Analysis of the genomes of African swine fever virus isolates from Cameroon

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Key words

Swine - African swine fever virus - Genomes - Analysis - Cameroon.

Summary

Restriction enzyme site mapping of genomes of African swine fever virus isolates obtained from different areas and at different times in Cameroon showed that the isolates obtained in 1982 and 1985 were indistinguishable. The genome of the 1987 virus isolate (CAM/87) differed from those of the other two isolates only when analyzed with the enzyme Asp718; the variable fragment that was about 100 bp larger in the CAM/87 virus genome occurred within the central region of the genome (89-91 kbp). But the DNA of the 1986 virus isolate (CAM/86) consistently differed from all the other isolates from the analysis with four restriction enzymes in two fragments occurring within the last 10 kbp from the right terminus and another occurring within the central region of the genome (89-91 kbp region). The fragment length variation at the right terminus between the CAM/86 ASF virus genome and the others was 400 bp, being larger in the CAM/86 ASF virus genome; the variation in the variable fragment in the central region was 100 bp and was still larger in the CAM/86 virus genome. Although, small differences were observed between genomes of these ASF virus isolates from Cameroon, they could be considered to belong to the same group based on the very similar restriction enzyme site maps of their genomes.

■ INTRODUCTION

African swine fever (ASF) is an acute, highly contagious and often fatal disease of domestic pigs (12, 13). It is caused by a large cytoplasmically located, icosahedral virus that contains a complex, linear double stranded DNA genome (1, 4). Although morphologically similar to and originally classified with the *Iridoviridae*, more detailed analysis has revealed that ASF virus genome and replication resembles that of the *Poxviridae* in many respects (1, 2, 11), and is presently placed in a separate family in which it is the only known member.

Distinct immunological types of ASF virus isolates have not been demonstrated using conventional laboratory methods such as neutralization assays because of the lack of neutralizing antibody in immune sera and the virulence of most ASF isolates for domestic pigs. Even though both radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) (5, 25) are known to detect anti-ASF virus specific antibody, none of these assays have yet been used to distinguish between virus isolates. Panels of monoclonal antibodies which recognize a number of ASF structural proteins have been used to study the antigenic variation of virus isolates (10, 18, 19) but they are not sufficiently sensitive to pick up minor antigenic differences between strains.

Restriction enzyme fragment analysis and restriction enzyme site mapping have been used to study the variability of the ASF virus genome and have been useful for epidemiological investigations. The genomes of ASF isolates from domestic pigs in Europe have been shown to be closely related to each other by restriction enzyme analysis (24, 26) and European isolates are closely related to isolates from outbreaks in domestic pigs in the Caribbean and to the isolate that caused the 1982 epizootic in Cameroon (24). By using eight restriction enzymes, Wesley and Tuthill (24) were able to classify nine ASF field isolates into four major groups. Group 1 consisted of the East African isolates HindeI and Uganda4, Group

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2 had the Tengani isolate from Malawi, Group 3 contained the Spencer isolate from South Africa and Group 4 consisted of the Lisbon/60 Madrid/75, Dominican Republic/78, Haiti/78 and the Cameroon/82 isolates also formed a subgroup 4. In contrast, African isolates of ASF virus collected from various hosts and from different geographical locations are genetically distinct from each other (7, 23, 26) and are also different from the Caribbean, the Cameroon/82 and European virus isolates (26).

It is postulated that the present enzootic state of ASF in domestic pigs in Cameroon is either due to re-infection of herds with different viruses from external sources or to the continuous circulation and persistence of the virus which was introduced in 1982 being maintained in a cycle of infection between pig herds. The aim of this study was to characterize virus isolates obtained from different regions and at different times in order to determine their relationship by restriction enzyme analysis.

■ MATERIALS AND METHODS

Virus isolates

Five isolates of ASF virus obtained from different regions and at different times were used in the study (table I).

