Arch Virol (2003)
DOI 10.1007/s00705-002-0946-8

Archives of Virology
Printed in Austria

Genotyping field strains of *African swine fever virus* by partial p72 gene characterisation

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Received July 17, 2002; accepted October 30, 2002
Published online January 16, 2003

Summary. A PCR-based sequencing method was developed which permits detection and characterization of *African swine fever virus* (ASFV) variants within 5 and 48 h, respectively, of receipt of a clinical specimen. Amplification of a 478 bp fragment corresponding to the C-terminal end of the *p72* gene, confirms virus presence with genetic characterization being achieved by nucleotide sequence determination and phylogenetic analysis. The method was applied to 55 viruses including those representative of the major ASF lineages identified previously by restriction fragment length polymorphism (RFLP) analysis. Results confirmed that the *p72* genotyping method identifies the same major viral groupings. Characterization of additional viruses of diverse geographical, species and temporal origin using the PCR-based method indicated the presence of ten major ASF genotypes on the African continent, the largest of which comprised a group of genetically homogeneous viruses recovered from outbreaks in Europe, South America, the Caribbean and West Africa (the ESAC-WA genotype). In contrast, viruses from southern and East African countries were heterogeneous, with multiple genotypes being present within individual countries. This study provides a rapid and accurate means of determining the genotype of field and outbreak strains of ASF and is therefore useful for molecular epidemiological clarification of ASF.

Introduction

African swine fever (ASF) is a highly lethal viral hemorrhagic fever of domestic pigs, the causal agent of which is an icosahedral double stranded DNA virus of the

family *Asfarviridae*, within the genus *Asfivirus* [10]. First described from Kenya in 1921 [27], it was subsequently found to exist in most countries in southern and eastern Africa, where ASF virus is maintained in an ancient sylvatic cycle primarily involving warthogs *(Phacochoerus* spp) and soft-shelled eyeless ticks of the *Ornithodoros* complex [19]. Although warthogs are distributed throughout the savanna belt from Cameroon to Sénégal [31], the presence of *Ornithodoros* species has been proven only in Sénégal, north of the ASF endemic area [23]. There is therefore no evidence that the sylvatic cycle occurs in West Africa. Export of ASF to Portugal from Africa in 1957 focused attention on the devastating effects of the disease and the difficulties of control and eradication [25]. Outbreaks occurred subsequent to this initial introduction in other European countries, in South America and in the Caribbean [2]. The disease disappeared from Europe (with the exception of Sardinia) in the mid 1990's, but re-emerged in Portugal in 1999.

Although pork production in Africa is negligible compared to other continents, pig numbers have increased markedly in the last decades, mainly because pigs provide a cheap source of protein and have minimal space requirements. In recent years, African swine fever (ASF) outbreaks have decimated domestic pig populations in Madagascar [14], West Africa [11, 28] and southern Africa, with devastating impact to both subsistence and commercial farmers. This is because there is presently no vaccine available with which to combat the disease. Control of ASF is, therefore, reliant on zoosanitary measures and slaughter of infected and in-contact animals.

ASFV was previously believed to be one of a limited number of viruses that lack neutralization sites [7], making control of the disease through vaccination unattainable. Recent studies have however demonstrated that neutralizing antibodies to virus protein 72 (VP72) occur, with corresponding epitopes being conserved across divergent strains [13, 38]. The neutralization mechanism associated with VP72 antibodies is one preventing virus attachment by inhibiting virus replication [13]. This major antigenic capsid protein makes up about 32% of the total protein mass of the virion and is antigenically stable [12, 24].

ASFV has a double-stranded DNA genome varying between 170 and 190 kbp in length, due mainly to deletions and insertions occurring within the left and right terminal regions of the genome. Length variation in the terminal ends occurs between different isolates [2, 9] and within strains following cell culture adaptation [2]. Although multiple ASFV antigenic types are known to exist [34], classification of viruses into discrete serological subtypes is not possible. Elucidation of the relationships of field strains is therefore dependent on genetic characterization and, so far, has been based primarily on restriction fragment length polymorphism analysis (RFLP) [2, 8, 35]. RFLP studies are, however, time-consuming and deliver relatively crude results, making it an impractical means of discerning viral relationships in an outbreak situation, where time is of the essence. Nucleotide sequence-based epidemiological studies on RNA viruses of veterinary importance are well established and usually involve characterization of immunodominant proteins (for recent examples see [15, 22, 30]). This is because nucleotide sequencing

when coupled with PCR is rapid and because capsid proteins tend to be variable and phylogenetically informative. In contrast, epidemiological studies of DNA viruses are usually RFLP-based. This is mainly due to the extremely low levels of variation at the nucleotide level, which necessitates screening of the entire genome or large portions thereof in order to obtain sufficient informative sites to allow differentiation [16, 33]. However, focused gene nucleotide sequencing has been shown to be useful for DNA viruses with moderate levels of variation [5, 18, 26, 37]. In these cases, sequencing of an immunogenic or surface protein provided results comparable to those obtained with complete genome characterization. As VP72 antigenicity is well-established [13, 38] and comparison of available complete gene sequences [6, 24, 28, 36] indicates some level of inter-strain variation, it was thought that this genomic region may be suitable for determining viral relationships of field strains. The aim of this study was therefore twofold: Firstly, to assess the usefulness of partial *p72* nucleotide sequences for discerning relationships previously identified by RFLP analysis, and secondly (upon method validation) to characterize field strains of diverse species, temporal and geographical origin in order to clarify genetic relationships of ASF viruses on the African continent.

