

# **Epidemiology of African swine fever virus in sylvatic and domestic cycles in Kenya**

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**A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy (Epidemiology)**

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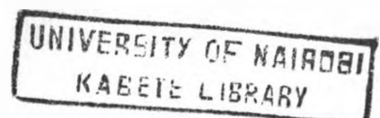
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## DECLARATION

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


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## **DEDICATION**

First, I dedicate this thesis to God for granting me 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

ANOVA	Analysis of Variance
ASF	African Swine Fever
ASFV	African Swine Fever Virus
CFT	Complement Fixation Test
CISA-INIA	Centro de Investigación en Sanidad Animal
CVO	Chief Veterinary Officer
CVL	Central Veterinary Laboratory
DVS	Director of Veterinary Services
DATD	Diallyltartardiamide
DNA	Deoxy-ribonucleic Acid
EDTA	Ethylenediaminetetraacetic acid
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GPS	Geographical Positioning System
HAD	Haemadsorbing
HCL	Hydrochloric Acid
HRPO	Horse radish Peroxidase
ILRI	International Livestock Research Institute
KNBS	Kenya National Bureau of Statistics
KS	Kansas State
KWS	Kenya Wildlife Service
LD	Lethal Dose
MS	Monkey Stable
OIE	Office International des Epizooties (World Organization for Animal Health)
OPD	Orthophenylenediamine
OVI	Onderstepoort Veterinary Institute
PCR	Polymerase Chain Reaction
PBS	Phosphate Buffered Saline
PE	Participatory Epidemiology
PRA	Participatory Rural Appraisal
RRA	Rapid Rural Appraisal
SD	Standard Deviation
USA	United States of America
USD	United States Dollar
W/V	Weight by Volume
W/W	Weight by Weight



## **ABSTRACT**

African swine fever (ASF) is a rapidly lethal epidemic disease of domestic swine that represents a constraint, as a result of high morbidity and mortality, to the development of the smallholder pig industry in sub-Saharan Africa. The first case of the disease in Africa was officially reported in Kenya in the 1920s. Outbreaks have been reported in the country since 2001. The current study was conducted in western, central and north eastern regions of Kenya with the purpose of understanding the epidemiology of African swine fever virus in both domestic and sylvatic transmission cycles of the disease. The objectives were to: 1) Characterize pig production systems in the major pig producing areas of Kenya; 2) Estimate the prevalence of ASF virus in contrasting pig production systems in Kenya; 3) Investigate reported outbreaks of ASF in the course of the study and assess possible causal relationships between current and recent outbreaks of ASF in the country; 4) Isolate and characterize ASFV in Kenya.

Western, Central and North Eastern regions of Kenya were selected for the study whereas Homabay, Kiambu, Thika, Kisumu, Kakamega, Busia, Machakos, Garissa and Mandera districts were selected from these regions as study sites. The study areas were selected because they represented contrasting pig production systems that were either free-ranging or stall-feeding or no pig keeping at all or were areas with frequent ASF outbreaks or were harboring wild and tick hosts. A systematic random sampling approach was used to select pig farming households. A questionnaire was used to collect data on potential risk factors for ASF from the 121 randomly selected pig-rearing households within the two contrasting pig farming regions; south-west Kenya was predominantly free-range while central region was stall-feeding. Northeastern region was selected as a control for wild

pigs that had no interaction with domestic pigs. In addition, blood and tissue samples were collected from domestic pigs, bush pigs, and warthogs and whole ticks were also collected in the study areas for diagnosis and ASFV isolation.

Farms and wildlife sampling points were geo-referenced for spatial analysis. Data from the questionnaire were analyzed using descriptive statistics; K-means cluster analysis for farm characterization, and Generalized Linear Regression Models for assessment of potential risk factors for ASFV infections. Biological samples were analyzed using Enzyme Linked Immunosorbent Assays (ELISA), Immunoblotting, virus isolation, Polymerase Chain Reactions (PCR), virus genetic characterization and population genetic techniques.

The best characterization of the pig farms was by size of land in hectares into small (6 hectares) and large (18 hectares) farm sizes and the number of pigs per herd into small (9 pigs) and medium scale (150 pigs) herds. The domestic cycle of virus transmission was influenced by poor management practices such as inadequate biosecurity measures within farms. There appeared to be existence of inapparent infections in domestic pigs as shown by high virus prevalence by PCR technique estimated at 29.2% (95% CI, 21.6, 39.9) in south-west Kenya and 3% (95% CI, 0, 5.5) in central Kenya. Genotyping of both domestic and bush pig viruses using p72 and p54 genotyping and CVR amino acid sequencing revealed homology suggesting sylvatic cycle of ASFV transmission involving the two species in south-west Kenya. On the other hand, some ASFV isolates from ticks and warthogs from central Kenya were similarly closely related to ASFV isolated from domestic pigs from the same locality, suggesting viral transmission between the ticks, warthogs and domestic pigs. The 5 outbreaks investigated occurred in 2010 and 2011 in

the study areas and were due to a single p72 genotype IX virus. These outbreaks were genetically and biologically associated with a closely related virus that has been present in areas around the Kenya-Uganda border region since 2006-2007. Characterization of viral isolates from domestic and bush pig in south-west Kenya by p72 and p54 genotyping showed homology to ASFV isolates from other parts of the East African region. However, on higher resolution by CVR amino acid sequencing of the same isolates complete homology was shown but was divergent from other isolates from Burundi and Tanzania from the same species. Overall, genetic characterization of the viral isolates from ticks, warthog, domestic and bush pigs revealed clustering with previous isolates from within the region isolated during the period 1959 to 2010, but were in a separate lineage from previous isolates from the southern Africa region.

Generally, epidemiology of ASFV in Kenya was influenced by pig production system, pig management practices, sylvatic reservoirs and virulent or avirulent virus strains in circulation. A further study is needed to assess possible phenotypic implications of ASF virus mutations, the extent to which outbreak patterns in Kenya and other countries are associated with the currently characterized group of viruses. The genetic relationships between bush pigs and domestic pigs and whether or not suspected hybridization between the two species that could have significant implications for disease epidemiology observed in south-west Kenya, does in fact occur should be looked at. Finally, assessment of impact of ASF on the pig value chain development in Kenya that was not covered in this study needs to be done.

## CHAPTER 1

### GENERAL INTRODUCTION

#### 1.1 Background

African swine fever (ASF) is a highly contagious and fatal haemorrhagic disease of domestic pigs. It was first described in Kenya, East Africa in 1910 (Montgomery, 1921), and soon afterwards in 1928 in South Africa and Angola (De Kock et al., 1940). The disease is caused by the African swine fever virus (ASFV) species, genus *Asfivirus* and that is the only member of the *Asfarviridae* family.

Wild Suidae, specifically bush pigs (*Potamochoerus larvatus*), warthog (*Phacochoerus africanus*) and giant forest hog (*Hylochoerus meinertzhageni*) are all known to be carriers of the ASFV (Anderson et al., 1998; Detray et al., 1961; Wilkinson, 1984). In the wild pig species the virus causes no apparent disease in bush pigs or warthogs (Coetzer and Tustin, 2004) and, at least in the case of warthogs, exists through 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. Currently there is no vaccine for ASF.

Pig production contributes appreciably to food security, particularly among the low income rural and urban populations. In Kenya, pork, a by-product of pig, is the cheapest source of animal protein in the rural villages in pig producing areas, the cost being USD 1.4 per Kg (Kagira et al., 2010b), which is considerably lower than the cost per Kg of other animal based proteins. The 2010 Kenya National Bureau of Statistics Consumer

Price Indices (CPI) showed a market price (USD) per Kg of 2.75, 3.75, 7 and 8 for beef, mutton, chicken and fish, respectively. Demand for pork is also constantly rising, especially for the tourist market in Kenya. Pigs provide a ready and regular source of income for farmers to meet family needs. They also play an important role in the social and cultural life of the communities.

African swine fever probably presents the most serious constraint to pig production in Africa. Where outbreaks of disease occur all animals are affected resulting in high mortality in naïve animals that can be up to 100%. The disease poses a threat to the food security and could possibly create social imbalance, as large and small scale producers may be affected differently. While the commercial producers can take protective measures for the long term, the small scale producers largely do not have such capacity, especially those that keep free-range pigs in villages. The disease is classified under the list A of World Organisation for Animal Health (OIE) ([http://www.oie.int/fileadmin/Home/eng/Health\\_standards/tahm/2.08.01 ASF.pdf](http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2.08.01_ASF.pdf)) diseases (OIE, 2012). List A diseases are “Transmissible diseases which have the potential for very serious and rapid spread, irrespective of national borders, which are of serious socio-economic or public health consequence and which are of major importance in the international trade of animals and animal products”( <http://www.oie.int/animal-health-in-the-world/oie-listed-diseases-2012/>).

The Kenya Department of Veterinary Services, in May 2007, reported to OIE (<http://web.oie.int/wahis/public.php?page=country&WAHIDPHSESSID>) suspected outbreaks of ASF in Central, Rift valley, Nyanza, and Western provinces of the country (OIE, 2007). During the outbreaks, eleven hemadsorbing viruses were isolated and found

identical to viruses responsible for ASF outbreaks that occurred in Uganda in 2003 by genotyping at specific loci (Gallardo et al., 2009a). This suggested trans-boundary spread of infection through domestic cycle transmission, either directly or indirectly via pig products or wastes. At the same time, Kenya Wildlife Services (KWS) reports (unpublished observations Ruma National Park Warden, 2005) confirmed bush pig-farmer conflicts around Ruma National Park in Homabay District, Kenya, an interface between farms (including piggery farms) and wild pigs. It was hypothesised that the bush pig and warthog populations, as known reservoirs of ASF, could have been responsible for some of the outbreaks observed in Kenya and the East African region in recent times.

## **1.2 Objectives**

The overall objective was to study the epidemiology of ASF with the ultimate goal of improving control of the disease in major pig producing areas in Kenya.

The specific objectives were to:

1. Characterize pig production systems in the major pig producing areas of Kenya;
2. Estimate the prevalence of ASFV in contrasting pig production systems in Kenya;
3. Investigate reported outbreaks of ASF in the course of the study and assess possible causal relationships between current and recent outbreaks of ASF in Kenya;
4. Isolate and characterize ASFV in Kenya.

### **1.3 Thesis structure**

Chapter 2 of this thesis reviews literature on pig production in Kenya as well as how ASF acts as a constraint. Epidemiology of the disease including aetiology, transmission, occurrence, distribution, clinical manifestation, diagnosis, virus characterization, disease prevention and control, is reviewed. An assessment of ASF surveillance in Kenya and gaps therein is discussed. The lessons from the literature review are presented at the end of the chapter.

This study characterised two pigs farming systems in Kenya; free-range and confined feeding systems, presented in Chapter 3, in terms of their production features. The aim was to identify production factors within each system that had bearing on enhanced risk of infection and spread of ASFV.

Assessment of ASFV prevalence in Chapter 4 using antibody and virus detection techniques was a proxy indicator of ASFV level of infection in pigs in the two farming systems as well as the sylvatic hosts. It was expected that where a higher risk of ASFV infection existed, this was accompanied by higher antibody or virus prevalence within the farming system or animal species studied.

Chapter 5 presents results of outbreak investigation conducted during the course of the study. The investigations used a trace back approach that looked at the potential causes of outbreaks. This particular study combined both farming practices as well as virus characteristics (both biological and molecular) to define causes of outbreaks in the areas studied in Western, Central and Rift valley provinces of Kenya thus epidemiology of ASF.

A further characterization of the viruses causing infections in the current study (Chapter 6) was used to identify viruses that are close to already characterised viruses associated with virulent disease in past outbreaks. Information generated was useful for predicting outbreak risks associated with Kenya virus isolates.

Potential risk factors that could contribute to the ASF outbreaks based on the data from chapters 3, 4, 5 and 6 are used to provide an improved overall understanding of the epidemiology of ASF in Kenya, in the final chapters. Strategies for mitigating the negative effects of the disease in the different pig farming systems in Kenya, including raising farmer and community awareness, are also presented at the end of the thesis.



## CHAPTER 2

### GENERAL LITERATURE REVIEW

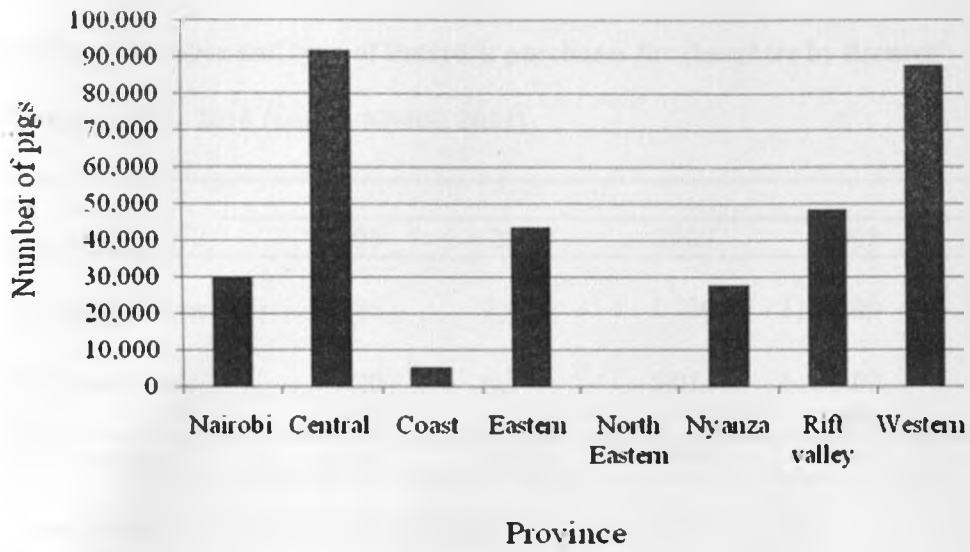
#### 2.1 Pig production in Kenya

Pig production and productivity in Kenya has been analysed in the recent past. The estimated meat production in Kenya is over 16,100 Metric Tonnes (Kiptarus, 2005). The current per capita meat consumption is estimated at about 10.8 Kg of red meat and 1.1 Kg of white meat (Government of Kenya, 2007). White meat, which includes poultry and pig meat, accounts for about 19% of the meat consumed in the country.

The pig population is estimated at 334,689 (Kenya National Bureau of Statistics, 2009). Central, Western and Rift valley Provinces holds the highest number of pigs compared to other provinces (Figure 2.1). Small-scale production constitutes about 70% of the total pig farmers in Kenya (Government of Kenya, 2007). A report by Kenya National Bureau of Statistics (KNBS) (<http://www.knbs.or.ke/publications.php>) on the number and types of livestock purchases for slaughter by licensed abattoirs in the country in the period 2005 and 2010 are shown in Table 2.1. During that period pigs constituted only about 3% of total number of livestock slaughtered. It is hypothesised that future increases in consumer incomes are expected to lead to increased demand for pork and other meat products. Thus, there is a huge scope for future increases in supply as long as economic growth can sustain higher pork demand.

Butchers in Western Kenya have been shown to have an average net income of USD 887 per annum from pig slaughter equating to a profit of USD 3.8 per pig (Kagira et al.,

2010b). The same study by Kagira *et al.* (2010b) identified ASF to have been the major cause of erratic number of pigs slaughtered. Table 2.1 shows unstable number of pig purchases for slaughter between the years 2005-2010, with a lower number in 2006 and 2007. These changes in figures are supported by observed outbreaks in the country reported to OIE (<http://www.oie.int/for-the-media/press-releases/detail/article>) in the same period. The outbreaks resulted in about 100% morbidity and mortality (OIE disease report, February, 2007) in the herds affected. African swine fever, thus, constitutes a constraint to pig production and productivity in Kenya. The extent and size of the disease problem needs to be comprehensively studied; in part this involves structured studies with the application of serological and nucleic acid diagnostics.



**Figure 2.1: Number of pigs produced in Kenya by provinces**

Source (Kenya National Bureau of Statistics, 2009)

**Table 2.1: Number and type of livestock purchases for slaughter by licensed abattoirs 2005 - 2010 (source KNBS, 2011)**

	2005	2006	2007	2008	2,009	'000 Head 2010*
Cattle and Calves	1,786	1,911	1,720	1,892.00	2,057	1,924
Sheep and Goats	4,220	4,775	5,014	5,425.00	5,716	6,163
Pigs	180	176	167	198	221	217
<b>Total livestock</b>	<b>6,186</b>	<b>6,862</b>	<b>6,901</b>	<b>7,515</b>	<b>7,994</b>	<b>8,304</b>

Source: Ministry of Livestock Development  
 \* Provisional

## 2.2 Epidemiology of African swine fever

### 2.2.1 Aetiology

African swine fever virus is a large, enveloped DNA virus that replicates primarily in cells of the mononuclear phagocytic system. The ASFV is an icosahedral cytoplasmic, double stranded DNA virus (Plate 2.1). Electron microscopic examination of structure of the virus particle (Carrascosa et al., 1982; Schloer, 1985) shows several concentric structures with an overall icosahedral shape. It is currently classified as the only member of a family called African swine fever-like viruses (*Asfarviridae*) (Dixon Linda et al., 2006).

The virus has the following features (Carrascosa et al., 1982; Schloer, 1985): ( 1) The inner region of the virus particles is a nucleoid that is surrounded by a 40–48 nm layer of core protein membrane covered by the capsid (an outer covering of protein that protects the genetic material of the virus); (2) the capsid has side-to-side dimensions of 172 to 191 nm and is built up by capsomers (basic subunits of the capsid) arranged in an hexagonal lattice; (3) computer-filtered electron micrographs of either negatively stained or freeze-dried and shadowed capsids reveal capsomers with a hexagonal outline and a hole in the centre, the inter-capsomer distance ranges from 7.4 to 8.1 nm; (4) the triangulation number of the capsid is estimated to be 189 to 217, indicative of 1892 to 2172 capsomers; (5) the extracellular ASFV particles have external membrane that resembles the host cell cytoplasmic membrane; and (6) cell associated virus measure from side-to-side 188 nm and vertex-to-vertex 212 nm.

The prolonged period during which ASF has been an enzootic disease in Africa has led to the selection of viruses of varying virulence, based on the number of 50% hemadsorption unit (HA50) or TCID50 required to produce 1 LD50 for swine (Pan and Hess, 1984). No distinct serotypes have been identified, but divergent genotypes have been identified by restriction enzyme analysis of the genomes of viruses obtained from different geographic areas over a long period of time (Pan and Hess, 1985). A comprehensive similar study on the genotypes circulating in both the sylvatic and domestic cycles in Kenya has not been done.

The virus is highly resistant to a wide pH range and to a freeze/thaw cycle and can remain infectious for many months at room temperature or when stored at 4°C (FAO, 2000). Virus in body fluids and serum is inactivated in 30 min at 60°C, but virus in unprocessed pig meat, in which it can remain viable for several weeks, can be inactivated only by heating to 70°C for 30 min (FAO, 2000). Although ASF virus can be adapted to grow in cells from different species, it does not replicate readily *in vivo* in any vertebrate species other than swine (Vallee et al., 2001). These virus characteristics make it possible to diagnose the presence of the virus in tissues.

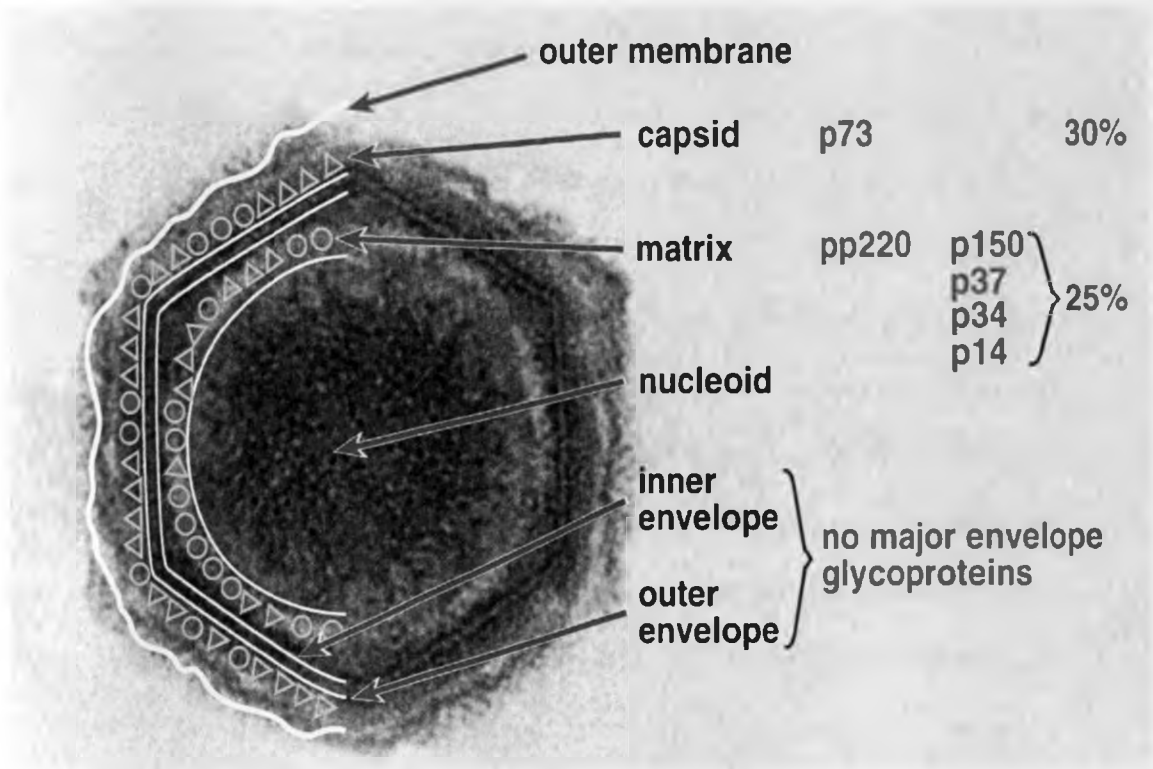


Plate 2.1: Electron microscope image of African swine fever virus (Dixon Linda et al., 2006)

### 2.2.2 Transmission of African swine fever

Naive pigs that come into contact with infected ones 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 remain viraemic for between 35 and 91 days following infection during which time they are able to infect the tick vector *Ornithodoros moubata*, which in turn is able to transmit the disease to domestic and wild pigs (Anderson et al., 1998). In addition, experimentally infected bush pigs can be re-infected with some strains of the virus but not others (Anderson et al., 1998). Haemadsorbing (HAD) ASFV, that bind to red blood cells, have been shown to be pathogenic compared to non-HAD isolates (Boinas et al., 2004). The mode of transmission of the ASFV in domestic and wild pigs and ticks in Kenya is not well understood. Infectivity and virulence of virus strains circulating in Kenya are equally not well understood.

The farm-level risk of sero-positivity to ASFV has been shown to be higher in free-range than in farms using partial or total-confinement (Mannelli et al., 1997). Levels of ASFV risk by pig production systems in Kenya have not been quantified. The presence of sero-positive pigs in herds where there is no detectable clinical disease has been shown (Thomson et al., 1979). A similar observation has not made in Kenya. It has also been demonstrated that wild suids, both warthogs and bush pigs, remain carriers of ASFV following infection (Anderson et al., 1998; Detray et al., 1961; Wilkinson, 1984). The significance of clinically in-apparent infections in both wild and domestic pigs and associations with the tick vector in the epidemiology of the disease remains unclear.



### 2.2.3 Occurrence and distribution

In Africa, ASF viruses appear to persist in at least three different cycles (Plowright et al., 1994; Thomson, 1985; Wilkinson and Paton, 1989).

- In a sylvatic association between wild pigs and especially warthogs and *Ornithodoros spp* ticks, the role of bush pigs is currently not well understood in this cycle;
- A cycle involving domestic pigs and *Ornithodoros spp*, this has not been documented in Kenya; and
- Maintenance of the virus in the domestic pig population independent of any role of wild pigs or ticks, which has been suspected from past outbreaks in Kenya (Gallardo et al., 2009a).

The disease has been observed in all breeds and types of domestic pigs and in the European wild boar. All age groups are equally susceptible. In Africa, the virus produces inapparent infection in 2 species of wild swine, warthog (*Phacochoerus africanus*) and bush pig (*Potamochoerus porcus*) and in the soft tick *O. moubata* (Jori and Bastos, 2009). When the disease was endemic in southern Spain and Portugal, a different species of soft tick, *Ornithodoros erraticus*, became infected with the virus (Sanchez-Botija, 1963). Several other *Ornithodoros spp* that are not usually associated with domestic pigs or wild suids have been infected experimentally. Quantification of virus prevalence in domestic pigs, wild pigs and *Ornithodoros spp* ticks has not been done in Kenya.

African swine fever has been reported in a large number of countries in sub-Saharan Africa, either as an enzootic disease or as sporadic epidemics in domestic pigs (Costard et al., 2009b). The first outbreak of the disease outside Africa was in Portugal in 1957; the outbreak was successfully eradicated. The second introduction in 1960 resulted in ASF becoming enzootic in Spain and Portugal and, subsequently (1978), in Sardinia. During the 1970s, it spread to the Caribbean and South America and serious, but limited, outbreaks in Europe occurred in Belgium (1985) and the Netherlands (1986). Rigorous detection and slaughter programs ended with the successful eradication of the disease from both Portugal (1993) and Spain (1995) (Costard et al., 2009b). Whereas a number of outbreaks in Kenya have been reported to OIE (<http://www.oie.int/for-the-media/press-releases/detail/article>) most recently since 2001, these outbreaks have not been investigated in detail.

#### **2.2.4 Clinical manifestation**

The first appearance of ASF is usually characterized by high mortality after a short febrile illness; pigs become depressed, stop eating, huddle together and, in the peracute form, may die before other clinical signs develop (McDaniel, 1979; Seifert, 1996). Initially, there is mucoid diarrhoea, while in some pigs it later becomes bloody. Almost all pigs that develop severe bloody diarrhoea die. Affected pigs spend much time lying down, but when aroused, they often eat limited amounts of food and frequently drink some water. Body temperature ranges between 40.5-42.2°C and hyperaemia of the skin is usually marked over the mouth, ear, under the belly, over the hindquarters and around the fetlocks, especially in white breeds (Greig, 1972; Seifert, 1996). Haemorrhages are also found in many internal organs, especially in lymph nodes. Pregnant sows often abort at

any stage of pregnancy soon after clinical signs develop. Petechial and ecchymotic haemorrhages may occur on the fetal membranes and skin of the aborted fetus (McDaniel, 1979).

An incubation period of 2-19 days leads to either peracute or acute ASF, which is characterized by high fever, thirst, lack of appetite, apathy, staggering gait, muscle tremors and dyspnoea with increased respiration (Seifert, 1996). The acute stage may last for seven days and the subacute up to 70 days. Generalized haemorrhages are associated with the acute form of the disease (Perez et al., 1998). During the peracute course of ASF, death may appear suddenly without clinical symptoms. In natural infections, only a few pigs in a group are initially clinically ill and the spread of the disease throughout the remainder of the herd may take several weeks with strains of low virulence, or only a few days with highly virulent viruses. Incubation periods vary from two to five days in experimentally infected pigs (Anderson et al., 1998; Hess, 1981; Seifert, 1996).

A chronic form of the disease may sometimes appear and last for 2-15 months. It is characterized by intermittent fever, emaciation, swelling of the joints and sheaths of the tendons, keratitis and atrophy of the bulbus (Seifert, 1996). Coughing often occurs in the chronic form. Diarrhoea and occasional vomiting may also be present. Morbidity can approach 100% and mortality can be above 80%. Chronically infected domestic pigs excrete the virus during the whole course of the disease (Seifert, 1996).

## **2.3 Diagnostics and characterization of African swine fever virus**

### **2.3.1 African swine fever diagnosis**

Active surveillance of ASF requires a combination of approaches including laboratory diagnosis. The ASFV diagnostic test standards defined by OIE (Wilkinson and Paton, 2004) focus on, first, identification of the agent using haemadsorption (Malmquist and Hay, 1960), antigen detection by fluorescent antibody test (Boal et al., 1969), detection of virus genome by conventional or real time polymerase chain reaction (PCR) (Aguero et al., 2003; Gonzague, 2002; King et al., 2003; Zsak et al., 1995), and inoculation of pigs, although the last option is not always practical.

The ASF virus grows best in pig macrophages derived from bone marrow or lung lavages (FAO, 2000). With many strains of ASF virus, the presence of virus in cell cultures can be demonstrated by adding red blood cells to the culture. These are attracted to the surface of infected cells, to which they cling and form "rosettes", a phenomenon known as haemadsorption. Some strains of virus do not cause red blood cells to adsorb to the surface of cells that they have infected, but dead cells in the culture will become obvious after a few days. The virus may be injected into pigs to demonstrate that it is capable of infecting pigs and causing disease.

Antibodies persist in recovered pigs for long periods after infection, sometimes for life, and a number of tests are available for detecting these antibodies, although only a few of them have been developed for routine use in diagnostic laboratories (Arias and Sanchez Vizcaino, 1992; Escribano et al., 1990; Pan et al., 1972; Pastor and Escribano, 1990). The most commonly used is the enzyme linked immunosorbent assay (ELISA) (Sánchez-

Vizcaíno et al., 1983; Vidal et al., 1997; Wardley et al., 1979). Confirmatory testing of ELISA-positive samples is carried out using an alternative test, such as immunoperoxidase staining or immunoblotting (Escribano et al., 1990; Pastor et al., 1989).

Viruses from Southern and East African countries are heterogeneous, with multiple genotypes based on the sequence of the p72 major surface capsid protein being present within individual countries (Bastos et al., 2003; Lubisi et al., 2007). Two major p72 virus variants are confined to four East African countries where they have caused numerous outbreaks between 1961 and 2001 (Lubisi et al., 2007). The viral strains vary in their ability to cause disease and severity of the symptoms, but at present there is only one recognised serotype of the virus detectable by antibody tests. 'Viral species' originally classified in the ASFV genus when it was formally defined are ASFV-Ba71V (culture adapted), ASFV-LIL20/1 and ASFV-LIS57 (International Committee on Taxonomy of Viruses, 2005).

Rapid and reliable detection of viral pathogens is critical for the management of the diseases threatening the economic competitiveness and future development of the pig industry in Africa. Both molecular and immunological techniques including ELISA, immunodot, radioimmunoassay, immunocytochemistry, microculture assay, DNA hybridization and PCR techniques have been developed for diagnosis of the disease. Specific protocols incorporating these methods have been adopted as standard diagnostic techniques prescribed by OIE (<http://www.oie.int/international-standard-setting/terrestrial-manual/access-online/>) for disease surveillance. This study provided an

opportunity to validate current ASF diagnostic tests for their suitability for Kenyan ASFV isolates.