Isolation of virus DNA

DNA was extracted from ASF virus isolated from the red cell fraction of infected pig blood essentially as described by Wesley and Tuthill (26), except that virus was banded on 25%-50% rather than 25%-60% sucrose gradients. In addition, virus preparations were treated with DNAse (50 µg/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.

Enzyme reactions

Restriction endonucleases were obtained from Boehringer, Mannheim and were used according to manufacturer's recommendations. End-labeling of ^{32}PdATP, using Klenow fragment of DNA polymerase, was performed using standard procedures (15).

Agarose gel electrophoresis and Southern blotting

DNA fragments were electrophoresed on 0.6% agarose (Sigma type II) gel in 40 mM Tris-acetate buffer (pH 8.0). The gels were dried and the bands were 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 hours.

Preparation of radioactively labeled DNA probes and hybridization

Plasmid clones of the Vero cell-adapted Spanish isolate of ASF virus DNA (14) were used as probes for hybridization. DNA was radioactively labeled (9) and denatured probes were hybridized overnight at 68°C in 6 x SSC, 5 x Denhardt's solution, 100 µg/ml denatured salmon sperm DNA and 0.5% SDS. After washing in 0.1 x SSC and 0.5% SDS at 68°C, filters were exposed to X-ray films for an appropriate time. Prior to rehybridization, radioactive probes were removed from filters by washing at 45°C first with 0.4 M NaOH for 30 min and then with 0.2 M Tris, 0.1 x SSC and 0.1% SDS for 30 min.

■ RESULTS

Restriction enzyme analysis

Four restriction enzymes namely BamHI, EcoRI, Asp718 and Xbal were used to digest DNA obtained from each virus isolate and the products were analyzed by agarose gel electrophoresis (figure 1). Genomes of isolates obtained in 1982 and 1985 were indistinguishable with the four restriction enzymes used. The fragment below the Asp718-C fragment in CAM/82 and CAM/85 virus genomes was the product of partial digestion and disappeared on complete enzyme digestion. The fragment size of the digested products of CAM/82 ASF virus DNA with each enzyme was also determined by agarose gel electrophoresis with lambda DNA fragments of known sizes as markers (table II). The total size of the CAM/82 ASF genome was found to be 172.9, 171.0, 172.4 and 170.5 kbp with BamHI, Asp718, Xbal and EcoRI respectively, giving a mean size of 171.4 kbp (table II). The 1986 and 1987 isolates differed from the others at the Asp718-N restriction fragment, which was 100 bp longer in these isolates than in the 1982 and 1985 isolates. In addition, the CAM/86 ASFV genome was 400 bp longer than the genomes of the other isolates within the BamHI-0, Asp718-H and EcoRI-N fragments (figure 1).

Restriction enzyme site mapping of ASFV genomes

Since similarity in size on its own is not a sufficient criterion to make conclusions on fragment homology, restriction enzyme sites mapping was carried out by cross-hybridization of cloned ASF virus DNA with Southern blots of BamHI and Asp718 restriction digests of virus DNA from the Cameroon isolates. The sequential order of the restriction fragments was deduced by cross-hybridization with both plasmid clones of the Vero cell-adapted isolate of ASF virus DNA (Ley *et al.*, 1984) and bacteriophage lambda DNA clones containing ASF virus DNA inserts from the Malawi isolate (Lil 20/1) (6). The results of the hybridizations from which the Asp718 and BamHI maps of the genomes were deduced are summarized in tables III and IV.

