Persistent domestic circulation of African swine fever virus in Tanzania, 2010-2017

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Abstract

Background

African swine fever (ASF) is a highly fatal viral hemorrhagic disease of domestic pigs that threatens livelihoods and food security. In Africa, ASF virus (ASFV) circulates in sylvatic (transmission between warthogs and soft argasid ticks) and domestic (transmission between domestic pigs) cycles, with outbreaks resulting from viral spill-over from sylvatic cycle. The present study investigated ASFV transmission patterns through virus genotyping.

Results

Genotypes II, IX, X, XV and XVI of ASFV were found to circulate and maintained by transmission between domestic pigs.

Methods

In addition to archived samples, tissue samples were collected from domestic pigs during outbreaks at different locations in Tanzania between 2015 and 2017 followed by nucleotide sequencing and phylogenetic analysis of B646L gene of ASFV.

Conclusion

Maintenance of ASFV in the domestic cycle was due to bleach of quarantine and transportation of affected pigs via major highways. Appropriate control measures including sanitary measures at the slaugher slabs and quarantine measures adherence are recommended to prevent ASF emergence and re-emergence in Tanzania. Transportation of pig and pig products for regional market should be controlled to prevent ASFV spreading to other states of the East African Community , as ASFV genotype II has spread beyond its geographical range.

Background

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), a DNA 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* act as vectors of ASFV contribute to viral maintenance within the sylvatic cycle and as well as in transmitting the virus 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, 24 genotypes of ASFV have been identified (7–9). All of the 24 ASFV genotypes have been described in African countries, South of the Sahara, 23 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 traditional geographical range. For instance, genotype I spread from West Africa to Europe, South America and the Caribbean (10). On the other hand, genotype II, which was known to circulate in Zambia, Malawi and Mozambique, spread to the Caucasus and afterwards to the European Union, Russia and China (11–15). Furthermore, genotype II ASFV has been introduced to Tanzania and Zimbabwe, where it was never known to circulate (16,17). Similarly, genotype IX which is restricted to Eastern Africa has been reported to spread to Western Africa (18). The spread of ASFV beyond African countries south of the Sahara and its traditional geographical boundaries poses a threat to the global pig industry, international trade market and food security. In 2018, the ASFV spread to China, a major pork producer, and afterwards, the virus has spread to South-East Asian countries of Vietnam and Cambodia (13–15).

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 (16,19–22). 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 (16,19–21,23). These outbreaks in other parts of Tanzania end up in Dar es Salaam due to transportation of infected pigs for sale and slaughter from other parts of the country to this main commercial capital (19,20). Many outbreaks have been reported in different parts of Tanzania between 2010 and 2017. The aim of this study was to investigate the ASFV transmission patterns through virus genotyping in order to understand the relationship between ASF outbreaks.

Results

Clinical signs and postmortem findings

Clinical signs observed in sick pigs included a high fever (>40 °C), anorexia, staggering gait, shivering and cutaneous congestion particularly on the outer side of the pinna, belly, limbs and genitalia (Fig. 1A). Pigs were dull and stayed together at one side of their pens (Fig. 1B). Abortion was observed in pregnant sows. At postmortem, the pericardial and thoracic cavities were filled with straw tinged fluid (Fig. 1C). In addition, postmortem findings included hemorrhages in the spleen, heart, kidneys and lymph nodes especially the gastrohepatic, thoracic, mesenteric and renal lymph nodes (Fig. 1D and 1F). Splenomegaly (enlargement of the spleen) and enteritis were also observed (Fig. 1E).

Molecular characterization of ASFV

A phylogenetic tree was constructed by the Neighbor-Joining method in order to determine the genetic relationship between the ASFV strains collected during 2015 and 2017, and previously sequenced Tanzanian ASFV strains available at GenBank (Fig. 2). The ASFV strains collected during this study (accession numbers MF437289 - MF437310) clustered with p72 genotypes II, IX and X (Table 1). Genotype II ASFV strains were collected from Southwestern, Central and Eastern Tanzania, genotype IX from Northwestern parts of Tanzania around Lake Victoria and genotype X from Northwestern, Northeastern and Central parts of Tanzania (Fig. 3).

