replicate to high titres in *O. moubata* (Greig, 1972; Kleiboeker *et al*., 1998a, 1998b).

1.3 African swine fever virus transmission

According to past field observations and recent genetic insights, the epizootic of African swine fever (Boshoff *et al*., 2007; Lubisi *et al*., 2005) is divided into 3 distinct parts namely; sylvatic cycle, intermediate enzootic (tick) cycle and domestic cycle (Plate2.1). The old enzootic

(sylvatic) cycle involve wild pigs (warthogs) and wild *Ornithodoros* ticks with accidental transfer to domestic pigs by ticks leading to sporadic outbreaks. Sylvatic and domestic pig cycles are common cause of ASF transmission in East Africa (Fasina *et al*., 2010).

Intermediate enzootic (tick) cycle involving domestic pigs and domestic *Ornithodoros* ticks acting as vectors and reservoirs with regular contamination of domestic pigs (Fasina *et al.*, 2010). The new epizootic (domestic) cycle is where the virus is transmitted by direct contact from infected to non-infected domestic pigs. It is characterized by direct transmission via pig movements and contacts and contaminated fomites or infected meat.

When naive pigs come into contact with infected pigs, they contract ASFV infection in 2-6 hours with virus detected in some tissues at 48 hours after initial contact (Ekue *et al*., 1989; Greig, 1972). Infected pigs are most dangerous during the incubation period of the disease, since they shed infective quantities of virus in their body fluids for up to 48 hours before developing clinical signs of disease. During the clinical stage of disease, when enormous amounts of virus are present in blood, secretions and excretions will result into infection. Pigs that recover may become carriers and shed virus for up to a month after the disappearance of clinical signs (Penrith *et al*., 2004).

In wild suids, ASF infection is characterized by low levels of virus in tissues and low or undetectable viraemia (Plowright, 1981). These levels of virus in adult suids are insufficient for transmission through direct contact between animals and/or indirect contact by ticks (Jori and Bastos, 2009).

In large pig populations, ASF virus can be maintained for long periods owing to the availability of a constant supply of susceptible pigs (Penrith *et al*., 2007, 2004). The virus has a remarkable

ability to survive for long periods in a protein environment, and therefore meat from pigs slaughtered in the infective stages of ASF or that die naturally of the disease provides a good source of virus (McKercher *et al*., 1978; Mebus, 1988).

More recent epidemiological studies in Kenya (Okoth *et al*., 2013) have shown a more complex epidemiology of ASF where a similar ASF virus was found in wild and domestic species in the same natural location. Association of a higher pig infection with proximity of pig farms to protected areas containing wild pigs has been demonstrated in south-west Kenya. The transmission of this virus between the two species is not well understood but ticks could play a major role (Okoth *et al*., 2013).

1.4 African swine fever control

Rigorous detection and slaughter programs ended with the successful eradication of the disease from both Portugal (1993) and Spain (1995) (Costard *et al.*, 2009b). Slaughter programs should be accompanied by compensation which could be very expensive especially in regions where the disease is endemic especially in Kenya and Uganda. Alternative ways should be followed in order to reduce disease incidence.

Development of strategies for control of ASF requires an understanding of risk factors involved, including vectors such as *O. moubata*. The role of ticks in ASFV transmission is not well understood in the East African region. It is hypothesized that elimination of *O. moubata* from human dwellings and pigsties could largely improve the control and prevention of ASF where ticks are involved in the epidemiology.

1.5 Objectives

The main objective was to investigate the potential role of *Ornithodoros spp.* in African swine fever epidemiology along Kenya Uganda border.

The specific objectives were:

i. To optimize tick ELISA for use in diagnosis of *O. moubata* exposure of domestic pigs in East Africa
ii. To determine and characterize the prevalence of exposure of pigs to *O. moubata* tick bites
iii. To assess risk factors associated with exposure of domestic pigs to tick bites

1.6 Hypothesis

i. Contact between domestic pigs and *O. moubata* ticks is not associated with ASFV maintenance in the environment, that results in outbreaks

ii. Farmer management practices are not associated with tick prevalence and thus ASFV prevalence.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Pig production

Pig keeping plays an important role of improving human livelihood in rural setting in developing countries as it is the source of income generation. Small-scale pig production constitutes about 70% of the total pig farmers in Kenya (Government of Kenya, 2007). Two pig management systems common in Kenya are free-range and confined feeding (Kagira *et al*., 2010a; Mutua *et al*., 2011; Wabacha *et al*., 2004; Wabacha *et a*l., 2001). In western Kenya, pigs are either tethered or kept in a mixed system characterized by free-range during the dry season and tethering during the rainy crop season (Okoth *et al*., 2013). In free-range system, pigs roam freely around the household and surrounding area, scavenging and feeding in the street, from garbage dumps or from neighboring land or forests around villages (FAO, 2010). Local Pig breeds commonly reared as they require minimal inputs in terms of family labour and feeding, perhaps an important motivation for farmers to raise pigs (Mutua *et al*., 2010).

Specific pig farming systems may define how healthy pigs contract ASF (Okoth *et al*., 2013), either through direct contact or through bite by vectors (soft ticks). Systematic studies that look at the pig production systems in East Africa vis-à-vis role of the management systems in level of exposure of pigs to ticks, as proxy to ASFV risk through tick transmission, have not been done to date. Knowledge of impact of pig management on tick exposure is useful in designing control strategies for ASF in smallholder systems in East Africa that may involve tick control.

2.2 *Ornithodoros* species of ticks

Ornithodoros is a genus of soft ticks belonging to the family *Agarsidae.* They are natural reservoirs and the biological vectors of ASF responsible for ASFV transmission. There are seven species of *Ornithodoros* tick depending on the ability to withstand the effects of desiccation. They include: *O. erraticus, O. moubata, O. coreaceu, O. turicata, O. puetoricensis O. parkeri* and *O. savignyi*. *O erraticus* and *O.moubata*. *Ornithodoros* are widely distributed in central, southern and eastern Africa, and the island of Madagascar (Roger *et al*., 2001). *O. erraticus* complex is distributed in Mediterranean basin and Middle East including the former Soviet Union states. They are the biological vectors of ASF responsible for ASFV spread in Europe. On the basis of both morphological and biological differences, there is further classification of *Ornithodoros moubata* into four species: *O. compactus, O. apertus, O. moubata* and *O. porcinus* (Walton, 1967). *O. moubata* and *O. moubata porcinus* are denoted as sylvatic ticks. These species have long life up to 15 years with strong resistance to starvation and persistence of infection for up to 5years (Rennie *et al*., 2001).

Transmission of ASF in sub-Saharan Africa involves both a sylvatic and a domestic (or urban) cycle. In sylvatic cycle, the virus is transmitted between warthog piglets (*Phacochoerus spp.*)and argasid (soft) ticks of the genus *Ornithodoros* inhabiting animal burrows leading to long-term persistence of ASFV in soft tick (Parker *et al*., 1969). Domestic (urban) cycle can occur between pigs, or between ticks and pigs. The type of pig farming systems in Africa (mostly traditional and semi-intensive) and presence of *Ornithodoros spp.* ticks infestation in pig sties, often complicates control efforts against ASF (Penrith *et al*., 2013). Other species adapt to domestic conditions and thus have been found in hen houses, cowshed or small ruminant buildings, pigsties and human dwellings with mud walls and floor (Rodhain, 1976; Walton, 1962). *O.*

porcinus colonizing pig pens share some morphological and genetic characteristics with *O. porcinus. Porcinus* (Walton, 1962). They are very closely related to the soft ticks that transmit *Borrelia duttonii,* the agent responsible for human tick-borne relapsing fever in Tanzania region (Fukunaga *et al*., 2001). This result suggests that the same *O. porcinus* ticks may be able to maintain and transmit both a human pathogen and an animal pathogen by shifting vertebrate hosts, depending on their availability in their habitat. This is a typical strategy of indiscriminate host feeders to increase the amount of potential hosts and may be interpreted as an adaptation to their endophilous lifestyle (Vial, 2009). This kind of lifestyle indicates how difficult it is to identify and control them.

O. apertus, a rare tick known only from two localities in Kenya and exclusively associated with African porcupines (hystrix); *O. compactus*, localized south of the Zambezi River and associated with several species of tortoises but never found in domestic areas.

O. moubata ticks are widely distributed in southern third of Africa, in South Africa with northward extensions through Mozambique to central Tanzania in the east and through southwest Africa into Angola on the west. This species is commonly found in warthog and porcupine burrows but also presents a domestic form inhabiting human dwellings (Walton, 1967). Walton also suggested that it was probably this species that infested domestic fowl houses in South Africa; *O. porcinus*, also widely distributed in the humid Central African Plateau, from central Kenya to central Mozambique, west to the eastern borders of Rwanda, Burundi and Malawi. *O. porcinus* is an abundant species in the bush, inhabiting warthog and porcupine burrows.

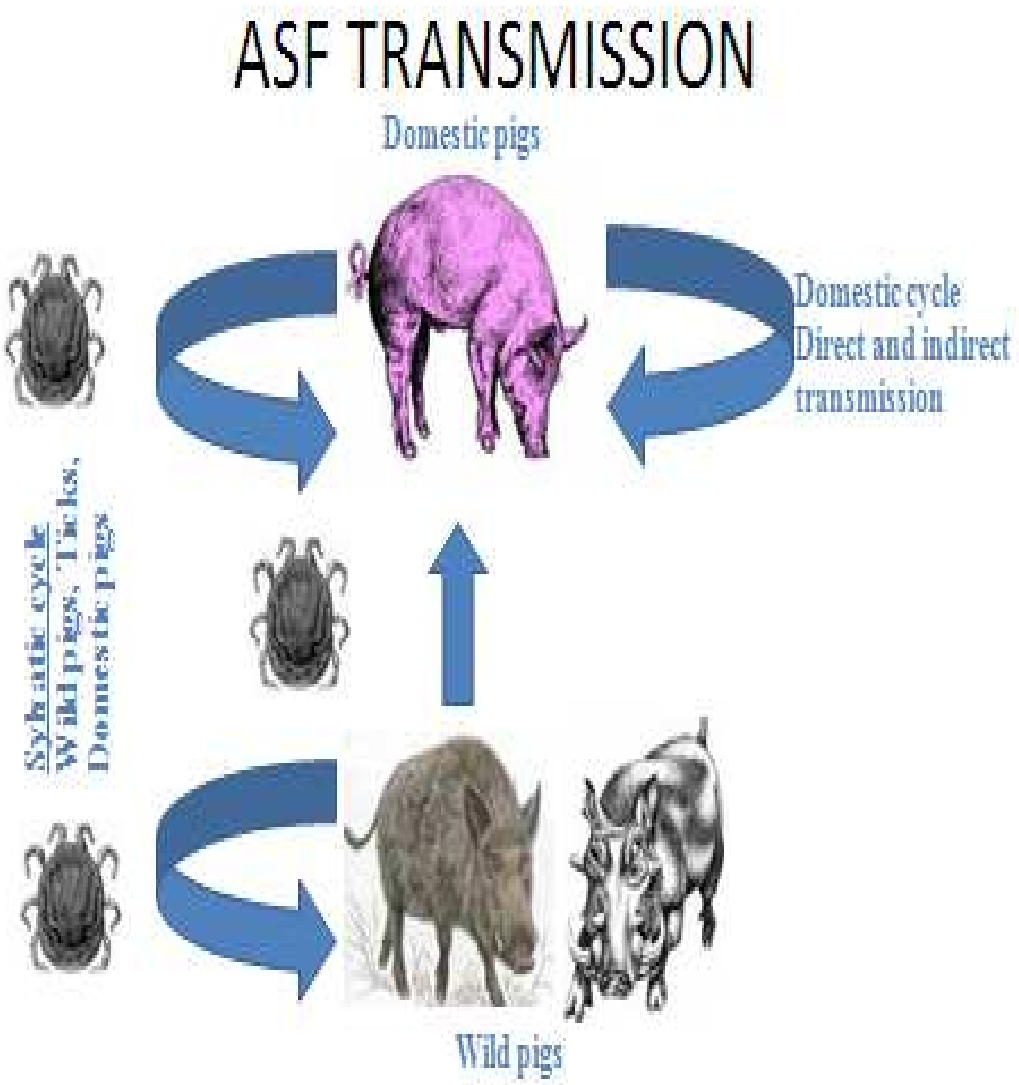


Plate 2.1: Schematic representation of ASFV transmission

2.3 Role of ticks in African swine fever epidemiology

Studies conducted in Spain identified *O. erraticus* as a vector and reservoir for the ASFV (Sanchez-Botija, 1963), which led to the discovery of ASFV in other *Ornithodoros spp* of ticks. This also led to the subsequent demonstration that *Ornithodoros spp* ticks are vital for the persistence of ASFV in its natural environments in Africa and that they are the likely initial source of the ASFV genotypes that now circulate in domestic pigs in Africa (Plowright *et al.*, 1969).