Table I

Description of ASF virus isolates from Cameroon used in restriction enzyme analysis

Isolate	Collection area	Date	Year isolated	Passage History	
				Pig	Leucocyte culture
CAM/82	Guzang, N.W. Province	Sept. 1982	1982	2	-
CAM/85	Mankon, N.W. Province	July 1985	1985	1	-
CAM/86	Limbe, S.W. Province	Dec. 1986	1986	1	-
CAM/87	Bafoussam, West Province	July 1987	1987	1	-

Table II

Sizes of ASF virus (CAM/82) DNA restriction fragment in kbp

DNA	Restriction Enzymes			
Fragments	BamHI	Asp718	Xbal	EcoRI
A	20.0	39.8	21.7 (2)*	30.2
B	19.1	23.2	16.4	13.9
C	17.6	16.8	13.0 (2)	11.0
D	13.35 (2)	15.0	11.5	10.7
E	10.8	14.3	9.5	10.3
F	-	13.3	8.7	9.4
G	9.4	9.4	8.4	8.9
H	8.5	9.0	8.0	7.2
I	7.9	8.5	6.6	6.9
J	7.2	5.7	4.4	6.5
K	6.8	3.7	4.1	5.7 (2)
L	6.4	2.8	3.8	5.3
M	6.1	2.4	3.3	4.8 (2)
N	4.6	2.3	3.2	4.4
O	4.3	2.0	3.1	3.4
P	4.1	1.3 (2)	2.8	3.1
Q	3.5	-	2.0	3.0
R	2.5	-	1.4	2.9
S	2.0	-	1.1	2.0
T	1.8	-	0.9	1.9
U	1.2 (2)	-	0.9	1.7
V	0.8	-	0.7	1.5
W	0.5	-	0.6	1.4
X	-	-	0.6	1.2
Y	-	-	-	0.7
Z	-	-	-	0.5
No. of fragments	24	17	26	28
Total size	172.9	170.8	171.4	170.5

* (2) indicates the existence of two fragments in the corresponding band

Table III

Mapping of Asp718 fragments from the genomes of ASF virus isolates from Cameroon

Probe*	Restriction enzyme fragment hybridising to probe		
	CAM/82	CAM/86	CAM/87
RK	A, H	A, H	A, H
SB	A, B, P	A, B, P	A, B, P
RC	A, B, D	A, B, D	A, B, D
RB	D, I, N, P'	D, I, N, P'	D, I, N, P'
RG	G, M, O	G, M, O	G, M, O
RC'	F, G	F, G	F, G
SD	F, J, L	F, J, L	F, J, L
RD	C, J, L	C, J, L	C, J, L
SE	C, E	C, E	C, E
RD'	H, K	H, K	H, K
LMw9	D, I, P	D, I, P	D, I, P
LMw10	D, G, I, P	D, G, I, P	D, G, I, P
LMw22	E, K	E, K	E, K

Because of the identical electrophoretic mobility of the restriction enzyme fragments of ASF virus DNA from CAM/82 and CAM/85, only the CAM/82 genome was mapped

* The radioactive DNA probe used; bacteriophage lambda (LMw9, LMw10, LMw22) clones of the Malawi (Lil 20/1) isolate are described elsewhere (6); rest of the probes are plasmid clones of the BA71-V ASF virus isolate (14)

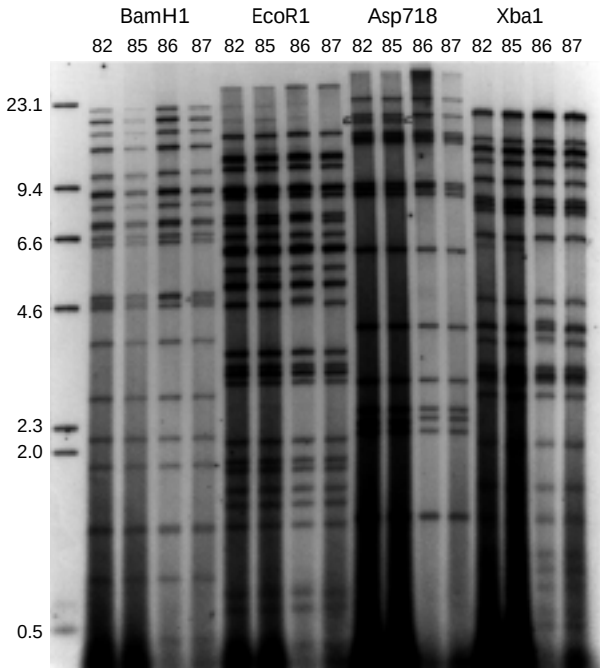

Figure 1: *Restriction enzyme analysis of ASF virus DNA from the CAM/82, CAM/85, CAM/86 and CAM/87 isolates using the enzymes BamH1, EcoRI, Asp718 and XbaI.*