Materials and methods

Viruses used in this study

Pig macrophage cell culture isolates representative of the geographical regions previously included in RFLP studies [2] were selected for this study. In addition, clinical specimens obtained from ASF outbreaks in Madagascar, West Africa and Mozambique were included in order to establish the genetic relatedness of these strains to each other. Geographical origin, sample source and collection date of the specimens are summarized in Table 1.

Nucleic acid extraction and genomic amplification

DNA was extracted directly from cell culture isolates or from 10% suspensions of ground clinical specimens by means of a silica/GuSCN method [3]. Complete *p72* gene sequences [6, 24, 28, 36] were used to design primers targeting genetically variable regions of the gene. Briefly, nucleotide and amino acid variability plots were generated in MEGA [20]. Results indicated the presence of two regions rich in non-synonymous mutations at amino acid positions 301–332 and 566–579, corresponding to the central and C-terminal regions of the gene, respectively. Analysis of the corresponding nucleotide variability plots revealed that the C-terminus region was flanked by completely conserved stretches of nucleotide sequence and therefore a suitable target for PCR primer binding. Two oligonucleotide primers termed P72-U (5′ GGCACAAGTTCGGACATGT 3′) and P72-D (5′GTACTGTAACGCAGCACAG 3′) were designed within these flanking regions following the guidelines of Rychlik [29]. Genomic amplification was performed in a 50 µl volume in the presence of 0.2 mM dNTP, 0.25 µM of each primer, 1U of thermostable DNA polymerase and 3 µl of DNA extract. The thermal profile followed 35 cycles of denaturation at 96 °C for 12 s, annealing at 50 °C for 20 s and extension at 70 °C for 25 s, after an initial denaturation step at 96 °C for 20 s.

Nucleotide sequencing and analysis

Amplification products of the expected 478 bp size were identified against a molecular weight marker, following elecrophoresis on a 1.5% agarose gel. Bands of the correct size were excised

Table 1. Summary of African swine fever viruses used in this study

Virus designation	Country of origin	Year of sampling	Reference	Genbank accession No
[1]Lisbon/57	Portugal	1957	This study	AF301537
[1]Dakar/59	Sénégal	1959	This study	AF301538
[1]Hinde II	Kenya	1959	This study	AF449480
[1]Lisbon/60	Portugal	1960	This study	AF301539
[1]Tengani/60[6]	Malawi	1960	This study	AF301541
RHO 61/1	Zimbabwe	1961	This study	AF449460
[1]Madrid/62	Spain	1962	This study	AF449461
[1]Katanga/63	DRC	1963	This study	AF301540
Uganda	Uganda	1965	Yu et al. 1996	L27499
[1]Kwh/12[6]	Tanzania	1968	This study	AF301546
[1]ANG/70	Angola	1970	This study	AF301542
BA71	Spain	1971	Lopez-Otin et al. 1990	M34142
[1]Val/76	Spain	1976	This study	AF449462
Malawi/1978	Malawi	1978	This study	AF270707
[1]Malta/78	Malta	1978	This study	AF301543
[1]Brazil/79	Brazil	1979	This study	AF302809
[1]DomRep/79	Dominican Republic	1979	This study	AF301810
NAM/1/80[6]	Namibia	1980	This study	AF504881
[1]CAM/82	Cameroon	1982	This study	AF301544
[1]BUR/1/84	Burundi	1984	This study	AF449463
[1]BUR/2/84	Burundi	1984	This study	AF449464
[1]ZOM/2/84	Malawi	1984	This study	AF449471
[1]CAM/4/85	Cameroon	1985	This study	AF301545
[1]ZAR/85	Spain	1985	This study	AF449465
[1]BEL/85	Belgium	1985	This study	AF449466
[1]HOL/86	Holland	1986	This study	AF449467
[1]Dedza	Malawi	1986	This study	AF449479
[1]Ourt/88/1[7]	Portugal	1988	This study	AF302811
[1]KAL/88/1	Zambia	1988	This study	AF449468
[1]JON/89/13	Zambia	1989	This study	AF449469
[1]KAV/89/1[7]	Zambia	1989	This study	AF449470
[1]BUR/90/1	Burundi	1990	This study	AF449472
NDA/1/90	Malawi	1990	This study	AF449473
[1]NUR/1/90	Sardinia	1990	This study	AF302813
[1]VICT90/1[7]	Zimbabwe	1990	This study	AF449474
[1]MOZ/94/1	Mozambique	1994	This study	AF270711
SPEC 265	Mozambique	1994	This study	AF270710
[1]UGA/1/95	Uganda	1995	This study	AF449475
[1]UGA/3/95	Uganda	1995	This study	AF449476
[2]IC/1/96	Côte d'Ivoire	1996	This study	AF302814
[2]IC/2/96	Côte d'Ivoire	1996	This study	AF302815
[3]IC/3/96	Côte d'Ivoire	1996	This study	AF504882

(*continued*)

Table 1 (*continued*)

