Comparison of genomes of African swine fever virus isolates from Cameroon, other African countries and Europe

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Key words

Swine - African swine fever virus - Genome - Cameroon - Europe - Africa.

Summary

A comparison of genomes of African swine fever virus (ASFV) isolates from Cameroon, other African countries and Europe by restriction enzyme analysis showed that those from Cameroon and Europe were very closely related except for the Lisbon/57 genome which had a longer left terminal region. Also, the genomes of isolates from Angola, Democratic Republic of Congo (DRC) and Senegal were similar to those from Cameroon and Europe, although the isolates from Angola and Katanga, in DRC, had a long left terminal region similar in size to the Lisbon/57. Isolates from East and Southeast Africa were genetically different from Cameroon and European isolates and also different from each other. Comparison of BamHI restriction enzyme site maps of the virus genomes of Cameroon and European isolates showed a variation in size of the BamHI-L fragment which maps to the middle of the genome, while a comparison between Cameroon, West and Southwest African isolates showed that there were variations in the central and both termini of the genome. Although small differences were observed between genomes of ASF virus isolates from Cameroon, Europe, West and Southwest Africa, they could be considered to belong to the same group based on the similar restriction enzyme site maps of their genomes. The results of this study also showed that the persistent outbreaks of ASF in domestic pig populations in Cameroon might have been due to the re-introduction of closely related virus isolates from infected areas outside the country.

■ INTRODUCTION

African swine fever (ASF) is an acute, highly contagious and often fatal disease of domestic pigs (17, 18). It is caused by a large cytoplasmically-located, icosaheral virus that contains a complex, linear double-stranded DNA genome (1, 7). Although morphologically similar to and originally classified with the Iridoviridae, more detailed analysis has revealed that ASF virus genome and replication resemble those of Poxviridae in many respects (1, 5), and the virus is at this time placed in a separate family (Asfaviridae) in which it is the only member.

It has been established by restriction enzyme analysis and mapping restriction enzyme sites on genomes, that ASF virus isolates obtained from different parts of Cameroon at different times were very similar to each other (4, 10). It was shown in previous studies that genomes of virus isolates obtained from outbreaks in domestic pigs in Europe were closely related to each other (6, 24, 25), and that some European isolates were closely related to those from outbreaks in domestic pigs in the Caribbean and the isolate that caused the ASF epizootic in Cameroon in 1982 (25). The origin of some outbreaks in Europe were traced back to the Iberian peninsula on the basis of epizootiological information and evidence from restriction enzyme analysis and restriction enzyme site maps of the genomes of the virus isolates (3, 26). In contrast, other African isolates of ASF virus collected from various hosts in different geographical locations over a long time span were genetically diverse (4, 9, 22, 23, 25). However, all the African isolates used by these workers were obtained from the eastern and southern parts of Africa. Outbreaks of ASF have been reported in other parts of the continent such as Benin, Senegal, Guinea Bissau, Nigeria and Côte d'Ivoire in the West, Central African Republic, Chad, Congo, Democratic Republic of Congo, Sao Tome, Angola and Namibia in the Southwest (2, 23, 27).

This study was therefore carried out to determine the genetic relationship between ASF virus isolates from Cameroon, other African countries and Europe by restriction enzyme site mapping of their genomes.

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■ MATERIALS AND METHODS

The ASF virus isolates used in the study are described in table I. Crossbred large White/Landrace pigs of 20-30 kg live weight were used to obtain infected red blood cells. Each pig was injected intramuscularly with 2 ml of original stock of each virus at a titer of 10^3-10^4 HAD_{50}/ml. When the animals developed a temperature of 40°C or more about 3-4 days postinoculation, infected blood was obtained from the jugular vein after anesthesia with Pentobarbitone-Na (Sagatal®, May and Baker). The blood was collected in one-liter plastic pots containing 100 ml of 7.5% EDTA to give a final concentration of 0.75% and the buffy coat was separated from red cells by centrifugation (5000 rpm for 30 min at 4°C). The extraction of ASF virus DNA from the infected pig red blood cells was then carried out as described by Wesley and Tuthill (25) on 25/50% rather than 25/60% sucrose gradients. In addition, virus preparations were treated with DNase (50 mg/ml) followed by 1% Tween 80 in order to remove contaminating DNA before loading onto sucrose gradients. Following lysis of virus with SDS and pronase, DNA was separated by phenol extraction. The restriction enzymes, BamHI, Asp718 and XbaI obtained from Boehringer Mannheim, were used to digest the virus DNA following manufacturer's recommendations. End-labeling of ^{32}PdATP, using klenow fragment of DNA polymerase, was performed using standard procedures (20). Electrophoresis of the digested and end-labeled products was performed on 0.6% agarose (sigma type II) gel in 40 mM Tris-acetate buffer (pH 8.0). The gels were dried and the bands visualized by autoradiography. DNA was transferred from wet agarose gels onto Hybond-N filters (Amersham) by the method of Southern (21) and was covalently attached to the filters by heating in an oven at 80°C for 2 h. Hybridization was carried out using plasmid clones of the Vero cell-adapted Spanish isolate of ASF virus DNA as probes (19), and the procedure was as described by Feinberg and Vogelstein (13). Preparation of Southern filters, radioactively labeled probes and hybridization to Southern filters were all procedures used in the construction of restriction enzyme site maps of the ASF virus isolates of the study.

