STUDIES ON EPIDEMIOLOGY AND SOCIO-ECONOMIC IMPACT ASSOCIATED WITH AFRICAN SWINE FEVER 2015 – 2017 OUTBREAKS IN TANZANIA

CLARA YONA

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EXTENDED ABSTRACT

African swine fever (ASF) is a highly fatal hemorrhagic disease of domestic pigs caused by ASF virus (ASFV) that can cause mortalities reaching up to 100%, depending on the virus strain. There have been sporadic ASF outbreaks in Tanzania that have affected food security and the livelihoods of pig farmers. Previous studies have reported the genetic nature viral strains that caused ASF outbreaks in Tanzania. The present study was conducted to investigate whether new or already described ASFV strains were involved during the November 2015 to June 2017 ASF outbreaks in different parts of Tanzania. In addition, the socio-economic impact and risk factors that are responsible for the occurrence and spread of ASF outbreaks in Tanzania were investigated. The study involved visits to slaughter facilities, pig farms and pig markets. Clinical signs were observed in pigs suspected with ASF prior to sample collection and postmortem examination was undertaken to the dead pigs. Tissue samples including spleen, lymph nodes and kidney were collected from a total of 124 dead pigs during reported outbreaks. A semi-structured questionnaire was used to investigate the ASF risk factors and its socio-economic impact to the farmers. The presence of ASFV in collected samples was detected by polymerase chain reaction (PCR) by partial amplification of the p72 (*B646L*) gene using *peste porcina Africana* (PPA1/2) primers. Genetic characterization was conducted in samples that were positive for ASFV by amplification, by nucleotide sequencing of the variable 3'-end of p72 (*B646L*) gene using primers p72U/D. During the survey, suspected outbreaks of ASF were reported in Kalambo, Ileje, Mbarali, Rungwe, Mbeya Municipality, Mbozi, Kongwa, Dodoma Municipality, Mpwapwa, Gairo, Temeke, Mvomero, Morogoro Municipality, Kibaha, Bukoba, Magu, Ngara, Babati, Mwanza, Manyoni and Kigoma districts. The clinical signs observed in affected pigs included sternal recumbency, cutaneous congestion on the outer side of the pinna, abdomen and limbs, inappetence, staggering gait, shivering,

hyperthermia and abortion in pregnant sows. Upon nucleotide sequencing and phylogenetic analysis, genotype II of ASFV was found in domestic pigs from Mbarali, Rungwe, Mbeya Municipality, Kalambo, Ileje, Mbozi, Kongwa, Dodoma Municipality, Mpwapwa, Gairo, Temeke, Mvomero, Morogoro Municipality and Kibaha districts, genotype IX in domestic pigs of Bukoba, Magu and Ngara districts while genotype X was found in samples collected from Babati, Mwanza, Manyoni and Kigoma districts. The spread of genotype II ASFV into Central and Eastern Tanzania, from southern highlands of Tanzania seems to be along the Tunduma-Dar es Salaam and Morogoro-Dodoma roads. This signifies a risk of further spread of genotype II of ASFV northwards along Dodoma-Mwanza road and to the neighboring countries. Sharing of farm equipment was found to be significantly associated with the spread of ASFV (OR=2.47, CI95%=1.4-99, P=0.023). The possible occurrence and spread of ASFV in Tanzania is within the domestic cycle rather than sylvatic cycle. The presence of ASF was found to lead to the financial losses, loss of income, unemployment, mental disturbance and poor livelihoods. Also respondents failed to meet medical expenses, farm labor expenses and school fees for their children. The disease poses a great threat to the pig industry and food insecurity. Further studies are recommended in order to fully sequence ASFV isolates obtained from the present study in so that to fully understand the genetic relatedness, evolution and epidemiology of ASFV in the country.

DECLARATION

I, Clara Yona, 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.

_____ _____

Clara Yona Date

(Candidate: Masters of Philosophy)

The above declaration is hereby confirmed

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

_____ _____

Prof. Hans J. Nauwynck Date

(Supervisor)

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DEDICATION

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LIST OF ABREVIATIONS 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
ds	double-stranded
DVO	District Veterinary Officer
EFSA	European Food Safety Authority
ELISA	enzyme-linked immunosorbent assay
FAT	fluorescent antibody test
FAO	Food and Agricultural Organization
HAD	hemadsoption test
kbp	kilo base pair
MGF	multigene families
OIE	World Organization for Animal Health
OR	odds ratio
ORF	open reading frame
PCR	polymerase chain reaction
SUA	Sokoine University of Agriculture
WAHID	World Animal Health Information Database

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

African swine fever (ASF) was first described in Kenya, East Africa, in the year 1921 (Montgomery, 1921). The first outbreak of ASF was retrospectively recognized to have occurred in the year 1907 (Plowright, 1969). The disease was confined to Africa mainly affecting domestic pigs of settlers until 1957 when it was first reported outside Africa in Portugal (Wilkinson, 1989). It is currently endemic in at least 26 African countries, south of Sahara. The disease can have severe socio-economic impact on people's livelihoods, food security affecting both regional and international trade, making ASF the main threat to both commercial and smallholder pig farmers (Penrith *et al*., 2013).

African swine fever is a highly fatal haemmorhagic viral contagious disease of domestic pigs caused by ASF virus (ASFV) (Dixon *et al*., 2005). The disease manifests as a hemorrhagic fever that can cause up to 100% mortalities in affected pigs depending on the strain (Costard *et al*., 2009). Pigs infected with ASFV gradually lose appetite and become depressed and cutaneous congestion is observed on the outer side of the pinna, abdomen and limbs, abortion in the pregnant sows, respiratory distress, vomiting, bleeding from the nose or rectum and sometimes diarrhea can also be observed (Penrith *et al*., 2004).

African swine fever virus contains a double-stranded deoxyribonucleic acid (dsDNA) and replicates in the cytoplasm of the infected cells (Dixon *et al*., 2012). The ASFV belongs to the family *Asfarviridae* and genus *Asfivirus* (Dixon *et al*., 2005), being the only member of this family and the only DNA arbovirus (arthropod-borne virus). Warthogs (*Phacochoerus africanus*) and bush pigs (*Potamochoerus* spp) are the natural hosts of ASFV which are

persistently infected by the virus with no apparent disease. Soft argasid ticks of the genus *Ornithodoros* act as vectors of ASFV.

1.2 African swine fever virus

1.2.1 Classification

The ASFV family name *Asfarviridae* is derived from African swine fever and related viruses (Dixon *et al.*, 2005). The ASFV particle has an icosahedral morphology, is enveloped and contains a dsDNA genome whose size varies between 170 to 193 kilo base pairs (kbp) containing between 150 and 167 open reading frames (ORF) (Yanez *et al*., 1995; Chapman *et al*., 2008; de Villiers *et al*., 2010). The difference in genome lengths is caused by insertion and/or deletion of ORFs of the multigene families (MGF).

African swine fever virus replicates in the cytoplasm of the infected cells, a similar characteristic to poxviruses. It also shares a similar genomic organization to poxviruses, for instance, the hairpins ends of the genome have inverted repeat sequences in the terminal positions (Sánchez-Vizcaino *et al.*, 2009). Phylogenetic analysis of ASFV made it possible to discriminate the asfarviruses into a different group other than the poxviruses and iridoviruses

1.2.2 Virion structure

African swine fever virus particles are organized as a complex multi-layered structure with a linear, covalently close-ended dsDNA (Fig. 1) (Sánchez-Vizcaino *et al.*, 2009). The virus particles consist of a nucleoprotein core structure with a diameter of 70-100 nm, which is surrounded by a lipid membrane covered by a capsid with a diameter ranging from 172-191 nm (Blasco *et al.*, 1989; Lubisi *et al*., 2005). The membrane lipids formed as a result

of budding off from the host cell plasma membrane and membrane proteins that are important for virus attachment into host cells (Sanz *et al*., 1985; Salas and Andrés, 2013).

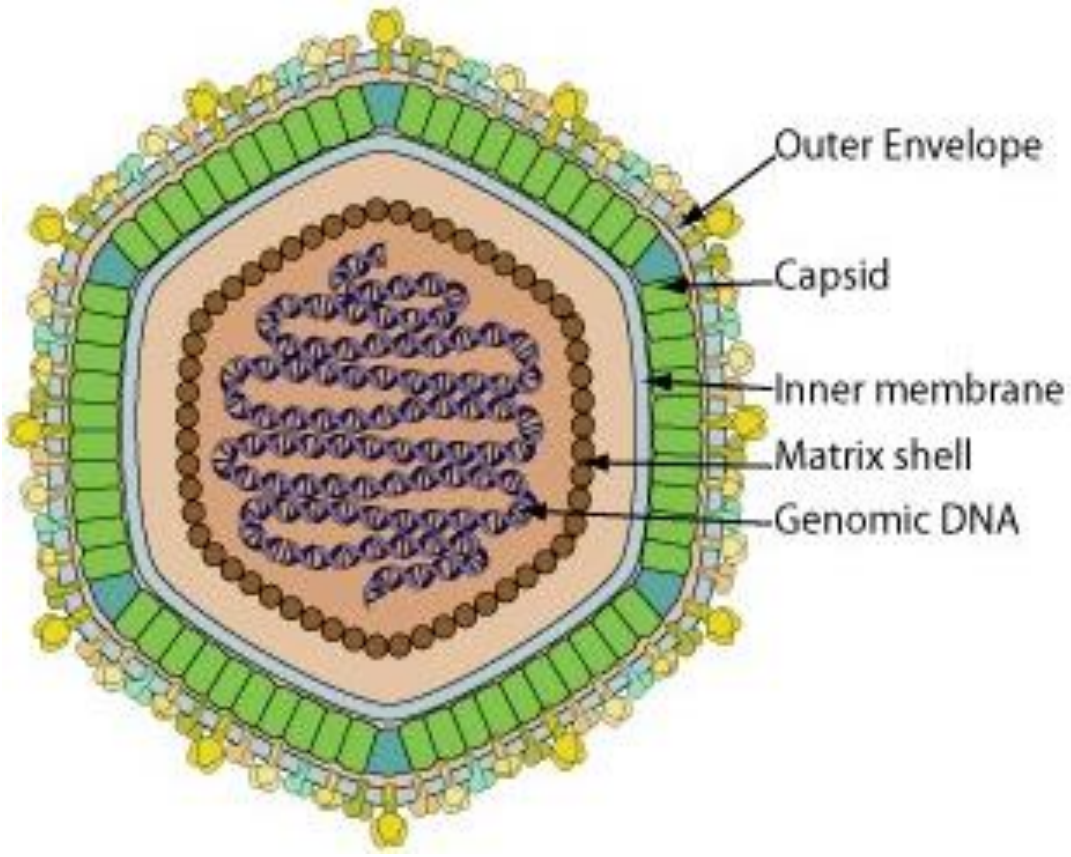

Figure 1: African swine fever virion **(**Source www. viralzone.expasy.org/ all by species /288.html)

1.2.3 The genome organization

The ASFV genome is a linear dsDNA consisting of a central conserved region (CCR) of about 125 kbp long and highly variable region located at the left and right ends of the genome having inverted complementary tandem repeats of about 35 and 25 kbp, respectively (Wesley and Tuthill, 1984; Blasco *et al*., 1989). The ASFV genome encodes for five multigene families, putative membrane and the secreted proteins, including enzymes that are required for virus replication and protein modification (Tulman *et al*., 2009). Variation of genome between the different ASFV isolates is due to gain or loss of MGFs 100, 110, 300, 360, 505/530 and family p22 which are located within the left

terminal 40 kbp and right terminal 20 kbp. Up to date only 12 ASFV complete genome sequences have been described (Chapman *et al*., 2008; de Villiers *et al*., 2010; Dixon *et al*., 2012).

1.2.4 Virus replication cycle

The ASFV replication occurs in the cytoplasm but early stages of replication have been described to occur in the nucleus (Salas and Andres, 2013). The virus enters the host cell via clathrin-mediated endocytosis involving viral proteins p12, p30, p54 and p72 (Alcami *et al*., 1989). The virus is released from endocytic vesicles into the cytoplasm through fusion of the virus envelope with that of the endocytic vesicles (Valdeira *et al*., 1998). The cellular receptors for the virus are cluster of differentiation 163 (CD163) molecules that are present on the surface of the cells. The ASFV has the preferential tropism to swine macrophages and monocytes. However, in the later stages of the disease other cells like dendritic cells, endothelia cells and other cell types can be infected (Minguez *et al*., 1988; Rodriguez *et al*., 1996; Dixon *et al*., 2005).

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). The DNA replication is initiated by the introduction of a single strand nick in the genome near to one or both termini. The exposed 3'-hydroxyl 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 *et al*., 2012). A putative DNA primase encoded by the *C962R* gene of ASFV may play a role in initiation of DNA replication or lagging strand DNA synthesis, suggesting for *de-novo* DNA 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 *et al*., 2012).