Virus designation	Country of origin	Year of sampling	Reference	Genbank accession No
[2]IC/576[5]	Côte d'Ivoire	1996	This study	AF504883
[1]BEN/1/97	Benin	1997	This study	AF302816
[3]MAD/1/98	Madagascar	1998	This study	AF270705
[3]NIG/1/98	Nigeria	1998	This study	AF302817
NIG/1/98	Nigeria	1998	Odemuyiwa et al. 2000	AF159503
[3]NIG-2[5]	Nigeria	1998	This study	AF504884
[3]NIG-6[5]	Nigeria	1998	This study	AF270714
RSA/1/98	South Africa	1998	This study	AF302818
[2]Togo/98[5]	Togo	1998	This study	AF449481
Awoshie/99[5]	Ghana	1999	This study	AF504885
BOT/1/99	Botswana	1999	This study	AF504886
[4]NIG/1/99	Nigeria	1999	This study	AF504887
RSA/1/99[6]	South Africa	1999	This study	AF302818
GAM/1/00	Gambia	2000	This study	AF449478
GHA/1/00	Ghana	2000	This study	AF504888
Ghana[5]	Ghana	2000	This study	AF504889

[1]Indicates isolates supplied by the Institute of Animal Health, Pirbright, UK; [2]Indicates viruses derived from clinical specimens supplied by LCPA, Bingerville, Côte d'Ivoire; [3]Indicates viruses derived from clinical specimens provided by AFSSA-Alfort, France; [4]Indicates clinical specimens provided by the Department of Virology, Ibadan, Nigeria; [5]Indicates virus characterization performed directly on clinical specimens; [6]Indicates viruses from warthog, *Phacochoerus* spp.; [7]Indicates viruses from *Ornithodoros* ticks

and purified according to manufacturer specifications (Machery Nagel). Nucleotide sequences were generated using a manual sequencing approach or alternatively with an automated sequencing approach. In the former, a radionucleotide is incorporated by T7 DNA polymerase (Sequenase version 2.0, USB) in the presence of 10% DMSO as previously described [1], whilst the latter involves fluorescent dye cycle sequencing (Perkin Elmer) on an ABI 377 sequencer (Applied Biosystems). Independent amplification and sequencing reactions were performed for each isolate with the p72-D and p-72U primers. Nucleotide sequences were aligned using DAPSA [17] and a homologous region of 415 nucleotides (nt) was identified for phylogenetic analysis. Unweighted pair-group arithmetic average (UPGMA), neighbor-joining (NJ), maximum parsimony (MP) and minimum evolution (ME) trees were constructed using MEGA 2 [21]. To determine the degree of character support at each node, data were resampled between 1000 and 10 000 times using the bootstrap method.

Results

Genomic amplification of diverse ASFV field strains

The *p72* gene primers detailed here successfully amplified over 100 ASFV isolates held in the Onderstepoort Veterinary Institute (OVI) virus bank (results not shown),

which are of diverse geographical, species and temporal origin. In addition, virus in five clinical specimens (Table 1) was also successfully detected and characterized, prior to isolation of virus from two of these samples ('Ghana' and 'NIG-6'), on porcine macrophage cultures. In some cases, no viable virus was present in the clinical specimens, but viral DNA was detected and characterised, eg. 'Togo/98', 'NIG-2' and 'IC/576'. Comparison of the nucleotide sequences derived from the clinical specimens and isolates made from these specimens following isolation and propagation in pig cell culture confirmed that no sequence changes were induced in this portion of the *p72* gene following cell culture.

Sequence variation

On sequencing 55 ASF viruses and comparing them with three published sequences it was found that 18 unique nucleotide sequence variants could be identified. Alignment and translation of these sequences revealed that 12 of the 138 amino acid sites (8.7%) were not completely conserved across the sequences compared (Fig. 1). The corresponding number of variable sites on nucleotide level was 59 of a total of 415 sites, or 14.2%. Of the 59 variable sites, 18 were singletons and the remaining 41 were parsimony informative. Most mutations (96%) occurred in the third base position arising primarily from transitions. Only 12 non-synonymous amino acid substitutions arose from the 59 point mutations (Fig. 1).

Suitability of the C-terminal region for molecular epidemiological studies

When comparing the 415 nt C-terminal region characterized in this study with the conserved and centrally located 236 nt region defined in a separate study [14] it was found that 10.9% and 6.3% of the nucleotide sites varied, respectively, for eight viruses common to both studies (results not shown). Amino acid variation was also higher for the C-terminal region than for the central region, indicating that the former target appears to be better suited to molecular clarification of viral relationships.

p72 gene relationships

Ten distinct viral groupings were identified by phylogenetic analysis (Fig. 2). Identical topologies were recovered using UPGMA, NJ, MP and ME, indicating that the data are robust and that the recovered phylogeny is unaffected by the underlying assumptions of the different methods used to infer the viral relationships. Five of the ten genotypes correspond to the same geographical groupings identified by RFLP analysis (Table 2). The major *p72* genotypes identified in this study were based on a minimum genetic distance of 0.96%, high levels of bootstrap support ($\geq$ 80%) and RFLP groups defined previously [2]. On this basis, any two viruses from distinct *p72* genotypes differ from each other at a minimum of four nucleotide sites across the genome region specified in this study. The validity of

