VIRAL GENETIC DIVERSITY, RISK FACTORS AND SOCIO-ECONOMIC IMPACT OF AFRICAN SWINE FEVER FROM SELECTED PARTS OF TANZANIA, 2019 AND 2022

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EXTENDED ABSTRACT

African swine fever virus (ASFV) is a double stranded DNA virus of the family *Asfaviridae* and genus *Asfivirus* that causes African swine fever (ASF). The disease can lead up to 100% mortality of infected domestic pigs causing farmers to incur enormous losses as a result of pig mortalities and loss of market for pigs and pig products. The disease is one of the major pig diseases in Tanzania, with outbreaks reported in different regions including Kigoma, Arusha, Dar es Salaam, Dodoma and Mbeya. Twenty-four (I-XXIV) p72 genotypes have been described so far in Sub-Saharan Africa. Previous studies have reported ASFV genotypes II, IX, X, XV and XVI to be associated with ASF outbreaks in Tanzania. Major factors responsible for the spread of ASF in Tanzania include illegal movement of pigs and swill feeding. This present study was conducted to: (i) diagnose and genotype ASFV from the 2019 ASF Ngara district outbreak western Tanzania, (ii) genotype different ASFV trains in ASF outbreaks between march 2021 and march 2022 located un Lake Zone (Kahama, Geita), northern (Mwanza and Katavi) and central Tanzania (Mpwapwa, Kongwa) and Morogoro in eastern Tanzania, and to (iii) determine the Ngara ASF outbreak related risk factors and socio-economic impact. Spleen, lymph nodes, tonsils and liver were collected from the outbreaks from pigs with ASF clinical signs including hyperthermia, sternal recumbence and cutaneous congestion on the limbs, abdomen and outer side of the pinna. Postmortem findings on affected pigs included splenomegaly, enteritis, and severe hemorrhages of gastrohepatic and mesenteric lymph nodes. Polymerase chain reaction (PCR) using *peste porcina Africana* (PPA 1/2) primers targeting the conserved part of *B646L* gene (coding for the p72 capsid protein) that generates a 257 base pairs amplicon was used for ASF diagnosis. Genotyping was done by nucleotide sequence analysis of the variable 3’ end of the *B646L* gene. African swine fever was confirmed in dead pigs by PCR.

Phylogenetic analysis found ASFV genotype II of ASFV in pigs samples from Kongwa (TAN/2021/Kongwa), Mpwapwa (TAN/2021/Mpwapwa) and Morogoro (TAN/2022/ Morogoro)

districts. Genotype X was found in domestic pigs samples from Kahama (TAN/2021/Kahama), Geita (TAN/2021/Geita) and Katavi (TAN/2022/Katavi) districts and genotype IX was found in Mwanza (TAN/2022/Mwanza). Phylogenetic analysis clustered ASFV from Ngara into genotype X. Presence of these genotypes indicate their relation with previous outbreaks in Tanzania indicating persistent circulation of the viruses. Feeding pigs of uncooked swill was shown to be significantly associated with the Ngara ASF transmission (OR=3.08, C.I.95%=1.06-8.99, P=0.0009). Occurrence of ASF outbreak resulted into loss of income and investment as most farmers kept pigs for the purpose of income generation. Food security was disturbed due to high pig mortality following occurrence of ASF outbreak. A total of 93 630 000 Tanzanian shillings (approximately 41 065 USD) was estimated to be lost as a result of pigs' mortality in 219 households. This calls for the need to educate farmers on the methods for effective control of disease and for the veterinary services to enforce regulation on movement of pigs and pig products between regions so as to prevent disease spread.

DECLARATION

I, Clementina Clement Kivumbi, 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 has neither been submitted nor being submitted concurrently in any other institution.

_____ _____

Clementina Clement Kivumbi Date

(MPhil candidate)

The declaration is hereby confirmed;

_____ _____

Prof. Gerald Misinzo Date

(Supervisor)

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DEDICATION

This work is dedicated to my brother, Matthew Clement Kivumbi for being my number one academic role model throughout my lifetime.

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LIST OF ABREVIATIONS AND ACRONYMS

°C	degree centigrade
ASF	African swine fever
ASFV	African swine fever virus
bp	base pair
C.I.	confidence interval
CD	cluster of differentiation
CPE	cytopathic effect
CSF	classical swine fever
CVR	central variable region
DAD	N,N'-diallytartardiamide
DNA	deoxyribonucleic acid
DRC	Democratic Republic of Congo
dsDNA	double-stranded deoxyribonucleic acid
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
et al	and others
FAT	fluorescent antibody test
FITC	fluorescein isothiocynate
g	gravitational constant
HAD	haemadsorption test
IPMA	immunoperoxidase Monolayer Assay
IPT	immunoperoxidase test
kbp	kilo basepairs
MGF	multigene families

MHC	major histocompatibility factor
mL	millilitre
MPhil	masters of philosophy
Mr	mister
Ms	miss
nm	nanometer
OIE	World organizational for Animal Health
P	P-value
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PDNS	porcine dermatitis and nephropathy syndrome
pH	potential of hydrogen
Prof	professor
SACIDS	SACIDS Africa Centre of Excellence for Infectious Diseases
TRS	tandem repeats sequence
TZS	Tanzanian shillings
URT	United Republic of Tanzania
USD	united states dollars
VERO	verda reno
µL	microliter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

African swine fever virus (ASFV) is the causative agent of a highly lethal and highly contagious disease namely, African swine fever (ASF). The virus is a large enveloped double stranded DNA virus whose genome length ranges from 170 to 194 kilobase pairs (kbp) (Alonso *et al*., 2018). The genome is complex and may encode up to 165 genes out of which there is a number of novel genes whose functions have not yet been described (Tulman and Rock, 2001). Depending on the virulence of the virus strain, the infective dose and the inherent susceptible host factors such as immunity, the disease mortality and morbidity rates can reach up to 100% (Penrith *et al.*, 2019). Outbreaks of ASF results into devastating economic losses as a result of pig and pig products trade restrictions and exceptionally high rates of animal losses due to resultant high mortality rates (Sánchez-Vizcaíno *et al*., 2012).

First description of ASF as a disease of domestic pigs was documented in 1928 in East Africa (Jori *et al*., 2013). The disease is currently endemic in sub-Saharan Africa due to both pig-to-pig transmission and virus persistence in the sylvatic cycle involving asymptomatically infected wild pigs and soft tick of the genus *Ornithodoros* (Jori *et al*., 2013). The main mechanism of ASFV transmission from wild pigs to domestic pigs in Africa is trough infected tick bites (Penrith *et al*., 2009). Initial reports of ASF outside Africa were in Portugal, Europe in 1957 and later on in 1960 (Garigliany *et al*., 2019). Later on ASF outbreaks occurred other countries of Europe including Italy (1967, 1980), France (1964, 1967), Malta (1978), Belgium (1985) and the Netherlands (1985, 1986) (Costard *et al.*, 2009). Today ASF has been eradicated in some parts of Europe including

the rest of Italy, except the Italian island of Sardinia where it has been endemic (Penrith *et al.,* 2009). The history of ASF in Southern America started with reports of the disease in Cuba in 1971, which was later on eradicated with difficulty. The disease was re-introduced in Cuba in 1980 following 1977-78 ASF outbreaks in Iberian Peninsula, which spread to Dominican Republic in 1978, Haiti in 1979 and eventual spread to Cuba (Penrith *et al.*, 2009). Following costly eradication measures, the disease was successfully eradicated in the Americas and the region is currently free of the disease (Penrith *et al.,* 2009). ASF was introduced in Georgia in 2007 and later on spread to Russia and Asia including China (Costard *et al*., 2013; Ge *et al*., 2018). Eradication of ASF in these regions has proved difficult as a result of complexity of sanitary, environmental and economic factors (Sánchez-Vizcaíno *et al*., 2012). There is no vaccine or treatment against ASF, and the only control measures include early detection, quarantine of infected animals and implementation of sanitary measures (OIE, 2012).