1.2.5 Physical and chemical properties of the virus

African swine fever virus is resistant to different physical and chemical actions. The virus is highly resistant to lower temperatures such as 2 ℃ or 4 ℃. The virus is susceptible to inactivation at lower pH below 3.9 or higher pH above 11.5 in serum free medium, the resistance of the virus tends to be increased by serum. For example, at pH 13.4, resistance lasts only for 21 hours without serum and 7 days with serum (Dixon *et al*., 2005). Also the virus is susceptible to different chemicals/disinfectants like ether and chloroform, and is inactivated by sodium hydroxide, hypochlorites, formalin, ortho-phenylphenol and iodine compounds (Dixon *et al*., 2005). The virus remains viable for long periods in blood, faeces and tissues; especially infected, uncooked or undercooked pork products. The ASFV can survive up to 11 days in faeces at room temperature, about a month in soiled pig pens, 70 days in the blood on wooden boards, 15 weeks in the putrefied blood or serum and 18 months in the blood at 4 °C (Penrith and Vosloo, 2009).

The long survivability of ASFV in meat and other products increases the spread of the virus in the unsanitary local pork markets. The virus can survive to about 15 weeks in chilled meat, about 300 days in cured hams and sausages and for about 15 years in the frozen carcasses (Penrith and Vosloo, 2009; Sánchez-Vizcaino *et al*., 2009).

1.2.6 Viral pathogenesis

The virus enters the host through oral, respiratory tract or through biting. The virus then goes through the tonsil or dorsal pharyngeal mucosa and to the mandibular or retropharyngeal lymphnodes from there the virus spread through viremia (Plowright *et al*.,

1994; Sánchez-Vizcaíno *et al*., 2009). In some cases the virus can go through bronchial, gastrohepatic or mesenteric lymph nodes (Costard *et al*., 2009). It has been shown in new-born piglets that primary viraemia are demonstrated approximately 8 hours post-infection, while secondary viraemia occurs 15 to 24 hours post-infection and after 30 h, virus may be found in almost all organs (European Food Safety Authority (EFSA), 2009).

The release of active substances including the cytokines, complement factors and arachidonic acid metabolites leads to impaired haemostasis that tend to cause massive destruction of macrophages hence plays a major role in the pathogenesis. The monocytes and macrophages secrete a range of soluble mediators including proinflammatory cytokines such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor alpha (TNF-α) (Unanue, 1993). The TNF-α induces vasodilation and activation of vascular endothelium, all of which alter the balance between procoagulant and anticoagulant activities and favour generation of microthrombi. It follows that, elevated systemic levels of TNF-α result in disseminated intravascular coagulation leading to extensive haemorrhages, shock, multiple organ failure and death.

Infected pigs suffer severe lymphopenia due to apoptosis of lymphocytes induced by production of pro-inflammatory cytokines by infected macrophages, usually suddenly during the initial-middle phase of disease. In chronic infection, an auto-immune component may occur, and resulting lesions are due to the deposition of immune-complexes in tissues such as kidneys, lungs and skin with their subsequent binding to complement (Blome *et al*., 2013). The ASFV incubation period is usually between 4-6 days and 6-8 days in subacute cases.

1.3 Epidemiology

1.3.1 Disease distribution

The disease was firstly reported in 1907 in Kenya (Montgomery 1921), and is still endemic in Africa, south of Sahara and in Sardinia (Italy). In 1957, ASF escaped out of Africa to Portugal where the mortalities rates reached up to 100% to pigs (Wilkinson, 1989). Later on, the disease spread to Spain, France, Italy, Malta, Belgium and the Netherlands.

In 1990s and 2000s, ASFV spread to other regions not naturally affected by ASF, including West African countries, where the virus was reported in 2010 in Ivory Coast, Nigeria, Togo**,** Ghana, Burkina Faso and Chad. The virus also spread to Madagascar in 1998 and Mauritius islands in 2007. The disease was re-introduced in the European continent in 2007, into the Republic of Georgia (Rahimi *et al.*, 2010; Rowlands *et al.*, 2008; Costard *et al.*, 2009; Sánchez-Vizcaino *et al.*, 2012), which then spread throughout the Caucasus region and the Russian Federation. By 2014, ASF had already spread to Ukraine, Belarus, Lithuania, Poland, Estonia and Latvia, affecting both the domestic pigs and wild boars (Gallardo *et al.*, 2009). It is feared that, ASF could spread to Western Europe and China, which are the major pork producers (Vergne *et al*., 2017).

1.3.2 Molecular epidemiology

So far 23 ASFV genotypes (I-XXIII) have been identified based on nucleotide sequencing of the variable 3'-end of the *B646L* gene that encodes for the capsid protein p72 (Gallardo *et al.*, 2009; Achenbach *et al.*, 2016). All 23 ASFV p72 genotypes have been reported to circulate in eastern and southern Africa. Outside Africa, Genotype I was the only genotype found in Europe, America, and the Caribbean (Nix *et al.*, 2006; Boshoff *et al.*, 2007; Bastos *et al.*, 2009), until the recent introduction of genotype II in Caucasus and

Malawi, Mozambique, Madagascar and Mauritius islands (Bastos *et al*., 2003; Boshoff *et al.*, 2007).

The ASFV circulating in Tanzania has been identified to be p72 genotypes II, IX, X, XV, and XVI (Lubisi *et al*., 2005; Wambura *et al*., 2006; Misinzo *et al*., 2011, 2012a, 2012b, 2014). Incursion and persistent circulation of a highly virulent p72 genotype II ASFV in Kyela in 2010 and later on in the southern highlands of Tanzania was described by Misinzo *et al*., (2012b). This genotype later on spread further to other parts and was reported in Mbeya, Iringa, Rukwa and Dar es Salaam. The ASFV genotype II in the southern highlands and other parts of the country is identical to the Georgia 2007/1 strain (Misinzo *et al.*, 2012b).

Different studies have identified genotype IX to be circulating in Northwestern part of Tanzania, Kigoma and Mwanza (Wambura *et al*., 2006). Genotype X, which is identical to the Kenya's isolate and XVI have been identified in the Northeastern part of Tanzania (Arusha, Machame, Rombo and Moshi and Longido) (Lubisi *et al*., 2005; Misinzo *et al*. 2012a). Genotype XV has been identified in Eastern part of Tanzania in Morogoro and Dar es Salaam regions (Misinzo *et al*., 2011).

African swine fever is a transboundary animal disease; therefore, there is an obligation to inform neighboring countries and the international community of its presence and persistence in some parts of the country. This is achieved through effective diagnosis and surveillance to allow timely intervention. Given the increase in new outbreaks and ASFV circulation among susceptible populations and for future eradication programs of the Food and Agricultural Organization (FAO) and the World Organization for Animal Health

(OIE), it is important to characterize ASF viruses and understand the factors that are associated with the occurrence of ASF in some areas in Tanzania.

1.3.3 African swine fever virus hosts

1.3.3.1 Domestic pigs

The virus can get into the domestic pigs population through domestic cycle or/and tick-domestic cycle. Once the virus is introduced to the domestic pig population ASF is typically associated with high morbidity and mortality rates and the rapid spread of outbreaks. In Africa, resistance to ASF infection has been observed with the proposed hypotheses involving an acquired immunity from previous exposure to lower doses of virus or to related viruses of the reduced virulence which may have been emerged from circulation in domestic pig population as stated by Penrith *et al.,* (2004). The sub-clinically infected, chronically infected or recovered pigs play an important role in the epidemiology of ASF persistence in endemic areas as well as for causing outbreaks or introduction into disease free areas via direct contacts or indirectly via tick bites or ingestion of contaminated formites or infected meat and products (Lubisi *et al*., 2005; Jori *et al*., 2013).

1.3.3.2 Wild suids

The virus has a range of wild suid hosts that include warthogs (*P. africanus*), bush pigs (*Potamochoerus* spp) and giant forest hogs (*Hylochoerus meinertzhageni*). In Africa, warthogs are considered as the most important vertebrate reservoir for ASFV due to their wide distribution and ecology (Lubisi *et al*., 2005; Sánchez-Vizcaino *et al*., 2012). This accelerates warthogs' contact with the *Ornithodoros* ticks that are living in the burrows but also with the domesticated pigs (Jori and Bastos 2009).

1.3.3.3 Soft ticks

Ornithodoros erraticus was first identified as a biological vector and reservoir for ASFV in Spain which then led to the discovery that ticks from the *O. moubata* complex play a role in the epidemiology of ASF in Africa. *Ornithodoros moubata* are a source of infection with ASFV for both domestic and wild pigs. The transmission of ASFV occurs during blood meals (Plowright *et al*., 1969), the infected pigs are able to retain the virus for a long period and transit to the susceptible hosts. In the absence of the viraemic hosts the persistence of ASFV infection is through transstadial, transovarial and sexual transmission in the *O. moubata*. *Ornithodoros moubata* are widely distributed in Southern and Central Africa and is thought to be absent in West Africa.

1.3.4 Epidemiological patterns of ASF

There are three epidemiological patterns of ASF depending on the presence of wild suidae and soft ticks (Lubisi *et al*., 2005; Sánchez-Vizcaino *et al*., 2012; Jori *et al*., 2013). These include Old enzootic (sylvatic cycle), intermediate enzootic and new epizootic (domestic cycle). The sylvatic cycle involves the warthog and the soft tick which they tend to act as the virus' reservoirs. African swine fever is transmitted to the domestic pig through tick bite. There is no vertical or horizontal transmission of the ASFV in warthogs. The virus is being maintained in soft argasid ticks (Thomson *et al*., 1980). Historically, in early studies in East and South Africa a high infection rate in the examined warthog populations was reported. In Tanzania (Serengeti), Wilkinson (1989) reported a prevalence of ASF antibodies of 100% and 50% in Kenya (Magadi). In Uganda (Queen Elisabeth National Park), a prevalence of 58% was reported by Plowright (1981). Penrith *et al*., (2004) studied the ASF seroprevalence in South Africa and reported it to be up to 90%.

The intermediate enzootic cycle involves the domestic pig and the soft argasid tick, *Ornithodoros* spp. These ticks tend to stay in the pig pens or crevices in human houses. Through biting a domestic pig, the ASFV gets transmitted to the domestic pigs from the ticks (Haresnape *et al.,* 1988; Wilkinson, 1989).

The new epizootic (domestic cycle) is restricted to the domestic pigs alone. The transmission can be through direct or indirect transmission routes, like direct contact of infected pigs and through infected meat, contaminated fomites or substances such as furniture, clothing, bedding, contaminated feeds with materials like urine faeces, saliva, and blood from the infected pig (Lubisi *et al*., 2005; Mur *et al*., 2012; Jori *et al*., 2013).

1.3.5 Transmission

1.3.5.1 Sylvatic cycle

In southern and eastern Africa, a sylvatic cycle has been well documented as the key transmission cycle involving the circulation of the virus between the warthogs (*P. africanus*) and soft ticks of the genus *O. moubata complex,* (Thomson, 1985; Plowright *et al.,* 1994), which historically are considered as the main source of outbreaks. The warthog remains asymptomatically infected for life but due to the absence of horizontal and vertical transmission between warthogs, the maintenance of infection is dependent on the ticks alone. The *O. moubata* colonies can maintain the ASFV infection for up to 15 months in the absence of blood meals (Plowright *et al.,* 1994), hence allowing subsequent transmission cycles with warthogs at the next farrowing season (Costard *et al.,* 2012). The presence of both warthogs and soft ticks in an area increases the infection rates of the wild suids (Plowright *et al.*, 1994; Jori and Bastos, 2009).

1.3.5.2 Tick-domestic pig cycle

This involves tick of *Ornithodoros* spp. and pigs. The ticks have frequently been found infesting pig pens. This has been mostly experienced in Africa and the Iberian Peninsula, where ticks tend to feed on pigs thus being involved in the ASFV transmission and long-term maintenance of ASF as it have been described previously. The *Ornithodoros* ticks can maintain ASFV infection for several months or even years after feeding on viraemic animals. This can cause the re-emergence of ASF outbreaks on previously infected farms due to the continued presence of ticks and even in farms with introduction of new pigs. For example in Madagascar, ASFV was isolated from ticks found on the farm that no pigs had been introduced for at least four years (Sánchez-Vizcaino *et al.,* 2009).

1.3.5.3 Domestic cycle

ASFV is transmitted through direct contacts and by formites once introduced into domestic pig populations (Penrith *et al.*, 2013) at local, regional and international levels. Lack of biosecurity practices and free movement of pigs for trade purposes highly contribute to the local spread of ASF in the endemic areas. ASFV is very resistant to inactivation, requires a heating at 60 for 20 min to be inactivated, thus having a lengthy persistence in tissues such as muscles, fat and bone marrow, pork products such as cured ham enabling it to remain infectious for several months (Mebus, 1988).

