MOLECULAR CHARACTERISATION AND ASSESSMENT OF EPIDEMIOLOGICAL RISK FACTORS OF AFRICAN SWINE FEVER VIRUS IN IRINGA REGION, TANZANIA

CHRISTOPHER DICKSON SIKOMBE

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ABSTRACT

A cross sectional study was carried out in Iringa Region involving two Districts of Kilolo and Iringa Municipal to determine the epidemiological risk factors of the African swine fever (ASF) outbreak and genotyping the circulating African swine fever virus (ASFV) during a hemorrhagic disease outbreak in 2012 in Iringa. A structured questionnaire was used to collect epidemiological risk factors from pig keepers. Eighty households that kept pigs and that experienced the disease in 2012 were involved. The epidemiological risk factors found were introduction of pigs into the herd before disease outbreak (OR=12.2578, $CI_{95\%}$=108.6596, *P*=0.0244), feeding of kitchen leftovers and consumption of pig meat (OR=35.6117, $CI_{95\%}$ =300.2233, *P*=0.001), duration of disease outbreak $CI_{95\%}$=14 -19 days, failure of the pig keepers and other stakeholders to adhere to quarantine condition and treatment of sick pigs during outbreak. Tissue samples were obtained from a total of 20 pigs. DNA extraction was done before performing polymerase chain reaction (PCR). Three sets of primers were used in this study to diagnose and genotype the ASFV. PCR of ASFV using diagnostic primers PPA1/PPA2 confirmed the disease outbreak to be ASF. Characterization of the virus was done by amplifying the variable part of *B646L* gene using primers p72U/p72D which showed that the circulating ASFV belonged to genotype II and 100% identical to the Georgia 2007/1 isolate and other Tanzanian ASFV that circulated in 2010 and 2011. Similarly, complete amplification of *E183L* gene using primers PPA89 and PPA722 showed that ASFV circulating in Iringa in 2012 belonged to group II of p54 genotypes clustering together with Georgia 2007/1 isolate. The results from the present study indicate that the ASFV isolate that was introduced into Tanzania in 2010 is still circulating in Iringa. Deliberate efforts have to be taken in order to save the pig industry from ASF devastation.

DECLARATION

I, Christopher Dickson Sikombe, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Christopher Dickson Sikombe Date

(MSc. One Health Molecular Biology student)

The above declaration is confirmed

_____ _____

Prof. Sharadhuri I. Kimera Date

(Supervisor)

_____ _____

Dr. Gerald Misinzo Date

(Supervisor)

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DEDICATION

To my beloved parents, the late Mr. Dickson Sikombe and Mrs Edina Nambela who did their best to send me to school. May Almighty God make them rest in peace, Amen. To my lovely wife Cecilia for her patience during time of study.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

APPENDIX

LIST OF ABBREVIATIONS AND ACRONYMS

ASF	African swine fever
ASFV	African swine fever virus
bp	base Pair
CCR	central conserved region
CI	confidence interval
CPE	cytopathic effect
CVR	central variable region
DIC	disseminated intravascular coagulation
DNA	deoxyribonucleic acid
dsDNA	double-stranded DNA
DVO	District Veterinary Officer
ELISA	enzyme -linked immunosorbent assay
EMPRES	Emergency Prevention System
FAO	Food and Agriculture Organization of United Nations
FAT	fluorescent antibody test
HAD	hemadsoption test
kbp	kilo base pair
min	minutes
ml	milliliters
OIE	Office International des Epizooties (World Organization for Animal Health)
OR	Odds ratio
ORF	open reading frame
PCR	polymerase chain reaction

SACIDS	Southern African Centre for Infectious Diseases Surveillance
sec	seconds
SUA	Sokoine University of Agriculture
USA	United States of America
WAHID	World Animal Health Information Database
µl	microlitres = 10^{-6} litres
°C	degree centigrade
x g	centrifuge rotor speed

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

African swine fever (ASF) is an infectious disease of the domestic pigs and other members of the family *Suidae*. ASF affects all age groups of *Suidae* family (Montgomery, 1921; OIE, 2005). The disease is caused by African swine fever virus (ASFV) which is an enveloped icosahedral arbovirus of the genus *Asfivirus* and the family *Asfarviridae* (Dixon *et al.,* 2000). Warthogs, bush pigs and the soft tick of the genus *Ornthodoros* are reservoir of the virus (Plowright *et al*., 1969; Thomson *et al.*, 1981).

ASF was first reported in Kenya in the year 1921 as an acute haemorrhagic fever which caused approximately 100% mortality in domestic pigs and it was proven that the disease outbreak occurred when domestic pigs came into close contact with wildlife species, in particular warthogs (Montgomery, 1921; Costard *et al.,* 2009).

ASF is endemic in most of Eastern and Southern African countries of which Tanzania is inclusive, where it is maintained either in a sylvatic cycle between warthogs (*Phacochoerus aethiopicus*) or bushpigs (*Potamochoerus* spp.) and soft tick vectors of the genus *Ornithodoros* or in a domestic cycle that involves domestic pigs with or without tick involvement (Rowlands *et al*., 2008; Vial *et al*., 2007; Jori *et al*., 2009, Penrith *et al*., 2012).

ASF is difficult to control because (i) no vaccine is available at the moment, (ii) ASFV infection does not elicit the production of neutralizing antibodies and (iii) the soft ticks

Ornithodoros spp which act as a vector of the disease are widely distributed and difficult to control (Boshoff, 2003).

ASF has a severe socio-economic impact, both in areas where it is newly introduced and where it is endemic and causes threat to food security at household and commercial levels due to the direct loss of pigs as well as resulting trade restrictions (Etter *et al*., 2011; Lubisi, 2005). For instance the ASF outbreak which occurred in Tanzania in 2011 killed 300,000 pigs which were approximately valued at US$240 million (Misinzo, 2012c).

In Tanzania the disease was first reported in 1914 (Empres, 2001). Several outbreaks of ASF occurred from the year 1962, 1987 and 1988 that were major epidemics with high economic loss outbreaks occurred in Mbeya, Arusha and Kilimanjaro regions (OIE, 2002; Wambura *et al*., 2006). Other outbreaks occurred at different regions such as Dar es Salaam and Morogoro in the year 2001, 2003 and 2004, Arusha in 2003, Kigoma (Kasulu and Kibondo Districts) in 2004, Mwanza in 2005, Turiani (Morogoro) and Dar es Salaam in 2008 and Longido (Arusha) in 2009 (Wambura *et al*., 2006; Misinzo *et al.,* 2011; Misinzo *et al.,* 2012a). Other outbreaks of the diseases occurred in Mbeya, Rukwa and Dar es Salaam Regions in 2010 (WAHID, 2012).

In 2011, the disease continued to spread to southern parts of Iringa Region (Ludewa district) and other districts of Mbeya Region (Chunya, Ileje, Rungwe and Mbarali). The disease was reported in Morogoro Region in February and July 2012 in Kilombero and Kilosa Districts respectively, and was also reported in Mbeya and Iringa Region in July 2012 (Misinzo *et al.,* 2012b).

1.2 Problem Statement and Justification of the Study

African swine fever has been sporadically occurring in different regions of Tanzania causing deaths in pig populations (Misinzo *et al.,* 2012a; Wambura *et al*., 2006). Iringa Region is among the regions where the disease is thought to be endemic and is the second largest pig producing region in Tanzania according to agricultural sample census of 2007/2008 the first being Mbeya Region. The outbreaks of disease in Iringa Region affected livelihoods of people keeping pigs as well as the nation as a result of direct loss of pigs due to mortality. The reasons for frequent recurrence of ASF in Iringa have not yet been determined, establishment of that reasons will lead to adequate control of the disease which was the purpose of this investigation. The hypothesis is that there are circulating ASFV strains and epidemiological risk factors contributing to frequent outbreak of the disease in Iringa Region. Thus, this study aimed at finding out the circulating strains of ASFV through genotyping and evaluating the epidemiological risk factors for the disease outbreaks. The results from the study will contribute to the design of disease control strategies and policy formulation.

1.3 Objectives

1.3.1 General objective

The main objective of this study was to characterize the of circulating ASFV using recent molecular tools with high precision, and to determine the epidemiological risk factors for frequent outbreaks of African swine fever in Iringa Region.

1.3.2 Specific objectives

(i) To determine the epidemiological risk factors for the outbreaks of the ASF in Iringa and Kilolo Districts.

(ii) To confirm the presence of ASF outbreak using PCR technique during the disease outbreak, and

(iii) To characterize the ASFV from the outbreak in Iringa and Kilolo Districts using PCR.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 African Swine Fever

African swine fever (ASF) is a notifiable highly contagious, fatal, and haemorrhagic disease of pigs (Montgomery, 1921). The mortality of the disease reaches 100% in the susceptible naive domestic pigs (Penrith and Vosloo, 2009). The disease can occur in different forms namely peracute, acute, sub-acute and chronic syndromes (Plowright *et al.*, 1994). The disease is caused by the complex DNA virus of the family *Asfarviridae* and the member of genus *Asfivirus* (Dixon *et al.*, 2000). ASF is defined by the world health organization as the highly contagious disease which can spread rapidly to cause a significant socio-economic impact on people's livelihoods, on national and international trade of animals and animal products and on food security and limits pig production in affected countries (Costard *et al.*, 2009; Sanchez-Vizcaino *et al*., 2009).

2.2 Aetiology

2.2.1 Classification

The causative agent is African swine fever virus (ASFV) which is an enveloped double stranded icosahedral arbovirus of the genus *Asfivirus* and the family *Asfarviridae.* It is the only known DNA arbovirus (Dixon *et al.*, 2000, 2005). The genome varies in length between 170 and 190 kbp depending on the virus isolate due to deletions and insertions occurring within the terminal regions of the genome and within a coding gene region within the central region of the genome termed the central variable region (CVR) (Lubisi, 2005; Sanchez-Vizcaino *et al*., 2009; Owolodun *et al.*, 2010; Dixon, 2012).

ASFV replicates in the cytoplasm of infected cells similar to the poxviruses and shares with the Poxviruses a similar genomic organization, like hairpins ends of the genome with inverted repeat sequences in terminal position (Sanchez-Vizcaino *et al*., 2009). Phylogenetic analysis made possible discrimination of the asfarviruses into a different group separate from the group of poxviruses and iridoviruses (Raoult *et al*., 2004).

2.2.2 Virus structure

Virus particles are organized as a complex multi-layered structure (Sanchez-Vizcaino et *al*., 2009). It has a linear, covalently close-ended double-stranded DNA genome (dsDNA), the virion consists of a glycoprotein core structure with a diameter of 70-100 nm that is covered by a capsid with a diameter ranging from 172-191 nm (Lubisi, 2005; Blasco *et al*., 1989). The genome consist of a central conserved region (CCR) of which has about 125kb long and highly variable region located left and right ends having inverted complementary tandem repeats of about 35kb and 25kb correspondingly (Wesley and Tuthill, 1984; Blasco *et al*., 1989).

The complete sequence of 12 ASFV isolate has been described (Chapman *et al.*, 2008; de Villiers *et al*., 2010; Dixon *et al*., 2012). The whole nucleotide sequence of the genome done from a Spanish isolate (BA71V) adapted to grow in tissue culture cells, showed that it is 170,101 nucleotide length and has 151 major open reading frame (ORF) (Yanez *et al*., 1995). Study from Malawi done to sequence more than half of the genome from a virulent field isolate has been published (Dixon *et al*., 1994; Yozawa *et al*., 1994) as well as comparisons of individual gene sequences from a number of different isolates. Virus replication requires proteins to be encoded, genes that are required for encoding proteins are believed to be 90 (Salas, 1999).

Genes which encode 15 structural proteins have been characterized of which two encode polyproteins p220 and p60. The protein p220 is processed into four virion proteins localized in the virus core which are p150, p37, p34 and p14 proteins while p60 is processed into two virion proteins found on the external membrane of the extracellular particle namely p12 and p24 (Simon-Mateo *et al.*, 1997, 1993; Sanchez-Vizcaino *et al.*, 2009). The envelope contains lipids such as glycolipids, also there carbohydrate which has been identified (Dixon, 2000).

2.2.3 Viral pathogenesis

The virus inters the host through oral, respiratory tract or through biting then goes through the tonsil or dorsal pharyngeal mucosa and to the mandibular or retropharyngeal lymphnodes from there the virus spread through viremia (Sánchez-Vizcaíno *et al.*, 2009; Plowright, 1994). In some cases the virus can go through bronchial, gastrohepatic or mesenteric lymph nodes. After its entrance the viremia is observed after three days (Costard *et al*., 2009).

Injuries on endothelium lead to haemorrhges and disseminated intravascular coagulation (DIC). Hemorrhages, effusions and oedema are preceded by thrombocytopenia, dysfibrinopaenia, coagulopathy and impaired vascular intergrity (Anderson *et al*., 1998; Lubisi, 2005, Hamdy and Dardiri, 1979). The incubation period is usually between 4-6 days (6-8 days in subacute cases) (Sánchez Botija, 1982).

2.2.4 Virus replication

Virus replication occurs in the macrophages and monocyte cells. The mode of entry of the virus is through receptor mediated endocytosis (Alcami *et al*., 1989) and is released from endocytic vesicles into the cytoplasm through fusion of the virus envelope with those of

the endocytic vesicles (Valdeira *et al*., 1998). The replication cycle is mainly cytoplasmic but early replication has been described to occur in nucleus (Rojo *et al.,* 1999; Salas and Andres, 2012, Malogolovkin *et al,* 2012).The role of the nuclear stage is not well understood to date but at this stage the nuclear organization is affected near replication site (Salas and Andres, 2012).

There is expression of the enzymes required for DNA replication immediately following virus entry into the cytoplasm from partially uncoated core. Prior to the cyoplasimic phase there is nuclear replication phase which occurs six hours post infection and diminishes to nearly zero after 12 hours post-infection (Rojo *et al.*, 1999; Dixon *et al*., 2012). Following the onset of DNA replication in the cytoplasm at about 6 hours post-infection a shift in pattern of virus gene transcription occurs (Salas *et al.,* 1986).