Outbreaks of the disease have been reported in a significant number of regions in Tanzania including Kigoma, Mwanza, Arusha, Kilimanjaro, Dar es Salaam, Morogoro, Dodoma, Rukwa, Iringa and Mbeya. Highest pig population in Tanzania is found in Mbeya region followed with Iringa, Ruvuma and Kilimanjaro regions (URT, 2012). Genotyping of ASFV is primarily done by nucleotide sequencing of variable 3'-end of the *B646L* gene encoding the p72 capsid protein. 24(I-XXIV) p72 genotypes have been described so far, with all of them being known to circulate in eastern and southern Africa (Quembo *et al.,* 2018). Molecular characterization of ASFV in Tanzania has so far associated the outbreaks with genotype II, IX, X, XV and XVI. The ASFV genotyping studies have helped in establishing the epidemiological patterns of the disease and helped in making informed decisions regarding prevention, control and eradication measures of the disease. Additionally, full-length sequencing of the *E183L* gene encoding the p54 transmembrane

protein and the central variable region (CVR) of the virus genome provide a high level of resolution in ASFV genotyping (Quembo *et al.*, 2018).

1.2 African swine Fever Virus

African swine fever virus was once assigned to the *Iridoviridae* family. However, it is currently classified as the sole member of the family *Asfaviridae* and genus Asfivirus (Alonso *et al*., 2018). The capsid of ASFV is icosahedral and is surrounded by the external dispensable lipid-containing envelope (Dixon *et al.,* 2012). The size of extracellular enveloped virions ranges from 175-215 nm in diameter. The core structure of the virion is a nucleoprotein, 70-100 nm in diameter, surrounded by an internal lipid bilayer. The virus has a large, linear, covalently closed-ended dsDNA of 170-194 kilobase pairs (kbp) (Alonso *et al*., 2018). The genome has a central variable region about 125 kbp long. Adjacent to the central variable region (CVR) both to the left and right sides a highly variable region of inverted complementary 6 tandem repeats of about 35 and 25 kbp correspondingly is located (Blasco and Lavega, 1989).

1.3 African Swine Fever Virus Physical and Chemical Properties

Infected pigs shed the virus in saliva, genital and nasal secretions, faeces, urine and tears (Mazur-Panasiuk *et al*., 2019). The virus is known to be resistant to various disinfectants with exception to ether and chloroform. Some chemicals that are able to inactivate the virus include sodium hydroxide, hypochlorites, formalin, ortho-phenylphenol and iodine compounds. Furthermore, it is stable in protein-rich environment over a wide range of temperature and pH (Dixon *et al.,* 2005). Therefore, it can survive in frozen, processed and putrefied meat without being inactivated over considerable time-periods. However, virus inactivation is achieved by cooking at 70 °C for 30 minutes (Beltran-Alcrudo *et al.*, 2017).

1.4 Primary Cells for ASFV Isolation

Porcine blood monocytes obtained from pig blood and tissue macrophages obtained by bronchoalveolar lavage are natural host cells of ASFV. They have been used as an *in vitro* system of choice for bulk production of virus ASFV isolates obtained from natural outbreaks, as wild-type ASFV were not found to propagate in conventional established cell cultures. Primary cells have been used since the beginning of studies on ASFV for propagation, infective assays and detection (Carrascosa *et al.,* 2011; De León *et al.,* 2013). Despite the use of primary cells being convenient for virus studies as they mimic the natural infection in in vitro system, there are limitations to it. Primary cells do not replicate in cell cultures. Therefore, they are difficult to produce in large quantities for studies with high demand like biochemical or structural studies (De León *et al.,* 2013). Batch-to-batch differentiation is another hindrance to using primary cells as it leads to lack of results reproducibility (De León *et al.,* 2013). However, some solutions to these drawbacks have been achieved through adaptation of ASFV isolates to grow in various established conventional cell lines (Rai *et al*., 2020).

1.5 Uses of Established Cell Lines for ASFV Culture

Adaptation of ASFV to grow in established cell lines has been helpful in solving limitations due to the inability to grow and batch-to-batch differences in ASFV natural host cells (Plowright *et al.,* 1969; Enjuanes *et al.,* 1976). Differences result from differences in levels of activation and differentiation between lots of primary cells and may prevent reproducibility of results. Some ASFV isolates have been adapted to grow in monkey derived cell lines such as Vero cells (Plowright *et al*., 1969: Enjuanes *et al*., 1976; Carrascossa *et al.,* 2011). Their ability to grow and be produced in sufficient amount has been useful on many biological, biochemical and structural studies of the virus. However,

they provide solutions to a limited degree as they can only be used with adapted isolates (De León *et al*., 2013).

COS.1 cells have been established and confirmed to have the ability to grow many ASFV isolates. The cells have been used in; construction of deletion mutants, bulk production of virus from poor quality samples collected in ASFV outbreaks and in production of ASFV cytoplasmic soluble antigens for indirect ELISA and IPT tests (De León *et al*., 2013; Rai *et al*., 2020).

1.6 Receptors for ASFV

Cells expressing CD163, CD203a, CD45 and MHCII have been shown to have the ability to be infected with ASFV. Maturation of monocytes to macrophages is associated with the up-regulation of expression of CD163, which is shown to increase ASFV infection. However, comparison of susceptibility to ASFV between cells that stably express CD163 and the parental cells showed no significant difference. This indicates that expression of CD163 alone is not enough to increase virus susceptibility (Lithgow *et al*., 2014). Expression of CD163 may be necessary but insufficient for infection and other molecules such as intracellular factors required for virus replication as well as those necessary for receptor complex may be necessary in the infection process (Galindo and Alonso, 2017). Cells expressing CD45, CD203a and MHCII were shown to be susceptible to ASFV infection but were also shown to not be preferentially infected.

1.7 African Swine Fever Virus Entry and Replication

The virus enters into cell through receptor-mediated endocytosis and micropinocytosis (Alonso *et al*., 2018). Following entry into the cell, uncoating of the virus takes place in the endosomal lumen at acidic pH. Released viral cores finally undergo degradation by

ubiquitin-proteasome system to release the viral DNA so as to start replication *(Alonso et al.,* 2018). The virus is then transported in association with the microtubular motor light chain dyein, to the perinuclear factory for DNA replication and virus assembly. Completely assembled new virions finally exit the cell through budding (Alonso *et al.,* 2018).

1.8 ASFV Virus Transmission Cycles

The virus circulates in four cycles; the domestic cycle, the pig-tick cycle, the sylvatic circle and the wild-boar habitat cycle (Chenais *et al*., 2019). Pigs contact the disease through direct contact with infected pigs or fomites in domestic pig cycle and get infected through infected tick bites in the tick-domestic pig cycle (Boinas *et al*., 2011). The sylvatic cycle involves asymptomatically infected ticks and wild pigs including bush pigs, warthogs which act as both reservoir and transmission vectors (Jori *et al*., 2013). In the wild boar-habitat cycle the Eurasian wild boars get infected directly through contact with other infected boars. Boars also indirectly get infected through contact with carcasses of infected pigs or contact with habitat contaminated by the carcasses (Chenais *et al*., 2019). In eastern and southern Africa, presence of warthogs (*Phacochoerus africanus*) and *Ornithodoros moubata* ticks maintain the presence of ASFV in nature (Costard *et al.,* 2009; Costard *et al.,* 2013). Domestic pigs and European feral pigs and wild boars (*Sus scrofa*) are susceptible to the disease and exhibit similar clinical signs and mortality rates. On contrary, African wild pigs: warthogs (*Phacochoerus aethiopicus/ Phacochoerus africanus*), giant forest hogs (*Hylochoerus meinertzhageni*) and bushpigs (*Potamochoerus porcus*) act as reservoir hosts of the virus and exhibit few or no clinical signs of the disease (Kleiboeker, 2002). *Ornithodoros moubata* and *O. porcinus* in sub-Saharan Africa and *O. erraticus* in Irebian peninsula act as both vectors and biological reservoirs of the virus (Bora *et al*., 2020). African swine fever virus infection is similar in both African wild pigs and *Ornithodoros* ticks in that it is highly inapparent. The infection in the soft ticks is characterized by high virus titer, a lifetime persisting infection and effective ability to pass

the infection to the offspring (Bora *et al*., 2020). These characteristics lead to speculation that ASFV is an insect virus. In contrast, the infection in African wild pigs is characterized by low virus titers, which indicate that mammalian species especially domestic swine represent “accidental hosts of the virus” (Kleiboeker, 2002). Warthogs live in subterranean burrows where they are normally heavily infested with *Ornithodoros* ticks and therefore maintain the sylvatic cycle of the virus. Transmission of the virus to domestic pigs is therefore most likely through infected *Ornithodoros* ticks feeding on domestic pigs (Penrith *et al*., 2019). Domestic pigs can also get infected with the virus through contact with contaminated materials and reservoir hosts which are asymptomatically infected or who have recovered from the infection (Kleiboeker, 2002). Also existence of asymptomatically infected wild pigs and *Ornithodoros* ticks can lead to introduction of the disease in areas where it was long eradicated (Kleiboeker, 2002).