Discussion

Several outbreaks of a highly fatal hemorrhagic disease affecting domestic pigs, suspected to be ASF based on clinical signs and postmortem findings, were reported in different parts of Tanzania between 2015 and 2017. In the present study, these outbreaks were confirmed to be ASF caused by ASFV belonging to genotypes II, IX and X (Fig. 2 and 3). Prior to 2015, the ASFV that caused ASF outbreaks in Tanzania clustered into genotypes II, IX, X, XV and XVI (Table 1) (19,21–23). The recent ASF outbreaks were caused by ASFV that were 100% genetically identical to previously reported viruses, for each of the genotype II, IX and X (Fig. 2). The identity of ASFV between previous and recent outbreaks and the pattern of disease spread strongly indicate domestic pig-to-pig transmission.

Prior to 2015, genotype II ASFV were reported during outbreaks in Southwestern and Eastern parts of Tanzania (Fig. 2 and 3) (16). Genotype II ASFV is thought to have been introduced into Tanzania in Kyela, a town in Southwestern Tanzania at the Tanzania - Malawi border following an outbreak in Karonga in 2010 (16). Since the introduction of genotype II ASFV, the virus spread within Southwestern parts of the country with occasional incursion into Eastern Tanzania (16). In the present study, we found that genotype II ASFV continued to circulate in previously reported areas and it spread into new areas of Central Tanzania (Fig. 3). Previously, ASF outbreaks in Eastern Tanzania were linked to outbreaks in Southwestern Tanzania due to transportation of live pigs for sale in the main commercial city of Dar es Salaam (16). The Southwestern part of the country is linked to Dar es Salaam by a major highway from Sumbawanga via Tunduma, Mbeya, Iringa and Morogoro (Fig. 3). Furthermore, the different ASF outbreaks between 2015 and 2017 due to genotype II ASFV occurred in various locations along and in the vicinity of the Morogoro - Dodoma highway, which branches off in Morogoro from the Tunduma - Dar es Salaam highway. The spread of the virus along these highways could be due to illegal transportation of infected domestic pigs from areas under quarantine, as described in previous reports (16,19).

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, Zimbabwe, Tanzania, the Caucuses region, Russia, Europe and Asia (13–15,24–26). If appropriate control measures of these genotype II viruses are not strictly enforced, we predict that this virus could possibly spread northwards and ultimately into bordering countries of Rwanda and Uganda, as these two countries are connected with Tanzania by major highways (Fig. 3). We recommend that stakeholders involved with ASF control be vigilant in order to prevent further spread of genotype II ASFV beyond Dodoma city, where it has reached.

In the present study, we found that ASFV genotype X circulated in Northeastern Tanzania, similar to other ASFV genotypes of in, similar to viruses that have been previously described (16,21). In addition, we found that genotype X ASFV has spread into new areas within Central Tanzania (Fig. 3). However, genotype IX was found restricted to Northwestern Tanzania, similar to what is reported in previous studies.

The occurrence and spread of ASF between different parts of Tanzania is likely due to bleach of quarantine imposed in areas affected with ASF. It is mostly likely 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 in affected farms and slaughter slabs and swill feeding increase the likelihood of ASFV spread at a given locality, as has been previously described (16,21).

Conclusions

Based on the results obtained from the present and previous studies, ASF occurrence and spread in Tanzania is primarily a result of virus maintenance within the domestic cycle. The spread of ASFV is mainly along major highways most likely due to transportation of pigs from affected to unaffected areas. Although certain viral genotypes seem to be geographically restricted into certain zones within Tanzania, genotype II seems to expand its geographical range northwards with likelihood of spreading to other states of the East African Community.

Methods

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 observation of pigs was 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. Tissues were temporarily stored at –20 °C before they were transported in ice cool boxes to the laboratory. 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 4 °C. The tissue supernatant was transferred into a cryovial and stored at –80 °C until DNA extraction.

Detection of ASF in pig samples

Aliquots (100 µL) of each of the homogenized tissue samples from the same pig were 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. (27).