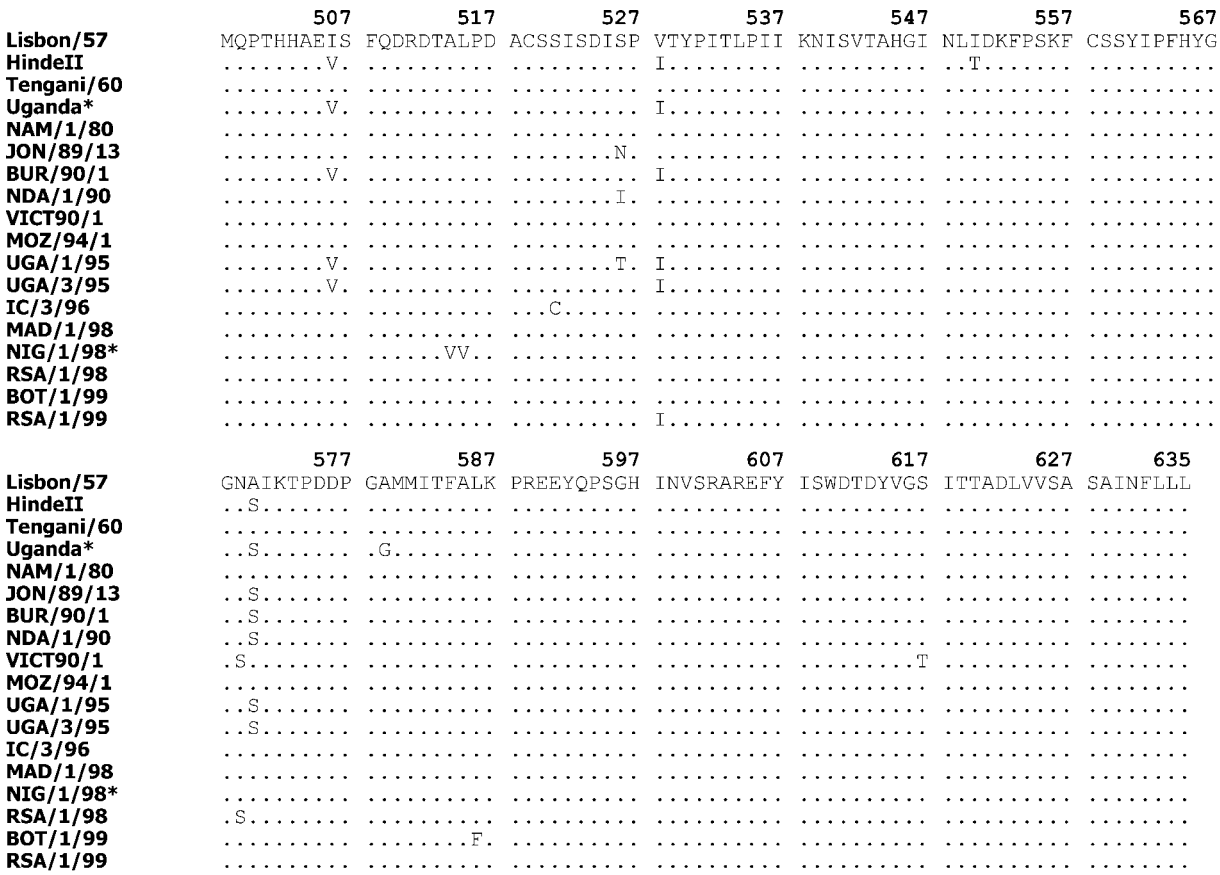

Fig. 1. Deduced amino acid sequence alignment of the C-terminal end of VP72 (positions 498–635). Sequences presented here are representative of the 18 unique nucleotide sequence types identified in this study. Dots indicate amino acids identical to that of the master sequence Lisbon/57

the *p72* genotypes defined in this study was strengthened by the incorporation of viruses identical to those used in the RFLP study, namely Lisbon/57, Lisbon/60, BA71, Malta/78 and Brazil/79. Where it was not possible to characterise exactly the same virus, viruses from the same locality were sourced and sequenced for comparative purposes (Table 2).

The largest and most homogeneous *p72* genotype, genotype I, comprised viruses from Europe, South America, the Caribbean and West Africa (Fig. 3), henceforth referred to as the ESAC-WA genotype. The combined RFLP and *p72* results indicate that a total of 24 countries have experienced ASF outbreaks caused by this genotype. Although no differences were observed at the nucleotide level within the C-terminal region of the *p72* gene of viruses from 20 of these countries, Nigeria, Côte d'Ivoire, Namibia and western Zimbabwe had virus variants distinct from that first introduced to Europe in 1957. Of the 12 African countries in which the ESAC-WA genotype is shown to be present, ten could have been possible