Ornithodoros ticks play a role as reservoir for ASFV. This was demonstrated when ASF re-emerged in Portugal in 1999 on a farm that had been affected in the past (Costard *et al*., 2009b). Infected ticks were found on the farm, suggesting that they had maintained the virus *(Costard et al*., 2009a). Pigs were the only domestic animal species always present in the occupied infested premises in the surveys and ticks were found in those premises populated by pigs at present or in the past (empty premises for more than 5 years as confirmed by regular visits to the farms) (Boinas *et al*., 2004). The experimental transmission was demonstrated from ticks collected over a year after the end of the outbreak, and infectious virus was isolated from ticks collected more than two years after an outbreak and maintained in the laboratory for a further three years. These results confirm that *O. erraticus* can transmit ASFV to pigs for over a year after the removal of infectious hosts and suggest that infectious virus can persist in tick populations for at least five years, indicating that the current quarantine period of six years is appropriate for areas where *Ornithodoros* ticks are known or suspected to occur. The period taken after an outbreak, before restocking, plays a role of pigs becoming infected by ASFV. Current guidance (FAO, 2009), suggests that restocking should initially occur at 10% of the original density and the animals then monitored for six weeks for clinical signs of ASFV before restocking occurs. It is known that

soft ticks are able to survive without feeding for periods of up to 5 years in the cases of large nymphs and adults (Fernandez, Garcia, 1970; Oleaga-Perez *et al*., 1990).

The life span of soft ticks can be of up to 15–20 years (Encinas-Grandes *et al*., 1993) and they can feed on alternative hosts to pigs, such as sheep and goats, rabbits, chickens and birds (Boinus, 1995). This can constraint tick control unless strategic regimen is adopted in all animals kept on the farm but it requires support. For instance, Portugal has been free of ASF since the single reoccurrence of 1999, and tighter quality control measures on meat during the last 10–15 years have placed increased economic pressure on pig farmers in Alentejo to protect their animals from tick bites in order to reduce the frequency of subcutaneous haemorrhages and haematomas.

A national campaign to inform farmers about the involvement of ticks in the transmission of ASFV has also been conducted by the Portuguese veterinary services, and in both Portugal and Spain where the re-use of premises with established tick population following an outbreak has been restricted (Arias and Sanchez-Vizcaino., 2002). As a result, some traditional pigsties have been abandoned or destroyed, probably reducing the number of farms on which *O. erraticus* could act as a reservoir of ASFV in the event of a future outbreak. Furthermore, although the European distribution of *O. erraticus* is limited to Portugal and Spain, other members of the species complex occur in parts of southern Europe, North Africa and the Caucasus (EFSA, 2010). Given the economic importance of ASFV and the extent to which *Ornithodoros* species can complicate its eradication, the potential role of these related *Ornithodoros* species in the epidemiology of ASFV must be clarified.

Infected pigs have been shown to remain viraemic for between 35 and 91 days following infection during which time they are able to infect the tick vector *O. moubata*. In turn the latter transmit the disease to domestic and wild pigs (Anderson *et al.*, 1998). Pigs are thought mostly to be accidental hosts. The role played by ticks in transmission of ASFV to domestic pigs is not well understood in East Africa.

Wildlife especially warthogs are believed to be responsible for maintaining of ASFV in many endemic areas in Africa. *O.porcinus. porcinus* ticks are found in warthog burrows in eastern and southern Africa (Plowright *et al*., 1994). The same study also showed that young warthogs less than one month old that were confined in burrows in which they were born develop viraemia that is high enough to infect *O. porcinus.porcinus* that feed on them. Another study showed that the proportion of infested warthog burrows and their numbers and stages found in individual burrows vary considerably, depending at least partly on the age and frequency of use of burrows with a distinct preference for porcine blood. In Tanzania and the Serengeti National Parks it was found that identifiable meals from burrow ticks were porcine, presumably warthog (Boreham and Greigy, 1976). Large numbers of *Ornithodoros.porcinus.porcinus* were also found in pigsties in Angola, Zaire and Malawi (Jori *et al*., 2007). This is good evidence that warthogs are involved in infection cycle between vertebrate and invertebrate hosts of ASFV. In Madagascar, *O.porcinus* was identified at three sites in Antananarivo province and also bush pigs were found on the island complicating ASF control (Roger *et al*., 2001) indicating that ticks can play a role in ASFV infections in domestic pigs.

ASF virus can be transmitted between *O. moubata* ticks by trans-stadial, sexual and trans-ovarian pathway, in contrast to *O. erraticus* in Europe where only trans-stadial transmission has been observed (Sánchez-Vizcaíno, 2006).

Transstadial transmission has been demonstrated by (Hess *et al*., 1989) who maintained laboratory colonies of *Ornithodoros* ticks from Zimbabwe that were already infected by ASFV and remained infected for at least 1 year. However no data are available on transmission rates between development stages.

Sexual transmission has been proved with a Ugandan isolate in *Ornithodoros* ticks of the *O. moubata* group with a male-to-female transmission rate of 87.6%. This finding may explain the 4- to 6-fold increase in infection prevalence between late nymphal stages and adults observed by (Plowright *et al*., 1974). Infected ticks excrete the virus in the salivary and coxal fluids (Kleiboeker *et al*., 1998a) that appear in the ventral body surface during and immediately after a blood meal, which is also often voided during or soon after feeding (Hoogstraal, 1956) , as well as in the saliva and female genital secretions (Greig, 1972).

Finally, transovarial transmission was also demonstrated by (Plowright *et al*., 1970) under field conditions on *Ornithodoros* ticks collected from warthog burrows in northern Tanzania, with a filial infection rate of 67-78%, and later, (Rennie *et al*., 2001) by laboratory experimental infections of *O. moubata* with a Zambian virus isolate originally collected from wild ticks, with a filial infection rate of 1.8-31.8%. ASFV persistence in ticks that present an extreme long life-span of 5-10 years without feeding makes it quite reasonable to consider that African *Ornithodoros* ticks act as natural reservoirs for ASFV. Regarding such modes of transmission, ASFV can be maintained in ticks without horizontal transmission involving swine, as well as ASFV multiplication leading to its long-term survival. The asymptomatic wild suids and the transmission among ticks allow a cycle which can be maintained indefinitely in Africa *(Parker et al*., 1969). These pathways could explain why ASF disease is endemic in Africa.

2.4 Correlation of tick exposure and virus prevalence

African swine fever transmission to domestic pigs is mainly caused by the bites of infected ticks or by the ingestion of tissues from acute-infected warthogs (Wilkinson and Paton, 1989). The wild swine in Africa can remain infected over a long period without showing symptoms of disease and thus can be considered as a natural reservoir. Although warthogs are natural hosts of the ASF virus, it has been well demonstrated that they can not transmit the virus directly to domestic pigs. It has been suspected that *Ornithodoros* ticks play some role in the ASF transmission. Infection in warthogs occurs basically in the burrows, where a strong symbiotic relation occurs with Argasid ticks. The infection is characterized by low levels of virus in the tissues, mainly in the lymphatic system and low or undetectable levels of virus in blood (Plowright, 1981) . Young warthogs are normally born uninfected but contract infection when bitten by *O. moubata* in the burrow, then develop a viraemia lasting for 2-3 weeks. This is sufficient to infect a proportion of ticks which feed on viraemic newborn warthogs (Thomson *et al*., 1980). Viral particles in warthog blood rarely exceed 102 Haemadsorbing (HAD50/ml) and progressively decrease thereafter. After this generalized phase of infection, the virus localizes in various superficial lymph nodes, with virus levels up to 106.6 HAD50 and animals remain infected for life (Rennie *et al*., 2001; Wilkinson and Paton, 1989). The virus has a predilection for lymph nodes of the head. Horizontal or vertical transmission does not occur in the warthog and maintenance of the virus within warthog populations is dependent on the soft tick *Ornithodoros moubata* which inhabits warthog burrows (Plowright, 1981).

The warthog-*O.moubata* cycle is virtually limited to areas where Argasid ticks are distributed and has been described in most of South and East African countries (Plowright *et al*., 1994). In southern Africa and some localities in eastern and central Africa the most likely explanation is

that live warthogs carry infected ticks onto land used for extensive foraging by pigs. Ticks would then transmit the virus through saliva and engorging or through contamination of skin wounds by excretions such as coxial fluid. Infected ticks could also be crushed against the skin or be eaten, thus releasing viruses and infecting orally or through discontinuities of the skin epithelia (Plowright *et al.*, 1994). Such studies have not been done in Kenya and Uganda hence tick distribution in domestic pig setting in the two countries is not documented.

The studies done in wildlife identified infestation of warthog burrows even in areas where the Argasid ticks is present-, are variable in terms of the numbers and stages of ticks found and the proportion of burrows infested, which might depend on warthog activity on those burrows. This was demonstrated in studies, (Okoth *et al*., 2013) where some areas in Central Kenya had seroprevalences in warthogs were observed but no Argasid ticks could be found in samples from some of the burrows. In Senegal for instance, it is likely that this relation does not occur since *O. moubata* is absent (Vial *et al*., 2007). This could be probably the reason why the circulation of ASF has never so far been demonstrated in warthogs outside Eastern and Southern Africa. This is an indication of *O. moubata* playing an important role in ASF transmission in Eastern and Southern Africa as the disease is endemic. Adult warthogs, as well as other mammalian hosts of the tick, which are able to wander freely into areas used for domestic swine farming, may act as efficient transporters of infected ticks from wild areas to domestic ones and initiate ASF outbreaks at intervals up to many months later (Parker *et al*., 1969). Domestic pigs especially those raised under free range could be infected once they scavenge at that area. Pigs may also become infected after being bitten by soft ticks, brought to human settlements with warthog carcasses or by ingestion of infected soft ticks. This hypothesis seems more plausible since soft ticks have been in some occasions found on warthogs bodies, outside their burrows (Horak *et al*.,

1983). Though bushpigs were demonstrated to be more efficient reservoirs for ASFV (Luther *et al*., 2007) they do not frequent burrows or caves that may be infested by *Ornithodoros* tick vectors. Thus it is very unlikely that bushpigs contribute to the infection of tick vectors because of their low probability of contacts. Warthogs on the other hand are the ones that play a big role in infecting tick vectors which in turn bite domestic pigs.