A risk of infection is also present when pigs have access to poor disposed carcasses, frozen insufficiently cooked or cured pork products. Formites such as contaminated clothing and shoes, equipment and vehicles (Mur *et al.,* 2012) impose the transmission possibility as ASFV can also persist in the environment for several days (Plowright *et al.,* 1994; Sánchez-Vizcaino *et al*., 2009). Penrith and Vosloo (2009) argue that there is no evidence that the ASF recovered pigs can become long-term virus carriers, while on the other hand

Sánchez-Vizcaíno *et al*., (2012) have demonstrated that both wild and domestic animals shed viruses thus play a significant role for persistence and spread of the disease in endemic areas.

1.5 Clinical signs

Depending on the virus virulence and host factors, the disease can vary in forms with different clinical manifestation (Blome *et al*., 2013). The disease can vary from a chronic form to an acute form where there are levels of morbidity and mortality reaching up to 100%. The incubation period of the disease ranges between 3-15 days (Plowright *et al*., 1994; Penrith *et al*., 2004). The onset of the diseases is marked by the sudden dullness and fever with high body temperatures up to 42 ℃ (Mebus, 1988). Other characteristics of the disease include cutaneous congestion on the outer pinna, the tail, distal extremities, ventral areas of chest and abdomen. Also anorexia, listlessness, cyanosis, incoordination, increased pulse and respiratory rate, vomiting, diarrhoea, which may be watery and sometimes bloody and abortion to pregnant sows may occur followed by death within 6-13 days after the onset of the disease (European Food Safety Authority (EFSA) 2009). The mortality rates with the virulent isolates can reach up to 100% (Blome *et al*., 2013). The less virulent isolates are characterized by weight loss, moist coughing, irregular remittent fever for up to one month, dyspnoea, with lower mortality rate ranging between 30-70% (OIE, 2012).

1.6 Pathology

The gloss lesions of the disease depend on the form of the disease and the isolate. The major gross pathological lesions in acute form include pronounced haemorrhages in the gastrohepatic and renal lymph nodes, petechial haemorrhages of the renal cortex, also in medulla and pelvis of kidneys, congestive splenomegaly, oedematous areas of cyanosis in

hairless parts. Also cutaneous ecchymoses on the legs and abdomen, excess of pleural, pericardial and/or peritoneal fluid, petechiae in the mucous membranes of the larynx and bladder, and on visceral surfaces of organs and oedema in the mesenteric structures of the colon and adjacent to the gall bladder (Plowright *et al*. 1994; Penrith *et al*. 2004; OIE, 2012). In chronic form, lymph nodes enlargement, focal caseous necrosis and mineralisation of the lungs can be observed (OIE, 2012).

1.7 Differential diagnosis

The ASF symptoms are often confused with other hemorrhagic diseases of swine. Diseases that present similar clinical signs include classical swine fever (CSF or hog cholera), porcine reproductive and respiratory syndrome (PRRS), erysipelas, pasteurellosis, salmonellosis, thrombocytopaenia purpura, Aujeszky‟s disease (or pseudorabies) in younger swine and other septicaemic conditions. Hence, the World Animal Health Organization (OIE) recommends for laboratory confirmation that is done either by the virus demonstration or virus antibody detection (OIE, 2008).

1.8 Laboratory confirmation

1.8.1 Samples for laboratory confirmation

Identification of the virus can be performed from whole blood in EDTA (0.5%) or heparin (10 IU/ml) anticoagulant from live sick animals. From dead animals, tonsils, spleen, lymph nodes or kidney samples (2-5 g) can be submitted if possible kept at 4 °C without freezing. Virus identification includes techniques such as hemadsorption test, fluorescent antibody test and PCR. Sera from suspect cases (8 to 21 days after infection) can also be collected for testing for the presence of antibodies by ELISA, indirect fluorescent antibody test, immunoblotting test and counter immunoelectrophoresis test. However, PCR is recommended by OIE for the confirmation of suspected ASF cases as a highly sensitive and rapid technique for ASFV detection (OIE, 2008).

1.8.2 Virus detection

1.8.2.1 Hemadsorption

The hemadsorption (HAD) test is based on the capacity of erythrocytes to adhere to the surface of pig monocyte or macrophage cells infected with ASFV (Malmquist and Hay 1960). The technique is specific to viruses which have hemadsorbing capacity. Very few 'non-hemadsorbing' isolates have been reported, of which most of them are avirulent (European Food Safety Authority (EFSA), 2009). Hemadsorption test is carried out by inoculating blood or tissue suspensions from the suspected pigs into the primary leucocyte cultures or into the alveolar macrophages cell cultures. The leucocyte cultures can be prepared from the pig's blood inoculated at the laboratory or from the field pig's collected blood. Hemadsorption test is a conclusive diagnostic test for ASF if the results are positive (OIE, 2012).

1.8.2.2 Fluorescent antibody test

Fluorescent antibody test (FAT) is used as an additional method to detect antigen in tissues of suspect pigs in the field or those inoculated in the laboratory. This technique can be used to detect non adsorbing isolates of the ASFV that cannot be detected by hemadsorption test. It can also be used to distinguish cytopathic effect (CPE) of ASFV from that of other viruses like Aujeszky's disease virus (OIE, 2008; Sánchez-Vizcaíno, 2009). The virus is detected using fluorescent microscope after staining the virus with antibodies that are conjugated with fluorescent dyes e.g. fluorescein isothiocynate (FITC), Texas Red or Alexa fluor.

1.8.2.3 Polymerase chain reaction

This technique is used to amplify 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 target the conserved region of p72 gene (Aguero *et al*., 2003). The technique allows detection of both nonhemadsorbing and low virulent ASFV and has a high specificity and sensitivity also is used in a wide range of circumstances and there is no need to isolate the virus when detection of the virus is ought to be done (Steiger *et al*., 1992; Bastos *et al*., 2003; OIE, 2008).

1.8.3 Antibody detection

1.8.3.1 Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) is a direct test that can detect antibodies to ASFV in pigs that have been infected by viruses of low or moderate virulence. The technique uses the combination of the specific antibodies that are conjugated with enzymes that are able to breakdown substrates into photometric products (Hamdy and Dardiri, 1979). ELISA is used to evaluate antibody in serum or fluid from tissues. It is recommended that a secondary confirmatory test such as immunoblotting test or the IFA is carried out. Commercial ELISA kits are available that show high levels of specificity and sensitivity. The sensitivity of the technique is decreased when the samples used are poorly preserved (OIE, 2008, 2012).

1.8.3.2 Immunoblotting

The technique is based on the antibody antigen binding principle. This test should be used as an alternative to the IFA test to confirm equivocal results with individual sera. This test is very specific and enables easier and more objective interpretation of the results and a can detect even weak-positive samples (Lubisi *et al*., 2005; Sánchez-Vizcaino *et al*., 2009). Viral proteins that induce specific antibodies in pigs have been determined. These polypeptides have been placed on antigen strips and have been shown in the immunoblotting test to react with specific antibodies from 9 days post infection.

1.8.3.3 Counter immunoelectrophoresis

Counter immunoelectrophoresis or immunoelectro-osmophoesis test can be carried out rapidly and specific antibody can be detected in some sera for 30 minutes after the test is set up. Counter electrophoresis requires the use of electrophoresis equipment (electrophoresis chamber, slide frames, gel cutter) and a 500 Volt constant-current power supply. Due to the low sensitivity of this test, it is only recommended for screening groups of pigs but not individual animals (OIE, 2008).

1.9 Control of African swine fever

There is no treatment available for ASF, thus leaving stamping out the only effective diseases control approach. Large gaps in knowledge concerning ASFV infection and immunity have hindered ASF vaccine development. Research progresses on the aspects of ASFV infection biology do provide new opportunities for ASF vaccine development with promising results by alternating the field viruses or recombinant DNA vaccines (Rock *et al*., 2017). Prevention of ASF depends on ensuring that neither infected pigs nor pig meat products are introduced into areas free of ASF, proper disposal of the carcasses and infected material by deep burial, disinfection of pig premises, strict import policy for animals and animal products, sanitary measures must be applied and includes control of movement and treatment of the waste food, proper disposal of waste food from aircraft or ships coming from infected countries (Costard *et al*., 2012). There is a need upon epidemiological investigation with tracing of possible sources of infection and immunity as a way forward into eradicating the disease (OIE, 2008; FAO, 2001; Penrith and Vosloo, 2009).

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CHAPTER TWO

2.0 Manuscript 1: African swine fever domestic pig cycle dominance in Tanzania, 2015-2017

Authors: Clara Yona, Merijn Vanhee, Edgar S. Simulundu, Mariam Makange, Hans J. Nauwynck and Gerald Misinzo

Affiliations:

SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Southern and Eastern Africa (SACIDS ACE), Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Tanzania. (C. Yona, M. Makange and G. Misinzo)

Department of Biotechnology, VIVES University College, Roeselare, Belgium. (M. Vanhee)

Department of Disease Control, School of Veterinary Medicine, University of Zambia, Lusaka, Zambia. (E. S. Simulundu)

Laboratory of Virology, Faculty of Veterinary Medicine, University of Gent, Gent, Belgium. (H. J. Nauwynck)

Article Summary Line: African swine fever spread in Tanzania is maintained through domestic pig to domestic pig transmission rather than virus emergence from sylvatic cycle

Running Title: Domestic transmission of ASFV in Tanzania

Keywords: African swine fever, African swine fever virus, *Asfarviridae*, genotype, domestic pig, domestic cycle, Tanzania.

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2.1 Abstract

African swine fever (ASF) is a highly fatal viral hemorrhagic disease of domestic pigs posing a threat to livelihoods and food security. In Africa, ASF virus (ASFV) is maintained by a sylvatic (transmission between warthogs and soft argasid ticks) with occasional spill over into domestic (transmission between domestic pigs) cycles. The aim of this study was to investigate whether recent ASF outbreaks were a result of virus spill from sylvatic cycle or spread within the domestic cycle. Tissue samples were collected from domestic pigs during outbreaks at different locations in Tanzania between 2015 and 2017 followed by partial nucleotide sequencing of the variable 3'-end of *B646L* gene of ASFV. Genotypes II, IX and X of ASFV, similar to those responsible for ASF outbreaks prior to 2015 were found, indicating domestic cycle virus spread and maintenance. Stakeholders' attitudes, practices and behaviors, uncontrolled pig movements, restocking, poor biosecurity measures and breach of quarantine were factors responsible for virus maintenance in domestic cycle.

2.2 Introduction

African swine fever (ASF) is a highly contagious viral hemorrhagic disease of domestic pigs whose mortality rates have been described to reach up to 100% (1). African swine fever is caused by ASF virus (ASFV), an arbovirus belonging to the *Asfivirus* genus and a sole member of the *Asfarviridae* family (2). The ASFV virion is enveloped, has an icosahedral morphology and contains a double-stranded DNA genome whose size ranges between 170 and 193 kilo base pairs depending on the isolate (3). Warthogs are reservoir hosts that are persistently infected with no obvious clinical disease, and soft ticks of the genus *Ornithodoros* are vectors for transmission of ASFV from sylvatic cycle to domestic pigs (4). Transmission of ASFV from sylvatic cycle to domestic pigs occurs either through a tick bite, feeding contaminated warthog carcasses to domestic pigs and/or contact with

warthog faeces (5). Once ASFV is transmitted to domestic pigs, the virus spreads between domestic pigs through contact between infected and susceptible pigs, feeding pigs with infected meat or via fomites such as contaminated clothing, shoes, equipment and vehicles (6).

The existence of the sylvatic cycle contributes to a rich genetic diversity of ASFV. Based on partial amplification and sequence analysis of the p72 (*B646L*) gene, 23 genotypes of ASFV have been identified (7,8). All the 23 ASFV genotypes have been described in African counties, south of Sahara, 22 of which are restricted to Eastern and Southern Africa (1,8). Genotypes I, II and IX of ASFV have been reported to spread beyond their south of Sahara geographical range. For instance, genotype I spread from West Africa to Europe, South America and the Caribbean (9). On the other hand, genotype II, which was known to circulate in Zambia, Malawi, and Mozambique, spread to the Caucasus, the Eastern territory of the European Union (Ukraine, Belarus, Lithuania, Poland, Estonia and Latvia) and Russia (10,11). Furthermore, genotype II ASFV has been introduced to Tanzania and Zimbabwe, where it was never known to circulate (12,13). Similarly, genotype IX which is restricted to Eastern Africa has been reported to spread to Western Africa (14). The spread of ASFV beyond African countries south of Sahara and its traditional geographical boundaries poses a threat to the global pig industry, especially to Western Europe and China, which are major pork producers (15).

