The pathogenicity of two groups of African swine fever virus isolates from Cameroon in domestic pigs

N.F. Ekue[1] P.J. Wilkinson[2]

Key words

Swine - Domestic animal - African swine fever virus - Pathogenicity - Cameroon.

Summary

A study carried out to determine the pathological relationships between two genetic groups of ASF virus isolates from Cameroon represented by the CAM/88 and CAM/86, demonstrated that both groups showed very similar clinical signs, gross lesions and virus titres in infected pigs. The clinical signs included a pyrexia first observed 3-6 days post inoculation, loss of appetite, listlessness, posterior incoordination, shivering and diarrhea. Other symptoms included dyspnea and lameness. The most frequently encountered lesions in all the pigs consisted of congestion of the lungs and hemorrhages in the kidneys and visceral lymph nodes. A pairwise comparison of the means of virus titres in similar organs of pigs infected with the two virus isolates showed no difference in virus titres ($p > 0.01$). The variation in the mean virus titres in organs was not affected by the virus isolates used ($p > 0.01$), implying no significant interaction between organs and the virus isolates. Finally, the overall mean virus titres in organs from pigs infected with the two virus isolates did not differ significantly ($p > 0.01$) but the virus titres varied significantly from one organ to the other ($p < 0.01$).

■ INTRODUCTION

In a previous study on the restriction enzyme analysis of genomes of ASFV isolates from Cameroon (8) it was shown that there are probably two genetically very closely related virus groups persisting within the pig population in the country; one group consists of the CAM/82, CAM/85 CAM/87 and CAM/88 ASFV isolates while the other includes the CAM/86 isolate only. The main differences between the two groups were variations in the size of one fragment occurring in the central region of the genome and two fragments in the right terminal region of the genome (8).

One of these isolates, CAM/82, has been previously used to infect pigs and the disease it produced was compared with that produced by other ASFV isolates from Malta, Dominican Republic and Brazil (9). It produced moderate lesions in infected pigs with ill-defined clinical signs. The mortality rate was low (33%) and there was clinical recovery of 7 of the infected pigs.

1. IRAD Nkolbisson, BP 2067, Yaounde, Cameroon, Tel/fax: +237 22 33 62; E-mail: iradpnva@iccnet.com

2. Institute of Animal Health, Pirbright Laboratory, Pirbright, Surrey GU24 ONF, United Kingdom

This study was carried out to record the responses of pigs infected with the CAM/88 and CAM/86 ASF virus isolates selected to represent the two groups circulating within the pig population in the country. The aim was to determine any pathological relationships between them to complement previous results from the genomic and antigenic studies.

■ MATERIALS AND METHODS

Crossbred Large White x Landrace pigs of 20-30 kg live weight were used in the study and they were divided into two groups of ten pigs each which were kept in two separate rooms in a large animal isolation compound. Before infecting the animals, pre-inoculation sera were collected. Each pig in group 1 was inoculated intramuscularly (i.m.) with $10^{2.6}$ HAD_{50} of the CAM/86 virus and each pig in group 2 was inoculated i.m. with $10^{2.1}$ HAD_{50} of the CAM/88 virus isolate.

The rectal temperature of each animal was recorded daily and clinical examinations also carried out each day. A blood sample for virus assay was collected in 5 ml glass vials containing EDTA

at a final concentration of 0.5% as anticoagulant from each animal every four days during the course of the infection. Post mortem examinations were carried out on every animal that died and the lesions observed were recorded. Samples of spleen, kidney, lung, tonsils, gastro-hepatic and mandibular lymph nodes were collected in sterile glass bottles for virus assay. The isolation, cultivation and assay of virus were carried out following standard procedures (12, 13, 21).

Any differences between the two isolates in terms of their virus titre in various organs from the infected pigs, within each group and between groups, were determined by the analysis of variance (ANOVA). The Mann-Whitney U-test was used to determine whether there was any difference in the duration of illness before death between the two groups of pigs using the statistical packages, "Minitab"[1] and "GLIM"[2].

■ RESULTS

Group 1 infected with the CAM/86 isolate of ASFV

Clinical signs

All ten pigs in the group (table I) became infected and the primary clinical sign was a fever of more than 40°C, first observed 3-6 days post inoculation (DPI). Other signs were evident from 7 DPI and those that were common to all the animals included loss of appetite, listlessness, incoordination in the hindquarters, shivering and diarrhea. At 11 DPI, five pigs developed lameness and had difficulty in breathing. Two pigs which became moribund at 14 DPI and 19 DPI were humanely killed with Sagatal® (May and Baker Ltd.). Most of pigs (8 of 10, 80%) died and this included the two pigs killed *in extremis*. The duration of clinical signs until death ranged from 6-16 days (median 10 days).

Gross lesions

A post mortem examination was performed on all dead pigs. The most frequently encountered lesions in all the pigs consisted of excess fluid in the thoracic and abdominal cavities, varying degrees of congestion of the lungs and hemorrhages in the kidneys and visceral lymph nodes which varied in the extent and severity in the different animals (table II). The gastro-hepatic lymph node was the most hemorrhage lymph node and resembled a blood clot in six out of eight pigs examined.