Table I

African swine fever virus isolates from Europe, Africa and Cameroon used in restriction enzyme analysis

Isolate	Area	Year	Passage history*
Europe			
Lisbon/57	Portugal	1957	BC4' P2
Lisbon/60	Portugal	1960	PBM2
Malta/78	Malta	1978	P1 of original lymph node
Sardinia/78	Sardinia	1978	PBL4
Sardinia/82	Sardinia	1982	PBL3, P1
Italy/83	Cavaller/cone	1983	P1
Montijo/84	Portugal	1984	P2
Belgium/85	West Flanders	1985	Buffy coat 1, P1
Holland/86	Near Hague	1986	P1 of original spleen sample
Africa			
Dakar/59	Senegal	1959	Spleen
Katanga/67	DRC	1967	No information
Angola/70	Luanda	1970	P3
Angola/72	Luanda	1972	No information
Mozambique/79	Mozambique	1979	No information
Malawi/84	Mchinji	1984	P1
Namibia/86-1	Omaruru	1986	BC2
Tanzania/87	Mbeya	1987	P2
Zambia/87	Nayamuseta	1987	P1
Cameroon			
CAM/82	Guzang, Northwest Province	1982	P2
CAM/85	Mankon, Northwest Province	1985	P1
CAM/86	Limbe, Southwest Province	1986	P1
CAM/87	Bafoussam, West Province	1987	P1
CAM/88	Bandjoun, West Province	1988	P1

NB: The various ASF virus isolates were obtained from spleen, blood and serum of infected pigs
* Legend: P = pig passage; BC = buffy coat passage; PBM = pig bone marrow passage; PBL = peripheral blood leukocyte passage

■ RESULTS

Restriction enzyme analysis of the genomes of European and Cameroon ASF virus isolates

Three restriction enzymes, BamHI, Asp 718, and XbaI, were used to digest the DNA obtained from the various ASF virus isolates and the products analyzed by agarose gel electrophoresis. The results showed that the genomes of Cameroon ASF virus isolates were very similar to those of the European isolates used in the study (figures 1, 2 and 3), although one fragment (BamHI-L, Asp 718-I) varied in length between the isolates. CAM/86 ASF virus isolate also differed from other isolates in the mobility of the Asp 718-H and BamHI-0 fragment.

Comparison of BamHI restriction enzyme site maps of genomes of European and Cameroon isolates of ASFV

The order of BamHI restriction enzyme fragments of the genomes of virus isolates from Europe and Cameroon used in the study were observed to be the same except for the Lisbon/57 virus isolate (figure 4). The right terminal fragment differed in size in the genomes of CAM/86 and Lisbon/57 virus isolates, but it was observed to be the same size in the genomes of the other virus isolates (figure 4). The BamHI-L fragment located in the central region of the genome showed a variation in size in the genomes of the virus isolates (figure 4). The sizes of the BamHI-L fragments are given in table II. The Lisbon/57 virus isolate had an additional 6 Kb sequence in the left region about 10 Kb from the left terminus of the genome and varied in the size of two fragments close to the right terminus (figure 4).

Cameroon and European ASFV isolates could be separated into four groups based on the variation in size of BamHI-L fragments