DNA replication is initiated by the introduction of a single strand nick in the genome near to one or both termini. The exposed 3′ OH group acts as a primer for DNA polymerase and DNA synthesis proceeds towards the genome termini. This generates an intermediate in which termini of nascent and template strands are self-complementary and fold-back to form a self-priming hairpin structure (Dixon, 2012).

A putative DNA primase (C962R) encoded by ASFV may play a role in initiation of DNA replication or lagging strand DNA synthesis suggestion for *de-novo* replication (Rojo *et al.,* 1999*).* The mature head to head concatemeric intermediates are resolved to unit length terminally cross-linked genomes, and packaged into mature virus particles in the cytoplasmic factory sites (Dixon, 2012).

2.2.5 Virus survival

The virus is highly resistant to low temperatures, inactivated by pH <3.9 or >11.5 in serum-free medium, Susceptible to ether and chloroform, can survive for long time in different environmental conditions, up to 11 days in feces (room temp), 1 month in soiled pig pens, 70 days in blood on wooden boards, 15 weeks in putrefied blood or serum and 18 months in blood at 4^0C. In pork products, the virus can survive for 15 weeks in chilled meat, 300 days in cured hams (Parma hams) and sausages which have not been cooked or smoked at a high temperature and can also remain for 15 years in frozen carcasses (Sanchez-Vizcaino *et al*., 2009; Penrith and Vosloo, 2009).

2.3 Epidemiology

2.3.1 Disease distribution

The disease is indigenous in Africa south of Sahara region where it was reported in 1921 in Kenya by Montgomery and currently the disease is endemic in these areas. The disease was first reported outside the African continent in Portugal in the year 1957 where it caused a mortality rate of 100% to pigs (Wilkinson, 1989). It was found in Spain (1960-1995), France (1964), Italy (1967, 1969, 1993), Malta (1978), Belgium (1985) and the Netherlands (1986), these countries were able to eradicate the disease with the exception of the Sardinia where the disease is endemic (Sanchez-Vizcaino *et al*., 2009; Penrith *et al.,* 2012).

During the 1990s and 2000s, there were changes in the epidemiology and distribution of the disease where by ASFV spread to other regions not naturally affected by ASF, these included West African countries, where the virus was first reported in Cote d'Ivoire (1996), Nigeria (1997), Togo (1997), Ghana (1999), Burkina Faso (2003) and recently, Chad (2010). It also spread to some islands including Madagascar in 1998 and Mauritius

in 2007. Notably, the disease was re-introduced in the European continent in 2007, via Georgia (Sanchez-Vizcaino *et al*., 2012; Costard 2009; Rowlands *et al*., 2008; Rahimi *et al.*, 2007, Rahimi *et al.,* 2010).

2.3.2 Molecular epidemiology

Genetic homogeneity across the C-terminal region of the *B646L* gene that encodes major capsid protein p72 is used to determine the genotype of the virus. There are 22 genotypes of African swine fever according to *p72* (Bastos *et al*., 2003; Lubisi *et al*., 2005; Boshoff *et al.,* 2007).

Genotype I occur to the area where the soft tick is not involved in the cycle that include Europe, western Africa, the Caribbean and South America while the remaining 21 genotypes appear in southern and East Africa where sylvatic cycle is involved (Bastos *et al*., 2003; Boshoff *et al.* 2007; Costard *et al.,* 2012). Genotype II is found in Georgia, Rusia federation, Mozambique, Madagascar, Zambia and Tanzania (Boshoff, 2007; Costard *et al*., 2012; Misinzo *et al.,* 2012b; Malogolovkin, 2012).

Study of ASFV genomes by restriction enzyme site mapping and by partial sequencing of the *B646L* gene encoding the major capsid protein p72 has shown that isolates from Europe, the Caribbean, South America, and West and Central Africa are alike as compared to isolates from South and East Africa which have higher genetic heterogeneity (Nix *et al*., 2006; Boshoff *et al.,* 2007; Bastos *et al*., 2009).

In Tanzania there were three genotypes identified namely X, XV and XVI (Misinzo *et al*., 2011; Lubisi *et al*., 2005). Study done by Misinzo *et al*. (2012b) described the highly virulent genotype II making a total of four genotypes of p72 ASFV circulating in

Tanzania. Current genotype II described is the similar to that identified in Georgia in 2007.

Genotype circulating in different regions of Tanzania as described by different studies shows that genotype XV is found in Morogoro and Dar es Salaam. Genotype X is found in Arusha and Manyara which is genetically the same like that of Kenya also in these regions there is genotype XVI (Misinzo *et al*., 2012a; Lubisi *et al*., 2005). In Mbeya Region the circulating genotype is XVI (Lubisi *et al*., 2005). Study done by Misinzo *et al*. (2012b) showed that genotype II is circulating in Mbeya, Ludewa, Kilombero and Sumbawanga.

2.3.3 Transmission

ASF is transmitted by direct contact with infected animals, bite by the infected ticks (*Ornithodoros spp.*) and consumption of the infected pig meat or indirect by contact with fomite or substances such as clothing, furniture, bedding, footwear or feed contaminated by material having virus such as urine, blood, faeces or saliva from infected pig. Other blood-sucking insects such as mosquitoes and biting flies also have been proven to transmit the virus mechanically (Penrith and Vosloo, 2009).

There are some deviations concerning the role of the chronic carrier animals.Penrith and Vosloo (2009) argue that there is no evidence that recovered pigs can become long-term virus carriers, while Sánchez-Vizcaíno *et al*. (2012) signify that carriers demonstrated in both, wild and domestic animals play a significant role for persistence and spread of the disease in endemic areas.

A review article done by Costard *et al.* (2009) showed that pigs may remain persistently infected for 6 months and during this time act as a source of transmission to susceptible pigs. A study conducted by de Carvalho Ferreira *et al.* (2012) showed that persistent infected animal can be viraemic and shed virus from oropharynx for at least 70 days post infection (Stegeman and Loeffen 2012).

2.3.4 Epidemiological pattern

There are three main types of the epidemiological patterns of African swine fever depending on the involvement of the wild *Suidae* and soft tick and their interaction (Lubisi, 2005; de Carvalho Ferreira *et al*., 2010, Sanchez-Vizcaino *et al*., 2012; Tejler, 2012; Jori *et al*., 2012). These are;

(i) Old enzootic cycle (sylivatic cycle) which involves the warthog and the soft tick which act as the reservoirs of the virus and the disease is transmitted to the domestic pig through tick bite as is the case with numerous outbreaks in eastern and southern African countries. There was a high infection rate in the warthog populations that were examined in east and South Africa regardless of geographical location. A study conducted by Wilkinson (1989), Heuschele and Coggins (1969) reported prevalence of ASF antibodies of 100% in Tanzania (Serengeti area) and 50% in Kenya (Magadi) respectively. A survey conducted in Uganda (Queen Elisabeth National Park) showed a prevalence of 58% in animals from 4 to 12 months, which was declining with age (Plowright 1981). A study done by Penrith *et al*. (2004) in the same area of South Africa showed that ASF seroprevalence ranged from 4% to 90%. There is no horizontal or vertical transmission of the ASFV between warthogs, the virus being maintained between warthog throughs *Ornithodorus moubata* tick (Thomson *et al*., 1980).

(ii) Intermediate enzootic cycle which involves the domestic pig and *Ornthodorus* ticks which stays in the pig pens or crevices in human houses. A good example is found in west-central Malawi (Haresnape *et al.,* 1988; Haresnape and Wilkinson, 1989).

(iii) New epizootic cycle or domestic cycle which is restricted to domestic pigs only. Transmission is through direct contact of infected pigs and indirect through contaminated fomites or infected meat (Lubisi *et al.*, 2005; Jori *et al*., 2012).

2.3.5 Host range

The virus has a range of hosts including African wild swine warthogs (*Phacochoerus aethiopicus*), bush pigs (*Potamochoerus* spp) and giant forest hogs (*Hylochoerus meinertzhageni*) which are reservoir hosts. Domestic pigs (*Sus domestica*), European wild boar, and American wild pigs develop a disease. Ticks of the genus *Ornithodoros* are considered as the natural arthropod host (Lubisi *et al.*, 2005; Sanchez-Vizcaino *et al*., 2012).

2.4 Diagnosis

2.4.1 Clinical diagnosis

The clinical manifestation of the disease depends on the virulence of the virus, the more virulent isolates produce peracute or acute haemorrhagic disease while less virulent isolates produce subacute or chronic forms. The incubation period of the disease ranges between 3-15 days followed by clinical manifestation of the disease as follows according to Plowright *et al*. (1994); Empress (2001); Penrith *et al*. (2004) and Sanchez-Vizcaino *et al*. (2009).

2.4.1.1 Peracute form

The animal may be found dead without showing any clinical signs or sometimes can show some signs before death like becomes recumbent with high fever, shade seeking, huddling together and rapid shallow breathing.

2.4.1.2 Acute form

The sick pig shows the symptoms like fever (40.5 - 42 °C); early leucopaenia and thrombocytopaenia (48-72 hours); huddling together; seek shade and are reluctant to move; reddening of the skin (white pigs), tips of ears, tail, distal extremities, ventral areas of chest and abdomen; anorexia; listlessness; cyanosis; incoordination within 24-48 hours before death; increased pulse and respiratory rate; vomiting; diarrhoea (sometimes bloody) and eye discharges may exist; soiling of the tail and perineum; abortion to pregnant sows may occur and death within 6 - 13 days or up to 20 days. Survivors are virus carriers for life and mortality rate often approaches 100%.

2.4.1.3 Subacute form

The animal losses weight, anorexia, moist coughing, irregular remittent fever for up to one month, dyspnoea, death may occur when animal is exercised, abortion to pregnant sows and the animal becomes sick for 30 - 45 days. Death occurs in a range of two weeks to months and mortality rate ranges from 30% to70%.

2.4.1.4 Chronic form

Growth retardation of growing pigs which have a long hairy coat, severe weight loss lameness caused by arthritis that can also become necrotic, irregular peaks of temperature, low mortality rate of less than 30%.

2.4.2 Pathological lesions

The gloss lesions of the disease depends on the form of the disease as describes here under (Plowright *et al*., 1994; Empress, 2001; Penrith *et al*., 2004; Sanchez-Vizcaino *et al*., 2009).

2.4.2.1 Peracute form

The carcass looks normal but in some circumstances may show skin flushing of extremities and the ventral abdomen in white pigs, congestion of organs and fluid accumulations in the body cavities. The histopathological lesions are infarcts of the spleen; severe necrosis and depletion of lymphocytes of both spleen and lymph nodes; massive infiltration of the bronchioles and alveoli by eosinophilic; exudates of the lungs and dilatation of the sinusoids and eosinophilic intranuclear inclusions in the liver.

2.4.2.2 Acute form

Grossly, visible lesions includes red to purplish cyanosis of the extremities and the ventral surface in white pigs, subcutaneous haemorrhage, cutaneous echymoses on the legs and abdomen, accumulation of straw-coloured blood fluid in body cavities (pleural, pericardial and/or peritoneal), congestion of the organs and hemorrhage of serosa surfaces, petechiae in the mucous membranes of the larynx and bladder, and on visceral surfaces of organs, splenomegaly, enlarged and congested lymphnode of the head and the gastrointestinal tract, failure of lungs to collapse when thoracic cavity is opened. Other lesions are oedema in the mesenteric structures of the colon and near to the gall bladder including wall of gall bladder and the trachea may be full of froth.

2.4.2.3 Subacute form

The dead pig shows interstitial pneumonia, enlarged lymph nodes, serofibrinous pericarditis and adhesions also joint and tendon sheath effusions with oedema of periarticular tissues.

2.4.2.4 Chronic form

Purulent or serofibrinotic arthritis, Lymphnodes draining affected organs are enlarged with a homogeneous firm consistency, there is lung hepatization of lobules and also fibrinous pericarditis.

It is difficult to differentiate ASF clinically from other haemorrhagic diseases of swine such as salmonellosis, classical swine fever, erysipelas, pasteurellosis or other septicaemic conditions. Therefore World Animal Health Organization (OIE) recommend for laboratory confirmation which is done by either virus demonstration or virus antibody detection (OIE, 2008). The OIE Manual recommends the confirmation of suspected cases of disease by using Polymerase Chain Reaction (PCR) which is highly sensitive and rapid technique for ASFV detection.

2.4.2 Laboratory diagnosis

2.4.2.1 Virus detection

(a) Fluorescent antibody test (FAT)

FAT assay is used as an additional method to detect antigen in tissues of suspect pigs in the field or those inoculated at the laboratory. This technique can be used to detect non adsorbing isolates of the ASFV that cannot be detected by hemadsoption test. It can also be used to distinguish CPE of ASFV to other viruses like Aujeszky's disease virus (Sánchez-Vizcaíno, 2006; OIE Manual 2008; Sánchez-Vizcaíno, 2009).

(b) Polymerase Chain Reaction (PCR)

This technique is used to amplify a highly conserved region of the ASFV genome using specific primers for *B646L* gene encoding the major capsid protein p72. Primers for diagnosis used are PPA1/PPA2 which targets the conserved region of *p72* gene (Aguero *et al*., 2003). The technique allows detection of both nonhaemadsorbing and low virulent ASFV and has a high specificity and sensitivity. It is also used in a wide range of circumstances and there is no need to isolate the virus when characterization of the virus is ought to be done (Steiger *et al*., 1992; Bastos *et al.,* 2003; OIE Manual, 2008).

(c) Hemadsoption test (HAD)

The haemadsorption test is based on the capacity of pig erythrocytes to adhere to the surface of pig monocyte or macrophage cells infected with ASFV as described by Malmquist and Hay (1960). The technique is specific to viruses which have hemadsorbing capacity and it is very specific. This is a conclusive diagnostic test for ASF if the results are positive (OIE Manual, 2012).

2.4.2.2 Antibody detection

Antibody detection is recommended to be used in areas where disease is endemic or where the disease is caused by the avirulent or low pathogenic strain of African swine fever virus.

(a) Enzyme linked immunosorbent assay (ELISA)

The technique uses a combination of specific antibodies and specific conjugated photometric antibodies enzyme linked as described by Hamdy and Dardiri (1979). It is used to evaluate antibody in serum or fluid from tissues. ELISA is mostly used to detect the disease in endemic areas or where there is low virulent isolates. It is preferable to

combine this technique with alternative serological test such as FAT or with antigen detection. The sensitivity of the technique becomes low when the samples used are poorly preserved (OIE Manual, 2008, 2012).