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. (28). Afterwards, the PCR products were subjected to automated dideoxynucleotide cycle sequencing using BigDye 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 were afterwards assigned with GenBank 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 nucleotide sequences were aligned with other Tanzanian ASFV nucleotide sequences available at GenBank using ClustalW. Phylogenetic analysis was performed using the Neighour-Joining method with 1000 bootstrap replications, and evolutionary distances were calculated by the Kimura 2-parameter method as implemented in MEGA 6.0 (29).

List Of Abbreviations

ASF - African swine fever

ASFV - African swine fever virus

BLASTN - Basic Local Alignment Search Tool for Nucleotides

DNA - deoxyribonucleic acid

p72 - capsid protein with molecular weight of 72 KDa

PCR - Polymerase chain reaction

Declarations

Ethics approval and consent to participate

Ethical approval for animals sampling was sought from the Ethical Committee of Sokoine University of Agriculture. Written consent to participate was obtained from farmers and veterinarians before sampling of tissues from slaughtered pigs.

Consent for publication

All authors read and approved the final manuscript. Consent for publication has been obtained from all authors.

Availability of data and material

The datasets generated and/or analysed during the current study are available in the GenBank repository, https//ncbi.nlm.nih.gov/genbank

Competing interests

The author's declare that they have no competing interests.

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Authors' contributions

CMY participated in sample collection, methods, data analysis and development of manuscript. MM performed laboratory analysis. CMY, MV, GM and HJN conceived the idea, analysed, interpreted data and revised the manuscript. GM and HJN secured funding. GM and ES critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1: Previously reported African swine fever virus (ASFV) representing different *B646L* (p72) genotypes and ASFV genetically characterized in the present study.

Figures

Isolate	Host species	Year of Isolation	Town	p72 gene Genbank accession number	p72 genotype	Reference
TAN/10/Kyela	Pig	2010	Kyela	JX391987	II	(22)
TAN/11/Ludewa	Pig	2011	Ludewa	JX391990	II	(22)
TAN/12/Ifakara	Pig	2012	Ifakara	JX391992	II	(22)
TAN/13/Iringa	Pig	2013	Iringa	KF834193	II	Unpublished
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	(23)
KIRW89/1	Warthog	1989	Kirawira	AY351514	X	(23)

TAN/Kwh12	Warthog	1968	Kirawira	AF301546	X	(28)
TAN 2004.1	Pig	2004	Kigoma	JX403648	X	Unpublished
TAN/09/Longido	Pig	2009	Longido	JX262383	X	(22)
TAN/13/Moshi	Pig	2013	Moshi	KF706360	X	(21)
TAN/13/Rombo	Pig	2013	Rombo	KF706361	X	(21)
TAN/13/Machame	Pig	2013	Machame	KF706362	X	(21)
TAN/13/Arusha	Pig	2013	Arusha	KF706363	X	(21)
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	2015	Kigoma	MF437289	X	This study
TAN/08/Mazimbu	Pig	2008	Mazimbu	GQ410765	XV	(16)
Tan/1/01	Pig	2001	Dar es Salaam	AY494552	XV	(23)
Tan/2003/01	Pig	2003	Arusha	AY494550	XVI	(23)