Table IV

Mapping of BamHI fragments from the genomes of ASF virus isolates from Cameroon

Probe*	Restriction enzyme fragment hybridising to probe	
	CAM/82	CAM/86
RK	B, O	B, O
HC	B	B
RJ	B, C	B, C
RA/SC	C, Q, I	C, Q, I
SI'	I, G	I, G
SB	G, A	G, A
SF/KB	A, W	A, W
RC	W, K, T, S	W, K, T, S
SA/KD	K, T, S, U, V, L	K, T, S, U, V, L
RB	V, L, E	V, L, E
RG	E, D	E, D
RC'	D, U'	D, U'
LMw9	E, L, S, T, U, V	E, L, S, T, U, V
SD	D, U', H	D, U', H
RD	H, D'	H, D'
RC'	D, U'	D, U'
SI	D'	D'
RP	D'	D'
LMw16	D', H'	D', H'
RH	H'	H'
SE	H', M, P	H', M, P
SH	P, N, R	P, N, R
HJ	R, J	R, J
RD	J, O	J, O

The restriction enzyme site map of the CAM/82 DNA is the same as CAM/85 and CAM/87

* The radioactive DNA probe used; LMw9 and LMw16 are bacteriophage lambda clones of the Malawi (Lil 20/1) ASFV isolate; rest of the probes are plasmid clones of the BA71 ASF isolate; RK is a clone which contains sequences from the terminal inverted repeat and therefore hybridizes to both terminal fragments

By comparison of the Asp718 and BamHI restriction enzyme site maps of the four genomes it was observed that fragment order for the five isolates was the same (figure 2). The Asp718-N fragment mapped to the central region of the genome (89-91 kbp). The size difference in this variable fragment between the genomes of CAM/86, CAM/87 and the other isolates was about 100 bp, with the fragment being larger in the former two isolates. The BamHI-0 and Asp718-H fragments mapped to the right terminus of the genome (12 kb from the right end) (figure 2). The variation in length in this region between the CAM/86 ASF virus DNA and the other isolates was found to be 400 bp.

In order to identify more precisely the regions in which differences were observed between the four isolates by restriction enzyme analysis, the virus genomes were further digested with HindIII and NcoI which produce more fragments. The digested products were blotted on to a filter after electrophoresis on 0.6% agarose gel and hybridized with ASF virus DNA clones containing inserts from the central and the right end of the genome. The restriction enzyme, NcoI, produced four fragments within the last 12 kb of the right terminus of the genome while HindIII produced five fragments within the same region (figure 3). A comparison of the right terminus of the genomes after hybridizing NcoI and HindIII Southern blots to the appropriate ASF virus DNA clones revealed that there were two variable regions lying within 167.5 kb and 169.5-172 kb from the right terminus of the genome. The variation in length of the first variable region designated as VR1 was 100 bp between the CAM/86 ASF virus DNA and the others. The variation in the second variable fragment, designated VR2, was 300 bp, being larger in the CAM/86 ASF genome.

Southern blots prepared from virus DNA fragments of the CAM/82, CAM/86 and CAM/87 ASFV isolates after digesting with the restriction enzymes Asp718, NcoI and HindIII were used to hybridize with the plasmid DNA clone, RB, which hybridizes with DNA fragments which map to the central region of the genome. By this method a fragment difference was observed between the genomes of CAM/86 and CAM/82 within the 89-91 kbp region and was about 100 bp in size. This third variable region was designated VR3 (figure 4) and was the same size in the CAM/86 and CAM/87 virus.