(b) Immunoblotting

This technique is based on the antibody-antigen binding principle and combines the resolution of the gel electrophoresis with the specificity of the immunochemical detection. It is sensitive and very specific that can detect even week positive samples (Sanchez-Vizcaino *et al*., 2009; Lubisi, 2005).

(c) Counter immunoelectrophoresis

This technique is not sensitive although it can give results within 30 minutes thus, recommended to be used in screening pools of sera and not individual samples (OIE, 2008).

2.5 Control of ASF

The control of ASF can be done through quarantine of the infected area, with prevention of all movement of animals and animal products and establishment of a zone of surveillance around the infected area. Slaughter with compensation of all affected and in-contact pigs after disease conformation. Proper disposal of the carcasses and infected material by deep burial also disinfection of pig premises. Detailed epidemiological investigation and tracing of possible sources of infection; watchful import policy for animals and animal products; proper disposal of waste food from aircraft or ships coming from infected countries. Efficient sterilization of garbage and avoid contact between pigs and soft tick vectors or their habitats (OIE Manual, 2009; Empress, 2001; Penrith and Vosloo, 2009).

African swine fever outbreaks have been occurring in Southern part of Tanzania killing a lot of pigs thereby causing high economical loss to pig keepers. The disease has no treatment therefore strategies and policy of the disease control should be developed which is possible by knowing the epidemiological risk factors and circulating ASFV.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Kilolo and Iringa municipality districts of Iringa Region located in the southern highland of Tanzania where outbreak of the disease was reported in year 2012. This region borders the dry belt of central Tanzania in the North and it is bordered in the South by Lake Nyasa. Iringa Region is located between latitudes 07º 05′ S and 12° 32′ S and longitude 33º 47′ E to 36° 32′ E. Iringa Region is contiguous with the Dodoma and Singida Regions in the North, Mbeya Region to the West, Morogoro Region in the East and Ruvuma Region in the South.

3.1.1 Iringa Municipal

Iringa municipal is one of the four districts of Iringa Region. It is located between latitude 7° 46′ S and longitude 35° 41′ E. The municipality covers an area of 162 square kilometres. It is situated on a plateau that ranges from 1500 metres to 2500 metres above sea level. Important geological features include numerous steep, rocky hills that punctuate the landscape and the little Ruaha river that runs through the municipality. Iringa municipal has a population of approximately 146 762 people in 2009 of which 76 429 were females and 70 333 were males. Indigenous inhabitants are Wahehe. Administratively the district has one division, 14 wards, 7 villages and 162 streets.

Forty percent of the population of the municipality depends on agriculture and livestock for their livelihood. The crops cultivated are maize, beans, potatoes, tomatoes and vegetables while livestock kept are cattle, goat, sheep, pigs and poultry. Pig keeping is

among of the activities practiced by households as a source of income with total number of pigs estimated to be about 5819.

3.1.2 Kilolo District

Kilolo district is one of the four district of Iringa region, borders to the North and East by the Morogoro region, to the South by Mufindi District and to the West with the Iringa rural District. The district Lies between the latitudes 7° - 8° 3′ S and longitudes of 34° - 37° E. The district covers an area of approximately 7881 km² of which 6803 km² is inhabited while the remaining part is covered by forest, mountains and water surfaces. Topographically the district has three landscape zones namely highlands, midlands and lowlands which are characterized with different climatic conditions.

Administratively the district is divided into three divisions, 22 wards and 106 villages. The district population is approximately 288 500 people and 50 677 households. Households in Kilolo depend on pig keeping for income generation with the number of pigs kept in Kilolo estimated at 22 065.

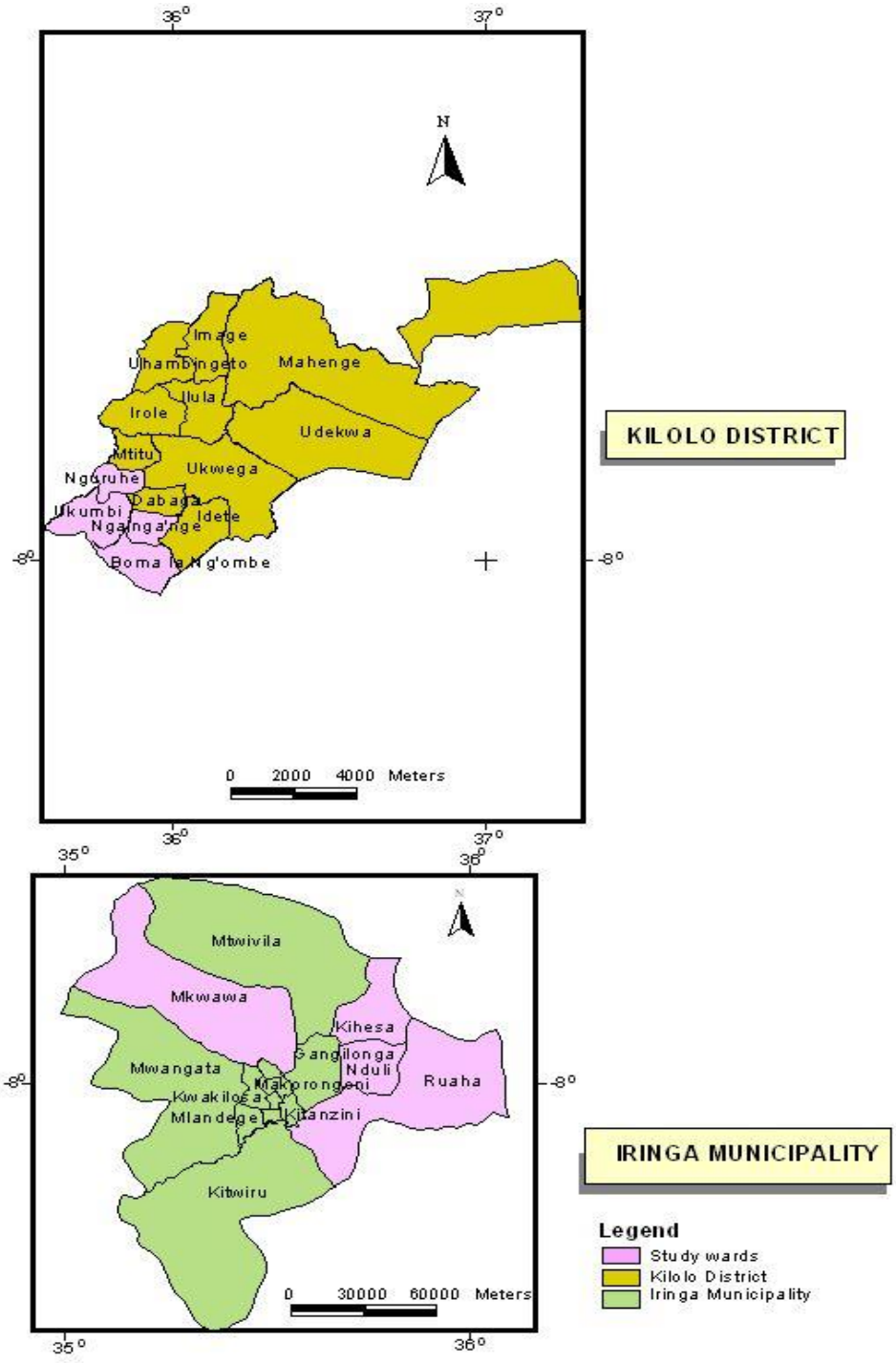

Figure 1: Map of Kilolo and Iringa municipality districts indicating the wards that were included in this study.

3.2 Study Design

A cross sectional study design was adopted where the target population was households that keep pigs and those that had experienced the disease during the 2012 outbreak. Questionnaires were administered to the household heads and blood samples were collected in pigs from the respective households.

3.3 Sampling Procedure

3.3.1 Sampling procedure for epidemiological risk factors

Purposive sampling approach was adopted in selection of the districts and wards based on the disease outbreak of 2012 while villages and streets were randomly selected out of the list of the affected areas. Two districts namely Iringa municipal and Kilolo District were selected. Eight wards were purposively selected from two districts. In this study, sampling units were households of which their herds were affected. The required sample size for pig keepers was 80 households selected randomly from 16 villages/streets.

3.3.2 Sample size

To estimate the required sample size for household interviewed for epidemiological factors of the disease outbreak a prevalence of 95% was assumed (to maximize the required sample size), with a required precision of 5% and an α-error of 5%. The formula that was used in determining sample size is shown below (Pfeiffer, 1999).

$$n = \frac{z_{\alpha}^{2} \times p(1-p)}{d^{2}} \quad \text{.....(1)}$$

Where;

n, is a required sample size

$Z^{2}\alpha$ is the percentile point relating to the required α error under the *z* distribution,

d^{2} is the absolute precision

Eighty household were interviewed that included 10% of estimated sample size which was 73 households.

3.3.3 Sampling procedure for molecular samples

Tissue samples from dead pigs which were suspected to have died in a course of the disease were taken. Samples taken were spleens, lymphnodes, liver and lung.

3.3.4 Sample storage and transportation

Chilled samples were transported in ice to Genome Sciences Laboratory, Faculty of Veterinary Medicine at Sokoine University of Agriculture where laboratory work was performed. The samples were stored at -80 °C until use.

3.4 Collection of the Epidemiological Risk Factors Data

A structured questionnaire was used as a tool to collect data for epidemiological risk factors of the disease from pig keepers. The questionnaire consisted of open and closed ended questions. Pre-testing of a questionnaire was done to respondents selected among the target population and appropriate amendments were incorporated to the final version of the questionnaires. Administration of the questionnaire was done by the researcher in Swahili language. A total of 80 households were interviewed. The focus was on the background information of respondent, knowledge of the disease, disease risk factors, disease mortalities, morbidities and action taken to contain the disease.

3.5 Confirmatory Diagnosis and Genotyping PCR

In this study, PCR was used as a method for diagnosis of the ASFV in tissue samples due to its specificity and sensitivity in disease detection (Aguero *et al.*, 2003). PCR and sequencing were used to perform the molecular characterization of ASFV

3.5.1 DNA extraction

Lymphnode, liver, lung and spleen were pooled and homogenised in a Ham's F-12 cell culture medium (Invitrogen) at ratio of 1 in 10. Thereafter DNA was extracted directly from the pooled samples using QIAmp Viral RNA Kit (City country) following manufacturer's instructions. Briefly, 560µl of prepared buffer AVL containing carrier RNA was added into 140µl of homogenized samples followed with pulse voltexing for 15s. The sample – AVL mixture was incubated at room temperature (25°C) for 10 minutes. Afterwards 560µl of ethanol (100%) was added, vortexed for 15s and centrifuged to pellet precipitated proteins. The supernatant were transferred onto the QIAmp spin column followed by centrifugation at 6000 x g for 1 minute to allow DNA binding into the column. The filtrate was collected in a 2ml collection tube and discarded. The column containing DNA was cleaned with buffers AW1 and AW2 followed by centrifugation at 6000 x g for 1 minute and 17,000 x g for 3 minutes respectively. DNA was finally eluted into 1.5ml eppendorf tube by addition of AVE buffer and centrifugation at 6000 x g. DNA was stored at -20°C until when PCR was performed.

3.5.2 PCR amplification of ASFV DNA

3.5.2.1 Diagnostic PCR.

Diagnostic PCR was performed to confirm the presence of the ASFV nucleic acid in the ASF – suspect samples obtained from Iringa region. In this study, the C-terminal region of the *p72* major capsid protein which encode for *B646L* gene was amplified using the primers; PPA1 (5'-AGTTATGGGAAACCCGACCC-3') and PPA2 (5'-CCCTGAATCGGAGCATCCT-3') which generate amplicon of 257 bp length (Aguero *et al*., 2003). DNA amplification was done in a total of 25µl using an AgPath-ID™ One-Step RT-PCR Kit (Invitrogen).

Table 1 : Master mix preparation for the diagnosis of ASFV

Reagent	Volume (µl) per reaction	Volume (µl) for 20 reactions
2x PCR Buffer with dNTPs	12.5	250
DNA polymerase	0.5	10
Forward primer (PPA1)(10nM)	1	20
Reverse primer (PPA2(10nM)	1	20
Nuclease free water	9	180

DNA amplification was done at 95 °C for 10 minutes as initial denaturation followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 62 °C for 30 seconds, extension at 72 °C for 1 minutes and a final extension at 72 °C for 7 minutes.

3.5.2.2 p72 genotyping PCR

The variable part of the *B646L* gene of ASFV was amplified using primers p72U-(5'GGCACAAGTTCGGACATGT3') and p72D – (5'GTACTGTAAC GCAGCACAG 3') primers as described by Bastos *et al.* (2003), which amplify a 478 bp of a C- terminal region of p72 protein. DNA amplification was done at 95 °C for 10 minutes as initial denaturation followed by 40 cycles of denaturation at 95 °C for 30 seconds, annealing at 48 °C for 30 seconds, extension at 72 °C for 1 minutes and incubation at 72 °C for 10 minutes.

3.5.2.3 p54 genotyping PCR

A full length of the *E183L* gene encoding the p54 protein was amplified in order to place the ASFV in major subgroups using the primers PPA722 (5'-CGAAGTGCATGTAATAAACGTC-3') and PPA89 (5'-TGTAATT TCATT GCGCCACAAC-3') that amply a 676 bp DNA fragment (Gallardo *et al*., 2009). DNA

amplification was done at 95 °C for 10 minutes as initial denaturation followed by 40cycles of denaturation at 95 °C for 30 seconds, annealing at 57 °C for 30 seconds, extension at 72 °C for 1 minutes and a final extension of 72 °C for 10 minutes.

3.5.3 Agarose gel electrophoresis of PCR product

Eletrophoresis of all PCR products was performed using 1.5% agarose gel against the 100bp ladder (Promega). DNA was stained using Gel Red (Biotium, USA) stain for visualization. Products were visualized and photographed through UV light to get results.