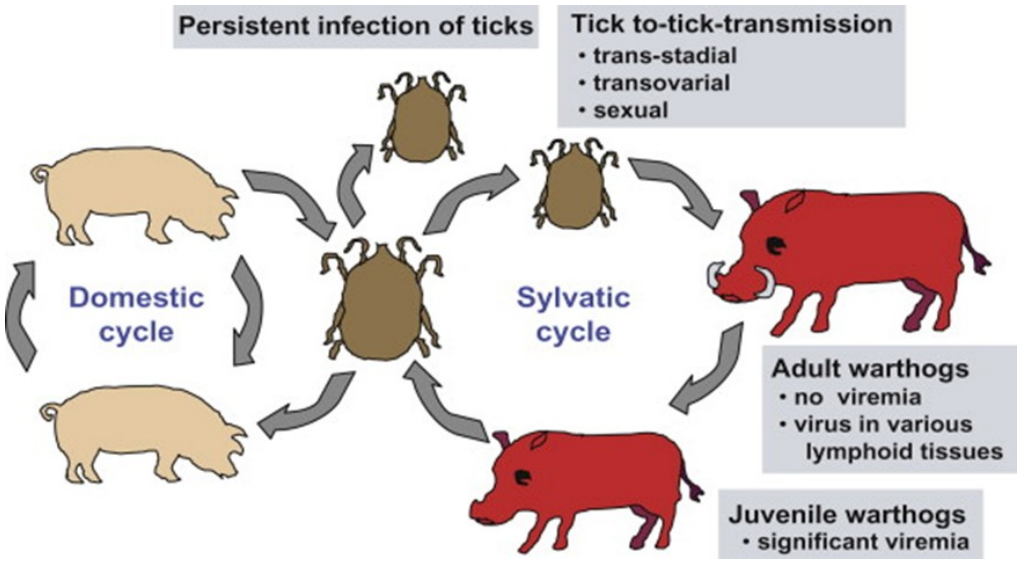

Figure 1.1: ASFV transmission cycle. African swine fever virus circulates in a sylvatic cycle of wild pigs and Ornithodoros ticks hosts. Infected ticks can transmit the virus between pigs through tick bites and can transmit the virus to other ticks sexually, transstadialy and transovarian. Domestic pigs can get infected through contact with contaminated carcasses, food, vehicles, equipment, and infected pigs and through infectedtick bites. (MacLachlan and Dubovi, 2010)

1.9 ASF Pathogenesis

Routes of ASFV entry into the host include tick bite, host respiratory tract and oral routes. The incubation period of the disease is dependent of the route of exposure. Inoculation of the virus through tickbite causes the disease incubation period of less than five days after inoculation. In contrast, incubation period following inoculation through direct contact varies from 5 to 15 days (Kleiboeker, 2002). The virus enters the host cells through clathrin-mediated endocytosis. It undergoes replication in the cytoplasm of host cells and the newly synthesized virions are released from the cell by budding (Gallardo *et al*., 2015).

1.10 African Swine Fever Clinical Signs

ASF outbreaks in disease-free areas can result in up to 95-100% mortality rates (Gallardo *et al*., 2015). Severity of the disease in domestic pigs ranges from peracute, acute, sub-acute and chronic depending on the virulence of the virus strain and the infective dose. Most virulent strains cause peracute and acute forms of the disease. Peracute ASF results from exposure to high doses of the highly virulent strains. Its mortality rates are very high and can reach up to 100% within a range of 1-4 days. In this form of the disease the pigs normally die before manifestation of clinical signs and before formation of characteristic lesions of the disease (Kleiboeker, 2002).

In acute ASF the incubation period ranges from 4-9 days post inoculation of highly virulent virus into the host. Acute ASF clinical signs include high fever (40.5–42°C), increased respiratory rate and hemorrhages, cyanosis and erythema in the skin (Kleiboeker, 2002). Hemorrhages occur also in internal organs, which normally become dysfunctional resulting into vomiting and hemorrhagic diarrhea (Gallardo *et al*., 2015). Death in acute ASF on average occurs within 6-13 days post-infection and mortality rate often approaches 100%.

Moderately virulent strains cause sub-acute ASF, which is characterized by less intense signs; slight fever, reduced appetite and depression and the mortality rate are lower (30–70%).

By contrast, chronic ASF is caused by moderately, and low virulent virus. Chronic ASF has a variety of signs including; loss of weight, irregular peaks of temperature, respiratory symptoms, necrosis in areas of skin, chronic skin ulcers, arthritis and takes months to develop normally resulting into low mortality rates (OIE, 2012).

1.11 ASF World Distribution

The disease has been endemic in most countries of sub-Saharan Africa since being firstly reported in Kenya in 1928 (Penrith and Vosloo, 2009). Initial occurrence outside of ASF outside Africa was reported in Portugal in 1957 (Jori *et al*., 2013). The disease then spread to other Europian countries including Spain, Italy, France, Belgium and Netherlands and to Caribbean in Dominican Republic and South American countries including Brazil, Cuba and Haiti (Garigliany *et al*., 2019). Eradication of ASF in America, the Caribbean and Europe has been achieved except Sardinia, Island in Italy since its introduction in 1982 (Rowlands *et al*., 2008).

Regions which have been successfully in eradicating the disease have applied drastic and very costly measures including testing and drastic sanitary measures (Costard *et al*., 2013). However, ASF remains endemic in the Caucasus and Eastern Europe since its reintroduction in 2007 and is present in the Russian Federation and Asia including China (Costard *et al.*, 2013; Ge *et al.*, 2018). Re-introduction of the disease and introduction mainly results from importation of infected pigs and pig products and by entry of contaminated vehicles, clothing and human food leftovers (Jori *et al*., 2013).

Following introduction of ASF in disease-free regions, its eradication is difficult because to date there is no vaccination and treatment against the disease (Borca *et al*., 2020). It is also difficult to be eradicated in regions with wild reservoirs (African bush pigs, warthogs and ticks of the genus *Ornithodoros*) of the ASF virus such as in sub-Saharan Africa (Boinas *et al*., 2011). Therefore these countries act as a large reservoir of the disease that can be transmitted to other disease free regions (Penrith and Vosloo, 2009). In Tanzania the disease outbreaks have been reported to occur sporadically in different regions including Dar es Salaam, Rukwa, Mbeya, Iringa, Arusha, Morogoro, Mwanza and Kigoma (Kimbi *et al.*, 2015). For 4 years from 1997 to 2001 no ASF outbreaks were reported in Tanzania. Appearance of the disease after many years of absence may be accounted by the persistence of the virus in the sylvatic cycle or by introduction of the virus by illegal importation of pigs and by swill feeding (Misinzo *et al*., 2014). This is because the pigs can get infected directly by contact with infected pigs or indirectly by getting into contact with contaminated feed, vehicles and gears (Wilson and Swai, 2013).

1.12 ASF Diagnosis

African swine fever is clinically similar to a number of swine diseases including: salmonellosis, erysipelas, pasteurellosis, Aujeszky's disease and porcine reproductive and respiratory syndrome. The disease can also not be differentiated from classical swine fever by both clinical signs and post-mortem findings (OIE, 2012). When ASF is suspected, spleen, tonsils, kidney and lymphnodes stored at 4°C and blood sample in EDTA should be submitted to the laboratory for definitive testing (OIE, 2012). Virus antigens can be detected in tissue smears and cryostat section by fluorescent antibody test. The viral gen2ome can be detected by polymerase chain reaction (PCR), which is a highly sensitive and specific test of ASF that can be used in a range of circumstances including when the samples are not suitable for virus isolation or for antigen detection. However, virus culture is useful for propagation of the agent to facilitate detection by PCR and antigen detection tests. Also, positive haemadsorption test during virus isolation is definitive for ASF

detection. Antibodies can be detected in pigs that have survived the infection 7-9 days post infection. Serological tests for detecting antibodies include immunoblotting test, the enzyme-linked immunosorbent assay (ELISA) and indirect fluorescent test IFA. The antibodies can persist for a lifetime therefore; serological tests are useful in detection of chronic ASF and detection of the virus in survivor pigs (OIE, 2012). Diagnosis of ASF and sanitary measures taken upon detection are key control methods of the disease since there is no vaccine and treatment against the disease up to date. Choice of the diagnostic method is dependent on the diagnostic capacity of the area and status of the disease (OIE, 2012).