### **2.3.2 Characterization of African swine fever virus**

Studies have characterized the ASFV using biological markers such as hemadsorption characteristics, plaque size, infectivity, virulence, antigenic determinants and genomic structure (Pan et al., 1988). At least 28 structural proteins have been identified in intracellular virus particles (Sánchez-Vizcaíno, 2006). More than a hundred infection proteins have been identified in infected porcine macrophages, and at least 37 of them react with sera from infected or recovered pigs (Letchworth and Whyard, 1984). Restriction endonuclease maps of the variable DNA regions of ASFV field isolates have shown that the changes in length are located in the terminal-inverted repetitions and in unique sequences close to the DNA ends (Blasco et al., 1989). More recent research shows that these regions contain multicopy gene families that represent virulence determinants (Chapman et al., 2008; Zsak et al., 1996). Some strains differ from others in several deletions located in the variable regions (de la Vega et al., 1990).

These virus characteristics discussed above may suggest a common source of infection for disease outbreaks (Wesley and Tuthill, 1984). At the same time, antigenic determinants can distinguish virus isolates (Pan et al., 1988; Whyard et al., 1985). Epidemiological studies of ASFV in Kenya have been limited. Viruses responsible for the 2006-2007 ASF outbreaks were genotyped at three loci (Gallardo et al., 2009a). Viruses from domestic pigs and warthogs and ticks from one locality have also been

genotyped (Gallardo et al., 2011b). However, phenotypic comparison of viruses circulating in Kenya with those from reference viral isolates has not been performed. In addition, the temporal patterns and spatial distributions of ASFV genotypes in Kenya have not been described in detail.

The ASFV complex in East Africa is composed of a biologically diverse virus population with genetically different members, which are only identified after cloning, genotyping and genome sequencing (de Villiers et al., 2010). The ASFV genome mapping has been used to distinguish as well as recognize similarities between virus isolates. Similarity in virus characteristics may suggest a common source of infection for disease outbreaks.

#### **2.4 Prevention and control**

It has been suspected that carriers among domestic pigs play a major role in maintaining the disease in the enzootic areas (Hess, 1981). Thus, although a detection and slaughter policy is the best means of control this is difficult in practice. The standard sanitary measures of quarantine, decontamination and carefully controlled and monitored restocking need to be more rigidly enforced than they are at present.

There is as yet no effective drug treatment or therapy for ASF. In the future, it may be possible to improve control of ASF by the use of effective novel vaccines, and/or by exploiting the natural resistance that appears to be present in some populations of domestic pigs (Penrith et al., 2004a). In the meantime, the difficulty is that the conventional control methods of 'stamping out' and destroying large numbers of pigs is unacceptable for economic and food security reasons and is in fact impossible to implement successfully in countries where resources in terms of both finance and

veterinary services are limited (Penrith and Thomson, 2004). The only viable alternative is farmer-based control, where the focus is on prevention. A directly transmitted disease like ASF can theoretically be controlled by stringent biosecurity measures e.g. confinement and disinfection on-farm (Food and Agriculture Organization of the United Nations et al., 2010), but the application of these in the areas most affected by ASF implies a change from traditional extensive low input husbandry to more intensive systems that place a higher demand on the producer for inputs (Penrith et al., 2007). Such a change may need to be consumer driven rather than depending entirely on disease prevention by government veterinary personnel for it to succeed. However, it hypothesised that with better knowledge of pig management systems and the epidemiology of the ASFV in Kenya, better disease management strategies can be developed.

## **2.5 African swine fever surveillance in Kenya**

The Department of Veterinary Services relies mainly on passive surveillance of ASF (Director of Veterinary Services, personal communication). In this system, information is gathered through disease outbreak reporting and the submission of diagnostic specimens to the Central Veterinary Laboratory in Nairobi. The level and timescale of submission of data and samples results in under-reporting in passive surveillance systems and means that the data collected are unrepresentative and cannot be used for either addressing disease outbreaks or longer term control strategies. Active surveillance for ASF has not been implemented in Kenya. As a first step in this process, collection of reliable, baseline population-based information on ASF, and the use of these data to plan disease control/eradication campaigns that will complement passive surveillance are required.



Use of characterization and diagnostic approaches can help in understanding the complex epidemiology of the disease, which can involve domesticated pigs, wild pigs and a tick and possibly dipteran flies (Penrith et al., 2004b) as vectors, and contaminated feed resources.

## **CHAPTER 3**

### **APPRAISAL OF PIG MANAGEMENT SYSTEMS IN WESTERN AND CENTRAL REGIONS OF KENYA**

#### **3.1 Introduction**

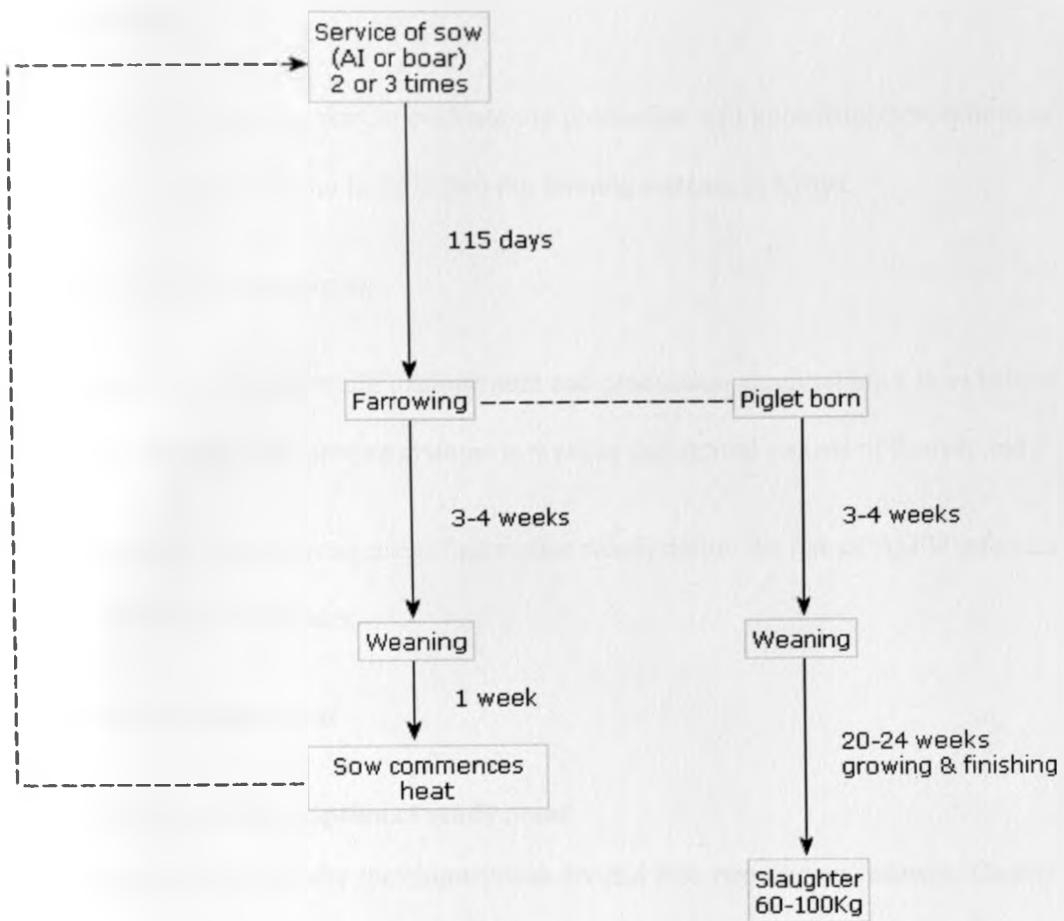
The ASF outbreaks that occurred in Nairobi in 1994 (OIE, 2001)( <http://www.oie.int/for-the-media/press-releases/detail/article/>) after an absence of officially recorded outbreaks of 30 years exhibited no evidence for the involvement of warhogs or ticks, and the disease was believed to have resulted from movements of infected pigs from other districts or regions within East Africa. Uncontrolled movement of pigs together with production practices present a risk for ASFV infection (FAO, 2010).

Two pig management systems are common in Kenya, free-range and confined feeding (Kagira et al., 2010a; Mutua et al., 2011; Wabacha et al., 2004; Wabacha et al., 2001). In free-range system, pigs roam freely around the household and surrounding area, scavenging and feeding in the street, from garbage dumps or from neighbouring land or forests around villages (FAO, 2010). Few arrangements are made to provide the pigs with housing. Depending on the local situation, pigs may be free-ranging for most of the year and penned during the rainy season. They may be housed at night in a small shelter, to protect them against theft and predators. Keeping scavenging pigs requires minimal inputs and low investment of labour, with no or limited money invested in concentrated feed or vaccines. Intensive system of pig production can be defined as the set of facilities and practices that are aimed at the production of pigs using the smallest area possible in system of absolute confinement (FAO, 2010; Rosero and Lukešová, 2008). Pigs are confined to a shelter, which can range from a simple pen made with local materials to

more modern housing. The pigs are completely dependent on their keeper for feed, and receive tree branches, leaves, crop residues, agricultural by-products or prepared feed. Smallholders raise pigs for both subsistence and commercial reasons. Confined animals under this system are at lower risk of encountering ASFV.

The extent and magnitude of the role of pig husbandry on the incidence and spread of ASF in the major pig production systems in Kenya has not previously been comprehensively studied. The management efficiency of these systems is equally not well documented. A typical pig production cycle takes 115 days between sow service and farrowing, 3 to 4 weeks between farrowing to weaning and 1 week from weaning to service. It typically takes 28 days lactation and a 5 day break to service and therefore it takes 144 days between services (Figure 3.1). In less efficient systems it is expected that the period between services is longer. This parameter is useful in determining efficiency of pig production and thus profitability, which in turn would have a bearing on farmers' affordability of disease control inputs.

This chapter describes and compares pig production systems and productivity based on pig management in two farming regions of Western and Central Kenya. The systems studied were those in which pig production was either free-range/tethered feeding and extensive, or stall-feeding and intensive, respectively. The chapter characterises and quantifies the production and productivity parameters of these two systems and also describes factors that would influence the epidemiology of African swine fever in the two regions.



**Figure 3.1: Pig production cycle in typical pig production systems**

Source: *Living Countryside* (2011)  
 ([http://www.ukagriculture.com/production\\_cycles/pigs\\_production\\_cycle.cfm](http://www.ukagriculture.com/production_cycles/pigs_production_cycle.cfm))

## **3.2 Objectives**

The overarching objective was to evaluate pig production and knowledge/perceptions of pig farmers of African swine fever in two pig farming systems in Kenya.

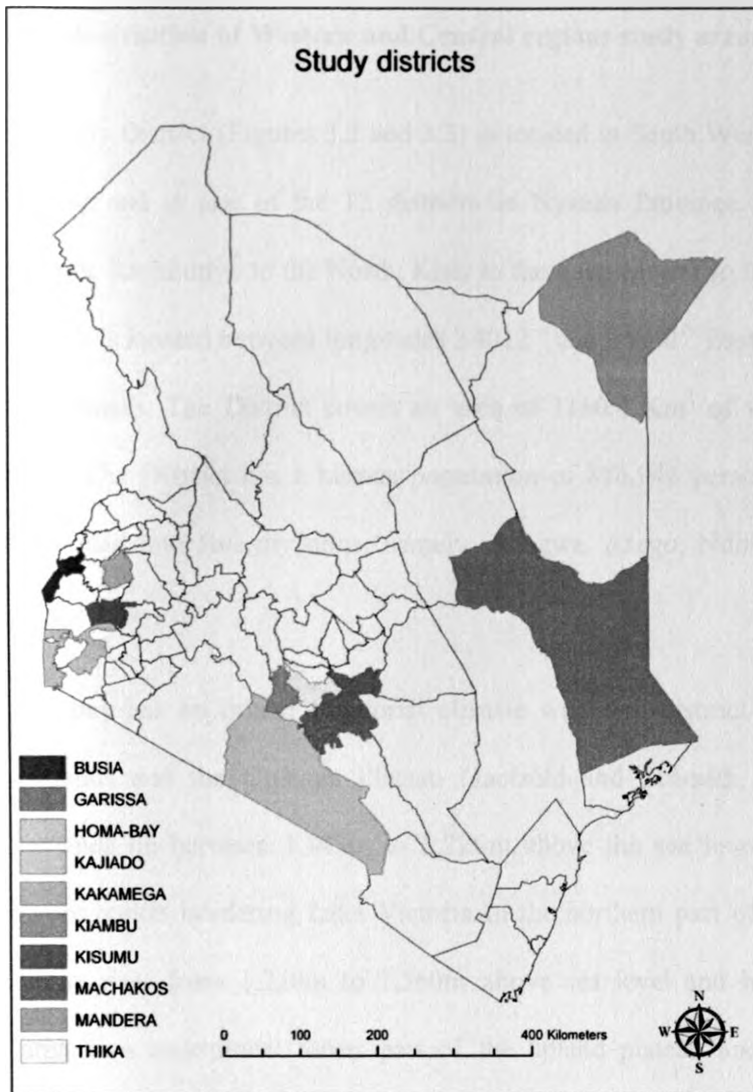
The specific objectives were to:

1. Assess and compare pig management and production characteristics in extensive and intensive pig farming systems in western and central regions of Kenya; and
2. Identify critical management factors that would define the risk of ASFV infection and disease outbreaks.

## **3.3 Materials and methods**

### **3.3.1 Selection and description of study areas**

For the purpose of the study the country was divided into two regions, namely, Central, and Western. Machakos, Kiambu, Thika and Kajiado districts represented the central region, and Homabay, District, the Western region. Homabay and Kiambu districts (Figures 3.2 and 3.3) were selected for detailed study because they represent free-range and intensive pig production systems, respectively. This chapter describes an analysis of domestic pig production systems conducted in Homabay (western region) and Kiambu (central region). Other study sites are described in the subsequent chapters.



**Figure 3.2: Map of Kenya showing the location of the study districts in Kenya, 2008-2011**

### 3.3.2 Description of Western and Central regions study areas

Homabay District (Figures 3.2 and 3.3) is located in South West Kenya adjacent to Lake Victoria and is one of the 12 districts in Nyanza Province. It borders the following districts: Rachuonyo to the North; Kisii to the East; Migori to the South; and Suba to the West. It is located between longitudes 34°12' and 34°40' East and latitudes 0°28' and 0°40' South. The District covers an area of 1160.4 Km<sup>2</sup> of which 29.5 Km<sup>2</sup> is under water. The District has a human population of 856,946 persons (KNBS, 2009) and is subdivided into five divisions, namely, Rangwe, Asego, Ndhiwa, Nyarongi and Riana (Figure 3.3).

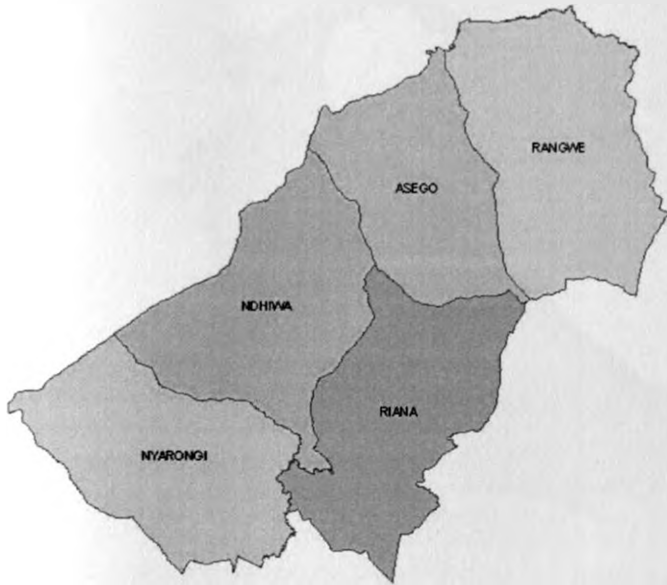
Homabay has an inland equatorial climate with two distinct regions, the Lake Shore Lowlands and the Uplands Plateau (Jaetzold and Schmidt, 1983). The Lake Shore Lowlands lie between 1,143m to 1,220m above the sea level and mainly comprise a narrow region bordering Lake Victoria in the northern part of the district. The Upland Plateau rises from 1,220m to 1,560m above sea level and has an undulating terrain. Kanyamwa escarpment forms part of the upland plateau and runs along the western border between Homabay and Suba Districts. At the end of the lake shore is a bay from which the district derives its name. The bay is skirted by a shoreline stretching about 16.5 km covering parts of Asego and Rangwe divisions. The district has 97,700 ha (84%) of arable land. This area is associated with smallholder pig production. The pigs are kept under free-range/tethering feeding systems at low density.

Kiambu District covers 2,500 Km<sup>2</sup> of the Central Highlands and comprises 5 administrative divisions (Figure 3.4). It is one of the most densely populated districts in

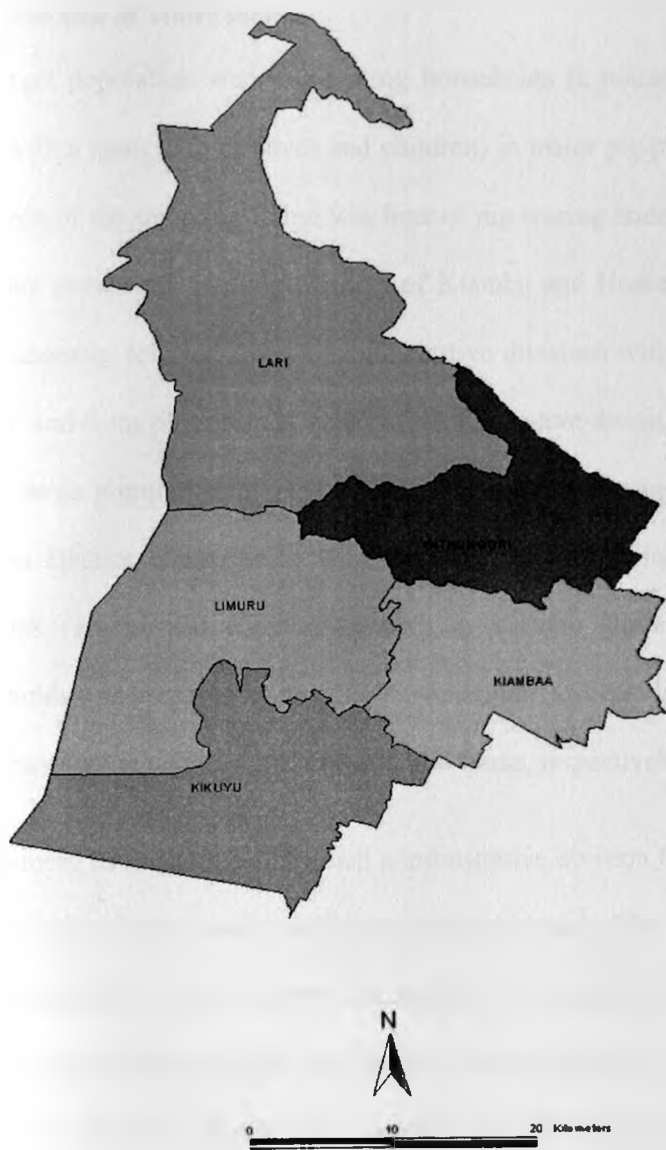
the Kenya highlands, having an estimated density of 649 persons per Km<sup>2</sup> (KNBS, 2009). Altitudes range from 1,400 m in the southeast to 2,400 m in the north. Rainfall is bimodal with the two peaks in April/May and October/November. Average rainfall is 1,100 mm per year. The most predominant soil type is nitisols (red Gikuyu loams). Mixed agriculture production systems including vegetable cultivation, poultry, pig and dairy production (zero-grazing) are used to maximise production given the typically small farm sizes and the high demand for produce in the city of Nairobi. Coffee, tea, pyrethrum, maize, beans and bananas are also grown.

The average farm-size is 1.1 ha per household of 4.8 people (Jaetzold and Schmidt, 1983). Most of the land is devoted to crop production (Stotz, 1983), the remainder being used as a dwelling place for humans and housing for livestock. Very few small-holdings have pastures.





**Figure 3.3: Map of Homabay District and the administrative divisions**



**Figure 3.4: Map of Greater Kiambu District and the administrative divisions**

### 3.3.3 Selection of study farms

The target population were pig-rearing households (a household being defined as one home with a man, wife or wives and children) in major pig producing regions of Kenya. The basis of the sampling frame was lists of pig-rearing households in all administrative divisions in two pig farming districts of Kiambu and Homabay. The study households were randomly selected from 4 administrative divisions with pigs out of 5 in Homabay District and from purposefully selected administrative divisions in Kiambu District. Due to the large population of pigs and higher number of pigs per household in greater Kiambu District compared to Homabay District, a decision was made to select two divisions (Tigoni and Kiambu Central) in Kiambu District. The list of pig-rearing households was prepared by the District Veterinary Officers in both districts. Kiambu and Homabay Districts had lists of 600 and 800 farms, respectively.

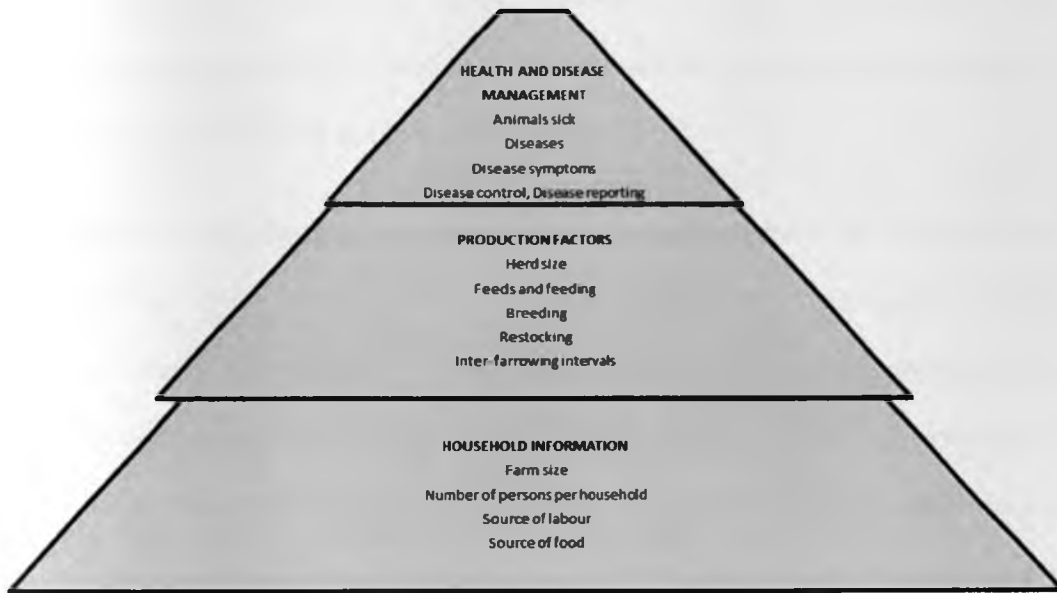
Pig-farming households within each administrative division (a subunit of a district) were selected using a systematic random sampling approach. This sampling approach selected the households at a fixed interval throughout the sampling transect after a random start. Out of a list of 600 and 800 pig-farming households every 7<sup>th</sup> and 20<sup>th</sup> household was selected in Homabay and Kiambu, respectively. The final number of household selected was limited by logistics and available financial resources. In total 40 farms were selected in the first period in April and May 2008 and 41 in the second sampling period in March 2009 in Homabay District. A total of 40 farms were selected in Kiambu district in the period January to April, 2010. From the selected list of farms all 40 were still operational in Kiambu District, but only 41 out of the 81 were found to keep pigs in Homabay District. The farms without pigs were replaced with neighbouring farms that had pigs.

The selected households were geo-referenced using Garmin Global Positioning System (GPS) 12XL unit (Garmin Olathe, KS, USA).

#### **3.3.4 Questionnaire survey of pig rearing households**

Data were collected through administration of questionnaires via personal interviews. Information was collected on 3 key areas (household information, production factors and health and disease management (Appendix 1). These aspects were brought together to form an information pyramid (Figure 3.5). The bottom layer of the pyramid defines the composition of farmer households, how they are organised, and general production capacity. The information was needed to assess the efficiency with which a household is capable of managing disease on-farm. The second layer covered the production factors that influence pig productivity and disease risk. The next layer covers data on animal health, which present as measure of risk for the occurrence of ASF and other diseases, e.g., parasitoses.

The triangulation method, which allows validation of information collected with data from at least two other sources or the other methods of data collection, was used to provide scientific rigour and validity. For example, farmer information was verified by local Veterinary and Para-veterinary professionals operating in the study areas.



**Figure 3.5: Information pyramid for data collection for the assessment of pig production and health**

### 3.3.5 Data handling and analysis

The household survey data were entered in prescribed forms in Microsoft© Office Access (Microsoft Corporation, 2003). Summaries of farm characteristics were performed using the GENSTAT statistical program and presented in tables and figures to describe the pig production and productivity types.

In addition, the farm characteristics were analysed using the hierarchical cluster analysis method (Ward, 1963). This is a collection of statistical methods, which identifies groups of samples that behave similarly or show similar characteristics. A hierarchy or tree-like structure was constructed to assess the relationship between entities (observations or individuals). Ordering was driven by how many observations could be combined at a time or what determines that the distance was not statistically different from 0 between two observations or two clusters. The Ward method used an analysis of variance approach to evaluate the distances between clusters. This method attempted to minimize the Sum of Squares of any two (hypothetical) clusters that could be formed at each step. Typical of properties of variance for statistical decision-making, this tends to create too many clusters, or clusters of small sizes because the more the observations are scattered, the larger the distance based on the sum of squares.

Cluster analysis was used in this study as a tool for identifying groupings of farms (farm types), given the values of a set of production and productivity indicator variables. The K-means cluster analysis procedure implemented within SPSS was used. The farm characteristics used were farm size, total number of animals per farm, numbers of sows, boars, weaners, non-weaner, piglets farrowed and piglets weaned.

## **3.4 Results**

### **3.4.1 Characterization of pig production and productivity**

Pig production and productivity characteristics analysed in this study were: Farm size, household size, family labour (proportion), hired labour (proportion), herd sizes, number of breeding sows, number of breeding boars, weaned piglets, non-weaned piglets, piglets farrowed during the last litter and piglets weaned during the last litter.

#### **3.4.1.1 Farm holding**

Mean farm holding (hectares) per household for 81 farms was analysed for Homabay District. The distribution of farm holding was skewed to the left with a mean value of 8 hectares and a range of 35 hectares (Figure 3.6). Most of the farms were clustered in farm sizes between 6 and 10 hectares. Mean farm holding (hectares) per household for the 40 farms in Kiambu District was 6 with the distribution skewed to the left with most of the farms clustered in farm sizes less than 1 hectare and a range of 80 hectares (Figure 3.6).

#### **3.4.1.2 Household sizes**

The Mean number of persons per household was 6 in Homabay and 4 in Kiambu (Table 3.1). The number of persons per households in Homabay had a wider distribution ranging from 1-20, whereas households in Kiambu ranged from 1-8 (Figure 3.7).

#### **3.4.1.3 Labour sources**

The labour source for most pig farms (99%) in Homabay was members of the household. Kiambu farmers (85%) mainly hired labour, with support from household members.

#### **3.4.1.4 Pig herd composition**

Mean number of pigs per farm was 2 in Homabay District (Table 3.1) with a distribution skewed to the left and ranged from 1 to 11. Kiambu had a mean number of 42 pigs per farm. The distribution of the pig numbers was skewed to the left (Figure 3.8). Pig herd range was 1 to 499 pigs per farm.

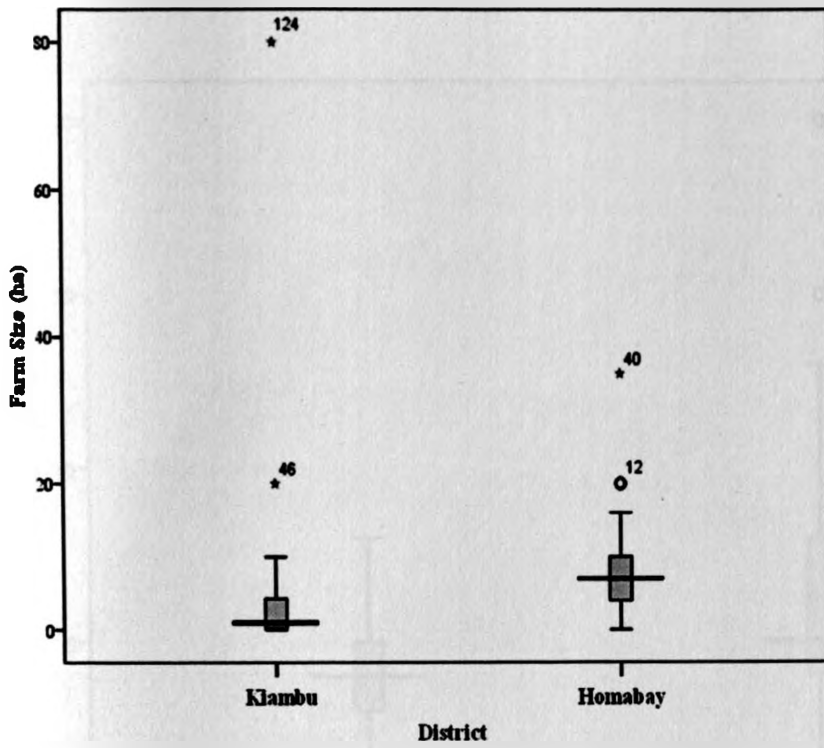
#### **3.4.1.5 Pig productivity**

The estimated mean weaning to service period of sows was 20 ( $\pm 10$ ) and 40 ( $\pm 10$ ) days in Kiambu and Homabay districts, respectively. The mean number of piglets farrowed per sow per litter was 13 and 5, and the average piglets weaned per sow per litter were 9 and 4 for Kiambu and Homabay districts, respectively (Table 3.1). These results were interpreted as lower production efficiency in Homabay District compared to Kiambu District. The pre-weaning piglet mortality was 9% and 19% in Homabay and Kiambu districts, respectively. The major cause of piglet mortality was reportedly due to being crushed by sows.