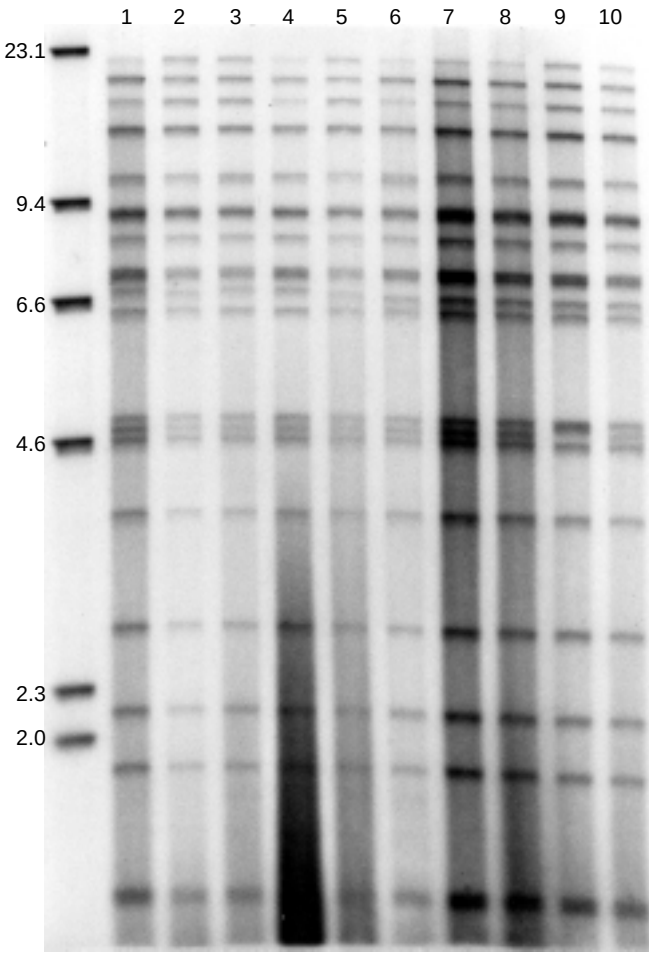

Figure 1: *Restriction enzyme analysis of genomes of Cameroon and European isolates of the African swine fever virus using BamHI. Lane 1: Malta/78; lane 2: Sardinia/78; lane 3: Sardinia/82; lane 4: Italy/83; lane 5: Montijo (Lisbon)/84; lane 6: Belgium/85; lane 7: CAM/82; lane 8: CAM/85; lane 9: CAM/86; lane 10: CAM/87. Sizes of molecular weight markers in Kbp are indicated.*

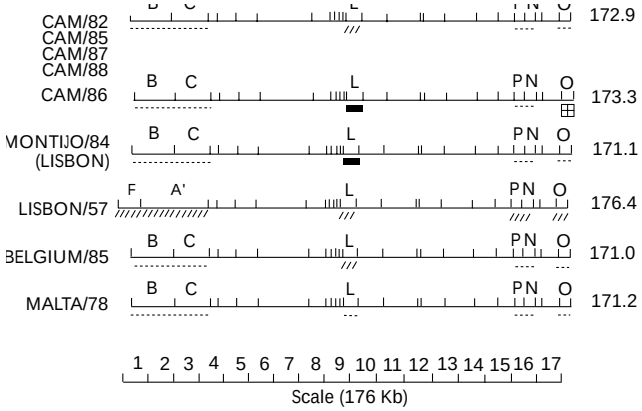

Figure 2: *Restriction enzyme analysis of genomes of Cameroon and European isolates of the African swine fever virus with the restriction enzyme Asp718. Lanes 1-10 contain ASFV isolates mentioned in the same order as in figure 1. Sizes of molecular weight markers in Kbp are indicated.*

even though their restriction enzyme fragment patterns were very similar (table III). Group 1 contained Belgium/85, CAM/82, CAM/85, CAM/87 and CAM/88 isolates. Group 2 included Montijo/84, Sardinia/78 and CAM/86, while Group 3 was made up of Malta/78, Sardinia/82 and Italy/83 isolates of ASFV. The genome of the CAM/86 virus isolate contained an additional variation in the right terminal fragment which differed from the other members of Group 2, so it could actually be placed in a subgroup (Group 2a) within Group 2.

Restriction enzyme analysis of the genomes of ASFV isolates from Cameroon and other African countries

Virus DNA prepared from isolates from Cameroon, Senegal, Democratic Republic of Congo (DRC), Angola, Namibia, Malawi, Zambia and Mozambique were digested with the restriction enzyme BamHI, and the digested products were analyzed by agarose gel electrophoresis. The restriction enzyme fragment patterns of genomes of virus isolates from the east and southeast of Africa (Tanzania, Malawi, Zambia and Mozambique) were very different from those of the genomes of Cameroon virus isolates and were also different from each other (figures 5 and 6).

The genome of the Dakar/59 virus isolate from Senegal in West Africa was very similar to the genomes of southwest African virus isolates and also similar to the genomes of viruses from Cameroon (figures 5 and 6). The Dakar/59 and southwest African isolates were genetically very different from the east and southeast African virus isolates (figures 5 and 6).

Comparison of BamHI restriction enzyme site maps of genomes of Cameroon and African isolates of ASFV

BamHI restriction enzyme site maps were constructed for the genomes of ASFV isolates from Senegal (Dakar/59), DRC (Katanga/67) and Angola (Angola/70, Angola/72). The order and sizes of restriction fragments were determined. The fragment order was the same for the genomes of ASFV isolates from Angola and DRC, but these two differed from that of the Dakar/59 isolate which showed a deletion of the BamHI-F fragment in the left terminus of the genome (figure 7). A comparison of restriction enzyme site maps showed that the fragment order of the genomes of Cameroon virus isolates differed from those of the two Angola viruses, Katanga/67 A and B, and Dakar/59 virus isolates (figure 7). The RK' plasmid clone which contains DNA from the