The presence of antibodies against *O. moubata* in domestic pigs suggests that soft ticks may be able to maintain ASFV within a domestic pig cycle (Ravaomanana *et al*., 2010). Studies that incorporate screening of pigs for exposure to tick bite therefore can serve as a proxy to demonstrate relationships between the exposure and virus prevalence.

2.5 Tick surveillance using *O. moubata* tick ELISA

Direct methods for tick surveillance are based on the capture and identification of specimens, either from the vegetation (dragging method) or from animal hosts in the area sampled. While these procedures are useful for the surveillance of ixodid ticks owing to their exophilous lifestyle and long feeding times, they will not work with Argasid ticks because they are endophilous/nidicolous and fast feeders. This means that vegetation dragging and the removal from animals are inefficient as direct methods for argasid surveillance; instead it is necessary to explore all possible tick refuges in the area sampled before such an area can be considered tick-free (Oleaga-Perez *et al*., 1990; Vial *et al*., 2006). Evidently, this is an impractical procedure for large-scale studies. Tick ELISA could be used for screening pig sera to identify those that are exposed to tick bites then carry out comprehensive tick control regime.

Serology against tick salivary protein has been used to evaluate the epidemiology of *Ornithodoros erraticus*, by ELISA (Canals *et al*., 1990). The test allowed for identification of pig

farms infested with *O. erraticus* in ASF-endemic area of Spain and permitted the application of specific control measures to avoid tick-pig contact on the tick-infested farms.

The antigen extract used in the ELISA test was salivary gland extract (SGE) obtained from *O. erraticus* ticks. The composition of SGE proved to be qualitatively similar in all the developmental stages of the tick and also to the salivary fluid secreted into the host *(Baranda et al*., 1997). Preparation of SGE is time-consuming and difficult to standardize and it may contain non-specific antigens giving rise to unexpected cross-reactivity. This prompted selection and characterization of a single antigen from SGE by purifying the four main antigens from both *O. erraticus* and *O. moubata* SGE and studied their diagnostic value, (Baranda *et al*., 2000). Regarding *O. moubata*, the best candidate for the serodiagnosis of infested animals was at its 20A1 antigen, which showed 50% identity in its N-terminus when compared to the TSGP1 salivary lipocalin of *Ornithodoros savignyi* (Baranda *et al*., 2000; Mans *et al*., 2003).

In a later study, it was showed that all the spots recognized on the *O. moubata* SGE in the 2D-Western blots by the a pool of sera from naturally infected pigs corresponded to different isoforms of the same protein identified by *denovo* sequencing as an artholoque of the *O. savignyi* TSGP1 lipocalin and was assumed to represent the 20A1 antigen (Oleaga *et al*., 2007).

Sera from experimentally infected pigs with different developmental stages of *O. moubata* were obtained. Blood samples were taken at seven days after the first infestation (primary response), and at seven days (secondary response) and 2.5 months (residual response) after the third infestation. Sera against different developmental stages of *O. erraticus* were also used. The pre-infestation sera (21 sera) were used as experimental negative controls (Baranda *et al*., 2000).

Regarding *O. moubata* field negative sera, pig samples were collected from Salamanca province (Spain), an area free of *O. moubata*. These sera included samples from farms free of and infested with *O. erraticus*, as detected with anti-*O. erraticus* SGE-ELISA (Perez-Sanchez *et al*., 1994). The pigs were living under free-range management system hence exposed to a wide range of ectoparasites. Collection of sera samples was done in regions free and infested with *O. erraticus* under free range pig management system to validate the *O. moubata* tick ELISA using a tick specific rtTSGP1 ELISA (Diaz-Martin et al., 2011). This was then used to test for antibodies to *O.moubata* salivary gland proteins. The rtTSGP1 ELISA kit was validated in Spain for detection *O. moubata* antibodies from known positives sera, potential positive and know negative sera obtained from the Institute of Natural Resources and Agrobiology Salamanca (IRNASA). The ELISA Kit involved the use of a recombinant form of omTSGP1 that had 100% sensitivity and 99.4% specificity at Cut -off (% SI) 7.53 (Diaz-Martin *et al*., 2011).

The study used tick exposure as a proxy for possibility of tick transmitting ASFV.

2.6 Tick control

The responsibility for control of serious transboundary diseases such as ASF mostly is directed to the Veterinary authorities, but there is no doubt that breakdowns will occur unless pig producers understand how ASF is transmitted and take all the necessary precautions to ensure that their own herds will not become infected (Wamwatila, personal communication).

Control of tick infestations and tick transmitted diseases can be difficult and frustrating. Reports from studies conducted (Stefanoff *et al*., 2012) showed that product failures are common and resistance is often touted as the reason for these failures. However, various biologic and ecologic factors are actually responsible for most perceived control failures. One area of specific

importance is that there has been a documented range expansion and increased density of several important tick species. The host, habitat and climatic factors have contributed to changes in tick distribution, density and seasonality. Increasing tick populations will cause significant problems by contributing to spread of tick transmitted diseases. To meet tick control challenges, comprehensive training of farmers is required on key issues namely:

i. Explain the factors that conspire to create higher tick populations in nature and increased risk of infestation to pigs

ii. Implement comprehensive tick control programs, including making recommendations on habitat and lifestyle modifications.

iii. Treat pigs effectively to prevent tick infestations through consistent use of acaricides and sound recommendations for avoiding heavily infested environments (Stefanoff *et al*., 2012).

Ornithodoros ticks feed mainly on animal species living in burrows, such as rodents and reptiles thus suspected to play an important in maintaining the local foci of the ASFV (and lead to endemicity in a region). However, they do not play an active role in the geographical spread of the virus. Pigs are mostly accidental hosts, from which the ticks can be infected. The epidemiological role played by soft ticks becomes important where pigs are managed under traditional system, including old shelters/sties with crevices. There is no well documented systematic monitoring of the occurrence of *Ornithodoros* ticks in European Union due to the limited available data on associated factors with the distribution of soft ticks. In addition, prediction of their potential distribution is difficult to construct. There is no single ideal solution to the control of ticks relevant for ASF. The integrated control approach is probably the most

effective. Vector and reservoir surveillance is an important component of such a strategy (EFSA, 2010).

Some species of *Ornithodoros* could adapt to domestic conditions and have been found in chicken pens, small ruminant sheds, pigsties and human dwellings with mud walls and floors (Rodhain, 1976; Walton, 1962). It has never been reported that soft ticks can move by itself outside buildings or its burrows but only by attaching to the host. Transfer can be explained either by the transfer of utensils contaminated with the parasite or by the passive transfer of soft ticks feeding on animals being moved. This could only be responsible for transfer over short distances, since the time of feeding is generally short, 10 to 30 minutes (Fernandez, Garcia, 1970), and possibly even shorter when the animal is in movement unless trapped in a skin fold. Identification of the geographical location of *Ornithodoros spp* can be achieved by determining exposure of domestic pigs to tick bites which may contribute to ease of their control as they tend to form stable foci.

Transboundary spread also occurs through movements of infected wildlife such as warthogs and bushpigs, together with the soft tick vector. The distribution of the latter may be affected by climate change or by spread to new habitats via the movement of warthogs. The recent creation of transnational protected areas across Africa is thought to expand the available habitats for wildlife and facilitate movements of wildlife disease reservoir species across borders (Zepeda *et al.*, 2001). This explains how control of *Ornithodoros* is very complex unless strategic control approach is adopted.

In regions and countries where tick-borne diseases are present, abundance, seasonality and distribution of the ticks can be assessed by catching ticks on the usual hosts and by collecting

methods of unfed ticks in the environment. Control and prevention of pathogens transmitted by ticks may be through the application of acaricide on the hosts or in the environment. Use of acaricide on pigs and their habitat can reduce the level of infestation in the premises but does not avoid the pigs of becoming infected by ASF virus if they are bitten by a virus infected tick. This could be due to the nidicolous life-style of the argasids, because it is not feasible to ensure that the acaricides reaches all places where the parasites hide (Astigarraga *et al*., 1995).

In addition, there could be inability of other farmers to buy drugs or lack of awareness that ectoparasites are a common problem in their farms. Studies conducted (Wabacha *et al*., 2001) demonstrated that 84% of the pig farmers use conventional acaricides. The commonly used drugs were amitraz- or cabaryl-based compounds and the interval of usage of these drugs was haphazard, and this could be the cause of poor parasite control. A substantial number of farmers also used non-conventional treatments, some of which have been reported in other studies (Ajala *et al*., 2007; Wabacha *et al*., 2004), but their efficacies have not been substantiated. Poor ectoparasite control especially tick control could be the contributing factor of ASF endemicity in most developing countries in sub-Saharan Africa

A more accurate surveillance system, combined with compulsory reporting, could therefore help control the spread of the disease. Developing this system would require development of resources for the local veterinary services. A risk-based surveillance approach, involving the awareness of the pig farming community, would allow more efficient control of the disease, but will require further analysis of risk factors for infection in East African Countries. A new public health policy regarding this issue, which includes a strategy of information dissemination about the disease and its risk factors among the pig farming community, is urgently needed.

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Area of study

The research was focused Busia County in western Kenya and eastern Uganda along the border region (Figure 3.1). Busia County in Kenya is located approximately 500 km from Nairobi on Latitude 0^0 136' and 0^0 North of the Equator. The Longitude 33^0 54' and 34^0 25' east of Greenwich meridian. It covers the 1, 261.3km^2 and is made up of six divisions which are Budalangi, Funyula, Matayos, Township, Butula and Nambale. The County lies within Lake Victoria Basin and has an altitude ranging between 1,130 and 1,375m above sea level (Kagira et al., 2010b; Mutua et al., 2011). Two Districts each from Busia and Uganda were selected using Geographical Information System (GIS) based on 2008 Kenyan and 2010 Ugandan administrative boundaries. This was followed by random selection of locations/sub-Counties, Sub-locations/Parishes and villages. In Busia County, Teso and Busia Districts were selected. In each District, four locations were randomly chosen. In Teso, the selected locations were: Akoreet, Moding, Amoni and Angorom while in Busia the selected locations were: Bunyala North, Bwiri, Nambuku and Lwanya. This was further followed by selection of four sublocations and narrowed down to two villages in each sublocation. The selected sublocations in Teso were: Okook, Apokor, Kajei and Alupe while in Busia were: Mundere, Busijo, Mango and Busende. The villages were: Abileng and Samma in Okook, Kakoit and Erotketom in Apokor, Ajonai and Dip in Kajei and finally ALupe B and Aget in Alupe. In Busia District, the villages were: Bwakama and Mundakaywa in Mundare, Busijo and Rudacho in Busijo, Buradi and Butemula in Mango and lastly Sigomere and Mukhweso in Busende.