**Table 3.1: Distribution of farm characteristics by district**

Farm characteristics	Study areas			
	Homabay District		Kiambu District	
	Mean	Standard Deviation	Mean	Standard Deviation
Farm size (hectares)	8.2	6.2	5.6	15.4
Household size	6.0	5.0	4.0	2.0
Family labor (%)	1.0	-	0.1	-
Hired labor (%)	0.0	-	0.9	-
Herd sizes	2.0	1.5	42.0	94.0
Breeding sows	1.0	0.7	5.0	9.6
Breeding boars	0.3	0.6	2.0	3.7
Weaned piglets	1.0	0.9	25.0	76.0
Non-weaned piglets	0.3	1.7	9.0	14.9
Piglet farrowed last litter	5.0	4.1	13.0	19.7
Piglets weaned current litter	4.0	4.0	9.0	11.6



**Figure 3.6: Distribution of farm sizes by district, 2009**

**\*Outliers**

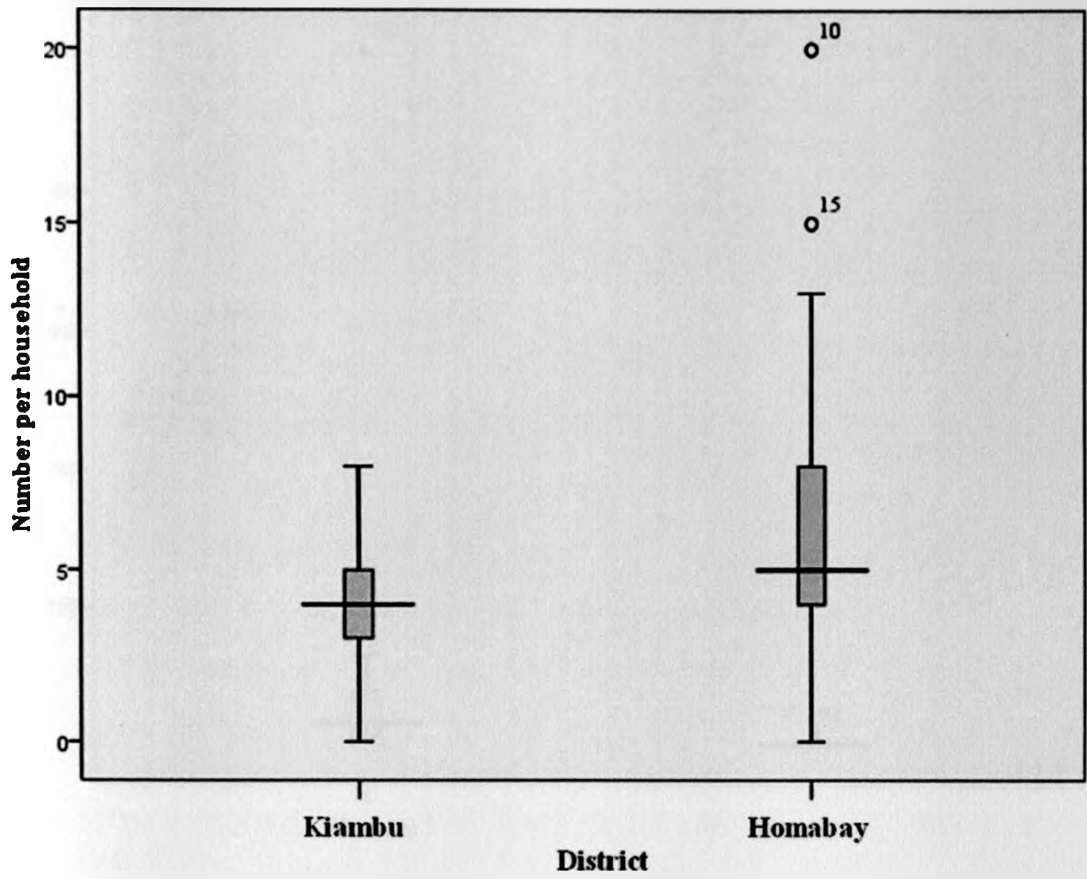


Figure 3.7: Distribution of number of persons per household by district, 2009

<sup>0</sup>Outlier

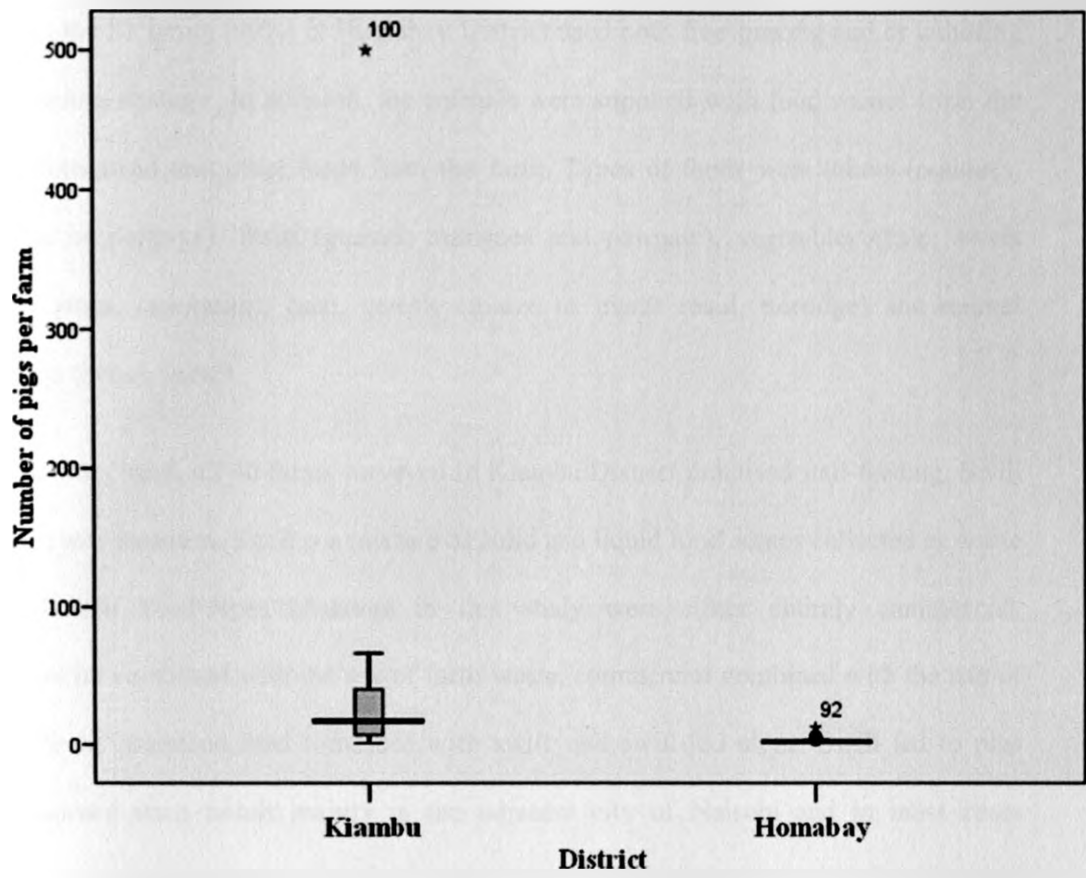


Figure 3.8: Distribution of number of pigs per farm by districts, 2009

\*Outliers

#### **3.4.1.6 Feeding**

Most of the 81 farms (99%) in Homabay District used both free-grazing and or tethering as a feeding strategy. In addition, the animals were supplied with food wastes from the same homestead and other feeds from the farm. Types of feeds were tubers (potatoes, cassava or peelings), fruits (guavas, mangoes and pawpaw), vegetables (kale, sweet potato vines, *machicha*), cane, cereals (maize or maize meal, porridge) and animal products (bones, meat).

On the other hand, all 40 farms surveyed in Kiambu District practised stall-feeding. Swill feeding was common. Swill is a mixture of solid and liquid food scraps collected as waste from hotels. Feed-types observed in this study were either entirely commercial, commercial combined with the use of farm waste, commercial combined with the use of swill, home produced feed combined with swill, and swill-fed alone. Swill fed to pigs was sourced from hotels mainly in the adjacent city of Nairobi and in most cases supplemented with commercial feeds. All farmers in Homabay and Kiambu Districts cited feeding of pigs as a major constraint to production.

#### **3.4.1.7 Breeding**

Homabay District had only 30% of the farms having breeding boars, whereas Kiambu District had 48% with a mean of 2 breeding boars per farm. The main breeding approaches in Homabay and Kiambu Districts were by use of a boar on-farm or, more frequently, contractual-breeding where a farmer would borrow a boar from the neighbourhood to mate his sows, or alternatively a sow taken to a boar when in heat. The main reason given by farmers for keeping sows rather than boars was because sows

guaranteed production of piglets to the farmers for finishing and selling or for restocking. Contractual breeding observed in this study clearly presents a risk of introduction of ASFV from infected to non-infected farms if the breeding animals are infected.

#### **3.4.1.8 Breeds**

The breeds kept in Homabay District were generally exotic crosses with locally adapted breeds. The breeds kept in Kiambu District included large white, landrace and their crosses.

#### **3.4.1.9 Stocking and restocking**

The restocking approach in 74% of the farms in Homabay District was through buying weaned piglets from neighbours and finishing them for market. It was observed that farmers with weaned piglets would loan them to neighbours to keep them until farrowing and then the donor farmer would be rewarded with a proportion of the piglets in return. This was to help the farmers reduce feed costs. A few farmers (26%) in Homabay District had breeding boars and sows that maintained their stocks. In Kiambu District, 47% of the farmers did their own breeding (typically through services of a boar on-farm) and selected replacement pigs.

#### **3.4.1.10 Housing**

An appraisal showed that all the 81 farms visited in Homabay District had none of their pigs housed. They were mainly tethered under trees for shade or alternatively house/cereal store eaves (Plate 3.1A). Similar appraisal in Kiambu District showed that all farms housed their pigs. In Kiambu pig housing showed that the pens were either earthen floor, raised slated wooden floors, and concrete floors without bedding (Figure

3.1B). Farmers with fewer pigs used earthen or wooden floors while those with larger herds housed their animal on concrete floors, normally without any bedding.

None of the farms in either Homabay or Kiambu Districts observed strict sanitary measures such as foot disinfection or restriction of visitors to the pig housing/shelters/sties.

#### **3.4.1.11 Disease control**

Farmers treated their animals with anthelmintics but acaricide treatment was not cited in any of the farms. There was a general belief in farms in Homabay through farmer interviews that pigs were resistant to diseases.



**Plate 3.1: Tethered pig in Homabay District (3.1A) and pigs reared in pens with concrete floor (3.1B) in Kiambu District**

*Source: Edward Okoth (2011)*



### 3.4.2 Typology of pig production systems

Two clusters were found to provide consistent differentiation according to the farming characteristics in Homabay District (Table 3.2). Cluster 1 exhibited small farm size (cluster centre of 6 hectares); Cluster 2 exhibited a larger farm size (cluster centre of 20 hectares). Analysis of variance (ANOVA) results derived from cluster analysis in Homabay shows that farm size provided the widest separation between clusters with an F value of 133.1 and  $p < 0.05$ . The Size of farm-holding is therefore a significant criterion for classifying the farms. Out of the 81 farms, 16 farms were found in cluster 1 and 65 in cluster 2.

Six clusters were identified based on farming characteristics for Kiambu District. The cluster centres are presented in Table 3.3. Analysis of variance (ANOVA) results from the cluster analysis showed that number of pigs per farm provided the greatest separation between clusters with an F value of 749.3 ( $p < 0.05$ ). Herd size, thus represented a significant criterion for classifying pig farms in Kiambu District. Out of the 40 farms surveyed, the majority were found in cluster 2 (10 farms) and cluster 3 (29 farms) and the rest were distributed in the remaining clusters. Cluster 2 was centred on 44 pigs per farm and cluster 3 had the least number of pigs centred at 9 pigs per farm.

**Table 3.2: Final farm cluster centers for pig farms in Homabay District, 2008**

<b>Farm Characteristics</b>	<b>Cluster</b>	
	<b>1</b>	<b>2</b>
Farm Size (ha)	18	6
Number of pigs per farm	2	2
Number of breeding sows	1	1
Number of breeding boars	0	0
Number of weaners	1	1
Number of piglets	0	0
Number of piglets farrowed	7	5
Number of piglets weaned	6	4
Number in cluster	16	65

**Table 3.3: Farm cluster centers for pig farms in Kiambu District**

Farm Characteristics	Cluster					
	1	2	3	4	5	6
Farm Size (ha)	80	2.54	2.18	10	0.25	5.5
Number of pigs per farm	56	44	9	150	140	500
Number of breeding sows	4	4	2	12	9	52
Number of breeding boars	0	2	1	1	1	6
Number of weaners	52	18	5	78	80	400
Number of piglets	0	20	1	59	50	45
Number of piglets farrowed	38	12	5	59	10	45
Number of piglets weaned	31	10	4	59	10	7
Number in clusters	1	10	26	1	1	1

### 3.5 Discussion

A description of the management and biosecurity practices in pig farming households is important to the investigation of their association with disease risk. Furthermore, the identification and characterization of different profiles of management and biosecurity practices enable development of specifically targeted recommendations for pig farmers to reduce the risk of disease in their herds. Pig farm characterization in relation to ASF risk has recently been performed in Madagascar (Costard et al., 2009a). In the study (Costard et al., 2009a) the risk for ASF to be introduced into a herd was influenced by multiple aspects of management and biosecurity practices. Important factors included pig sales and purchases and feeding regimes.

The data obtained in this study shows that Homabay District had smallholder pig farms with an average of 8 hectares of land. The average size of land was 8 times more in Homabay than in Kiambu District that had mode of farm size less than 1 hectare and average of 5 hectare, when the outlier farms were censored. Farm size was shown to be the best criterion for classifying farms in Homabay District. Large farm sizes could explain why free-grazing and tethering were prevalent in Homabay. Where farm sizes were large the 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 and or wild pigs, contaminated faecal materials and other excreta shed in pasture by the pigs scavenging in the farms. In addition, it was observed that same farms had uncultivated sections providing an ideal environment for bush pigs at night. Farmers with smaller land holdings

tended to partially confine the animals by tethering to prevent them from destroying neighbors' crops. These 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 minimised. Other studies on characterization of farming practices and ASF risk in Sardinia have shown that extensive pig farming and ASF were spatially co-distributed (Mannelli et al., 1997) pointing to the fact that extensive system present as a risk for ASF.

It was also observed that the mix of tethering and free-grazing of the pigs depended on presence or absence of crops on the farm in Homabay District. Pigs were tethered when crops were on the farms and the animals roamed more freely after harvest. Another study in Western Province of Kenya also observed that tethering of pigs was frequent (>50%) during the planting (91%), growing (90%) and crop harvesting seasons (78%) (Mutua et al., 2011). It was speculated that alternating free-range grazing and tethering could result in variability in the incidence of ASFV infections that could probably lead to seasonal patterns of occurrence. However, due to the design of the study, it was not possible to obtain data for testing this hypothesis. Sampling in Homabay District was done at two sampling periods, though not with the objective of looking at seasonal patterns of ASFV infections.

Breeding of sows was by boars kept on-farm, borrowed from neighbors or sows introduced to boars off-farm. Restocking was similarly diverse through piglets farrowed on-farm, animal loans from neighbors or local purchases. Both animal loans for breeding

and restocking could contribute to transmission of ASFV between farms through direct pig to pig contact.

No acaricide application was practiced in any of the farms. Therefore the role of ticks in the epidemiology of ASFV in Kenya is a possibility that requires further investigation. However, there is currently no evidence for the occurrence of *Argasid* ticks on farms except in warthog burrows.

Homabay District was characterized by inefficient production due to the period taken between sows farrowing and weaning (twice the time relative to a typical value for weaning to service of 3-4 weeks). However, the Homabay situation represents a low input system; where there are no sties built for pigs and the animals are mainly let out to forage freely with minimum feed supplementation. Low inputs may probably lead to high virus prevalence. This is because farmers could not spend money on disease control including regular deworming, ectoparasite control and housing. Parasite control could prevent contact of pigs with the tick *O. moubata*.

Small farm sizes in Kiambu dictated that the pigs had to be stall fed. Other studies (Wabacha et al., 2001) have shown comparable farm size to the current study with a median farm size of 1 ha. The same study (Wabacha et al., 2001) showed that all pig farmers fed their animals in small stalls throughout the year.

Qualitative rapid assessment of constraints associated with feeding showed majority of farmers, in both Kiambu and Homabay districts, cited feed as expensive; most farmers used swill, which is a cheaper alternative to commercial feeds or freely grazed pigs, for this reason. In Kiambu District, use of swill was common (69% ), as a supplement to

commercial feeds. Swill feeding of food scraps, which may contain imported animal products, is a very important means by which ASF and other serious transboundary animal diseases such as foot-and-mouth disease (FMD), swine vesicular disease and classical swine fever (CSF) may be introduced into a farm or a country (FAO, 2000). Thus swill, if it contains pork products that are infected with ASFV, and is not boiled before feeding to pigs, present as a risk for ASF. Free-range pigs in Homabay District sometimes caused serious damage to crop in farms. The feed constraint could explain the small number, on average, of pigs kept per farm in Homabay District. Most farmers loaned out piglets to neighbours for keeping as a strategy for maximising utilisation of feed resources.

Whereas the difference in the average numbers of members per household was statistically insignificant in both Homabay and Kiambu districts, the households in Homabay District provided labour for pig production. The higher production efficiency level in Kiambu District, could explain why the farmers hired labor, since profits were presumably greater. Cluster analysis showed that farms in Kiambu were better characterized by herd sizes. Relatively, farmers kept more pigs, and this could also explain affordability of labor and production efficiency in Kiambu District. These results led to the conclusion that pig farming in Homabay is of low-input with an income-generating objective, a result similar to other findings (Kagira et al., 2010b). On the other hand this study classified pig farming system in Kiambu to be generally of medium-input with an income-generating objective.

Poor housing, sanitation and lack of biosecurity were prevalent features of all the farms analysed in this study and presented a major risk for spread of ASF. Breeding, restocking

and lack of housing were observed to be biosecurity risks for ASFV infections. Farmers took their sows to be mated off-farm or loaned their boars for mating. Restocking also involved buying or loaning pigs from other farms. These two activities presented as a risk for transferring disease from infected farms to clean farms. Lack of housing enhanced pig-pig contact and keeping pigs in earthen floors made it difficult to clean or disinfect pig shelters/premises thus enhancing risk of viral persistence and transmission. Lack of restriction for visitors to the piggery units enhanced chances for spread of the virus through direct or indirect contact.

It can therefore be concluded that pig management practices might be associated with the risk of ASFV in the two pig producing systems studied in Western and Central regions of Kenya.



## **CHAPTER 4**

### **AFRICAN SWINE FEVER VIRUS PREVALENCE IN DOMESTIC AND SYLVATIC CYCLES IN KENYA**

#### **4.1 Introduction**

Kenya reported several outbreaks occurring late in the year 2010 in the following districts: Kisumu, Kakamega, Busia, Thika and Kiambu. A total of 19 African countries, including Kenya, (eastern: Tanzania, and Uganda; western: Togo, Nigeria, Benin, Burkina Faso, Ghana, and Guinea Bissau; southern: Madagascar, Zambia, Malawi, Angola, and Mozambique; and central: Central African Republic, Democratic Republic of Congo, Republic of Congo, Chad, and Cameroon) reported ASF outbreaks during the same year to OIE (<http://www.promedmail.org>). Given the increasing magnitude of the ASF problem in Africa as pig population increase and more particularly in Kenya where the demand for pig meat is growing, new information on the prevalence of the ASFV in the various species and locations is urgently required.

Although ASF is one of major diseases in need of epidemiologic surveillance in Kenya, no structured surveys have been conducted. A problem of underreporting of disease in the country is possible and therefore existence of a gap between declarations and reality of disease prevalence. To date, no data are available on the epidemiology of ASF in Kenya. Data available from OIE reports does not include risk assessments and prevalence studies, especially persistence of the disease post outbreaks. Therefore, this study was designed to fill that information gap and document the seroprevalence of ASF in Kenya.

Such information is needed to clearly define risk points for the disease and thus point out where to focus control efforts. This chapter reports an assessment of the prevalence of ASFV antibody and virus, as proxy for ASF risk, in both domestic and sylvatic hosts and in two pig production systems; free-range and confined feeding.

## **4.2 Objectives**

The main objective was to estimate the prevalence of ASFV in domestic and sylvatic hosts. The specific objectives were to:

1. Determine the prevalence of ASFV in ticks, domestic pigs and wild pigs; and
2. Compare the prevalence of the ASFV in different regions of Kenya.

## **4.3 Material and methods**

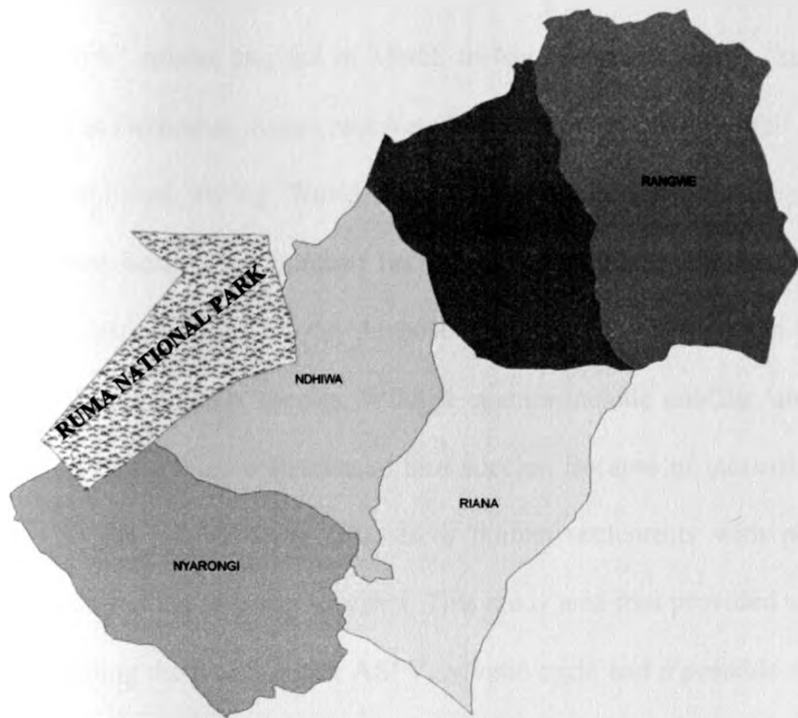
### **4.3.1 Description of study sites**

The study areas were described and displayed in Chapter 3. Briefly the country was divided into three regions, namely, Central, Western and North Eastern. Machakos, Kiambu, Thika and Kajiado districts represented the central region, Homabay District, the Western region, and Garissa and Mandera Districts the North Eastern Region. Homabay and Kiambu Districts were selected because they represented free-range and intensive pig production systems, respectively. Two study sites, Ruma National Park in the Western region and Kapiti Plains Ranch in the Central region (Figures 4.1 and 4.2) were purposely selected to represent a bush-domestic pig and warthog-domestic pig interfaces, respectively. Garissa and Mandera were selected for desert warthog sampling.

There is little, if any, pig farming in North Eastern; however a potential wildlife reservoir, the desert warthog occurs there.

Ruma National Park (Figure 4.1) is a wildlife protected area approximately 120 Km<sup>2</sup>. It is located on the southern part of Lambwe Valley in Suba District, only 10 kilometres east of Lake Victoria. The Lambwe valley has been described previously (Allsop and Baldry, 1972). It lies between latitudes 34010'' and 34025'' east and longitudes 0030'' and 0050'' south. The park is bordered to the west by Gwassi, to the north by the Gembe and Ruri hills and to the east by Kanyamwa escarpment along the border with Homabay District. The entire Lambwe valley occupies a total of 350 Km<sup>2</sup>. The Lambwe floor is approximately 1230 metres above sea level. The park hosts a range of wild animals including bush pigs. Lambwe River traverses the valley from the southern side originating near Miriya Hill (Luwa Tonga Valley) northwards entering Lake Victoria near white rock point. The bank of the river provides an environment for bush pig nesting (KWS staff, personal communication). The vegetation of Ruma National Park consists mainly of savannah grasslands and woodlands, interspersed by extensive acacia thickets, a suitable environment for the bush pigs.

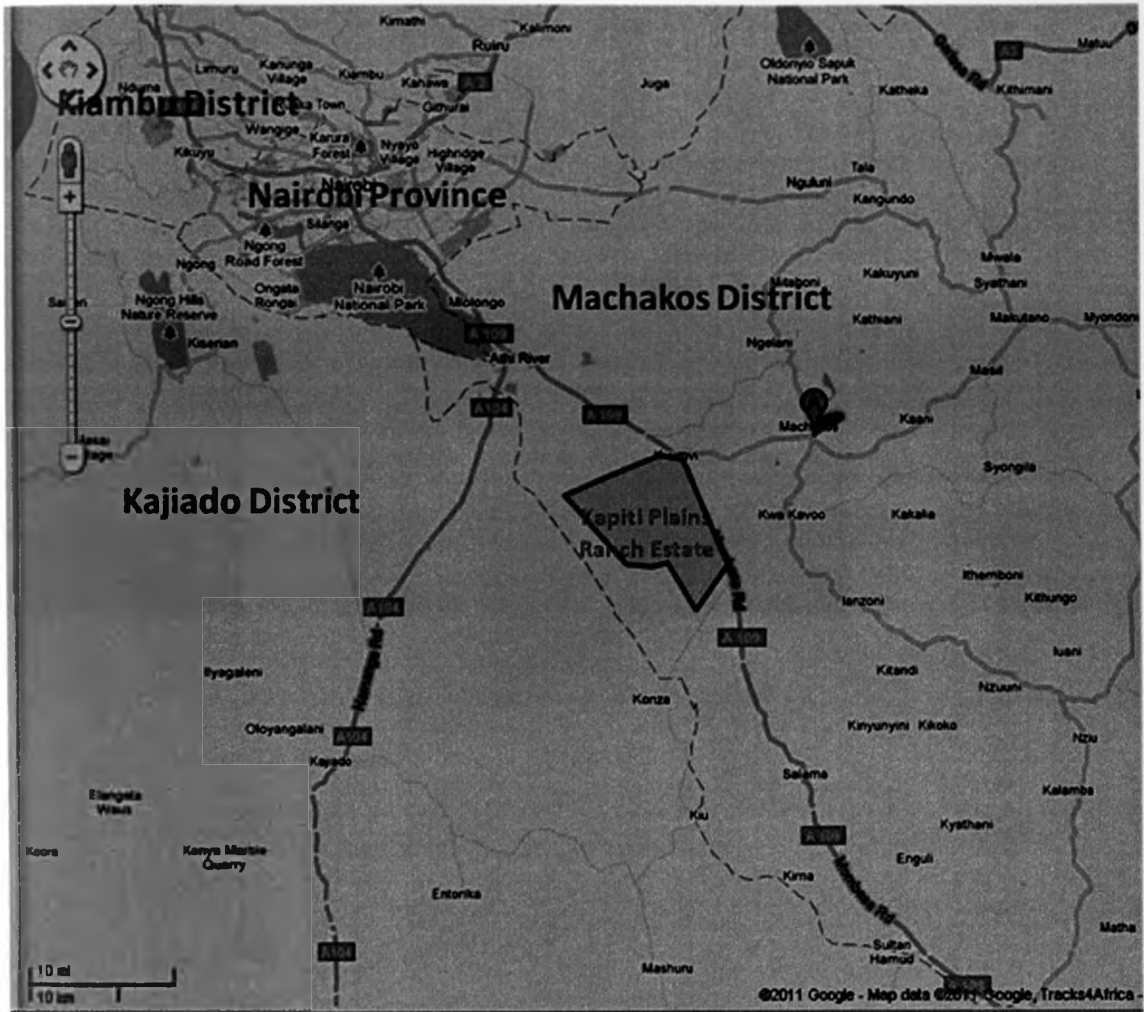
Ruma National Park is the home of the only population of globally scarce Roan Antelope remaining in Kenya. Farmers around the park keep cattle, sheep, goats and domestic pigs. Incursions of bush pigs into farms neighbouring the park have been reported (KWS, 2008, 2009). Ruma National Park and the surrounding farms thus presents a good site for the study of possible bush pig-domestic pig interaction.



**Figure 4.1: Map of Homabay District showing administrative divisions and the location of Ruma National Park, 2008**

*Source: International Livestock Research Institute*

Kapiti Plains Ranch Estate (Figure 4.2) is a 13,000-hectare ranch located in Machakos District, about 65 Km south-east of Nairobi. The rainfall of the area is divided into a "long rains" season that fall in March to May followed by the "short rains" season in October to December. Kapiti receives about 550mm average rainfall each year. The ranch was established during World War II and used in the development of the highly productive Boran (*Bos indicus*) beef cattle breed. Kitengela and Kapiti plains connect Nairobi National Park to the Amboseli Ecosystem. Kapiti ranch currently hosts both livestock and wildlife species. Wildlife species include giraffes, antelopes, wildebeests, warthogs and a wide collection of bird species. Because of increasing pressure for land, some of the neighbouring areas have human settlements with piggery comprising a component of the farming activities. This study area thus provided an appropriate site for investigating the warthog-tick ASFV sylvatic cycle and a possible warthog-domestic pig interface in ASFV transmission.