■ DISCUSSION

Restriction enzyme analysis of genomes of ASF virus isolates obtained from different areas and at different times in Cameroon carried out with the restriction enzyme BamHI, EcoRI, Asp718 and Xbal, showed that the genomes of isolates obtained in 1982 and 1985 were indistinguishable (figure 1). The genome of the CAM/87 virus differed from those of the other two isolates when analyzed with Asp718 at the Asp718-N fragment (figure 2). But the DNA of the 1986 isolate (CAM/86) consistently differed from all others at the right terminal fragment (figure 1) when analyzed with the four restriction enzymes used in the study. Another difference in fragment mobility was observed in the CAM/86 virus DNA with the enzyme Asp718 at the Asp718-N fragment position. By comparing the Asp718 and BamHI restriction enzyme site maps of the CAM/82 and CAM/86 virus isolates, the fragment order in both isolates was observed to be the same (figure 3). Restriction enzyme site mapping of the genomes of the CAM/85 and CAM/87 virus isolates was not carried out because the restriction enzyme fragment patterns were indistinguishable from that of the CAM/82 isolate (figure 1).

The variable fragment Asp718-N in the CAM/86 ASFV DNA mapped to the central region of the genome while the variable fragments, Asp718-H and BamHI-0 in the same genome mapped to the right terminus (figure 2). Similar results showing the pre-