1.12.1 Haemadsorption test

Most ASF strains can cause hemadsorption; a tendency of pig erythrocytes to adhere to infected pig monocytes and macrophages (Carrascosa *et al.*, 2011). Positive haemadsorption test is when formation of erythrocytes rosettes around infected pig primary leucocytes occurs and it is definitive for ASFV detection. However, the test is limited to detection of only the hemadsorbing ASFV strains. A very small number of the non-haemadsorbing strains have been isolated which were mostly avirulent but some produced acute ASF (OIE, 2012). A negative hemadsorption (HAD) test plus cytopathic effect (CPE) consisting of reduction in number of adherent cells may be from infection by a non-hemadsorbing virus, cytotoxicity of the inoculum or Aujeszky's disease virus. Presence of non-haemadsorbing virus can be detected by use of FAT test, Immunoperoxidase test or PCR test (OIE, 2012).

1.12.2 Antigen detection tests

1.12.2.1 Fluorescent antibody test (FAT)

In FAT, antibodies against the virus, conjugated with fluorescein isothiocyanate (FITC) are used in detection of ASFV in tissues and viscera of suspect animals. It can also be used in detection of non-haemadsorbing virus in HAD negative monolayers (OIE, 2012). This test is not recommended for detection of infectious agents in chronic and sub-chronic because

formation of antigen-antibody complexes reduces the test sensitivity by blocking the antigens making them undetectable by the test. However, FAT test is quick, easy and sensitive for detection of the virus in pigs that were infected with high virulent strains and died before formation of antibodies (Kleiboeker, 2002). Observation of the test results is done using a fluorescent microscope and positive FAT results plus clinical signs and indicative lesions provide presumptive diagnosis of African swine fever.

1.12.3 Serological tests

1.12.3.1 Enzyme-linked immunosorbent assay

Antibody-ELISA is a highly specific, sensitive and cost effective test and is the prescribed for international trade (OIE, 2012). The test is not recommended for detection of the highly virulent ASF viruses, since they cause death of pigs before formation of antibodies. It is recommended for detection of moderate and low virulent virus because of formation of antibodies which are not fully virus neutralizing. Antibodies can persist for a very long time up to a lifetime and they can be detected by the antibody-ELISA test in serum and fluid from tissues (Kleiboeker, 2002). In endemic areas ELISA-positive samples should be confirmed by any of the confirmatory tests such as immunoblotting, IFA test and immunoperoxidase staining. In the study by (Gallardo *et al*., 2015). The OIE prescribed serological tests were shown to be accurate, specific and sensitive in detection of the virus in all epidemiological settings. However, the OIE recommended ELISA was shown to not detect some of the East African strains of ASF virus (Gallardo *et al*., 2015).

1.12.3.2 Immunoblotting

In this test soluble virus proteins are prepared and are electrophoresed through acryl-amide/N,N’-diallytartardiamide (DAD) gels. Then the proteins are transferred to the nitrocellulose membrane by electrophoresis. Afterwards the membrane is dried and the side

on to which the proteins were electrophoresed is labeled. Then the membrane is cut into strips, which are approximately 4cm long and 0.5cm wide. The strips are then labeled on the side to which the proteins were electrophoresed. Then these strips can be used in detection of antibodies in sera of pigs which are convalescent and are in acute ASF (OIE, 2012).

1.12.3.3 Indirect fluorescent antibody test

Antibodies in sera, plasma and tissue exudates can be detected using indirect antibodies labeled with fluorescein isothiocyanate (FITC). The test has high sensitivity and specificity and positive samples show specific fluorescence in the cytoplasm of infected cells under fluorescent microscope. It is used for confirmation of ASF in inconclusive ELISA results for sera from endemic areas. It is also used for confirmation of the disease for sera from non-endemic areas that are positive in ELISA (OIE, 2012).

1.12.4 Detection of ASFV genome by polymerase chain reaction

Detection of the virus genome by PCR is done using primers targeting a highly conserved and well-characterized region within the p72 coding region (Beltran-Alcrudo *et al.,* 2017). This is the most rapid, sensitive and specific ASFV detection technique relative to other antigen detection methods. All p72 genotypes including both haemadsorbing and non-haemadsorbing isolates can be detected by PCR using DNA extracted even in samples that are too degraded for virus isolation. It is a detection method of choice in per acute, acute and chronic forms of the disease due to its ability to detect highly, moderately and low virulent ASF strains.

Both conventional and real-time PCR methods for ASF detection have been developed and validated (OIE, 2012). Furthermore, a multiplex real time PCR has been developed for

simultaneous detection and differentiation of ASF with diseases of similar clinical signs including classical swine fever (CSF) and porcine dermatitis and nephropathy syndrome (PDNS) (King *et al*., 2003).

1.12.5 Molecular epidemiology of ASFV

Twenty four (I-XXIV) ASFV genotypes have been identified by nucleotide sequencing of the variable 3'-end of the *B646L* gene encoding the p72 capsid protein. Further subtyping of these genotypes into subgroups has been achieved by additional full-length sequencing of the *E183L* gene encoding the p54 transmembrane protein, analysis of the tetramer amino acid repeats encoded by the CVR of the virus genome and the tandem sequence repeat located between the *173R* and *1329L* region (Quembo *et al.*, 2018). Historically, all genotypes have been known to circulate in eastern and southern Africa while only genotype I was known to circulate in Europe, South America, the Caribbean and West Africa (Costard *et al*., 2013). Genotype IX was later on described in West Africa and genotype II spread to Russian Federation, Caucasus region and Asia (Costard *et al*., 2013; Galindo and Alonso, 2017; Garigliany *et al*., 2019; Taylor *et al*., 2020).

Viruses that have caused ASF outbreaks in Tanzania belong to *B646L* p72 genotype II, IX, X, XV and XVI (Lubisi *et al*., 2005; Misinzo *et al*., 2014; Yona *et al*., 2020). Initial report of the highly virulent genotype II identical to the Georgia isolate 2007/1 in Tanzania was in the 2010 ASF outbreaks in Kyela district, Mbeya. Later on, the genotype spread to other parts of the country including Dar es Salaam, Mbeya, Iringa and Rukwa (Misinzo *et al*., 2012). Outbreaks in northwestern parts of Tanzania, Kigoma and Mwanza have been identified with genotype IX (Wambura *et al*., 2006). Genotype X identical to Kenyan isolate and XVI have been identified in northeastern Tanzania regions (Arusha, Machame, Rombo and Moshi and Longido) (Lubisi *et al*., 2005; Misinzo *et al*., 2012). Furthermore,

eastern regions of Tanzania (Morogoro and Dar es Salaam) outbreaks have been identified with genotype XV (Yona *et al*., 2020).

Molecular characterization has been a tool of understanding the ASFV isolates in outbreaks and their possible source. Information obtained from ASFV genotyping is useful in understanding the number of genotypes associated with outbreaks and provides scientific based information regarding the outbreaks to stakeholders. Therefore, helps in making well-informed decisions regarding relevant control and eradication measure to be taken (Lubisi *et al*., 2005).

1.12.6 Prevention and control of African swine fever

Pig keeping industry is one of the fastest growing industries in most African countries providing both employment opportunities and food security (Fasina *et al*., 2020). However, ASF outbreaks have proved to be one of the major constraints in pig farming resulting from high pig mortalities and hindrance in pig and pig products trade (Bastos *et al*., 2003). In some regions pig keeping has attained substantial growth and replaced major economic activities. For example, in Rombo district, Kilimanjaro pig keeping has replaced coffee farming in many households as a means of generating income (Misinzo *et al*., 2014). Therefore, ASF outbreaks occurred in Tanzania have highly affected the livelihood of pig farmers and contributed to food and nutritional insecurity (Misinzo *et al*., 2014).

