Molecular epidemiology of African swine fever in East Africa

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Summary

African swine fever (ASF) a lethal, viral hemorrhagic disease of domestic pigs, first reported from East Africa in 1921, is still widespread in this region. In order to assess field heterogeneity at the regional level, nucleotide sequences corresponding to the C–terminal end of the *p72* gene were determined for 77 ASF viruses of diverse temporal and species origins occurring in eight East African countries. The number of sites completely conserved across all East African sequences was 18.3 % and 14.2 % on nucleotide and amino acid level, respectively. Phylogenetic analysis of a homologous 404 bp region revealed the presence of fourteen East African genotypes, of which ten appear to be country specific. A novel East African, pig-associated, homogeneous virus lineage linked to outbreaks in Mozambique, Zambia and Malawi between 1978 and 2001 was identified. In addition, genotype I (ESACWA) viruses were identified in East African sylvatic hosts for the first time which is significant as this genotype was previously thought to be restricted to the West African region where it occurs only in domestic pigs. The presence of discrete epidemiological cycles in East Africa and recovery of multiple genotypes affirms the epidemiological complexity of ASF in this region.

Introduction

African swine fever (ASF) is a viral disease of domestic pigs that causes a lethal peracute or acute hemorrhagic fever, or a less virulent chronic disease [21]. It was first described from East Africa in 1921 [18], but subsequently identified in southern, central and West Africa [21]. The aetiological agent, ASF virus (ASFV), is a large enveloped icosahedral arbovirus of the *Asfivirus* genus in the family *Asfarviridae* and has a linear, covalently close-ended, double-stranded DNA genome, 170 – 190 kbp in size [6].

The disease is indigenous to the African continent where it circulates in one of three distinct cycles: (1) an ancient sylvatic cycle involving eyeless argasid ticks of the *Ornithodoros* genus and wild suids such as warthogs (*Phaecochoerus aethiopicus*) and bushpigs (*Potamochoerus porcus*), (2) a tick – domestic pig cycle and (3) a domestic pig cycle that occurs in the absence of ticks [21]. In the sylvatic cycle, the natural hosts of the virus are wild African suids, which become sub-clinically infected, and argasid ticks, which become infected and transmit the virus when feeding on wild or domestic pigs [21;22;24;28]. Once the virus is introduced into a naïve herd, horizontal transmission occurs swiftly among domestic pigs, a factor that was readily appreciated following the introduction of the disease to Europe in 1957 [21].

Although eradicated from most of Europe, ASF remains a disease of worldwide relevance as many countries within and outside the African continent have suitable hosts for ASFV in their pig and wild suid populations. In addition, the threat of virus maintenance posed by diverse globally distributed argasid ticks of the *Ornithodoros* genus is significant [9;13]. The fact that the virus has high morbidity, is shed in all excretions of clinically ill domestic pigs [18], is extremely resistant to harsh environmental conditions [7;23], and that there is no vaccine to protect against the disease [29], makes potential ASF introduction to naïve pig herds a serious threat.

In the event of an ASF outbreak, stamping out and movement restriction are the main control measures undertaken and hinge on the rapid laboratory confirmation of ASFV. Initial detection is usually followed by molecular characterization of outbreak strains in order to identify the possible source of the virus, thereby preventing further introductions. PCR amplification and sequencing of the *p72* gene coding for the major capsid protein is increasingly being used to distinguish viruses from recent outbreaks in sub-Saharan Africa [1;2;8] and 10 distinct viral genotypes are presently known to occur in this region [1].

The epidemiology of ASF in East Africa is complex. Not only is there evidence for a sylvatic cycle, but domestic pig cycles have also been described [10;21]. Despite the known deviations from the more classical epidemiological cycles no extensive molecular database comprising ASFV strains from different host species is available which would assist in clarifying the epidemiology of the disease in this region. This study aims to address this shortcoming by extensive sampling and *p72* gene characterization of East African viruses of diverse species and temporal origins so that a comprehensive regional database can be established that will be useful for future outbreak eventualities and that will provide epidemiological insights into historical and contemporary outbreaks of the disease in this region.

Materials and Methods

Study area and samples

For the purpose of this study, East African countries are defined as those occurring east of latitude 20°00E and south of latitude 5°00N, but excluding Namibia, South Africa, Botswana and Zimbabwe. This area encompasses the following eight countries: Burundi, Democratic Republic of Congo, Kenya, Malawi, Mozambique, Tanzania, Uganda and Zambia. A total of 77 viruses of diverse species and temporal origin from these eight East African countries were sequenced and characterized in this study, with the majority of viruses being supplied by the World Reference Laboratory, Institute for Animal Health (IAH), Pirbright. Additional strains were isolated at the Onderstepoort Veterinary Institute (OVI), Agricultural Research Institute (ARC) from clinical material supplied by the respective departments of veterinary services (summarized in Table 1).

Virus isolation

Primary swine macrophage cultures were prepared in 96 well plates as described by Malmquist & Hay [16] with slight modifications. Inoculum containing 10 % (w/v) of sample material in wash buffer consisting of phosphate buffered saline (PBS), antibiotics (Penicillin, Streptomycin, and Neomycin) and normal bovine serum (NBS), was inoculated on the cells in 10 fold dilutions. The cells were examined daily for cytopathogenic effect or haemadsorption. Viruses from positive wells were harvested and stored at -70^0C.