Hemorrhages were also frequently observed on the ventricles of the heart. Extensive bleeding into the subscapular region was observed in one pig probably as a result of a failure in the clotting mechanism, when the animal was bled two days before it died. There was one pig with slight splenomegaly and a dark colored spleen (table I). Hemorrhages in the gall bladder and small and large intestines were also seen in two pigs. The liver appeared normal in all the pigs.

Viremia and virus titres in the tissues

Virus was first detected in the blood at 3 DPI and maximum titres ranging between $10^{7.0}$-$10^{9.2}$ HAD_{50} /ml were recorded between 3-6 DPI in all the animals (table III). Infectious virus could not be detected in the blood of the two animals, which survived infection at 30 DPI and 45 DPI. The virus titres in the tissues collected from each animal during autopsy were also recorded. Highest titres were recorded in the spleen and ranged between $10^{4.3}HAD_{50}$ /gm in one pig which died 15 days after the onset of fever and $10^{7.3}$ HAD_{50} /gm in another which died 6 days after the onset of fever (table IV).

Group 2 infected with the CAM/88 isolate of ASFV

Clinical signs

All ten pigs in this group (table V) became infected and the primary clinical sign was a fever of more than 40°C. Other signs became evident in the animals at 7 DPI and they included loss of appetite, posterior incoordination, dyspnea and diarrhea (which was blood-stained in two pigs). Three pigs became moribund at 12 DPI and another at 24 DPI and all were killed humanely with Sagatal. Most of the pigs (9 out of 10, 90%) died and these included the four pigs killed *in extremis*. The duration of illness before death ranged between 7-12 days (median 8 days).

Gross lesions

A post mortem examination was performed on all nine pigs, which died or were killed during the course of the infection. The frequent lesions included varying degrees of lung congestion hemorrhages in the kidney (table V) and visceral lymph nodes with the gastro-hepatic lymph node resembling a blood clot (table VI). Excess fluid in the abdominal and thoracic cavities was observed in two pigs while hemorrhages in the stomach wall, gall bladder and small and large intestines were evident in two other pigs. Extensive bleeding into subscapular region was observed in one case

Table I

Distribution and severity of pathological changes in the organs of pigs dead following infection with the CAM/86 isolate of ASF virus

Pig No.	DPI	DPP	Lung	Heart		Kidney	Spleen		Liver
			Congestion*	Hemorrhage*	Fluid	Petechiae*	Enlarged	Dark*	
RM 15	10	6	4+	3+	3+	3+	1+	1+	0
RM 16	13	9	3+	0	0	3+	0	2+	0
RM 17	12	9	3+	3+	0	2+	0	0	0
RM 19**	19	16	3+	2+	1+	2+	0	0	0
RM 20	16	12	3+	2+	0	2+	0	0	0
RM 22	11	16	3+	3+	0	3+	0	0	0
RM 23	19	15	3+	2+	0	2+	0	0	0
RM 24**	14	11	3+	2+	1+	4+	0	0	0

DPI: Days post inoculation; DPP: Days post pyrexia; * Lesions are scored from 1+ to 4+; ** Animals killed *in extremis*

1. Minitab, U.S. Inc., 1985.

2. GLIM 3.77 Update 0, 1985, Royal Statistical Society, London.

Table II

The severity of hemorrhage in lymph nodes collected at necropsy from dead pigs following infection with the CAM/86 isolate of ASF virus

Pig No.	DPI	DPP	Degree of hemorrhage* in lymph nodes							
			Latera	Mandibular	Bronchial	Prescapular	Gastro	Mesenteric	Iliac	Prefemoral
RM 15	10	6	4+	4+	4+	4+	4+	4+	4+	4+
RM 16	13	9	0	1+	0	0	4+	4+	0	0
RM 17	12	9	0	0	1+	0	4+	0	0	0
RM 19**	19	16	1+	0	0	4+	1+	1+	1+	0
RM 20	16	12	1+	1+	0	0	4+	1+	1+	0
RM 22	11	6	4+	4+	4+	4+	4+	4+	4+	4+
RM 23	19	15	1+	1+	1+	0	4+	0	0	0
RM 24**	14	11	0	0	0	0	1+	0	0	0

* 0: no change; 1+: slight reddening; 3+: 50% of the lymph node hemorrhagic; 4+: represents a lymph node resembling a blood clot
** Animals killed *in extremis*

Table III

Viremia in pigs infected with the CAM/86 isolate of ASFV (log_{10} HAD_{50} /ml)

Pig No.	Days post inoculation											
	3	6	10	16	6	20	23	27	30	34	41	45
RM15	9.2	9.2	dead									
RM16	9.2	9.2	7.5	dead								
RM17	9.0	9.2	6.0	dead								
RM18	7.5	9.0	7.0	5.5	5.2	4.2	3.0	NVD	NVD	NVD	NVD	
RM19	7.0	8.5	7.0	dead								
RM20	7.2	9.0	6.5	dead								
RM21	9.2	9.0	7.2	6.2	4.2	5.5	5.5	5.0	4.2	3.0	NVD	
RM22	9.0	8.0	dead									
RM23	9.2	7.0	6.0	dead								
RM24	9.2	7.0	6.0	dead								
Mean	8.5	8.5	6.6	5.8	4.7	4.8	4.2					