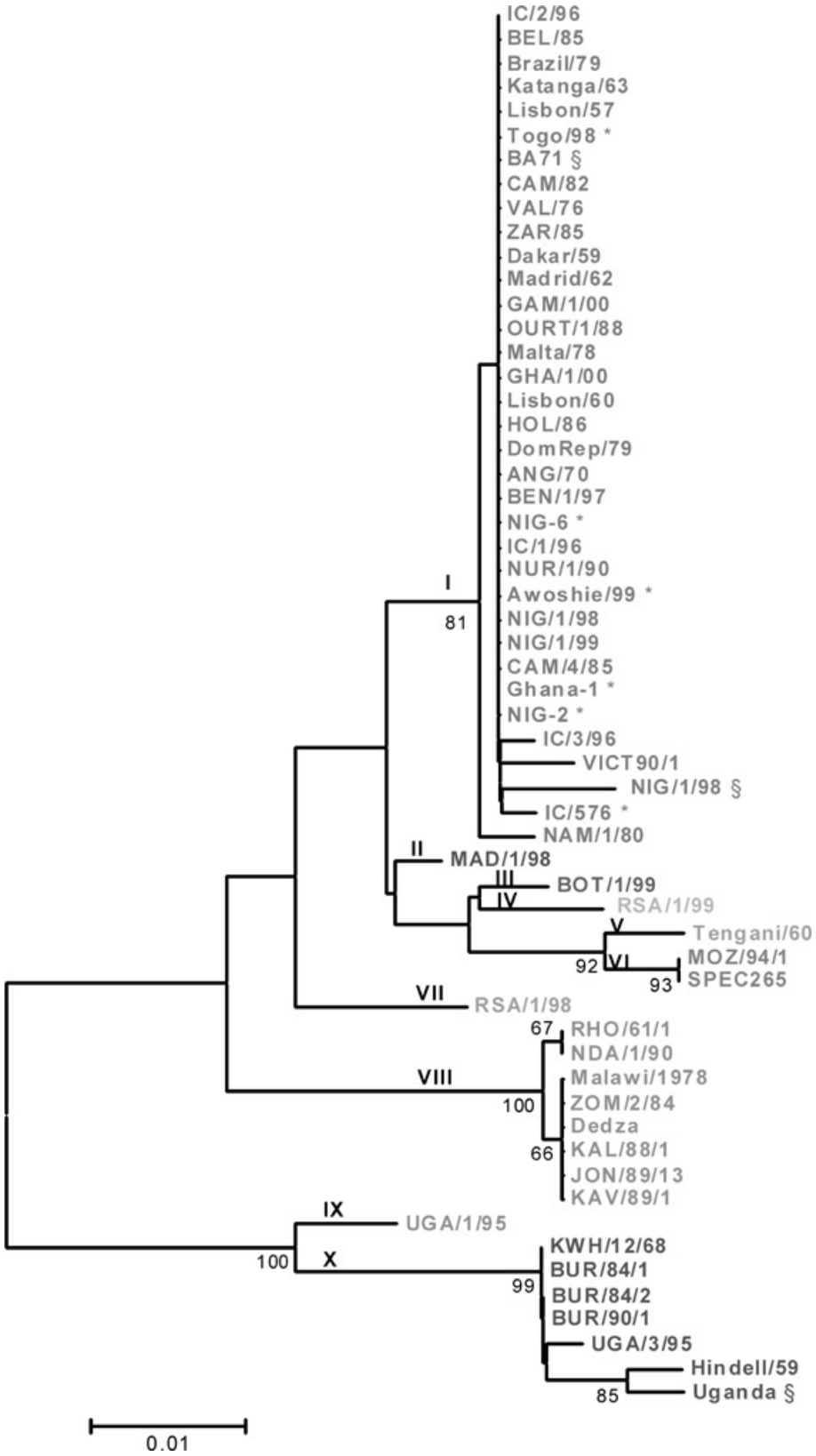
IC/2/96
BEL/85
Brazil/79
Katanga/63
Lisbon/57
Togo/98 *
BA71 §
CAM/82
VAL/76
ZAR/85
Dakar/59
Madrid/62
GAM/1/00
OURT/1/88
Malta/78
GHA/1/00
Lisbon/60
HOL/86
DomRep/79
ANG/70
BEN/1/97
NIG-6 *
IC/1/96
NUR/1/90
I
Awoshie/99 *
81
NIG/1/98
NIG/1/99
CAM/4/85
Ghana-1 *
NIG-2 *
IC/3/96
VICT90/1
NIG/1/98 §
IC/576 *
NAM/1/80
II
MAD/1/98
III
BOT/1/99
IV
RSA/1/99
V
Tengani/60
92
VI
MOZ/94/1
93
SPEC265
VII
RSA/1/98
67
RHO/61/1
NDA/1/90
VIII
Malawi/1978
100
ZOM/2/84
Dedza
66
KAL/88/1
JON/89/13
KAV/89/1
IX
UGA/1/95
100
X
KWH/12/68
BUR/84/1
99
BUR/84/2
BUR/90/1
UGA/3/95
Hindell/59
85
Uganda §
0.01

Table 2. Comparison of RFLP and partial *p72* nucleotide sequencing for identifying the major African swine fever virus groupings/genotypes

RFLP study (Blasco et al. 1989)		*p72* genotyping (this study)	
Genotype	Representative countries	Genotype	Representative countries
I	***Uganda***, 'Rhodesia'	IX	***Uganda***
II	***Portugal***, Madeira, ***Spain***, Italy, ***Malta***, ***Sardinia***, Cuba, ***Brazil***, Haiti, ***Angola***, ***DRC***	I	***Portugal***, ***Spain***, ***Sardinia***, ***Malta***, Holland, Belgium, ***Brazil***, Dominican Republic, ***Angola***, ***DRC***, Nigeria, Gambia, Ghana, Benin, Côte d'Ivoire, Senegal, Togo, Cameroon, western Zimbabwe, Namibia
III	***Malawi***	V	***Malawi***
IV	***Mozambique***	VI	***Mozambique***
V	***Tanzania***	X	***Tanzania***, Burundi, Uganda, Kenya
		II	Madagascar
		III	Botswana
		IV	South Africa
		VII	South Africa
		VIII	Zambia, Zimbabwe, Malawi

Countries common to the corresponding genotypes of the two studies are indicated in bold and italic, DRC: Democratic Republic of the Congo

sources of virus export as they have the virus variant identical to that introduced into Europe.