Extraction and genomic amplification of viral DNA

DNA was extracted from 100µl aliquots of virus samples or tissue sample homogenates using a silica/guanidium-based nucleic acid extraction method [3]. A diagnostic PCR was used to confirm the presence of ASF viral DNA using ASF-1 and ASF-2 primers and protocols prescribed for ASF diagnosis in Chapter 2.1.12 of the 2000 edition of the OIE Manual of Standards for Diagnostic Tests and Vaccines, whilst

p72 genotyping was achieved by PCR using P72-U and P72-D primers [1] which amplify a 478bp C-terminal region of the *p72* gene. All PCRs were conducted in a final volume of 50µl, containing 1 X buffer (Roche), 2.5 U *Taq* polymerase (Roche), 0.5 µM of each primer and 200 µM dinucleotide triphosphates (dNTPs) (Roche). Amplification of the C-terminal region of *p72* was achieved following 40 cycles of denaturation at 96 °C for 12 s, annealing at 50 °C for 20 s and extension at 70 °C for 90 s.

Genomic characterization and phylogenetic analysis

Amplification products were electrophoresed on a 1.5 % agarose gel against a 100 bp DNA marker (Promega) and visualized by UV irradiation and ethidium bromide staining. Amplicons of the correct size were excised from the agarose gel and purified using the NucleoSpin Extract 2 in 1 kit (Macherey-Nagel) according to the manufacturer's specifications. Nucleotide sequences were generated with an ABI Prism 310 Genetic Analyzer (Applied Biosystems) using Big Dye v.3.0 cycle sequencing kit ready mix, and aligned using the DAPSA programme [11]. Thirty five ASF viruses representative of each of the ten previously identified *p72* genotypes [1] were included for phylogenetic analysis purposes, bringing the total number of viruses used in this study to 102. A homologous region of 404 nucleotides was used for Minimum Evolution (ME), Maximum Parsimony (MP) and Maximum Likelihood (ML) methods of phylogenetic analysis. The HKY 85 nucleotide substitution model [12] with parameters recovered from the Akaike Information Criterion of Model Test [25] was used for ML analysis in PAUP [26]. For MP, equal weighting and successive weighting schemes were investigated. A ME tree was inferred with MEGA v 2.1 [14], employing the Tamura–Nei nucleotide substitution model [27] with a gamma distribution shape parameter of 0.86. Genotypes were assigned following previously defined criteria [1].

Results

Trees with comparable topology were obtained with all methods of phylogenetic inference. A total of seventeen *p72* genotypes were consistently recovered (Fig. 1), of which fourteen occur within the East African region (Fig. 2). Genotypes I-X correspond to those identified previously [1], whilst genotypes XI-XVII are reported here for the first time and are therefore regarded as novel (Table 2). Genotype I (also referred to as the ESACWA genotype), initially identified in pig isolates from Europe, South America, the Caribbean islands and West Africa [1], was found in this study to be present in East African sylvatic hosts such as bushpigs and ticks. Similarly, genotype V, X and XII viruses were recovered from both domestic pigs and from wild vertebrate and invertebrate hosts, indicating that the argasid tick vector moves readily between wild and domestic vertebrates within the regions in which these genotypes occur (Fig. 2). These four genotypes I, V, X and XII were shown to be present in three, one, four and two East African countries, respectively (Fig. 2), with some of these genotypes having a field presence of more than four decades (Table 2). Genotypes XI, XIII and XIV appear to be associated exclusively with a sylvatic cycle as these viruses, which were collected in Zambia between 1983 and 1986, were all of tick (from warthog burrow) origin. Genotypes II, VI, VIII, IX, XV, XVI and XVII comprised exclusively domestic pig strains. Out of these genotypes, II and VIII were confined to three countries each, and circulated for between 10 and 20 years in the field, genotype IX caused two temporally unrelated outbreaks in domestic pigs in 1995 and 2003, while the remaining genotypes were restricted to one country each and were associated with a single epizootic (and sometimes a single virus). The molecular phylogeny further revealed the presence of three distinct evolutionary groups (labeled A-C in Fig. 1). Viruses sharing a common evolutionary history fall within the following genotype clades:

(A) Genotypes I-VII and XVII (77 % bootstrap support)

(B) Genotypes VIII and XI-XVI (63 % bootstrap support)

(C) Genotypes IX and X (99 % bootstrap support)

The 404 nucleotide region sequenced was A-T rich (57.3 %) with a transition:transversion (si/sv) ratio of 2.2 (ML estimate from PAUP). There were 74 variable sites (Fig. 3), of which 50 were parsimony informative and 24 were singletons. On amino acid level, 19 of the 134 codon sites were variable and 6 of these variable sites were parsimony informative. Levels of mean intra-genotypic variation ranged from 0 % (genotypes II, VI, IX and XVI) to 0.7 % (genotype XII), whilst mean inter-genotypic levels of variation ranged from 0.9 % (between genotypes V and VI) to 7.9 % (between genotypes V and X). The maximum level of sequence divergence between any two isolates was 8.3 %.