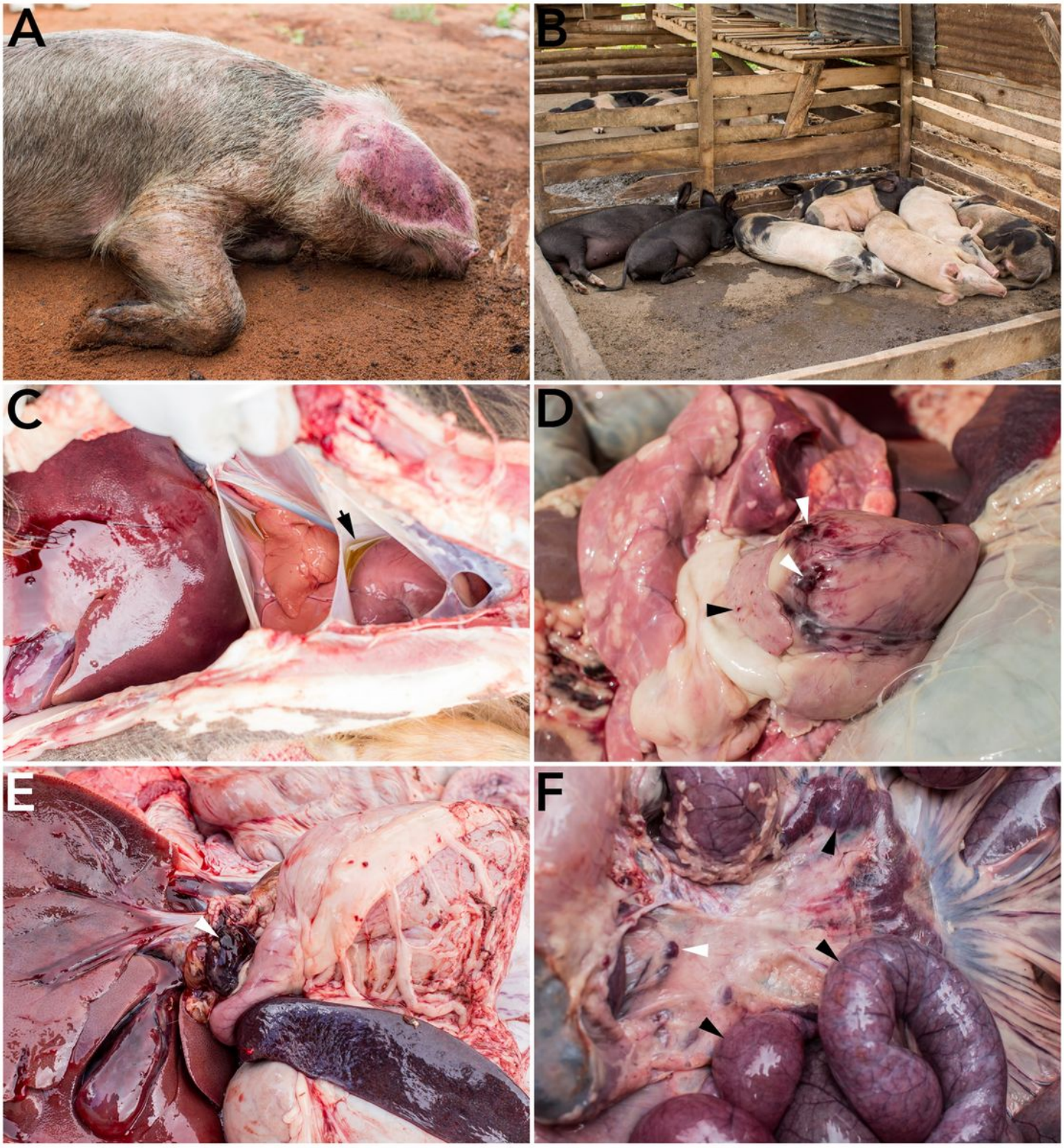

Figure 1

Clinical signs and postmortem findings observed in domestic pigs with African swine fever. (A) Ventral recumbence and cutaneous congestion especially on the outer side of pinna and (B) loss of appetite, lateral recumbence and a tendency to stay together at one side of the pen were observed in pigs with African swine fever. At postmortem, (C) the pericardial and thoracic cavities were filled with straw tinged fluid (indicated by an arrow), (D) hemorrhages of the heart (indicated by an arrow) especially at the

atrioventricular junctions, (E) hemorrhages of the gastrohepatic lymph node (indicated by an arrow) and (F) enteritis and hemorrhages of the mesenteric lymph nodes (indicated by an arrow head).