A number of sporadic ASF outbreaks have been reported since 2000 in different parts of Tanzania, associated with ASFV genotypes II, IX, X, XV and XVI (12,16–19). There appears to be a geographical restriction of the ASFV genotypes in Tanzania with genotype II being restricted to Southwestern Tanzania, genotype IX to Northwestern Tanzania, genotypes X and XVI to Northeastern Tanzania and genotype XV to Eastern Tanzania

(12,16–18,20). These viruses tend to end up causing outbreaks in Dar es Salaam due to transportation of pigs for sale and slaughter from other parts of the country to this main commercial capital (16,17). Many outbreaks have been reported in different parts of Tanzania between 2010 and 2017. The aim of this study was to investigate the genetic nature of ASFV responsible for the ASF outbreaks in different parts of the country that occurred between 2015 and 2017.

2.3 Materials and methods

2.3.1 Study area, sampling and sample processing

Samples were collected from domestic pigs following reports of suspected ASF outbreaks in different locations within Tanzania between 2015 and 2017. Samples were collected from Mwanza, Manyoni, and Bukoba districts in the year 2015, Kigoma, Babati, Ngara, Magu, Mbeya Municipality, Rungwe and Mbarali districts in the year 2016 and Kalambo, Ileje, Mbozi, Kongwa, Dodoma, Mpwapwa, Gairo, Mbagala, Mvomero, Morogoro Municipality and Kibaha districts in the year 2017. Clinical and postmortem examination of pigs were performed prior to sampling. Tissue samples including spleen, lymph nodes, lungs and kidney were collected from domestic pigs at slaughter slabs or from those that were found dead from suspected ASF. Approximately, 1 g of each tissue sample was homogenized in 3 ml of sterile phosphate-buffered saline (PBS), followed by centrifugation of the homogenate at 6000 *g* for five minutes at room temperature. The tissue supernatant was transferred into a cryovial and stored at -80 °C until DNA extraction.

2.3.2 Detection of ASF in pig samples

Aliquots (100 µl) of each of the homogenized tissue samples from the same pig was pooled before conducting DNA extraction. DNA was extracted from the supernatant of pooled

homogenized tissues using a QIAamp nucleic acid extraction kit (Qiagen, Hilden, Germany), following the manufacturer's instructions. The presence of ASFV DNA was detected by polymerase chain reaction (PCR) using the ASF diagnostic primer set PPA1 and PPA2 that partially amplify the *B646L* (p72) gene as previously described by Aguero *et al.* (21).

2.3.3 Genetic characterization of ASFV

Genetic characterization of ASFV was conducted in samples confirmed with ASFV by partial nucleotide amplification of the *B646L* (p72) gene using primers p72U and p72D. as previously described by Bastos *et al.* (9). Afterwards, the PCR products were subjected to automated dideoxynucleotide cycle sequencing using Big Dye Terminator Cycle sequencing kit version 3.1 (Applied Biosystems, Foster City, CA) and generated chromatograms were read by Sequence Scanner version 1.0 software (Applied Biosystems, Foster City, CA). The obtained nucleotide sequences were submitted to GenBank and assigned with accession numbers (Table 1). The similarity search of the obtained nucleotide sequences against other ASFV sequences at GenBank database was performed using BLASTN version 2.6.0. The ASFV were aligned with other Tanzanian ASFV nucleotide sequences available at GenBank using ClustalW. Phylogenetic analysis was performed using the Neighbour-Joining method with 1000 bootstrap replications, and evolutionary distances were calculated by the Kimura 2-parameter method as implemented in MEGA 6.0 (22).

Table 1: Summary of Tanzanian African swine fever virus (ASFV) isolates used for the construction of phylogenetic tree based on partial *B646L* (p72) gene sequences.

Isolate	Host species	Year of Isolation	Town	p72 gene Genbank accession number	p72 genotype	Reference
TAN/10/Kyela	Pig	2010	Kyela	JX391987	II	(19)
TAN/11/Ludewa	Pig	2011	Ludewa	JX391990	II	(19)
TAN/12/Ifakara	Pig	2012	Ifakara	JX391992	II	(19)
TAN/13/Iringa	Pig	2013	Iringa	KF834193	II	(23)
TAN/16/Mbarali	Pig	2016	Mbarali	MF437296	II	This study
TAN/16/Tukuyu	Pig	2016	Tukuyu	MF437295	II	This study
TAN/16/Uyole	Pig	2016	Uyole	MF437294	II	This study
TAN/17/Kalambo	Pig	2017	Kalambo	MF437304	II	This study
TAN/17/Ileje	Pig	2017	Ileje	MF437301	II	This study
TAN/17/Mbozi	Pig	2017	Mbozi	MF437303	II	This study
TAN/17/Kongwa	Pig	2017	Kongwa	MF437299	II	This study
TAN/17/Dodoma	Pig	2017	Dodoma	MF437309	II	This study
TAN/17/Mpwapwa	Pig	2017	Mpwapwa	MF437307	II	This study
TAN/17/Gairo	Pig	2017	Gairo	MF437302	II	This study
TAN/17/Mbagala	Pig	2017	Mbagala	MF437300	II	This study
TAN/17/Mazimbu	Pig	2017	Mazimbu	MF437306	II	This study
TAN/17/Mzumbe	Pig	2017	Mzumbe	MF437310	II	This study
TAN/17/Morogoro	Pig	2017	Morogoro	MF437305	II	This study
TAN/17/Kibaha	Pig	2017	Kibaha	MF437308	II	This study
TAN 2005.1	Pig	2005	Mwanza	JX403640	IX	Unpublished
TAN/15/Bukoba	Pig	2015	Bukoba	MF437290	IX	This study
TAN/16/Magu	Pig	2016	Magu	MF437297	IX	This study

TAN/16/Ngara	Pig	2016	Ngara	MF437293	X	This study
KIRT89/4	Tick	1989	Kirawira	AY351513	X	(20)
KIRW89/1	Warthog	1989	Kirawira	AY351514	X	(20)
TAN/Kwh12	Warthog	1968	Kirawira	AF301546	X	(9)
TAN 2004.1	Pig	2004	Kigoma	JX403648	X	(16)
TAN/09/Longido	Pig	2009	Longido	JX262383	X	(19)
TAN/13/Moshi	Pig	2013	Moshi	KF706360	X	(18)
TAN/13/Rombo	Pig	2013	Rombo	KF706361	X	(18)
TAN/13/Machame	Pig	2013	Machame	KF706362	X	(18)
TAN/13/Arusha	Pig	2013	Arusha	KF706363	X	(18)
TAN/16/Babati	Pig	2016	Babati	MF437298	X	This study
TAN/15/Mwanza	Pig	2015	Mwanza	MF437291	X	This study
TAN/15/Manyoni	Pig	2015	Manyoni	MF437292	X	This study
TAN/15/Kigoma	Pig	2016	Kigoma	MF437289	X	This study
TAN/08/Mazimbu	Pig	2008	Mazimbu	GQ410765	XV	(12)
Tan/1/01	Pig	2001	Dar es Salaam	AY494552	XV	(20)
Tan/1/01	Pig	2003	Arusha	AY494550	XVI	(20)

2.4 Results

2.4.1 Clinical signs and postmortem findings

Clinical signs observed in sick pigs included a high fever, anorexia, staggering gait, shivering and cutaneous congestion particularly on the outer side of the pinna, belly, limbs and genitalia (Fig. 2). Pigs were dull and stayed together at the corners of their pens (Fig. 2). Abortion was observed in pregnant sows. At postmortem, the pericardial and thoracic cavities were filled with straw tinged fluid. In addition Postmortem findings included hemorrhages in the spleen, heart, kidneys and lymph nodes especially the gastrohepatic, thoracic, mesenteric and renal lymph nodes (Fig. 2). Splenomegaly (enlargement of the spleen) and enteritis were also observed.

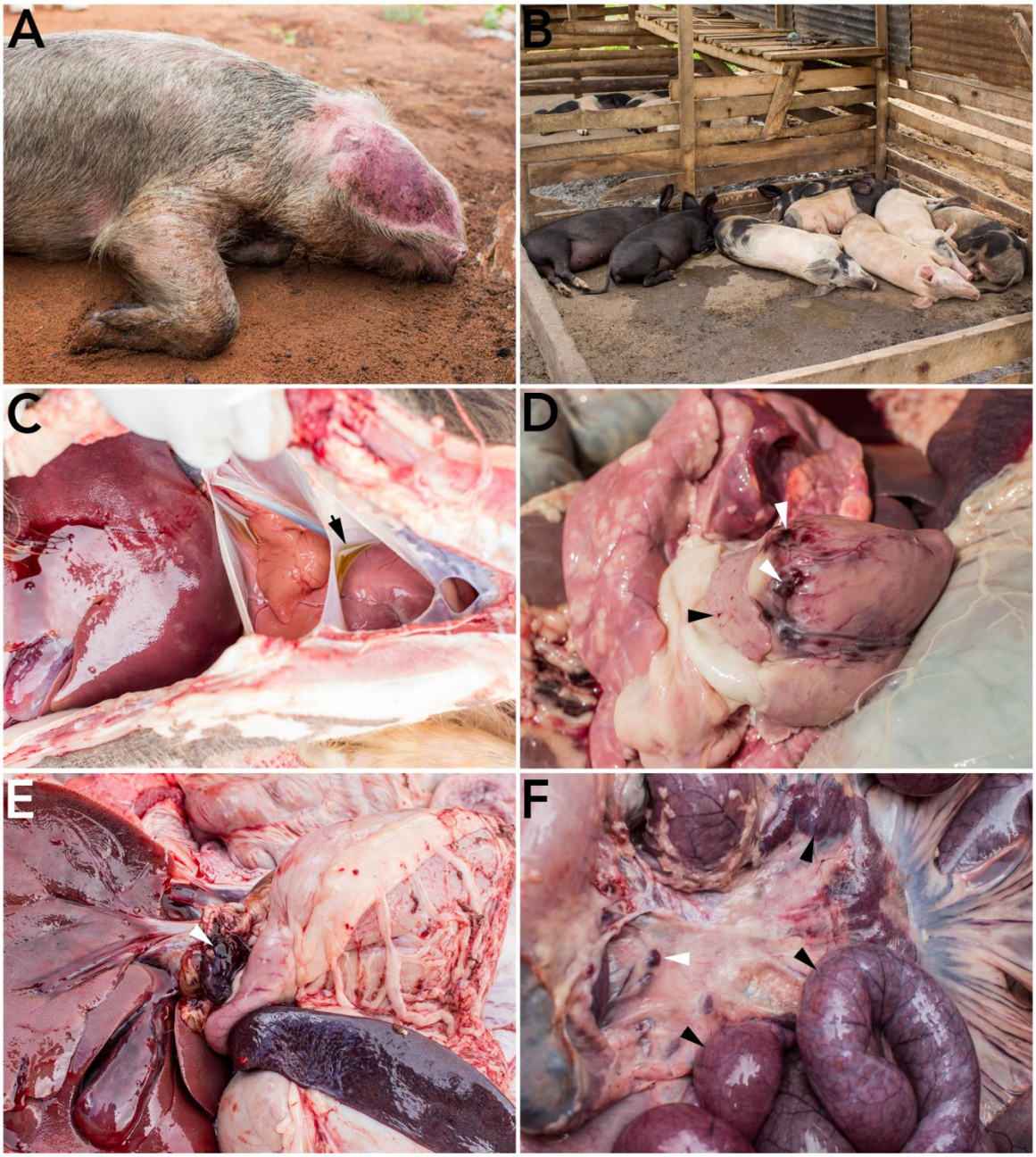

Figure 2: Clinical signs and postmortem findings in domestic pigs with African swine fever. Cutaneous congestion of the outer side of the pinna was observed in pigs with ASF (A) and domestic pigs with ASF cuddled together in their pens (B). At postmortem, the pericardial and thoracic cavities were filled with straw tinged fluid, hemorrhages of the heart, lymph nodes and enteritis were observed (C-F). Hemorrhages are indicated by arrow heads.

2.4.2 The ASFV genotypes

A phylogenetic tree (Fig. 3) was constructed by the Neighbor-Joining method to determine the genetic relationship between the 2015 and 2017 Tanzanian isolates, and the previously identified ASFV genotypes that have been previously characterized in Tanzania (Table 1). The ASFV collected from Southwestern (Uyole, Tukuyu, Mbarali, Kalambo, Ileje, Mbozi), Central (Dodoma, Kongwa, Mpwapwa, Gairo) and Eastern (Morogoro, Mazimbu, Mbagala, Kibaha Tanzania clustered into genotype II (accession numbers MF437294-96, MF437299 and MF437300-09). The ASFV from Northwestern Tanzania (Magu, Bukoba, Mwanza Ngara, Kigoma (accession numbers MF437289-91, MF437293 and MF437297) clustered into genotype IX and X. Genotype X was also found in Central Tanzania (Manyoni) and Northeastern (Babati) Tanzania (accession number MF437292 and MF437298) (Fig. 3 and 4).