Discussion

The presence of seven more East African genotypes than was previously identified [Bastos et al. 2003] was revealed by the molecular phylogeny. Genotypes I, V, X, XI, XIII and XIV are examples of viruses that are present within a sylvatic cycle (occurring either within eyeless tampans or sylvatic vertebrate hosts, or both), half of which have also caused outbreaks in domestic pigs. Genotype XII which comprised two viruses isolated 10 years apart from a tick and domestic pig may be an example of a pig-tick cycle but this requires confirmation by more intensive screening of sylvatic vertebrates within Malawi and Zambia. The pig-to-pig cycle is however classically exemplified by genotype VIII, which has been in active circulation for at least 23 years with the 39 isolates originating from three countries. A genetic feature of a domestic pig cycle appears to be a pronounced lack of genetic variation, as both genotype I in West Africa (where it has only been isolated from domestic pigs) and genotype VIII in East Africa have extremely low levels of intratypic variation (0.2 % and 0.1 %, respectively), with most isolates being identical to each other. Although sample sizes of the remaining genotypes are inadequate to permit speculation on the epidemiological cycles into

which they may be classified, the results indicate that all three ASF epidemiological cycles appear to exist in East Africa.

The East African region undoubtedly has far more ASFV genotypes than West Africa, which only contains one genotype in the ten countries previously screened [1]. Southern Africa is more genotype rich, having five domestic pig genotypes (when Mozambique is excluded from the results) [5]. While many of the East African genotypes are apparently country specific (V, VI, IX, XI, XIII, XIV, XV, XVI and XVII), others (I, II, VIII, X and XII) are not restricted by national boundaries. In addition, most countries within the East African region have more than one genotype within their borders. Zambia is particularly genotype rich with eight genotypes being identified, followed by Mozambique with four, Malawi and Tanzania with three, and Kenya and Uganda with two each.

The ASFV introduced to Europe through illicit movement of pig products [17] and which is widespread throughout West Africa [1] may have its origins in East Africa as this study revealed it to be present in the natural sylvatic hosts as far back as 1961. As the classical ASFV transmission mode involving a sylvatic cycle could not be proven in West Africa [21], it is likely that the disease was introduced from the East of the continent. The more widespread distribution of the ESACWA genotype identified in this study makes this genotype the most successful and extensively distributed genotype (being present in 22 countries) described to date.

The low levels of intratypic genetic diversity within the large and homogeneous genotype VIII necessitates an investigation into a more variable gene region in order to clarify within genotype relationships. In addition, the possibility that genotype I, which was previously believed to be confined to West Africa, originated from East Africa should be confirmed through sequencing of an alternative and more informative gene region. By focusing on typing ASFV from domestic pigs in East and Central African countries, where

genotype I is present in the sylvatic hosts, it may also be possible to trace the route of entry of this virus into West Africa.

Both the sylvatic and domestic pig cycles appear to play an important role in the epidemiology of ASF in East Africa. The existence of multiple genotypes within countries, trans-boundary distribution of genotypes between countries and regional genotype richness adds to the complexity of ASF epidemiology in East Africa. As genotyping in this study was based on partial characterization of the gene coding for the immunodominant protein VP72, future vaccination campaigns could utilize this information when formulating vaccine for specific countries, since immunizing pigs with antigens from viruses distantly related to those with which they are challenged offers less protection [4; 19]. These factors are important considerations that need to be taken into account for effective control of the disease in East Africa.

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Figure Legends

Fig. 1 Minimum evolution tree depicting the 17 ASFV *p72* genotypes from East Africa (labeled I-XVII) and the three main evolutionary lineages (labeled A-C). Bootstrap values > 50% are indicated next to the relevant node and were obtained following 10 000 replications.

Fig.2 Geographical distribution of the 17 major African Swine Fever virus genotypes identified by partial *p72* genotyping

Fig. 3: Nucleotide sequence alignment of the 49 unique sequences identified in this study and in previous studies. Only variable sites are presented with relevant variable site numbers being shown in bold above master sequence IC3/96. Dots indicate nucleotide sites identical to that of the master sequence, and superscript numbers 1 through 4 indicate those viruses included in the studies of Bastos et al. 2003, Odemuyiwa et al. 2000; Bastos et al. 2004 and Yu et al. 1996, respectively.