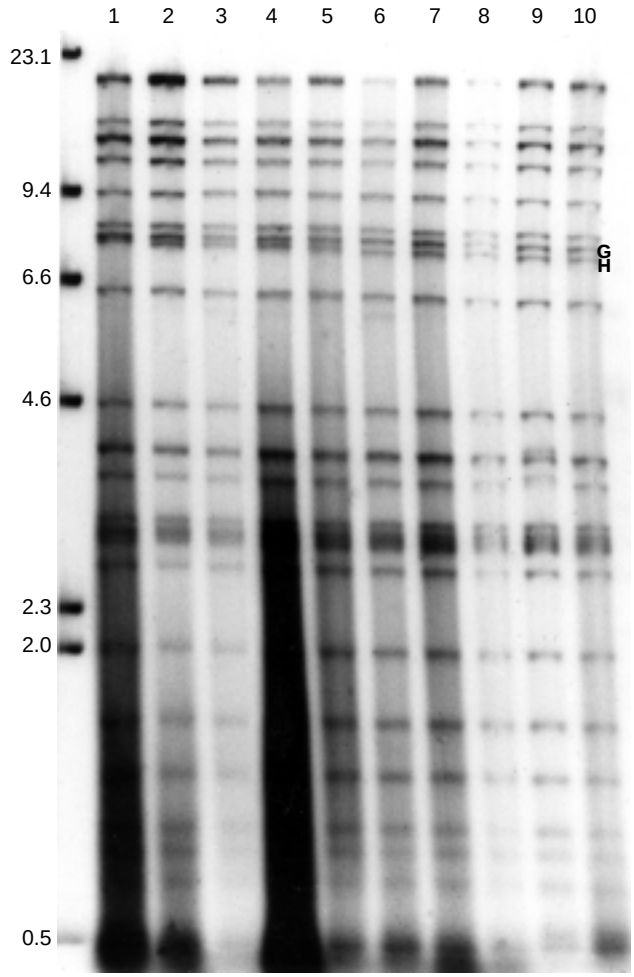

Figure 3: *Restriction enzyme analysis of genomes of Cameroon and European isolates of the African swine fever virus with the restriction enzyme XbaI. Lanes 1-10 consist of ASFV isolates mentioned in the same order as in figure 1. Sizes of molecular weight markers in Kbp are indicated.*

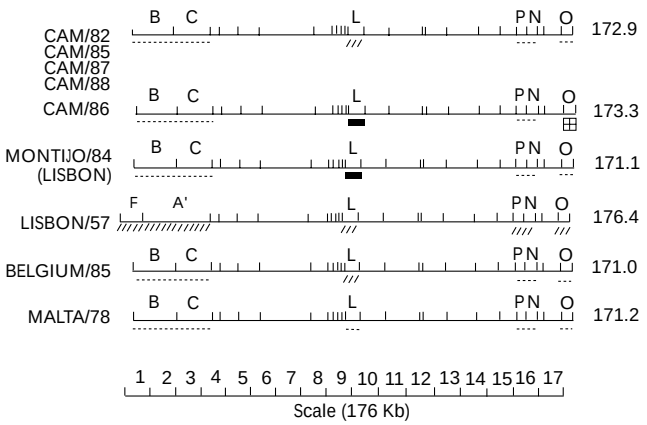

Figure 4: *Comparison of BamHI restriction enzyme site maps of genomes of Cameroon and European isolates of African swine fever virus. Variable regions in the different genomes are indicated. Restriction site maps of the genomes of virus isolates from Europe taken from Wilkinson* et al. *(unpublished results).*

Table II

Sizes of BamHI-L restriction enzyme fragments in genomes of isolates of African swine fever virus from Cameroon and Europe (in Kbp)

Belgium/85	6.4	Sardinia/78	6.5	Malta/78	6.6
CAM/82	6.4	Montijo/84	6.5	Sardinia/82	6.6
CAM/85	6.4	CAM/86	6.5	Italy/83	6.6
CAM/87	6.4				
CAM/88	6.4				

Table III

Distribution of isolates of African swine fever virus from Cameroon and Europe into groups based on the sizes of BamHI-L restriction enzyme fragments

Group 1	Group 2	Group 3
Belgium/85	Montijo/84	Malta/78
CAM/82	Sardinia/78	Sardinia/82
CAM/85		Italy/83
CAM/87	**Group 2a**	
CAM/88	CAM/86	

left terminus of the BA71V Spanish isolate (19) hybridized to two fragments in the genome of the Katanga/67 virus isolate, BamH1-A' and BamH1-F (figure 8). This resulted in the construction of two alternative restriction enzyme site maps for the genome of Katanga/67 ASFV isolate (figure 7). The sizes of the genomes were determined to be 167.4 Kbp for the Dakar/59, 178.2 Kbp for Katanga/67 and 177.3 Kbp for the virus isolates from Angola (Angola/70, Angola/72).