**Figure 4.2: Map of Kapiti Plains Ranch in relation to Nairobi National Park, Kajiado District, Kiambu District and City of Nairobi**

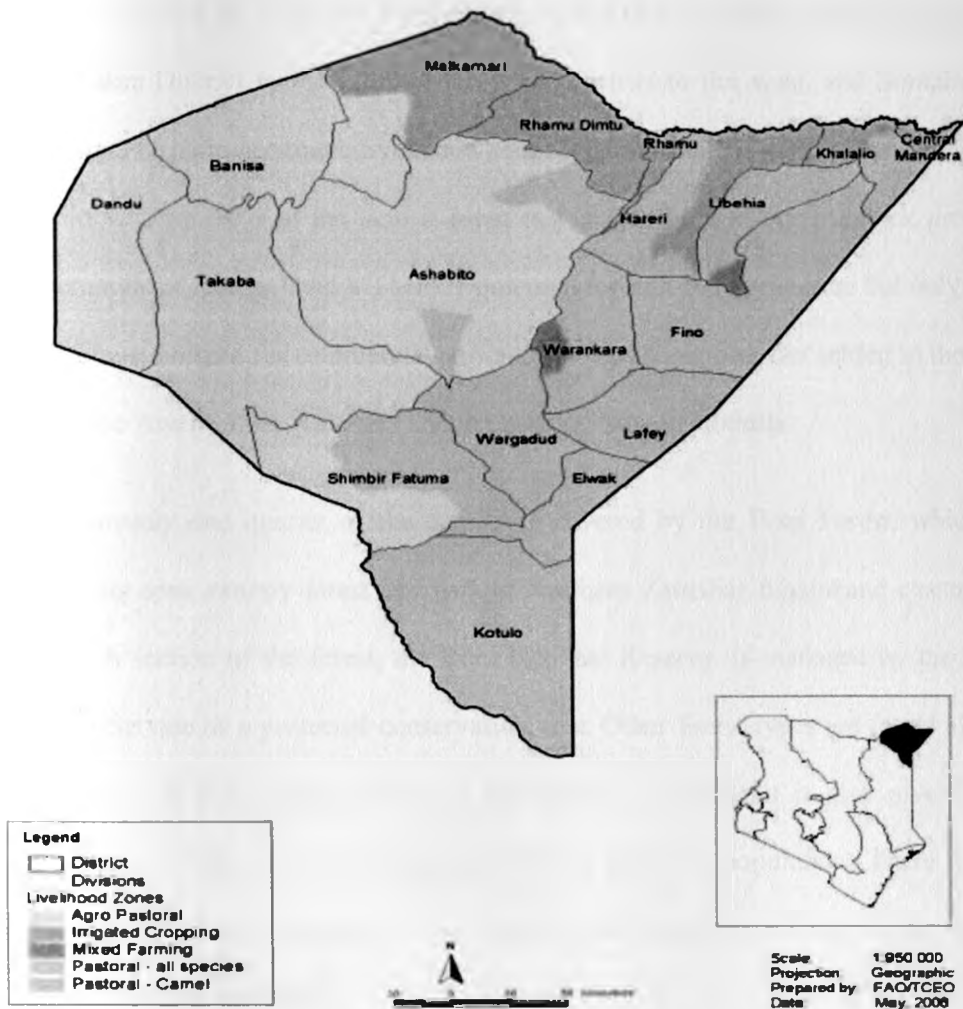
*Source: Modified from Google Earth 2012*

The larger Mandera District (Figure 4.3) is one of the arid districts of Kenya with an erratic mean annual rainfall of 255mm, mean temperatures of 28°C and a projected population of 1,025,756 persons (KNBS, 2009). It shares borders with Ethiopia to the North, Somalia to the East and the Wajir District of Kenya to the South. Mandera, Takaba and Elwak are the only gazetted urban centres and accommodate majority of the peri-urban poor.

The greater Mandera district has an area of 26,470 Km<sup>2</sup>. There are three main livelihood zones in the district, i.e., a pastoral economy zone in the East and agro-pastoral economy zone in the West and an irrigated cropping zone in the North along the Daua River. Over 81% of households in the district derive their livelihoods solely from livestock (FAO, 2007).

The district is prone to unpredictable climate changes, leading to either severe droughts or heavy rains. The main livelihood is livestock production, with irrigated farming confined to the riverine belt where 6,500 hectares is utilised out of 10,500 hectares of potential irrigable land. Some opportunistic rain-fed farming is also practiced in the Agro-pastoral livelihood zone.

River Daua that stretches for 150km along the border with Ethiopia provides water either by direct flow or digging when the riverbeds are dry. Malka Mari National park lies along the Daua River on the Kenya Ethiopia border in the extreme North-east of Kenya on the Mandera plateau. The park has a population of desert warthogs (*Phacocherus aethiopicus*).



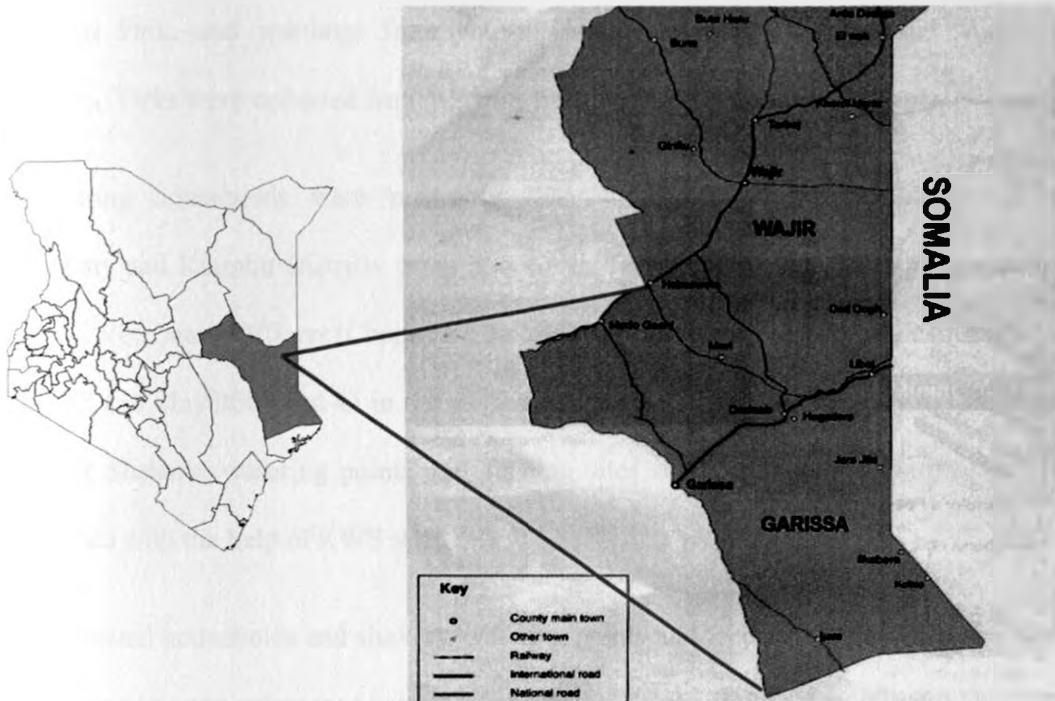
**Figure 4.3: Map of Mandera District showing administrative divisions and livelihood zones**

*Source: Food and Agriculture Organization of the United Nations (2006)*



Garissa District (Figure 4.4) covers an area of 33,620 Km<sup>2</sup> and, according to the population census of 2009, has a population of 623,060. It borders Wajir District to the north, Lamu District to the south, Tana River District to the west, and Somalia to the northeast. The main economic livelihood of the inhabitants is pastoralism and subsistence agriculture. Over 80% of the land is most sustainably suitable for livestock production (Government of Kenya, 2005). There is potential for rain-fed agriculture but only a small proportion is utilized for commercial agriculture. Local communities settled in the district include the Awer hunter-gatherers and the pastoral Somali Abdalla.

Approximately one quarter of the district is covered by the Boni Forest, which is an indigenous open canopy forest and part of Northern Zanzibar-Inhambane coastal forest mosaic. A section of the forest, the Boni National Reserve, is managed by the Kenyan Wildlife Service as a protected conservation area. Other forest types are found along the Tana River on the western border of the district. The district is also pivotal in the conservation of the critically endangered Hirola antelope population. There is also a population of desert warthogs in the district, with majority of them in the Boni and Arawale National Reserves.



**Figure 4.4: Map of Kenya showing location of Garissa District**

*Source: Modified from Wikimedia*

### **4.3.2 Animal Sampling**

Domestic pigs were sampled from Homabay and Kiambu Districts, bush pigs from Ruma National Park, and warthogs from Kapiti Plains Ranch and Garissa and Mandera Districts. Ticks were collected from warthog burrows at the Kapiti Plains Ranch.

Pig-rearing households were randomly selected from administrative divisions in Homabay and Kiambu Districts using lists of pig-rearing households obtained from the District Veterinary Officers (Chapter 3). In total 40 farms were selected in the first period in April and May 2008 and 41 in the second sampling period in March 2009 in Homabay District. Shelters, watering points and feeding sites of bush pigs and warthogs were identified with the help of KWS staff.

The selected households and shelters, watering points and feeding sites of bush pigs and warthogs were geo-referenced using Garmin Global Positioning System (GPS) 12XL unit (Garmin Olathe, KS, USA).

Domestic pigs from the selected farms were physically restrained prior to sampling. All pigs belonging to the selected households were sampled for whole blood. Bush pigs in Ruma National Park and warthogs in Kapiti Plains Ranch, Garissa and Mandera were captured using specially designed trapping nets and restrained chemically using Azaperon 40mg/ml (Stresnil®-P/M; Mallinckrodt) at a dose of 0.5 mg/Kg body weight before sampling for blood. Some animals were killed humanely using a capture bolt stunner for tissue sampling.

Ticks were collected from warthog burrows using a long-handle spade (Plate 4.1A and 4.5B). The soils scooped out of the burrows were carefully sorted to separate ticks, which were stored in perforated tubes (Plate 4.1C).

All pigs (all sexes and ages) in each of the selected farms were sampled. Domestic pig samples (blood/serum) were collected between April 2008 and April 2010, from 146 pigs (74 in period 1 and 72 in period 2) in Homabay District and 117 in Kiambu, Machakos and Kajiado Districts. Initially, 12 warthogs were sampled in Kapiti Plains Ranch in August 2008 and a repeat sampling was performed between January and April, 2009, when 36 warthogs were captured and sampled for blood and serum. One warthog from Kapiti Plains Ranch was stunned using a captive bolt stunner before slaughtering for tissue sampling. The tissues sampled were brain, lymph nodes (intra-mammary, parotid pre-scapular, uterine, peri-renal and mesenteric lymph nodes), heart, kidney, liver, lungs, salivary glands and spleen. To complement the ASFV study in warthogs, serum samples from desert warthogs collected from North Eastern Province (Garissa and Mandera Districts) were obtained from KWS. A total of 81 serum samples (29 collected in 2006, 21 in 2007 and 31 in 2008) were screened for antibodies to ASFV. A total of 8 bush pigs in Ruma National Park were captured and sampled for whole blood and serum and out of the 8 one was sacrificed for tissue sampling. Tissue collected were spleen, Kidney, lungs, heart and lymph nodes.

The samples (blood, serum, tissues and ticks) were shipped to the European Union (EU) ASF reference Laboratory in Spain (CISA-INIA) where the diagnostic and genetic characterizations were performed.

#### **4.3.3 Sample collection, storage and analysis**

Blood was collected from the subcutaneous abdominal or ear veins using BD Vacutainer® needles (gauge x length: 21 x 1-1/2 inch) into 10 ml BD Vacutainer® glass serum tube and 4.5 ml 15% EDTA tubes (Becton, Dickinson and Company, United Kingdom). Non-EDTA blood was allowed to clot and serum separated. Both serum and EDTA blood aliquots were dispensed into 2 ml cryo-vials (Greiner bio-one, Germany) and stored at minus 20°C. The samples were shipped to the European Union ASF reference laboratory in Spain (CISA-INIA) on dry ice according to standard protocols for shipping infectious biological materials.

Another aliquot of blood in EDTA was transferred rapidly onto filter papers (Michaud et al., 2007) for DNA analysis. Any pigs displaying signs of clinical ASF during the study were sacrificed for tissue sampling.

Sampled ticks from warthog burrows were stored in perforated tubes and sent to the laboratory for tick identification, classification of different instars, and PCR detection of ASFV.



4.1A



4.1 B



4.1 C

**Plate 4.1: Warthog burrow at Kapiti Ranch, Machakos District(4.5 A); Scooping soil from warthog burrow using a long-handle spade (4.5 B); Sorting ticks (*Ornithodoros spp*) from soil collected from a warthog burrow (4.5 C).**

#### **4.3.4 African swine fever virus diagnosis**

##### **4.3.4.1 Antibody detection**

The ASFV antibody detection was performed using OIE-approved serological tests. The tests consisted of an initial screening of sera by enzyme-linked immunosorbent assay (OIE-ELISA), followed by an immunoblotting assay to confirm doubtful results. Briefly, both conventional ELISA and immunoblotting assays were performed using a lysate of MS stable monkey kidney cell line (ECACC, 91070510) infected with ASFV E70MS48 as the antigen and protein-A conjugated to HRPO as the indicator. Both procedures were carried out following the protocols described in the OIE Manual of Diagnosis (<http://www.oie.int/manual-of-diagnostic-tests-and-vaccines-for-terrestrial-animals/>).

##### **4.3.4.1.1 Antigen preparation for ELISA**

The ELISA antigen was prepared from ASFV infected cells grown in the presence of pig serum (Escribano et al., 1989). Monkey stable (MS) cells were infected at a multiplicity of infection of 10 with adapted virus, and incubated in medium containing 2% pig serum. The cells were harvested at 36–48 hours post-infection, when the cytopathic effect was extensive. The cells were then washed in PBS and sedimented at 650 g for 5 minutes, the cell pellet washed in 0.34 M sucrose in 5 mM Tris/HCl, pH 8.0, and centrifuged to pellet cells. These steps were carried out on ice. The cell pellet was re-suspended in 67 mM sucrose in 5 mM Tris/HCl, pH 8.0 (1.8 ml per 175 cm<sup>2</sup> flask), and left for 10 minutes with agitation after 5 minutes. The Nonionic detergent (Nonidet P-40) was added to a final concentration of 1% (w/v), and left for 10 minutes (with agitation after 5 minutes) to lyse the cells. Sucrose was added to a final concentration of 64% (w/w) in 0.4 M Tris/HCl, pH 8.0, and followed by centrifugation at 1000 g for 10 minutes to pellet

nuclei. The supernatant was collected and EDTA added (2 mM final concentration), beta-mercaptoethanol (50 mM final concentration) and NaCl (0.5 M final concentration) in 0.25 mM Tris/HCl, pH 8.0, and incubated for 15 minutes at 25°C. Centrifugation was then performed at 100,000 g for 1 hour at 4°C over a layer of 20% (w/w) sucrose in 50 mM Tris/HCl, pH 8.0. The band was removed immediately above the sucrose layer and used as the ELISA antigen. The antigen was stored at -20°C.

#### **4.3.4.1.2 ELISA test procedure**

The ELISA test was performed accordingly (Arias and Sanchez Vizcaino, 1992; Pastor and Escribano, 1990). Microtitre ELISA plate(s) were coated with ASFV antigen by adding 100 µl of the pre-titrated dilution of antigen in 0.05 M carbonate/bicarbonate buffer, pH 9.6, to each well. The plates were incubated at 4°C for 16 hours (overnight) and then washed five times with 0.05% Tween 20 in PBS, pH 7.2. The test sera and positive and negative control sera were diluted 1/30 in 0.05% Tween 20 in PBS, pH 7.2, and 100 µl of each diluted serum added to duplicate wells of the antigen-coated plate(s). The plates were incubated at 37°C for 1 hour on a plate shaker and then washed five times with 0.05% Tween 20 in PBS. A 100 µl of protein-A/horseradish-peroxidase conjugate (Pierce) was added to each well at the pre-titrated dilution in 0.05% Tween 20 in PBS. The plates were incubated at 37°C for 1 hour, and then washed five times with 0.05% Tween 20 in PBS.

Substrate was prepared by adding hydrogen peroxide to the substrate solution (0.04% orthophenylenediamine (OPD) in phosphate/citrate buffer, pH 5.0) at the rate of 10 µl/25 ml, and 100 µl of substrate added to each well.



The plates were incubated at room temperature for approximately 6–10 minutes (before the negative control begun to develop colour). The reaction was stopped by adding 100  $\mu$ l of 1.25 M sulphuric acid to each well.

#### **4.3.4.1.3 Reading the ELISA results**

Positive sera had a clear yellow colour and could be read by eye, but to ensure that all positive sera were identified, the absorbance in each well was read spectrophotometrically, at 492 nm in an ELISA reader. Using the OPD substrate, serum was considered to be positive if it had an absorbance value of more than twice the mean absorbance value of the control negative sera on that plate.

#### **4.3.4.1.4 Preparation of antigen strips for immunoblotting test**

Immunoblotting technique was done accordingly (Escribano et al., 1990; Pastor et al., 1989).

Cytoplasmic soluble virus proteins were prepared as described in Section 4.3.4.1.1. The proteins were electrophoresed through 17% acryl-amide/N, N'-diallyltartardiamide (DATD) gels with appropriate molecular weight standards. The proteins were transferred on to a 14  $\times$  14 cm<sup>2</sup> nitrocellulose membrane by electrophoresis at a constant current of 5 mA/cm in transfer buffer (20% methanol in 196 mM glycine, 25 mM Tris/HCl, and pH 8.3). The membrane was dried and labeled on the side on to which the proteins were electrophoresed. One strip was cut from the edge of the filter and was used to perform the immunoblotting procedure described below.

Chloranaphthol substrate solution was prepared immediately before use by dissolving 6 mg of 4-chloro-1-naphthol in 2 ml of methanol and adding this solution slowly to 10 ml

of PBS with stirring. The white precipitate that was removed by filtration through Whatman No.1 filter paper. Then 4  $\mu$ l of 30% hydrogen peroxide were added.

#### **4.3.4.1.5 Immunoblotting test procedure**

The antigen strips were kept with the labeled side uppermost during the immunoreaction procedure. The strips were incubated in blocking buffer (2% nonfat dried milk in PBS) at 37°C for 30 minutes with continuous agitation. Preparation of 1/40 dilutions of test sera and positive and negative control sera in blocking buffer was then done. The antigen strips were incubated in the appropriate serum at 37°C for 45 minutes with continuous agitation. One antigen strip was incubated in positive control serum and one in negative control serum. The strips were washed four times in blocking buffer with the final wash performed for 5 minutes with continuous agitation. Protein-A/horseradish-peroxidase conjugate was added at pre-titrated dilution (1/1000 dilution) in blocking buffer to all antigen strips. The strips were further incubated at 37°C for 45 minutes with continuous agitation then washed four times in blocking buffer with the final wash done for 5 minutes with continuous agitation.

The substrate solution was prepared, and added to the antigen strips, and incubated at room temperature for 5–15 minutes with continuous agitation. The reaction was stopped with distilled water when the protein bands were suitably dark.

#### **4.3.4.1.6 Reading the immunoblotting test results**

Positive sera reacting with more than one virus protein in the antigen strip gave a similar protein pattern that had the same intensity of colour as the antigen strips stained with positive control serum.

#### **4.3.4.2 Virus detection**

##### **4.3.4.2.1 Sample preparation**

Extraction of DNA was done directly from serum, blood or 10% suspensions of ground tissues or ticks using a nucleic acid extraction kit (Nucleospin/ Machery-Nagel-Cultek) following the manufacturers' recommendations.

For organ and tissue samples, a 1/10 homogenate of the material in PBS was first prepared, then centrifuged to clarify at 12,000 g for 5 minutes. Extraction of DNA from the resultant supernatant fluid was then done.

Extraction for control samples used 1/10 tissue homogenates (from the same tissue as the samples to be analysed). For both the negative and positive control, 200 µl of a homogenate of ASFV-negative and positive tissues, respectively, were used, with both controls processed together with the test samples.

The test sample (200 µl) was pipetted into a 1.5 ml microcentrifuge tube, and 200 µl of binding buffer and 40 µl of proteinase K were added. These were mixed immediately and incubated for 10 minutes at 72°C. The 1.5 ml microcentrifuge tube was briefly centrifuged to remove drops from the inside of the lid.

Isopropanol (100  $\mu$ l) was added to the sample tube. The High Pure filter tube was placed in a collection tube and the sample pipetted in the upper reservoir and centrifuged for 1 minute at 8000 rpm (the centrifugation step was repeated with blood samples if any sample remained in the filter tube). The collection tube was discarded and the filter tube placed into a clean collection tube.

Inhibitor Removal Buffer (500  $\mu$ l) was added to the upper reservoir and centrifuged for 1 minute at 8000 rpm. The collection tube was discarded and the filter tube was placed into a clean collection tube. Wash buffer (450  $\mu$ l) was added to the upper reservoir and centrifuged for 1 minute at 8000 rpm. The collection tube was discarded and the washing step repeated. The collection tube was then discarded and the filter tube placed into a clean collection tube, followed by centrifugation for 10 seconds at 13000 rpm to remove residual wash buffer. The collection tube was discarded and the filter tube placed in a clean 1.5 ml microcentrifuge tube. For the elution of nucleic acids, 50  $\mu$ l of pre-warmed (70°C) sterile water was added to the upper reservoir and centrifuged for 1 minute at 8000 rpm. The DNA was either used immediately or stored at -20°C for future use.

The reagents and buffers used for the PCR amplification assay are shown in Appendix 2.

#### 4.3.4.2.2 PCR amplification assay

A PCR assay using the ASF diagnosis primers PPA1/PPA2, which generates an amplicon of 257 base pairs within the p72 protein (Aguero et al., 2003) was used to confirm the presence of ASFV DNA. The tick samples were analysed in pools using diagnostic PCR (Aguero et al., 2003) and specific-tick PCR (Basto et al., 2006). Since tick homogenates may contain PCR inhibitors, which may result in false negative results, an internal control (IC), which is a 498 bp fragment was amplified from a plasmid containing a DNA fragment from the *sdbA* gene of *Clostridium thermocellum* using primers specific for the foreign DNA but with the diagnostic primers sites (72ARs and 72ARas) as 5' overhanging ends, was used in the first round of tick-specific nested PCR.

The PCR reaction mixture was prepared in a sterile 1.5 ml microcentrifuge tube as described below for each sample. The reaction mixture was prepared in bulk for the number of samples to be assayed allowing for one extra sample.

Reaction mixture: Nuclease-free or sterile distilled water (17.375  $\mu$ l), 10  $\times$  PCR Buffer II (2.5  $\mu$ l), magnesium chloride 25 mM (2  $\mu$ l), dNTP mix 10mM (0.5  $\mu$ l), primer 1, 20 pmol/ $\mu$ l (0.25  $\mu$ l), primer 2, 20 pmol/ $\mu$ l (0.25  $\mu$ l), AmpliTaq Gold DNA polymerase 5 U/ $\mu$ l (0.125  $\mu$ l) and in the case of tick specific PCR the IC plasmid.

The PCR reaction mix (23  $\mu$ l) was added to the required number of 0.2 ml PCR tubes. Extracted sample template (2 $\mu$ l) was added to each PCR tube including a positive reaction control (2  $\mu$ l of ASFV DNA) and a negative reaction control (2  $\mu$ l of distilled water) for each PCR run.

All the tubes were placed in an automated thermal cycler and the following programme was run:

- One cycle at 95°C for 10 minutes for initial denaturation of the DNA;
- 40 cycles at 95°C for 15 seconds, 62°C for 30 seconds and 72°C for 30 seconds;
- One cycle at 72°C for 7 minutes;
- Sample held at 4°C.

The PCR tubes were removed at the end of the programme and 2.5 µl of 10× loading buffer added to each tube.

All the samples were loaded in a 2% agarose gel in TAE buffer containing ethidium bromide at a final concentration of 0.5 µg/ml. Marker DNA was then added to one lane on each side of the gel. The gel was run at a constant voltage of 150-200 volts for about 30 minutes.

#### **4.3.2.2.3 Evaluating the p72 PCR amplification assay results**

The gel was examined over a UV light source. In a positive sample, a discrete band was present that co-migrated with the PCR product of the positive control. Calculation of the size of the PCR products in the test samples and the positive control was by reference to the standard markers. The PCR product of the positive control has a size of 257 base pairs. No bands were seen in the negative control.

The specificity of the amplicons obtained was confirmed using the BsmAI (Aguero et al., 2004) restriction endonuclease. For this assay, a total of 5 µl of amplified DNA product

was incubated for 2.5 hours at 55°C in a final volume of 20 µl digestion mix: 2 µl of 10 × buffer, 1 µl of BsmAI (5 U/µl) and 12 µl of sterile distilled water. The samples were then run in a 3% agarose gel as described above. The restriction pattern included two fragments of 173–177 and 84–80 base pairs in the positive samples.

#### **4.3.4.2.4 Virus isolation**

Pig (blood, serum and tissues) and tick samples were shipped to the High Security Laboratory (HSL) OIE reference laboratory for ASF (Centro de Investigación en Sanidad Animal (CISA – INIA)) in Spain, for virus isolation and characterization.

Cultures were used for the isolation of virus in samples recovered from naïve domestic pigs as previously described (Malmquist and Hay, 1960). Briefly, primary leukocyte culture cells from infected pigs were seeded into 96-well tissue culture grade microtitre plates (200 µl; 300,000 cells per well) in homologous swine serum, and incubated in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C. Three-day cultures were infected at a multiplicity of infection (moi) 1:10 with serum, blood or 10% suspensions of ground tissues or ticks in phosphate buffered saline supplemented with 5 µg/ml gentamycin sulphate (BioWhittaker) and incubated for 24 hours at 37°C. After inoculation, a preparation of 1% homologous red blood cells in buffered saline was added to each well. The plates were examined for haemadsorption over a 6 day period. The samples were blind passaged three times.

#### 4.3.5 Data analysis

Summary statistics was performed using the GenStat<sup>®</sup> data analysis program.

Prevalence was calculated using the formula:

$$\text{Prevalence} = a/a + b$$

Where *a* is the number of animals with ASFV and *b* are those animals without the virus.

To test the null hypothesis that prevalence of ASFV in one cohort is equal to prevalence in the second cohort ( $P_1=P_2$ ), a pooled sample proportion (*p*) was calculated to compute the standard error of the sampling distribution (Dunn and Clark, 2009).

$$p = (p_1 * n_1 + p_2 * n_2) / (n_1 + n_2)$$

Where *p*<sub>1</sub> is the sample proportion from population 1, *p*<sub>2</sub> is the sample proportion from population 2, *n*<sub>1</sub> is the size of sample 1, and *n*<sub>2</sub> is the size of sample 2.

The standard error (SE) of the sampling distribution difference between two proportions was computed using the formula:  $SE = \text{sqrt} (p * (1 - p) * [ (1/n_1) + (1/n_2) ] )$

Where *sqrt* is square root, *p* is the pooled sample proportion, *n*<sub>1</sub> is the size of sample 1, and *n*<sub>2</sub> is the size of sample 2.

The test statistic was a z-score (*z*) defined by the following equation:  $z = (p_1 - p_2) / SE$

Where *p*<sub>1</sub> is the proportion from sample 1, *p*<sub>2</sub> is the proportion from sample 2, and *SE* is the standard error of the sampling distribution.



Investigation of risk information obtained from questionnaire administration was analysed using descriptive methods and a regression analysis using a Poisson model in GenStat® to quantify and assess the role of production factors such as feeding and types of feed, breeding approaches, restocking of new pigs, tick control, grazing management and proximity to protected areas containing wild pigs, in explaining whether a farm had ASFV positive animals or not. The response variable (what was considered positive by any of the confirmatory diagnostic tests) was the count of positive animals per farm and the fitted variable were a constant and the production factors.

The regression equation was as follows:

$$\text{Log}_e(Y) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3$$

Where,  $Y$  is the count of ASFV infection,  $e$  is the base of the natural logarithm ( $e = 2.71828$ ),  $\beta$  the model coefficient, and  $\beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \dots$  were the predictor variables.

In the case of Homabay District, regression analysis was done and involved fitting the following variables into a Poisson model; sampling period (sampling was done at two sampling points in the same area), feeding sources, breeding, tick control, distance/distance categories to Ruma National Park and herd size as an offset, to explain count of ASFV infection on-farm. The model was fitted using a backward elimination method. A conservative approach was used where the p value was set at 0.1 to give the variables a chance to be retained in the model. The fitted Poisson model was used to predict count and rates of ASFV on-farm given the set of fitted variables.

## 4.4 Results

### 4.4.1 Prevalence of ASFV in domestic pigs in free-range and stall-feeding systems

Virus in blood and serum samples was detected in 35 domestic pig samples from 146 animals in Homabay District using the p72 PCR for a prevalence of 24% (95% CI, 17, 31) (Table 4.1). No sero-positive pigs were detected using the OIE-ELISA. Majority of farms with ASFV positive pigs according to PCR analysis were from Ndhiwa Division (33%), and Nyarongi Division (60%), and they either bordered or were located close to Ruma National Park. No virus could be isolated after three passages in macrophages from PCR positive samples.

Of the total 117 pigs sampled from Kiambu, Machakos and Kajiado Districts (central region), only 3 pigs tested positive on the p72 PCR analysis for a prevalence of 3% (95% CI, 0, 5) (Table 4.1). Like in Western Region, none of the samples collected from pigs were positive for ASFV antibody using the OIE-ELISA. All the 3 positive samples in central region were from Machakos and Kajiado Districts, and none from Kiambu District. The ASFV positive pigs were from farms that bordered the Kapiti Plains, which is divided into ranches that contain populations of warthogs. The prevalence, as assessed by PCR, was significantly ( $P < 0.05$ ) higher in western (30%) compared to central region (3%).

**Table 4.1: Prevalence of African swine fever virus based on PCR assay in Western and Central regions of Kenya, 2008-2010**

Region	Number of pigs	Number positive	Prevalence Proportion (%)	95% Confidence Interval for Mean	
				Lower Bound	Upper Bound
Western	146	35	24	17	31
Central	117	3	3	0	5
<b>Total</b>	<b>263</b>	<b>38</b>	<b>14</b>	<b>10</b>	<b>19</b>

#### **4.4.2 Prevalence of ASFV in wild pigs**

##### **4.4.2.1 Warthogs**

The 12 and 36 warthogs sampled at Kapiti Plains Ranch in 2008 and 2009, respectively, were all positive for antibody by both OIE-ELISA and immunoblotting. However, the p72 PCR assay detected only 10% (5/48) of the adult savannah warthogs as being positive for the virus in blood and no virus was detected in the samples of 81 desert warthogs. All tissues (tonsils, lymph nodes, lung, heart, spleen, liver and kidney) collected from a single warthog were virus negative by PCR.

Retrospective analysis of serum samples of desert warthogs (*P. aethiopicus*) from Mandera and Garissa districts (North Eastern region) demonstrated sero-prevalence of 93% (27/29), 95% (20/21) and 97% (30/31) in the years 2006, 2007 and 2008, respectively, for antibody by OIE -ELISA. Analysis of the same samples by p72-PCR for virus detection was negative.

Of the 5 warthog samples identified as positive for ASFV by PCR, only one animal was positive in both blood and serum.

##### **4.4.2.2 Bush pigs**

Eight bush pigs were sampled in Ruma National Park but only one bush pig was sampled for tissues and the kidney sample was found to be positive for virus by p72-PCR. All the eight bush pig serum and blood samples were negative by p72-PCR, OIE-ELISA and immunoblotting. Tissues were collected from only one of the eight bush pigs captured, due to the ethical and conservation limitation related restrictions to sacrificing of these relatively uncommon animals within a National Park.