LIV13/33/P
ZAIRE
KIMAKIAI/KEN/B
KIMAKIAII/KEN/B
Lis57/PORT/P
Dakar59/SEN/P
CAM/82/CAM/P
Brazil/79/C
LIV10/11/ZAM/T(W)
LIV9/35/ZAM/T(W)
LIV12/17/ZAMT(W)
LIV5/4/ZAM/T(W)
LIV5/40/ZAM/T(W)
LIV9/31/ZAM/T(W)
ZAM01/1/P
ZAM01/2/P
ZAM01/3/P
ZAM01/5/P
ZAM02/1/P
ZAM01/4/P
MAD/1/98/MAD/P
LUS93/1/ZAM/P
MOZ/2002/2/P
MOZ/2002/1/P
BOT/1/99/BOT/P
RSA1/99/RSA/W
MOZ94/1/MOZ/P
SPEC265/MOZ/P
Tengani60/MAL/W
MAL2002/1/P
RSA1/98/RSA/P
CHJ89/1/ZAM/P
LIL90/1/MAL/P
DED91/1/MAL/P
MCHINJI075/MAL/P
DED89/1/MAL/P
LIL89/1/MAL/P
BAN91/1/MAL/P
CHM88/1/ZAM/P
THY90/1/MAL/P
MALAWI/1978/P
MPO89/1/ZAM/P
KANA89/1/ZAM/P
KAC91/2/MAL/P
MCH89/1/MAL/P
MOZ2001/1/P
CHK89/2/ZAM/P
YEL88/4/ZAM/P
DOWA/MAL/P
KAV89/1/ZAM/P
KAL88/1/ZAM/P
ZON88/1/ZAM/P
MPI89/1/ZAM/P
MCH89/3/MAL/P
GUL88/1/ZAM/P
NGE92/1/MAL/P
NKZ88/1/ZAM/P
ZAW88/1/ZAM/P
Dezda/MAL/P
MAN89/2/ZAM/P
JON89/13/ZAM/P
TEN89/1/ZAM/P
SAL92/1/MAL/P
ZOM2/84/MAL/P
KLI88/2/ZAM/P
CHG88/1/ZAM/P
SIY91/2/MAL/P
PHW88/1/ZAM/P
TMB89/1/ZAM/P
NDA1/90/MAL/P
KAB6/2/ZAM/T(W)
MZI92/1/MAL/P
MFUE6/1/ZAM/T(W)
TAN1/01/P
TAN/2003/1/P
TAN/2003/2/P
SUM14/11/ZAM/T(W)
NYA1/2/ZAM/T(W)
UGA2003/1/UGA/P
UGA1/95/UGA/P
Magadi w/hog 9/KEN/W
BARTLETTII/KEN/W
KIRT89/2/TAN/T(W)
KIRT89/3/TAN/T(W)
KIRT89/4/TAN/T(W)
BUR90/3/P
KILLEAN III/KEN/W
KILLEAN II/KEN/W