The remaining nine *p72* genotypes identified in this study originate from southern and East Africa (Fig. 3) where an ancient sylvatic cycle occurs. In these countries, greater genetic variation was observed, with multiple genotypes being recorded in South Africa, Malawi, Zimbabwe and Uganda. Seven of the nine genotypes were represented by viruses from a single country. In common with the ESAC-WA genotype in which 12 neighbouring countries share a common genotype, between three and four countries in the eastern and southern regions of the continent had genotypes VIII and X within their borders. The *p72* genotyping results further indicate that the 1996–2000 outbreaks occurring in West Africa were genetically related to each other, whilst those occurring in Madagascar, South Africa and Botswana in the same time period were caused by genetically distinct viruses and were therefore unrelated.

Fig. 2. Minimum evolution tree depicting *p72* gene relationships of African swine fever viruses from Africa, Europe, South America and the Caribbean. Ten genotypes (labelled *I–X*) corresponding to different geographical regions are indicated. § indicates sequences from published sources, whilst * denotes sequences obtained directly from clinical samples

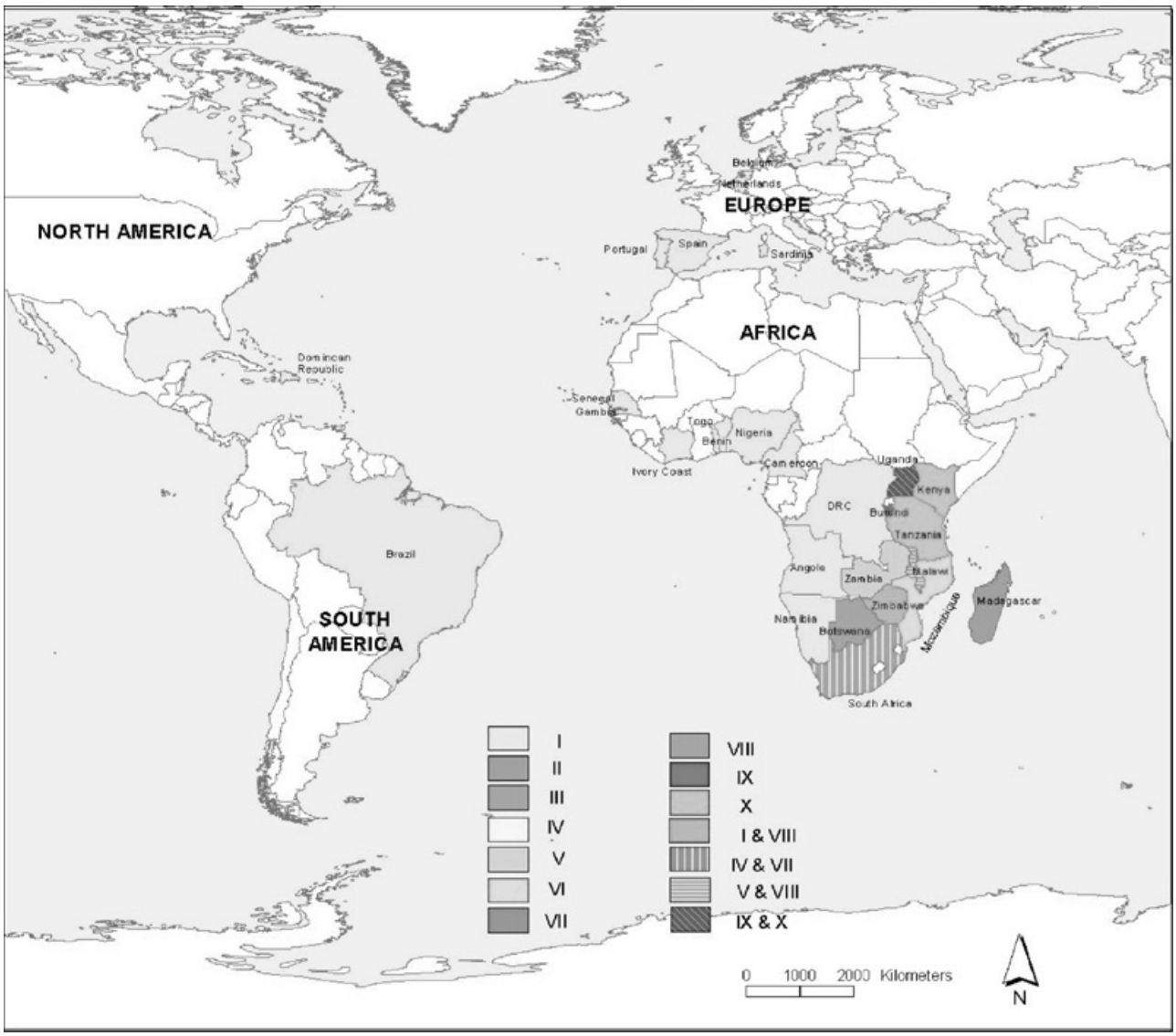

Fig. 3. Geographical distribution of African swine fever virus *p72* genotypes

Discussion

Partial nucleotide sequencing of the C-terminal end of the *p72* gene of 55 ASF viruses originating from Africa, Europe, South America and the Caribbean, permitted the identification of 10 major viral groupings. By comparing the sequencing results with those obtained previously by RFLP analysis [2], it was shown that the sequencing approach detailed in this study is capable of resolving ASF virus relationships to the same degree, but is superior in terms of cost and speed. This is primarily due to the ability to characterize viruses directly from clinical specimens thereby obviating the need for virus isolation. Identification of the major ASF viral grouping or genotype can be achieved within 48 hours of receipt of a clinical

specimen following the *p72* PCR-sequencing approach, as opposed to the 5–6 day period usually required to generate restriction enzyme profiles. This means that field epidemiologists rapidly obtain valuable information on the possible source of outbreaks, which helps in devising control strategies and ensuring that the introduction does not recur.