#### 4.4.3 Prevalence of ASFV in ticks

*Ornithodoros moubata* ticks were collected from warthog burrows in two sampling periods in 2008 and 2009. The ticks were pooled and grouped based on stage of development. The ticks were classified into nymphal instars 1-3 (N1-N3), 4-5 (N4-N5) and adults. A summary of the analysis is presented in Table 4.2. Of the 285 pools tested by both conventional PCR and tick-specific PCR, 14% (41/285) and 22% (62/285) were positive by the methods, respectively; the difference was significant ( $P < 0.05$ ). There was also a significant chance ( $p < 0.05$ ) that tick-specific PCR had a higher proportion. A total of 20 ASF viruses were isolated from the 103 ASFV positive tick pools. The analysis of differences in prevalence between all the nymphal stages was insignificant ( $p > 0.05$ ).

**Table 4.2: Infection by ASFV in different developmental stages of *O. moubata* ticks from Kapiti Plains Ranch, 2008-2009**

Stage of tick development	No of ticks collected	Diagnostic PCR		Specific-tick PCR		Virus Isolation
		No of pools tested	No positive (%)	No of pools tested	No positive (%)	No positive (%)
*N1-N3	891	116	15 (13)	116	19 (16)	7 (37)
**N4-N5	485	80	9 (11)	80	18 (22)	4 (22)
ADULT	200	89	17 (19)	89	25 (28)	9 (36)
<b>TOTAL</b>	<b>1576</b>	<b>285</b>	<b>41 (14)</b>	<b>285</b>	<b>62 (22)</b>	<b>20 (32)</b>

**Key:**

\*N1-N3 = Nymph stage 1 to 3

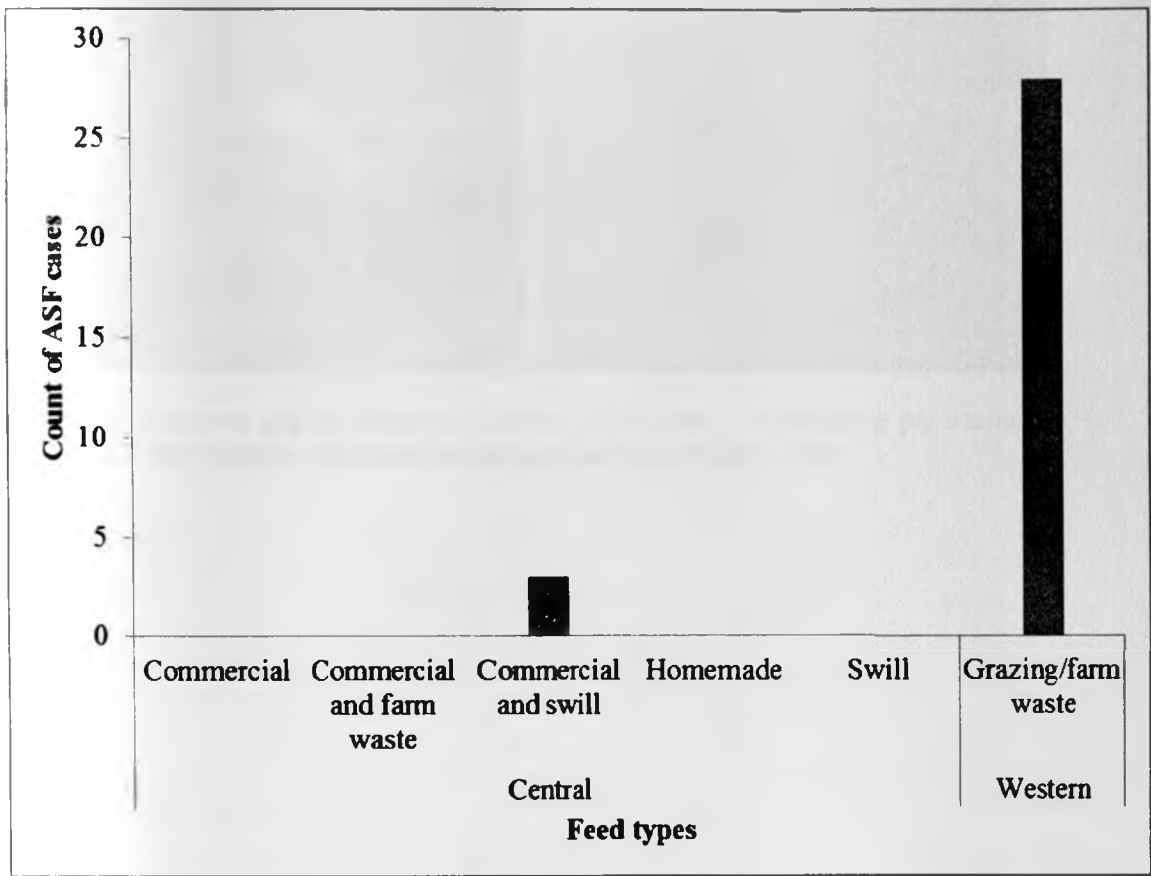
\*\*N4-N5 = Nymph stage 4 to 5

#### 4.4.4 Prevalence ASFV based on management system

The count of ASFV cases on-farms as they relate to the various feeding systems is shown in Figure 4.5. Commercial feed supplemented with swill (associated with 3% of pigs) and free-grazing supplemented with household food wastes (associated with 30% of pigs) were the feed-types associated with ASFV infections as compared to the other feed-types. Free range foraging, scavenging and tethering were all common husbandry practices in Homabay District, Western Region. Examples of tethering in Homabay District and scavenging in Kisumu District are presented in Plate 4.2A and 4.2B. Feeding of swill was the most prevalent in Kiambu District, in Central Region.

When a general analysis of ASFV risk was performed, with individual animal data, it was found that most of the risk variables were similar in farms within the two farming systems studied but different between the systems. In this case, farms had similar characteristics in herd structures, pig breeds, health care measures, pig management

approaches and pig productivity, depending on their location. There was similarity between feeding, labour source and location. Given our knowledge on the role of feeding systems in the epidemiology of the ASFV, feeding was thus adopted in the comparative analysis of animal level disease risk between the two pig production systems.



**Figure 4.5: Count of ASFV positive animals for Central and Western regions by type of pig feed on-farm**





**Plate 4.2: Tethered pig in Homabay district (4.2A) and a scavenging pig along the shores of Lake Victoria, Kisumu feeding on fish waste (4.2B), 2008**

#### 4.4.5 Spatial characterization of ASFV prevalence

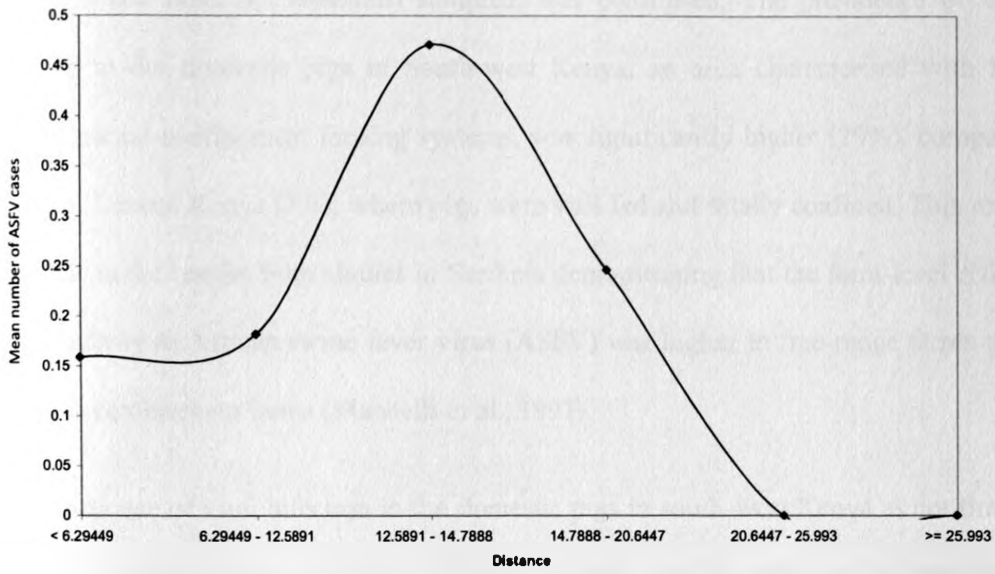
Most of the farms that had ASFV positive pigs were from Ndhiwa, Kobama and Nyarongi divisions, which border or are closer to Ruma National Park and where bush pig conflict with farmers had been reported. The rest of the farms in the other divisions located furthest away from the park were negative for ASF. The tissue from one bush pig out of eight sampled from the park was PCR positive for ASFV.

The Poisson model demonstrated that period of sampling and distance from Ruma National Park explained presence or absence of ASFV infection, and this was statistically significant ( $P < 0.05$ ). The model estimates for farm size and distance categories is shown in Table 4.3. A higher disease count was found in distance range between 6 Km and 16 Km away from the park (Figure 4.6). The risk of ASF was non-significant in farms less than 6 Km. The ASF risk was also not significant at more than 16 Km from the park and since there were more pigs at this distance range, this prediction was interpreted as a reduction in ASFV infection risk as a result of increased distance from the park.

**Table 4.3: Poisson regression estimates of parameters**

Variable	Level	$\beta$	Std. Error	95% Wald Confidence Interval		Wald Chi-Square	Hypothesis Test	
				Lower	Upper		Df	P value
(Intercept)		-8.271	1.2203	-10.663	-5.879	45.939	1	0
[Period=1.00]	P1	3.045	0.5482	1.971	4.12	30.858	1	0
[Period=2.00]	P1	0 <sup>a</sup>	.	.	.	.	.	.
[DistCate< 6.29]	D1	2.083	1.2249	-0.318	4.484	2.892	1	0.089
[DistCate=2.00]6.29 - 12.59	D2	2.715	1.1009	0.558	4.873	6.084	1	0.014
[DistCate=3.00] 12.59 - 14.79	D3	3.039	1.146	0.793	5.285	7.034	1	0.008
[DistCate=4.00] 14.79 - 20.64	D4	2.151	1.1628	-0.128	4.43	3.421	1	0.064
[DistCate=5.00]20.64 - 25.99	D5	1.317	1.4477	-1.52	4.155	0.828	1	0.363
[DistCate=6.00] >= 25.99	D6	0 <sup>a</sup>	.	.	.	.	.	.
Farm Size		0.001	0.0386	-0.075	0.077	0.001	1	0.979
(Scale)		1 <sup>b</sup>						

Key: a. Set to zero because this parameter is redundant      b. Fixed at the displayed value.



**Figure 4.6: Predicted mean cases of ASFV occurrence in farms in Homabay District relative to distance from Ruma National Park**

#### 4.5 Discussion

In this study, the presence of ASFV in Kenyan populations of domestic pigs, warthogs, bush pigs and ticks (*O. moubata*) sampled, was confirmed. The prevalence of viral infection in the domestic pigs in South-west Kenya, an area characterised with free ranging partial-confinement feeding systems, was significantly higher (29%), compared to that of Central Kenya (3%), where pigs were stall fed and totally confined. This result is similar to the results from studies in Sardinia demonstrating that the farm-level risk of sero-positivity to African swine fever virus (ASFV) was higher in free-range farms than in partial-confinement farms (Mannelli et al., 1997).

The prevalence of viral infection in the domestic pigs in south-west Kenya as confirmed p72-PCR diagnosis was very high (29.2%), and was not accompanied by observable clinical signs in the positive animals. This result suggests a number of possible explanations, including:

1. The PCR positive animals were in an early infection phase;
2. Infection with inapparent disease due to factors e.g.
  - Host factors confer resistance/tolerance to the virus; and
  - Viral factors such as low virulence could lead to infection without apparent disease.

These explanations are not mutually exclusive and some combination of factors may apply.

Two samplings were performed in Western Kenya at different time-points (March to April 2008 and February to March 2009). The results from both studies revealed a similar epidemiological picture, specifically high ASFV prevalence with no obvious clinical disease and antibodies against the virus were also not detected by OIE-ELISA. It was expected that animals in the same or different herds would be at various stages of sub-clinical and clinical presentation of ASF and that acute symptoms (i.e. pig deaths) or morbidity would have occurred between the two sampling points. However this was not apparent from direct observations of the same animals and farmers did not report any disease. This result, thus, appear to rule out the hypothesis that animals originally sampled in Homabay were undergoing early phase infections.

Observation of inapparent infection in domestic pigs has previously been made in northern Mozambique (Penrith et al., 2004a). In the Mozambican study, genetic resistance or viral virulence was not an explanation for the epidemiological picture observed.

A phenomenon of suspected hybridization of domestic pigs and wild African pigs has been reported in different parts of Africa, where free-ranging female domestic pigs (*Sus scrofa*) are mounted by male wild pigs (*Potamochoerus spp*). This suggests genetic closeness of the two pig species. Further scientific evidence is needed to support the reality of these observations. If this phenomenon occurs it may contribute to resistance/tolerance to ASF infection, as inherited genetic characteristic of *Potamochoerus spp*. The possibility of introgression of bush pig genetic material into the domestic pig population could not be ruled out in the current study.

Absence of detectable antibodies in infected Homabay pigs and the fact that the animals were ASFV positive but without clinical disease manifestations, suggests that innate factors in the animals, or a cellular immune response without antibodies could be responsible. Immune responses mediating protection against ASFV are poorly understood. Several studies have shown that virus-specific antibodies delay the onset of clinical signs and reduce the level of viremia, thereby protecting the pigs from lethal infections (Onisk et al., 1994). Anti-ASFV antibodies may influence the course of the clinical disease but they have never been found to neutralize the virus (Gomez-Puertas et al., 1996).. Recent developments in understanding of cellular defence mechanisms in the context of *in vivo* responses to ASFV infection in pigs, and on the induction of certain cytokines warrant further investigation (Tulman and Rock, 2001).

The failure of the 'indigenous' pigs to seroconvert observed in this study supports previous findings documenting a low incidence of detectable serological responses to ASFV in East Africa (Gallardo et al., 2009a; Gallardo et al., 2011b; Perez-Filgueira et al., 2006). Recent studies have demonstrated that the formal diagnostic techniques result in serological diagnosis with high sensitivity, specificity and confidence, and are adapted to all epidemiological situations. The OIE-ELISA test is also a viral extract that contains multiple antigens. This suggests that there could be an actual failure of the pigs to seroconvert leading to absence of anti-ASFV antibodies in the blood.

An alternative explanation for the low seropositivity may possibly be related to the host phenotype/genotype. There is evidence that pigs from Western Kenya exhibit some introgression of genetic material from the Asian centres of wild boar domestication (Ramirez et al., 2009) and are therefore not identical to European and West African pig

breeds, from which the diagnostic tests have been validated. In consonance with the pattern observed in the indigenous pigs, previous findings indicate that the viraemia in bush pigs generally lasted longer than in warthogs in absence of seroconversion due to B lymphocyte apoptosis indicating that antibody mediated immunity may not be critically important for bush pig survival (Anderson et al., 1998; Oura et al., 1998). The result also suggests a possible role for T cells. Peptides expressed by an ASFV genomic library have been shown to be reproducibly recognized by lymphocytes from immune pigs (Jenson et al., 2000). The presence of ASFV encoded antigenic epitopes recognized by T cells supports the concept that cellular immunity to the virus may play an important role in resistance to ASF in the absence of antibodies (Jenson et al., 2000). However, despite the likely role of T-cells in immunity, this does not fully explain the failure of the pigs sampled to produce detectable ASFV-specific antibodies.

Virus populations with variable virulence, immunogenicity and infectivity to pigs can be generated by passaging African swine fever viral isolates in cell culture (Pan, 1992). Variability of viral strains belonging to the same cluster and genetic changes observed in viruses isolated between outbreaks although perhaps not directly involved could suggest additional changes elsewhere in the viral genome resulting in changes in virulence of viral strains circulating at the Kenyan study sites. Data indicate that the highly conserved NL-S gene of ASFV, while nonessential for growth in swine macrophages *in vitro*, is a significant viral virulence factor and may function as a host range gene (Zsak et al., 1996). Deletion of NL-S has been shown not to affect viral growth in primary swine macrophages or Vero cell cultures *in vitro*, but the null mutant, E70/43, exhibited a marked reduction in pig virulence. In contrast to revertant or parental E70 where



mortality was 100%, all E70/43-infected animals survived infection. Genomic coding sequences have also been shown to provide information concerning the genetic variability of African swine fever virus isolates that differ in pathogenicity (Chapman et al., 2008). Avirulent isolates compared with the virulent strains were shown to have deletions of 8–10 kbp that encode six copies of the multigene family (MGF) 360. These results indicate that the inapparent disease in virus positive pigs in this study could also be due to an avirulent strain of virus circulating in south-west Kenya.

Statistical analysis showed that distance from the park significantly explained the count of ASFV cases on-farm. The risk of ASF was non-significant in farms less than 6 Km. This range of distance had no cultivated land that was the attraction for bush pigs leading to their incursions into the farms for food. There was thus limited interaction between bush pigs and domestic pigs at this distance range from the park. The Lambwe Valley where the farms were supports large numbers of tse tse fly (*Glossina pallidipes Austeni*) and infections of *Trypanosoma brucei rhodesiense*. The ASF risk was also not significant at more than 16 Km from the park interpreted as reduction of ASFV infection risk as a result of increased distance from the park. Distances more than 16 Km were not in the reach of the wild pigs and therefore no interactions with domestic pigs were possible.

The high sero-prevalence of ASFV was observed in warthogs in this study (up to 100% sero-positivity) by sero-diagnosis is comparable to studies in warthogs in Serengeti region of Tanzania (Heuschele and Coggins, 1969). Infection of neonate warthogs occurs in burrows containing infected argasid ticks and is characterised by detectable viraemia for up to 11 days, becoming undetectable in blood after 33 days (Jori and Bastos, 2009;

Penrith et al., 2004b). In long term infections of warthogs the virus has a predilection for lymph nodes of the head (Plowright, 1981), animals remain infected for life (Wilkinson and Paton, 1989) and antibodies against the virus persist in blood for several years (Gallardo et al., 2006). These facts could explain the high prevalence observed in this species.

The 36 warthogs sampled at Kapiti Plains Ranch in 2009 were 100% positive by both OIE and INGENASA-ELISA and immunoblotting. However, PCR assay using the ASF diagnosis primers PPA1/PPA2 showed positivity in serum and in blood. This result was associated with virus positive warthog piglets and adults sampled in this study. Whereas earlier studies detected viraemia in young animals, there was no evidence for subsequent viraemias in the blood of older animals (Thomson, 1985). A recent study (Gallardo et al., 2011b) showed that ASFV could be genotyped directly from adult warthog sera. This observation was again consistent with the findings in the current study on viraemia in adult warthogs. There are few studies that have confirmed presence of ASFV in adult warthogs in their natural habitat except, in rare cases, in lymph nodes (Penrith et al., 2004b). However, young pre-weaned animals have been shown to have ASFV viraemia at levels considered sufficient for transmission to piglets in the burrows.

Analysis of 8 bush pig blood and serum samples by PCR assay using the ASF diagnostic primers PPA1/PPA2, both OIE and INGENASA-ELISA and immunoblotting showed negative results. However, PCR detected a positive result from a bush pig kidney sample, from the only bush pig whose tissues were sampled. Information on prevalence of ASF in bush pigs is scarce (De Tray, 2008; Haresnape et al., 1985; Jori et al., 2007). This is probably because bush pigs are elusive nocturnal creatures, difficult to capture.

Nevertheless, it has also been suggested that ASFV circulates only occasionally or at low levels in bush pigs (Jori et al., 2007). Earlier study reported “low frequencies” of the virus in bush pigs in Kenya (De Tray, 2008), but without supporting figures. The result suggested sequestration of the virus in some internal organs of bush pigs and that the virus is frequently not detectable in blood in long term infections.

The bush pig data needs further investigation in the context of the low success rate of sero-diagnostics in domestic pigs. If ASF screening is only based on serum and blood samples then such cases of virus infections in tissues can be missed during diagnosis. This means that an ASFV positive animal can be certified as virus free (false negative) yet have viruses sequestered in the tissues. There is further need to confirm if indeed sequestered virus in domestic pig tissues can be transmitted to in-contact pigs. It will also be important to understand if there is a specific window within which this transmission can occur between pigs. Other studies have shown that infectiousness of foot and mouth disease virus in a cow can only occur for 1.7 days (Charleston and Rodriguez, 2011).

The findings on prevalence of ASFV in bush pigs were comparable as well as contrasting with those of domestic pigs. There were neither antibodies nor virus that could be detected in either blood or serum of the bush pigs sampled in this study, yet the virus could be detected in the tissue of one bush pig. This was in contrast to what was observed in domestic pig blood and serum where virus was routinely detected, but similar in that there were no antibodies. The ASFV detectable in tissues of bush pigs and frequently in blood of domestic pigs within the localised area that was not accompanied by obvious signs of clinical disease in the theoretically susceptible domestic pig hosts also raised a

further question. This was whether a jump of the virus from a bush pig to domestic pig could be the explanation for avirulence and lack of antibody response.

Bush pig incursions into some of the PCR positive farms had been reported to KWS by pig farmers indicative of possible interaction between the wild pigs and domestic pigs in the same locality. The farmers were unable to describe any clinical disease observed in pigs that could be related to ASF. No ASF-like outbreaks had been observed or reported to the veterinary professionals operating in the district. These observations explain in-apparent infections in the domestic pigs. However, in comparison, all farmers interviewed in Central Region could describe ASF-like symptoms in pigs and ASF-like outbreaks had been observed and reported to veterinary authorities in the region.

Slight differences were found in the % positivity between pools of three tick stages (N1-N3; N4-N5; Adult) by diagnostic PCR, and specifically-adapted PCR for detection of ASFV in ticks, and virus isolation. The first two diagnostics had high prevalence in adult ticks, whereas the efficiency of virus isolation was equivalent in both N1-N3 and adult ticks. These findings showed that all stages of ticks had ASFV and that the viruses could be isolated from the tick samples. The result confirms that *Ornithodoros spp* ticks have the potential to spread the virus to domestic pigs in Kenya. A study (Gallardo et al., 2011b) has also shown that ASFV that was genotyped directly from domestic pigs in a slaughter house in Central Kenya were within p72 genotype X and genetically similar to viruses from ticks collected from Kapiti Plains Ranch.

The prevalence of ASFV in ticks was 14%, 22% and 32% using conventional PCR, Tick specific PCR and Virus isolation, respectively. These results showed virus isolation as the

most sensitive test. The virus prevalence in tick contrasted with those of other studies in Malawi, where infection rates of 1-3% were commonly reported in *Ornithodoros* ticks infesting pigpens (Haresnape et al., 1988), and which was similar to rates reported for wild ticks inhabiting warthog burrows (Plowright, 1981). However, the results could compare with other (Haresnape and Wilkinson, 1989) that showed that infection rates could reach 11-24% after an ASF outbreak occurring a few months ago.

## **CHAPTER 5**

# **INVESTIGATION OF AFRICAN SWINE FEVER OUTBREAKS IN WESTERN AND CENTRAL KENYA, 2010-2011**

### **5.1 Introduction**

African swine fever (ASF) is a highly contagious and fatal viral disease of domestic pigs. It was first described in Kenya, East Africa (Montgomery, 1921). According to the OIE ASF has occurred in most of the East and Southern African countries for the last 6 years. No case was reported in Botswana, Swaziland and Sudan and no information is available for Zimbabwe, Lesotho, Ethiopia, Somalia and Eritrea. For some countries like Sudan, Somalia and Eritrea, pig production is not highly developed leading to low risk of ASF; no outbreaks have been recorded in the countries.

Tanzania reported an outbreak in free-range domestic pigs in the Mbeya region in 2011. The 31 affected premises were all backyard farms around built-up areas and they probably used swill feed. Since then other cases have been reported in the central and coastal regions of the country. Uganda with the largest and fastest growing pig production in Eastern Africa has reported several outbreaks in the same period with the latest in Gulu District and neighbouring areas that has now claimed lives of at least 600 pigs since April, 2011.

The consequences of ASF outbreaks in many countries are catastrophic, with major economic losses in developing countries, and considerable social effects may result: the loss of employment for farm workers, the loss of a major source of income for farmers,

the loss of a major source of high quality and cheap protein for poor communities, and the consequences for traditional ceremonies (for which pigs are often required, as is seen in Cameroon and Côte d'Ivoire) (el Hicheri et al., 1998; Nana-Nukechap and Gibbs, 1985).

In Kenya outbreaks of ASF were reported in 1954 in Laikipia District, 1958 in Kiambu District, and in 1964 on a farm in Trans-Nzoia District (source: Kenya Department of Veterinary Services). In 1994 further outbreaks of ASF occurred after an absence of 30 years (O.I.E, 2001). From August 2001, 11 farms in Kiambu, Thika and Nairobi districts comprising a total of 9000 pigs were affected (source: Kenya Department of Veterinary Services). Outbreaks started again in May 2006 in Busia District. More outbreaks were experienced from November 2006 to early 2007 in Uasin Gishu, Nakuru, Kisumu, Nairobi and Kiambu districts (OIE disease report, February, 2007).

The Department of Veterinary Services relies mainly on passive surveillance of ASF (DVS, personal communication). In this system, information is gathered through disease outbreak reporting and the submission of diagnostic specimens to the Central Veterinary Laboratory in Nairobi by field veterinarians. The outbreaks reported in Kenya have never gone through a comprehensive outbreak investigation process that can generate information that is required for the development of disease control strategies in the country or the region.

Outbreak investigations can help identify the source of on-going outbreaks and prevent additional cases. Even when an outbreak is over, a thorough epidemiologic and environmental investigation increase knowledge of a disease and prevent future

outbreaks. However, in many outbreaks, the number of cases available for study is limited, and therefore, the statistical power of the investigation is limited. If detection of the outbreak is delayed, useful clinical and environmental samples may be very difficult or impossible to obtain.

This chapter reports case study of suspected ASF outbreaks in Western and Central Kenya reported between December 2010 and February 2011. The outbreaks were defined as sudden onset of a clinical disease resulting in unusually high mortality in pigs.

## **5.2 Objectives**

The main objective was to investigate origin of current outbreaks. The specific objective was to identify the potential sources to infection of the pigs and the causes.

## **5.3 Material and methods**

Outbreaks of ASF occurred in Busia, Kakamega, Kisumu, Kiambu and Thika Districts (Figure 3.2). An additional suspected outbreak was reported earlier on 15<sup>th</sup> September, 2010, in Kiambu District, but initial samples taken from the affected animals were negative according to the laboratory testing.

This study applied principles of outbreak investigations that have been well described (Kelsey et al., 1986; Lessard, 1988; Martin et al., 1987). These principles, briefly, involves examination of both affected and unaffected animals with the goal of identifying factors associated with the occurrence of disease. By following a series of systematic steps, an unbiased, organized assessment of the problem can be made, and the likelihood of understanding a disease outbreak is increased.



In the current study, samples were collected from animals in the affected farms for laboratory confirmation of disease. In addition, viruses associated with the recent outbreaks were compared with those that occurred between 2003 and 2008 in Kenya and Uganda (Fasina et al., 2010; Gallardo et al., 2009a).

### **5.3.1 Investigation procedure**

Investigations of the outbreaks were conducted sequentially as outlined below:

1. Study of case reports as presented by Veterinary Officers in the outbreak areas to confirm the existence of the outbreak;
2. Affected farms were visited and observations made on clinically ill pigs, apparently healthy pigs within the same herd, and dead pigs. The information was used to verify the clinical diagnosis and determine the aetiology of the disease;
3. Based on the field observations, a case definition was developed;
4. Using the case definition, cases were searched and data on the cases collected;
5. Conclusions on causal agent, mode of transmission, sources and population risk factors were drawn to explain the causes or the determinants of the outbreak based on clinical, laboratory, epidemiological, and environmental evidence.

### **5.3.2 Outbreak reports**

The study relied on primary information relayed from pig farmers to Veterinary Officers in the field to confirm the existence of an outbreak. The primary information indicated that pigs were dying in greater numbers than normal and within a short period of time. In all the sites studied for the outbreaks, the Field Veterinary Officers first conducted their

own investigation, then wrote reports and notified the Central Veterinary Laboratory in Kabete. The Director of Veterinary Services (DVS) upon receiving the reports gave permission for further studies of the outbreak.

### **5.3.3 Case definitions**

A suspected case was a domestic pig that manifested at least two of the following signs: dullness, inappetence, flushing of the skin, recumbence and death during the period of 22 December, 2010 to 31 December 2011.

A confirmed case was a suspected case that tested positive to ASF by indirect antibody ELISA, immunoblotting technique, and viral detection using the standard p72 PCR assay.

### **5.3.4 Data collection**

The affected areas were visited within one week of receiving permission for study and with the assistance of the District Veterinary Officers the farms were identified. Information collected included:

- 1) Identification: name of household owner, species affected and address of contact. This information was used to avoid duplication of enumerated cases and maintain communication with the farmers when more information was needed.
- 2) Demography: age and sex of animal and farm location. This information was used to describe the characteristics and distribution of cases.
- 3) Clinical signs: the symptoms and signs in pigs, the date of onset, the duration of disease and results of diagnostic procedures. These are the data that confirmed

true cases, provided the pattern of clinical manifestations, and also the distribution of cases by time.

4) Suspected risk factors: history of exposure to some factors before disease developed. The timing of interest was incubation period where aetiology was known or suspected. A question about contacts with other pigs with similar clinical symptoms was asked.

#### **5.3.4 Sample collection**

All live, dead and in-contact animals in the same household were sampled for blood and/or tissues using the approaches already described in Section 4.3.3. Necropsy was done on selected sick pigs or on pigs that died.

#### **5.3.5 Diagnosis**

The primary confirmatory diagnosis of ASF was made from blood and tissues (liver, spleen, brain, lymph node, heart and lung). Laboratory investigation on the samples was then performed including ASFV antibody detection as described in Section 4.3.4.1, virus detection and isolation as described in Section 4.3.4.2, and virus genetic characterization as described in Section 6.3.5.2 and histopathology.

#### **5.3.6 Spatial characterization of past ASF outbreaks**

ArcMap module in ArcGIS Desktop was used to create a map of districts in Kenya highlighting the districts that have reported ASF outbreaks in the 10 years starting from 2001.