BUR1/84/P
BUR2/84/BUR/P
UGA3/95/UGA/P
KWH/12/TAN/W
BUR90/1/BUR/P
KIRW89/1/TAN/W
DAVIS/KEN/W
Magadi w/hog1/KEN/W
GASSON/KEN/P
TRENCH/KEN/W
HINDEI/KEN
KILLEAN I/KEN/W
Hinde II/KEN/P
DOIG/KEN/W
I
II
III
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A
B
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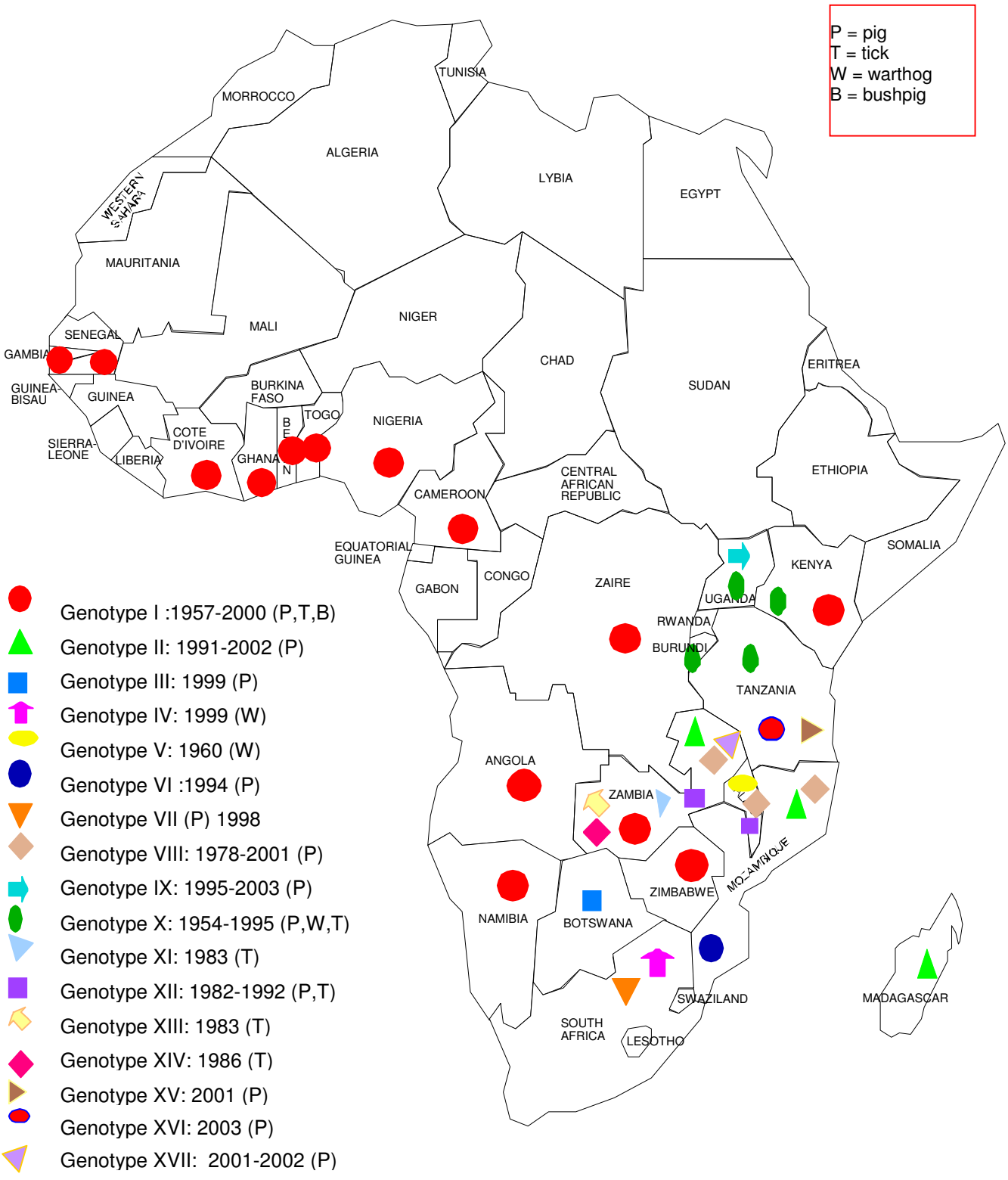
P = pig
T = tick
W = warthog
B = bushpig
TUNISIA
MORROCCO
ALGERIA
LYBIA
EGYPT
WESTERN SAHARA
MAURITANIA
MALI
NIGER
CHAD
SUDAN
ERITREA
SENEGAL
GAMBIA
GUINEA-BISAU
GUINEA
BURKINA FASO
TOGO
NIGERIA
SIERRA-LEONE
LIBERIA
COTE D'IVOIRE
GHANA
B E N
CAMEROON
CENTRAL AFRICAN REPUBLIC
ETHIOPIA
SOMALIA
KENYA
EQUATORIAL GUINEA
GABON
CONGO
ZAIRE
UGANDA
RWANDA
BURUNDI
TANZANIA
ANGOLA
ZAMBIA
ZIMBABWE
MOZAMBIQUE
NAMIBIA
BOTSWANA
SWAZILAND
SOUTH AFRICA
LESOTHO
MADAGASCAR
Genotype I :1957-2000 (P,T,B)
Genotype II: 1991-2002 (P)
Genotype III: 1999 (P)
Genotype IV: 1999 (W)
Genotype V: 1960 (W)
Genotype VI :1994 (P)
Genotype VII (P) 1998
Genotype VIII: 1978-2001 (P)
Genotype IX: 1995-2003 (P)
Genotype X: 1954-1995 (P,W,T)
Genotype XI: 1983 (T)
Genotype XII: 1982-1992 (P,T)
Genotype XIII: 1983 (T)
Genotype XIV: 1986 (T)
Genotype XV: 2001 (P)
Genotype XVI: 2003 (P)
Genotype XVII: 2001-2002 (P)