Attempts to prevent ASF through vaccination have not resulted in production of effective and safe vaccine so far (Wang *et al*., 2018). This is mainly because of complexity of the virus genome encoding more than 160 polypeptides most of which are responsible for invading and weaken host immunity (Sang *et al*., 2020). Due to high variability of ASFV isolates identified thus far, attenuated live vaccines produced have led to protection against

homologous isolates (Galindo and Alonso, 2017). Inoculation of antibodies into pigs from ASFV-infected and recovered pigs has shown to offer protection against homologous ASFV through viremia reduction and delay of onset of the disease clinical signs (Galindo and Alonso, 2017; Borca *et al*., 2020). Cross protection between genotypes has not been widely studied with few reports on cross protection between certain genotypes (Arias *et al*., 2017). Therefore, due to unavailability of ASF vaccine, control and prevention of the disease relies on early detection followed by drastic sanitary measures (Galindo and Alonso, 2017). On cases of ASF outbreaks, virus spread to disease free regions can be prevented by quarantine of infected live pigs and pig products followed by sanitary measures such as burning and burying. Strict biosecurity measures is also an effective way to prevent human involvement in spread of the disease (Arias *et al*., 2017). Access to pig keeping premises should be restricted to only essential visitors like workers and veterinarians. There should be facilities for changing cloths to cloths that do not leave the premises so as to prevent entry of virus through contaminated cloths. Countries should ensure that farmers, field veterinarians and relevant authorities are trained on how to detect ASF, keep records and follow regulations aimed at controlling the disease (Penrith and Vosloo, 2009; Beltran-Alcrudo *et al*., 2017).

1.13 Problem Statement and Justification

Outbreaks of deaths of pigs with clinical signs suggestive of ASF such as haemorrhages visible on the ears and flanks occurred in different locations in Tanzanian including Ngara, Mwanza, Geita, Katavi, Kongwa Mpwapwa, Kahama and Morogoro between 2019 and 2022. Tissue samples from the dead domestic pigs were collected for ASF detection and ASFV molecular characterization. Molecular characterization studies of the virus have highly contributed in understanding the virus epidemiology. Previous ASFV genotyping studies conducted in Tanzania have associated ASF outbreaks with *B646L* (p72) genotype

II, IX, X, XV and XVI. In Tanzania, ASF is endemic in domestic pigs and is maintained in the sylvatic cycle involving warthogs and *Ornithodoros moubata* ticks. This study will contribute in understanding the relatedness of ASFV associated with the mentioned ASF outbreak with other ASFV in the GenBank. The study findings will also determine the association between risk factors and ASF occurrence and its impact on livelihood of small-scale pig keepers. In turn, this will help in making informed decisions regarding risk management of the disease.

1.14 Study Objectives

1.14.1 General objective

Assessment of the viral genetic diversity, risk factors and socio-economic impact of African swine fever from selected parts of Tanzania, 2019 to 2022.

1.14.2 Specific objectives

i. To genetically characterize ASFV from domestic pigs in Ngara during the 2019 ASF outbreaks, and
ii. To identify and quantify the associated risk factors and evaluate socio-economic impact.
iii. To investigate the genetic diversity of ASFV strains involved in the ASF outbreaks between march 2021 and march 2022 in different parts of Tanzania.

CHAPTER TWO

2.0 Manuscript 1: An assessment of the epidemiology and socio-economic impact of the 2019 African swine fever outbreak in Ngara, western Tanzania

CHAPTER THREE

3.0 Manuscript 2: Co-circulation of genotype II, IX and X African swine fever virus during 2021 and 2022 outbreaks in Tanzania

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3.1 Abstract

African swine fever (ASF) caused by ASF virus (ASFV) is a leading cause of domestic pigs mortalities globally. This study was conducted to investigate the genetic diversity of ASFV involved in reported ASF outbreaks between March 2021and March 2022 in Tanzania. Tissue samples were collected from dead domestic pigs following the reported ASF outbreaks in different cities located in central, eastern, northern (Lake Zone) and western Tanzania. Confirmation and genetic characterization of ASF was done by partial nucleotide amplification of the *B646L* (p72) gene using PPA1/2 and p72U/D primers, respectvely. Genotype II of ASFV was found in domestic pig samples from central and eastern Tanzania. Genotype IX and X were found in domestic pig samples from Lake Zone, while genotype X was also found in domestic pig samples from western Tanzania. These ASFV genotypes were similar to previously reported genotypes in Tanzania, Malawi, Burundi, Democratic Republic of the Congo, Kenya and Uganda. The similarities of the ASFV strains responsible for the 2021 and 2022 outbreaks to previously described Tanzanian ASFV strains suggest maintenance of the virus in the domestic pig cycle. Co-circulation of three different ASFV genotypes in different geographical zones of Tanzania may indicate re-emergence of the virus from different sources. Furthermore, the similarity of ASFV genotypes to those previously reported in neighboring countries underscore the transboundary nature of ASFV spread. Zoosanitary measures and quarantine enforcement are recommended in order to prevent ASFV domestic circulation in Tanzania.

Keywords: African swine fever, African swine fever virus, genotypes, domestic pigs, Tanzania.

3.2 Introduction

African swine fever (ASF) is a highly deadly and contagious viral hemorrhagic disease of domestic pigs and Eurasian wild boars of all ages and so far ASF does not have a cure or vaccine (Borca *et al*., 2020). It is caused by ASF virus (ASFV) (Alonso *et al.,* 2018). The virus is a large, enveloped double stranded DNA virus whose genome is 170-194 kilo base pairs (kbp) long and a sole member of a family *Asfarviridae,* genus *Asfivirus*. ASFV is the only known DNA arbovirus, hosted and transmitted by soft ticks of the genus *Ornithodorus* (Alonso *et al.,* 2018). The virus also asymptomatically infects African wild pigs (warthogs and bush pigs) and thus they act as reservoir hosts of the virus normally with low viremia (Boinas *et al*., 2011).

ASFV is found in bodily fluids of the infected pigs (tears, blood and urine) and faeces, and can spread rapidly in pig herds through direct contact between susceptible and infected domestic pigs. Pigs get infected through infected tick bites, and contact with contaminated objects like farm equipment, boots and vehicles (Kleiboeker, 2002). Clinical signs of the disease include cutaneous congestion, noticeably on the flanks and ears, high fever (40.5 - 42°C), abortion in pregnant sows, staggering gait, loss of appetite, bloody diarrhea, straw tinged urine and vomiting. Disease severity in domestic pigs ranges from highly fatal disease that can result up to 100% pig mortality to subclinical forms of disease depending on the virulence of the virus strain and the infective dose (Kleiboeker, 2002).

African swine fever has so far been described in Europe, Asia, South America and Africa. The first description of the disease was done in Kenya in 1921 (Montgomery, 1921). ASF was restricted in Africa until its initial report in Europe, Portugal in1957 where it was first reported outside Africa continent (Costard *et al.,* 2013; Jori *et al*., 2013). The disease then

spread to other European countries including Spain, Italy, France, Belgium and Netherlands and to Caribbean in Dominican Republic and South American countries including Brazil, Cuba and Haiti (Garigliany *et al*., 2019). Costly and drastic sanitary measures including culling of all infected animals enabled eradication of ASF in America, the Caribbean and Europe except Sardinia island in Italy where ASF is endemic since its introduction in 1978 (Rowlands *et al*., 2008). Following the introduction of the African swine fever virus in Georgia in 2007, the virus has widely spread into Europe and Asia, including largest pork-producing countries including the Russian Federation and China thus posing a great threat to food security worldwide (Ge *et al*., 2018). Transboundary nature of ASF is mainly a result of human activities including illegal international transportation of infected pigs and pig products and entry of contaminated vehicles, fomites and human food leftovers in disease-free regions (Jori *et al*., 2013).

ASF is endemic in sub-Saharan Africa where it is maintained in wild host reservoirs (African bush pigs, warthogs and ticks of the genus *Ornithodoros*) (Boinas *et al*., 2011). Sub-Saharan countries therefore act as a large reservoir of the disease that can be transmitted to other disease free regions (Penrith and Vosloo, 2009). In Tanzania ASF outbreaks have been of both sporadic and endemic in nature due to persistency of the domestic cycle (Misinzo *et al*., 2014; Kimbi *et al.*, 2015).