### **5.3.7 Limitation of the outbreak investigations**

Response to outbreaks depended mainly on the information relayed from the Directorate of Veterinary Services before an active investigation was done. The process to investigation took one week to one month after initial outbreaks and the duration taken between the onsets of outbreaks to the time a definitive diagnosis of ASF was made took a maximum of 6 weeks. This meant that in some cases, e.g. in Kisumu and Thika, the number of animals sampled were few because by the time of investigation there were very few animals surviving the outbreak. In other cases dead animals had been disposed of which meant that carcasses were not available for conducting a thorough post-mortem examination. In this regard, comprehensive post-mortem examinations were done for animals found dead in Busia and Kakamega districts only. The other post-mortem reports recorded depended on the Field Veterinary Officer reported observations.

Following outbreaks in all the outbreak areas in this study, pig farmers sold their pigs in a rush to avoid losses. This resulted in very few in-contact animals being available for sampling to evaluate their carrier status.

Because of the level of biosecurity needed on-farm during the outbreaks and level of resources available, e.g., enough changes of personal protective equipment per day, it was not possible to visit many other farms after visiting an infected farm within a day. This meant that only a few farms were investigated at a time.

## **5.4 Results**

### **5.4.1 Outbreak reports**

A summary of data from outbreak reports by Veterinary Officers in affected districts is presented in Table 5.1. This documents the estimated population of pigs in each district, the number of pigs affected and number that died during the outbreak.

**Table 5.1: Summary of reported outbreak locations in Kenya between December 2010 and February 2011**

Reporting Date	Location		Total pigs at risk per location	Number of pigs affected	Number of dead pigs	Morbidity (%)	Mortality (%)
	District	Administrative location					
22/12/2010	Kisumu East	Kondele	1500	1000	800	67	53
23/12/2010	Kakamega	Municipality	200	200	182	100	91
18/01/2011	Busia	Bumala	655	655	590	100	90
28/01/2010	Kiambu	Municipality	10000	11	6	0.01	0.01
30/01/2011	Thika	Makongeni	200	100	100	50	50

## **5.4.2 Primary outbreak investigation in Kakamega District**

### **5.4.2.1 Characteristics of affected farms**

Two farms that reported ASF outbreaks were investigated. One farm kept free ranging pigs and in the other the pigs were confined. The pigs affected in the village with free-ranging feeding had 150 pigs and the farm with animals confined in sties had 50 pigs. The confined animals were fed swill from hotels in Kakamega town.

The Morbidity rates based on farmer observations on clinical signs was 100% in both farms with mortality of 99% in free-range pigs and 68% in confined pigs. All ages and sexes were affected.

### **5.4.2.2 Clinical signs and necropsy findings**

The main clinical signs observed by farmers and veterinary Officers in the affected animals were a sudden onset of disease manifesting as dullness, anorexia and a very high body temperature of 42<sup>o</sup>C. These initial clinical signs were followed by dyspnoea, ataxia, frothing at the mouth, recumbency and death. Duration of signs lasted 24-48 hours prior to death.

Post mortem findings by investigator revealed carcasses in generally good body condition, but with hyperaemic skin containing visible ecchymotic haemorrhages, ascitis, petechiation/ecchymosis of small intestines, stomach, liver and kidneys, hydrothorax, congested liver and lungs and froth in the trachea. The most significant gross change was haemorrhage in several organs.

#### **5.4.2.3 Laboratory investigation**

Tissues (liver, spleen, brain, lymph node, heart and lung) from 2 necropsies and 3 sera were confirmed as positive for ASF. Laboratory test results for outbreak samples are presented in Table 5.2.

#### **5.4.2.4 Potential risk factors**

Free-ranging and scavenging pigs were considered to be at risk because they may have eaten ASFV-contaminated feed, faeces, or could have come into direct contact with infected pigs.

The confined pigs were reportedly fed swill from hotels in town, which may have been contaminated with ASFV-infected pork products.



**Table 5.2: Laboratory test results from African swine fever outbreak samples in Kakamega District in 2010**

<b>Diagnostic test</b>	<b>Number of samples tested</b>	<b>Number positive</b>	<b>Percent Positive</b>
Immunoblotting	12	1	8
INGENASA-ELISA	12	1	8
OIE-ELISA	12	1	8
PCR	31	9	29
Virus isolation	20	3	15

### **5.4.3 Primary Outbreak investigation in Kisumu District**

#### **5.4.3.1 Characteristics of affected farms**

The pigs affected in Kondele, Kisumu, were mainly free-ranging pigs that scavenged for food. A slaughter slab for pigs was located in the same area and sourced pigs from other locations outside the district for slaughter. Farms with confined pigs were not affected.

An estimate of the pig population at risk was 1500, out of which 1000 were free-ranging and 500 confined. The morbidity rate in free ranging pigs was based on farmers' observation of clinical signs and was estimated at 67% and the mortality was 53%. All ages and sexes were affected.

#### **5.4.3.2 Clinical signs and necropsy findings**

The main signs observed by the farmers in the affected animals were a sudden onset of dullness and anorexia followed by recumbency and death within 24-48 hours. Pregnant sows that were affected were reported to have aborted.

Necropsy findings by investigator revealed carcasses in good general body condition, hyperaemic skin with visible haemorrhages, ascitis, petechiation/ecchymosis of small intestines, stomach, liver and kidneys, hydrothorax, congested liver, renal lymph nodes and lungs and froth in the trachea.

#### **5.4.3.3 Laboratory investigation**

A total of 7 pigs were sampled for blood and tissues. Of the 7, tissues (liver, spleen, brain, lymph node, heart and lung) from 3 animals were confirmed positive for ASFV by p72-PCR and virus isolation and, 3 out of the remaining 4 were confirmed positive by

indirect antibody ELISA and immunoblotting on sera. Laboratory test results for outbreak samples are presented in Table 5.3.

#### **5.4.3.4 Potential risk factors**

The first case of ASF was discovered reportedly in a slaughter slab in Kondele through a post-mortem examination. Ante mortem examination showed no signs on this animal. Free-ranging and scavenging pigs could have had prior contact with the affected pig which had been sourced earlier from the neighbouring Bondo District. The affected pigs may have also fed on infected pork waste from the Kondele slaughter slab. Since the pigs roamed freely, healthy pigs could also have come into contact with infected pigs leading to further spread. The infected pigs did not come into contact with any confined pigs, and no cases of ASF were reported in this group of pigs.

**Table 5.3: Laboratory test results from African swine fever outbreak samples in Kisumu District in 2010**

<b>Diagnostic test</b>	<b>Number of samples tested</b>	<b>Number positive</b>	<b>Percent Positive</b>
Immunoblotting	4	3	75
INGENASA-ELISA	3	3	100
OIE-ELISA	3	3	100
PCR	29	27	93
Virus isolation	28	26	93

#### **5.4.4 Primary outbreak investigation in Busia District**

##### **5.4.4.1 Characteristics of affected farms**

The pigs affected in Bumala Division, Busia District were mainly free-ranging animals.

An estimate of the pig population at risk was 655 and all were free-ranging. A morbidity rate of 100% and mortality rate 90% were estimated based on farmer observation. Because most of the pigs were free-roaming, the exact percentage of dead pigs could not be accurately quantified.

The farm investigated had a pig herd of 15 (comprising 1 breeding female, 2 piglets each 4 months old, 2 piglets each 3 months old and 10 were 2 month old). The farm was located next to a slaughter slab and practiced free-range pig management system. Morbidity within this herd was 100% with mortality of 60% based on farmer observation.

##### **5.4.4.2 Clinical signs and necropsy findings**

The main signs observed by the farmer, in the affected animals, were a sudden onset of dullness and anorexia followed by recumbency and death in two days, consistent with observations in the cases in Kisumu and Kakamega. The investigator examined one clinically sick animal found on the affected farm and the following signs were observed: high body temperature of 42<sup>0</sup>C, dyspnoea, ataxia, frothing in the mouth, and recumbency. The animals died 24-48 hours after onset of clinical signs.

Necropsy was performed on 2 piglets found dead on the farm and tissue samples were also taken. One piglet, representing one of a litter of 10 that were born approximately 2 months prior to the outbreak had the following gross description:

1. The piglet was a 4.5 kg, black and white, female, 2 months old, of mixed breed in fair to good body condition with mild autolysis. A small amount of soft, dark brown faeces adhered to the skin of the perineum. A small amount of light tan foamy fluid oozed from both nostrils.
2. The abdomen contained approximately 10.0 ml of dark red watery fluid. Bilaterally, the peri-renal lymph nodes were diffusely dark red and a small amount of dark red fluid oozed out on cut section (this was presumed to originate from a haemorrhage). The prescapular, cranial, cervical and mediastinal lymph nodes contained variably sized, irregular, poorly demarcated dark red areas that encompassed up to three quarters of the lymph node. This change was predominantly confined to the medulla on section.
3. The stomach was empty. Within the fundic mucosa were approximately ten well demarcated, dark red, round to linear depressions with sharp edges, the largest of which measured 0.6 cm in diameter and 2.0 cm x 0.3 cm (ulcers). The small and large intestine contained a moderate amount of soft to watery grey-green digesta. The spleen was normal in size, shape and consistency.
4. Within the subcutaneous tissue of the right thorax between the 4<sup>th</sup> to 8<sup>th</sup> ribs were approximately one dozen, well demarcated, round to irregular dark red areas (haemorrhage).

The main gross findings were:

1. Peri-renal Lymph nodes had acute, and severe presumptive haemorrhage,

2. Prescapular, cranial cervical and mediastinal lymph nodes had acute, multifocal and mild to moderate presumptive haemorrhage,
3. Stomach, fundus had acute, multifocal, mild mucosal ulceration,
4. Subcutis, right thorax had acute, multifocal, mild haemorrhage.

The second piglet had the following gross description following necropsy:

1. The piglet was a 5.0 kg, 2 month old, female, mixed breed in good body condition with mild autolysis. Attached to the outside of the lower right lip and the inside of both pinnae were approximately one dozen black ticks. There were numerous hair fleas throughout the hair coat.
2. The gastro-splenic and peri-renal lymph nodes were diffusely dark black and oozed a moderate amount of red-black fluid on section (haemorrhage). The mesenteric lymph nodes contained dozens of irregular, poorly demarcated, dark red area ranging in size from 0.2 cm to 0.5 cm diameter. This was again suggestive of haemorrhage.
3. Over the surface of all liver lobes were approximately ten, poorly demarcated, white, radiating, slightly sunken areas (capsular fibrosis). The stomach contained a moderate amount of green-brown, fibrous ingesta. The small and large intestine contained a moderate amount of grey-green, watery to pasty digesta.
4. Within a primary bronchus of the right lung lobe was a single white worm approximately 1.2 cm in length. This was probably a larval ascarid.

The main findings were:

- The gastro-splenic and peri-renal lymph nodes had acute, diffuse and severe haemorrhage.
- The mesenteric lymph node had acute, multifocal and moderate haemorrhage (presumptive).
- The liver exhibited capsular, chronic, multifocal and mild fibrosis
- Lungs had a mild ascarid nematode infection.
- The Body had a mild infestation with ectoparasites.

#### **5.4.4.3 Laboratory investigation**

All the tissues collected (liver, spleen, brain, lymph node, heart and lung) from the 2 necropsies were confirmed to be ASF positive by p72-PCR. All 4 sera collected from surviving pigs were negative for ASF by indirect antibody ELISA; the duration post infection before sample collection was not clear. The sera were, however, positive for ASFV by virus detection using p72-PCR. Laboratory test results for outbreak samples are presented in Table 5.4.

#### **5.4.4.4 Potential risk factors**

The pigs were, generally, free roaming and had contact with other village roaming pigs (around Bumala market) that were all reported to have died. In addition, a pig slaughter slab was located across a main highway from Busia to Kisumu on the opposite side of the affected farm, approximately 200 m from the farm. Disposal of slaughter waste was in a pit that was not secured and could be a source of contaminated waste. Dogs, humans and other stock could freely pass from the slaughter area to this farm.



**Table 5.4: Laboratory test results from African swine fever outbreak samples in Busia District in 2011**

<b>Diagnostic test</b>	<b>Number of samples tested</b>	<b>Number positive</b>	<b>Percent Positive</b>
Immunoblotting	6	0	0
INGENASA-ELISA	6	0	0
OIE-ELISA	6	0	0
PCR	28	22	79
Virus isolation	22	0	0

## **5.4.5 Primary Outbreak investigation in Kiambu District**

### **5.4.5.1 Characteristics of affected farms**

Primary ASF outbreak investigations were performed in Kiambu District, Municipality Division. The pigs affected in the peri-urban area of Kiambu town were confined and stall-fed. The pigs were fed commercial feeds. An estimate of the pig population at risk in the district was 10, 000.

Two farms were investigated one with 5 pigs and the other with 6 pigs. Morbidity was 100% (5/5 and 6/6) and mortality 55% (3/5) and 30% (3/6) in both farms, respectively, based on farmer observation.

### **5.4.5.2 Clinical signs and necropsy findings**

The main signs observed by farmers in the affected animals were a sudden onset of symptoms comprising reddening of the skin, dullness and anorexia. These signs were followed by recumbence and death in 24-48 hours.

Post mortems were not performed because the dead animals had been disposed of by the time of investigation. Further analysis relied on samples collected by the local Veterinary Officer 6 days before the investigation and samples collected from surviving pigs by the investigator.

### **5.4.5.3 Laboratory investigation**

A pig serum sampled from one farm and 2 pigs sera sampled from a second farm were sero-negative by indirect antibody ELISA, but positive for ASFV by virus detection using p72-PCR. Laboratory test results for outbreak samples are presented in Table 5.5.

#### 5.4.5.4 Potential risk factors

The results from the preliminary investigation were consistent with the conclusion that the outbreak was caused by ASFV. A new pig had recently (a week prior to the onset of disease) been brought into the first farm, while a sow in a second farm had been taken out to a neighbouring farm for mating. Both of these animals, the newly imported pig and the impregnate sow died. This was followed by other subsequent deaths among the in-contact pigs.

**Table 5.5: Laboratory test results from African swine fever outbreak samples in Kiambu District in 2010**

<b>Diagnostic test</b>	<b>Number of samples tested</b>	<b>Number positive</b>	<b>Percent Positive</b>
Immunoblotting	3	0	0
INGENASA-ELISA	3	0	0
OIE-ELISA	3	0	0
PCR	8	5	63
Virus isolation	5	0	0

## **5.4.6 Primary Outbreak investigation in Thika District**

### **5.4.6.1 Characteristics of affected farms**

Primary ASF outbreak investigations were performed in 2 peri-urban informal settlements around Thika Town. The pigs affected represented both free-ranging and confined animals. The areas affected were the Makongeni and Sewage sub-urban informal settlements.

### **5.4.6.2 Clinical signs and necropsy findings**

The main signs observed in the affected animals were a sudden onset of signs comprising reddening of skin, dullness and anorexia followed by recumbency and death in 24-48 hours. Morbidity of 50% (100/200) was reported in the farms investigated with fatality rate of 100% (100/100), based on farmers' reports.

Post mortem findings from 1 dead pig revealed a carcass with a good general body condition, hyperaemic skin with visible ecchymotic haemorrhages of the skin, ascitis, petechiation/ecchymosis of serosal surfaces of small intestines, stomach, liver and kidneys, hydrothorax, congested liver and lungs and froth in the trachea.

### **5.4.6.3 Laboratory investigation**

Tissues (liver, spleen, brain, lymph node, heart and lung) that were sampled for virology from a pig necropsy in Sewage area were positive for ASFV by virus detection using p72-PCR. Blood from 6 pigs (3 from Makongeni and 3 from Sewage locations) each comprised two that were positive for ASFV by virus detection using PCR, but sera from the same pigs were negative by indirect antibody ELISA. Laboratory test results for outbreak samples are presented in Table 5.6.

#### **5.4.6.4 Potential risk factors**

The farms were close to a Thika Town Council garbage dumping site located adjacent to a shrubby range area. Wild pigs (bush pigs and warthogs) were reported to frequent the garbage site where the roaming domestic pigs also fed on dumped waste. A number of pigs were sold to a slaughter house in the nearby Muranga District, due to fear of them dying and the farmers thereby incurring losses. This posed a risk for additional spread of the disease.

**Table 5.6: Laboratory test results from African swine fever outbreak samples in**

**Thika District in 2011**

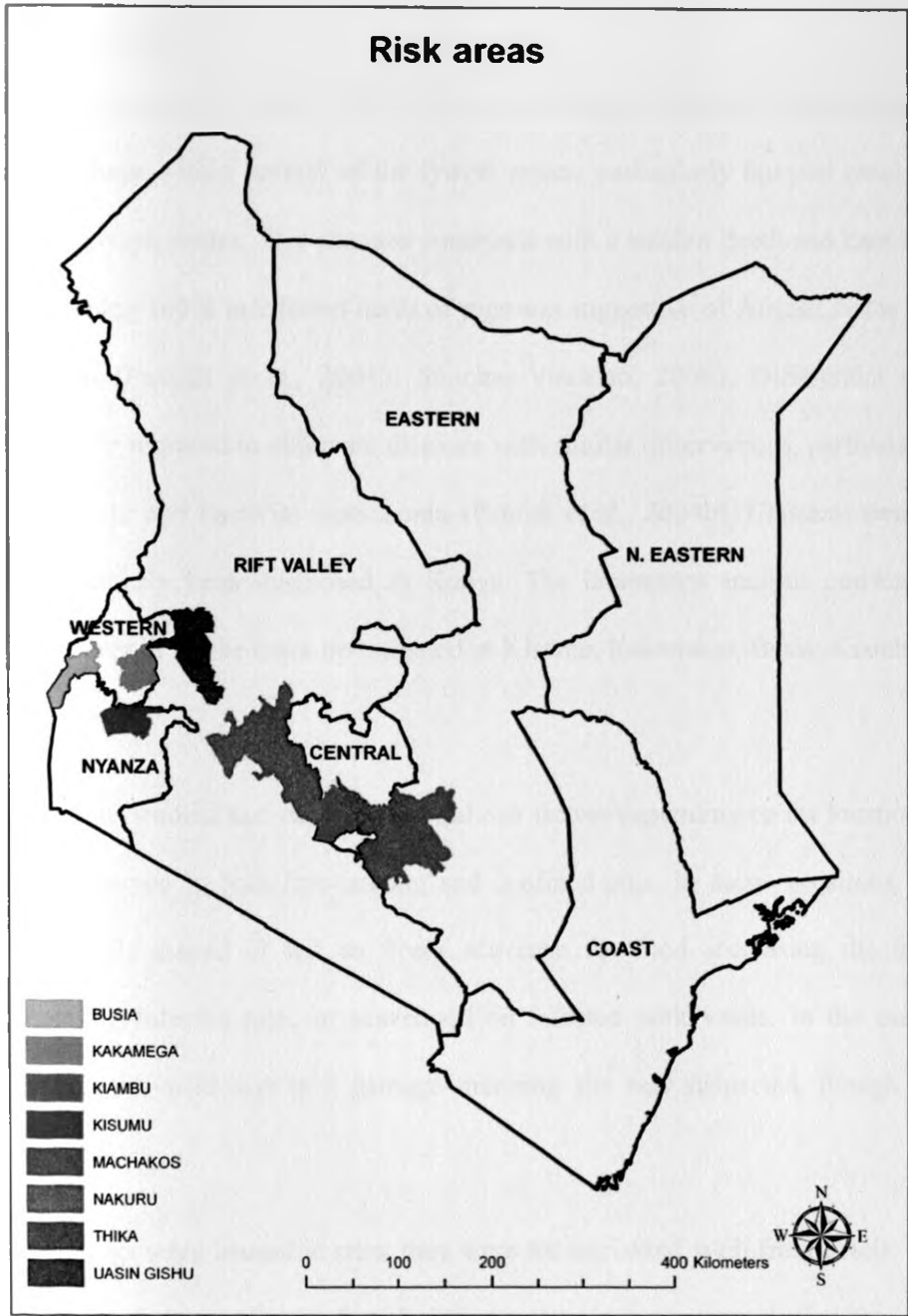
<b>Diagnostic test</b>	<b>Number of samples tested</b>	<b>Number positive</b>	<b>Percent Positive</b>
Immunoblotting	5	0	0
INGENASA-ELISA	5	0	0
OIE-ELISA	5	0	0
PCR	16	11	69
Virus isolation	11	0	0

#### **5.4.7 African swine fever risk areas**

An outbreaks risk map for Western and central Kenya was prepared using information on outbreaks during the last ten years starting in 2001. These areas are presented in Figure 5.1. The districts affected were Busia, Kakamega, Kisumu, Uasin Gishu, Nakuru, Kiambu and Thika districts. These locations are clustered in areas with high pig densities (Figure 2.1) in Kenya (KNBS, 2009) and spatially correlate with locations of major highways and urban centres in Kenya.







**Figure 5.1: A map showing documented ASF outbreak districts in Kenya**

*Source: International Livestock Research Institute*

## 5.5 Discussion

The most prominent feature observed at post mortem in the pigs in the case studies was haemorrhage within several of the lymph nodes, particularly the peri-renal and gastro-hepatic lymph nodes. This changes combined with a sudden death and case fatality rates approaching 100% in affected herds of pigs was suggestive of African swine fever (ASF) infection (Penrith et al., 2004b; Sánchez-Vizcaíno, 2006). Differential diagnosis is potentially required to eliminate diseases with similar observations, particularly classical swine fever and bacterial septicaemia (Penrith et al., 2004b). Classical swine fever has not previously been diagnosed in Kenya. The laboratory analysis confirmed African swine fever in all the cases investigated in Kisumu, Kakamega, Busia, Kiambu and Thika Districts.

The farms studied had various potential risk factors depending on the location; outbreaks were observed in both free-ranging and confined pigs. In some situations, the animals were freely-grazed or left to freely scavenge for food increasing the likelihood of contacting infected pigs, or scavenged on infected pork waste. In the case of Thika, contact with wild pigs at a garbage dumping site was suspected, though not directly observed.

Where pigs were housed in sties, they were fed uncooked swill from hotels, which could contain pork wastes from infected pigs. An observation was made that farmers sold sick pigs to butchers to avoid incurring losses due to ASF. Pork from these pigs could end up as swill for feeding other healthy pigs.

Most of the pigs consumed in the affected areas were from slaughter slabs from within the localities, with the implication that infected pork products may have ended up in household/restaurant food and subsequently transmitted to other pigs in the swill.

There were no biosecurity practices, e.g. foot baths, observed in the affected farms and thus workers in the farms and visitors may have carried ASFV to the farms.

One farm in Bumala, Busia was situated next to a slaughter slab and also had free roaming pigs making it possible for the animal to come into contact with infected tissues which were not disposed of in a biosecure manner. The disposal pits were easily accessible. In addition, all the slaughter slabs observed in the study were unfenced and thus dogs and other animal could not be prevented from contacting infected waste tissues or condemned carcasses.

Moving pigs between farms for restocking or mating presents a risk of introduction of new cases into clean farms. This was clearly observed in the Kiambu District outbreaks.

The rapid spread of the virus among pigs could indicate that the disease may have been maintained in the border districts either in contaminated pork products (given that feeding of swill and pork products was incriminated in the outbreak farms), or in live carriers pigs that survived the outbreaks before. The swine fever virus is highly contagious, and spreads rapidly in pig populations by direct or indirect contact (Costard et al., 2009b). This DNA virus is highly stable and persists for long periods of up to three months in pig products and the environment (Penrith et al., 2004b).

Outbreaks risk for Western and Central Kenya in the last ten years is found in Busia, Kakamega, Kisumu, Uasin Gishu, Nakuru, Kiambu and Thika districts. These locations are clustered in areas with high pig densities in Kenya (KNBS, 2009). This result shows the potential risk that ASF poses on pig production and productivity, and that outbreak poses major risk to a big population of pigs. The outbreaks observed here resulted in immense economic losses that was observed in the entire pig value chain. Farmers, pig traders and butchers were all affected.

The confirmation of suspected ASF outbreaks took more than two weeks in all the areas studied. The delay in the response to outbreaks by the department of Veterinary services was due to the more time needed by Field Veterinary Officers to investigate, sample pigs and transport of the samples to the Central Veterinary Laboratory in Kabete, Nairobi. The submitted samples were forwarded to the International Livestock Research Institute for shipments facilitation to the ASF reference laboratory in Spain. Quick response to outbreaks, including putting in place quarantine measures, requires quick recognition of the disease. Rapid approaches for the diagnosis of the disease are needed.

There was detection of antibodies in pigs sampled in Kisumu and Kakamega and not in pigs in the rest of outbreak areas. Pigs that survive natural infection usually develop antibodies against ASFV from 7–10 days post-infection and these antibodies persist for long periods of time (Arias et al., 1993). Where the disease is endemic, or where a primary outbreak is caused by a strain of low virulence, the investigation of new outbreaks should, therefore, include the detection of specific antibodies in serum or extracts of the tissues submitted. However, in case of an early infection, the antibodies

may not be detected, and this could explain the reason for lack of antibody detection in some pigs from outbreak areas in this study.

CHARACTERIZATION OF AFRICAN SWINE  
FEVER VIRUS

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## CHAPTER 6

# MOLECULAR CHARACTERIZATION OF AFRICAN SWINE FEVER VIRUSES IN KENYA

### 6.1 Introduction

There have been several officially documented outbreaks of ASF in Kenya the most recent being in 2006-2007 and in 2010 to 2011. The later outbreaks were discussed in Chapter 5. The Department of Veterinary Services reported suspected outbreaks of African swine fever in Central, Rift valley, Nyanza, and Western provinces of the country to the OIE in December 2006 and May 2007. A common feature of the affected pigs was that they ranged freely in search of food. The specific outbreak locations were in Kiambu, Eldoret, Nakuru, Kisumu and Busia districts and affected 1,011 out of an estimated 13,601 pigs out of which 630 died. The EU-OIE reference laboratory at CISA-INIA, Spain, tested and confirmed samples from the affected areas as positive for ASFV (Gallardo et al., 2009a) and February, 2007 and March 2011 OIE Disease Reports

The epidemiology of ASF in East Africa is complex. Not only is there evidence for a sylvatic cycle, but a domestic pig cycle and a pig-tick cycle have also been described (Haresnape, 1984; Penrith et al., 2005). The presence of all three cycles requires molecular characterization of the ASFV strains. This would assist in clarifying whether one or more viruses are circulating and to distinguish viruses causing outbreaks that are geographically or temporally related. This would further allow the description of country- or zone-specific epidemiological patterns related to vertebrate and arthropod hosts and leading to different virulence or pathogenic effects. Molecular characterization can point

to the direction of source of infection or relationship between disease outbreaks (Pan et al., 1988; Wesley and Tuthill, 1984; Whyard et al., 1985).

Numerous field isolates of diverse species were characterized using partial p72 nucleotide sequences of ASFV (Bastos et al., 2003), as well as distinct temporal and geographical origins, in order to clarify genetic relationships of ASFV. This study used a similar approach.

This chapter presents genetic characterization of viruses in this study.

## **6.2 Objectives of the study**

The specific objectives were to: 1) conduct an analysis of the genotypes of viruses in domestic and wild pigs in Kenya; 2) Investigate associations between the viruses isolated in different locations in Kenya.

## **6.3 Materials and methods**

### **6.3.1 Study areas**

The study was conducted in Busia, Kisumu, Kakamega, Homabay, Thika, Machakos, Kajiado and Kiambu Districts, Ruma National park and Kapiti Plains Ranch as described and displayed in Chapter 3. Homabay District was selected because it represents a predominantly free-range smallholder pig production system and lies in close proximity to the Ruma National Park that has a population of bush pigs (*Potamochoerus spp*) (Plate 6.1). The exact number of wild suids in the park was difficult to quantify, but was conservatively estimated at 100.

Kajiado, Machakos and Kiambu districts are characterized by predominantly stall-feeding pig production system. Kiambu District was one of the focal areas for the ASF outbreaks

witnessed in Kenya between 2006 and 2007. The district hosts 2 main slaughter houses in the country, Farmers choice and Ndumboini that receive pigs from many parts of the country. The transportation of pigs to these abattoirs was suspected to be responsible for some of the outbreaks that occurred in the district.

Kapiti Plains Ranch (described in Chapter 4) hosts a population of warthogs (that was estimated at 300) and is located in the central region of the country. The other districts (Kisumu, Kakamega, Busia and Thika) in this study were those where outbreaks occurred and specific outbreak investigations were conducted as described in the previous chapter.





**Plate 6.1: Bush pig (*Potamochoerus larvatus*) captured in Ruma National Park in 2008.**

*(Source: Edward Okoth)*

### **6.3.2 Selection of pig farming households**

With the help of the District Veterinary Officers from Department of Veterinary Services of the Ministry of Livestock Development, a list of pig farming households was obtained. Pig farms within each division in Homabay and Kiambu Districts were selected on a random basis. A total of 81 farms were selected in Homabay and 40 in the central region of Kenya.

Where outbreaks occurred, DVS staff alerted the investigator and helped in identifying the affected farms for investigation.

### **6.3.3 Spatial information**

Pig farming households were geo-referenced using Garmin Global Positioning System (GPS) 12XL unit (Garmin Olathe, KS, USA). The GPS data were downloaded using Ozi Explorer and exported into ArcGIS® 9.3 (ESRI, California, USA) for analysis. The GPS data were used to estimate distances from locations with wild pig populations needed for spatial characterization and statistical modeling of ASF risk. Accordingly distances (Km) from pig farming households to the park was calculated and used in regression model.

### **6.3.4 Animal sampling**

Ticks, domestic and bush pigs were sampled according to the methods described in Chapters 3 and 4. Briefly, the ticks were collected from warthog burrows and stored for analysis. Pigs and warthogs were sampled for blood and serum and one animal from each species was sacrificed for necropsy and tissue sampling. All samples were stored at -20°C before laboratory analysis.

A severe haemorrhagic disease in domestic pigs was reported in December 2010 by District Veterinary Officers in Kisumu Central and Kakamega Central Districts in western Kenya. Following these reports, an investigation was conducted as described in Chapter 5. Using ASF OIE-prescribed diagnostic procedures, analysis of the samples collected from the outbreak farms confirmed that the animals were infected with ASFV. These samples were also used in the virus characterization study. These were performed on isolated viruses when possible or on infected tissues when virus isolation was unsuccessful.