	111222	2355667778	11111 8899900133	1111122222 4568900111	2222222222 1233444456	2222223333 6678990123	3333333333 4455566788	3333333334 8888899990
	2679024457	9902031285	6713658025	4859817357	9814035681	4705146246	2517836234	5678935680
IC/3/96[1]	TGCTATAGAA	GTCCCAGTGA	GCGTGCATCA	TTGTCCCCAG	ACCTGTCGCT	TGGCCTCCAT	CGCGTCTTTA	TCGGCTCTTA
Lisbon/57[1]	....	......C...	....	....	....	....	....	....
LIV10/11/ZAM/T(W)	....	......C...	....	........G.	....	....	....	....
VICT90/1[1]	....	......C...	....	........G.	....	....	....A.....	....
LIV9/35/ZAM/T(W)	......T...	......C...	....	........G.	....	....	....	....
LIV5/40/ZAM/T(W)	......T...	T.....C...	....	...A......	....	....	....	....
LIV9/31/ZAM/T(W)	....	......C...	....	...A......	....	....	....	....
NAM/1/80[1]	....	......C...	....	....	.T.C......	....	....	....
ZAM021/P	....	......C.A.	....	....	....	....	.......AT	CG.T......
AF159503[2]	....	..TG..C...	....	....	....	....	.........T	....
ZAM014/P	......C...	......C.A.	.......A..	....	....	....	....	....
ZAM013/P	....	......C.A.	....	....	....	....	....	....
MAD/1/98[1]	...C......	......C.T.	....	..A.......	G.........	....	....	....
BOT/1/99[1]	....	......C.T.	......G...	..A.......	G..C......	.C........	T.....C...	....
RSA/1/99W[1]	....	......C.T.	..A...G...	..A.......	G..C......	......TT..	T.........	....
MOZ/94/1[1]	....	......C.T.	...C....T.	..A.T.....	G..C......	C......T..	T.........	....
MAL2002/1/P	....	......C.T.	...C......	..A.T.....	G.........	C......T..	T.........	..A.......
Tengani/60[1]	....	......C.T.	...C.T....	..A.T.....	G.........	C......T..	T.........	..A.......
MOZ/1960[3]	....	......C.T.	...C.T....	..A.T.....	G......A..	C......T..	T.........	....
RSA/1/98[1]	....	......C.T.	....	..A.....G.	G.TCT.....	...T......	..T...C...	....
NYA1/2/ZAM/T(W)	.......A..	......C.T.	A.........	.......T.T	GT...C....	....	..T...C...	.......C..
TAN/2003/2/P	....	......C.T.	A...T.....	.........T	GTTC.C...C	....	..T...C...	....
TAN101/P	.......A..	.C....C.T.	AT..T.....	.........T	GTT..C..TC	.......T..	..T...C...	....
SUM14/11/ZAM/T(W)	.......A..	......C.T.	A...T.....	.........T	G..C.C...C	....	..T...CGC.	.GC.G.....
MZI921/MAL/P	.......A..	.....GC.T.	A...T.....	.........T	G..C.C...C	....	..T..TC...	....
MFUE6/1/ZAM/T(W)	.C.....A..	.....GC.T.	A...T.....	.........T	G..C.C...C	....	..T..TC...	.......GG.
KAB6/2/ZAM/T(W)	.......A..	......C.T.	A...T.....	.........T	G..C.C...C	....	..T..TC...	..A.......
NDA/1/90[1]	.......A..	......C.T.	T...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
SAL921/MAL/P	....T.TA..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
TMB89/1/ZAM/P	.......A..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A....G.C
PHW88/1/ZAM/P	.......A..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A....G..
JON/89/13[1]	.......A..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
CHG881/ZAM/P	G......A..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
KLI882/ZAM/P	.......A.C	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
SIY91/2/MAL/P	..G....A..	......C.T.	A...T.....	......T..T	G.T..C...C	....	..TA.TC...	..A.......
UGA/1/95[1]	.....C..G.	....T.CCT.	C.A.T....T	C.A......T	G..C.C....	..A..C...C	.AT...C...	.....C....
MWHOG9/KEN/W	.....C..G.	....T.CCA.	C.A.T.G..C	..A..T...T	G..C.C....	..A......C	.AT...CC..	.....C....
BARTLETT2/KEN/W	.....C.AG.	....T.CCAT	C.A.T.G..C	..A..T...T	G....C....	..A.....GC	.AT...CC..	.....C....
GASSON/KEN/P	.....C..G.	....T.CCAT	CTA.T.G..C	..A..T...T	G....C....	..A.....GC	.AT...CC..	.....C....
DOIG/KEN/W	.....C..G.	....T.CCAT	CTA.T.G..C	..A..T...T	G..C.C....	..A.T...GC	.AT...CC..	.....C....
HindeII/59[1]	.....C..G.	....T.CCAT	CTA.T.G..C	.CA..T...T	G..C.C....	..A.T...GC	.AT...CC..	.....C....
Uganda[4]	.....C..G.	....T.CCAT	CTA.T.G..C	..A..T...T	G..C.GG...	..A.T...GC	.AT...CC..	.....C....
KILLEAN1/KEN/W	.....C..G.	....T.CCAT	C.A.T.G..C	..A..T...T	G..C.C....	..A.T...GC	.AT...CC..	.....C....
HINDE1/KEN	......C.G.	....T.CCAT	CTA.T.G..C	..A..T...T	G..C.C....	..A.T...GC	.AT...CC..	.....C....
TRENCH/KEN/W	......CAG.	....T.CCAT	CTA.T.G..C	..A..T...T	G..C.C....	..A.T...GC	.AT...CC..	.....C....
DAVIS/KEN/W	.....CT.G.	....T.CCAT	C.A.T.G..C	..A..T...T	G..C.C....	..A....TGC	.AT...CC..	.....C....
MWHOG1/KEN/W	.....C..G.	....T.CCAT	C.A.T.G..C	..A..T...T	G..C.C....	..A....TGC	.AT...CC..	.....C....
UGA/3/95[1]	.....C..G.	....T.CCAT	C.A.T.G..C	..A..T...T	G..C.C....	..A.....GC	.AT...CC..	.....C....
KIRW89/1/TAN/W	.....C..G.	....T.CCAT	C.A.T.G..C	..A..T...T	G..C.C....	..A.....GC	.AT...CC..	.....CA...