In Uganda, Tororo and Busia- Uganda Districts were selected. In each District, four subcounties were chosen. In Tororo, the selected subcounties were: Mella, Kwapa, Magola and Lyolywa while in Busia-Uganda, the sub-counties were: Lumino, Buhehe, Masinya and Buteba. Sublocations in Uganda are named as Parishes. Four parishes selected were; Hasyule, Buhehe, Masinya and Mawero in Busia. In Tororo, the Parishes were: Amoni, Morukebu, Magola and Poyem. The villages were: Nebololа B and Bukani in Hasyule, Bwolia A and Bwani in Buhehe, Bunyukhe and Bulekya in Masinya, Alupe and OkameAmagoro in Mawero. In Tororo district, the villages were: Katanya and Aterait in Amoni, Kangura East and Osera in Morukebu, Paloto A and Poniara in Magola and Nyemera A and Poyem A in Poyem.

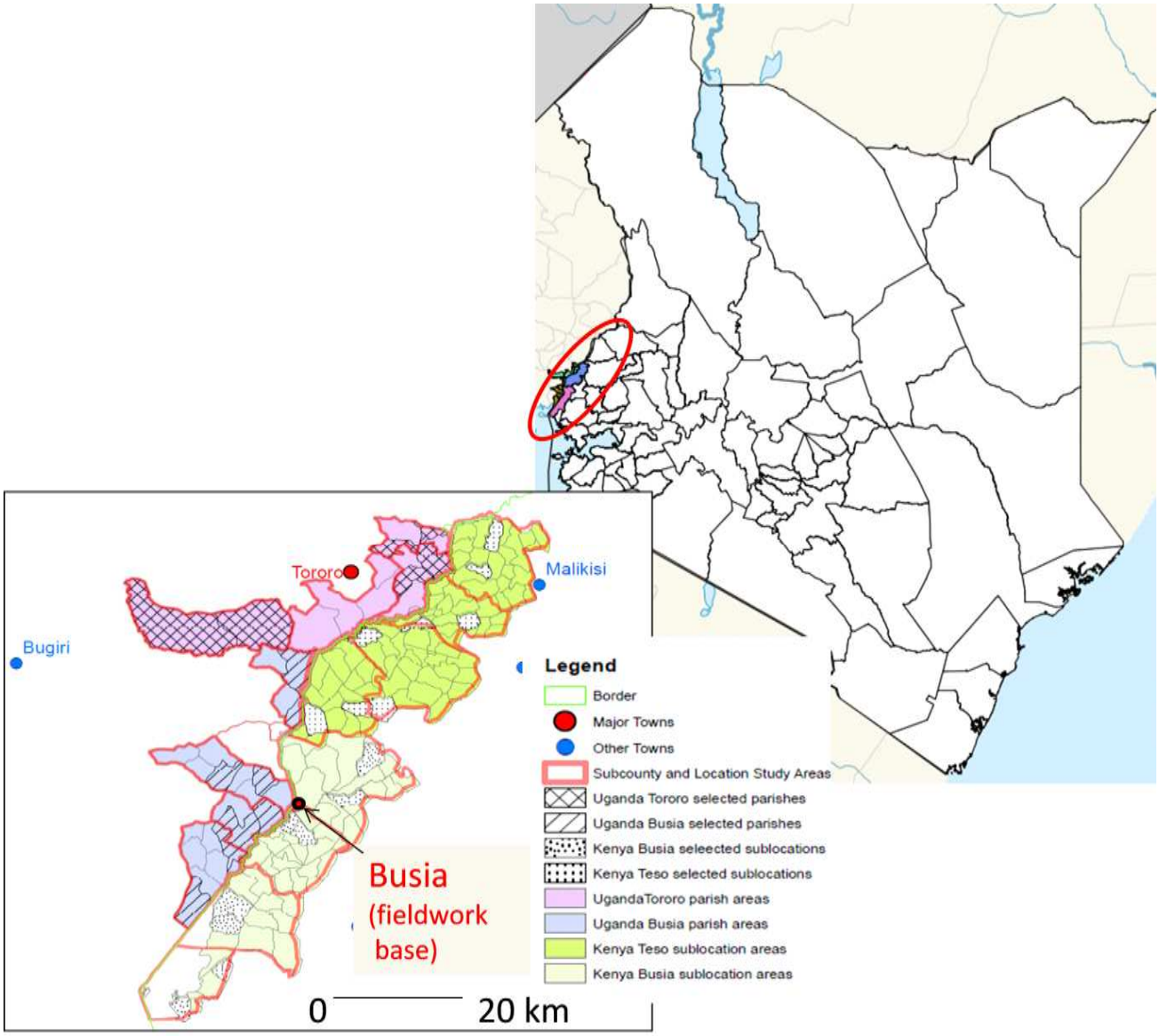


Figure3. 1: Map showing the study site along Kenya-Uganda border

3.2 Sample size determination

Sample size was determined using the following formula (Dohoo *et al*., 2009)

$$n = \frac{(1.96)^2 P(1-p)}{L^2}$$

Where L is the required precision (+ or – error around estimate) - 5%,

P is the anticipated prevalence or proportion of attribute- 30% (Okoth *et al*., 2013) and

The estimate is at the 95% level of confidence.

Then the required sample size (n) is 320 households.

To determine the number of pigs to be sampled in each household, all pig categories (piglet, weaner, sow and boar) were included in selection having a maximum of four pigs in each household. Piglets from three months were selected for sampling (pigs below 3 months not sampled to avoid mortalities when collecting blood from jugular vein).

The breeds reared were categorized into local and crosses/ exotic

3.3 Sampling strategy

The research study was conducted along the Kenya Uganda border in order to understand the trans-boundary dynamics of ASF transmission which has implications on food security and trade as demonstrated in previous outbreaks (Okoth *et al*., 2013). International Livestock Research Institute (ILRI) in collaboration with Ministry of Livestock through the Director of Veterinary services (DVS) - Kenya and The Ministry of Livestock and the local administration in Uganda,

facilitated the ethical procedures to allow sampling activities along Kenya Uganda border. A stratified random sampling approach was used. The sampling frame was; all pig farming households along the Kenya-Uganda border and the sampling unit was the pig. The study used local administration to identify the pig keeping households within the study area.

A multistage sampling approach was used. In the first stage eight administrative locations were randomly selected from Busia County; four each from Teso and Busia districts in Kenya. On the Ugandan side, eight administrative sub-counties were selected; four each from Busia and Tororo districts. The next stage randomly selected two sub locations / Parishes from each Location / sub-county in Kenya and Uganda, respectively, making a total of eight sub-locations/ Parishes selected along the border. The third stage was a random selection of two villages from each sub-location/ Parish. The fourth stage was random selection of twenty pig keeping households from each selected village totaling to six hundred and forty (640) pig keeping households selected for the cross-sectional study. There was an additional sampling in 43 pig keeping households in Busia County because there was a rumour of ASF outbreak in Totokakile sublocation (Totokakile and Onyunyur B villages) leading to a total of 683 pig keeping households in the study.

Finally, a maximum of 4 pigs were selected for sampling from each household. A total of 1085 pigs were selected for the initial cross-sectional sampling.

3.4 Data collection

3.4.1 Household survey

A cross sectional survey was conducted in selected pig keeping households in Busia County and Uganda side. A survey in Busia County was conducted starting from July 2012- August 2012. While in Uganda, the survey was conducted from September- November 2012. Three hundred and twenty (320) pig keeping households from Busia and Uganda were visited. Since there was a rumour of ASF outbreak in Busia County during the time of our survey, 43 pig keeping households were also sampled making a total of 683 households in the research. Structured questionnaire was administered to the selected households. Face to face household interviews were done where general household information, production factors, health and disease management (tick control) was investigated

3.4.2 Characterization and selection of variables

Data on household (respondent's gender, age and level of education and occupation), animal level variables (breed, age, sex, body measurements, and disease status), pig herd structure (pig categories, numbers in each household) and pig management (feeding regimes, parasite control) were collected.

The categorical variables were divided into several levels. For instance: pig breed (cross and exotic), respondent education (none, primary, and secondary and above), feed management (confined and free range), tick control (yes and none).

Tick control practice in Kenya is done by acaricide application either by dipping or spraying. Tick control factor considered in the study was whether the pig farmer practices tick controls on his pig farm or not

Factors considered under feed management were whether pigs were tethered, housed or free range. Pigs tethered during planting season and released to roam freely after harvesting crops were placed in free range category since this management system was likely to increase the risk of tick exposure in pig herd while scavenging for food

3.4.3 Pig sampling

Pigs from the selected farms were physically restrained using a pig catcher prior to sampling. Blood was collected from the jugular veins using BD Vacutainer® needles (gauge x length: 21 x 1-1/2 inch) into 10 ml BD Vacutainer® glass serum tube (Becton, Dickinson and Company, United Kingdom). Blood was allowed to clot and serum separated. Serum were dispensed into 2 ml cryo-vials (Greiner bio-one, Germany) and stored at minus 20^{o}C. The samples were transported to International Livestock Research Institute (ILRI), Nairobi for laboratory analysis.

3.5 Optimization of rtTSGP1 ELISA and field sample analysis

To determine the prevalence of tick exposure in Busia and Uganda, serum specimen collected from 1085 domestic pigs (595 from Busia County and 490 from Uganda) in the 4 pig-farming districts were tested for antibodies to *O. moubata* salivary gland proteins using a tick specific rtTSGP1 ELISA (Diaz-Martin *et al*., 2011). The rtTSGP1 ELISA kit was validated in Spain for detection *O. moubata* antibodies from known positives sera, potential positive and know negative sera obtained from the Institute of Natural Resources and Agrobiology Salamanca

(IRNASA). The ELISA Kit involved the use of a recombinant form of omTSGP1 that had 100% sensitivity and 99.4% specificity at Cut -off (% SI) 7.53 (Diaz-Martin *et al*., 2011).

The study used tick exposure as a proxy for possibility of tick transmitting ASFV.

To optimize tick ELISA for use in diagnosis of *O. moubata* exposure to domestic pigs in East Africa, various ELISA plates (Immulon 1B, Immulon 4 HBX, Polysorp and Maxisorp) assessed for performance. The plates were each coated with 100 ng of rtTSGP1 antigen followed by several dilutions of reagents and serum samples and washing using 0.05% Tween 20 in PBS pH 7.2 (TPBS), at room temperature. Two different substrates (OPD) Ortho-phenilen-diamine, (SIGMA P-1526) and 3,3′,5,5′-tetramethylbenzidine (TMB) were also tested to choose the best substrate for carrying out the ELISA and to check for presence of background across entire plates (to generate a stronger signal that differentiates the "positive value" from the "negative value").

Further optimization using known serum samples from young warthogs associated with burrows with infested ticks that tested positive for exposure for *O. moubata* antibodies were also tested (Okoth *et al.*, 2013).