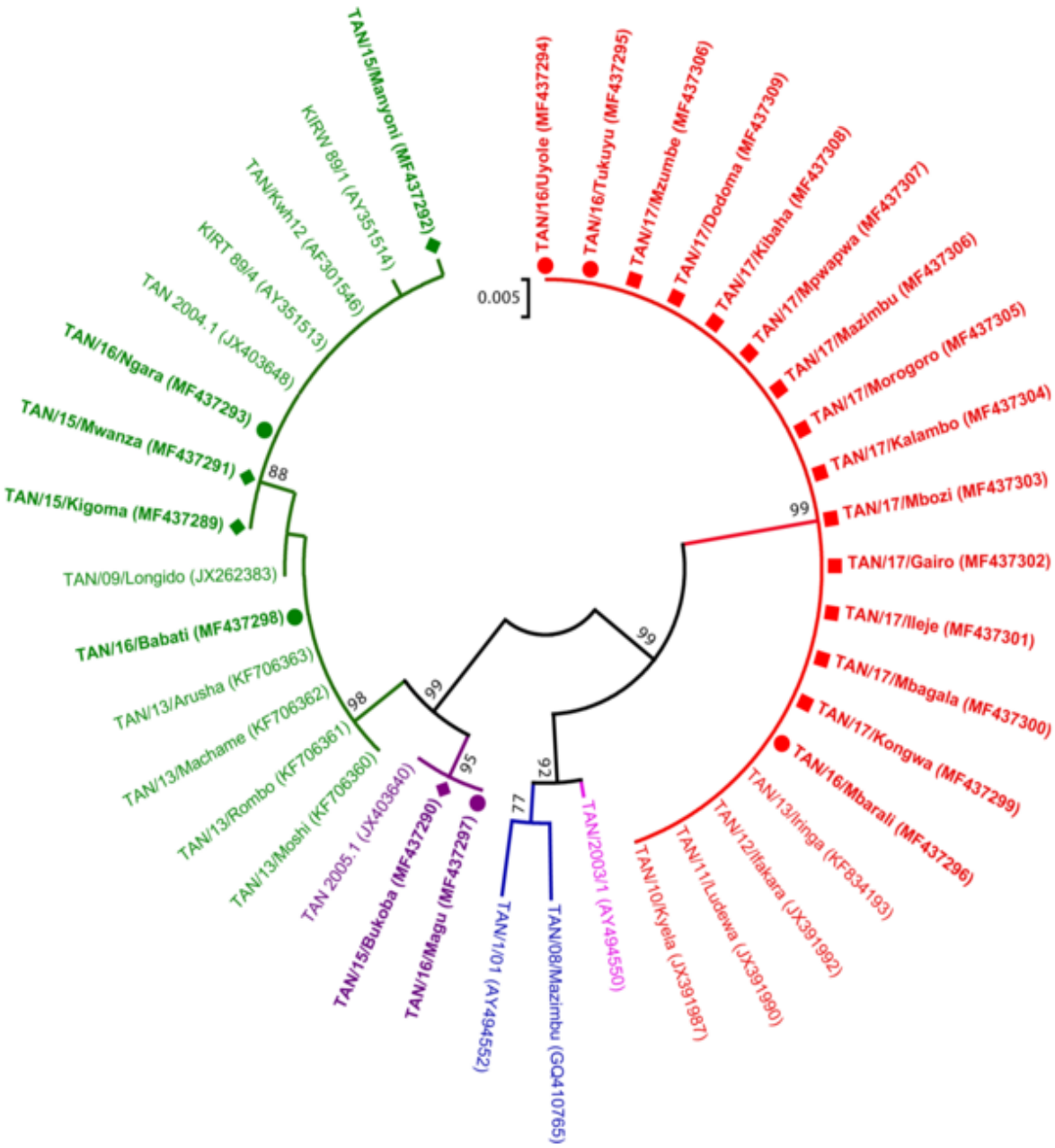

Figure 3: Phylogenetic relationship of African swine fever viruses (ASFV) from Tanzania. The ASFV collected during 2015, 2016 and 2017 are indicated by square, circle and diamond, respectively. Genotype II, IX, X, XV and XVI are shown using red, purple, green, black and blue colors, respectively. Phylogeny was inferred following 1,000 bootstrap replications and node values show percentage bootstrap support. Scale bar indicate nucleotide substitution per site. The GenBank accession numbers for the different *B646L* (p72) gene are indicated in parenthesis.

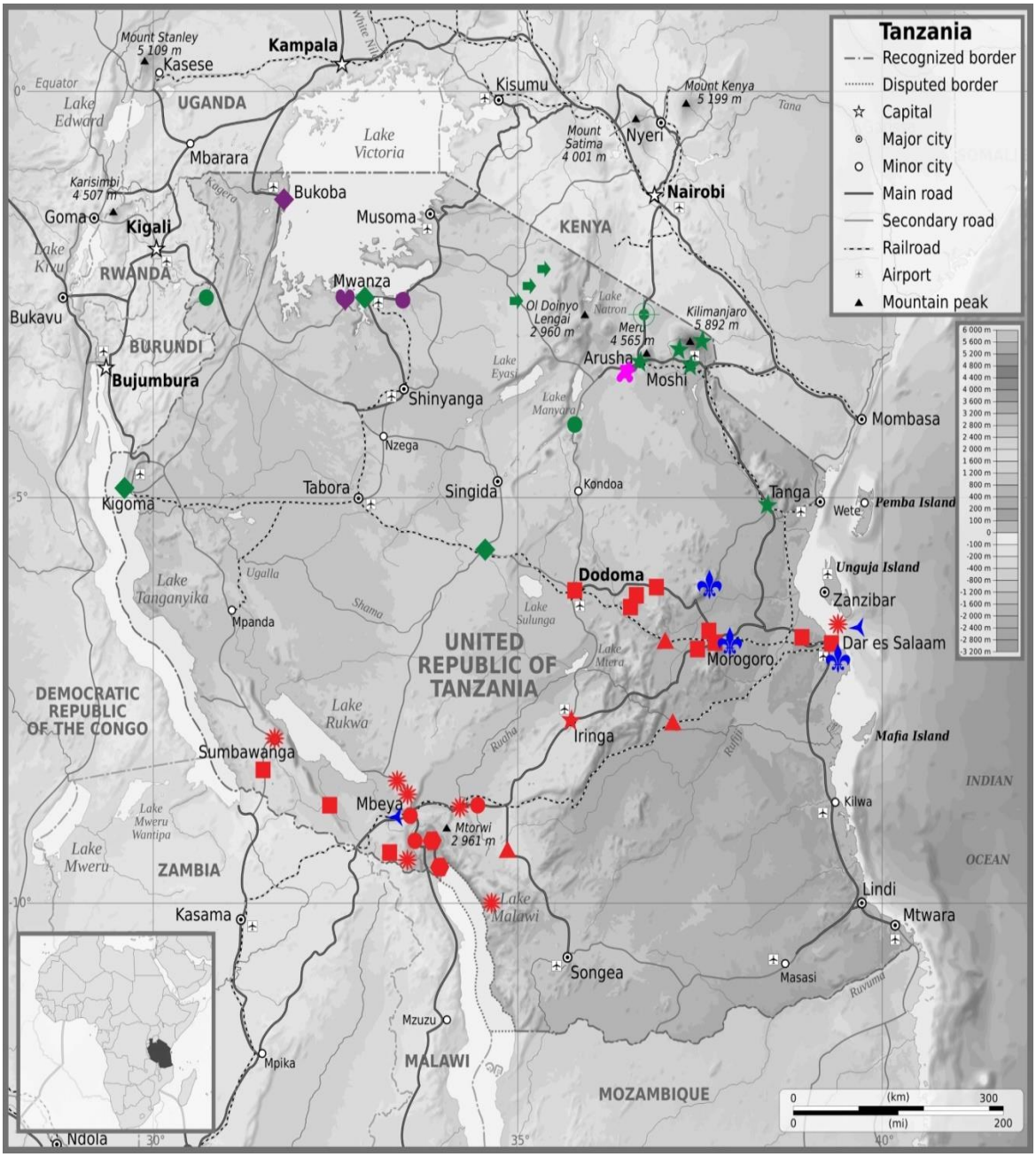

Figure 4: Map showing sampling district affected by ASF in Tanzania. Africa swine fever outbreaks were reported in Southwestern, Eastern, Central, Northeastern and Northwestern Tanzania caused by ASFV genotypes II (red), IX (purple), X (green), XV (blue) and XVI (pink). The ASFV strains collected in Tanzania between 1968 and 2017 are indicated using different symbols; 1968 (➡), 1989 (➡), 2001 (⅄), 2003 (✖), 2005 (♥), 2008 (⦸), 2010 (⬢), 2011 (✹), 2012 (▲), 2013 (★), 2015 (◆), 2016 (●) and 2017 (■).

2.5 Discussion

Several outbreaks of hemorrhagic disease in domestic pigs suspected to be ASF based on clinical signs and postmortem findings were reported in different parts of Tanzania between 2015 and 2017. Prior to 2015, several ASF outbreaks were confirmed and the ASFV responsible for these outbreaks have been described to cluster into ASFV genotypes II, IX, X,XV and XVI (16,18–20).

The present study was conducted to investigate whether new or already described ASFV were responsible for the ASF outbreaks that occurred between 2015 and 2017in different parts of Tanzania (Table 1). Nucleotide sequencing and phylogenetic analysis of ASFV collected from the different parts of Tanzania between 2015 and 2017 clustered the virus into genotypes II, IX and X with each of the genotype being 100% identical to previously reported ASFV (Fig. 4).

Prior to 2015, genotype II of ASFV was reported during outbreaks in Southwestern and Eastern parts of Tanzania (Fig. 3 and 4). Genotype II viruses were introduced from Malawi in 2010 into Kyela, a district in the Southwestern Tanzania, at the border between Tanzania and Malawi (12). Since its introduction, the virus has primarily circulated in Southwestern Tanzania with occasional spread into Eastern Tanzania (12). In the present study we found genotype II viruses in Southwestern, Central and Eastern parts of Tanzania (Fig. 4). The patterns of spread of genotype II of ASFV seem to occur along the Sumbawanga-Tunduma-Mbeya-Iringa-Morogoro-Dar es Salaam highway and its branch to Morogoro-Dodoma highway (Fig. 4). The spread of the virus along these highways could be due to smuggling of live pigs, pork or pork products from areas that experience outbreaks to major cities for sale, illegal transportation of pigs from areas under quarantine have been previously described to spread ASFV within Tanzania (12,16). Therefore, illegal

transportation of pigs should be banned and maximum quarantine adherence should be met with law enforcements advancing, as it has been observed in propagating the spread of ASF in Tanzania.

Genotype II ASFV is highly virulent and has been reported to spread beyond its traditional geographical boundaries of Malawi, Mozambique and Zambia into Madagascar, Mauritius islands, Zimbabwe, Tanzania, the Caucuses region, Russia and Eastern Europe (10,24,25). If appropriate control measures of these genotype II viruses are not put in place, we predict that this virus could spread to Northern parts of Tanzania and ultimately into bordering countries of Burundi, Rwanda, Uganda and Kenya. Hence, prior precautions should be taken in unaffected areas throughout the country and by the neighboring countries towards this posed threat.

In the present study the circulation of genotype X of ASFV in Northeastern Tanzania is shown, similar to what was described by (12,18). The closeness of these districts with wildlife areas of Northern Tanzania argues the possible spreads of genotype X of ASFV from warthogs. A closer investigation on this argument is of crucial importance. In addition, genotype X ASFV was found in Northwestern and Central Tanzania, indicating the spread of these viruses beyond their traditionally geographical boundary. However, genotype IX was found within Northwestern parts of Tanzania, similar to what is reported in previous studies, indicating restriction in the traditional geographical areas. The restriction of genotype X in the North poses a threat to further spreads in new areas that has not reported.

The occurrence and spread of ASF between different parts of Tanzania is likely due to breach of quarantine imposed in areas with ASF. Pig traders smuggle and transport pigs or

pig meat from areas affected with ASF, where the prices are lower, into unaffected areas. Poor biosecurity measures at farms and slaughter slabs increase the likelihood of ASFV spread at a given locality. In addition, swill feeding and feeding of kitchen leftovers spreads ASFV as has been previously described (12,18). Additionally, raising ASF awareness amongst stakeholders and adherence to biosecurity practices at all critical control points are encouraged

2.6 Conclusion

Based on the results obtained from the present study, ASF occurrence and spread in Tanzania between 2015 and 2017 was confirmed among domesticated pigs. The new ASFV that caused ASF reported outbreaks during the study have been identified to be close related to each other and to the previous isolated ASFV. In addition, some ASFV genotypes are restricted to certain zones, although genotype II and X are spreading beyond their traditional geographical range. It is therefore recommended that further study should be conducted to understand the epidemiology of ASFV that will end the threats posed by ASF outbreaks in Tanzania and neighboring countries. Also, ASF spread in Tanzania to be controlled in order to avoid spreads of ASFV within the country and beyond Tanzanian boundaries. Law enforcement, adherence to quarantine and biosecurity measures are encourage upon controlling further ASF outbreaks and spreads to new areas.