In common with previous RFLP studies, the results obtained here suggest that all European, Caribbean and South America field isolates are related [2]. This study showed that the most likely source of the infection for Europe was one of the African countries along the historical west-coast shipping route between Africa and Europe, stretching from Angola in the south to as far north as Sénégal. Due to the homogeneity in the *p72* sequences of the ESAC-WA genotype, it was not possible to discern the exact origin of the virus exported from Africa or to differentiate between outbreaks occurring as early as 1959 and as recently as 2000 in West Africa. Given the epidemiological importance of the disease in countries affected by this genotype it is imperative that alternative molecular methods be investigated that may provide higher levels of resolution, within a similar time period.

In countries where the sylvatic cycle plays a crucial part in the epidemiology of the disease, sufficiently high levels of variation were observed with which to distinguish between viruses. Genetic variation between ASFV strains from different *p72* genotypes was as high as 9.4% at the nucleotide sequence level, indicating that *p72* is a suitable candidate for molecular epidemiological studies. In the East and southern African regions, *p72* genotyping is a valuable means of distinguishing between viruses causing outbreaks that are geographically or temporally related. It was clearly shown that ASF outbreaks occurring in Uganda in 1995 were caused by two different viruses (UGA/1/95 and UGA/3/95) whilst the 1984 and 1990 outbreaks in Burundi were caused by identical viruses. Co-circulation of two ESAC-WA virus variants (eg. IC/1/96 and IC/3/96) within what was believed to have been a single epizootic was also confirmed for the 1996 Côte d'Ivoire outbreak. The PCR-sequencing method approach detailed here is therefore suitable for molecular epidemiological studies of ASF as it provides rapid and reliable identification of the major virus groups and is not reliant on virus isolation. The successful application of this technique has generated sequence data for ASF viruses of diverse geographic and temporal origin and represents a first step in the establishment of a regional reference sequencing database. In addition, it has provided some indication of disease complexity on the African continent where at least ten distinct genotypes occur.

Acknowledgements

We wish to express our sincere thanks to the Food and Agriculture Organization (FAO) and the International Atomic Energy Agency (IAEA) of the UN for financial support and permission to publish these results. We gratefully acknowledge B. Phologane and C. I. Boshoff for laboratory assistance, O. C. Phiri for providing valuable information on some of the viruses used in this study and an anonymous reviewer for helpful comments.

References

1. Bastos ADS (1998) Detection and characterization of foot-and-mouth disease virus in sub-Saharan Africa. Onderstepoort J Vet Res 65: 37–47
2. Blasco R, Aguero M, Almendral JM, Viñuela E (1989) Variable and constant regions in African swine fever virus DNA. Virology 168: 330–338
3. Boom R, Sol CJ, Salimans MMM, Jansen CL, Wertheim-van Dillen PME, van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28: 495–503
4. Borca MV, Irusta P, Carrillo C, Afonso CL, Burrage T, Rock DL (1994) African swine fever virus structural protein p72 contains a conformational neutralizing epitope. Virology 201: 413–418
5. Chou S (1992) Molecular epidemiology of envelope glycoprotein H of human cytomegalovirus. J Infect Dis 166: 604–607
6. Cistué C, Tabarés E (1992) Expression in vivo and in vitro of the major structural protein (VP73) of African swine fever virus. Arch Virol 123: 111–124
7. Dimmock NJ (1993) Neutralization of animal viruses. Curr Top Microbiol Immunol 183: 1–149
8. Dixon LK, Wilkinson PJ (1988) Genetic diversity of African swine fever isolates from soft ticks (*Ornithodoros moubata*) inhabiting warthog burrows in Zambia. J Gen Virol 69: 2981–2993
9. Dixon LK, Baylis SA, Vydeingum S, Twigg SRF, Hammond JM, Hingamp PM, Bristown C, Wilkinson PJ, Smith GL (1993) African swine fever virus genome content and variability. Arch Virol 7: 185–199
10. Dixon LK, Costa JV, Escribano JM, Rock DL, Viñuela E, Wilkinson PJ (2000) Family Asfarviridae, In: Van Regenmortel MHV, Fauquet CM, Bishop DHL, Carestens EB, Estes MK, Lemon SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle CR, Wickner RBFA, Murphy CM, Fauquet DHL, Bishop SA, Ghabrial AW, Jarvis GP, Martelli MD (eds) Virus taxonomy: Seventh Report of the International Committee on Taxonomy of Viruses. Summers Academic Press, San Diego, pp 159–165
11. El-Hicheri K, Gomez-Tejedor C, Penrith M-L, Davies G, Douati A, Edoukou GJ, Wojciechowski K (1998) L'épizootie de peste porcine africaine de 1996 en Côte d'Ivoire. Rev Sci Tech 17: 660–673
12. García-Barreno B, Sanz A, Nogal ML, Viñuela E, Enjuanes L (1986) Monoclonal antibodies of African swine fever virus: Antigenic differences among field virus isolates and viruses passaged in cell culture. J Virol 58: 385–392
13. Gómez-Puertas P, Rodríguez F, Oviedo JM, Ramiro-Ibáñez F, Ruiz-Gonzalvo F, Alonso C, Escribano JM (1996) Neutralizing antibodies to different proteins of African swine fever virus inhibit both virus attachment and internalization. J Virol 70: 5689–5694
14. Gonzague M, Roger F, Bastos A, Burger C, Randramparany T, Smondack S, Cruciere C (2001) Isolation of a non-haemadsorbing, non-cytopathic strain of African swine fever in Madagascar. Epidemiol Infect 126: 453–459
15. Grieser-Wilke I, Fritzemeier J, Koenen F, Vanderhallen H, Rutili D, De Mia GM, Romero L, Rosell R, Sanchez-Vizcaino JM, San Gabriel A (2000) Molecular epidemiology of a large classical swine fever epidemic in the European Union in 1997–1998. Vet Microbiol 77: 17–27
16. Hamel AL, Lin LL, Sachvie C, Grudeski E, Nayar GP (2000) PCR detection and characterization of type-2 porcine circovirus. Can J Vet Res 64: 44–52
17. Harley EH (2001) DAPSA. DNA and protein sequence analysis, version 4.91. Department of Chemical Pathology, University of Cape Town, South Africa