3.5.1 rtTSGP1 ELISA protocol

Microtitre ELISA plates were coated with 100 ng of rtTSGP1 antigen, diluted in 100 µl of 0.05 M carbonate/bicarbonate buffer, pH 9.6. to each well. The plates were then incubated at 4°C for 16 hours (overnight). The plates were then washed five times with 200 µl/well of 0.05% Tween 20 in PBS pH 7.2 (TPBS), at room temperature. Plates were coated using 200 µl/well of 1% BSA in PBS, 1 hour at 37^{o}C on a plate shaker and then washed five times with 200 µl/well of

0.05% Tween 20 in PBS pH 7.2 (TPBS). Test sera, positive and negative control sera were diluted 1/300 in 0.05% Tween 20 in PBS, and 100 µl of each diluted serum added to duplicate wells of the antigen-coated plate. Four pairs of each positive and negative control serum were added to wells in different parts of the plate, hence 40 sera tested in duplicate on one plate. The plates were then incubated for 1 hour at 37 ºC. Plates were then loaded with the conjugate by adding 100 µl/well of anti-pig IgG-horseradish peroxidase (SIGMA, A5670), diluted 1/5000 in 0.05% Tween 20 in PBS and incubated for 1 hour at 37^{o}C then washed five times with TPBS.100 µl of (OPD) Ortho-phenilen-diamine, (SIGMA P-1526) substrate solution was added in each well and plates incubated at room temperature for approximately 3-5 minutes (before the negative control begins to be coloured). The time necessary for the colour to develop depended on both the temperature of the substrate when added to the wells, and the room temperature. The reaction was stopped by adding 100 µl of 3N sulphuric acid to each well.

3.5.2 Reading the ELISA results

Positive sera had a clear yellow colour and could be read by eye after adding the OPD substrate, but to ensure that all positive sera were identified, the absorbance in each well was read spectrophotometrically, at 492 nm using BioTek Synergy HT ELISA reader. Serum was considered to be positive if it had an absorbance value of greater than two standard deviations from the mean absorbance value of the control negative sera.

3.5.3 Calculation of serological index (SI).

The following formula was used:

$$[(NC-S)/(NC-PC)]\times 100,$$ (Diaz-Martin *et al.*, 2011)

Where:

- NC and PC represent the negative and positive control values, respectively, and S stands for each sample serum values.
- The threshold using SI for a positive was an SI value greater than mean SI of the negative sera added to twice the standard deviation.
- Negative value SI was a value less than Mean SI of the negative sera added to twice the standard deviation.
- Positive value SI was a value greater than Mean SI of the positive sera added to twice the standard deviation.

For the threshold using optic density (OD):

- Negative value was Optic density less than Mean optic density of the negative sera added to twice the standard deviation.
- Positive value was Optic density greater than Mean optic density of the negative sera added to twice the standard deviation.

3.6 Data handling and analysis

Field and laboratory data were recorded and entered into a database using Microsoft Access 2000 (Microsoft Corporation) and statistical analyses were conducted using Statistical Package for Social Sciences (SPSS) (IBM SPSS Statistics for Windows, Version 21.0)

Data analysis was done at two levels. The first part involved tick exposure and farm level characteristics. The second part involved regression analysis using the variables in part one to generate pig tick exposure prediction model. This model was used to select factors that

significantly explain the outcome (pig exposure to tick bite in the pig herd) that are important risk factors.

Regression analysis was done in SPSS (IBM SPSS Statistics for Windows, Version 21.0). The analysis used factors as animal level variables (breed and number of pigs), farm management practices (feeding management and tick control)

The model was fitted using logistic regression since the dependent variable was dichotomous/ binary. Backward elimination method was used to build the logistic model. The analysis provided a method of identifying the important factors thought to influence pig exposure to tick bites in pig herd.

Cross tabulation (association tests) was done to examine the interrelation between exposures to ticks and previous suspected ASF like outbreaks in pig keeping households to check for if there was interaction. To determine how strong the interaction between tick exposure and previous ASF outbreaks, Phi and Cramer's V was used at (CI 95%, $P<0.05$)

The model was also tested for goodness of fit using Phi and Cramer's V at (CI 95%, $p<0.05$) to determine how strong the association between tick exposure and significant risk factors.

CHAPTER FOUR

4. RESULTS

This chapter reports the results from household and pigs survey in western Kenya and eastern Uganda. The farms and households were characterized in the four districts of Busia and Teso (Kenya) and Busia and Tororo (Uganda) and associations drawn between exposures of domestic pigs to tick bites.

4.1. Household characteristics

Descriptive statistics characterized respondents and household using variable that included age, sex, gender, level of education, household economics, pig management and pig herd structures.

4.1.1. Household gender

A total of 683 pig-keeping households were visited in the study area. Female respondents were 60% of the total number of respondents from these households. This was interpreted as a balanced gender ratio in the study (Table 4.1) and thus gender as variable was considered an indicator of contributions by gender to pig husbandry in the study region. The women were either heads or wives within the households (Table 4.1). Women headed households were 15.5% of the total female respondents.

Table4. 1: Respondents gender and position within household

			Respondent position within household				Total
			Household head	Other (specify)	Son/daughter	Wife	
Respondents Gender	Female	Count	22	3	6	111	142
		% within Resp. Gender	15.50%	2.10%	4.20%	78.20%	100.00%
	Male	Count	83	4	14	3	104
		% within Resp. Gender	79.80%	3.80%	13.50%	2.90%	100.00%
Total		Count	105	7	20	114	246
		% within Resp. Gender	42.70%	2.80%	8.10%	46.30%	100.00%

4.1.2. Respondents level of education

In both Kenya and Uganda 87% of the respondents in the study area had undergone some level of education (Table 4.2). Majority of the pig farmers (78%) had attended primary school level of education and above though literacy level was much higher within education category in Uganda compared to Kenya (90% of respondents had attended secondary level of education in Uganda while only 10% in Kenya). Literacy was considered to influence decisions at the farm level especially on disease control.

Majority of respondents practiced farming as their source of income which accounted for 82.12% S (78% in Kenya and 84% in Uganda).

Table4. 2: Respondent's education levels by country

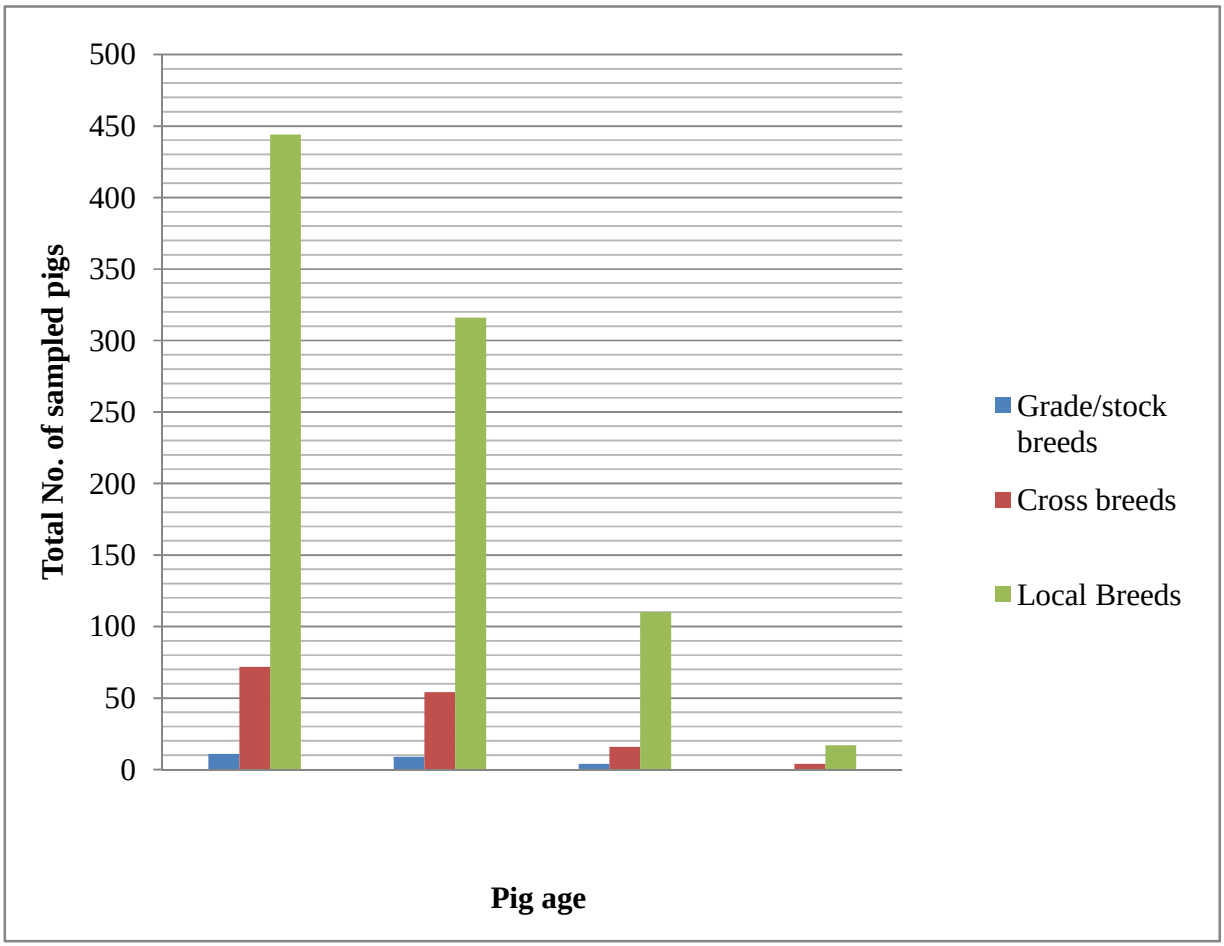
			Respondent Education			
			None	Primary	Secondary and above	Total
Country	Kenya	Count	14	79	15	108
		% within Country	13.0%	73.1%	13.9%	100.0%
		% within education	18.9%	23.0%	10.0%	19.0%
	Uganda	Count	60	264	135	459
		% within Country	13.1%	57.5%	29.4%	100.0%
		% within education	81.1%	77.0%	90.0%	81.0%
Total		Count	74	343	150	567
		% within Country	13.1%	60.5%	26.5%	100.0%
		% within education	100.0%	100.0%	100.0%	100.0%

4.2. Pig production and management

4.2.1. Herd structure

The survey showed that pig farming was of a small scale type where farmers owned on average 3 (CI 2.95, 3.31) pigs. Categories of pigs kept by the farmers were piglets (below 3 months), weaners, breeding sows (pregnant or furrowed) and breeding and castrated boars. Few farmers kept breeding boars and therefore bred their sows using borrowed boars. Farmers kept local and cross breeds. The local breeds were described by farmers as well adapted to the environment where as the pure-breeds and their crosses were less well adopted. The commonly reared breed was local as compared to cross breed. In Busia County, Kenya, local breeds accounted for 84.5% (876/1037) and only 15.5% (161/1037) was accounted for by cross-breeds. In Busia and Tororo districts in Uganda, local breeds accounted for 92.1% (715/776) and 8% (61/776) were accounted for by cross-breeds.