Molecular studies of ASF virus have thus far described twenty four ASFV genotypes (I-XXIV) based on partial *B646L* (coding for the p72 major capsid protein) gene sequencing (Boshoff *et al*., 2007; Gallardo *et al*., 2009; Quembo *et al.*, 2018). Further sequence analysis of full-length gene *E183L* (coding for p54 envelope protein) and central variable region (CVR) within the *B602L* gene is used in clustering isolates into groups and subtypes

(Lubisi *et al.,* 2007; Gallardo *et al.,* 2009). Thirty one subgroups of ASFV have been identified by sequence analysis of the especially discriminative genetic marker, the *B602L* gene based on variations in the encoded tetrameric amino acid repeats (Nix *et al.,* 2006). Studies on incidence and distribution of ASFV genotypes in different outbreaks have given insights on means of spread of disease helping to come up with effective prevention and control measures.

To date, the 24 ASFV genotypes described are all known to circulate in southern and eastern Africa while genotype I was circulating in the Caribbean, South America, West Africa and Europe (Costard *et al*., 2013). The latest genotype XXIV was described in Ethiopia. Transboundary transmissions of the virus led to introduction of genotype IX to western Africa and genotype II to the Republic of Georgia in 2007 and subsequently spreading into Armenia, Azerbaijan, and Russia (Costard *et al*., 2013). The highly virulent p72 genotype II ASFV, identical to the Georgia 2007/1 isolate has been described to circulate persistently in Tanzania. In addition to genotype II, genotypes IX, X, XV and XVI have been described in ASF outbreaks in Tanzania (Lubisi *et al.,* 2005; Misinzo *et al.*, 2010; Misinzo *et al.,* 2014; Yona *et al*., 2020).

In this study, molecular characterization of the ASFV isolates from the ASF outbreaks between March 2021 and March 2022 in western, northern (Lake Zone) and eastern Tanzania was performed. Confirmatory diagnosis of ASF was done based on partial amplification of the p72 gene using polymerase chain reaction (PCR). Furthermore, genotyping was done based on nucleotide amplification and sequencing of the variable 3'-end of the *B646L* gene encoding p72 protein.

3.3 Materials and Methods

3.3.1 Sampling for ASFV genotyping

Outbreaks of ASF in Kahama, Mwanza and Geita located in Lake Zone (northern Tanzania), Katavi located in western Tanzania, Mpwapwa and Kongwa located in central Tanzania and Morogoro located in eastern Tanzania were reported between March 2021 and March 2022. Tissue samples including spleen, lymph nodes, lung and kidney were collected from 10 dead domestic pigs from each location before being transported to the laboratory under a cold chain. Upon reaching the laboratory, tissue samples from each domestic pig were prepared by chopping one gram of each tissue followed by pooling into 5 mL sterile phosphate-buffered saline (PBS). The pooled homogenized tissues were centrifuged at 6 000 *g* for five minutes at room temperature. Afterwards, the tissue supernatant was transferred into cryovials and stored at -80 °C until DNA extraction.

3.3.2 DNA Extraction and ASF detection

Frozen aliquots (100 µL) of preserved tissue supernatant were allowed to thaw and genomic DNA extraction was performed using a commercial nucleic acid extraction kit (Qiagen, Hilden, Germany), according to instructions provided by the manufacturer. Detection of ASFV DNA was done using primers PPA1 and PPA2 to amplify a 257 base pairs fragment of the C-terminal region of p72 major capsid protein encoded by *B646L* gene (Aguero *et al*., 2003).

3.3.3 Molecular characterization of ASFV

Genotyping was done by nucleotide sequence analysis of the variable 3’ end of the *B646L* gene. Amplification of the C-terminal region of the *B646L* gene was done using p72U and p72D primers, expected to produce a PCR product of 478bp (Bastos *et al.,* 2003).

Sequencing was done using dideoxynucleotide cycle sequencing using a Big Dye Terminator kit v3.0 (Applied Biosystem Foster City, CA). Products from the cycle sequencing reaction were purified by ethanol precipitation and separated by capillary gel electrophoresis on an ABI 3730xl DNA analyser (Applied Biosystems, Foster City, CA). Primers including p72U and p72D were used for dideoxynucleotide cycle sequencing. The quality of the sequencing of PCR products was determined by visual examination of chromatograms using Sequence Scanner software (Applied Biosystems, Foster City, CA). The nucleotide sequences were submitted to GenBank and assigned with accession numbers (Table 1). The similarity search against other ASFV sequences at GenBank database and their alignment with other Tanzanian ASFV nucleotide sequences was done using BLASTN. Phylogenetic analysis was performed using the Maximum Likelihood method with 1,000 bootstrap replications, and evolutionary distances were calculated by the Kimura 2-parameter method as implemented in MEGA X (Kumar *et al*., 2018).

3.4 Results

3.4.1 Clinical Signs and Postmortem Findings

Sick domestic pigs in visited farms showed ASF clinical signs including high fever (41-42 °C), staggering gait, anorexia and cutaneous congestion of the ears, belly and genitalia. Pathological lesions suggestive of ASF were observed during post-mortem including; enteritis, enlargement of spleen (splenomegaly), petechiation of the kidney cortex and hemorrhages in lymph nodes, especially the mesenteric and hepatogastric lymoh nodes.

3.4.2 ASFV genotypes

A phylogenetic tree (Fig. 1) was reconstructed based on *B646L* (p72) gene sequences. The nucleotide sequences from a single location had 100% identity after phylogenetic analysis,

genotype II of ASFV was found in domestic pig samples from Kongwa, Mpwapwa located in central Tanzania, and Morogoro located in eastern Tanzania. Genotype X was found in domestic pig samples from Kahama and Geita located in Lake Zone (northern Tanzania) and Katavi located in western Tanzania. Genotype IX was found in Mwanza located in Lake Zone (northern Tanzania).

3.5 Discussion

African swine fever outbreaks have been frequent in Tanzania in recent years due to a persistent domestic pig cycle (Yona *et al*., 2020). This study confirms ASF outbreaks in eastern, central, northern (Lake Zone) amd western Tanzania between March 2021 and March 2022. Furthermore, the present study determined the genetic characteristics of ASFV. We report the co-circulation of ASFV belonging to genotype II, IX and X in the different locations where domestic pigs sampling was conducted. These genotypes have been reported in previously described ASF outbreaks in Tanzania, and neighboring countries. To date, genotype II, IX, X, XV and XVI have been reported in Tanzania (Misinzo *et al*., 2014; Yona *et al*., 2020). In the present study, genotype II ASFV was found in domestic pigs from Mpwapwa, Kongwa and Morogoro districts located in eastern and central Tanzania.

Genotype II ASFV have been previously reported to persist in different locations along the Tunduma-Dar es Salaam and Morogoro-Dodoma highways since the introduction of the genotype in 2011, possibly from Karonga in Malawi (Yona *et al*., 2020). These results indicate that genotype II ASFV has not yet spread northwards beyond Dodoma, to locations connected to Rwanda, Burundi, Uganda and Kenya via highways from Dodoma.

Genotype X caused ASF outbreaks in domestic pigs from Kahama and Geita located in Lake Zone (northern Tanzania) and Katavi located in western Tanzania. This ASFV genotype has been previously reported during the 2015, 2016 and 2019 ASF outbreaks in Mwanza located within the Lake Zone (northern Tanzania) and Ngara located in western Tanzania (Yona *et al*., 2020: Kivumbi *et al*., 2021). The ASFV strains found in domestic pigs from Kahama, Geita and Katavi were identical to previously reported ASFV strains from Burundi (BUR/18/Rutana) and Kenya (Kenya 1950) (Hakizimana *et al*., 2020). This indicates possible geographical restriction of genotype X viruses to north-western Tanzania and the neigbouring country of Burundi.

The ASFV strain that was recovered from domestic pigs in Mwanza (Lake Zone in northern Tanzania) belonged to genotype IX, and was highly identical to previously reported ASFV strains from Uganda, (Ug12.Kabale1) (Atuhaire *et al*., 2013). . Genotype IX ASFV were found to co-circulate with genotype X ASFV in domestic pigs from the Lake Zone (northern Tanzania). Co-circulation of three different ASFV genotypes in different geographical zones of Tanzania may indicate re-emergence of the virus from different sources and/or ASFV cycles. Tanzania has a vast of National Parks such as Selous game reserve in Morogoro and Burigi in western Tanzania where the reservoir and asymptomatic hosts including warthogs infested with ticks reside (Bora *et al*., 2020). Molecular studies revealed ASFV genotype X infection in Warthog and ticks in Kirawira in 1968 and 1989, respectively confirming presence of ASFV in the sylvatic cycle in Tanzania (Lubisi *et al*., 2005). Prevention of virus spill over from the sylvatic cycle to domestic pigs is crucial in the control of ASF spread and prevention coupled with adherence to zoosanitary measures to prevent pig to pig transmissions in the domestic ASFV cycle (Misinzo *et al*., 2014). However, ASF is a transboundary disease and Tanzania is bordered

by countries where ASF is endemic. Some of the latest ASF outbreaks in countries bordering Tanzania include genotype X that was responsible for the 2018 ASF outbreak in Burundi, genotype IX that was responsible for the 2012 ASF outbreak in Uganda and 2009 in Democratic Republic of the Congo (Hakizimana *et al*., 2020; Atuhaire *et al*., 2012; Gallardo *et al*., 2011).