18. Ishikawa K, Tsutsui M, Tagushi K, Saitoh A, Muramatsu M (1996) Sequence variation of the gC gene among pseudorabies virus strains. Vet Microbiol 49: 267–272
19. Kleiboeker SB, Scoles GA (2001) Pathogenesis of African swine fever virus in *Ornithodoros* ticks. Anim Health Res Rev 2: 121–128
20. Kumar S, Tamura K, Nei M (1993) MEGA. Molecular Evolutionary Genetics Analysis, version 1.0. Pennsylvania State University, USA
21. Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2. Molecular Evolutionary Genetics Analysis software, version 2.0 Pennsylvania State University, USA
22. Le Gall G, Arnauld C, Boilletot E, Morisse JP, Rasschaert D (1998) Molecular epidemiology of rabbit haemorrhagic disease virus outbreaks in France during 1988 to 1995. J Gen Virol 79: 11–16
23. Leeson HS (1952) The recorded distribution of *Ornithodoros moubata* (Murray) (Acarina). Bull Entomol Res 43: 407–411
24. López-Otin C, Freije JMP, Parra F, Mendez E, Viñuela E (1990) Mapping and sequence of the gene coding for protein p72, the major capsid protein of African swine fever virus. Virology 175: 477–484
25. Manso Ribeiro J, Rosa Azevedo JA, Texeira MJO, Braco Forte MC, Rodrigues Ribeiro AM, Oliveira E, Noronha F, Grave Perreira C, Dias Viagrio J (1958) Peste porcine africaine provoquée par une souche different (Souche L) de la souche classique. Bull Off Int Epiz 50: 516–534
26. Mbayed VA, Lopez JL, Telenta PF, Palacios G, Badia I, Ferro A, Galoppo C, Campos R (1998) Distribution of hepatitis B virus genotypes in two different pediatric populations from Argentina. J Clin Microbiol 36: 3362–3365
27. Montgomery RE (1921) On a form of swine fever occurring in British East Africa (Kenya Colony). J Comp Pathol 34: 159–191
28. Odemuyiwa SO, Adebayo IA, Ammerlaan W, Ajuwape ATP, Alaka OO, Oyedele OI, Soyelu KO, Olaleye DO, Otesile EB, Muller CP (2000) An outbreak of African swine fever in Nigeria: Virus isolation and molecular characterization of the VP72 gene of a first isolate from West Africa. Virus Genes 20: 139–142
29. Rychlik W (1993) Selection of primers for polymerase chain reaction. In: White BA (ed) PCR protocols: Current methods and applications. Humana Press Inc, Totowa, NJ, pp 31–40
30. Sangare O, Bastos ADS, Marquardt O, Venter EH, Vosloo W, Thomson GR (2001) Molecular epidemiology of serotype O foot-and-mouth disease virus with emphasis on West and South Africa. Virus Genes 22: 345–351
31. Skinner JD, Smithers RHN (1990) The mammals of the southern African subregion, 2nd edn. University of Pretoria Press, Pretoria
32. Tabares E, Fernandez M, Salvador-Temprano E, Carnero ME, Sanchez-Botija C (1981) A reliable enzyme linked immunosorbent assay for African swine fever using the major structural protein as antigenic reagent. Arch Virol 70: 297–300
33. Takayama M, Takayama N, Inoue N, Kameoka T (1996) Application of a long PCR method of identification of variations in nucleotide sequences among varicella-zoster virus isolates. J Clin Microbiol 34: 2869–2874
34. Vigario JD, Terrinha AM, Moura Nunes JF (1974) Antigenic relationships among strain of African swine fever. Arch Ges Virusforsch 45: 272–277
35. Wesley RD, Tuthill AE (1984) Genome relatedness among African swine fever virus isolates by restriction endonuclease analysis. Prev Vet Med 2: 53–62
36. Yu M, Morrissy CJ, Westbury HA (1996) Strong sequence conservation of African swine fever virus p72 protein provides the molecular basis for its antigenic stability. Arch Virol 141: 1795–1802

37. Zhang YJ, Deng JH, Rabin C, Gao SJ (2000) Molecular epidemiology of an outbreak of fulminant hepatitis B. J Clin Microbiol 38: 2975–2981
38. Zsak L, Onisk DV, Afonso CL, Rock DL (1993) Virulent African swine fever virus isolates are neutralized by swine immune serum and by monoclonal antibodies recognizing a 72-kDa viral protein. Virology 196: 596–602

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