Figure 4.1 Herd structure



4.2.2. Feeding management

Majority of pig farmers practiced free range, tethering or mixed (free range and tethering) (Figure4.2 and Plate 4.1). In the free range system, pigs were released in the morning to roam and scavenge throughout the day for feed and returned in the evening for shelter and feed supplementation with kitchen wastes and water or confined during the day and released to scavenge overnight. Other farmers tethered their pigs around the homestead especially during planting season and released them during harvesting and post harvesting periods to roam until the next planting season. Farmers mentioned that tethering of pigs was practiced to confine animals and prevent conflict with neighbors. Where all land had been cultivated due to small land sizes giving no room for pigs to roam freely, the farmers had no option but to tether their pigs. Tethered pigs were fed potato tubers or vines, cassava or their peelings and fruits such as mangoes, guavas, papaws and avocados. Vegetables such as cabbage, kales and cereals e.g. maize meal were also fed to pigs. Majority of farmers specialized in selling their piglets at the age of 2 months. Main reasons given were due to feeding constraints and economic gains.

Figure4.2: Feeding regimes

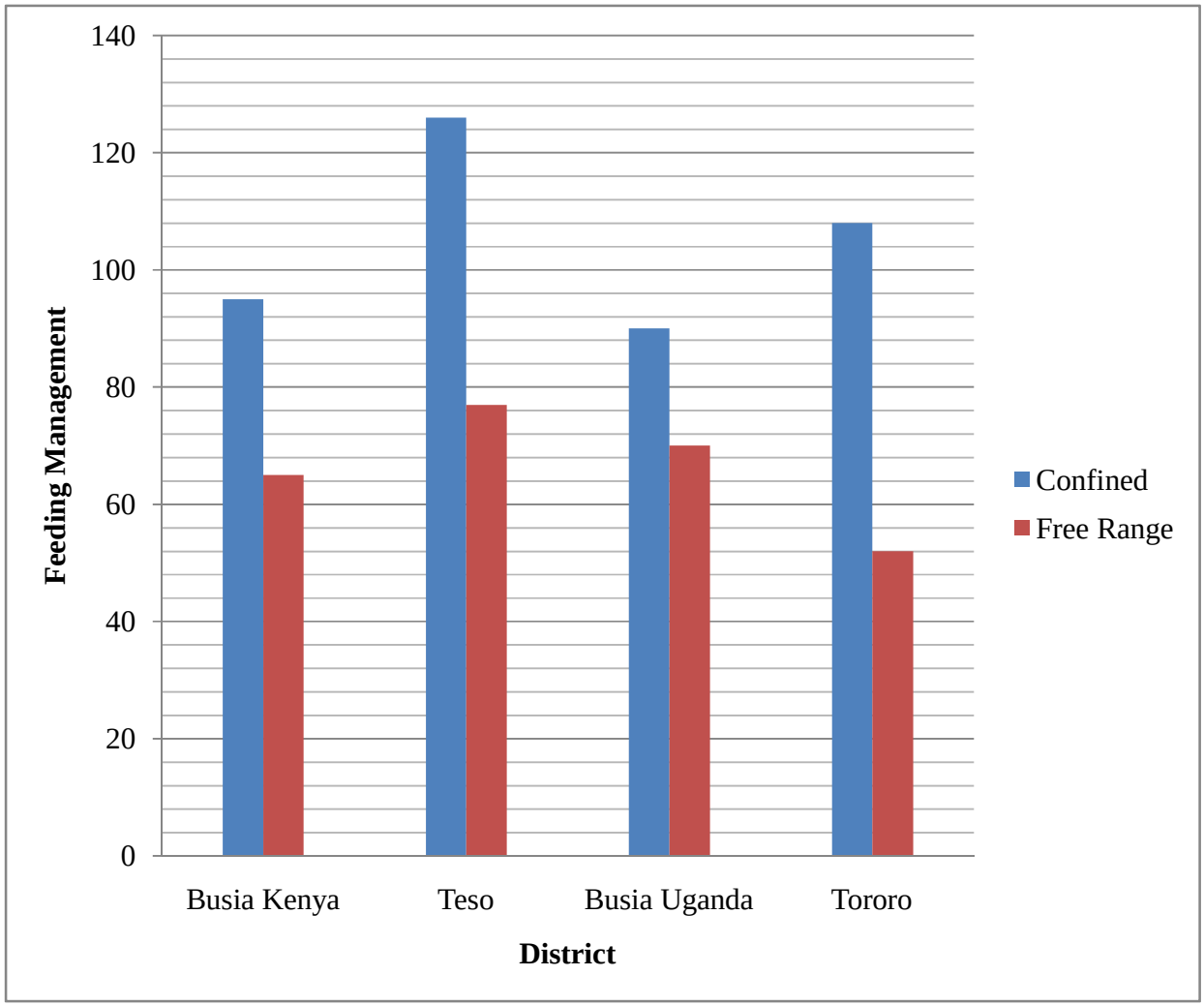
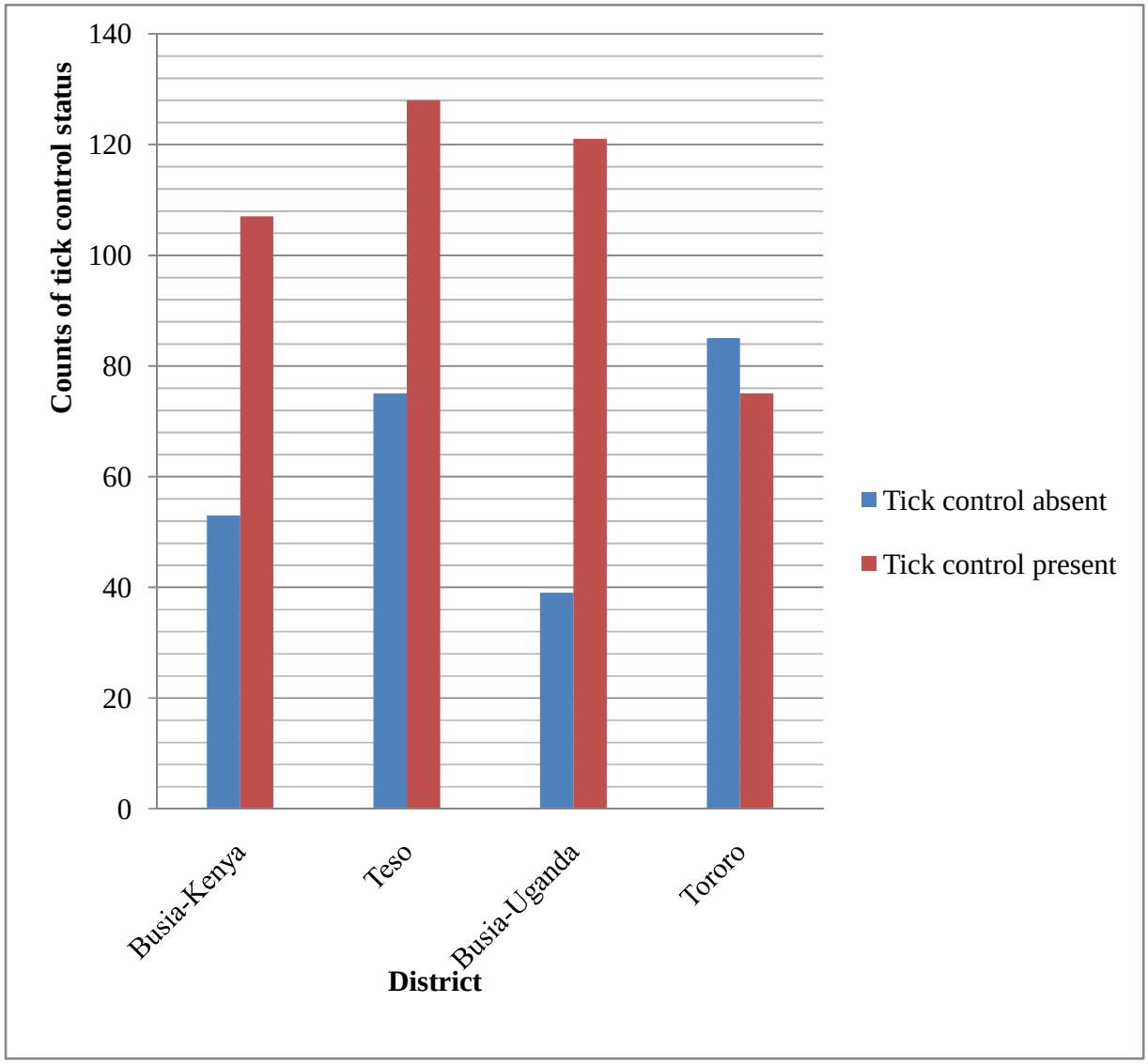


Plate 4.1: (a) Traditional pig house, Tororo (b) Pig feeding on garbage, Busia, Uganda (c) Tethered pig under a tree, Teso

4.2.3. Tick control

Generally out of 683 households 64 % of farmers (66% in Kenya and 62% in Uganda) said they controlled ticks in their farms. Parasite control involved farmers self-treating own pigs (41%) and or contracting animal health service providers for the service (10%) and the rest using non-conventional methods including mud baths/wallowing that was perceived by farmers as one of the method for tick control but in the reality it is not. This is because the actual act of mud bathing/wallowing was not done by the pig owner rather dictated by the pigs themselves. When farmers were asked about the frequency of conventional tick control using acaricides the control regimes mentioned included once, daily, weekly, monthly and every three months and more. Respondents mentioned that the frequency of acaricide application depended on visibility of external parasites on the pigs by the farmers. The % acaricide applications in the four districts studied were a 67%, 63%, 76% and 47 % in Busia (Kenya), Teso, Busia (Uganda) and Tororo, respectively (Figure 4.3).

Figure 4.3Tick control status



4.3. Optimization of tick ELISA for use to assess for *O. moubata* exposure

Immulon 1B ELISA plate was found to give the least background when used with OPD substrate hence both chosen as the best plate and substrate combination for serology to determine presence of antibodies against *O. moubata*.

Further optimization using known serum samples from young warthogs associated with burrows with infested ticks that tested positive for exposure for *O. moubata* antibodies (Okoth *et al.*, 2013) were also shown to be optimum as positive controls for the tick ELISA.

4.4. Risk of pig exposure to tick bites

4.4.1. Tick bite prevalence

The study confirmed that domestic pigs in the study area were exposed to *O. moubata* tick bites. Prevalence of pig exposure to tick bites was 17% (181/1085) in the whole study area. Kenya had the highest prevalence of 22% and Uganda 10%. The exposure varied between study districts, with prevalence (%) of 5, 10, 15 and 31 in Tororo-Uganda, Busia-Kenya, Busia-Uganda and Teso-Kenya, respectively. When exposure between pig breeds was compared, there was 7% exposure prevalence in crosses compared to 18% in local breeds with the difference being significant at 95% confidence (odds of exposure was 0.109 and 0.237 (Uganda and Kenya respectively) more exposure in local breeds compared to cross breeds). High exposure to *O. moubata* in local breeds could be attributed to large numbers of local pigs in the study (more than 80%) and pig management systems in the region where less attention is given to local pigs in terms of investment. Local pig breeds are allowed to roam freely around the household and surrounding villages to scavenge for food (FAO., 2009). In addition, some local breeds were

housed at night in small shelters to protect them against theft and predators. Their shelters (pens) were made of simple local materials (wooden/ mud walls and mud floors) as compared to better housing (concrete houses without crevices and cracks) where exotic breeds are raised. Lack of confinement and poor housing is a major risk for *O. moubata* exposure (Boinas *et al*., 2004).