A comparison of restriction enzyme site maps showed that the fragment order of the genomes of Cameroon virus isolates differed from those of the two Angola viruses, Katanga/67 and Dakar/59 virus isolates (figure 7).

No fragment size variations were observed within the BamHI-L fragment, which maps to the central region of the genome for DNA of CAM/82, CAM/85, CAM/87, CAM/88, Angola/70, Angola/72 and Dakar/59 virus isolates. Virus isolates from Cameroon with the exception of CAM/86 had BamHI-L fragments, which were the same in size as those of the genomes of the two viruses from Angola and the one from Senegal (figure 7).

Variations were also observed in the Angola viruses, Katanga/67 and Dakar/59 isolates in the left terminal region, the region 10-35 Kb from the left terminus, the region about 10-20 Kb from the right terminus and the right terminus of the genome (figure 7). The right terminal fragment was the same in the genomes of Angola and Katanga/67 isolates, but differed from that of the Dakar/59 isolate (figure 7), and the difference was about 100 bp. The genomes of Katanga/67 and Dakar/59 virus isolates did not differ within the region about 10-20 Kb from the right terminus of the genome.

The virus genome of the Lil 20/1 isolate from Malawi (8) was very different from those from Cameroon and other isolates from the west and southwest of Africa by restriction enzyme site mapping, and it showed a marked difference in the order of the restriction enzyme fragments compared with the other virus isolates (8).

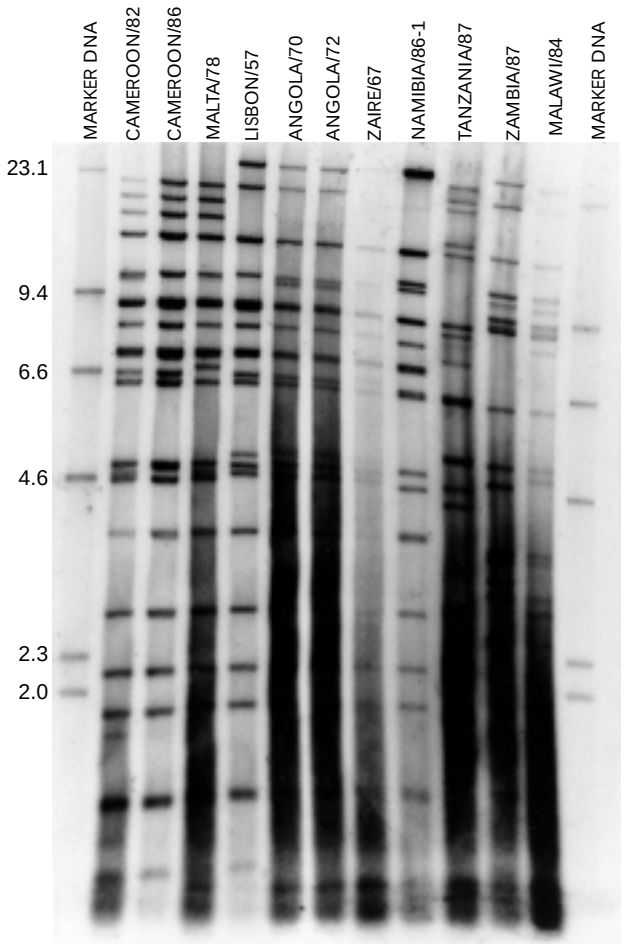

Figure 5: *Restriction enzyme analysis of genomes of African swine fever virus isolates from Europe, Cameroon and other African countries with the restriction enzyme BamHI. The figure shows positions and sizes of the molecular weight markers in Kbp.*