TABLE 1: Summary of the *African swine fever virus* isolates characterised in this study

Virus name	Country of origin	Town / District	Year of isolation	Species of origin	GenBank Accession number
BAN 91/1	Malawi	Bangula, Lower Shire	1991	*Sus scrofa*	AY351501
Bartlett II	Kenya	Timau	1959	*Phaecochoerus aethiopicus*	AY351532
BUR 90/3	Burundi	Muyinga	1990	*Sus scrofa*	AY351525
CHG 88/1	Zambia	Chaguza, Katete, Eastern Province	1988	*Sus scrofa*	AY351552
CHJ 89/1	Zambia	Chiphanje, Petauke, Eastern Province	1989	*Sus scrofa*	AY351519
CHK 89/2	Zambia	Chikuwe, Chipata, Eastern Province	1989	*Sus scrofa*	AY351526
CHM 88/1	Zambia	Chambula, Petauke, Eastern Province	1988	*Sus scrofa*	AY351520
DED 89/1	Malawi	Chiphazi, Dedza District	1989	*Sus scrofa*	AY351502
DED 91/1	Malawi	Mtenden Campus, Dedza	1991	*Sus scrofa*	AY351503
Davis	Kenya	Nanyuki	1959	*Phaecochoerus aethiopicus*	AY351527
Doig	Kenya	Kiganjo	1957	*Phaecochoerus aethiopicus*	AY351528
DOWA	Malawi	Moya, Dowa	1986	*Sus scrofa*	AY351509
Gasson	Kenya	Nanyuki	<1961	*Sus scrofa*	AY351529
GUL 88/1	Zambia	Gulumule, Katete, Eastern Province	1988	*Sus scrofa*	AY351521
Hinde I	Kenya	Nanyuki	1954	Suid	AY351530
KAB 6/2	Zambia	Livingstone game park, south Zambia	1983	Tick *	AY351522
KAC 91/2	Malawi	Kachendere Seminary, Chisengu, Mchinji	1991	*Sus scrofa*	AY351504
KANA 89/1	Zambia	Kangwero farm 17, Katete, Eastern Province	1989	*Sus scrofa*	AY351523
Killean I	Kenya	Nanyuki	1959	*Phaecochoerus aethiopicus*	AY351550
Killean II	Kenya	Nanyuki	1959	*Phaecochoerus aethiopicus*	AY351551
Killean III	Kenya	Nanyuki	1959	*Phaecochoerus aethiopicus*	AY351531
Kimakia I	Kenya	UK	1961	*Potamochoerus porcus*	AY351533
Kimakia II	Kenya	UK	1961	*Potamochoerus porcus*	AY351534
KIRT 89/2	Tanzania	Kiriwira	1989	Tick *	AY351511
KIRT 89/3	Tanzania	Kiriwira	1989	Tick *	AY351512
KIRT 89/4	Tanzania	Kiriwira	1989	Tick *	AY351513

KIRW 89/1	Tanzania	Kiriwira	1989	*Phaecochoerus aethiopicus*	AY351514
KLI 88/2	Zambia	Kalinda, Petauke, Eastern Province	1988	*Sus scrofa*	AY351553
LIL 89/1	Malawi	Mlozi, Lilongwe District	1989	*Sus scrofa*	AY351505
LIL 90/1	Malawi	Kafere diptank, Lilongwe	1990	*Sus scrofa*	AY351510
LIV 5/40	Zambia	Livingstone Game Park, south Zambia	1982	Tick *	AY351536
LIV 5/4	Zambia	Livingstone Game Park, south Zambia	1983	Tick *	AY351537
LIV 9/31	Zambia	Livingstone Game Park, south Zambia	1983	Tick *	AY351538
LIV 9/35	Zambia	Livingstone Game Park, south Zambia	1983	Tick *	AY351539
LIV 10/11	Zambia	Livingstone Game Park, south Zambia	1983	Tick *	AY351535
LIV 12/17	Zambia	Livingstone Game Park, South Zambia	1983	Tick *	AY351524
LIV 13/33	Zambia	Livingstone Game Park, South Zambia	1983	Tick *	AY494560
LUS 93/1	Zambia	Nawande farm, Lusaka district, Lusaka Province	1991	*Sus scrofa*	AY351563
Magadi w/hog 1	Kenya	Magadi	1959	*Phaecochoerus aethiopicus*	AY351548
Magadi w/hog 9	Kenya	Magadi	1959	*Phaecochoerus aethiopicus*	AY351565
MAL 2002/1[1]	Malawi	Mpemba Quarantine Camp	2002	*Sus scrofa*	AY494553
MAN 89/2	Zambia	Mangulu, Katete, Eastern Province	1989	*Sus scrofa*	AY351562
MCH 89/1	Malawi	Kachebere Seminary, Mchinji	1989	*Sus scrofa*	AY351506
MCH 89/3	Malawi	Chisikwa diptank, Lilongwe District	1989	*Sus scrofa*	AY351507
Mchinji 075	Malawi	Mchinji	1987	*Sus scrofa*	AY351508
MFUE 6/1	Zambia	Mfue, Luangera National Park	1982	Tick *	AY351561
MOZ 2001/1[5]	Mozambique	Zambezi, Quilemane	2001	*Sus scrofa*	AY351516
MOZ 2002/1[5]	Mozambique	Northern Nampula region	2002	*Sus scrofa*	AY351517
MOZ 2002/2[5]	Mozambique	Northern Nampula region	2002	*Sus scrofa*	AY351518
MPI 89/1	Zambia	Mpima Seminary, Kabwe, Central Province	1989	*Sus scrofa*	AY351540
MPO 89/1	Zambia	Mpoka, Petauke, Eastern Province	1989	*Sus scrofa*	AY351541
MZI 92/1	Malawi	Euthini, Mzinda District, north Malawi	1992	*Sus scrofa*	AY351543
NGE 92/1	Malawi	Ngerenge diptank, Karonga District	1992	*Sus scrofa*	AY351544