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Michael Madege (VIC, Mwanza), Dr. Obed N. Malangu (VIC, Arusha), Dr. Omari Nkullo (DVO, Kongwa) and Dr. Godbless E. Luhunga (DVO, Gairo) for their assistance in sample collection. This research was supported by a TEAM Project “Improving Livelihoods through the control of short cycle stocks” to Sokoine University of Agriculture and the University of Gent from the Flemish Interuniversity Council (VLIR-UOS) and the SACIDS Africa Center of Excellence for Infectious Diseases of Humans and Animals in Southern and Eastern Africa. Grant no. from the Government of the United Republic of Tanzania …. the World Bank.

Biography

Clara Yona is currently a Masters of Philosophy (MPhil) candidate in the Department of Veterinary Microbiology, Parasitology and Biotechnology at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture in Morogoro, Tanzania. Her research interests are on the epidemiology of African swine fever in Tanzania.

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Corresponding author: Gerald Misinzo, SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Southern and Eastern Africa (SACIDS ACE), Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3297, Morogoro, Tanzania. Tel: +255 767 058 805; E-mail: gerald.misinzo@sacids.org

CHAPTER THREE

3.0 Manuscript 2: Determinants of African swine fever occurrence and associated socio-economic impact in selected parts of Tanzania

Yona, C[1], Vanhee, M[2]., Makange, M[1]., Nonga, H. E[3]., Komba, E. V. G[3]., Lupindu, A. M[3]., Mlangwa, J. E. D[3]., Nauwynck, H. J. and Misinzo, G[1].

[1]*Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.*

[2]*Department of Biotechnology, VIVES University College, Campus Roeselare, Belgium.*

[3]*Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.*

[4]*Laboratory of Virology, Faculty of Veterinary Medicine, University of Gent, Gent, Belgium,*

Keywords: African swine fever, Africa swine fever virus, Risk factor, Epidemiology, Socio-economic, Tanzania

Corresponding author: Gerald Misinzo, SACIDS Africa Centre of Excellence for Infectious Diseases of Humans and Animals in Southern and Eastern Africa (SACIDS ACE), Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3297, Morogoro, Tanzania. Tel: +255 767 058 805; E-mail: gerald.misinzo@sacids.org

Target Journal: Tropical Animal Heath and Production

3.1 Abstract

African swine fever (ASF) is a highly fatal viral hemorrhagic disease of domestic pigs. It is caused by ASF virus (ASFV). The disease has severe impact to food security and livelihoods as some ASFV strains cause mortalities reaching up to 100%. Despite the occurrence of several ASF outbreaks in Tanzania the risk factors and the socio-economic impact associated with ASF in Tanzania have not been proper investigated. This study was carried out to assess the risk factors and socio-economic impact that are associated with the occurrence of ASF in Tanzania. Questionnaires were administered to 124 respondents to collect information on demography characteristics of the respondents, pig management system, general information on pig health and ASF, ASF mortality, ASF morbidity, ASF risk factors and socio-economic impact of ASF. Descriptive statistics and univariable and multivariable logistic analyses were performed to determine the possible risk factors associated with ASF. It was found that the risk factors associated with the occurrence and spread of ASF included the lack of biosafety at slaughter slabs, uncontrolled movement of pigs, sharing of farm equipment and breach of quarantine. Sharing of farm equipment was found to be statistically significant factor (OR=2.47, $CI_{95\%}$=1.4-99, *P*=0.023) for the spread of ASF. The ASF outbreak has been found to be associated with financial loss, loss of income, unemployment, decreased labor in farm activities, mental disturbance, poor livelihoods, food insecurity and threats to the pig industry. Implementation of simple control approaches especially adherence to quarantine, biosafety measures and restriction of pig movements are encouraged in order to prevent further spread of ASF in Tanzania.

3.2 Introduction

African swine fever (ASF) is a highly fatal viral contagious disease of domestic pigs caused by ASF virus (ASFV) of the genus *Asfivirus*, family *Asfarviridae* (Denyer et al. 1998; Dixon et al. 2005). The disease manifests as a hemorrhagic fever that can cause up to

100% mortalities in affected pigs (Costard et al. 2009). Pigs infected with ASFV gradually lose their appetite and become depressed (Denyer et al. 1998; Penrith et al. 2004). The virus contains a double stranded DNA and replicates in the cytoplasm of the infected cells (Dixon et al. 2012). This virus is the only known DNA arthropod-borne virus (arbovirus). Warthogs and bush pigs are the natural hosts of ASFV which are persistently infected with no apparent disease. Soft argasid ticks of the genus *Ornithodoros* act as vectors of ASFV.

African swine fever was first described in Kenya, Eastern Africa in the year 1921 (Montgomery, 1921). The first outbreak of ASF was retrospectively recognized to have occurred in the year 1907. The disease was confined to Africa mainly affecting domestic pigs in settler's farms until 1957 when it was first reported outside Africa in Portugal (Wilkinson, 1989). It is currently endemic in at least 26 African countries in south of Sahara. The disease was first reported in Tanzania in 1914 (FAO, 2001) followed by sporadic outbreaks of ASF in different parts of the country (Wambura et al. 2006). Since the introduction of a new strain of ASFV in Kyela, in year 2010, has shifted the trend of ASF from sporadic nature to persistent outbreaks (Lubisi et al. 2005: Wambura et al. 2006: Misinzo et al. 2011, 2012, 2014). Globally, the ASF virus is present in Africa, Italy (Sardinia), Georgia, Latvia, Poland, Ukraine, Russia and some Caribbean countries, with an increasing risk of spreading to ASF-free countries of Western Europe, America and China (Vergne et al. 2017).

The disease can have a severe socio-economic impact on people's livelihoods, food security and both regional and international trade, making ASF the main threat to both commercial and smallholder pig farmers (Penrith, 2013). Several studies have been carried out in Tanzania to understand the dynamics of disease transmission, molecular epidemiology and control. Some indication of risk factors and socio-economic impact

assessment have been established in few studies, however, detailed and proper investigation has not been done. This study was carried out to investigate the risk and socio-economic impact (mortalities and revenue foregone) of ASF outbreaks in Tanzania.

3.3 Materials and methods

3.3.1 Study area

The present study was conducted in Tanzania which is located on the East side of Africa (Figure 5). The country lies between latitudes 1º and 12º south of the Equator and 29º and 41º East of Greenwich. It is bordered by Kenya and Uganda to the North, Burundi, Rwanda and Democratic Republic of Congo in the West, Zambia, Malawi and Mozambique to the South with the Indian Ocean on the East. Tanzania mainland area is about one million square kilometers with 39 percent of the total area used for grazing. During the study, breeding system, husbandry practices (disease prevention, housing and feeding systems) on the local, cross and exotic breeds were investigated. The pig management systems in the study area are semi-intensive farming and free ranging system. The study was carried in Songwe, Njombe, Rukwa and Morogoro regions. The study locations were chosen based on reports of occurrences of ASF based on the reports from DVOs and farmers.

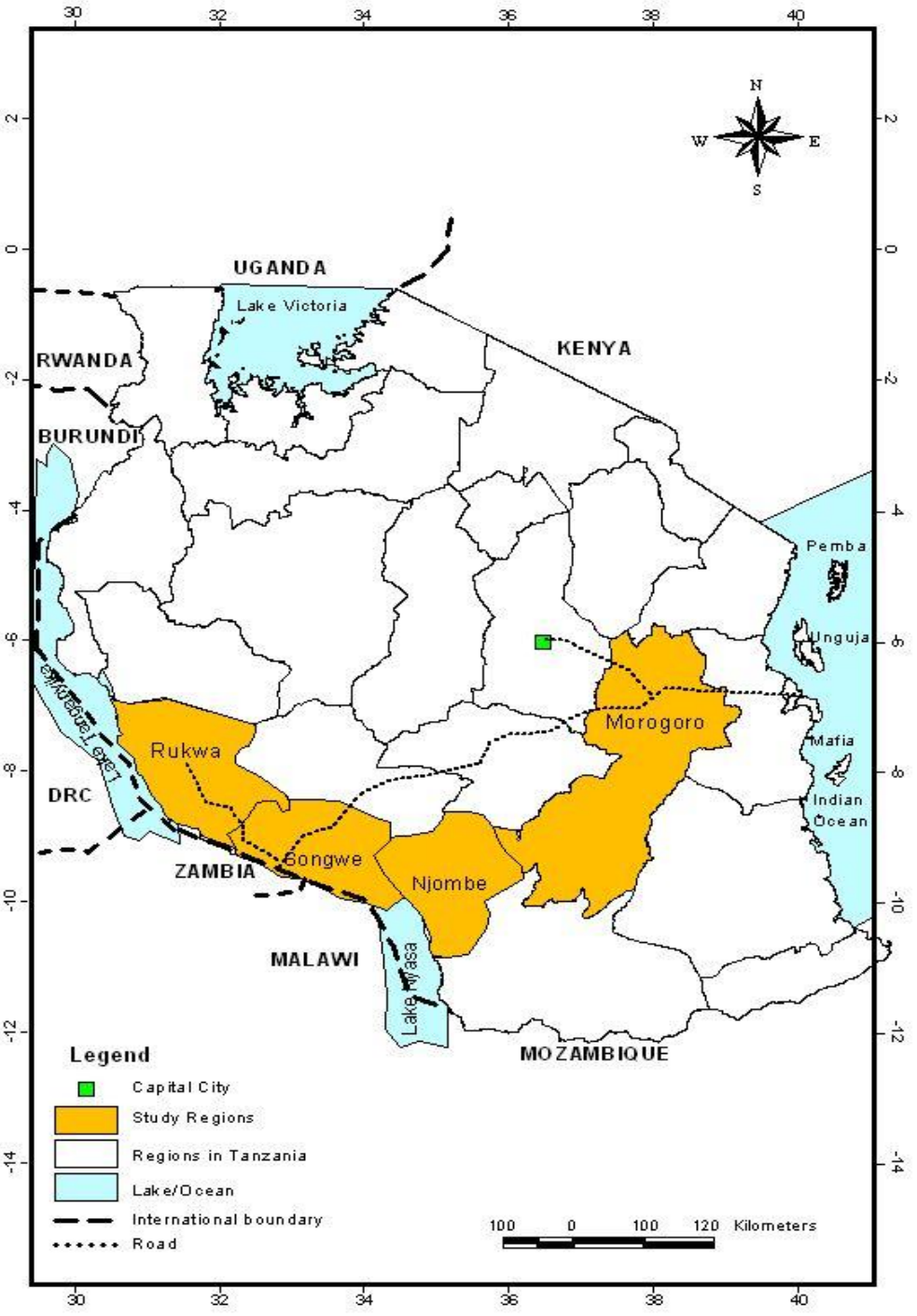

Figure 5: Map of Tanzania showing the locations where the ASF risk factors and socio-economic impact study was carried out.

3.3.2 Study design

The primary sampling units were the districts and households were the secondary sampling units, with a one year old pig herd. Purposive sampling approach was adopted in selection

of the study districts and wards while villages and households were randomly selected out of the list of the districts that had experienced ASF. The reason for the procedures used to arrive to this sampling was to have a detailed and proper investigation on the risk factors and socio-economic impact associated with the ASF outbreaks in Tanzania. This strategy fits proper investigation on the study objectives as less biasness was presented and provided a clear explanation of the study interest. The studied districts had experienced ASF outbreaks before and were again having outbreaks during the study period. For the investigation of ASF risk factors, five districts namely Kalambo, Ileje, Gairo, Makambako and Njombe were selected with recorded geographical references. Gairo district was selected for investigating the socio-economic impact associated with the occurrence of ASF as a representative district, also preliminary survey showed high ASF awareness in Gairo district. The two Gairo district's villages (Bwawani and Chagani) that were involved in the study of risk factors were not involved in the socio-economic impact. This should not bring confusion in the ASF morbidities and mortalities in Gairo district.