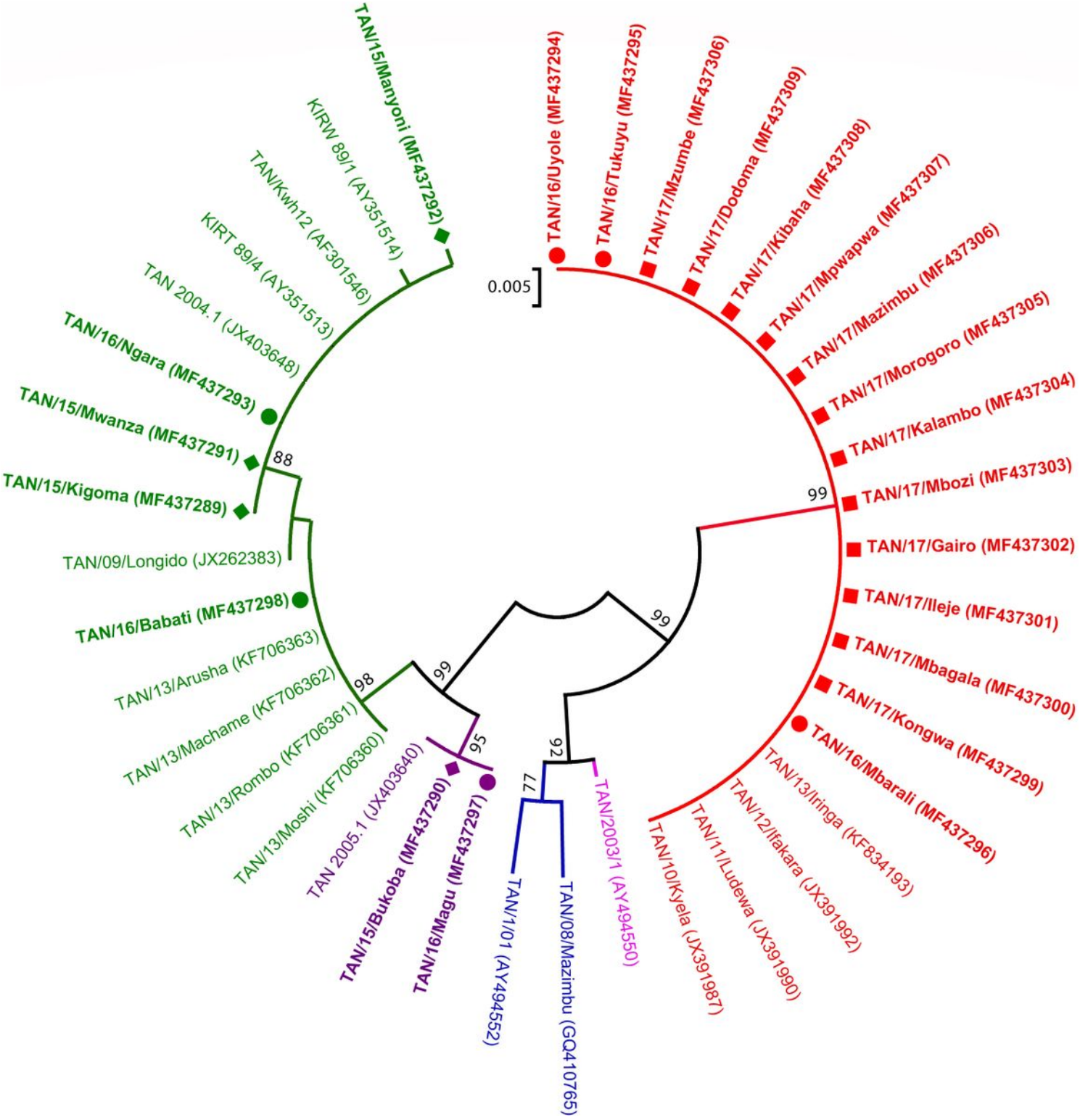

Figure 2

Phylogenetic relationship of African swine fever viruses (ASFV). The ASFV which were collected in the present study and during 2015, 2016 and 2017 are indicated by square, circle and diamond, respectively. Genotype II, IX, X, XV and XVI are labeled in red, purple, green, blue and pink respectively. Phylogeny was

inferred following 1,000 bootstrap replications and node values show percentage bootstrap support. Scale bar indicates nucleotide substitution per site. The GenBank accession numbers for the different B646L (p72) gene are indicated in parenthesis.