NKZ 88/1	Zambia	Nyankonzi, Petauke, Eastern Province	1988	*Sus scrofa*	AY351554
NYA1/2	Zambia	Kalumo	1986	Tick *	AY351555
PHW 88/1	Zambia	Phwata, Chipata, Eastern Province	1988	*Sus scrofa*	AY351567
SAL 92/1	Malawi	Chiripa diptank, Salima District	1992	*Sus scrofa*	AY351546
SIY 91/2	Malawi	Sinyala diptank, Lilongwe	1991	*Sus scrofa*	AY351566
SUM 14/11	Zambia	Sumbu National Park	1983	Tick *	AY351542
TAN/1/01[2]	Tanzania	Dar Es Salaam	2001	*Sus scrofa*	AY494552
TAN/2003/1[2]	Tanzania	Arusha	2003	*Sus scrofa*	AY494550
TAN/2003/2[2]	Tanzania	Arusha	2003	*Sus scrofa*	AY494551
TEN 89/1	Zambia	Tenesi, Petauke, Eastern Province	1989	*Sus scrofa*	AY351556
THY 90/1	Malawi	Comforzi farm, Thyolo District	1990	*Sus scrofa*	AY351545
TMB 89/1	Zambia	Tembo, Petauke, Eastern Province	1989	*Sus scrofa*	AY351557
Trench	Kenya	Mweiga	1959	*Phaecochoerus aethiopicus*	AY351547
UGA2003/1[4]	Uganda	Maria Village, Masaka District	2003	*Sus scrofa*	AY351564
YEL88/4	Zambia	Yelani, Petauke, Eastern Province	1988	*Sus scrofa*	AY351558
ZAM01/1[3]	Zambia	Lusaka	2001	*Sus scrofa*	AY494554
ZAM01/2[3]	Zambia	Kafue	2001	*Sus scrofa*	AY494555
ZAM01/3[3]	Zambia	Mazabuka	2001	*Sus scrofa*	AY494556
ZAM01/4[3]	Zambia	Namwala	2001	*Sus scrofa*	AY494557
ZAM01/5[3]	Zambia	Monze	2001	*Sus scrofa*	AY494558
ZAM02/1[3]	Zambia	Kyiundi Ranch	2002	*Sus scrofa*	AY494559
Zaire	DRC	NK	NK	NK	AY351515
ZAW 88/1	Zambia	Gulumule, Katete, Eastern Province	1988	*Sus scrofa*	AY351559
ZON 88/1	Zambia	Zondola, katete, Eastern Province	1988	*Sus scrofa*	AY351560

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TABLE 2 Distribution, field presence and intra-genotypic variation of the major African swine fever virus *p72* genotypes using data of 141 virus sequences from this study and that from previous studies (Lopez-Otin *et al*. 1990; Yu *et al*. 1996; Odemuyiwa et al. 2000; Bastos et al. 2003; Bastos et al. 2004).

Genotype	Representative countries	Presence in the field	Species affected	No. of viruses	No. of countries	Mean intragenotypic nucleotide variation
I	Zambia, Kenya, Zaire, Cameroon, Ghana, Senegal, Nigeria, Gambia, Benin, Côte d'Ivoire, Togo, Angola, Zimbabwe, Namibia, Portugal, Brazil, Spain, Sardinia, Malta, Holland, Belgium, Dominican Republic	1957 - 2000	Bushpig Domestic pig Tick (warthog)	51	22	0.2 %
II	Mozambique, Zambia, Madagascar	1991 - 2002	Domestic pig	5	3	0.0 %
III	Botswana	1999	Domestic pig	1	1	--
IV	Republic of South Africa	1999	Warthog	1	1	--
V	Malawi, Mozambique	1960	Domestic pig Warthog	4	2	0.4 %
VI	Mozambique	1994	Domestic pig	3	1	0.0 %
VII	Republic of South Africa	1998	Domestic pig	1	1	--
VIII	Zambia , Malawi and Mozambique	1978 - 2001	Domestic pig	39	3	0.1 %
IX	Uganda	1995 - 2003	Domestic pig	2	1	0.0 %
X	Uganda, Burundi, Tanzania, Kenya	1954 - 1995	Domestic pig Tick (warthog) Warthog	22	4	0.6 %
XI	Zambia	1983	Tick (warthog)	1	1	--
XII	Malawi and Zambia	1982 -1992	Domestic pig Tick (warthog)	2	2	0.7 %
XIII	Zambia	1983	Tick (warthog)	1	1	--

Genotype	Representative countries	Presence in the field	Species affected	No. of viruses	No. of countries	Mean intragenotypic nucleotide variation
XIV	Zambia	1986	Tick (warthog)	1	1	--
XV	Tanzania	2001	Domestic pig	1	1	--
XVI	Tanzania	2003	Domestic pig	2	1	0.0 %
XVII	Zambia	2001-2002	Domestic pig	6	1	0.6 %