3.3.3 Collection of ASF risk factors data

A semi-structured questionnaire (Appendix 1) was used to collect data for epidemiological risk factors of the disease. The questionnaire consisted of open and closed ended questions. Pre-testing of a questionnaire was done to respondents who were afterwards not included in the study. Administration of the questionnaire was done by the researcher in Swahili, as this is the commonly spoken language to 124 respondents. A total of 945 pig sample were analyzed during the study of socio-economic impact. The focus were on the demography characteristics of the respondents, pig management system, general information on pig health and ASF, ASF mortality, ASF morbidity and ASF risk factors. Variables that were thought to be associated with the presence of ASF (the dependent/outcome variable) were included in the questionnaire, with prior on the analysis of their biological sense (Appendix

1). ASF cases were established through clinical signs and symptoms observed prior to the reporting of the disease and confirmed by polymerase chain reaction in the laboratory (data not included in this paper). Flock dynamics were managed by considering the off take, live born and brought inn pigs before, during and after the ASF outbreaks so as to avoid their influences in the morbidity and mortality estimates during the study.

3.3.4 Socio-economic impact of ASF

The socio-economic impact of ASF to the respondents (producers, traders and retailers) was assessed. A study in Gairo district, in Morogoro region was conducted upon observing the total loss that has been encountered by farmers and businessmen during 2017 ASF outbreaks in 10 villages from March to June, where ASF peaks were recorded. Gairo district was selected as a representative of the five districts involved in this study. Financial loss calculations were assessed by calculating the approximated pigs selling prices, with the exchange rate of 1 USD = 2200 Tshs. Respondents were assessed in the forms of activities that they are source of their income, the primary and secondary one. Financial records of respondents were roughly estimated before, during and after the outbreaks. Also during the study, social life of the respondents were observed and analyzed to understand the possible social impact brought by ASF outbreaks.

3.3.5 Data analysis

Epidemiological data were entered and validated in Excel spreadsheets. All statistical data analyses were performed using SPSS version 21.0 (IBM Corp 2012). Descriptive statistics; particularly frequencies, percentages, means and counts from the multiple responses analysis were used to determine distributions and magnitudes of the variables among the respondents. The encounter of a disease was modeled as the dependent variable and run against the ASF risk factors as the independent using the uncondional logistic regression.

Pairwise association of independent variables was tested using chi-square test. The statistical significance was established at 95% confidence interval and critical p-value of 0.05.

3.4 Results

3.4.1 Demographic characteristics and distribution of respondents

In this study a total of 124 respondents were interviewed, owning a total of 945 pigs and or those involved in the pig chain (traders, retailers). There are some questions that respondents were not aware of or not comfortable, causing the total number of respondents to vary in some variables. Demographic information of the respondents is detailed in Table 2. The results show that most respondents (67.7%) were male with the age $\leq$ 40 years and the majority had primary education (77.4%). About 80% of the respondents were married with 0% divorced. It was also established that 79% of the respondents were the pig owners. However 56.4% of the respondents were keeping poultry along with pigs.

Table 2: Socio-demographic characteristics of respondents

Demographic information	Category	Number (%) of respondents in the study					
		Kalambo (n=24)	Ileje (n=24)	Gairo (n=24)	Makambako (n=37)	Njombe (n=15)	Total n (%)
Sex	Male	15 (63)	20 (83)	14 (58)	25 (68)	12 (80)	84 (67.7)
	Female	9 (37)	4 (17)	10 (42)	12 (32)	3 (20)	40 (32.3)
Age (years)	≤40	24 (80)	18 (72)	8 (36)	15 (58)	5 (29)	74 (60)
	≥40	6 (20)	7 (28)	14 (64)	11 (42)	12 (71)	50 (40)
Level of education	Non formal education	2 (9)	0 (0)	0 (0)	3 (15.8)	3 (16.7)	8 (6.5)
	Primary level	18 (78)	10 (71.4)	40 (78.4)	15 (78.9)	13 (72.2)	96 (77.4)
	Secondary	2 (9)	3 (21)	8 (15.7)	0 (0)	2 (11.1)	15 (12.1)
	Tertiary	1 (4)	1 (7.1)	3 (5.9)	1 (5.3)	0 (0)	5 (4)
Marital status	Single	3 (16.7)	6 (15)	5 (19.2)	2 (10)	4 (20)	20 (16.1)
	Married	15 (83.3)	32 (80)	18 (69.2)	18 (90)	16 (80)	99 79.8)
	Widow/Widower	0 (0)	1 (2.5)	1 (3.8)	0 (0)	0 (0)	2 (1.6)
	Separated	0 (0)	1 (2.5)	2 (7.7)	0 (0)	0 (0)	3 (2.4)
Pig ownership	Yes	25 (92.6)	12 (54.5)	8 (53.3)	36 (92.3)	17 (80.9)	98 (79)
	No	2 (7.4)	10 (45.4)	7 (46.7)	3 (7.7)	4 (19)	26 (20.9)
Other livestock kept	None	8 (21)	5 (22.7)	2 (7.7)	4 (18.2)	1 (6.25)	20 (16.1)
	Poultry	21 (55.3)	12 (54.5)	18 (69.2)	8 (36.4)	11 (68.8)	70 (56.4)
	Cattle	1 (2.6)	3 (13.6)	1 (3.8)	6 (27.3)	2 (12.5)	13 (10.5)
	Sheep/Goat	8 (21)	2 (9)	5 (19.2)	4 (18.2)	2 (12.5)	21 (16.9)

3.4.2 General pig husbandry in study areas

General information on the pig husbandry was recorded. The most pig breeds kept were the local breed 440 (46.5%) followed by the exotic breed 279 (29.5%). The most pig keeping system observed in the studied districts was semi-intensive system 45.1% (n=56) as shown in Table 3. Most of the pig pens were constructed using the local available materials like woods, bamboo trees and logs. Some of the pig pens were constructed using bricks and were cemented. Additionally, most respondents claimed to never observe ticks having access to the pig pens 106 (85.4%).

Table 3: General pig husbandry

Parameter	Variable	Number of respondents (%)
Management system	Free ranging system	27 (21.7)
	Semi-intensive system	56 (45.1)
	Intensive system	41 (33)
Tick access to pig pens	Yes	18 (14.5)
	No	106 (85.4)
Breeds	Local	440 (46.5)
	Cross	226 (23.9)
	Exotic	279 (29.5)
Sex	Male	367 (38.8)
	Female	578 (61.2)

3.4.3 General information on ASF and animal health

General information on pig health and ASF are summarized in Table 4. African swine fever was reported to be most disease affecting pigs 47% (n=58). However helminthes and mange were other common diseases of pigs. Results showed the most ASF affected breed to be the local breed 48% (n=60), and the most affected group to be adults 54% (n=67).

Inappetence was the major reported ASF clinical sign 56% (n=70), however, the majority of respondents don't keep health records 54% (n=67).

The majority of respondents 66.1% (n=82) reported to hear about ASF before, from which fellow farmers were the source of information 38.3% (n=45). Wet season was mostly reported by the respondent 60% (n=74) to be the season that is associated with ASF outbreaks. During ASF outbreaks, quarantines were imposed so as to contain the disease. The majority of respondents 59.6% (n=50) did not adhere to the quarantine conditions. Most of the respondents 58.1% (n=72) did not know what to do to contain ASF.

Table 4: General information on pig health and ASF (n=124)

Parameter	Variable	Number of respondents (%)
Breeds affected by ASF	Local	60 (48.4)
	Cross	35 (28.2)
	Exotic	29 (23.4)
Keeping health records	Yes	57 (46)
	No	67 (54)
Common disease affecting pigs	Helminthes	26 (21)
	ASF	58 (47)
	Mange	40 (32)
Heard about ASF before	Yes	82 (66.1)
	No	24 (33.9)
Heard from	Veterinary officer	32 (25.8)
	Media	5 (4)
	Other farmers	45 (36.3)
Group most affected with ASF	Adults	67 (54)
	Growers	25 (20)
	Weaners	20 (16)
	Piglets	12 (10)
Have you encountered ASF	Yes	97 (78)
	No	27 (22)
Have your neighbor encountered ASF	Yes	80 (65)
	No	44 (35)
Clinical signs of ASF	Inappetence	70 (56)
	Cutaneous congestion	5 (4)
	Shivering	14 (11)
	Others (death)	35 (28)
Season of ASF	Wet	74 (60)
	Dry	50 (40)
Adherence to quarantine	Yes	50 (40.4)
	No	74 (59.6)
Action taken to pigs during ASF outbreaks	No actions	72 (58.1)
	Slaughtered	7 (5.6)
	Sold	10 (8.1)
	Treatment	16 (12.9)
	Disinfection	19 (15.3)
Did you report to the livestock office the occurrence of ASF	Yes	88 (70.1)
	No	36 (29.9)

3.4.4 ASF morbidity and mortality

A total number of 945 pigs were recorded in the five districts. About 709 pigs were affected with ASF making a 75% ASF morbidity rate. Out of the 709 ASF affected pigs, 668 pigs died from ASF, with an average of 72.2% ASF mortality rate as indicated in Table 5.

Table 5: ASF morbidity and mortality of pigs during outbreaks of 2017 in sampled districts

District	Village	Total pigs in a village	ASF affected pigs	No. of pigs died of ASF	Crude ASF mortality (%) rate
Kalambo	Kalambo	125	98	80	64
	Legezamwendo	70	65	60	85.7
Ileje	Isongole	200	140	140	70
	Msia	38	27	20	52.6
Gairo	Bwawani	100	67	80	80
	Chagani	65	60	60	92.3
Makambako	Kilimahewa	89	80	76	85.3
Njombe	Kifanya	180	110	99	55
	Mjimwema	50	40	33	66
	Chaugingi	28	22	20	71.4
Total		945	709	668	

3.4.5 Risk factors of ASF infection

The summary of the analysis of possible risk factors that associate with the presence of ASF are as presented in Table 6. A total of 14 risk factors were assessed and the result indicated that sharing of farm equipment was found to be statistically significant factor (B=2.473, OR=11.54, $CI_{95\%}$=1.4-99, *P*=0.023) for spread of ASF.

Table 6: Results of multivariable logistic regression analysis assessing the possible risk factors associated with the occurrence of ASF.

Variable (Risk factor)	B	S.E.	Sig.	Exp(B)	95% C.I. for Exp(B)	
					Lower	Upper
Breeds	-3.023	1.131	0.407	0.049	0.005	0.446
Contact	0.55	0.606	0.364	1.733	0.529	5.683
Pig management	0.403	0.354	0.255	1.496	0.747	2.995
Ticks	-1.407	0.66	0.333	0.245	0.067	0.894
Maize brans/ Rice polishes	-1.174	0.563	0.37	0.309	0.102	0.932
Leftovers	2.087	1.125	0.164	8.057	0.889	73.035
Warthogs	0.163	0.56	0.771	1.177	0.393	3.527
Source of stock	-1.282	0.699	0.066	0.277	0.071	1.091
Pig introduction	2.643	3.347	0.43	14.052	0.452	2.675
Breeds	-2.98	1.087	0.251	0.051	0.006	0.428
Breach of quarantine	-1.075	0.582	0.065	0.341	0.109	1.068
Neighbors encountered ASF	-1.196	0.55	0.075	0.302	0.103	0.889
Sharing of farm equipment	2.473	1.087	0.023	11.854	0.382	3.468
Lack of biosafety	-1.335	0.676	0.048	0.263	0.038	0.991
Constant	3.767	3.211	0.241	43.253		

B- Coefficient for the constant, S.E-Standard error, Sig-Significance level, Exp(B)-Exponential for B

3.4.6 Socio-economic impact

3.4.6.1 ASF mortality in Gairo

The socio-economic impact was studied in the Gairo district, in Morogoro region. This was conducted upon observing the total loss that has been encountered by the farmers and businessmen during ASF outbreaks in 10 villages. A total number of 1725 pigs died in a period of four months as indicated in Table 7.

Table 7: ASF mortality observed during outbreaks in Gairo district

Village	March	April	May	June	Total
Kibedya	0	60	47	0	107
Chigela	0	120	38	0	158
Rubeho	42	468	58	0	568
Nongwe	0	0	25	0	25
Chagongwe	0	0	26	0	26
Gairo	53	238	0	0	291
Magoweko	177	215	0	0	392
Mkalama	41	0	0	0	41
Ukwamani	23	31	0	0	54
Msingisi	0	63	0	0	63
Total	336	1195	194	0	1725

3.4.6.2 Financial loss impact

A financial loss of about Tanzanian shillings 401,120,000 was calculated, this being approximately to USD 182,320 (Exchange rate: 1 USD = 2200) (Table 8).