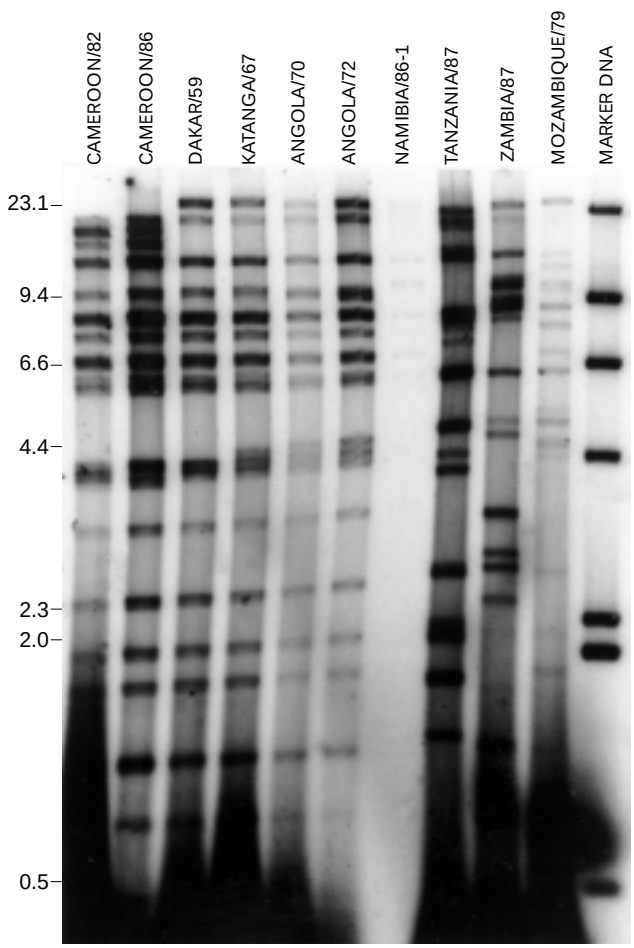

Figure 6: *Restriction enzyme analysis of genomes of African swine fever virus isolates from Cameroon and other African countries with the enzyme BamHI. Molecular weight markers are indicated.*

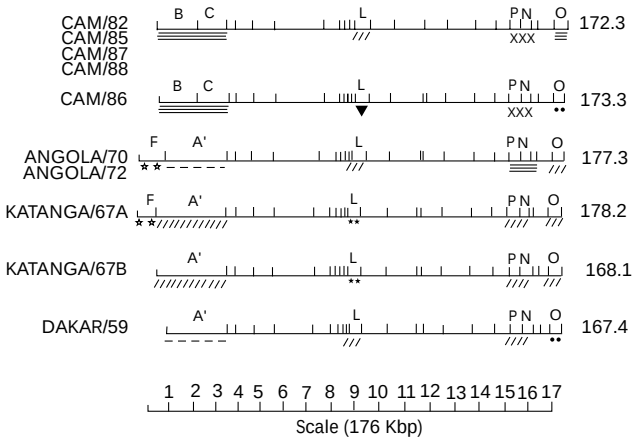

Figure 7: *BamHI restriction enzyme site maps of virus genomes of Cameroon, Angola, Katanga/67 and Dakar/59 isolates of the African swine fever virus. The variable regions in each genome are indicated.*

■ DISCUSSION

Restriction enzyme analysis of genomes of ASF virus isolates from Cameroon, other African countries and Europe showed that isolates from Cameroon and Europe were very closely related, except for the Lisbon/57 genome which had a longer left terminal region than the others. Also, the genomes of isolates from Angola, DRC, and Senegal were similar to those from Cameroon and Europe. Virus isolates from Angola and Katanga had a long left terminal left region similar in size to the Lisbon/57 isolates. Isolates from East and Southeast Africa were genetically different from Cameroon and European isolates and also different from each other.

Comparison of BamHI restriction enzyme site maps of the virus genomes of the Cameroon and European isolates showed a variation in length of the BamHI-L fragment, which maps to the middle of the genome (figure 4). By comparing the variation in the BamHI-L fragment size, Cameroon and European isolates were separated into four groups: Group 1 contained the Belgium/85, CAM/82, CAM/85, CAM/87 and CAM/88 isolates, while Group 2 had the Montijo/84 and Sardinia/78 isolates with a subgroup 2a containing the CAM/86 isolate only. The CAM/86 virus isolate was put into a subgroup (Group 2a) because of the additional difference in size of the right terminal fragment despite the equality in size of the BamHI-L fragment with the other members of Group 2. Group 3 was made up of the Malta/78, Sardinia/82 and Italy/83 virus isolates (tables II and III).

Other workers have reported similarity in the restriction enzyme fragment patterns of genomes of ASF virus isolates obtained from outbreaks in domestic pigs in Europe, the Caribbean and the 1982 ASF outbreak in Cameroon (24, 25), and the results presented in this study were similar to their findings about the genomes of isolates from Cameroon and Europe. Although the Lisbon/57 virus