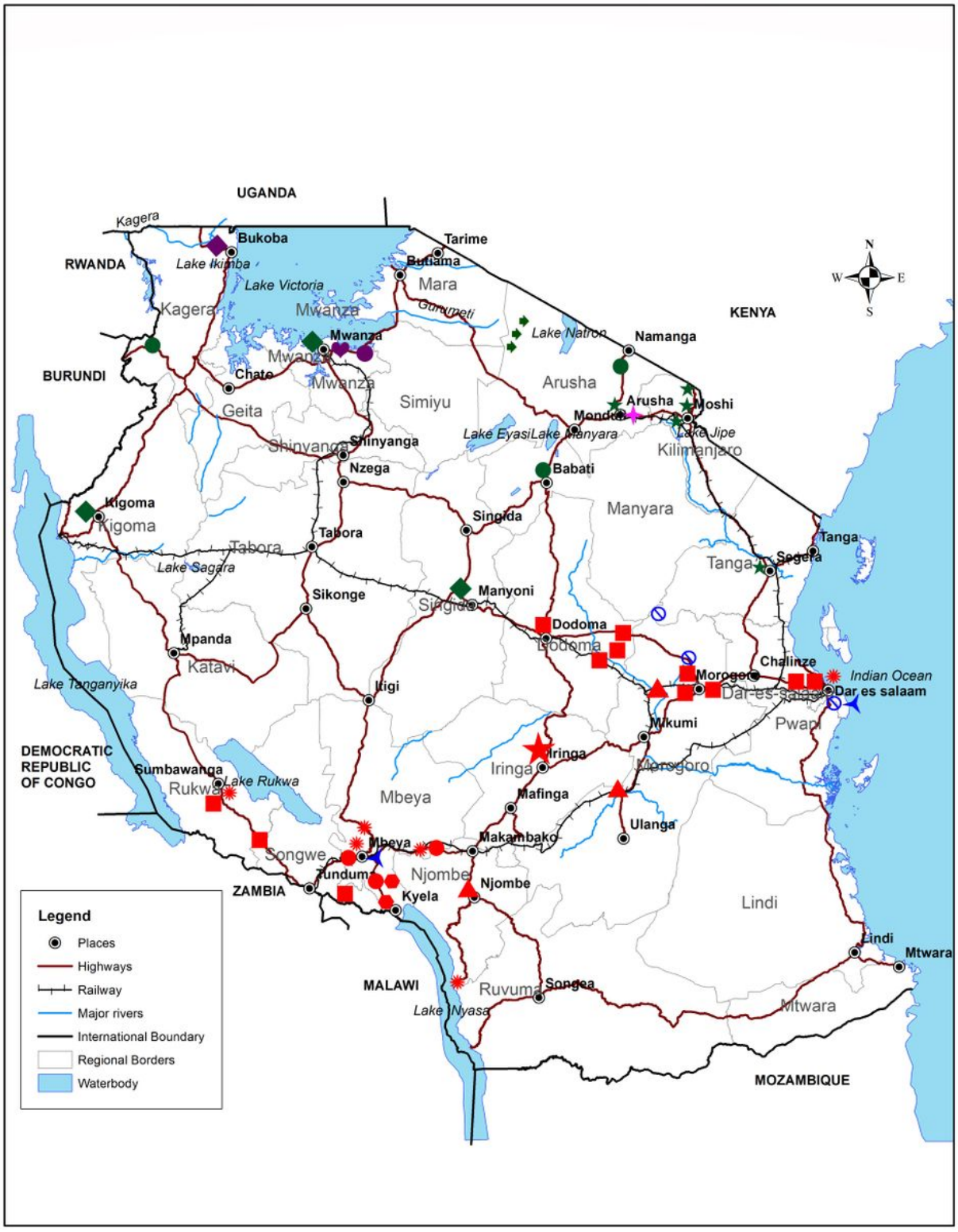

Figure 3

Map of Tanzania showing reported African swine fever outbreaks and ASF virus (ASFV) genotypes between 2010 and 2017. 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; Due to technical limitations, these symbols are only available as a download in the supplemental files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- fig3symbols.jpg