There was mixed feed regime in the four studied regions. These were free range, tethering and a mixture of both depending on either planting or harvesting season. Mixed tethering and free range depended on presence or absence of crops on the farms. Tethering was done when crops were on the farms and free-ranging done after harvesting. Few farmers provided housing for their pigs and the common pig houses were local mud houses. Majority of pigs (61%) were confined (tethered or housed) compared to 39% free ranging. Comparison of exposure of confined and free grazing pigs showed 15.6% in confined pigs while 18.4% in free range pigs. Assessment of association of exposure and tick control using a Pearson Chi-square test in a 2x2 table was not significant ($P>0.05$) interpreted as tick control having no effect on tick exposure. Similar assessment of association of exposure with respondents education category was significant ($P<0.05$), this result interpreted as education level explaining whether a household implemented control or not.

4.4.2. Regression analysis of tick exposure

Further analysis was done to identify factors which could potentially influence exposure of domestic pigs to tick bites. The factors fitted in the model were: pig breed, number of pigs per household, respondent's level of education, respondent's occupation, feed management and tick control**.** The categorical variables were pig breed (cross and exotic), respondent education (none,

primary, and secondary and above), feed management (confined and free range), tick control (yes and none).

The dataset was divided into two (Kenya and Uganda) as it was thought there could be some difference in terms of practices in the two countries according to experts which could lead to confounding of other multiple explanatory variables. Factors found to be significant in Uganda were: Pig breed, feed management and tick control. In Kenya, the significant factors were pig breed, respondent education and respondent occupation, number of pigs per household and tick control. The results from the model are shown in (Table 4.4 and 4.5).

The results showed the following:

Respondent education, respondent occupation and cross breed reduced the odds of exposure to tick bites while number of pigs per household increased exposure to tick bites.

No tick control reduced the odds of tick exposure in Uganda while in Kenya; it increased the odds of tick exposure 1.7 times. This was interpreted as tick control being effective in Kenya and non-effective in Uganda

Table4. 3: Binary logistic regression analysis of tick exposure for Uganda

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for	
								Lower	Upper
Step 1[a]	Pig breed (cross)	-2.215	1.019	4.719	1	.03	.109	.015	.805
	Feed management (confined)	-1.015	.287	12.554	1	.00	.362	.207	.635
	Tick control (none)	-.673	.324	4.320	1	.04	.510	.270	.962
	Constant	-1.290	.197	42.903	1	.00	.275		

Table4. 4: Binary logistic regression analysis of tick exposure for Kenya

		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for Lower	Upper
Step 1[a]	Pig breed (cross)	-1.441	0.394	13.398	1	0.00	0.237	0.109	0.512
	Respondent education category			14.012	2	0.00			
	Respondent education category (none)	-1.093	0.422	6.707	1	0.01	0.335	0.147	0.767
	Respondent education category (Primary)	-1.036	0.283	13.416	1	0.00	0.355	0.204	0.618
	Occupation category (Farming)	-0.853	0.272	9.847	1	0.00	0.426	0.25	0.726
	No of pigs per household	0.15	0.051	8.775	1	0.00	1.162	1.052	1.283
	Tick control (None)	0.53	0.241	4.844	1	0.03	1.699	1.06	2.723
	Constant	-0.184	0.337	0.3	1	0.58	0.832		

4.4.3. Association of tick exposure and African swine fever outbreaks

Associations of tick exposure and previous ASF like outbreaks on farm were also investigated. There were no farms that had experienced outbreaks among those sampled in Kenya though their neighbours had been reported to have had outbreaks. In this regard associations between tick exposure and outbreaks could not be drawn from Kenya. In Uganda there was an association between exposure and ASF outbreaks with significant Chi-square ($P<0.05$) and (Cramer's V value of 0.079) interpreted as ticks could play a role in the ASF outbreaks.

CHAPTER FIVE

5. DISCUSSION

Virus cycles are recognized to occur in endemic African setting referred to as pig-tick cycle and a cycle in which the virus persists in domestic pigs without sylvatic host (Penrith *et al*., 2004). The occurrence of a limited number of positive domestic pigs to *O. moubata* complex antibodies raises the hypothesis that occasional contacts between domestic pigs and soft ticks are possible and that the arthropods could contribute to ASFV maintenance in the environment, in areas where the tick remains present since they establish stable foci (Penrith *et al.*, 2004). The occurrence of positive domestic pigs to *O. moubata* complex antibodies has not been investigated in East Africa and therefore the role of ticks in the epidemiology of ASF as observed in Kenya (Okoth *et al*., 2013) is not well understood. Control of ASF will need this understanding. The current study surveyed household characteristics and risk factors for tick exposures to support the understanding.

The study shows equal participation of both genders in pig production. This was indicative of the role pigs play in livelihoods of the rural poor and especially women and children who are generally marginalized. The current study shows a number of women headed households (15.5%) are keeping pigs and a balanced distribution in pig keeping roles to both genders (42%) of women participating in pig production. This result compares to studies in western Kenya that show many smallholder pig systems are managed by women (Mutua *et al*., 2010). Improvements in productivity can be achieved through disease control (and especially ASF vector control). In addition, improved service delivery along the value chain can reduce waste and inefficiency leading to improved quality of the final product, thereby adding value that translates in increased

employment and income to women to support their families. This puts to task the role of development partners to focus in the development of the pig sector to improve livelihoods especially through empowering women. Other studies have also shown that pigs are traditionally owned by women (Muys and Westenbrink, 2004), who play crucial roles in both domestic and economic life of the society (Damisa and Yohanna, 2007).

To assess the influence of risk factors on tick exposure, an analysis was done first on a total data set that included both data from Kenya and Uganda. The explanatory variables (number of pigs per household, feeding management, tick control, respondent's education and occupation) included in the models did not show any influence on exposure outcome. However when data from each country was subjected to individual analysis, significant risk factors were identified for each country. Pig breed and no tick control were found to be significant risk factors in Kenya-Busia County and Uganda. There were other factors which were found to be significant in one country but insignificant in the other. For instance in Uganda, feed management was significant while in Kenya: respondent' education, respondent's occupation and number of pigs per household were found to be significant. This suggested heterogeneity between and homogeneity within countries with country being a confounder in the analysis. This further suggested difference in pig management and pig production in the two countries. The significant risk factors influencing tick exposure in both countries is further discussed.

Literacy was considered in this study to influence decisions at the farm level and especially disease control and thought to influence information take up and retention to implement disease control strategies such as vector control on pig farms. Farmers with higher levels of education were thought to be likely to take up information through extension services and were able to assess other important information regarding pig husbandry. This study regards that the level of

education observed in the study area is adequate to influence uptake of new innovations for improved animal productivity. Agricultural extension is a powerful tool with a rich potential to empower and support rural livelihoods (Anon, 1999; Rola *et al.*, 2002). Examples of extension system weaknesses have been reported in Kenya (Muyanga and Jayne, 2006). Improvement of knowledge of pig management need to be considered in future planning and strengthening of extension networks, particularly in designing field training manuals to help in improving pig sector and poverty reduction in rural setting that takes into account the illiterate that are likely to be women who are often denied access to education. This would enable them to equally participate in economic activities and pig based livelihoods.

Examples of approaches that encourage greater knowledge retention and more sustainable farming practices include farmer participatory research (FPR) (Escalada and Heong, 1993) and the farmer field school (FFS) (Kenmore, 1991; Van de Fliert, 1993). These approaches require farmers' hands-on participation in small, trainer-facilitated groups. The FPR and FFS approaches, unlike media campaigns, can be expensive -both in time and in related training costs especially when a large number of farmers are to be reached. Therefore, the participatory learning approaches will rely on interpersonal channels and group methods of interaction as practical mechanisms for information and knowledge diffusing to willing farmers more quickly. Farmers in the study may rely heavily on informal farmer-to-farmer interaction channels for broad and rapid diffusion of new farming knowledge and information awareness and facilitating learning among the larger group of "untrained" farmers to improve their pig management practices (including vector control) hence reduce ASF risk.

Majority of respondents practiced farming as their source of income which accounted for 82.12%. Those farmers that had off-farm sources of funds were thought to have the ability to

better support the cost of disease control on farm or ability to afford biosecurity measures (Wamwatila, Personal communication). In this study off farm income was not associated with less risk of tick exposure.

Pig breeds were mainly indigenous (80%) as opposed to exotic breeds. Use of improved pig breeds in developing countries present farmers with a major challenge, as the breeds require intensive management for them to realize their full production potential. Those who kept improved breeds showed less risk in tick exposure, probable due to the fact that they invested more to maintain the pigs.

Farmers kept indigenous breeds for their tolerance to diseases and to utilize feed of low nutrient density to produce good quality meat and perform well even without very sophisticated management. Large pig population was found to increase exposure to tick bites especially in Busia County (Kenya). This could be attributed to large numbers of local breeds in the study as they required little attention and investment to manage (in terms of feeding, labour and management practices) and also their tolerance to diseases and parasites

Pigs were not permanently confined but were both free ranged and tethered depending on the season exposing themselves to diseases and vectors. Other studies have also shown high risk of disease and ectoparasites in free range farms than in total or partial confinement farms (Mannelli *et al*., 1997). Lack of provision of housing by most farmers is a manifestation of low-input traditional system of pig farming, which is common in most developing countries (Hide, 2003; Nsoso *et al.*, 2006). This could be a manifestation of rural poverty. In addition, a large pig population could act as tick source of feed hence high risk of exposure considering the nature of *O. moubata* to feed for few minutes and dropping from the body of the host. In the study, pigs

were raised both on free range and tethering depending on the season of the year. Pigs were confined during planting season or when crops were on their farms and left to roam after harvesting crops from the field. The results were in agreement with other studies (Mutua *et al*., 2011; Okoth *et al*., 2013). These studies showed that farm size and seasons (planting/ harvesting) were the best criterion for classifying farms in Busia and Uganda. Where farm sizes were large, farmers tethered the pigs in pastures or left them to roam freely because presumably there was available space. Free- range foraging of pigs created the possibility of the pigs coming into contact with other ASFV infected domestic pigs. Farmers with smaller land holdings tended to partially confine the animals by tethering to prevent them from destroying neighbors‟ crops. Farmers also tended to use all the land for farming, leaving no room for pig free-range foraging or extensive pig production during the crop planting seasons. It was envisaged that this would impact on the epidemiology of the disease in small size farms since the probability of animal interaction with disease reservoirs, vectors and excreta from infected pigs was minimized.

Local pig breeds are well adapted to their environment making them tolerant to diseases and parasites. This can be reflected in reduced mortalities in local pigs than in exotic pigs incase a disease outbreak or parasite infestation including *O. moubata* occurs. High exposure in local breed in the study could be attributed to large numbers of local pigs surviving an ASF outbreak making them susceptible to vector *O. moubata* bites.

Tick control generally involves a combination of several techniques which include vector/tick control by use of acaricide, and controlled grazing management. In Kenya, the current tick control is by intensive weekly dipping or spraying all year round. The analysis showed that farms in Kenya where tick control was not practiced were about two times at risk of pigs being exposed to tick bites while in Uganda, there was reduced exposure on farms where tick control was not

practiced. This was interpreted as tick control could reduce risk of tick exposure if appropriate tick control strategies are implemented. In Uganda tick control had no effect, though it was speculated that other factors could explain this result other management measures in an integrated tick control approaches.