3.6 Nucleotide Sequencing and Analysis

Sequencing was performed using p72U, p72D, PPA89 and PPA722 primers using the BigDye terminator v.3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufacturer's instructions and using ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, USA). Sequence analysis was done using sequence scanner v1.0 software (Applied Biosystem). Sequences obtained were aligned using CLUSTAL W while phylogenetic analysis was performed using MEGA 5.1 using the neighbour-joining algorithm method (Tamura *et al.*, 2011). Nodal support was evaluated by 1000 bootstrap replications.

6.7 Data Analysis

Epidemiological data were entered and validated in Excel spreadsheets and exported to computer software Epi Info (Centre for Disease Control and Prevention), where by descriptive statistics like frequency distribution and percentages were analyzed. The presence of the disease (encounter of a disease) was modeled as the dichotomous and run against independent epidemiological risk factors using the unconditional logistic regression. Initial screening of the epidemiological risk factors was done by univariable

uncondional logistic regression. Pairwise associations of independent variables were tested using two-tailed chi-squire test.

Variables which were associated with the presence of the disease at $P \leq 0.4$ were carried forward for multivariable unconditional logistic regression. Backward selection procedure was used in order to reduce the number of variables in the model.

All variables with $P \leq 0.05$ were removed and then were retested. Finally odds ratio, *P*-value and 95% confidence interval were reported.

CHAPTER FOUR

4.0 RESULTS

4.1 Results for Epidemiological Risk Factors of a Disease

In this study a total of 80 respondents were interviewed to find out the risk factors which attributed to the occurrence of the ASF in Iringa region. Out of respondents interviewed 86.3% (n=69) were males and13.8% (n=11) were females.

Pig keeping system in Iringa was intensive system whereby pig keepers did not allow their pigs to roam outside irrespective of the season. Most of the pig pens were constructed using local available materials like woods, bamboo trees and logs. Few pens were constructed using bricks and were cemented. In most cases the roofs of these pens were made using thatch grass and a few using iron sheets.

The disease started in April 2012 in Kilolo District (Mwatasi village) and spread to Iringa municipal in May 2012 (Donbosco Street) (Fig. 2) where the transmission revealed to be introduction of new pigs bought from pig business men who brought the pigs from Kilolo District. Report from Kilolo District showed that the disease started in May showing that there was a delay in reporting of the disease occurrence at the district level.

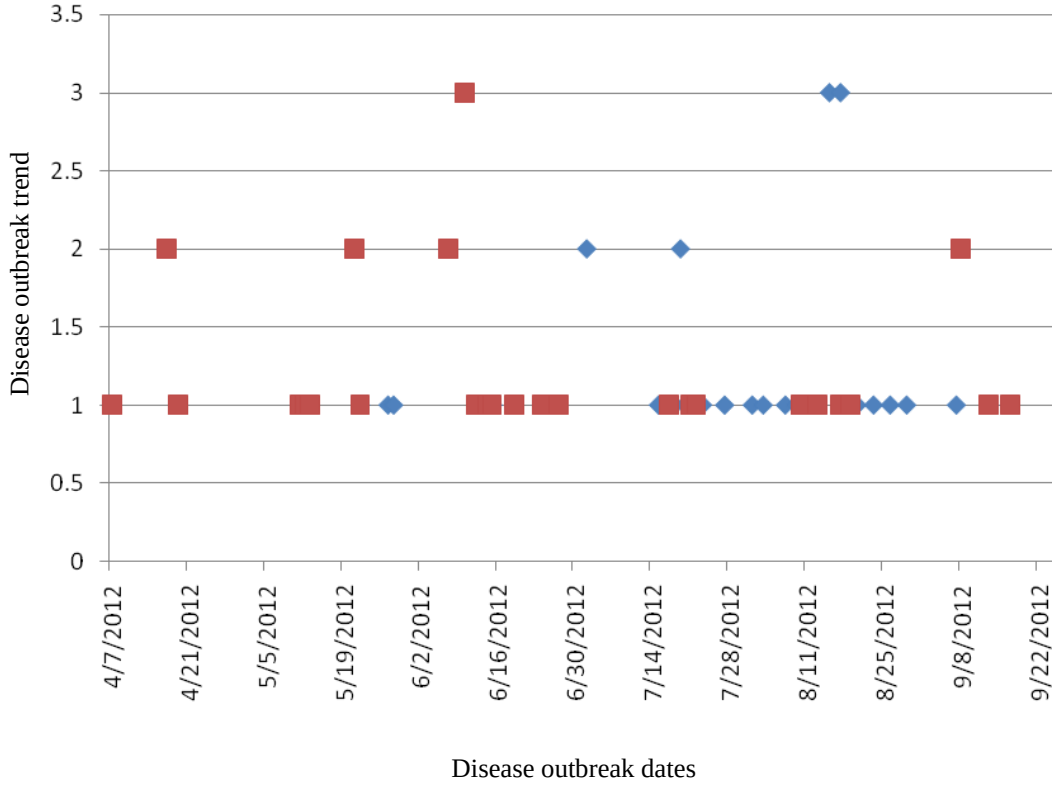

Figure 2: Disease outbreak trend in two districts of Iringa region red dots and blue dots showing outbreaks trend in Kilolo district and Iringa municipal respectively.

4.1.1 Respondent characteristics

Respondent's characteristics are presented in Table 2. The Table showed that most of the respondents 58.8% (n=47) attended primary education that is primary level 5-7/8, followed by ordinary secondary education 26.3% (n=21), advanced secondary education 6.3% (n=5), 2.5% (n=2) attended school above advanced secondary education and there was only 1.3% (n=1) illiterate respondent. Moreover, (82.2% (n=66) respondents were married and only 11.3% (n=9 were single.

Table 2: Respondent's characteristics

Category	Frequency	Percentage
Not gone to school	1	1.3
Primary level (1 - 4)	4	5
Primary level (5 - 7/8)	47	58.8
Secondary (1 - 4)	21	26.3
secondary (5 - 6)	5	6.3
Above secondary 6	2	2.5
Marital status		
single	9	11.3
Married	66	82.2
Widow	3	3.8
Divorced	1	1.3
Separated	1	1.3
Occupation		
Crop farmer	51	63.8
Livestock keeper	14	17.5
Employee	12	15
Trader	3	3.8

4.1.2 Knowledge on the disease

The questionnaire interview results show that many pig keepers were not aware of the disease (Table 3).

Table 3: Knowledge of African swine fever in Iringa 2012

Category	Number of respondents	Number of positive responses(Yes)	Percentage of positive responses
Do you know African swine fever	80	9	11.30
Have you or your neighbour ever encountered ASF	80	1	1.30

4.1.3 Marketing channels

From the study carried out in Iringa (Kilolo District and Iringa Municipal) pig marketing channel comprises of pig producers, pig traders who are involved in live pig or pig meat business and consumers. It was shown that pig traders move to different areas where pigs are kept and buy the pigs from homes of the pig keepers. Out of 80 pig keepers

interviewed 100% said they sell their pigs at their home places for the purpose of slaughtering. Pig keepers sell their pigs direct to traders or sometimes they slaughter and sell meat to consumers. On the other hand traders buy pigs from one district and transport them to another district especially from Kilolo to Iringa municipality. There is no proper market place for selling live pigs in both districts.

In Iringa municipality there is an official pig slaughtering house where all pig traders send their pigs for slaughter. In areas far from the slaughter house, pigs are slaughtered at open places. In Kilolo District there are no official areas where the pigs are slaughtered. Pigs are slaughtered at homes of the traders or homes of pig keepers.

4.1.4 Pig feeding

The results from interview done to pig keepers showed that feeding of pigs in area where the study was done consisted of mainly kitchen leftovers, maize bran, rice polish, local forage and rarely restaurant leftovers. It was found that 98.8% (n=79) of pig keepers feed their pig with kitchen leftovers, 85% (n=68) feed maize bran, 12.50% (n=10) feed rice polish, 22.50% (n=18) feed forage and only 0.038% (n=3) pig keepers feed hotel leftovers (Fig. 3).

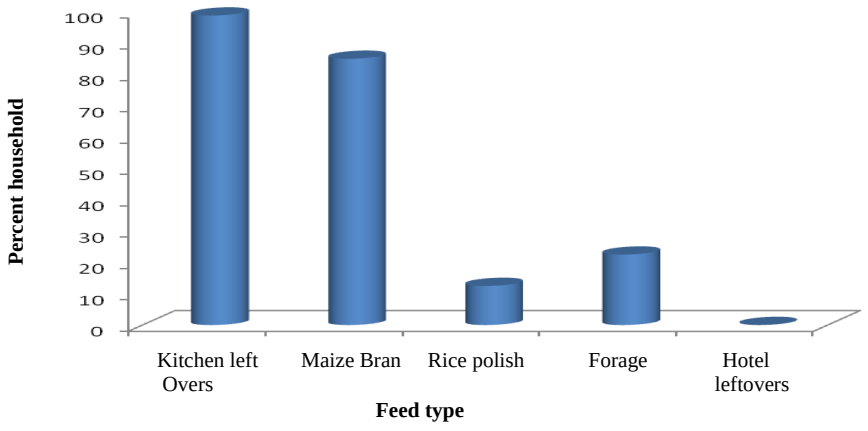

Figure 3: Feeding systems of pig in Iringa

4.1.5 Morbidity and mortality

According to the interview a total herd size was 872, where 776 pigs died and 96 pigs survived the disease which made the average mortality rate of the disease in two districts to be 90.38%. Pigs which contracted the disease were 767, having 87.95% morbidity rate as per table 4. It showed that some of pigs died without showing clinical signs which was difficult to be noticed by the owner.

Table 4: **Morbidity and mortality of pigs during outbreak in Iringa region**

District	Village	Herd size	Morbidity	Mortality	Mortality %
Iringa Municipal	Bwawani	37	35	35	94.59
	Donbosco	72	59	59	81.94
	Ipogolo	31	31	31	100.00
	Kigonzile	94	87	87	92.55
	MsikitinI	170	140	140	82.35
	Nduli	51	45	45	88.24
	Ngeleli	52	46	46	88.46
	Semtema	33	33	33	100.00
Kilolo	Idasi	21	20	20	95.24
	Kihesamgagao	70	63	63	90.00
	Masasali	56	46	46	82.14
	Mdeke	58	45	47	81.03
	Mwatasi	17	17	17	100.00
	Ng'uruhe	35	33	33	94.29
	Wangama	27	22	22	81.48
	Winome	48	45	45	93.75
Total		872	767	776	90.38

4.1.6 Outbreak pattern of the disease

The disease outbreak started in April and May in Kilolo District and Iringa Municipal respectively. The disease outbreak in April started with few cases and went increasing until August thereafter number of cases started decreasing. The disease outbreak cases were few in September (Fig. 4).

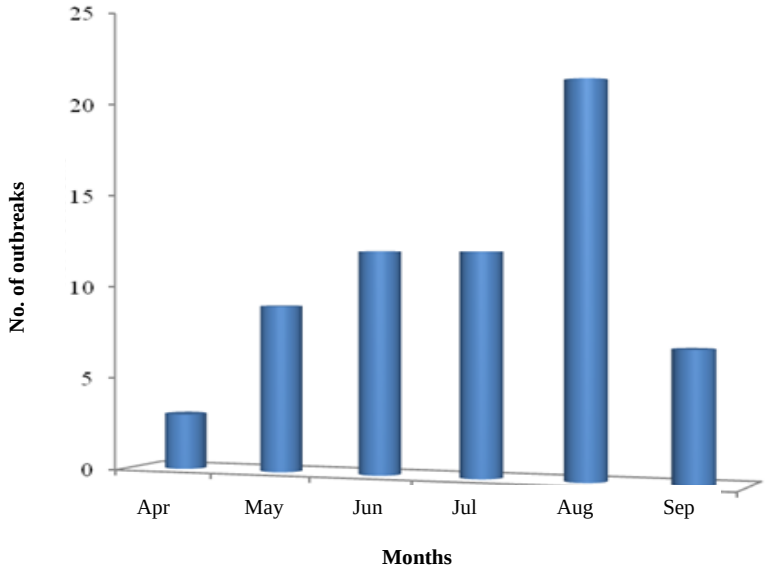

Figure 4: Number of outbreaks in Iringa

4.1.7 Outbreak duration

The duration of outbreak per herd was, on average 16.3 (SD ±10.6) days and the standard deviation of the mean was 10.6 which gave the confidence interval at 95% of ± 2.58. The true population mean was 14-19 days at 95% confidence interval.

4.1.8 Adherence to quarantine conditions and treatment of sick pigs

During the outbreak, the quarantine was imposed so as to contain a disease. It was unfortunate majority of livestock keepers 66.3% (n=53) did not observe the quarantine conditions and 36.7% (n=27) observed the quarantine conditions. On the other hand treatment of sick pigs was done during the outbreak by livestock extension officers or pig keepers. It showed that 68.75% (n=44) of pig keepers treated their pigs and 31% (n=20) did not treat sick pigs (Table 5).

Table 5: Adherence to quarantine conditions and treatment of sick pigs

Category	No	Yes	Percentage No	Percentage Yes
Treatment of sick pigs	20	44	31	68.75
Adherence to quarantine conditions	53	27	63.3	36.7

4.1.9 Univariable and multivariable analysis

Epidemiological risk factors which were included in univariable logistic regression are

- Animal owner/attendant to have contact with the neighbourhood infected farm, presence of ticks on pigs/premises,
- sharing equipment with other pig keepers,
- rats having access to store or pig pens,
- wild birds to have access to pig pens,
- disease presence to the neighbour keeping pigs,
- disposal of dead pigs, presence of wild pigs/warthogs,
- treatment of swills,
- source of stock,
- tendency of hunting wild pigs/warthogs,
- pig butcher/abattoir around,
- introduction of pigs into the herd before disease outbreak,
- feeding kitchen leftovers and consumption of pig meat,
- source of maize bran/rice polish and
- System of pig management (Table 6).