Table 8: Financial losses associated with ASF mortality in Gairo

Appx. size of the pigs	Approximate (Kg)	No. of dead pigs	Approximate price	Total loss (Tshs)
Large	Adult (60-120)	956	350,000	3,346,000,000
Medium	Weaners & Growers (25-50)	401	120,000	48,120,000
Small	Piglets (5-15)	368	50,000	18,400,000
Total		1725		401,120,000

3.5 Discussion

The presence of ASF was confirmed in the laboratory (data not indicated in this paper). The ASF mortality rates are reported to be ranging from 52.6% to 92.3%, with the average of 72.2% in the five studied districts. In this study possible risk factors associated with the ASF occurrences were studied. These factors were animal owner/attendant to have contact with the neighborhood 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 neighbor 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.

Sharing of farm equipment was found to be a risk factor that is significantly associated with the presence of disease in Tanzania (P=0.023). Prior to introduction of ASF in a neighboring farm, spread of ASF might be accelerated by the habit of sharing farm equipment among different farms as also reported by Saka et al. (2010) and Fasina et al. (2010). In addition to this, there are other confounders that are likely to influence this factor, example roaming of pigs that are kept in semi-intensive systems and visitation from one farm to another during ASF outbreaks. There is a need of educating farmers and all stakeholders on ASF especially the means of spread of ASFV, so that one can avoid sharing of equipment between different farms.

The lack of biosafety and/or poor practices during slaughtering showed a statistical significance of P-value=0.048 association with the presence of ASF. However, this risk factor analysis is not of trust as p-value is approximately to 0.05. Respondents are not

meeting the set biosafety measures. Also it was observed that during various activities when dealing with pigs, only few individuals adhered to the biosafety precautions. These activities tend to associate with the occurrences and spread of ASF in various parts that were studied. Proper biosafety practices during slaughtering whether at slaughter slabs and farms will minimize the possible spread of the ASFV to other places. However, proper slaughtering points should be identified, for completely assurance of biosafety adherence.

In this study, it was clearly observed that there was a failure of the pig keepers and other stakeholders to adhere to the quarantine conditions. Breaching of quarantine measures was indicated by the majority of respondents 59.6% (n=74), with most of the respondents 58.1% (72) to not take any actions. Moreover, slaughtering of pigs during the quarantine period is done secretly and in areas that are not authorized. Sequantially these activities can lead to the environmental contamination and hence precipitating ASF's spread. These factors have been reported in the studies done by Owolodun et al., (2007) and Costard et al., (2009) to be associated with the presence of ASF. Biosecurity measures have to be considered when slaughtering pigs and during performing various activities with the pig. Generally, in Tanzania quarantine and trade restrictions are set up with poor implementations as the compensatory mechanism is not observed. Hence, making the pig keepers to sell the pigs and pig meat during ASF outbreak. Quarantine must be set up whenever there are suspected ASF outbreaks and punishments should be provided to those not adhering to. This will help the maintenance of the virus in the area reported with the outbreaks and minimize the possible spreads to the neighboring areas.

Pig farming is one of the fastest growing livestock activities African countries in the south of Sahara region as a means of increasing food security, income generating and employment. However, ASF has on several occasions hampered this (Bastos et al. 2003).

ASF outbreaks deteriorate livelihoods of smallholder farmers and people that are involved in pig industry. In this study, an approximate of USD 182,320 loss is presented in the Gairo district, Morogoro region. The government loses a lot of money as a source of revenue from its people.

A number of socio-economic impacts were raised by the respondents and the interviewer, this data were quantified in including: loss of income as 56 percent of the respondents depend on the pig keeping and related activities. Also, unemployment, mental disturbance, and poor livelihoods were raised. Medical and education expenses were couldn't be well met as parents failed to pay the school fees and to pay medical expenses. Additionally, some respondents had loans from banks, due to ASF outbreaks they failed to pay loan that were taken to start businesses and also failure to pay the farm labor by most of the farmers. ASF is posing a threat to the country's economy and also food security especially protein.

However, there is a need of incorporating ASF and animal health knowledge into the formal and informal education systems especially the primary level as majority of respondents 77.4% (99) attended primary level of education. This will enhance the sharing of proper information about ASF and animal health in the country.

There is a need to raise awareness about ASF in all over the country. The only few designated state veterinarians cannot cover all of the districts and administrative areas effectively, the role of veterinary paraprofessionals in the rapid surveillance and diagnosis is extremely important. Hence, these individuals should be trained in disease recognition, rapid diagnosis, outbreak control, disease management and associated biosecurity under the supervision of competent veterinarians as stated by Fasina et al. (2010). This will help in the general practices and actions taken before, during and after ASF outbreaks, adherence to the biosafety measures and quarantine when there is an outbreak.

3.6 Conclusion

Following ASF outbreaks in Tanzania a number of socio-economic impacts have been presented in the livelihoods of pig keepers and all key stakeholders. From this study it can be concluded that ASF is a major threat to Tanzania pig industry. The occurrences and spreads of ASF in different parts of Tanzania are portrayed to be associated with sharing of farm equipment. Additionally, poor biosafety measures, breaching of quarantine conditions, actions taken during ASF outbreaks, bad abattoir practices and uncontrolled and/or illegal movement of pigs should be taken into consideration as most probable ASF risk factors. Stamping out of pigs is the only present control approach as there is no vaccine for ASFV. Advanced law enforcement, knowledge, attitudes and practices on ASF should be taken into account for possible eradication of ASF in Tanzania and prevent further spread to the unaffected areas and neighboring countries.

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Biography

Clara Yona is currently a Masters of Philosophy (MPhil) candidate in the Department of Veterinary Microbiology, Parasitology and Biotechnology at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture in Morogoro, Tanzania. Her research interests are on the epidemiology of African swine fever in Tanzania.

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CHAPTER FOUR

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

From the finding of this study it can be concluded that:

i. African swine fever poses a devastating threat to the pig keepers and all key stakeholders of the pig industry in Tanzania.

ii. The African swine fever virus p72 genotypes II, IX and X were found to be circulating between 2015 and 2017 during ASF outbreaks in Tanzania.

iii. The most virulent p72 genotype II of ASFV that was isolated in the southern, central and eastern Tanzania was 100% similar to the Georgia 2007 isolate that has also been isolated in Zambia, Madagascar and Mozambique.

iv. Sharing of farm equipment was found to be a statistically significant risk factor to be associated with the presence of ASF in Tanzania.

v. African swine fever transmissions are mostly probably accelerated by transport of live pigs and storage of pig products during quarantines for resale after quarantines are lifted.

vi. African swine fever spread beyond traditional geographical areas is through road infrastructure development, for example Sumbawanga-Tunduma-Mbeya-Iringa-Morogoro-Dar es Salaam highway and its branch to Morogoro-Dodoma highway road.

4.2 Recommendations

From the conclusions drawn, it is therefore recommended that:

i. Further studies on the ASF epidemiology and associated socio-economic impact especially through the value chain should be carried out to fully understand the disease.

ii. Early diagnosis and confirmation of ASF using field or near-field deployable diagnostic tests such as lateral flow devices and loop-mediated isothermal amplification of DNA are highly recommended in order to reduce the time between disease outline and quarantine.

iii. A study on fully genome sequencing of the ASFV isolates discovered in Tanzania is recommended in order to determine ASFV relatedness with other ASFV full genomes and their virulence for vaccine development.

iv. The responsible Ministry which is the Ministry of Agriculture, Livestock Development and Fisheries is hereby advised to make integrative and collaborative approaches for quick ASF diagnosis, surveillance and control strategies for its eradication.

APPENDICES

Appendix 1: Questionnaire

ASSESSMENT OF RISK FACTORS AND SOCIO-ECONOMIC IMPACT ASSOCIATED WITH PERSISTENCE OF ASF

Geo Ref …….. .. Q. NO.....

PART A: GENERAL INFORMATION

A1: Today's date	
A2: Time now	
A3: Name of interviewer	
A4: Region	
A4: District	
A5: Ward	
A6: Village	

PART B: BIO-DATA

B1: Respondent's name	
B2: Respondent's sex	❖ Male ❖ Female
B3: Respondent's Age	
B4: Level of education	❖ None ❖ Primary ❖ Secondary ❖ Higher education (Tertiary)
B5: Marital status of respondent:	❖ Single ❖ Married ❖ Widow/ Widower ❖ Divorced ❖ Separated
B6: Do you keep pigs	❖ Yes ❖ No
B7: Which other animals do you keep?	❖ None ❖ Poultry ❖ Cattle ❖ Pigs/Goats

PART C: INFORMATION OF AFRICAN SWINE FEVER

C1. General information and animal health status

C1. 1: Number of years lived in this village/area*:*	
C1. 2: Number of years keeping pigs (current herd)	
C1. 3: Type of Breeds	❖ **Local breed** ❖ **Cross breed** ❖ **Exotic breed**
C1. 4: Do you keep health records for your pigs?	❖ **Yes** ❖ **No**
C1. 5: If no give reasons	
C1. 6: What are the common diseases affecting your pigs?	
C1. 7: Which disease kills most?	
C1. 8: What age group that is most affected	
C1. 9: Have you heard of ASF before?	❖ **Yes** ❖ **No**
C1. 10: Where did you hear it from?	❖ Veterinary officer ❖ Media ❖ Other farmers ❖ Government offices ❖ Others specify
C1. 11: What are the disease's signs?	
C1. 12: Have your pigs encountered the disease?	❖ **Yes** ❖ **No**
C1. 13: Disease occurs in which season of the year?	

C2. Disease morbidity

C2. 1: Have you ever encountered case of African swine fever in your herd?	**Yes ( ) No ( )**
C2. 2: If yes, what was the date when pigs started and stopped suffering	
C2. 3: How many pigs were you having before outbreak?	❖ Adult boar/sow ❖ Growers ❖ Weaners ❖ Piglets
C2. 4: How many pigs got sick?	❖ Adult boar/sow ❖ Growers ❖ Weaners ❖ Piglets

C3. Disease mortality

C3. 1: How many pigs died?	❖ Adult boar/sow ❖ Growers ❖ Weaners ❖ Piglets
C3. 2: What actions did you take to prevent losses?	❖ No actions ❖ Slaughtered ❖ Sold ❖ Treatment ❖ Disinfection ❖ Others specify…….
C3. 3: If you used disinfectant, where was the source?	
C3. 4: What were the disinfecting procedures used?	

C4. Disease risk factors

C4. 1: Where is your source of stock?	❖ Fellow Farmers ❖ Research projects ❖ Commercial farmers ❖ NGO'S ❖ Own source
C4. 2: System of pig management	❖ Free ranging system ❖ Tethering system ❖ Semi-intensive system ❖ Intensive system
C4. 3: What type of feed do you feed your pigs?	❖ Kitchen leftovers ❖ hotel leftovers ❖ Maize bran ❖ Rice polish ❖ Others specify……
C4. 4: If kitchen left over, do you include pig meat?	**Yes ( ) No ( )**
C4. 5: If hotel left over does that hotel save pig products?	**Yes ( ) No ( )**
C4. 6: Do you treat the swills?	**Yes ( ) No ( )**
C4. 7: If Maize bran or Rice polishes, where is the source? ……..	
C4. 8: Did you introduce pigs into the herd before disease outbreak?	**Yes ( ) No ( )**
C4. 9: If yes, when …….. and where did you bring the pig(s) from?	

……..……	
C4. 10: Have you or animal attendant have contact with infected neighborhood pig farms?	**Yes ( ) No ( )**
C4. 11: Are there any ticks observed on pigs or premises? (*Ornithodorous* genus)	**Yes ( ) No ( )**
C4. 12: Do you share farm equipment with other pig keepers**?**	**Yes ( ) No ( )**
C4. 13: If yes which equipments?	
C4. 14: Are rats having access to store or pig pens?	**Yes ( ) No ( )**
C4. 15: Do wild birds enter pig pens?	**Yes ( ) No ( )**
C4. 16: Do any of your neighbor keeping pigs have encountered the disease?	**Yes ( ) No ( )**
C4. 17: How do you dispose the dead pigs?	
C4. 18: Are there any wild pigs around? **(***Phacochoerus africanus) Ornithodorous*	**Yes ( ) No ( )**
C4. 19: If yes do you have a tendency of hunting wild pigs?	**Yes ( ) No ( )**
C4. 20: Where do you sell your pigs ……. and for what purpose …….	

C4. Veterinary services

C4. 1: Did you report the occurrence of the disease to the veterinary officer?	**Yes ( ) No ( )**
C4. 2: Which services were you given?	❖ **Treatment of sick pigs** ❖ **Advice** ❖ **Others specify ……**
C4.3: Were you satisfied with the service given?	**Yes ( ) No ( )**
C4. 4: Did you adhere to quarantine conditions?	**Yes ( ) No ( )**
C4. 5: Did other people adhere to quarantine conditions?	**Yes ( ) No ( )**
C4. 6: Were you informed about the opening the quarantine?	**Yes ( ) No ( )**
C4. 7: Were you told when to restock the pigs?	**Yes ( ) No ( )**
C4. 8: Were you told the source of stock?	**Yes ( ) No ( )**

THANK YOU