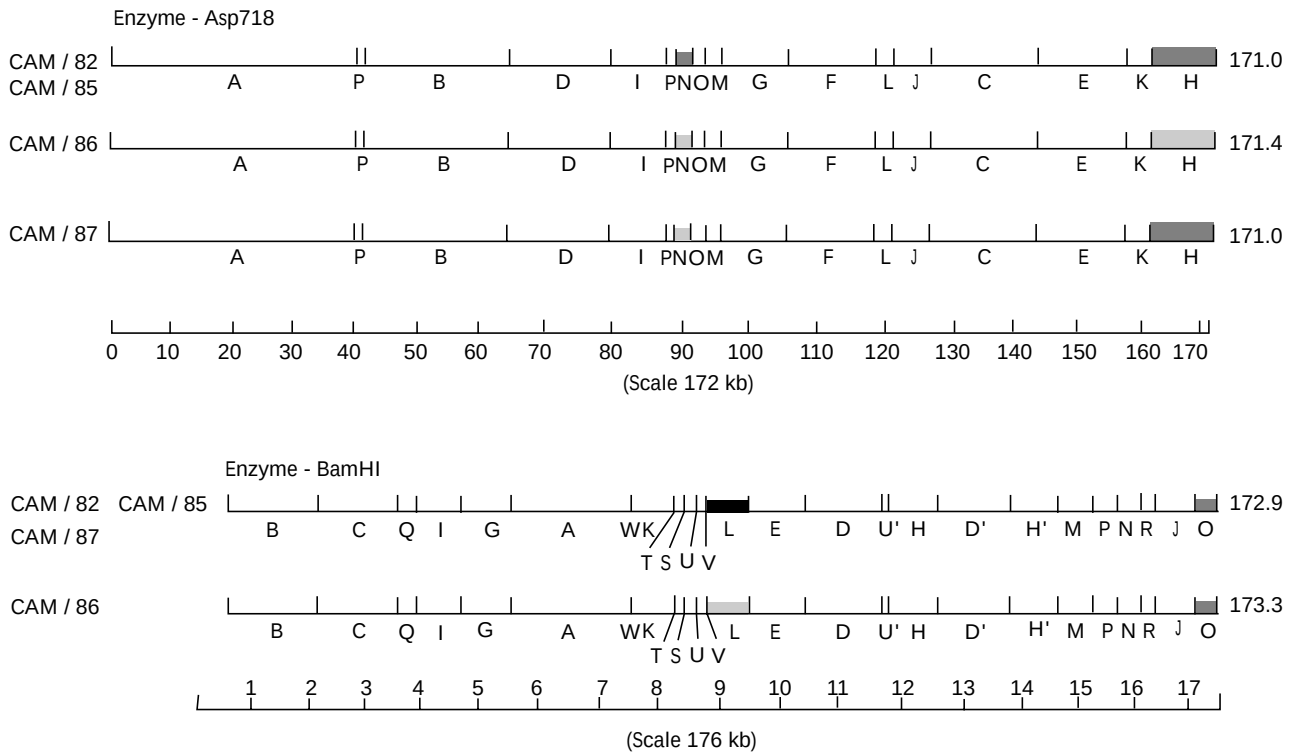

Figure 2: *Comparison of the BamHI and Asp718 restriction enzyme site maps of the genomes of isolates from four different areas in Cameroon. Similarities between genomes are indicated. Dashed lines indicate fragments that are exactly conserved in length between isolates. Black and dotted areas indicate the variable regions in the different genomes.*

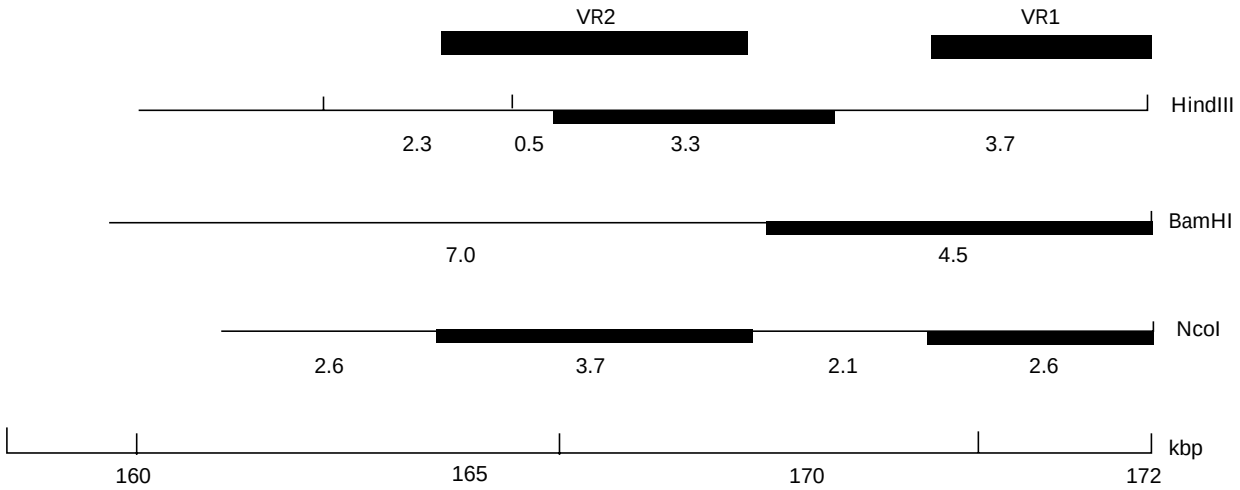

Figure 3: *Restriction enzyme site maps of DNA fragments from the right terminus of the CAM/86 ASFV genome showing the variable regions, VR1 and VR2. Thick lines indicate variable regions as observed with the corresponding restriction enzyme.*