Table 6: Univariable unconditional logistic regression analysis of risk factors of ASF in Iringa region, Tanzania 2012

Variable/Risk factor	OR	CI	P-Value
Animal owner or attendant to have contact with neighbourhood farm	0.5322	2.34	0.4036
Presence of ticks on pigs/premises	0.4839	5.69	0.5638
Sharing equipments with other pig keepers	87183.96	87183.96	0.9745
Rats having access to store or pig pens	248840.84	248840.84	0.9662
Wild birds to have access to pig pens	0.74	7.60	0.7982
Disease presence to the neighbour keeping pigs	2.14	6.5	0.1783
Disposal of dead pigs	224509.38	224509.38	0.9688
Source of stock	1.23	3.99	0.7254
Pig butcher/abattoir around	244761.48	244761.48	0.9707
Treatment of swills	87183.96	87183.96	0.9745
Introduction of pigs into the herd before disease outbreak	6.36	6.36	0.0836
Feeding kitchen leftovers and consumption of pig meat	26.74	214.90	0.002

Variables selected for multivariable unconditional logistic analysis were animal owner or attendant to have contact with neighbourhood infected farm, disease presence to the neighbour keeping pigs, introduction of pigs into the herd before disease outbreak, feeding kitchen leftovers and consumption of pig meat (Table 7). Variables which were statistically significant associated with the occurrence of the disease were introduction of pigs into the herd before disease outbreak (OR=12.2578, $CI_{95\%}$=108.6596, *P*=0.0244) and feeding of kitchen leftovers and consumption of pig meat (OR=35.6117, $CI_{95\%}$ =300.2233, *P*=0.001).

Table 7: Multivariable unconditional logistic analysis of risk factors associated with ASF Iringa region, Tanzania 2012

Variable/Risk factor	OR	CI	P-Value
Animal owner or attendant to have contact with neighbourhood infected farm	0.5237	0.5237	0.5248
Disease presence to the neighbour keeping pigs	2.7121	11.242	0.169
Introduction of pigs into the herd before disease outbreak	12.2578	108.6596	0.0244
Feeding kitchen leftovers and consumption of pig meat	35.6117	300.2233	0.001

4.2 ASF Diagnosis

4.2.1 Gross pathology

Postmortem examinations were performed on dead pigs that were suspected to have died from ASF. Postmortem lesions observed included the congestion and enlargement of the spleen, hemorrhagic enteritis and severe hemorrhages of mesenteric and gastrohepatic lymph nodes (Fig. 5).

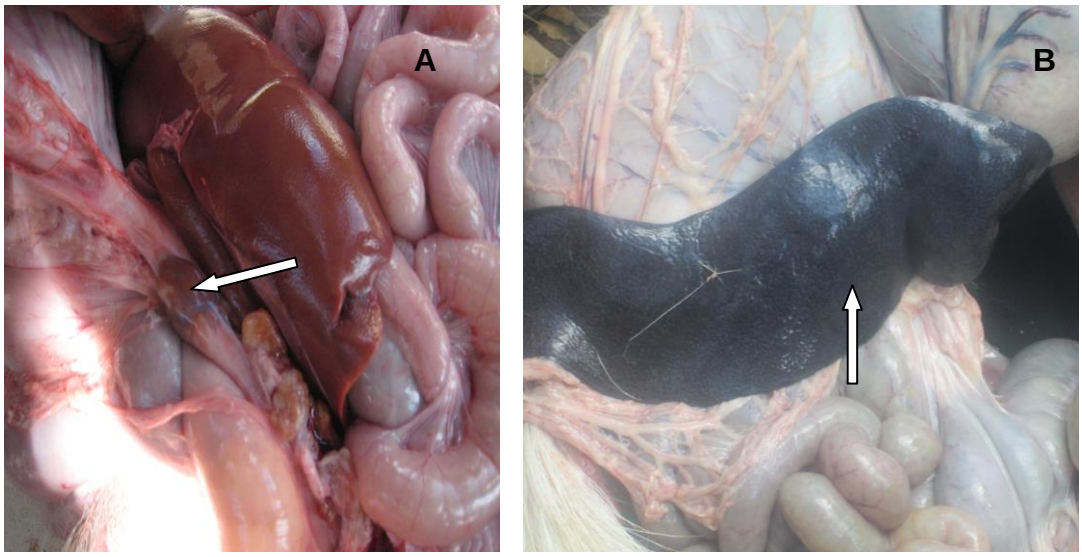

Figure 5: Postmortem pictures of dead pig showing congestion of the gastrohepatic lymphnode (A) and enlargement of the spleen (B) shown by arrow.

4.2.2 Confirmatory diagnosis

In this study, 20 tissue samples were taken from pigs suspected dying of ASF for detection of presence of the disease. Diagnosis of ASF was done using PCR employing diagnostic primers PPA1/PPA2. Out of 20 samples tested 11 samples were PCR positive. Following amplification of ASFV DNA, single major amplicon of approximately 257 bp was generated in 11 positive samples (Fig. 6).

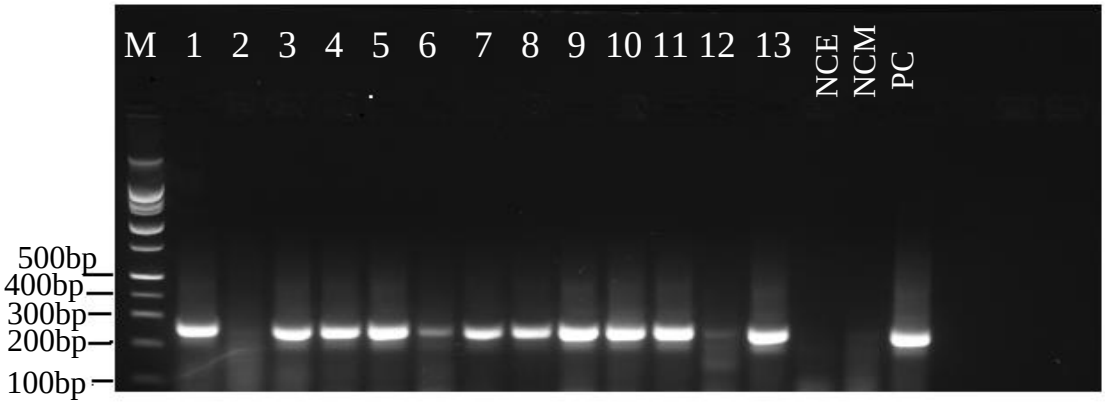

Figure 6: An agarose gel after performing ASFV PCR and electrophoresis. Marker was loaded on lane M while samples 1-13 were loaded lanes 1to 13. NCE = Negative control for extraction, NCM = Negative control for master mix and PC positive control ASFV DNA from Ifakara added into a master mix.

4.3 P72 Genotyping

The *B646L* gene was sequenced and blasted against sequences in a GeneBank (Table 8). Blast of the sequence obtained from Iringa ASFV showed that the virus was 100% identical to ASFV from Georgia 2007 outbreak, Tukuyu (TAN/10/Tukuyu), Kyela (TAN/10/Kyela), Ludewa and Chunya (TAN/11/Chunya) from Tanzania 2010 to 2011

outbreak Circulating Iringa ASFV was classified by comparing with other representative genotypes from each 22 already identified genotypes (Boshoff, 2007) available in GeneBank.

Table 8: Summary of the African swine fever virus (ASFV) isolates p72 genotype used in construction of phylogenic tree.

Isolate	Country	Host species	Year of out break	Town/Province	p72 gene GeneBank accession number	References
Ang72	Angola	pig	1972	Not known	FJ174378	Gallardo *et al*., 2009
Nig01	Nigeria	pig	2001	Not known	FJ174382	Gallardo *et al*., 2010
Georgia2007/1	Georgia	pig	2007		AM999764	Rowlands *et al*., 2008
TAN/12/Iringa	Tanzania	pig	2012	Iringa	KF834193	This study
TAN/10/Kyela	Tanzania	pig	2012	kyela	JX391987	Misinzo *et.al*.,2012
TAN/10/Tukuyu	Tanzania	pig	2012	Tukuyu	JX391988	Misinzo *et.al*.,2012
TAN/11/Chunya	Tanzania	pig	2012	Chunya	JX391989	Misinzo *et.al*.,2012
TAN/11/Ludewa	Tanzania	pig	2012	Ludewa	JX391990	Misinzo *et.al*.,2012
Warmbaths	South Africa	Tick	Not known	Not known	AY261365	unpublished
RSA/1/99/W	South Africa	Warthog	1999	Not known	AF449477	Bastos *et al*., 2003
Mal/2002/1	Malawi	pig	2002	Mpemba quarantine camp	AY494553	Lubisi *et al*., 2005
RSA/1/98	South Africa	Warthog	1999	Not known	AF302818	Bastos *et al*., 2003
MOZ/A-98	Mozambique	pig	1998	Tete	AY274452	Bastos *et al*., 2003
Malawi1978	Malawi	pig	1978	Not known	AF270707	Bastos *et al*., 2003
Kirt89/2	Tanzania	Tick	1989	Kiriwara	AY351511	Lubisi *et al*., 2005
Ug03H.1	Uganda	pig	2003	Hoima	FJ154428	Gallardo *et al*., 2009
Ken06.Kis	Kenya	pig	2006	Not known	FJ154440	Gallardo *et al*., 2009
Ug64	Uganda	pig	1964	Not known	FJ174383	Gallardo *et al*.,2009
MZI/92/1	Malawi	pig	1992	Euthini, MzindaDistric,North Malawi	AY351543	Lubisi *et al*., 2005
MFUE6/1	Zambia	Tick	1983	Sumbu National park	AY351542	Lubisi *et al*., 2005
NYA/1/2	Zambia	Tick	1989	Kalumo	AY351555	Lubisi *et al*., 2005
Tan/1/01	Tanzania	pig	2001	Dar es salaam	AY494552	Lubisi *et al*., 2005
TAN/08/Mazimbu	Tanzania	pig	2008	Mazimbu	GQ410765	Misinzo *et.al*., 2011
TAN/08/Mabibo	Tanzania	pig	2008	Mabibo	GQ410768	Misinzo *et al*., 2011
TAN/2003/1	Tanzania	pig	2003	Arusha	AY494550	Lubisi *et al*., 2005
TAN/2003/2	Tanzania	pig	2003	Arusha	AY494551	Lubisi *et al*., 2005
ZIM/92/1	Zimbabwe	pig	1992	Gweru Midlands	DQ250119	Boshoff *et al*.,2007
NAM/1/95	Namibia	pig	1995	Windhoek	DQ250122	Boshoff *et al*.,2007
RSA/3/96	South Africa	pig	1996	Pienaarsrivier	DQ250127	Boshoff *et al*.,2007
Lillie	South Africa	Tick	1979	Not known	DQ250109	Boshoff *et al*.,2007
RSA/1/96	South Africa	pig	1996	Gravelotte	DQ250125	Boshoff *et al*.,2007
SPEC/245	South Africa	pig	1992	Louis Trichardt	DQ250117	Boshoff *et al*.,2007
sum/1411	Zambia	Tick	1983	Sumbu National park	AY351561	Lubisi *et al*., 2005

Phylogenic analysis of these sequences showed that the Iringa ASFV belonged to B646L genotype II along with ASFV from Georgia (Georgia 2007/1), Tukuyu (TAN/10/Tukuyu), Kyela (TAN/10/Kyela),Ludewa and Chunya (TAN/11/Chunya) (Fig. 7).

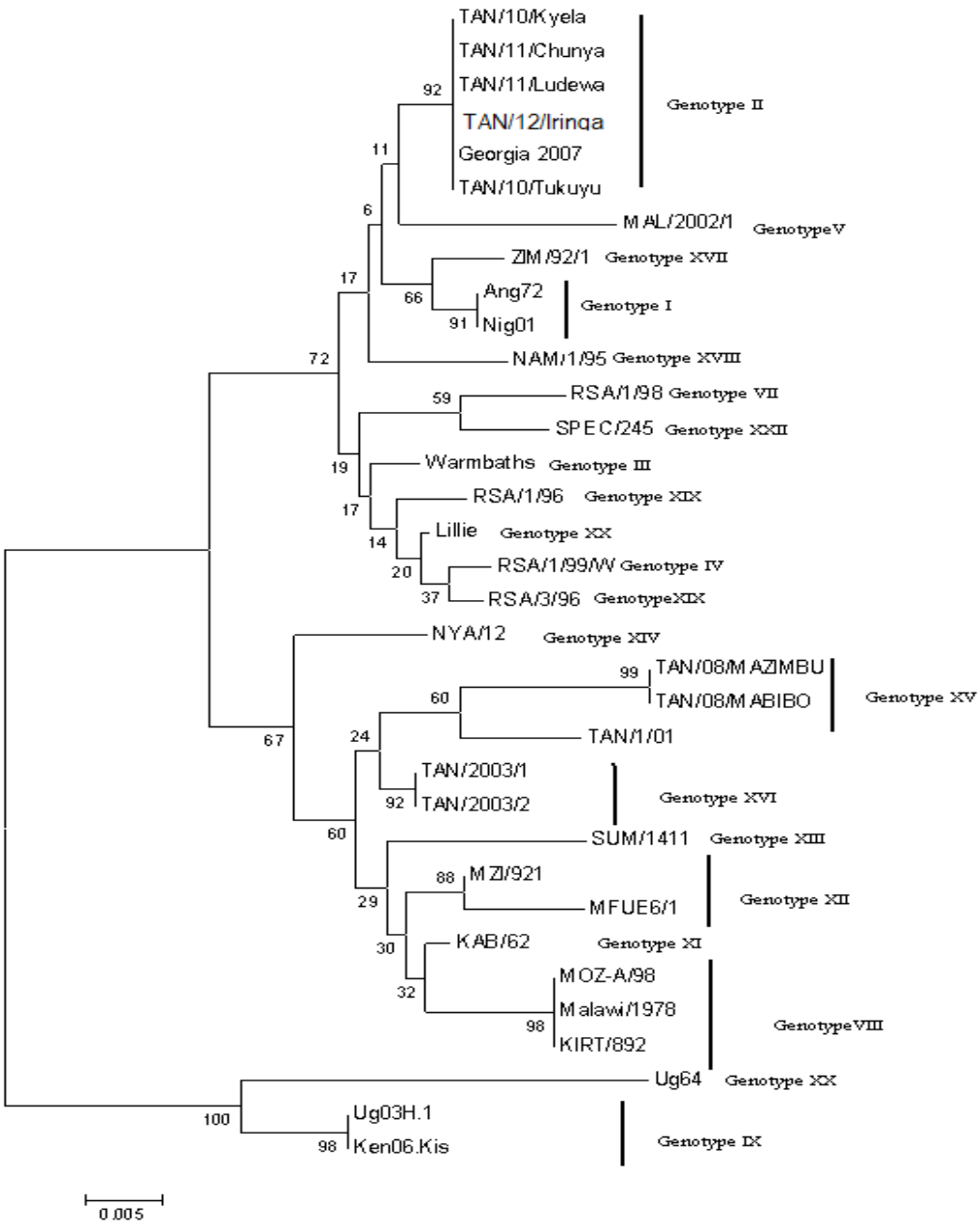

Figure 7: Phylogenetic tree constructed using Neighbour-joining algorithm showing the position of Iringa ASFV as compared to other P72 genotypes from GeneBank database.