Association of tick exposure and previous ASF like outbreaks on farm observed in this study highlights the potential of ticks in maintenance and transmission of ASF in the study area. No study has associated ticks with outbreaks in East Africa though genetically similar viruses have been characterized in tick and in pigs in one general location in Central Kenya (Gallardo *et al*., 2011). Few studies have directly associated ticks with outbreaks. A study in Portugal in 1999 showed that ASF re-emerged on a farm that had been affected in the past (Costard *et al*., 2009b). Infected ticks were found on the farm, suggesting that they had maintained the virus. This result further suggests the importance of inclusion of tick control in ASF control programs in East Africa that is currently nonexistent.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1. CONCLUSIONS

The following conclusions were made from the study:

i. Combination of Immulon 1B ELISA plate and OPD substrate optimized performance of the rtTSGP1 ELISA

ii. This study confirmed the presence of pig exposure to tick bites that suggests the potential of the ASFV vector *O. moubata* to transmit ASFV to naïve pigs via bites. The overall prevalence of pig exposure was 17% with Busia County having the highest prevalence of 22% and Uganda 10%.

iii. Risk factors for tick exposure were different in the two countries in the study reflecting the difference in policies and production practices in Kenya and Uganda.

iv. In Kenya, cross breeds, respondent education and occupation reduced the odds of exposure to tick bites whereas number of pigs per household and lack of tick control on farm increased the odds of exposure.

v. In Uganda, exotic breeds and feed management by confining reduced the odds of exposure to tick bite. Pig breed and tick control were significant in both countries. Pig breed (cross) reduced the odds of exposure. No tick control was confounded by other practices and showed no negative effect on exposure.

vi. Outbreaks, where they occurred in farms in Uganda that were studied, had association with tick exposure

6.2. RECOMMENDATIONS

i. Further study is recommended to assess the role of the ticks in the maintenance of ASFV by determining the virus prevalence in the ticks, infectiousness of the tick viruses and association of these viruses with other outbreak viruses in the East African region.

ii. Poor ectoparasite control especially tick control could be the contributing factor of ASF endemicity in most developing countries in sub-Saharan Africa. Assessment and recommendation of appropriate tick control regimes is thus required.

iii. Owing to the relatively low levels of education of some farmers, it is important for extension practitioners to develop more intensive interventions that engage farmers directly in the knowledge discovery process.

iv. Improved farmer extension services on pig management to enhance knowledge of farmers in high risk areas on techniques that reduce risk of ASF should be promoted.

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APPENDIX

QUESTIONNAIRRE

Barcode:

SECTION 1: GENERAL INFORMATION

1.1 Name of Enumerator_____1.1.2 Date (DD/MM/) ___/___/12. 1.1.3 Language of administration_____

1.1.4 District _____1.1.5 Division/County _____ 1.1.6 Location_____

1.1.7 Sub-Location_____1.1.8 Village ((LC1)_____

1.1.9. GPS READING

Location of household: Latitude (N)……..... Longitude (E)…..….....Altitude……..……...

SECTION 2: HOUSEHOLD INFORMATION

2.1 Details of household head and respondent

Respondent's Name	**Gender**	**Age (yrs)**	**Occupation**	**Level of education**	**Education of best educated HH member**	**Tribe**	**Position in HH**

2.2 If **Position in HH** is not HH head, then give details of HH head

HH head's Name	**Gender**	**Age (yrs)**	**Occupation**	**Tribe.**	**Level of education**

SECTION 3: PIG HUSBANDARY/FARM CHARACTERISTICS

3.1. How many pigs does the Household have?

Category	**No. of grade /crosses**	**No. of local breeds**
Piglets (1-3 months)		
Weaners (>3 months)		
Sows (Pregnant or farrowed)		
Breeding boars		
Castrated boars		
Total		

3.2 Did you purchase any pigs in the last year? If yes please provide the following information on the number of pigs purchased.

Category	No. of pigs	Unit price
Piglets		
Weaners		
Sows		
Breeding boars		
Castrated boars		

3.3 How do you keep your pigs?

1= Tethered 2=Free Range 3=Housed 4= Other (specify

- If housed, are the pigs confined in the house all the time?

  1=Yes 2=No

SECTION 4: SOCIO-ECONOMIC CHARACTERISTICS

- Household size: Number of people in the Household_____
- Number of Adults in a household working on the farm_____
- Number of dependants in the household (Children, disabled and elderly)_____
- Does any member of the household have another job or source of income?

  1=Yes 2=No

- List the sources of income for the respondent and other household members?

Income source	**Amount per year (Shs)**
Wages/salaries	
Sale of livestock or livestock products	
Remittances from relatives	
Sale of crop produce	
Renting of land	
Trader/Business	
Government Pension	
Casual labour	
Other (specify)	

SECTION 5: PERSPECTIVES/EXPERIENCES

- When did you first start keeping pigs on this farm? (Month/Year)_____
- Is there a period when you stopped keeping pigs **1=Yes** **2=No**
- If yes, why had you stopped? _____
- When was the last time you had pigs apart from the current one? (Month/Year) _____

5.5. Why do you keep pigs?

1= Home consumption 2= Income/cash 3= Culture 4= Hobby 5 Security/ mobile bank 6= Other (Specify)

5.6. Why do you think it was a good idea?

1	Easy to look after	**4**	Returns are high with low inputs	8.Ease of sale
2	Viable/profitable Enterprise	**5**	They produce many piglets/Multiply faster	9.Other (Specify)
3	Require small space	**6**	Grow faster	

SECTION 6: PIG FEEDING

6.1. What do you feed your pigs on?

1=Commercial pig feeds (including pellets**)**	**6=**House hold food left overs
2=Home mixed feeds	**7=**Swill
3=Purchased maize/flour	**8=**Crop residues from farm
4=By products from food processing	9=Grass
5=By products from brew	

6.2. **If swill, how often do you buy? _____**

1= Not at all **2=**Daily **3=** Weekly **4=**Monthly

6.3 Where do you get swill from?

1=Hotel/restaurant	2=Institutions (e.g. hospitals)	3=Neighbours, other villagers	4=Other (specify)

6.4. Does the swill or house hold food left overs ever contain pork products or pig offal and slaughter waste?

1=definitely no pork	2= do not	3= sometimes contain	4=always contain pork

products	know	pork products	products

6.5. Do you treat the swill in any way before feeding your pigs?

1=boil feed that may have pork products	2= treat feed that may contain pork products	3= make Ugali	4= mix various feed sources	5=Not treated

SENCNTION 7: **PIG HEALTH**

7.1. Do you give any other supplements to the pigs? (Vitamins, minerals) 1= Yes 2=No

7.2. What supplements do you give? List up to 4 options

1=Fish(omena, mokene)	2=Vitamins	3=Others (Specify)

7.3 Do you treat these pigs for external parasites?

1=Yes 2=No

7.4 How do you treat them?

1=Mud baths/wallow	2=Vet	3=Self treatment	4=supervised dipping/spraying

7.5. How often?

1= Weekly	2=Fort nightly	3=Monthly	4=Every 3 months	5=Every 6 months

7.6. When was the last treat treatment (month/year) _____

7.7. Have these pigs ever been sick in other ways? 1=Yes 2=No

7.8 The last time your pigs were sick, what symptoms did they have?

1=Diarrhoea	2=lack of appetite	3=dullness	4=swaying gait	5=skin flash	6=respiratory problems	7=Sudden death
8=Vomiting	9=Coughing	10=Shivering	11=Foaming at mouth			

7.9 Did you hear or see other farmers who had similar symptoms as your pigs?

1=Yes 2=No

7.10. Do you have a name for the disease the pigs had? _____

7.11. Did you go to anyone for help with the disease?

1=Yes (go to 7.15) 2=No

7.12. Who did you seek help from?

1=HH member	2=local leader	3=neighbour	4=relative	5=friend	6=other farmer	7=pig trader	8=livestock development officer includes (NAADS)
9=Livestock Development Officer includes DVO	10=NGO	11=Farmer organisation/self help group	12=Youth group	13=School	14=church	15=Private provider	16=other (specify

7.13. Who gave you the best help? (Please give contact details)

1=first and last name	2= village name	3= phone number	4= distance (<1km, 1-5km)

7.14 Did you report the disease to the veterinary authorities?

1=Yes 2=No

7.15. If you reported to vet authorities, how did you report?

1=mobile	2= physically	3= phone number	4= distance (<1km, 1-5km)

SECTION 8: CONSTRAINTS

8.1. What pig health constraints do you face?

1=frequent health treatment needed 2=cost of disease treatment 3=risk of pig deaths 4= ASF impact (mark only if mentioned specifically, then omit ASF awareness question) 5=other (specify)

8.2. Have you heard of a pig disease called ASF?

1=Yes 2=No

8.3. When was the most recent ASF outbreak that you have heard about? (Month or year)_____

8.4. Where was the outbreak? (Village/District and Distance from your farm)_____

8.5. Have you ever had pigs that got sick or died from ASF?

1=Yes 2=No

8.6. How many ASF outbreaks have you had on your farm since you started keeping pigs? (E.g. 1, 2, 3-5, 5-10, more than 10)_____

8.7. When was the most recent ASF outbreak that you have had on your farm? (Month or year)_____

8.8. Who detected the disease?

1=Husband/HH 2= Wife 3=Daughter 3=Son

4=Labourer 5=other (specify)

8.9. Who attended to the sick pigs?

1=Husband/HH 2= Wife 3=Daughter 3=Son

4=Labourer 5=other (specify)

8.10. When there is an outbreak of ASF what do you do?

1=Reported to vet authorities	2=Reported to NGO	3=Reported to NAADS	4=Reported to private service provider
5= Self medicated	6=Slaughtered	7= Got advice from Agrovet	8 Never sought for help
9=Sold	10. Other (specify)		

8.11. How many of your pigs died from the recent ASF outbreak? _____

Category	Piglets	Weaners	Sows	Boars
No. of pigs				

8.12. How many of your pigs survived in the most recent outbreak? _____

8.13. How did you know about the most recent outbreak?

1=own pigs got sick or died 2= neighbours pigs got sick or died 3= Heard about outbreak from someone (got to ASF outbreak information) 4= others (specify)

8.14=Has ASF affected your pig farming in other ways?

1=Yes 2=No

8.15. In what other ways has ASF affected your pig farming?

1=closure of pig market 2=-did not restock for some time 3=no pigs available for restocking 4=sold pigs early 5=good sales price due to pig scarcity after outbreak 6=other

SECTION 9: BIOSECURITY

9.1. Do you ever use disinfectant on your farm? 1=Yes 2=No

9.2. What type do you use? _____

9.3When do you use disinfectants?

1=clean pig house	2=wash hands e.g. after animal handling	3=dead animal disposal	4=clean shoes of visitors to pig farm	5=other household use	6=other (specify)

9.4. How often?

1= Always	2=regularly	3=irregularly

9.5. Give reasons for not using disinfectants?

1= cash constraint	**2=** I don't know how to use.	**3=**I don't know what to use	**4=**= I don't know that I need to use it	**5=**Never heard about disinfectant

9.6. How often do you get visitors to your farm (including neighbours, relatives, friends, others)

1=most days	2= a few times each week	3= a few times each month	4=less than once a month	5= very rarely	6=never