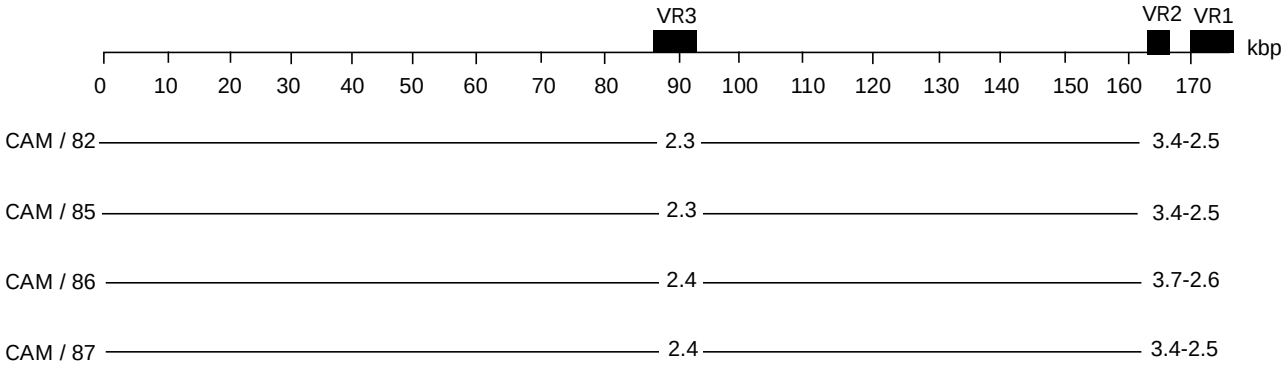

Figure 4: *Variable regions in the genomes of ASFV isolates from Cameroon.*

sence of a central and right terminal variable regions were obtained by Wilkinson *et al*. (unpublished results) on the restriction enzyme analysis and site mapping of DNA from some European ASF virus isolates. A variable central region of the ASFV genome was also observed by Sumption *et al*. (22) in virus isolates from pigs in Eastern Zambia and Malawi. The central variable region, VR3 (figure 4), in the present study corresponded to the VR4 region in the genome of the Malawi (Lil 20/1) and Zambian ASFV isolates (22). This variation in the central region was not observed by Blasco *et al.* (3) who reported a conserved 125 kbp central region of the genome from SalI restriction enzyme site mapping. Wilkinson *et al*. (unpublished results) used BamHI to map the genomes and Asp718 was used in addition to BamHI in this study. Both enzymes cut the virus genome more frequently in the central region than SalI that was used by Blasco *et al*. (3). Hence it was possible to identify small length variations in the middle of the genome.

Other restriction enzymes such as HindIII and NcoI which produce more than 30 fragments were used in mapping the variable regions observed in the Cameroon ASFV genome. A comparison of the right terminus of the genomes of CAM/82 and CAM/86 ASFV isolates showed the presence of two variable regions located 164-169.5-172 kbp from the end (figure 3), making a total of three variable regions in the Cameroon ASF virus DNA. The two variable regions, VR2 and VR1, corresponded to the VR7 and VR1 regions which are the last two variable regions to the right end in the genome of the Malawi (Lil 20/1) and the Zambian ASFV isolates (22). These observations differ from those of Blasco *et al*. (3) who described only one variable region in the right terminus of the ASFV genome that spans from 13-16 kb from the terminus.

The variation between genomes of various ASF isolates has generally resulted from differences in the length of restriction enzyme fragments in the variable regions located within 40 kb from the left terminus, 25 kb from the right terminus in the terminal inverted repetitions close to the ends (3, 7, 8, 22, 24) and in one fragment in the middle of the genome (18, 22). Terminal inverted repeat sequences consisting of multiple sets of tandem repeats of total length 2.4 kb have been characterized in the genome of ASF virus (20). Small variations in the length of fragments located within 2.4 kb from both termini probably resulted from variation in the number of tandem repeats within the terminal inverted repeats as has been observed in the poxvirus genomes (16). Such tandemly repeated sequences were also analyzed for two other variable

regions, one located 25 kb from the right terminus and the other in the middle of the genome (Dixon *et al.*, unpublished results). It is possible that variation in length of the terminal regions of the genome of ASFV isolates from Cameroon could also be explained by variation in the number of repeats present within the terminal inverted repetitions.