P54 genotyping

P54 sequencing has been substantiated to be the valuable additional method, intermediate resolution and molecular epidemiological tool for typing ASFV viruses (Gallardo *et al*., 2009). The sequence obtained was compared to 32 p54 sequences of ASFV available in GenBank to group the circulating TAN/12/Iringa (Table 9).

Table 9: Summary of the reference sequences used for the p54 gene analyses

Isolate	Country	Host species	Year of outbrak	Town/ Province	p54 gene GeneBank accession number	p54 geno type	Refferences
Z85	Spain	Pig	1985	Zaragoza	FJ174396	Ia	Gallado *et al*., 2009
Lisbon57	Portugal	Pig	1957	Lisbon	FJ174420	Ia	Gallado *et al*., 2009
Mafra86	Portugal	Pig	1986	Mafra	DQ028319	Ia	Unpublished
Coimbra87	Portugal	Pig	1987	Coimbra	DQ028319	Ia	Unpublished
Barrancos93	Portugal	Pig	1993	Barrancos	DQ028318	la	Unpublished
Almodovary99	Portugal	Pig	1999	Almodovar	DQ028315	la	Unpublished
Almodovary99 /NEI	Portugal	Pig	1999	Almodovar	DQ028317	la	Unpublished
Georgia2007	Georgia	Pig	2007	Not Known	AM999765	II	Unpublished
Brazil78	Brazil	Pig	1978	Rio Jaineiro	FJ238535	la	Gallado *et al*., 2009
kat67	Zaire	Pig	1967	Katanga	FJ174423	Ib	Gallado *et al*., 2009
CV97	Cape verde	Pig	1997	Not Known	Fj174427	Ib	Gallado *et al*., 2009
CV98	Cape verde	Pig	1998	Not Known	FJ174428	Ib	Gallado *et al*., 2009
Nig01	Niger	Pig	2001	Not Known	FJ174426	Ib	Gallado *et al*., 2009
MZUKI/1997	South Africa	Tick	1979	Mkuzi	AY261362	Id	Complete genome
Moz64	Mozambiqu e	Pig	1964	Not Known	FJ174422	Vb	Gallado *et al*., 2009
MwLil 20/1	Marawi	Tick	1983	Chalaswa	FJ174425	VIII	Gallado *et al*., 2009
Kenya 195	Kenya	Pig	1950	Not Known	AY261360	Xb	Complete genome
pr96/4	South Africa	Tick	1979	Kruger N.park	AY261363	Xxa	Complete genome
LILLIE	South Africa	Tick	1979	Not Known	X84888	XXb	Sun *et al*. (22)
Ken06.Bus	Kenya	Pig	2006	Busia	FJ174446	IX	Gallado *et al*., 2009
ken06.kis	Kenya	Pig	2006	kisumu	FJ174447	IX	Gallado *et al*., 2009
RSA/95/1	S.Africa	Pig	1995	Hoedspuit	DQ250123	XX	Boshoff *et al.*, 2007
CAM/85/4	Cameroon	Pig	1985	Not Known	EU874322	Ia	Bastos *et al*., 2003
CAM/02/4	Cameroon	Pig	1982	Guzang	AF301544	Ia	Bastos *et al*., 2003
GHANA/02/1	Ghana	Pig	2000	Not Known	EU874328	Ia	Bastos *et al*., 2003
BOT/99/1	Botswana	Pig	2008	sherwood	AF504886	III	Bastos *et al.*, 2003
TAN/12/Iringa	Tanzania	Pig	2012	Iringa	KF834194	II	This study
NG70	Angola	Pig	1997	Not nown	FJ174424	I	Gallado *et al*.,2009
Ug03H.1	Uganda	Pig	2003	Hoima	FJ154428	IX	Gallado *et al*.,2009
portalegre 90	Portugal	Pig	1990	portalegre	DQ028323	Ia	Unpublished
ori93	Italy	Pig	1995	Sardinia	FJ174412	Ia	Gallado *et al*.,2009
CAM/02/3	Ghana	Pig	1985	Not Known	EU874325	Ia	bastos *et al*., 2003

The results obtained from this study show that the Iringa isolate clusters together with the Georgia isolate from 2007 outbreak (Fig. 8).

Figure 8: Neighbour-joining tree depicting ASF outbreak viruses p54, Iringa ASFV clustered together with isolate from Georgia. Bootstrap values were obtained through 1000 replications.

CHAPTER FIVE

5.0 DISCUSSION

5.1 General discussion

In this study the epidemiological risk factors and molecular genotypes of ASFV during the year 2012 outbreak were investigated. The average mortality rate of the disease during the outbreak in the two District of Iringa was 90.38%. Mortality rate was 90.01% in Iringa Municipality and 89.74% in Kilolo District. The mortality rate seems to be very high in this outbreak showing that the form of the disease was acute despite that the disease is endemic in the area.

The disease started in April 2012 in Kilolo District (Mwatasi village) and spread to Iringa Municipal in May 2012 (Donbosco Street) where the transmission revealed to be introduction of new pigs bought from pig business men who brought the pigs from Kilolo District. The disease outbreak started in April in Kilolo District with few outbreaks and spread to Iringa Municipal in May, in August number of disease cases increased dramatically and went down in early September, the outbreak ended in September with few outbreaks.

5.2 Univariable and Multivariable Analysis of Epidemiological Risk Factors

In this study different epidemiological risk factors of the disease were studded in Iringa Region involving two Districts namely Kilolo and Iringa Municipality. These factors were animal owner/attendant to have contact with the neighbourhood infected farm, presence of ticks on pigs/premises, sharing equipment with other pig keepers, rats having access to store or pig pens, wild birds to have access to pig pens, disease presence to the neighbour keeping pigs, disposal of dead pigs, presence of wild pigs/warthogs, treatment

of swills, source of stock, tendency of hunting wild pigs/warthogs, pig butcher/abattoir around, introduction of pigs into the herd before disease outbreak, feeding kitchen leftovers and consumption of pig meat, source of maize bran/rice polish, system of pig management, duration of disease outbreak and adherence to quarantine.

Feeding kitchen leftovers and pig meat consumption was highly associated with the presence of disease in pig farms (*P*=0.001).Those pig keepers who consumed pig meat during the outbreak their pigs were found affected. Contributing to inter herd transmission of ASF. The reasons for the outbreak were pig keepers and sellers not to adhere and observe to quarantine conditions during disease outbreak in Iringa. During the study it was found that pig meat sellers pass from one house to another selling pig meat at low prices (2000Tshs.-3000Tshs as compared to normal price 5000Tshs.) and there were calling this meat different local names like mchicha (amaranthus spp). This meat possibly came from infected pigs because inspection was not done and if the kitchen leftovers fed to naïve pigs it would cause infection to pigs. However, feeding the pigs uncooked swills may also contribute to the spread of the disease if are contaminated.

Studies done by Misinzo *et al.* (2012a), Wambura *et al*. (2006) in Tanzania and Tjeler, (2012) in Uganda found that feeding of swills/wastes contributed on the spread of the disease which is the same to this study. On the other hand the results from Malawi by Allaway *et al.* (1995) found that feeding of the kitchen leftovers was not a potential risk factor in the spread of the disease which is contrary to this study.

Introduction of the pigs in the herd was the second epidemiological risk factor associated with disease outbreak (P=0.0244), this factor contributed to both introduction of the disease to new areas and spread of the disease. In this study it was shown that

introduction of pigs from Mufindi District made the disease to be transmitted to Kilolo District and then from Kilolo the disease was introduced to Iringa municipal. The reasons for introduction and spread of disease are pig keepers buying pigs from pig sellers without knowing the health status of the animals and introduce them in their herds thereby if the pigs introduced are sick may cause other pigs to suffer, introduction of pigs for breeding purposes where the farmer may borrow a boar or the sow may be send to the boar for mating when in heat then if there is infection can lead to the spread of the disease, farmers tend to get pigs from their neighbours as a source of stock without knowing if the animals from neighbours are healthy or not. The result from this study is supported by the result reported by Tjeler (2012).

Studies done by Saka *et al.* (2010) and Fasina *et al*. (2012) revealed the risk factors of ASF to be sharing of the implements, means of transportation for inputs and animals and presence of neighbourhood infected farm and the presence of pig abattoir in pig rearing communities. Poor biosecurity practices, bad abattoir practices, pig management system(free ranging pigs), and open live pig markets were reported to be risk factors (Owolodun *et al.,* 2007; Costard *et al.,* 2009; Owolodun *et al.,* 2010).

In this study, it was clearly shown that failure of the pig keepers and other stakeholders to adhere to quarantine condition set by the DVOS was a risk factor for introduction and spread of the disease. Selling and transportation of the pig and pig product leads to the spread of the disease since it is not known that pigs or pig products transported are disease free or they are infected, pigs may be transported while in incubation period which upon stress of transportation and introduction in new herd can reactivate the virus and cause the disease to in-contact pigs. Study done by Gallardo *et al*. (2011) found that 49% of clinically health pigs were PCR positive for ASF. Moreover, slaughtering of pigs

during quarantine period is done secretly and in areas which are not authorized which sequentially can lead to environmental contamination and hence precipitating the disease spread. In Iringa and Tanzania in general quarantine and trade restriction are setup during ASF outbreaks but the implementation is very poor also the compensatory mechanism is not observed which make the pig keepers fraught to sell pigs or pig meat during the disease outbreak.

This study also found that duration of the disease outbreak is one of the risk factors of disease spread between herds. The confidence interval of the disease duration was 14 to 19 days which is a long period for a disease to stay in a herd. The consequence of the disease to stay for a long time in the herd leads to disease spread in a sense that pig keepers or animal attendants continuing to handle such pigs may have contamination and if they get into contact with another neighbours herds they can transmit the disease which is contrary if the disease had a short outbreak duration. In addition veterinarians and livestock extension officers who provide services can transmit the disease between infected herds and none infected herds due to contamination, this study found that treatments of sick pigs were done during disease outbreak making the possibility of transmitting the disease between herds due to the same instrument used for treatment.

5.3 Disease confirmation and charactrerisation of ASFV

This study confirmed that the hemorrhagic disease with high mortality rate to pigs in Iringa was ASF after performing PCR using diagnostic primers PPA1/PPA. Confirmation of ASF using PPA1 and PPA2 primers have been used elsewhere (Aguero *et al.*, 2003). Two regions of genomic fragments of the ASFV were analysed to characterize the virus causing the outbreak of ASF in Iringa in 2012. The variable terminal end of the p72 encoded by the *B646L* gene and gene encoding the p54 were amplified by PCR followed

by sequencing. The sequences obtained were compared with other sequences available in gene GenBank database to evaluate their relationship.

Sequence analysis of p72 genome region from Iringa isolate showed that the virus belonged to genotype II clustering together with the ASFV from (Georgia 2007/1), Tukuyu (TAN/10/Tukuyu), Kyela (TAN/10/Kyela), Ludewa and Chunya (TAN/11/Chunya). The outbreak in Georgia occurred in 2007 and it was suspected that the disease came from Madagascar through imported meat product (Rowlands *et al.,* 2008). The outbreak of the ASF in Tukuyu, Ludewa, Kyela and Chunya occurred in 2010 to 2011 (Misinzo *et al*., 2012b). The results suggest that the same virus which is affecting pigs in Iringa and Georgia is similar and points to a possibility of this virus to have been introduced to Tanzania and Georgia from the same source. ASFV genotype II found in this study was different from all other previous genotypes ASFV that caused different outbreaks in Tanzania in 2001, 2003, 2004 and 2008 (Misinzo *et al.,* 2011; Wambura *et al.*, 2006).

Iringa ASFV has homology to ASFV which was found in Madagascar (MAD 1/98), Mozambique (Moz 1/02, Moz 2/02, Moz 1/03) and Zambia (Lus 1/93) (Rowlands *et al*., 2008; Bastos *et al.,* 2003) suggesting that Iringa 2012 ASFV was introduced from one of these southern African countries.

Sequence analysis of ASF Iringa genome isolate by *E183L* gene encoded by p54 clustered together with 2007 Georgia isolate in genotype II. The data generated using the p54 genes were consistent with the p72 genotyping information showing the ASFV from Iringa clustring in genotype II.

The results from the present study indicate that the ASFV isolate that was introduced in 2010 is still circulating in Iringa. Lack of quarantine enforcement and swill feeding remain the most important factors for the persistence of ASF in the region. Deliberate efforts have to be taken in order to save the industry from ASF devastation.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

African swine fever still poses the devastating deaths to pigs in Iringa and other regions of southern highlands of Tanzania. Since 2001 to date the disease has been circulating among pig farms making it to be endemic in these regions.

Assessment of risk factors of the disease was done through questionnaire whereby it was found that the risk factors for the introduction and spread of the disease are introduction of pigs into the herds, feeding of kitchen leftovers with pig meat consumption, failure of the pig keepers and other stakeholders to adhere to quarantine condition, treatment of sick pigs during outbreak and duration of disease outbreak.

The mortality rate of the disease was very high approximately 90.38% which caused economical loss to pig keepers.

This study found the circulating ASFV in Iringa Region with reference to available 22 genotypes as denoted by p72 protein to be genotype II, the virus found is the same to that genotyped by Misinzo *et al*. (2012) in 2010-2012 outbreak in other regions of Tanzania which is 100% similar to that characterized in Georgia in 2007. The virus has also reported to be found in other countries like Mozambique, Madagascar and Zambia.

Reporting system of disease was seen to be a problem in the study area that there were delays in reporting of the disease occurrence which may make the disease control difficult.