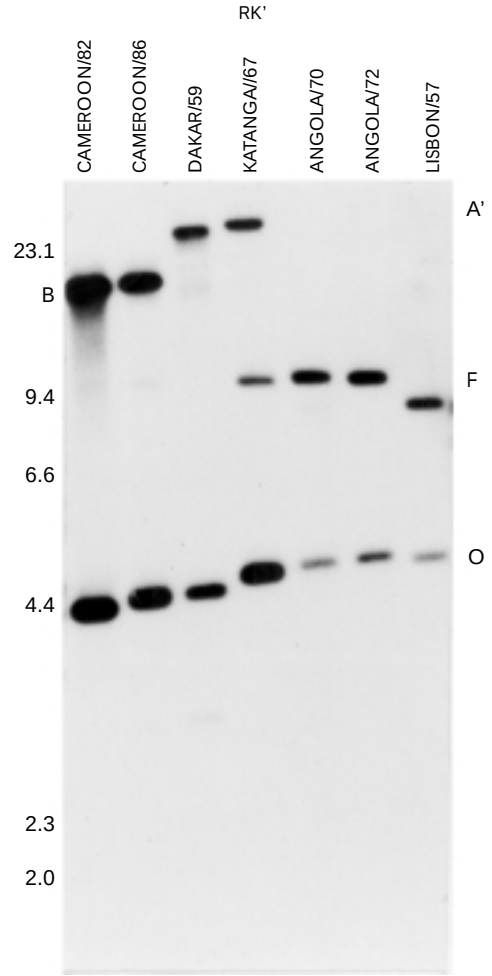

Figure 8: *Hybridization of the plasmid DNA clone RK' with BamHI digests of CAM/82, CAM/86, Dakar/59, Katanga/67, Angola/72 and Lisbon/57 isolates of the African swine fever virus. Molecular weight markers in Kbp are indicated.*

isolate showed a similar restriction enzyme fragment pattern to those of the genomes of the Cameroon isolates, differences in fragment sizes were observed at both ends of the genome (figure 4); it had an additional 6 Kb sequence at a position about 10 Kb from the left terminus (24).

A comparison of the genomes of ASF isolates from Cameroon with those of isolates from other African countries by restriction enzyme analysis showed that the virus isolates from Senegal (Dakar/59), DRC (Katanga/67), Angola (Angola/70, Angola/72) and Namibia (Namibia/86-1) were all similar to the genomes of Cameroon and European ASFV isolates (figure 5). The genomes of virus isolates from East and Southeast Africa were genetically very different from isolates from Cameroon, Europe, West and Southwest African countries, and were also very different from each other (figures 5 and 6). The RK' plasmid clone which contains DNA from the left terminus of the BA71V Spanish isolate (19) hybridized to two fragments in the genome of the Katanga/67 virus isolate, BamHI-A' and BamHI-F (figure 8). This resulted in the construction of two alternative restriction enzyme site maps for the genome of the Katanga/67 ASFV isolate (figure 7). Two possible explanations could be given for this phenomenon.

First, it is possible that the virus DNA was extracted from two virus populations in the Katanga/67 virus isolate which differed in the left terminal fragment of their genomes as observed by the cross-hybridization with the RK' plasmid clone (figure 8). In the Katanga/67B virus population, a deletion may have occurred near the end of the genome which was the same size as the BamHI-F fragment and also removed the Sal I site between fragments F and A'. The genome map would therefore have a left terminal fragment the same length at the A' fragment and no F fragment (figure 7). The Katanga/67A virus population would have the BamHI-F fragment as the left terminal fragment since the F fragment in the Katanga/67B DNA was of reduced intensity compared to those in other virus isolates (figure 8). This indicates that the F fragment is present in a reduced molar ratio supporting the hypothesis that two virus populations were present in the Katanga isolate. The other possible explanation is that the Katanga/67 virus probably has sequences in the BamHI-A' fragment which were either the 360 multigene family or tandem repeats closely related to those in the terminal inverted repetitions as observed in the BA71V strain of ASFV (14), and these hybridize with the RK' plasmid DNA clone. The Lisbon/57 and Angola viruses did not show such hybridization with the RK' plasmid clone, hence they do not have these sequences within the BamHI-A' fragment.

However, a comparison of restriction enzyme site maps of genomes of Cameroon and west and southwest African isolates showed that there were variations in the central region and in both termini of the genomes (figure 7). The order of restriction enzyme fragments was the same only for the virus isolates from Angola and the Katanga/67A, and the same for the Katanga/67B and Dakar/59 isolates (figure 7). The order of restriction enzyme fragments of the genome of the Lil20/1 isolate from Malawi (8) was very different from those of the other virus isolates. However, Dixon (8) observed that some BamHI fragments were conserved or were similar in length both in the Lil 20/1 and European isolates. These fragments were generally located in the right terminal 50 Kb, indicating that this region contained (by comparison) the most conserved sequences of the genome.

The genetic diversity of ASF virus isolates from the east and southeast of Africa has been reported by other workers (3, 4, 9, 22). It is also known that in these areas the virus is present in warthogs, soft ticks and domestic pigs (3, 4, 15, 16, 27). It is therefore possible that the genetic diversity observed in virus isolates from these areas may reflect the selective pressure imposed by the genetically different hosts in the area. On the other hand, the similarity of isolates from domestic pigs in Cameroon, Europe, the west and southwest of Africa indicated that all these domestic pig isolates may have originated from a single introduction of the ASF virus from a wildlife source into the domestic pig population. Once introduced into this population the virus was readily transmitted between pigs.