3.6 Conclusion

In this study three ASFV genotypes II, IX and X were found to cause ASF outbreaks between March 2021 and March 2022 in eastern, northern and western Tanzania. Phylogenetic analysis indicated close relationship of these genotypes with previously reported genotypes from ASF outbreaks both within Tanzania and neighboring countries. All ASF outbreaks are devastating as the disease has no cure or vaccine and the sanitary measures to contain disease are costly in terms of not only loss of pigs but also loss of pig products markets (Fasina *et al*., 2010).

The community at large needs to understand the importance of adhering to biosecurity measures taken so as to prevent ASF transmission. Engagement actors in the pig value chain such as producers, producer associations, traders, brokers, butchers and retailers especially should be well educated on the disease control and prevention measures. The government can help in ASF prevention and control by routinely surveying the disease occurrence and strict enforcement of regulations put forward in prevention of ASF transmission between regions. Compensation following compulsory slaughter of ASF infected pigs is recommended whenever outbreaks occur as it enables them to adhere to biosecurity measures against ASF spread (Fasina *et al.*, 2010; Fasina *et al*., 2020).

To prevent transboundary ASF transmission, implementation of restriction of illegal transportation and importation of pig and pig products should be adhered to. Sanitary measures that prevent introduction of ASFV from human activities including tourism and food importation should be implemented. Control of ASF is a collective impact of all the mentioned preventive measure.

3.7 Conflict of Interest Statement

The authors declare that they have no personal or financial relationships that may have inappropriately influenced them in writing this article.

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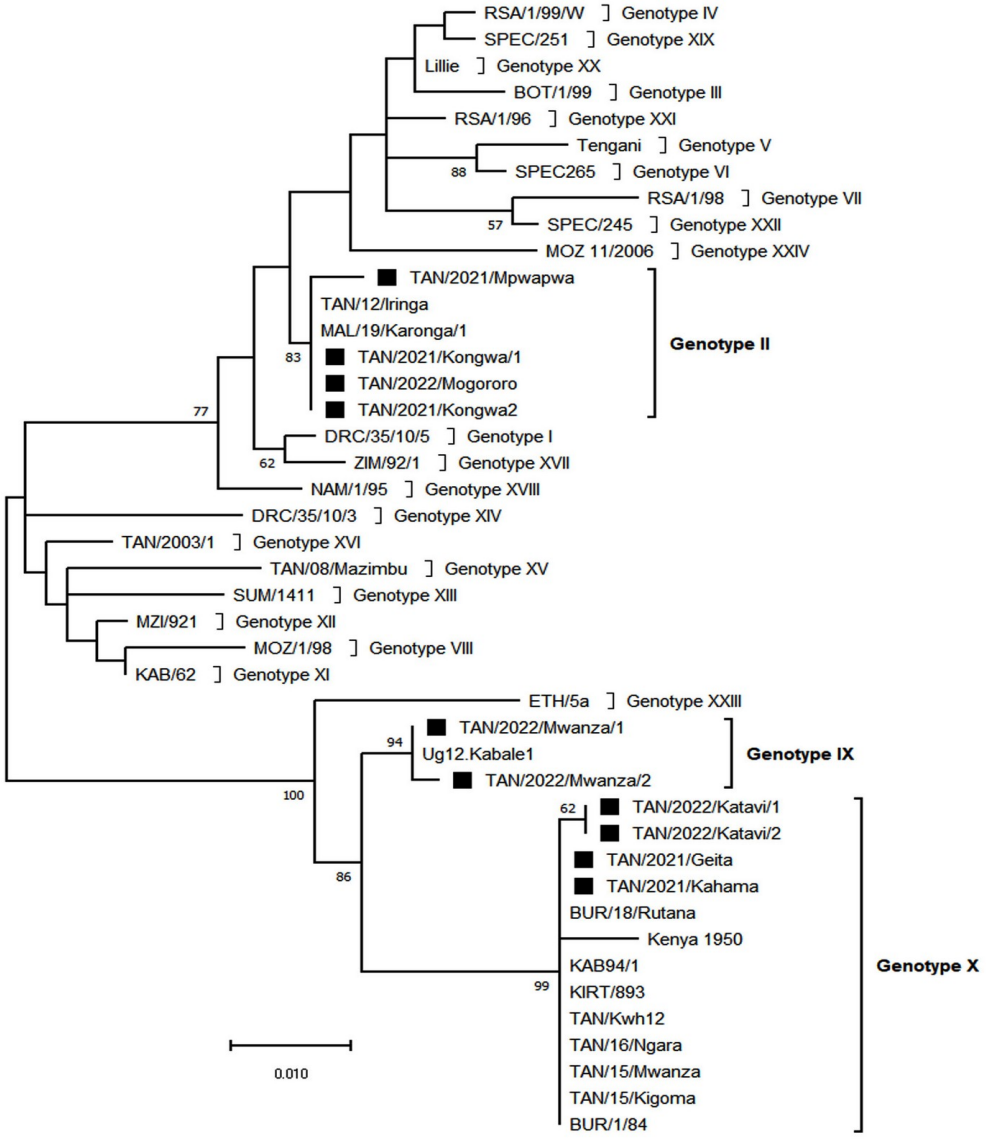

Figure 3.1: Evolutionary relationships of representative strains of African swine fever virus based on the Maximum Likelihood phylogeny of the partial p72 gene sequences. The phylogenetic analysis was performed using MEGA X (http://www.megasoftware.net) and the Kimura 2-parameter substitution model, determined by a model selection analysis. Phylogeny was inferred following 1,000 bootstrap replications, and the node values show percentage bootstrap support (only the values above 50% are shown). The square black spots indicate the African swine fever virus sequence from this study and the scale bar indicates nucleotide substitution per site.

Table 3.1: 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	Country	P72 Genbank accession number	P72 genotype	Reference
DRC/35/10/5	Pig	2010	NK	DRC	KX121552	I	(Malumba-Mfumu *et al*., 2017)
TAN/2021/Mpwapwa	Pig	2021	Mpwapwa	Tanzania	ON419321	II	This study
TAN/2022/Morogoro	Pig	2022	Morogoro	Tanzania	ON419319	II	This study
TAN/2021/Kongwa1	Pig	2021	Kongwa	Tanzania	ON419318	II	This study
TAN/2021/Kongwa2	Pig	2021	Kongwa	Tanzania	ON419320	II	This study
MAL/19/Karonga/1	Pig	2021	Karonga	Malawi	MW856068	II	(Hakizimana *et al*., 2021)
TAN/12/Iringa	Pig	2012	Iringa	Tanzania	KF834193	II	(Sikombe,2013)
RSA/1/99/W	Pig	1999	NK	South Africa	AF449477	III	(Bastos *et al*., 2003)
BOT/1/99	Pig	1999	NK	Botswana	AF504886	III	(Bastos *et al*., 2003)
Tengani	Warthog	NK	Tengani	Malawi	AF301541	V	(Bastos *et al*.,2004)
SPEC265	Pig	1994	NK	Mozambique	AF270710	VI	(Bastos *et al*., 2004)
RSA/1/98	NK	1998	NK	South Africa	AF302818	VII	(Bastos *et al*., 2004)
MOZ/1/98	Pig	1998	Tete	Mozambique	AF270705	VIII	(Batos *et al*., 2003)
TAN/2022/Mwanza/1	Pig	2022	Mwanza	Tanzania	ON419324	IX	This study
TAN/2022/Mwanza/2	Pig	2022	Mwanza	Tanzania	0N419325	IX	This study
Ug12.Kabale1	Pig	2012	Kabale	Uganda	KC990890	IX	(Atuhaire *et al*., 2013)
TAN/2021/Kahama	Pig	2021	Kahama	Tanzania	0N419317	X	This study
Kenya 1950	Pig	1950	NK	Kenya	AY261360	X	Unpublished
TAN/2021/Geita	Pig	2021	Geita	Tanzania	ON419316	X	This study
TAN/2022/Katavi/1	Pig	2022	Katavi	Tanzania	ON419322	X	This study
TAN/2022/Katavi/2	Pig	2022	Katavi	Tanzania	ON419323	X	This study
BUR/18/Rutana	Pig	2018	Rutana	Burundi	MK829709	X	(Hakizimana *et al*., 2020)
TAN/Kwh12	Warthog	1968	Kirawira	Tanzania	AF301546	X	(Bastos *et al*., 2003)
KAB94/1	Pig	1994	NK	Kenya	AY972163	X	(Phologane *et al*., 2005)
KIRT/893	Ticks	1989	Kirawira	Tanzania	AY351512	X	(Lubisi et al., 2005)
TAN/16/Ngara	Pig	2016	Ngara	Tanzania	MF437293	X	(Yona *et al*., 2020)
TAN/15/Mwanza	Pig	2015	Mwanza	Tanzania	MF437291	X	(Yona *et al*., 2020)