It was found that most of people do not abide to quarantine conditions, all stake holders should make sure that there is no violation of the quarantine conditions.

6.2 Recommendations

i. Further study should be carried out to investigate the source of the disease since this study failed to quantify the source of the disease given that this disease have been circulating in southern highland from 2010.

ii. Early reporting and detection should be emphasized in order to contain the disease. Moreover, slaughter police should be put in function to compensate those affected.

iii. Quarantine conditions should be adhered to. This may help to control the spread of the disease from one area to another. Implementation and means of reinforcing quarantine should be put in place also compensation policy which is in place must work. Animal check points should be established to make sure that animals transported are inspected.

iv. The responsible Ministry which is the Ministry of livestock and fisheries development should make the follow up and take necessary action for disease which is going on, since there is no any in depth and immediate solutions taken to contain the disease. The activities are left to DVOS only who do not have enough fund and power.

REFERENCES

Aguero, M., Fernandez, J., Romero, L., Sanchez-Mascaraque, C., Arias, M. and Sanchez-Vizcaino, J. M. (2003). Highly sensitive PCR assay for routine diagnosis of African swine fever virus in clinical samples. *Journal of Clinical Microbiology* 41: 4431 – 4434.

Allaway, E., Chinombo, D., Edelsten, R., Hutchings, G. and Sumption, K. (1995). Serological study of pigs for antibody against African swine fever virus in two areas of southern Malawi. Revue Scientifique et Technique Office. *Internationale des Epizooties* 14(3) : 667–676.

Alcami, A., Carrascosa, A. L. and Vinuela, E. (1989). The entry of African swine fever virus into Vero cells. *Virology* 171: 68 – 75.

Anderson, E. C., Hutchings, G. H., Mukarati, N. and Wilkinson, P. J. (1998). African swine fever virus infection of the bushpig (Potamochoerus porcus) and its significance in the epidemiology of the disease. *Veterinary Microbiology* 62 (1): 1198 1 – 15.

Bastos, A. D. S., Arnot, L. F., Jacquier, M. D. and Maree, S. (2009). A host species-informative 1220 internal control for molecular assessment of African swine fever virus infection rates in the African sylvatic cycle Ornithodoros vector. *Medical and Veterinary Entomology* 23(4): 399 – 409.

Bastos, A. D. S., Penrith, M. L., Crucière, C., Edrich, J. L., Hutchings, G., Roger, F., Couacy-Hyman, E. and Thomson, G. R. (2003). Genotyping field strains of African swine fever virus by partial p72 gene characterisation. *Archives of Virology* 148: 693 – 706.

Boshoff, K. (2003). The utilisation of strategic analysis and planning by occupational therapy services. *Australian Occupational Therapy Journal* 50(4): 252 – 258.

Boshoff, C. I., Bastos, A. D. S., Gerber, L. J. and Vosloo, W. (2007). Genetic characterisation of African swine fever viruses from outbreaks in Southern Africa. *Veterinary Microbiology* 121: 45 – 55.

Blasco, R., Delavega, I., Almazan, F., Aguero, M. and Vinuela, E. (1989). Genetic variation of African swine fever virus-variable regions near the ends of the viral DNA. *Virology* 173: 251 – 257.

Chapman, D. A. G., Tcherepanov, V., Upton, C. and Dixon, L. K. (2008). Comparison of the genome sequences of nonpathogenic and pathogenic African swine fever virus isolates. *General Virology* 89: 397 – 408.

Costard, S., Mur, L., Lubroth, J., Sanchez-Vizcaino, J. M. and Pfeiffer, D. U. (2012). Epidemiology of African swine fever virus. *Journal of Virus Research* doi:10.1016/j.virusres.2012.10.030).

Costard, S., Wieland, B., de Glanville, W., Jori, F., Rowlands, R., Vosloo, W., Roger, F., Pfeiffer, D. U. and Dixon, L. K. (2009). African swine fever: how can global spread be prevented? Philosophical Transactions of the Royal Society of London. Series B. *Biological Sciences* 364(1530): 2683 – 2696.

de Carvalho, F. H. C., Weesendorp, E., Elbers, A. R. W., Bouma, A., Quak, S., de Villiers, E. T., Gallardo, C., Arias, M., da Silva, M., Upton, C., Martin, R. and Bishop, R. P. (2010). Phylogenomic analysis of 11 complete African swine fever virus genome sequences. *Virology* 400: 128 – 136.

de Carvalho, F. H.,C., Weesendorp, E, Elbers, A. R., Bouma, A., Quak, S., Stegeman, J. A., and Loeffen, W. L. (2012). African swine fever virus excretion patterns in persistently infected animals: A quantitative approach. *Veterinary Microbiology* 160(4): 327- 359.

Dixon, L. K., Alonso, C., Escribano, J. M., Martins, C., Revilla, Y., Salas, M. L. and Takamatsu, H. (2012). *Asfarviridae* Virus Taxonomy: *Ninth Report of the International Conference committee on Taxonomy of Viruses.* Elsevier. pp. 153 – 162.

Dixon, L. K. G. M., Chapman, D. D., Netherton, C. L. and Upton, C. (2010). African swine fever virus replication and genomics. *Virus Research Journal* doi:10.1016/j.virusres.2012.10.020).

Dixon, L.K., Escribano, J.M., Martins, C., Rock, D.L., Salas, M.L. and Wilkinson, P.J., (2005). *Virus Taxonomy. VIII. Report of the ICTV*. (Edited by Fauquet, C. M., Mayo, M. A., Maniloff, J., Desselberger, U. and Ball, L. A.), Elsevier Academic Press, London. pp. 135 – 143.

Dixon, L. K., Costa J. V., Escribano, J. M., Rock, D. L., Venuela, E. and Wilkinsons, P. J. (2000). *Family Asfaviridae. In: Virus Taxonomy, Seventh Report of the International Committee on Taxonomy of Viruses*. Academic Press, London. 165pp.

Dixon, L.K., Twigg, S.R.F., Baylis, S.A., Vydelingum, S., Bristow,C., Hammond, J.M. and Smith, G.L. (1994). Nucleotide-sequence of a 55 kbp region from the right end of the genome of a pathogenic African swine fever virus isolates (Malawi Lil20/1). *Journal of General Virology* 75: 1655 – 1684.

Food and Agriculture Organization (2001). Transboundary animal diseases [http://www.fao.org/docrep/003/Y2283E/Y2283E00.HTM] site visited on 25/8/2012.

Etters, E. M. C., Seck, I., Grosbois, V., Jori, F., Blanco, E., Vial, L., Akakpo, A. J., Bada-Alhambedji, R., Kone, P. and Roger, F. L. (2011). Seroprevallance of African swine fever in Nigeria. *Emerging Infectious Disease Journal* 17(1): 49 – 54.

Fasina, F. O., Shamaki, D., Makinde, A. A., Lombin, L. H., Lazarus, D. D., Rufai, S. A., Adamu, S. Agom, S. D., Pelayo V., Soler, A., Simon, A., Adedeji, A. J., Yakubu, M. B., Mantip, S., Benshak, A. J., Okeke, I., Anagor, P., Mandeng, D. C., Akanbi, B. O., Ajibade, A. A. Faramade, I., Kazeem, M. M., Enurah, L. U., Bishop, R., Anchuelo, R., Martin, J. H. and Gallardo, C. (2010). Surveillance of African swine fever in Nigeria 2006-2009. *Transboundary and Emerging Diseases* 57: 244 – 253.

Food and Agriculture organization (2001). *Manual on Preparation of African Swine Fever Contingency Plan.* FAO Animal Health Services. Animal health manual No. 11. Animal Production and Health Division, Rome, Italy. 70pp.

Gallardo, C., Okoth, E., Pelayo, V., Anchuelo, R., Martín, E., Simón, A., Llorente, A., Nieto, R., Soler, A., Martín, R., Arias, S. and Bishop, R. (2011). African swine fever viruses with two different genotypes, both of which occur in domestic pigs, are associated with ticks and adult warthogs, respectively, at a single geographical site. *Journal of General Virology* 92: 432 – 444.

Gallardo, C., Soler, A., Simón, A., Martín, E., Martín, R., Pelayo, V., Okoth, E., Bishop, R., Sánchez, M. A., De mia, G., Fasina, F. O., Sánchez-vizcaíno, J. M. and Arias, M. (2010). *African swine fever Threat: Evaluating Diagnostic Tools with ASFV Circulating Strains*. Annual Meeting of National African Swine Fever Laboratories, Pulawy, Poland.

Gallardo, C., Reis, A. L., Kalema-Zikusoka, G., Malta, J., Soler, A., Blanco, E., Parkhouse, R. M. E. and Leitão, A. (2009). Recombinant antigen targets for Serodiagnosis of African 1320 Swine Fever. *Clinical and Vaccine Immunology* 16(7): 1012 – 1020.

Gonzalez, A., Talavera, A., Almendral, J. M. and Vinuela, E. (1986). Hairpin loop structure of African swine fever virus-DNA. *Nucleic Acids Research* 14: 6835 – 6844.

Hamdy, F. M. and Dardiri, A. H. (1979). Enzyme linked immunosorbent assay for diagnosis of African swine fever. *Veterinary Record* 105: 445 – 446.

Haresnape, J. M. and Wilkinson, P. J. (1989). A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from three villages in the ASF enzootic area of Malawi following an outbreak of the disease in domestic pigs. *Epidemiology Infectious* 102(3): 507 – 522.

Haresnape, J. M., Wilkinson, P. J. and Mellor, P. S. (1988). Isolation of African swine fever virus from ticks of the *Ornithodoros moubata* complex (Ixodoidea: Argasidae) collected within the African swine fever enzootic area of Malawi. *Epidemiology Infectious* 101(1): 173 – 185.

Heuschele, W. P. and Coggins, L. (1969). Epizootiology of African swine fever virus in warthogs. *Bulletin of Epizootic Disease Africa* 17: 179 – 183.

Jori, F. and Bastos, A. D. S. (2009). Role of wild suids in the epidemiology of African swine fever. *EcoHealth* 6: 296 – 310.

Jori, F., Vial, L., Penrith, M. L., Perez-Sanchez, R., Etter, E., Albina, E., Michaud, V. and Roger, F. (2012) Review of the sylvatic cycle of African swine fever in sub-Saharan Africa and the Indian Ocean. *Journal of Virus Research* doi:10.1016/j.virusres.2012.10.005.

Lubisi, B.A., Bastos, A.D.S., Dwarka, R.M. and Vosloo, W. (2007). Intra-genotypic resolution of African swine fever viruses from an East African domestic pig cycle: A combined *p72*-CVR approach. *Virus Genes* 35: 729 – 735.

Lubisi, A. B. (2005). Molecular epidemiology of African swine fever in East Africa. Dissertation for Award of MSc Degree University of Pretoria, South Africa, 132pp.

Lubisi, B. A., Bastos, A. D. S. Dwarka, R. M. and Vosloo, W. (2005). Molecular epidemiology of African swine fever in East Africa. *Archive Virology* 150: 2439 – 2452.

Malogolovkin, A., Yelsukova, A., Carmina, G., Tsybanov, S. and Kolbasov, D. (2012). Molecular characterization of African swine fever virus isolates originating from outbreaks in the Russian Federation between 2007 and 2011. *Veterinary Microbiology* 158: 415 – 419.

Malmquist, W. A. and Hay, D. (1960). Haemadsorption and cytopathic effect produced by African swine fever virus in swine bone marrow and buffy coat cultures. *American Journal of Veterinary Research* 21: 104 – 108.

Misinzo, G., Kasanga, C. J., Ngeleja, C. M., Masambu, J., Kitambi, A. and Doorsselaere, J. (2012a). African swine fever Virus, Tanzania. *Emerging Infectious Diseases* 181(2): 2081 – 2083.

Misinzo, G., Jumapili, F., Ludosha, M., Mafie, E., Silialis, J., Mushi, R., Viaene, W. and Doorsselaere, J. (2012b). Genotyping of African swine fever virus from a 2009 outbreak in Tanzania. *Research Opinions Animal Veterinary Science* 2(5): 334-338.

Misinzo, G. (2012c). Deadly pig fever sweeps across Tanzania. SUA convocation newsletter, issue No.9987640028.p.33.

Misinzo, G., Magambo, J., Masambu, J., Yongoro, M. G., Van Doorsselaere, J. and Nauwynck, H. J. (2011). Genetic characterization of African swine fever viruses from a 2008 outbreak in Tanzania. *Transboundary Emerging Diseases* 58: 86 – 92.

Montgomery, R. E. (1921). On a form of swine fever occurring in British East Africa (Kenya colony). *Comparative Pathology* 34: 159 – 191.

Nix, R. J., Gallardo, C., Hutchings, G., Blanco, E. and Dixon, L. K. (2006). Molecular epidemiology of African swine fever virus studied by analysis of four variable genome regions. *Archive Virology* 151: 2475 – 2494.

OIE (2005). *Meeting of OIE Working Group on Wildlife Diseases*. Report No. 4. Paris, France. 47pp.

OIE (2008). African swine fever. In: Manual of diagnostic tests and vaccines for terrestrial animals mammals, birds and bees. [http://www.oie.int/e ng/normes/ mmanual/2008/pdf/2.08.01_ASF.pdf] site visited on 24/6/2012.

OIE (2009). Event Summary: African swine fever, Mauritius. [http://www.oie.int/wa his/public.php?page=eventsummary&thiscountrycode=MUS&reportid=63 69] site visited on 24/7/2013.

OIE (2012). WAHID interface animal health information African swine fever [athttp://web.oie.int/wahis/public.php] site visited on 20/6/2013.

Owolodun, O. A., B., Yakubu, J. F., Antiabong, O. K., Adefalujo, M. E., Ogedengbe, E. Obishakin, T. and Shamaki, D. (2007). Molecular assessment of African swine fever in North Central Nigeria. *Bulletin Animal Health Production Africa* 55: 96 – 103.

Owolodun, O. A., Bastos, A. D. S., Antiabong, J. F., Ogedengbe, M. E., Ekong, P. S. and Yakubu, B. (2010). Molecular characterization of African swine fever viruses from Nigeria (2003-6) recovers multiple virus variants and reaffirms CVR epidemiological utility. *Virus Genes* 41(3): 361 – 368.