The results of this study have shown that the persistent outbreaks of ASF in domestic pig populations in Cameron may have been due to the reintroduction of closely related virus isolates from infected areas outside the country. The possible role of soft ticks and warthogs in the transmission and persistence of the disease in the three main pig-producing provinces of Cameroon had also been investigated (11) and there was no evidence of the presence of these reservoir hosts of ASFV in these provinces.

Acknowledgments

The authors would like to thank Dr. L. Dixon for providing the DNA clones of the lil 20/1 ASF virus isolate used in this study, Mr. G. Hutchings and Miss B. Lade for excellent technical assistance and Mr. I. Hughes for his help in the isolation compound. Our gratitude also goes to Dr. E. Vinuela who provided the

plasmid clones of the BA71-V ASF virus isolate used in this study. Finally, we thank the Institute of Agricultural Research for Development (IRAD), Cameroon, and the Overseas Development Administration (ODA), presently known as Department for International Development (DFID), for providing the funds.

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Reçu le 24.07.2000, accepté le 12.03.2001

Résumé

Ekue N.F., Wilkinson P.J. Comparaison des génomes d'isolats de virus de la peste porcine africaine provenant du Cameroun, d'autres pays africains et d'Europe

Une comparaison des cartes de restriction enzymatique des génomes d'isolats de virus de la peste porcine africaine (ppa) provenant du Cameroun, d'autres pays africains et d'Europe a montré que les souches du Cameroun et d'Europe étaient apparentées, à l'exception du virus Lisbon/57 dont la région terminale gauche du génome était plus longue. Par ailleurs, les génomes d'isolats d'Angola, de la République démocratique du Congo (Rdc) et du Sénégal étaient semblables à ceux du Cameroun et de l'Europe. Cependant, ceux d'Angola et du Katanga, en Rdc, avaient une longue région terminale gauche semblable à celle du Lisbon/57. Les souches d'Afrique de l'Est et du Sud-Est étaient génétiquement différentes de celles du Cameroun et d'Europe et différaient aussi entre elles. La comparaison des cartes de restriction obtenues par digestion de virus du Cameroun et d'Europe avec l'enzyme BamH1 a montré des différences de la taille du fragment BamHI-L qui est localisé au milieu du génome, alors qu'en comparant les isolats provenant du Cameroun, d'Afrique de l'Ouest et du Sud-Ouest les différences observées étaient situées au centre et aux extrémités du génome. Malgré ces petites variantes entre les génomes d'isolats de virus de la ppa provenant du Cameroun, d'Europe, d'Afrique de l'Ouest et du Sud-Ouest, on peut considérer qu'ils appartiennent au même groupe étant donné la grande ressemblance entre les cartes de restriction enzymatique de leurs génomes. Les résultats de cette étude ont également montré que les foyers récurrents de la ppa chez les populations porcines domestiques du Cameroun pouvaient être dus à la réintroduction de souches apparentées du virus à partir des zones infectées situées hors du pays.

Mots-clés : Porcin - Virus peste porcine africaine - Génome - Cameroun - Europe - Afrique.

Resumen

Ekue N.F., Wilkinson P.J. Comparación de genomas de aislamientos del virus de la Fiebre porcina africana en Camerún, otros países africanos y Europa

Una comparación de los genomas de aislamientos del virus de la Fiebre porcina africana (ASFV) en Camerún, otros países africanos y Europa, mediante un análisis de restricción enzimática, mostró que aquellos de Camerún y Europa se encuentran estrechamente relacionados, excepto el genoma Lisbon/57, el cual presentó una región terminal más larga. De igual manera, los genomas de aislamientos de Angola, la República Democrática del Congo (DRC) y Senegal fueron similares a los de Camerún y Europa, a pesar de que los aislamientos de Angola y Katanga, en DRC, presentaron una región terminal izquierda larga, similar en tamaño a la de Lisbon/57. Los aislamientos de Africa sudeste y este fueron genéticamente diferentes de los de Camerún y Europa, así como también diferentes entre ellos. La comparación de los mapas de sitios de restricción enzimática BamHI de los genomas virales de aislamientos de Camerún y Europa, mostraron una variación en tamaño del fragmento BamHI-L, localizado en el medio del genoma, mientras que la comparación entre aislamientos de Camerún, el oeste y sudoeste Africano mostraron que había variaciones en el centro y ambas terminales del genoma. A pesar de que se encontraron pequeñas diferencias entre los genomas de aislamientos de virus ASF de Camerún, Europa y Africa del oeste y sudoeste, estos podrían considerarse como pertenecientes al mismo grupo, basándose en la similitud de los mapas de los sitios de restricción enzimática. Los resultados de este estudio también mostraron que los brotes persistentes de ASF en las poblaciones suinas de Camerún pueden haber sido provocados por la re introducción de aislamientos de virus íntimamente relacionados desde áreas infectadas desde fuera del país.

Palabras clave: Cerdo - Virus de la peste porcina africana - Genoma - Camerún - Europa - Africa.