TAN/15/Kigoma	Pig	2015	Kigoma	Tanzania	MF437289	X	(Yona *et al*., 2020)
BUR/1/84	Pig	1984	Gitega	Burundi	AF449463	X	(Bastos *et al*., 2003)
KAB/62	Ticks	1983	Livingstone Game Park	Zambia	AY351522	XI	(Lubisi *et al*., 2005)
MZI/921	Pig	1992	Mzinda	Malawi	AY351543	XII	(Lubisi *et al*., 2005)
SUM/1411	Ticks	NK	Sumbu Park	Zambia	AY351542	XIII	(Lubis *et al*., 2005)
DRC/35/10/3	Pig	2010	Ngaliema	DRC	KX121550	XIV	(Mulumba *et al*., 2017)
TAN/08/Mazimbu	Pig	2008	Mazimbu	Tanzania	GQ410765	XV	(Misinzo *et al*., 2012)
TAN/2003/1	Pig	2003	Arusha	Tanzania	AY494550	XVI	(Lubisi *et al*., 2005)
ZIM/92/1	Pig	1992	Gweru	Zimbabwe	DQ250119	XVII	(Boshoff *et al*., 2007)
NAM/1/95	Pig	1995	Windhoek	Namibia	DQ250122	XVIII	(Boshoff *et al*., 2007)
SPEC/251	Pig	1996	Ellisras	South Africa	DQ250118	XIX	(Boshoff *et al*., 2007)
Lillie	Pig	NK	NK	South Africa	DQ250109	XX	(Boshoff *et al*., 2007)
RSA/1/96	NK	1996	Gravelotte	South Africa	DQ250125	XXI	(Boshoff *et al*., 2007)
SPEC/245	NK	NK	Louis Trichardt	South Africa	DQ250117	XXII	(Boshoff *et al*., 2007)
ETH/5a	Pig	2011	Bahir Dar	Ethiopia	KT795361	XXIII	(Achenbach *et al*., 2017)
MOZ 11/2006	Tick	2006	Gorongosa National Park	Mozambique	KY353990	XXIV	(Quembo *et al*.,2018)

CHAPTER FOUR

4.0 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

4.1 Discussion

African swine fever outbreaks in domestic pigs have been persistent in in recent years in Tanzania (Yona *et al*., 2020). This study reports on the responsible ASFV genotypes for the 2019-2022 ASF outbreaks in eastern, central, northern (Lake Zone) and western Tanzania. It also reports on the risk factors and socio-economic impact of the 2019 ASF outbreak in Ngara district, western Tanzania. Previous ASFV molecular characterization studies have reported genotype II, IX, X, XV and XVI in Tanzania (Misinzo *et al*., 2014; Yona *et al*., 2020). In this study ASFV clustering to *B646L* p(72) genotype II, IX and X were found to the causative agents of the respective ASF outbreaks. ASFV Genotype II was responsible for outbreaks in Mpwapwa, Kongwa and Morogoro districts located in eastern Tanzania. The genotype was also the cause for the 2017 and 2019 ASF outbreaks in Iringa and Morogoro all in central Tanzania (Yona *et al*., 2020). TAN/2021/Kongwa, TAN/2021/Mpwapwa and TAN/2022/Morogoro ASFV strains clustered most closely with TAN/12/Iringa and MAL/19/Karonga/1. Genotype X caused ASF outbreaks in Kahama and Geita located in northern Tanzania and Katavi and Ngara located in western Tanzania. The ASFV genotype X was also reported during the 2015 and 2016 ASF outbreaks in Mwanza and Ngara all in western Tanzania (Yona *et al*., 2020; Kivumbi *et al*., 2021). TAN/2021/Kahama and TAN/2022/Katavi ASFV strains showed that they are closely related to isolates from Burundi, BUR/18/Rutana and Kenya, Kenya 1950 (Hakizimana *et al*., 2020). The strain TAN/2022/Mwanza belonging to ASFV genotype IX clustered most closely with the isolates from Uganda, Ug12.Kabale1 (Atuhaire *et al*., 2013).

Close relationship between this study genotypes and previous genotypes reported from both inside and outside Tanzania, indicate persistent circulation of the virus in both domestic cycle and possible introduction from neighboring countries. Presence of the virus in the sylvatic cycle and possible spill over to the domestic cycle is also a possibility. ASFV persistence in the sylvatic cycle in Tanzania is maintained by presence of asymptomatic hosts including warthogs infested with ticks in national parks such as Selous game reserve in Morogoro and Burigi in western Tanzania where the reservoir and asymptomatic hosts including warthogs infested with ticks reside (Bora *et al*., 2020). Presence of ASFV in the sylvatic cycle in Tanzania has been reported with ASFV genotype X has being found in Warthog and ticks in Kirawira in 1968 and 1989, respectively (Lubisi *et al*., 2005).

The Ngara risk factors and socio-economic impact study in Ngara district showed significant association between feeding pigs with uncooked swill and ASF spread. Other studies have shown movement of people between farms and sharing of equipment as these factors associated in ASF outbreaks (Fasina et al., 2010; Yona, 2017). Association of these risk factors from this study and previous studies indicate lack of adherence to the disease control measures by pig value chain actors including pig traders, brokers and farmers. The Ngara 2019 ASF outbreak was shown to cause significant financial loss of about 41,065 USD as a result of pig mortality.

4.2 Conclusion

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

i. Findings of the study indicate persistence of circulation of the virus within the country and possible introduction from other countries.

ii. ASFV genotype II, IX and X were associated with the ASF outbreaks in years 2019, 2021 and 2022 respectively in Tanzania.

iii. The genotypes have been shown to be closely related to previous ASFV genotypes from both Tanzania and neighboring countries.

iv. The risk factors and socioeconomic impact findings from Ngara district outbreak showed association between feeding pigs with uncooked swill and ASF spread and highlighted on the resulted high pig mortality.

v. The Ngara ASF outbreak resulted in high financial losses estimated from the monetary values of the dead domestic pigs. A total of 93 630 000 Tanzanian shillings (approximately 41 065 USD) was estimated to be lost as a result of pigs' mortality in different households.

4.3 Recommendations

i. All ASF outbreaks are devastating as the disease has no cure or vaccine and the sanitary measures to contain disease are costly in terms of not only loss of pigs but also loss of pig products markets.

ii. The community at large needs to understand the importance of adhering to biosecurity measures taken so as to prevent ASF transmission.

iii. Engagement actors in the pig value chain such as producers, producer associations, traders, brokers, butchers and retailers especially should be well educated on the disease control and prevention measures.

iv. The government can help in ASF prevention and control by routinely surveying the disease occurrence and strict enforcement of regulations put forward in prevention of ASF transmission between regions. Compensation following compulsory

slaughter of ASF infected pigs is recommended whenever outbreaks occur as it enables them to adhere to biosecurity measures against ASF spread.

v. To prevent transboundary ASF transmission, implementation of restriction of illegal transportation and importation of pig and pig products should be adhered to. Sanitary measures that prevent introduction of ASFV from human activities including tourism and food importation should be implemented. Control of ASF is a collective impact of all the mentioned preventive measure.

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