The ASFV isolates obtained in 1982, 1985 and 1987 came from areas within a radius of 200 km in the North West and West Provinces and the genomes of these isolates were indistinguishable by restriction enzyme analysis and mapping. The CAM/86 ASFV isolate whose genome showed small variations from others in the centre and the right terminal regions came from the coastal town of Limbe in the Southwest Province situated about 265 km from the West Province. Although small differences were observed between the genomes of these ASFV isolates from Cameroon, they could still be said to belong to the same group based on the very similar restriction enzyme site maps of their genomes. Therefore, we could possibly conclude that the enzootic state of ASF in the domestic pig population in Cameroon could be due to the continuous circulation of the virus of the 1982 epizootic that is being maintained in a cycle of infection between the herds or to the possible reintroduction into Cameroon of virus isolates which are very closely related to those present within the country.

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Retour au menu

Résumé

N.F. Ekue, P.J. Wilkinson Analyse du génome des isolats du virus de la peste porcine africaine au Cameroun

L'analyse de la carte de restriction enzymatique du génome du virus de la Ppa, provenant de différentes zones du Cameroun à différentes périodes, a été effectuée. Tout d'abord, elle a montré que les isolats obtenus en 1982 et 1985 n'étaient pas distinguables. Ensuite, le génome de l'isolat du virus de 1987 (CAM/87) différait des deux précédents uniquement lors de son analyse à l'aide de l'enzyme Asp718 ; le fragment variable, plus large d'environ 100 bp, se trouvait dans la région centrale (89-91 kbp) du génome. Enfin, l'Adn de l'isolat du virus de 1986 (CAM/86) différait de celui des trois autres lors de l'analyse avec quatre enzymes restrictives. La différence se trouvait dans deux fragments, l'un situé dans les derniers 10 kbp de l'extrémité droite du génome et l'autre dans sa région centrale (89-91 kbp). La variation de la longueur du fragment à l'extrémité droite était de 400 bp, celle du fragment de la région centrale de 100 bp ; les deux fragments étaient plus larges dans le génome du virus CAM/86. Bien que de légères différences aient été observées entre les génomes de ces différents isolats, ils peuvent être considérés comme appartenant à un même groupe car ils présentent une grande similitude dans la configuration de la carte de restriction enzymatique.

Mots-clés : Porcin - Virus de la peste porcine africaine - Génome - Analyse - Cameroun.

Resumen

N.F. Ekue, P.J. Wilkinson Análisis de los genomas de aislamientos de virus de la peste porcina africana en Camerún

Un mapa de los sitios de restricción enzimática de los genomas de aislamientos virales de la PPA, obtenido en diferentes áreas y momentos en Camerún, mostró que no fue posible distinguir los aislamientos obtenidos en 1982 de aquellos obtenidos en 1985. El genoma del aislamiento viral obtenido en 1987 (CAM/87), difirió de aquellos otros dos aislamientos únicamente cuando se analizó con la enzima Asp718; el fragmento variable, el cual fue aproximadamente 100 kbp mayor en el genoma del virus CAM/87, se presentó dentro la de la región central del genoma (89-91 bp). Sin embargo, el ADN aislado en el virus de 1986 (CAM/86) difirió consistentemente de todos los otros aislados del análisis con cuatro enzimas de restricción en dos fragmentos, que se dieron dentro de los últimos 10 kbp de la zona terminal derecha y otro dentro de la región central del genoma (región 89-91 kbp). La variación en la longitud del fragmento en la zona terminal derecha entre el genoma del virus CAM/86 ASF y los otros, fue de 400 bp, siendo mayor en el genoma del virus CAM/86 ASF; mientras que la variación en el fragmento variable de la región central fue de 100 bp y fue aún más grande en el genoma del virus CAM/86. A pesar de que se observaron pequeñas diferencias entre los genomas de los aislamientos de virus AFS de Camerún, éstos pueden considerarse como pertenecientes al mismo grupo, basado en la similitud de los mapas de los sitios de restricción enzimática de los genomas.

Palabras claves: Cerdo - Virus de la peste porcina africana - Genomas - Análisis - Camerún.