Penrith, M. L., Thomson, G. R. and Bastos, A. D. S. (2004). *African Swine Fever. In: Infectious Diseases of Livestock with Special Reference to Southern Africa*. (Edited by Coetzer, J. A. W. and Tustin, R. C.), Oxford University Press, London, UK. pp. 1087 – 1119.

Penrith, M. L., Vosloo, W., Jori, F. and Bastos, A. D. S. (2012) African swine fever virus eradication in Africa. *Journal of Virus Research* doi:10.1016/j. virusres.2012.10.011).

Penrith, M. L. and Vosloo, W. (2009). Review of African swine fever. Transmission spread and control. *South Africa Veterinary Association* 80(2): 58 – 62.

Pfeiffer, D. V. (1999). *Veterinary Epidemiology*. An introduction. Epicentre, Newzland. 345pp.

Plowright, W. (1981). *African swine fever. In: Infectious Diseases of Wild Mammals.* (Edited by Davis, J. W., Karstad, L. H. and Trainer, D. O.), Ames, State University Press, Iowa Iowa. 190pp.

Plowright, W., Parker, J. and Pierce M. (1969). African swine fever virus in ticks (*Ornithodoros moubata*, Murray) collected from animal burrows in Tanzania. *Nature* 221: 1071 – 1073.

Plowright, W., Thomson, G. R. and Neser, J. A. (1994). *African Swine fever. In Infectious Diseases of Livestock with Special Reference to Southern Africa*. (Edited by Coetzer, J. A. W., Thomson, G. R. and Tustin, R. C.), Cape Town Oxford University Press, Oxford. 599pp.

Rahimi, P., Sohrabi, A., Ashrafihelan, J., Edalat, R., Alamdari, M., Masoudi, M., Mostofi, S. and Azadmanesh, K. (2007). Emergence of African swine fever virus, Northwestern Iran. *Emergence Infectious Disease* 16(12): 1946 – 1948.

Rahimi, P., Sohrabi, A., Ashrafihelan, J., Edalat, R., Alamdari, M., Masoudi, M., Mostofi, S. and Azadmanesh, K. (2010). Emergence of African swine fever virus, northwestern Iran. *Emerging Infectious Diseases* 16: 1946 – 1948.

Raoult, D., Audic, S., Robert, C., Abergel, C., Renesto, P., Ogata, H., La Scola, B., Suzan, M. and Claverie, J. M. (2004). The 1.2-megabase genome sequence of Mimivirus. *Science* 306: 1344 – 1350.

Rojo, G., Garcia-Beato, R., Vinuela, E., Salas, M. L. and Salas, J. (1999). Republiction of African swine 858 fever virus DNA in infected cells. *Virology* 257(2): 524 – 536.

Rowlands, R. J. V., Michaud, L., Heath, G., Hutchings, C., Oura, W., Vosloo, R., Dwarka, T., Onashvili, E., Albina, E. and Dixon L. K. (2008). African swine fever virus isolate, Georgia, 2007. *Emerging Infectious Diseases* 14: 1870 – 1874.

Saka J. O., Adesehinwa, A. O. K. and Ajala, M. K. (2010). Incidence of African swine fever disease and its associated implications on pig production in Lagos State, Nigeria. *Bulgarian Journal of Agricultural Science* 16(1): 80 – 90.

Salas, M. L., Rey-Campos, J., Almendral, J. M., Talavera, A. and VinÄuela, E. (1986). Transcription and translation maps of African swine fever virus. *Virology* 152: 228 – 240.

Salas, M. (1999). *African Swine Fever Virus* (*Asfarviridae*). In: Encyclopedia of Virology. (Edited by Allan Granoff, R. G. W.), Academic Press, London. 38pp.

Salas, M. L. and Andrés, G. (2012). African swine fever virus morphogenesis. *Virus Respondent* doi:10.1016/j.virusres.2012.09.016.

Sanchez, B. C. (1982). African swine fever: New developments. *Revue Scientifique et Technique* 1(4): 1065 – 1094.

Sánchez-vizcaíno, J. M. (2006). *African Swine Fever Diseases of Swine*. (Editors by Straw, B., D'Allaire, S., Mengeling, W. and Taylor, D.), Iowa State University, USA. 298pp.

Sanchez-Vizcaino, J. M., Marinez-López, B., Marinez-Avilés, M., Martins, C., Boinas, F., Vial, L., Michaud, V., Jori, F., Etter, E., Albina, E. and Roger, F. (2009). *Scientific Review on African Swine Fever*. Scientific report submitted to EFSA. 141pp.

Sanchez-Vizcaino, J. M., Mur, L. and Marinez-Lpez, B. (2012). African swine fever. An epidemiological update. *Transboundary and Emerging Diseases* 59(1): 27 – 35.

Simon-Mateo, C., Andres, G., Almazan, F. and Vinuela, E. (1997). Proteolytic processing in African swine fever virus: evidence for a new structural polyprotein, Journal *of Virology* 62(71): 5799 – 5804.

Simon-Mateo, C., Andres, G. and Vinuela, E. (1993). Polyprotein processing in African swine fever virus-a novel geneexpression strategy for a DNA virus. *EMBO Journal* 12: 2977 – 2987.

Stegeman, J. A. and Loeffen, W. L. A. (2012). African swine fever virus excretion patterns in persistently infected animals: A quantitative approach. *Veterinary Microbiology* 160: 327 – 340.

Steiger, Y., Ackermann, M., Mettraux, C. and Kihm, U. (1992). Rapid and biologically safe diagnosis of African swine fever virus infection by using polymerase chain reaction. *Journal Clinical Microbiology* 30(1): 1 – 8.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* 28: 2731 – 2739.

Tejlar E. (2012). *Outbreaks of African Swine fever in Domestic Pigs in Gulu District.* Uganda. 85pp.

Thomson, G. R., Gainaru, M., Lewis, A., Biggs, H., Nevill, E., van der Pypekamp, M., Gerbes, L., Esterhuysen, J., Bengis, R., Bezuidenhout, D. and Condy, J. **(**1981). The relationship between ASFV, the warthog and *Ornithodoros species* in southern Africa. In *ASF, Eur8466 EN. Proceedings of CEC/FAO Research Seminar* (Edited by Wilkinson P. J.). Sardinia, Italy. pp. 85 – 100.

Thomson, G. R., Gainaru, M. D. and Van Dellen, A. F. (1980). Experimental infection of warthog (Phacocherus aethiopicus) with African swine fever virus. *Onderstepoort Journal of Veterinary Research* 47: 19 – 22.

Valdeira, M. L., Bernardes, C., Cruz, B. and Geraldes, A. (1998). Entry of African swine fever virus into Vero cells and uncoating. *Veterinary Microbiology* 60: 131–140.

Vial, L.,Wieland, B., Jori, F., Etter, E., Dixon, L. and Roger, F. (2007). African swine fever virus DNA in soft ticks, Senegal. *Emerging Infectious Diseases* 13: 1928 – 1931.

WAHID (2012). Interface-OIE World Animal Health Information Database. African swine fever Tanzania. [http://web.oie.int/wahis/publicc.php?page=single_report&pop=1&r eportid=11508] site visited on 24/6/2012.

Wambura, P. N., Masambu, J. and Msami, H. (2006). Molecular diagnosis and epidemiology of African swine fever outbreaks in Tanzania. *Veterinary Research Communication* 30: 667 – 672.

Wesley, R.D. and Tuthill, A. E. (1984). Genome relatedness among African swine fever virus field isolates by restriction endonuclease analysis. *Preventive Veterinary Medicine* 2: 53 – 62.

Wilkinson, P. J. (1989). *African Swine Fever Virus. In: Virus Infections of Vetebrates: Virus Infections of Porcines*. (Edited by Penjaert, M. B.), Elsevier, Amsterdam. 35pp.

Yanez, R. J., Rodriguez, J. M., Nogal, M. L., Yuste, L., Enriquez, C., Rodriguez, J. F. and Vinuela, E. (1995). Analysis of the complete nucleotide-sequence of African swine fever virus. *Virology* 208: 249 – 278.

Yozawa, T., Kutish, G. F., Afonso, C. L., Lu, Z. and Rock, D. L. (1994). 2 Novel Multigene families, 530 and 300, in the Terminal variable regions of African Swine Fever virus genome. *Virology* 202(2): 997 – 1000.

APPENDICES

Appendix 1: Questionnaire for Pig Keepers

Q. NO.....

EVALUATION OF EPIDEMIOLOGICAL RISK FACTORS IN THE AFRICAN SWINE FEVER OUTBREAK IN IRINGA, 2012

INTERVIEW SCHEDULE FOR INDIVIDUALS

Part A: Location And Administrative Issues

A1: Time now ☐ Hr ☐ Minutes

A2: Name of interviewer: ☐

A3: Today's date: Day Month Year

A4: Name of Village: ☐ Geo-ref: S ☐ E ☐

A5: Name of Ward: ☐

A6: Name of Division: ☐

A7: District's name: ☐

Part B: Respondent's Characteristics

B1: Name of Respondent: [ ]

B2: Sex of Respondent: 1 Male ( ) 2 Female ( )

B3: Age in years: …… years

B4: Level of education:

1 Not gone to school ( ) 2 Primary 1-4 ( ) 3 Primary 5-7/8 ( )

4 Secondary 1-4 ( ) 5 Secondary 5-6 ( ) 6 Beyond secondary 6 ( )

B5: Primary occupation:

1 Crop farmer ( ) 2 Livestock keeper ( ) 3 Employee ( ) 4 Trader ( )

B6: Secondary occupation:

1 Crop farmer ( ) 2 Livestock keeper ( ) 3 Employee ( ) 4 Trader ( )

Others specify ……

B7: Marital status of respondent:

1 Married ( ) 2 Never married ( ) 3 Widow ( )

4 Divorced ( ) 5 Separated ( ) 6 widower ( )

B9: Which of the following do you keep?

1 Chicken ( ) 2 Cattle ( ) 3 Pigs ( ) 4 Do not keep any livestock ( )

Part C: Information of African swine fever

C1. General information

C1.1: Number of years lived in this village/area*:* ☐

C1. 2: Number of years keeping pigs ☐

C1.3: Do you know African swine fever?

Yes 1( ) No 2( )

C1.4: Have you ever or your neighbor encountered African swine fever?

Yes 1( ) No 2( )

C1.5: If yes when was it ……..

C1.6: Breeds of the pigs.

Local breed 1 ( ) Cross breed 2 ( ) Exotic breed 3 ( )

C2.Disease morbidity

C2.1: Have you ever encountered case of African swine fever in your herd?

Yes 1 ( ) No 2 ( )

C2.2: If yes, what was the date when pigs started suffering ……

What was the date when pigs stopped suffering ……

C2.3: How many pigs were you having before outbreak?

ADULT BOAR	ADULT SOW	GROWERS	WEANERS	PIGLETS

C2.4: How many pigs got sick?

ADULT BOAR	ADULT SOW	GROWERS	WEANERS	PIGLETS

C3 Disease mortality

C3.1: How many pigs died?

ADULT BOAR	ADULT SOW	GROWERS	WEANERS	PIGLETS

C3.2: What actions did you take to prevent losses?

No actions 1( ) Slaughtered 2 ( ) Sold 3 ( ) Treatment 4 ( ) Disinfection 5( ) Others specify…….

C3.3: Where was your source of the disinfectant …….

C4.Disease risk factors

C4.1: Where is your source of stock?

Fellow Farmers 1( ) Research projects 2 ( ) Commercial farmers 3 ( ) NGO'S 4( ) Own source 5( )

C4.2: System of pig management

Free ranging system 1 ( ) Tethering system 2 ( ) Semi-intensive system3 ( ) Intensive system 4 ( )

C4.4: What type of feed do you feed your pigs?

Kitchen leftovers 1 ( ) hotel leftovers 2 ( ) Maize bran 3 ( ) Rice polish 4( ) Others specify……

C4.5: If kitchen left over have you consumed pig meat?

Yes 1( ) No2 ( )

C4.6: If hotel left over does that hotel save pig products?

Yes 1( ) No2 ( )

C4.7: Do you treat the swills?

Yes 1( ) No2 ( )

C4.8: If Maize bran or Rice polish where do you get them? ……..

C4.9: Did you introduce pigs into the herd before disease outbreak?

Yes1 ( ) No2 ( )

C4.10: If yes, when …….. and where did you bring the pig(s) from? …….

C4.11: Have you or animal attendant have contact with infected neighbourhood pig farms? Yes 1 ( ) No2 ( )

C4.12: Are there any ticks observed on pigs or premises?

Yes1 ( ) No2 ( )

C4.13: If yes which one among of these shown? ……

C4.14: Do you share farm equipment with other pig keepers?

Yes1 ( ) No2 ( )

C4.15: If yes which equipments? ……..

C4.16: Are rats having access to store or pig pens?

Yes1 ( ) No2 ( )

C4.17: Do wild birds enter pig pens?

Yes1 ( ) No2 ( )

C4.18: Do any of your neighbor keeping pigs have encountered the disease?

Yes1 ( ) No2 ( )

C4.19: How do you dispose the dead pigs?

Deep burial 1( ) throw away 2 ( ) Others specify ……

C4.20: Are there any wild pigs around?

Yes1 ( ) No2 ( )

C4.21: If yes do you have a tendency of hunting wild pigs?

Yes1 ( ) No2 ( )

C4.22: Where do you sell your pigs ……. and for what purpose …….

C4. Veterinary services

C5.1: Did you report the occurrence of the disease to the veterinary officer?

Yes 1( ) N o2 ( )

C5.2: Which services were you given?

Treatment of sick pigs1 ( ) advice2 ( ) Others specify ……

C5.3: Were you satisfied with the service given?

Yes1 ( ) No2 ( )

C5.4: Did you adhere to quarantine conditions?

Yes1 ( ) No2 ( )

C5.5: Did other people adhere to quarantine conditions?

Yes1 ( ) No2 ( )

C5.6: Were you informed about the opening the quarantine?

Yes1 ( ) No2 ( )

C5.7: Were you told when to restock the pigs?

Yes1 ( ) No2 ( )

C5.8: Were you told the source of stock?

Yes1 ( ) No2 ( )

J21 Time now: Hr Minutes

THANK YOU FOR YOUR TIME AND RESPONSES