### **6.3.5 African swine fever molecular characterization**

#### **6.3.5.1 Virus isolates**

The ASFV characterization was done on the following samples:

1. Random virus isolates from samples collected from the Farmers Choice slaughter house, Central Kenya, in 2005;
2. Virus isolates from ticks collected from Kapiti Plains Ranch in 2008 and 2009. The ticks were used as proxy for infections in young warthogs.
3. Virus in blood samples from two domestic pigs in Homabay District;
4. Virus in kidney tissue from a bush pig in Homabay District;
5. Virus isolates from ASF outbreaks in Kiambu, Thika, Busia, Kakamega and Kisumu Districts that occurred between the months of November 2010 to February 2011. Three haemadsorbent ASFV isolates from Kisumu

(Ken10/Kis027 and Ken10/Kis028) and Kakamega (Ken10/KAKFA1) were selected for ASFV genotyping purposes.

#### **6.3.5.2 Virus characterization**

Viruses isolated were characterised at CISA-INIA using molecular techniques as described before (Boshoff et al., 2007). A two-step genetic characterization approach was used in which initially, P72 and P54 gene sequencing was used to delineate genotypes. This was followed by higher resolution dissection of viral relationships by central variable region characterisation of the 9RL ORF (locus B602L). Viruses isolated from previous outbreaks in Kenya (Gallardo et al., 2009b) were compared with viruses isolated in the current study.

#### **6.3.5.3 Polymerase Chain Reaction and nucleotide sequencing**

For genetic characterization, PCR was performed on nucleic acid extracted from selected ASFV positive samples using specific primers to amplify three independent regions from the ASFV genome; i) 478 base pairs within the 3' end of p72 gene were amplified using primers p72-U/D (Bastos et al., 2003); ii) The full length p54-gene encoding the VP54 protein was amplified using the primers PPA89/722 (Gallardo et al., 2009a); and iii) 485 base pairs of central variable region (CVR) within B602L gene located between positions 145413 and 145964 on the reference ASFV genome (Chapman et al., 2008; de Villiers et al., 2010), was amplified using primers CVR1 (5' ACTTTGAAACAGGAAAC(AT)AATGATG 3', binding site 145342–145364) and CVR2 (5' ATATTTTGTAATATGTGGGCTGCTG 3' binding site 145997–146017), previously described (Gallardo et al., 2011b). Primer binding sites and the predicted product size of B602L gene were based on the Kenya 1950 ASFV genome (Accession

No. AY261360). Amplicons of the expected size were excised and purified by Quiaex gel extraction (QUIAGEN) and cloned into a pGMT easy vector according the manufacturer's instructions. The nucleotide sequence of the purified products was determined using primers specific for the pGMT vector (SP6/T7) using an automated 3730 DNA analyzer (Applied Biosystems). The Homabay bush pig and domestic pig sequences were submitted to GenBank.

#### **6.3.5.4 Data Analysis**

Analysis of sequence data was performed with Chromas ([www.technelysium.com.au](http://www.technelysium.com.au)), BioEdit ([www.mbio.ncsu.edu/BioEdit/BioEdit.html](http://www.mbio.ncsu.edu/BioEdit/BioEdit.html)), and ClustalX version 1.83 ([www.clustal.org](http://www.clustal.org)). For the tandemly repeated amino acid sequences (TRS) analyses within the CVR nucleotide sequence, deduced amino acid sequences were manually aligned with gaps being inserted to optimize the alignment. Two different datasets were employed in this study for phylogenetic analyses conducted using MEGA version 4.0 (Kumar et al., 2001): i) A p72- gene dataset comprising 279 taxa in which sequences generated in this study from Kenyan viruses were compared with ASFV sequences available in GenBank; and ii) p54 sequences corresponding to Kenya ASFV viruses characterized in this study compared with a dataset comprising 114 taxa with ASFV p54 gene sequences available in GeneBank. Neighbor joining (NJ) and minimum evolution (ME) p72 and p54 trees were constructed employing the p-distance nucleotide substitution model as implemented in the MEGA v4.0 program. The robustness of the ME tree was tested using the Close-Neighbor-Interchange (CNI) algorithm at a search level of 1. To determine the degree of statistical support for each node in the resulting trees, data were re-sampled 1000 times using the bootstrap method.

## **6.4 Results**

### **6.4.1 Virus isolates**

The Kenyan isolates selected for genotyping after virus isolation are presented in Appendix 3. The table also shows the geographical location and host/reservoir origin of the isolates. No viral isolate was obtained from serum and blood of ASF sero-positive adult warthogs; however genotypes were obtained by PCR amplification of three genes, p72, p54, and the region encoding the central variable region (CVL) of locus B602L from the blood of adult warthogs. The viruses characterized were 2 from slaughter house pigs, 3 from warthogs, 10 from ticks, 3 from free-range pigs and 3 from outbreaks in domestic pigs (Appendix 3).

### **6.4.2 Genetic characterization of viruses of domestic pigs and bush pigs in Homabay District**

Phylogenetic trees based on the 3' end of the gene encoding the C-terminal end of the p72 protein and the full length p54-gene of 3 Kenyan ASFV viruses characterized in this study illustrating the genetic relationship with other ASF viruses belonging to ASFV p72 and p54 genotypes X is shown in Figure 6.2. The trees of 279 (p72) and 114 (p54) taxa were inferred using the Minimum Evolution (ME) method following initial application of a Neighbor-Joining algorithm. The percent replicate trees, in which the associated taxa clustered together by bootstrap analysis (1000 replicates), is shown adjacent to the nodes.

Phylogenetic sub-trees based on the 3' end of the p72 gene and the full length p54-gene of 3 Kenyan ASFV viruses characterized in this study showed that the domestic pig (Ken08DP/Ndhiwa and Ken08DP/Nyarongi) and bush pig (Ken08BP/HB) virus isolates clustered together suggesting a possible association of infections between the two species

in south-west Kenya (Figure 6.1). The tree derived from the 3' end of the p72 gene showed that these viruses clustered together with other isolates from Kenya, and also Tanzania, Burundi, Uganda and Zambia as shown in Table 6.1. In addition, the viruses clustering with the Kenyan isolates in this study originated from hosts/reservoirs that included warthogs, ticks and domestic pigs (Table 6.1). The full p54-gene characterization showed the domestic and bush pig isolates clustered together with a *Sus scrofa* isolate from Burundi. These results suggested a possible association of tick, domestic and wild pig infections (i.e., both sylvatic and domestic cycles) in this region.

Amino acid sequencing of the tetrameric repeats that constitute the central variable region (CVR) of the B602L gene identified in viruses belonging to CVR subgroup XXVI previously described (Nix et al., 2006), was also performed. Sequences generated in this study indicated amino acid sequence homology between the domestic and bush pigs of the CVR encoded within the B602L ASFV gene from Homabay District. However, the amino acid repeat sequence was divergent from the other isolates from Burundi and Tanzania that are within same p72 cluster (Table 6.2). This suggested divergence by mutation at the B602L locus of the South-west Kenyan virus isolates subsequent to their separation from the Burundi and Tanzania isolates.

#### **6.4.3 Molecular Characterization of viruses isolated from domestic pigs, warthogs and ticks in Central Kenya**

Phylogenetic sub-trees based on the 3' end of gene encoding the p72 protein of 20 Kenyan ASFV viruses isolates from ticks and 12 virus isolates from domestic pigs sampled from the Central region of Kenya formed 3 main clusters (Figure 6.2) within p72 genotype X. Of the 3 clusters, 2 were composed mainly of tick virus isolates from Kapiti

Plains Ranch, and 1 cluster was composed of both the tick isolates and domestic pig 2005 virus isolates from the Farmers Choice slaughterhouse in Kiambu District in 2005. Three viruses from adult warthogs that were genotyped from blood samples from Kapiti were of genotype IX.

Phylogenetic trees based on full length p54-gene of the 32 Kenyan ASFV viruses from central Kenya clustered into two main subgroups, one having a mixture of domestic pig and tick ASFV isolates and the other containing only tick ASFV isolates (Figure 6.2).

The viruses from Central Kenya did not cluster with other regional isolates used for comparisons in this study (Figure 6.2).

The results indicated that some ASFV isolates from ticks and warthogs were closely related to ASFV isolated from infected domestic pigs from the same general locality. The three adult Kapiti warthogs (Ken08WH/4, Ken08WH/5 and Ken08WH/8) were different and were classified in genotype IX according to p72 and corresponded to the virus that caused the outbreaks in 2006-2007 and 2010-2011 (Appendix 3).

#### **6.4.4 Characterization of virus associated with recent ASF outbreaks in Kenya**

The Kenyan ASF viruses associated with the 2010-2011 outbreaks exhibited 100% sequence identity both among one another and with the previously characterized viruses obtained from outbreaks in Uganda and Kenya in 2006 and 2007 falling within the domestic pig-associated p72 and p54 genotype IX (Figure 6.2).

In an attempt to determine the source of the outbreak at higher resolution, the CVR sequences within the B602L gene of viruses from Kenya 2010 was determined (Gallardo



et al., 2009b). The type of aminoacid tetramer repeats (AAABNABBNABBaaBBNABNaBA) identified in the Kisumu, Kakamega, Busia, Kiambu and Thika isolates was related to CVR subgroup XXIV (Gallardo et al., 2009b) that is also present in isolates associated with earlier outbreaks in Uganda and Kenya. However, differences were identified in the number of tetrameric amino acid repeats. When the tetrameric repeats within the B602L gene from the 2010-2011 Kenyan outbreak viruses were compared with viruses included within the CVR sub-group XXIV, viruses characterized showed a minor change in the insert of a single internally located tetrameric repeat (CADT) which was present in viruses obtained during the second wave of outbreaks that occurred in Western and Central Kenya in late 2006 and 2007 and in Uganda in 2007.

**Table 6.1: African swine fever virus isolates from domestic pigs and bush pigs (highlighted) from Homabay District and other regional isolates present within the same p72 gene cluster**

Isolate name	Origin	Date	Host species	p72 genotype	p72 gene GenBank accession number
BUR/1/84	Muyinza, Burundi	1990	<i>Sus scrofa</i>	X	AY351
BUR/2/84	Muyinza, Burundi	1990	<i>Sus scrofa</i>	X	AY351
BUR/90/1	Muyinza, Burundi	1990	<i>Sus scrofa</i>	X	AY351
BUR90/3	Muyinza, Burundi	1990	<i>Sus scrofa</i>	X	AY351525
KAB94/1	Zambia	1983	<i>Ornithodoros spp</i>	X	AY351
<b>Ken08BP/HB</b>	<b>Homabay, Kenya</b>	<b>2008</b>	<b><i>Potamochoerus larvatus</i></b>	<b>X</b>	JN590917
<b>Ken08DP/Ndhiwa</b>	<b>Homabay, Kenya</b>	<b>2008</b>	<b><i>Sus scrofa</i></b>	<b>X</b>	JN590918
<b>Ken08DP/Nyarongi</b>	<b>Homabay, Kenya</b>	<b>2008</b>	<b><i>Sus scrofa</i></b>	<b>X</b>	JN590919
Kilean/2	Nanyuki, Kenya	1959	<i>Phacochoerus aethiopicus</i>	X	AY351551
Kilean/3	Nanyuki, Kenya	1959	<i>Phacochoerus aethiopicus</i>	X	AY351531
KIRT89/3	Kiriwa, Tanzania	1989	<i>Ornithodoros spp</i>	X	AY351512
KIRT89/4	Kiriwa, Tanzania	1989	<i>Ornithodoros spp</i>	X	AY351513
KIRW89/1	Kiriwira, Tanzania	1989	<i>Phacochoerus aethiopicus</i>	X	AY351514
TAN/kwh12	Tanzania	2002	<i>Phacochoerus aethiopicus</i>	X	AF301546
UGA/3/95	Uganda	1995	<i>Sus scrofa</i>	X	AY351

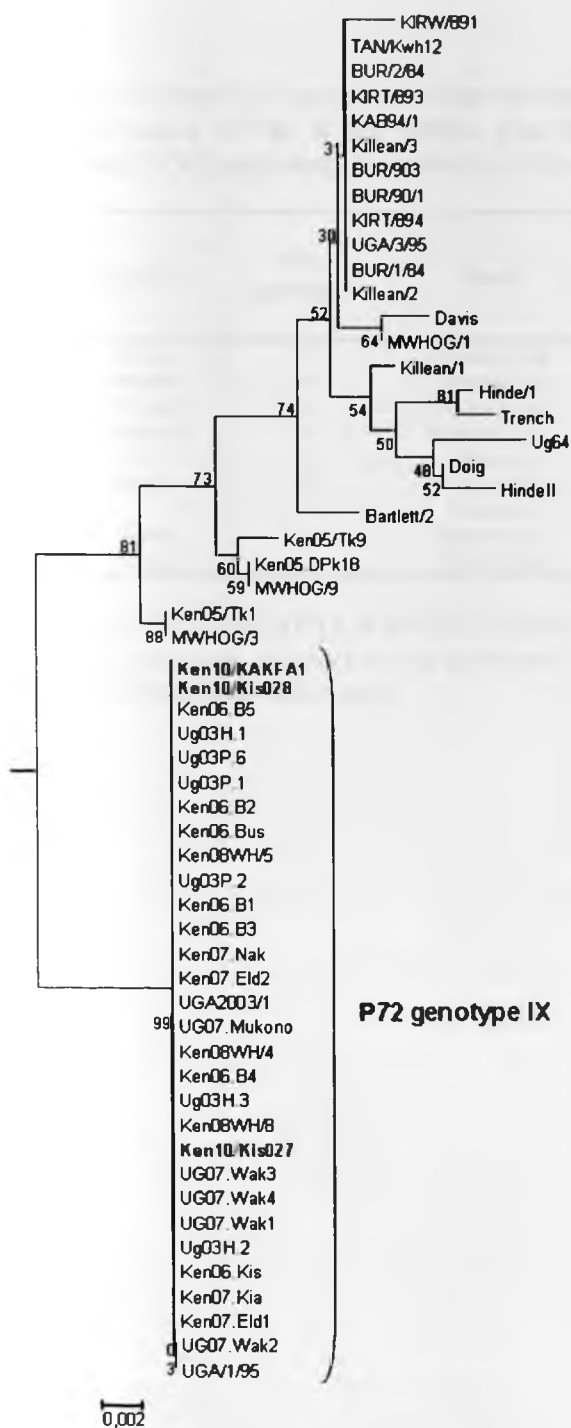
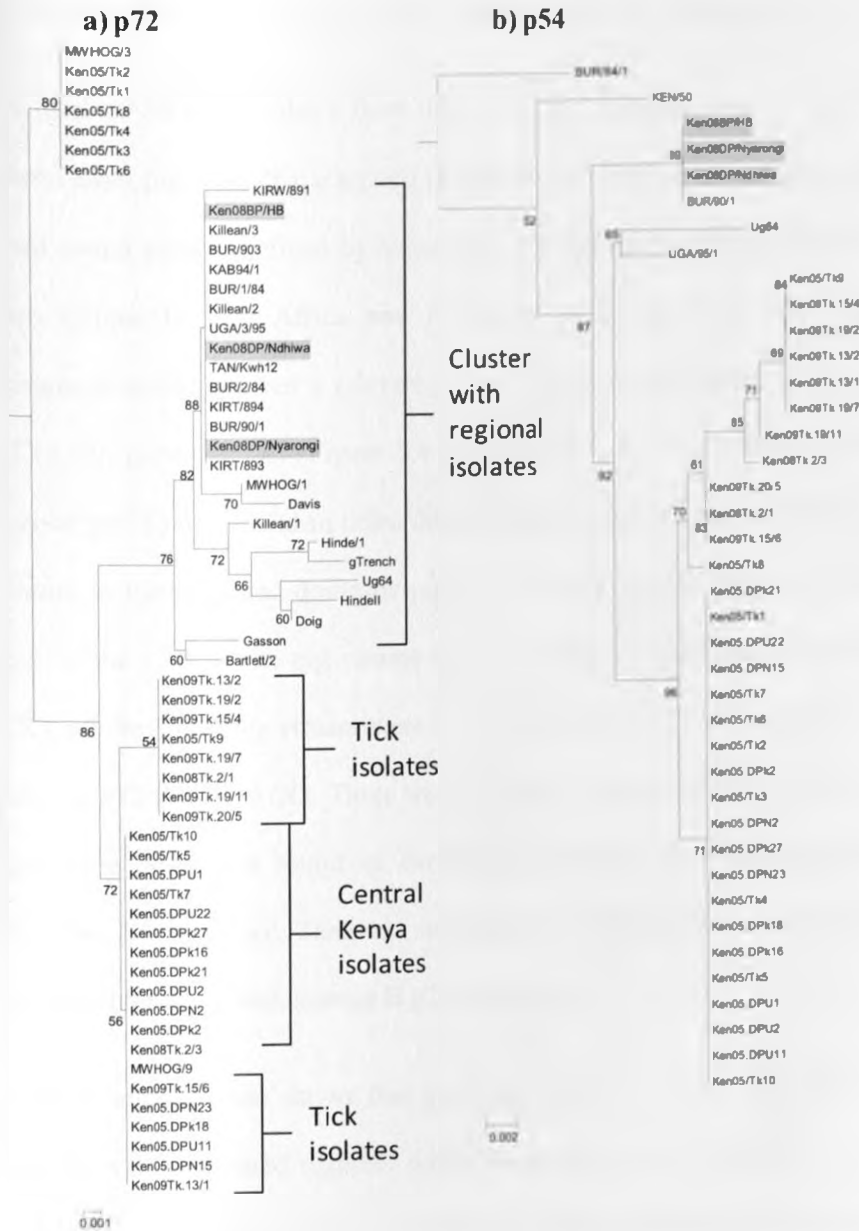


Figure 6.1: P72 genotyping with ASFV Kenya 2010 isolates highlighted (UG=Uganda Isolates, Ke=Kenya isolates)

**Table 6.2: Amino acid sequence of the tetrameric repeats that constitute the central variable region (CVR) of the B602L gene identified in viruses belonging to CVR subgroup XXVI previously described by Nix *et al.* 2006**

Isolate	Country	P72 genotype	Host	CVR aminoacid sequence	No repeats	CVR Genbank accession no.
1	Kenya	X	Domestic pig	AAA---BNAAAAAAAAAABA	17	AM259421.1
2	Burundi	X	Domestic pig	AAA---BNAAAAAAAAAABA	17	AM259424.1
1	Burundi	X	Domestic pig	AAA---BNAAAAAAAAAABA	17	AM259423.1
			Bushpig			
<b>BP/HB</b>	Kenya	X	( <i>Potamochoerus larvatus</i> )	AAA---BN-----AAAABA	11	JN590917
<b>DP/Ndhiwa</b>	Kenya	X	Domestic pig	AAA---BN-----AAABA	11	JN590918
<b>DP/Nvarongi</b>	Kenya	X	Domestic pig	AAA---BN-----AAAABA	11	JN590919

**Key:** A (CAST); B (CADT); N (NVDT). Dashes indicate gaps introduced manually to enable similarities between sequences to be more easily visualized. Sequences generated in this study are indicated in bold and underlined.



**Figure 6.2: Phylogenetic sub-trees based on a) C-terminal end p72 protein and b) full length p54-gene of 3 Kenyan ASFV viruses characterized illustrating the genetic relationship with other ASF viruses belonging to ASFV p72 and p54 genotype X. The Kenyan ASFV viruses characterized in this study are marked in grey.**

#### **6.4.5 Genetic relationships of ASF viruses in Kenya with regional virus genotypes**

A total of 20 virus isolates from ticks, 17 from domestic pigs, 3 from warthogs and 1 from bush pig were characterized (Appendix 3). The viruses analyzed here cluster into two major groups, defined by evolutionary lineage and genotype (Figure 6.3). Group A are unique to East Africa and B unique to south Africa. The result from virus characterization showed a mixture of two p72 genotypes found in this study; genotype (IX) and genotype (X) (Figure 7.4 and Appendix 3). The results also showed that p72 genotype (X) was found in ticks, domestic pigs and bush pig and p72 genotype (IX) was found in warthogs and domestic pigs. All the tick viruses fell into p72 genotype (X), 3 out of the 17 domestic pig viruses were p72 genotype (IX) and the rest in p72 genotype (X), all the 3 warthog viruses were in p72 genotype (IX) and finally, the 1 bush pig virus was in p72 genotype (X). There was no Kenyan isolates in this study that clustered with genotype X viruses found in evolutionary lineage B associated with isolates from Malawi, Mozambique, Tanzania and Zambia. However, there are early (1959) Kenyan isolates clustering with lineage B p72 genotype X.

Further analysis also shows that genotype (IX) in warthogs and domestic pigs in the current study clustered together with viruses that caused outbreaks in Uganda in 1993, 2003 and 2007 (Figure 6.2), in Kenya in 2005, 2006, 2007, 2008 and 2010 and the Democratic Republic of Congo in 2009.

The p72 genotype (X) in the current study does not cluster with viruses that have been shown to cause outbreaks in Kenya in the recent past and also with p72 genotype X

viruses in Lineage B (Figure 6.3). However, the viruses cluster with p72 genotype X ASFV isolates that caused outbreaks in Burundi in 1990 and Uganda in 1995.

One virus strain (p72 genotype IX) characterized in the current study present a risk of ASF in naïve pigs given that they are associated with recent outbreaks and was highly infectious and highly virulent.

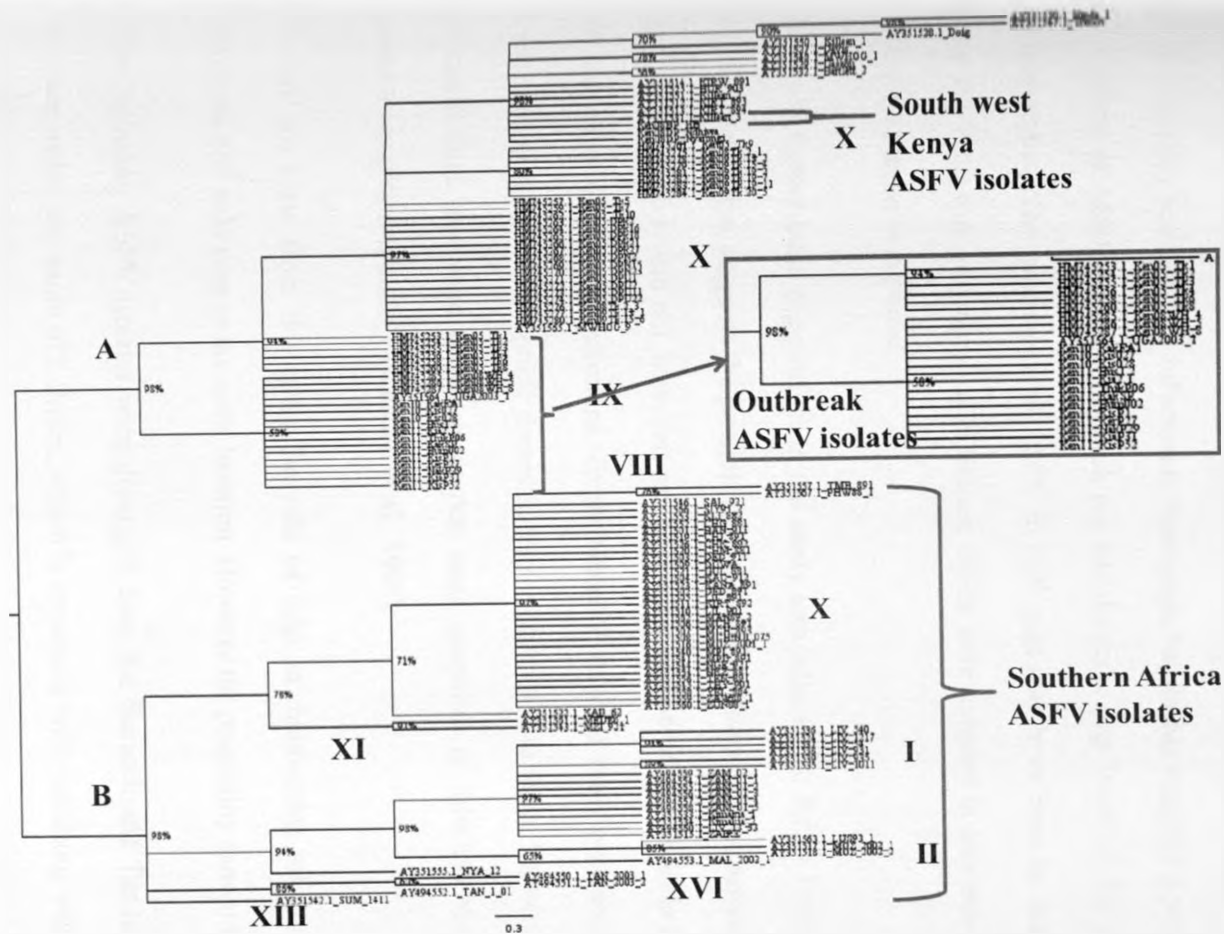


Figure 6.3: Phylogenetic subtrees based on C-terminal end p72 protein of 3 Kenyan south west Kenya and 2010-2011 outbreak ASF viruses characterized illustrating the genetic relationship with other ASF viruses belonging to ASFV p72 and two main evolutionary lineages. The Kenyan ASFV viruses characterized in this study are shown.



## 6.5 Discussion

This study documents the presence of ASFV infection in bush pigs, in their natural habitat, in South-west Kenya. Susceptibility of bush pigs to ASF was first demonstrated in 1921 in Kenya (Montgomery, 1921) and ASFV was subsequently isolated from several bush pigs in the country (Detray, 1963). These studies did not demonstrate any direct association of the virus infections or ASF outbreaks with domestic pigs. The current study, not only confirms the infection in bush pigs, but further suggests a possible direct association of ASFV infections in bush pig and domestic pigs based on the similarity of the genotypes. The prevalence of ASFV in bush pigs may have been an under-estimate since the virus was only detected in tissues, which were sampled in only one of the eight bush pigs that were captured.

Reported farmer-bush pig conflict in the study area adjacent to Ruma National Park in South-west Kenya suggests the possibility of direct or indirect contact between the 2 pig species. This study did not, however, establish the direction of any possible transmission of the observed ASFV infections. Experimentally infected bush pigs were shown to transmit the virus to susceptible domestic pigs in contact with them, but acutely infected domestic pigs that were known to secrete large quantities of infective virus, failed to make a reciprocal transfer (Anderson et al., 1998).

It was not clear from this study the role of ticks in maintaining infections between domestic and wild pigs in the same location. However the possibility cannot be ruled-out.

The Homabay ASFV isolates were divergent from the Burundi and Tanzanian isolates that are within the same p72 cluster, which is consistent with mutations within the CVR

subsequent to isolation of South-west Kenyan virus isolates from those from Burundi and Tanzania, compared in this study. It was also demonstrated that the Kenya 2010 ASF viruses characterized showed a minor change in the insert of a single internally located tetrameric repeat (CADT), relative to those responsible for earlier outbreaks in Uganda and Kenya which was also present in viruses obtained during the second wave of outbreaks that occurred in Western and Central Kenya in 2006-2007 and in Uganda in 2007. This may, perhaps indicate either a direct relationship between the viruses responsible or reoccurrence of the same mutation.

The existence of two p72 genotype clusters of viruses in Central Kenya was confirmed. One of the clusters was closely related to ASFV isolates from domestic pigs taken for slaughter in the same region (Gallardo et al., 2011b). The p72 genotype IX type virus from warthogs in Kapiti Plain Ranch isolated in this study has been associated with recent ASF outbreaks in Kiambu District (Gallardo et al., 2011b).

The results from this study strongly suggest association of the 2010-2011 outbreaks with a closely related virus that has been circulating in pig populations in areas around the Kenya-Uganda border region and also in central Uganda since 2003 (Gallardo et al., 2011b; Gallardo et al., 2009b). The rapid spread of the virus among pigs and the per-acute and acute clinical manifestation of the disease also indicated similarity with earlier outbreaks. The virus may have been maintained in the border districts either in contaminated pork products (given that feeding of swill and pork products was incriminated in the outbreak farms), or in live carriers pigs that survived the earlier outbreaks. More extensive sampling and characterization of viruses in Uganda-Kenya border districts, ideally by whole genome sequencing rather than using three polymorphic

Proxy genes is required to generate baseline information that will allow pinpointing of the source of any future outbreaks.

The p72 genotype IX, in this study, was associated with virulent ASF outbreaks in Kenya in 2010 and 2011 and thus it presents a real risk for future outbreaks. It is also closely associated with both tick and warthog viruses. Given that the role of ticks in the epidemiology of ASFV in Kenya is not well understood, the extent to which this viral strain can spread through tick biting domestic pigs cannot be underscored.

Given that there was no Kenyan isolates in this study that fell into genotype X found in evolutionary lineage B (Figure 6.4) associated with isolates from Malawi, Mozambique, Tanzania and Zambia, the result suggest an evolutionary separation of ASFV genotype X associated with tick, domestic pig and wild pig infections in Kenya. However, the earlier genotype X viruses isolated in 1959 cluster together with ASFV p72 genotype lineage B isolated recently, suggesting that this separation occurred probably in the last 50 years and is characterized by a southward spread of the virus strain given the temporal patterns observed. These results showed the urgent need to prevent trans-boundary spread of Southern, Central and West African viral strains into East Africa and *vice versa* that can further complicate the epidemiology of the disease in the region.

## **CHAPTER 7**

### **GENERAL DISCUSSION**

The current study identified 3 key broad factors that could influence the epidemiology of ASFV in Kenya. These were pig production and management practices defined by farm level characteristics, spatial location of pig farms to disease risk areas and infection and virulence characteristics of viruses circulating in Kenya.

Before animal production systems can be improved, existing systems of production must be fully defined and understood. One of the recent attempts to map livestock production systems over the whole of Africa and South Asia was by the International Livestock Research Institute (ILRI) (Kruska et al., 2003; Thornton et al., 2002). Geospatial datasets of human population density, vegetation cover, temperature and irrigation, combined with selected FAO livestock distribution databases, were used in an initial attempt to quantify livestock production systems in the developing world. The resulting map depicted areas that are environmentally suitable for a given type of livestock production system together with estimated densities of poor livestock keepers.

Livestock production systems have previously been classified according to a number of criteria, including integration with cropping, animal-land relationships, agro-ecological zones, intensity of production, and type of product (Otte and Chilonda, 2002). Livestock systems are defined as those in which greater than 90% dry matter fed to the livestock in these systems comes from rangelands, pastures, annual forages and purchased feeds and less than 10% of the total value of production comes from non-livestock farming

activities (Sere and Steinfeld, 1996). The same authors also define mixed systems as those in which more than 10% of the dry matter fed to livestock comes from crop by-products and/or stubble or more than 10% of the value of production comes from non-livestock farming activities. A farming system approach, wherein a system is defined as a group of farms showing similar structure and function (Ruthenberg, 1980), has been widely used to classify livestock production systems (Jahnke, 1982; Wilson, 1995).

A general typology of smallholder livestock systems has been suggested (McDermott et al., 1999) that takes into account the gradients from extensive pastoral to landless, from subsistence to market-oriented, from livestock-dominated to crop-dominated and from mixed to specialized systems. The authors suggest two typologies with broad classification axes, namely degree of intensification of the livestock activity and importance of livestock activity within the household economy. From an intervention perspective, the authors further suggest the use of such typologies based on disease risk and ability to provide and sustain disease control inputs to inform policy makers.

Studies in Kenya have shown that pig production systems, in rural western Kenya, although less so in central Kenya, are typically characterized as being low-input with an income-generating objective (Kagira et al., 2010a). Free-range pig farming is common, with sixty five per cent of the pigs tethered and housing not provided in 61% of farms. The pigs are kept to provide cash for household needs. On the other hand, studies in central Kenya (Wabacha et al., 2004) demonstrate that pig production in peri-urban areas is characterised by farms keeping crossbreed pigs with a significant genetic component from the 'Large White' or 'Landrace' breeds. Most farmers are engaged in 'farrow-to-

finish' pig-production and most (60%) do not keep a breeding boar, a constraint that is also true for Western Kenya. The pigs are stall-fed the year round.

Whereas these studies (Government of Kenya, 2005; Kagira et al., 2010a; Kagira et al., 2010b; Kiptarus, 2005; Wabacha et al., 2004) characterised pig production systems and the pig industry in Kenya, they did not link the production system typologies with disease risks and the economics of control, especially as regards ASF. The current study went further to delineate farm types within the same production system. Characterization of pig farming households using K-means cluster analysis used farm level factors that included composition of farmer households, how they are organised and general production capacity, factors that influence pig production and disease risk and ASFV status of animals. Using this approach the farms were defined by farm and herd sizes in Homabay and Kiambu districts, respectively.

An initial investigation of risk factors for ASF in Homabay District including feeding regime, breeding strategies, restocking of pigs, tick control, foraging management and proximity to the Ruma National Park was performed. Domestic pigs in south-west Kenya were fed cereals and cereal food wastes, vegetables and fruits, in addition to free range foraging. Feeding of pork or other pig products was not cited by any farmer interviewed. Family food was sourced on-farm or locally and cooked and left over after a meal fed to pigs. The factors mentioned above did not directly pose significant risk for introduction of new viral infections.

Statistical analysis of risk of ASFV suggests that production approaches such as feeding and feed types present as risks for ASFV infection with partial confinement significantly

explaining risk. This study showed that farms with positive ASFV cases were those that either allowed free-range foraging of pigs or those that were fed uncooked swill mixed with commercial feeds. Swill feeding, in particular, was implicated in that it was the main source of infected pork getting to clean uninfected pigs. Farmers who fed purely commercial feeds, cooked swill, home-made feed and household waste had no ASFV cases. Farmers, who fed swill only, as observed in this study, cooked the swill to reduce risk of disease. Swill feeding was implicated in the incursion of ASF in Portugal in 1957 (Costard et al., 2009b) and thus is an important management risk in the spread of ASFV.

As regards the role of farm sizes in defining risk of ASF, it was observed that farmers with large farm sizes modified their production practices to include free-grazing or tethering in pastures. Large farms were more common closer to the park because of a sparse human population resulting from infestation of tsetse flies carrying human-infective trypanosomiasis in this area (Wilde, 1989). These factors can explain why farms that were closer to the park had a higher chance of having pigs infected with ASFV. The ASFV genetically characterised in these pigs were found to be closely related to one also found in a bush pig in the same locality.

The higher number of pigs per household within a system that is predominantly stall feeding could explain the low level of virus prevalence (3%) in Kiambu District. These farmers, because of high outputs from larger herd sizes, were able to put housing structures to confine the pigs.

Protected natural reserves are the homes of wild suids that are asymptomatic carriers of ASFV. Risk of ASFV infection explained by proximity to Ruma National Park was found

to be significant ( $P < 0.1$ ) for distance less than 6 Km from the park but insignificant at  $P < 0.05$ . This finding was explained by the fact that the predicted counts were weighted by number of animals at this range of distance from the park and the distance range (less than 6 Km) had fewer pigs. The Lambwe Valley where the park is located supports large numbers of *Glossina pallidipes Austeni* and infections of *Trypanosoma brucei rhodesiense* (Allsopp et al., 1972; Otieno and Darji, 1985; Turner, 1986; Wilde, 1989). This probably explains fewer farmer settlements close to the park and thus large farm sizes associated with free-range production of pigs. However, this study shows a range of distance (6-15 Km) from the park to be significant ( $P < 0.05$ ) and thus association with ASFV infections in domestic pigs. It was speculated that this range of distance associated with ASFV infections coincides with range of bush pig incursions from the park into farms, based on farmer observations, creating direct or indirect contact with domesticated pigs. A study conducted by the author in 2010 in Uganda (unpublished data), used a radio collar device on a bush pig to determine distances travelled during the night into farms by the bush pig. The result showed that the bush pig travelled a total of 20 Km, on average, per night. This result confirms that there is possibility of bush pig movement covering the distances from the park as observed in Homabay District.

Although undoubtedly important historically in the initial transfer of ASFV into domestic pigs, the current role of wild suids is not well understood especially in the case of elusive and nocturnal bush pigs (Jori and Bastos, 2009). More comprehensive data on bush pig populations, distribution and movement could lead to improved understanding of the potential risk based on the probability of contact with domestic pigs.



On the other hand, given the feeding nature of warthogs, the route of contact with domestic pigs could only be through hunted warthog meat being fed to pigs, though this observation was not confirmed directly in the current study. The pig farms in rangelands with warthog population can get infected through ticks that maintain infection in the warthog population. These pigs end up in slaughter houses in Kiambu District and pork products fed to pigs would then lead to outbreaks.

To date, the use of empirical methods has failed to produce an effective vaccine against ASF, partly due to the complexity of the virus and its ability to manipulate the host immune system, and also limited understanding of what types of immune response protect against infection (Dixon-Linda et al., 1999). Antibody responses alone probably do not protect pigs and while the presence of antibodies against ASFV is good way to detect exposure to the virus; this is not an indicator of immunity. Pigs that have recovered from ASF remain infected for periods of 6 months or more (Oura et al., 2005; Wilkinson, 1984). Prevalence studies are suggested to be important pointers in informing disease control strategies, especially in identifying species to focus for control. This study confirmed the presence of ASFV in domestic pigs, warthogs, bush pigs and ticks sampled in various regions of Kenya. These results showed that the risk of ASF in the country is both through domestic and sylvatic cycles. The impact of vaccination under this situation needs further investigation.

Though the prevalence of viral infection in the domestic pigs in south-west Kenya as confirmed PCR diagnosis was very high (29.2%), it was not accompanied by observable clinical signs in the positive animals. This result revealed that future prevalence studies must take into consideration the actual epidemiological situations in the field, especially

whether the infection risks are actually associated with outbreaks. This study also clarified that passive surveillance approaches alone cannot identify a clear epidemiological situation in Kenya, but must be accompanied by laboratory diagnostics support. The prevalence studies also identified the need a for understanding of the virus ecology, host genetics and a closer look at the sensitivity of serological tests, in the context of the findings of this study.

Outbreak investigations helped identify the source of on-going outbreaks, and information needed to prevent additional cases. Earlier investigations showed that disease outbreaks occurred when domestic pigs came into close contact with wildlife species, particularly warthogs (*P. Africanus*) (Montgomery, 1921). The first spread of ASF outside Africa was to Portugal in 1957 as a result of waste from airline flights being fed to pigs near Lisbon airport. In Spain *O. erraticus* was identified as a vector and reservoir for the virus (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 are the likely initial source of the ASFV genotypes that now circulate in domestic pigs in Africa (Plowright et al., 1969). *Ornithodoros spp* ticks are widely distributed in the savannah regions of southern and eastern Africa (Plowright et al., 1994).

This study does not link outbreaks investigated to the involvement of wild suids, except in an outbreak investigation done in Thika District that showed the domestic pigs, warthogs and bush pigs shared food wastes from a dump site. Wild suids, and warthogs in particular, and with less certainty bush pigs, are believed to be important for the maintenance of ASFV in many endemic areas (Penrith et al., 2004b). *Ornithodoros*

*porcinus* and *O. moubata* have regularly been found in warthog burrows where they are clearly involved in maintenance of ASFV (Plowright et al., 1994). In the case of bush pigs, the infection dynamics in natural conditions are not as clear. Experimentally infected bush pigs have been shown to transmit ASFV to susceptible domestic pigs in contact with them, but bush pigs exposed to acutely infected pigs that are known to excrete large quantities of infective virus, failed to become infected (Anderson et al., 1998). Recent studies in Madagascar did not detect any anti-ASFV or anti-tick antibodies in sampled bush pigs but the study suggested that the wild suids could still play a role in the epidemiology of ASF ((Ravaomanana et al., 2011).

Transmission of ASFV through direct contact between domestic pigs can occur for up to 30 days after infection, or for 8 weeks in the case of contact of a naïve animal with the blood of an infected animal (Wilkinson and Paton, 1989). The virus can persist in tissues for several months and exposure of pigs to carcasses or insufficiently cooked pork can result in infection (Wilkinson and Paton, 1989). Contaminated pig pens in the tropics were shown to remain infectious to domestic pigs for up to 3 days, but not for 5 days (Montgomery, 1921). Given this scenarios, a breach in biosecurity as a result of poor pig management easily results in outbreaks. Farm management was the major risk factor associated with outbreaks as demonstrated by the outbreak investigation results presented in Chapter 5. These factors included free-ranging/scavenging, feeding swill, poor control of pig movements and poor biosecurity on-farm, among others.

The domestic cycle outbreaks observed in this study could have also involved carrier pigs that survived ASFV infections that were eventually able to transmit the disease to healthy susceptible pigs. Pigs that suffer chronic infections have been shown to transmit ASF for

one to two months after initial infection and ASFV may be recovered from tissues of these pigs for up to about six months (Wilkinson, 1983). However, improved management practices could have eliminated this. The results from 2010-2011 outbreak investigation in the current study suggests the existence of carrier pigs in Kenya that are capable of maintaining infectivity of the pigs between outbreaks, since a small percentage of PCR positive survivors was detected in the affected herds on several farms.

The molecular characterization data was used to create an understanding of the possible infectiousness and virulence of the isolates in this study that had relevance in predicting their potential as risk for infection, spread and cause of mortalities in domestic pigs. This study appears to demonstrate three distinct viral transmission scenarios: One in south-west Kenya involving p72 genotype X, possibly associated with bush pigs, which apparently does not result in detectable clinical disease in local domestic pigs; the other warthog/tick associated virus involving p72 genotype IX that has been associated with recent outbreaks involving acute disease in western and central Kenya; and group X viruses isolated in ticks in this study shown to cluster together with viruses isolated from domestic pigs in a slaughter house in Kiambu District in 2005 where the pigs sampled exhibited no clinical signs according to farmers' choice veterinarians or postmortem lesions. The animals did not show antibody response (Gallardo et al., 2011b). The genotype X in this study is thus apparently not associated with disease, but further investigation is needed to confirm this observation.

Phylogenetic trees based on the 3' end of the gene encoding the C-terminal end of the p72 protein and full-length p54-gene of selected domestic pig and bush pig ASFV viruses were generated to illustrate the genetic relationships. These shows that the two sets of

viruses were distinct by disease phenotype clustered together. A further analysis of predicted amino acid sequence of the tetrameric repeats that constitute the central variable region (CVR) of the B602L gene belonging to CVR subgroup XXVI identified in the Homabay samples showed homology between the domestic and bush pig viruses. These results suggested a common source of ASFV infection or viral transmission between the two pig species in extreme south-west Kenya. Bush pigs have been shown to transmit the virus directly to domestic pigs (Anderson et al., 1998) and this may explain a possible role of bush pigs in this epidemiology, but this requires further investigation.

There is good evidence that warthogs are involved in a complex cycle of infection between the vertebrate and invertebrate hosts of ASFV. Although warthogs are natural hosts of the ASF virus, it has been demonstrated that they are unable to transmit the virus directly to domestic pigs (Thomson, 1985). The current study provided data demonstrating that Genotype IX from warthogs in Kapiti Plains Ranch was genetically similar to and fell within the p72 genotype IX that is associated with recent ASF outbreaks in Kenya and Uganda (Gallardo et al., 2011b). Poaching of warthogs that are eaten by pig farming households could be a possible source of infection in domestic pigs.

This study also provided data demonstrating the similarity of virus isolates from ticks and with those of domestic pigs in central Kenya interpreted as ticks being associated with infections in the domestic pigs.

These results indicate a possible current role of a sylvatic cycle involving bush pigs, warthogs and ticks in the epidemiology of the disease in Kenya. Other studies (Boshoff et al., 2007; Lubisi et al., 2005) also confirmed that ASFV genotypes in East Africa were

circulating in both sylvatic and domestic cycles. However, the timescale of virus transfer from the sylvatic cycle to domestic pigs requires further investigation.

It is interesting to note that the only Kenyan ASF virus for which a complete genome sequence is currently available (Chapman et al., 2008; de Villiers et al., 2010) was an isolate obtained in the 1950s that is also classified in p72 genotype X, but was, unlike the viruses in the same genotype described in this study, highly virulent in domestic pigs. It cannot be ruled out that changes resulting in significant mutations can result in the genotype X viruses in this study becoming more virulent in the future.

Pan and Hess (1984) demonstrated that haemadsorbing and non-haemadsorbing ASF viruses can be classified into 3 groups: highly infectious and highly virulent; highly infectious and moderately virulent; and slightly infectious and slightly virulent. This classification was based on the number of 50% haemadsorption unit (HA50) or tissue culture infectious dose (TCID50) producing 1 lethal dose (LD50) for swine, the number of HA50 or TCID50 producing one 50% pig infectious dose (PID50), and the number of PID50 required for each LD50. This classification showed the virulent virus (group 1) required less than or equal to 10 virus units (HA50 or TCID50) for 1 PID50 and LD50 (highly infectious and highly lethal), respectively, and had a ratio of 1.0 for PID50/LD50, i.e., all infected pigs died from acute African swine fever. Low virulence of viruses especially p72 genotype X isolates from south-west Kenya can also be explained in the context of virulence determinants both in the virus and the host.

Potential for spread of viruses circulating in West Africa could spell more risk for ASF in East Africa and Kenya in particular. A study has shown new incursions into Central

Africa of viral strains that were found in West Africa (Gallardo et al., 2011a). African swine fever virus p72 genotype IX, associated with outbreaks in eastern Africa, is co-circulating in the Democratic Republic of Congo with West African genotype I. Data suggest that viruses from eastern Africa are moving into western Africa, increasing the threat of outbreaks caused by novel viruses in this region (Gallardo et al., 2011a). The earlier genotype X viruses isolated in 1959 clustered together with ASFV p72 lineage recently circulating in the southern Africa region that is in a separate lineage from those in this study, suggesting that this separation occurred probably in the last 50 years and is characterized by a southward spread of the virus strain given the temporal patterns observed. These results showed the urgent need to prevent trans-boundary spread of viral strains into regions they have not been before. These new incursion have a potential for devastating mortalities that can lead to major economic losses to farmers.

## CHAPTER 8

### CONCLUSIONS AND RECOMMENDATIONS

#### 8.1 Conclusions

1. The general characteristics of pig farms showed that: i) Majority of pig farming households had small to medium farm sizes (1 to 10 ha) with the households (65%) supporting labour on the farms instead of hired labour; ii) Farms in Homabay District generally held a lower number of pigs per household (average of 2) compared to Kiambu District (average of 23); and iii) The efficiency of pig production measured by weaning to service intervals, number of piglets farrowed and weaned, identified Kiambu District farms as being more efficient than those of Homabay, a factor associated with low inputs in Homabay compared to Kiambu. Cluster analysis of farm characteristics showed that pig farm-types in Kiambu were generally best described by total number of animals and number of breeding females, but farm size was specifically an important characterization criterion for farms in Homabay District. These factors had the largest F-values and provided the best separation between farm clusters using K-means cluster analysis method.
2. The main breeding approaches in Homabay and Kiambu were use of a boar on-farm or contractual-breeding with the latter presenting as a risk for the spread of ASFV. Breeds of pigs kept were both exotic pig breeds and their crosses.
3. Both the sylvatic and domestic cycles of ASFV transmission were confirmed in this study as important in the epidemiology of ASF in Kenya. Genotyping of both domestic and bush pig viruses using p72 and p54 genotyping and CVR amino



acid sequencing revealed homology suggesting sylvatic cycle of ASFV transmission involving the two species in south-west Kenya. On the other hand, some ASFV isolates from ticks and warthogs from central Kenya were similarly closely related to ASFV isolated from domestic pigs from the same locality, suggesting viral transmission between the ticks, warthogs and domestic pigs. A single viral genotype was also confirmed to cause outbreaks through a domestic pig cycle transmission. However, the timescales of the most recent introduction of ASFV from the sylvatic cycle to domestic pigs and hence the current importance are unclear.

4. There appeared to be existence of inapparent infections in domestic pigs as shown by high virus prevalence by PCR technique estimated at 29.2% (95% CI, 21.6, 39.9) in south west Kenya and 3% (95% CI, 0, 5.5) in central Kenya in the apparent absence of frequent outbreaks of disease. (Aguero et al., 2003)
5. The laboratory testing of samples collected from ASF outbreaks confirmed the presence of ASFV by p72-PCR in Busia, Kakamega, Kisumu, Kiambu and Thika Districts. All the cases investigated represented localised outbreaks based on the high number of cases reported over a short period.
6. The outbreaks in this study were caused by a single p72 genotype IX virus genetically closely related to a virus that has been present in areas around the Kenya-Uganda border region for a long period. The outbreaks were associated with each other given the sequence of spatial and the temporal patterns seen in the epidemiological units affected. This suggested that the spread of ASF was via transportation of pigs and pig products between districts via roads.

7. Management practices observed on the affected farms e.g. free-grazing, free-scavenging, feeding of swill, total lack of biosecurity measures on-farm, lack of rational management of breeding, restocking and biosecurity of slaughter slabs, were all potentially implicated in the ASF outbreaks in 2010-2011 and domestic cycle of virus transmission.
8. Sequence analysis of the region encoding tetrameric repeats in the CVR of the B602L viral gene suggests possible mutation in the genes of the south-west Kenyan virus isolates from domestic and bush pigs following presumed separation from virus isolates from Burundi and Tanzania which are similar at the p72 and p54 loci. Whereas, Burundi and Tanzania virus isolates fell within same p72 genotype cluster X as the south-western Kenya isolates, they are associated with outbreaks, the latter is not. The separation partly explained lack of virulence observed in domestic pigs in south-west Kenya.
9. Overall, genetic characterization of the viral isolates from ticks, warthog, domestic and bush pigs revealed clustering with previous isolates from within the region isolated during the period 1959 to 2010, but were in a separate lineage from previous isolates from the southern Africa region. This conclusion suggested that both the sylvatic and domestic cycles within the region have contributed to disease in domestic pigs in the region although the precise timescale was unknown.
10. Generally, the epidemiology of ASFV in Kenya was influenced by pig production system, pig management practices, sylvatic reservoirs necessary for persistence of the virus in pigs and virulent or avirulent virus strains in circulation. More broadly

feeding systems defined risk of ASF in both free-range and stall feeding at multiple sites in different regions of Kenya.

## 8.2 Recommendations

The following recommendations can be made based on this study:

1. The two types of viruses circulating in Kenya that belong to the p72 genotypes IX and X are proxies for genetic divergence that require further investigations to demonstrate genes that could be responsible for the virulence of the viruses, ideally based on complete genome sequences of key isolates.
2. Complete ASFV genome sequences are required to clarify difference underpinning the two major p72 viruses exhibiting different virulence in the field.
3. A more comprehensive understanding of risk of ASF requires a longitudinal study that examines relevant factors at multiple time-points. A cross-sectional approach to evaluating risks, as used in this study, results in identification of risk factors that are applicable specifically at the time of the study, but may miss others and fail to capture the dynamics of those identified.
4. A comprehensive longitudinal study of pig value chains incorporating pig genetics, feed input and health constraints should be performed in the East African region to generate information on the socio-economic impact of control of ASFV and other diseases of pigs, relative to other factors in pig production systems. This kind of study can inform the development of a comprehensive policy document that governs the pig sub-sector.

5. Further characterization of bush and domestic pig viruses and level of pig species contact in multiple areas within the region to establish extent of genetic relationships and investigate the importance of *Potamochoerus spp* in the transmission of ASFV to domestic pigs.
6. Investigation of the virulence of the viral strains in this study is suggested. This can be performed either through introduction of naive animals to the ASFV positive farms, or experimental infection of naive pigs with viral isolates derived from this study. This level of prevalence of ASFV in some cases in the absence of clinical symptoms, implying possible tolerance/resistance of domestic pigs to ASFV has not been reported previously in Eastern Africa.
7. More extensive sampling and characterization of viruses in border districts within the East African countries is required to generate information for improved understanding of the extent to which disease outbreaks in Kenya and Uganda are as the result of transboundary movement of ASFV.
8. The phenomenon of suspected hybridization of domestic pigs and wild African bush pigs needs to be investigated using modern genetic tools such as mitochondrial sequencing, analysis of chromosome-specific VNTRs and application of SNP genotyping. If confirmed this will provide an explanation of the ASFV tolerance phenotype observed in some domestic pigs.
9. A numbers of factors were identified in this study that if modified represent interventions that could alter the epidemiology of ASFV and mitigate the impact of ASF. These factors were:

- a) Implementation of biosecurity on-farm to prevent introduction of infections to clean farms through human visitors or contaminated feed;
- b) Strict implementation of a quarantine policy following outbreaks to prevent spread of ASFV combined with more rapid confirmation of ASF using diagnostics; and confinement of pigs to prevent transmission of ASFV by contact with other pigs (domestic and wild); this should be encouraged through provision of financial incentives from the Government through development organisations;
- c) Government compensation of farmers to encourage stamping out of ASFV through slaughter;
- d) Management of slaughter houses and slabs as quarantine areas, with appropriate control measures to restrict entry of stray animals, e.g., dogs and implementation of biosecurity. The slaughter-houses should be located away from main roads and highways to prevent vehicles carrying infections from these pig concentration points.
- e) Introduction of artificial insemination or progressive breeding to reduce use of contract breeding, which is a risk for spread of diseases from infected to clean farms; and,
- f) Improved Farmer extension services on pig management, to enhance knowledge of farmers in high risk areas on techniques that reduce risk of ASF.

10. Whereas comprehensive policies are available that govern the pig sub-sector, it is important that a policy document targeted at the farmers and private and public sectors is produced that directs the development of the sub-sector using a pig value-chain approach. Such a document would address the constraints associated with pig genetics, feeding and pig health in relation to their impacts on the entire value chain. The ultimate goal would be to enhance the socio-economic impact of the sub-sector on small-scale pig farmers in Kenya.

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# APENDICES

## APPENDIX 1

### QUESTIONNAIRE USED FOR PIG FARM CHARACTERIZATION

#### General Information

Form number \_\_\_\_\_ Date \_\_\_\_\_

Province \_\_\_\_\_ District \_\_\_\_\_ Division \_\_\_\_\_

Village \_\_\_\_\_ Site \_\_\_\_\_ GPS \_\_\_\_\_

#### Household information

Name of farmer \_\_\_\_\_ Farm size (ha) \_\_\_\_\_ Number in HH \_\_\_\_\_

Source of labour (Tick)

Family \_\_\_\_\_ Hired labour \_\_\_\_\_ Other \_\_\_\_\_

#### Herd Information

Pig Breed (s)	Number in the farm

Number of breeding females \_\_\_\_\_ Number of breeding males \_\_\_\_\_

Number of weaned piglets \_\_\_\_\_ Number of non weaned piglets \_\_\_\_\_

#### Feeding strategy (tick)

Free range \_\_\_\_\_

Partial confinement \_\_\_\_\_

Total confinement \_\_\_\_\_

Others \_\_\_\_\_

Type of feed (List) and source

Feed	Source of feed

**Production data**

Weaning-to-service interval (months) \_\_\_\_\_ Inter-farrowing interval (months) \_\_\_\_\_

Number of live-born piglets \_\_\_\_\_ Number of piglets weaned per litter \_\_\_\_\_

Piglet sick past 3 months \_\_\_\_\_ Piglets dead past 3 months \_\_\_\_\_

**Health Information**

Number of animals sick past 3 months \_\_\_\_\_

Number of animals dead past 3 months \_\_\_\_\_

Disease/Disease symptoms observed	Number affected	Age affected	Number dead

Disease control method (List) and frequency

Disease control	Frequency

**Animal level health data**

Animal ID \_\_\_\_\_ Sex M \_\_\_ F \_\_\_ Breed \_\_\_\_\_ Age \_\_\_\_\_

Source of animal:

Born on-farm \_\_\_\_\_ from neighbour \_\_\_\_\_ from same district \_\_\_\_\_

from another district \_\_\_\_\_. If from another district, which district \_\_\_\_\_

Has any disease been observed in this animal recently? Yes \_\_\_ No \_\_\_

If yes, what were the disease symptoms \_\_\_\_\_

Tentative diagnosis \_\_\_\_\_

Was the disease reported? Yes \_\_\_ No \_\_\_

If yes, name the title or authority disease reported to \_\_\_\_\_

Was the animal treated? Yes \_\_\_ No \_\_\_

If yes, who treated the animal? Vet \_\_\_ AHA \_\_\_ Farmer \_\_\_ Others \_\_\_\_\_

Which drug(s) was used? \_\_\_\_\_

Comments \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## APPENDIX 2

### STOCK SOLUTIONS FOR PCR AMPLIFICATION ASSAY

1. Nuclease-free sterile water.
2. AmpliTaq Gold DNA polymerase, 10× PCR Buffer II, and magnesium chloride
3. PCR nucleotide mix containing 10 mM of each dNTP
4. Primers derived from p72 at a concentration of 20 pmol/μl:

Primer 1 sequence 5'-AGT-TAT-GGG-AAA-CCC-GAC-CC-3' (forward primer);

Primer 2 sequence 5'-CCC-TGA-ATC-GGA-GCA-TCC-T-3' (reverse primer).

5. 10× Loading buffer: 0.2% xylene cyanol, 0.2% bromophenol blue, 30% glycerol.
6. TAE buffer (50×) for agarose gel: Tris base (242 g); glacial acetic acid (57.1 ml); 0.5 M EDTA, pH 8.0(100 ml); distilled water (to 1 litre).
7. Marker DNA: 100 base-pair ladder.

### APPENDIX 3

#### KENYAN ASFV ISOLATES FROM CURRENT STUDY USED FOR GENOTYPING AND VIRUS CHARACTERIZATION

Isolate name	Origin	Year	Host species	p72 genotype	p72 gene GeneBank accession number
Ken05/Tk1	Kapiti Plains Ranch, Machakos district	2005	<i>Ornithodoros porcinicus</i>	X	HM745253
Ken05/Tk2	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745254
Ken05/Tk3	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745255
Ken05/Tk4	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745256
Ken05/Tk5	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745257
Ken05/Tk6	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745258
Ken05/Tk7	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745259

Isolate name	Origin	Year	Host species	p72 genotype	p72 gene GeneBank accession number
Ken05/Tk8	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745260
Ken05/Tk9	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745261
Ken05/Tk10	Kapiti Plains Ranch, Machakos district	2005	<i>O. porcinicus porcinicus</i>	X	HM745262
Ken05/DPk2	Farmers choice slaughter house, Kiambu district	2005	<i>Sus scrofa</i>	X	HM745263
Ken05/DPk16	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745264
Ken05/DPk18	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745265
Ken05/DPk21	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745266
Ken05/DPk27	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745267
Ken05/DPN2	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745268
Ken05/DPN15	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745269
Ken05/DPN23	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745270
Ken05/DPU1	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745271



Isolate name	Origin	Year	Host species	p72 genotype	p72 gene GeneBank accession number
Ken05/DPU2	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745272
Ken05/DPU11	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745273
Ken05/DPU22	Farmers choice slaughter house, Kiambu district	2005	<i>S. scrofa</i>	X	HM745274
Ken08WH/4	Kapiti Plains Ranch, Machakos district	2008	<i>Phacochoerus africanus</i>	IX	HM745285
Ken08WH/5	Kapiti Plains Ranch, Machakos district	2008	<i>P. africanus</i>	IX	HM745286
Ken08WH/8	Kapiti Plains Ranch, Machakos district	2008	<i>P. africanus</i>	IX	HM745287
Ken09Tk.2/1	Kapiti Plains Ranch, Machakos district	2008	<i>O. porcinicus porcinicus</i>	X	HM745275
Ken09Tk.2/3	Kapiti Plains Ranch, Machakos district	2008	<i>O. porcinicus porcinicus</i>	X	HM745276
Ken09Tk.13/1	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcinicus porcinicus</i>	X	HM745277
Ken09Tk.13/2	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcinicus porcinicus</i>	X	HM745278
Ken09Tk.15/4	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcinicus porcinicus</i>	X	HM745279
Ken09Tk.15/6	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcinicus porcinicus</i>	X	HM745280

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Isolate name	Origin	Year	Host species	p72 genotype	p72 gene GeneBank accession number
<b>Ken09Tk.19/2</b>	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcunicus porcunicus</i>	X	HM745281
<b>Ken09Tk.19/7</b>	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcunicus porcunicus</i>	X	HM745282
<b>Ken09Tk.19/11</b>	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcunicus porcunicus</i>	X	HM745283
<b>Ken09Tk.20/5</b>	Kapiti Plains Ranch, Machakos district	2009	<i>O. porcunicus porcunicus</i>	X	HM745284
<b>Ken08BP/HB*</b>	Homabay, Kenya	2008	<i>P. larvatus</i>	X	JN590917
<b>Ken08DP/Ndhiwa*</b>	Homabay, Kenya	2008	<i>S. scrofa</i>	X	JN590918
<b>Ken08DP/Nyarongi*</b>	Homabay, Kenya	2008	<i>S. scrofa</i>	X	JN590919
Ken10/Kis027	Kisumu, Kenya	2010	<i>S. scrofa</i>	IX	
Ken10/Kis028	Kisumu, Kenya	2010	<i>S. scrofa</i>	IX	
Ken10/KAKFA1	Kakamega, Kenya	2010	<i>S. scrofa</i>	IX	