NVD: no virus detected (< 1.0 HAD_{50} /ml)

Table IV

Virus titre in tissues of pigs collected after necropsy at various times after infection with the CAM/86 insolate of ASFV

Pig No.	DPI	DPP	Virus titre (log_{10} HAD_{50} /gm)					
			Spleen	Kidney	Lung	Tonsil	Gastro-hepatic L. N.	Mandibular L. N.
RM 15	10	6	7.25	4.75	7.25	6.75	2.25	7.75
RM 16	13	9	7.25	6.25	4.75	4.25	6.25	5.25
RM 17	12	9	6.25	4.25	4.25	6.25	4.25	7.75
RM 19**	19	16	5.25	3.0	4.0	2.25	3.25	2.25
RM 20	16	12	6.0	4.0	4.75	3.0	6.25	5.75
RM 22	11	6	7.0	6.25	6.0	5.25	6.0	6.25
RM 23	19	15	4.25	4.0	4.25	2.75	3.75	3.25
RM 24	14	11	6.75	5.75	5.25	2.75	3.25	2.75

** Animals killed *in extremis*

Table V

Distribution and severity of pathological changes in the organ of dead pigs following infection with the CAM/88 isolate of ASFV.

Pig No.	DPI	DPP	Lung	Heart		Kidney	Spleen		Liver
			Congestion*	Hemorrhage*	Fluid	Petechiae*	Enlarged	Dark*	
RM 25	12	8	3+	3+	0	3+	0	0	0
RM 26**	12	7	3+	3+	0	3+	0	0	0
RM 27**	12	8	0	2+	0	3+	0	infarct	0
RM 28	12	7	2+	2+	0	3+	0	0	0
RM 29	14	10	3+	3+	2+	2+	0	0	0
RM 30	13	9	3+	3+	0	2+	0	0	0
RM 31**	24	21	3+	3+	3+	1+	1+	0	0
RM 32	12	6	2+	3+	0	3+	1+	0	0
RM 33**	12	9	3+	2+	0	3+	0	0	fibrin.

* Lesions scored from 1+ to 4+
** Animals killed *in extremis*

Table VI

The severity of hemorrhage in the lymph nodes collected at necropsy from dead pigs following infection with the CAM/88 isolate of ASF virus

Pig No.	DPI	DPP	Degree of hemorrhage*							
			Lateral retropharyngeal	Mandibular	Bronchial	Prescapular	Gastro-hepatic	Mesenteric	Iliac	Prefemoral
RM 25	12	8	3+	3+	4+	1+	4+	1+	1+	1+
RM 26	12	7	1+	1+	1+	1+	4+	1+	1+	1+
RM 27	12	8	1+	1+	0	1+	4+	1+	1+	1+
RM 28	12	7	3+	3+	1+	1+	4+	1+	1+	1+
RM 29	14	10	0	0	0	0	4+	0	4+	0
RM 30	13	9	0	1+	0	0	4+	0	3+	0
RM 31	24	21	0	0	3+	0	1+	0	0	0
RM 32	12	6	1+	1+	1+	1+	3+	3+	1+	1+
RM 33	12	9	1+	1+	1+	1+	4+	3+	1+	1+

* 0: no change; 1+: slight reddening; 3+: 50% of the lymph node hemorrhagic; 4+: represents a lymph node resembling a blood clot

probably resulting from clotting failure when the pig was bled two days before it died. Two other pigs showed a slight enlargement of the spleen including infarcts in one of them. The liver appeared normal in all but one pig, which had fibrin around the liver (table V).

Viremia and virus titres in the tissues

Virus was first detected in the blood at 3 DPI in all the pigs and maximum titres ranging between $10^{7.2}$-$10^{9.2}$ HAD_{50} /ml were recorded between 3-6 DPI (table VII). Infectious virus could not be detected in the blood of one pig, which survived the infection at 34 DPI. Virus titres in tissues collected from each animal during autopsy were recorded. Highest titres were recorded in the lung and ranged between $10^{4.0}$ HAD_{50} /gm in one pig, which died at 24 DPI and $10^{7.0}$ HAD_{50} /gm in two others, which died at 12 and 14 DPI (table VIII).

There was no significant difference ($p > 0.01$) between the means of virus titres in different organs infected with the CAM/86 ASFV isolate. A difference in virus titre ($p < 0.01$) was observed between the tonsil, lung and gastro-hepatic lymph node from pigs infected with the CAM/88 virus isolate; the lung and the gastro-hepatic lymph node contained higher virus titres than the tonsil. However, no organ in this group responded very differently from the rest.

A pairwise comparison of means of virus titres in the same organs in pigs infected with the two virus isolates showed that there was no difference in virus titres in organs of pigs infected with the two virus isolates ($p > 0.01$). No difference was observed between the duration of illness produced in infected pigs by the two isolates ($p > 0.01$).

An analysis of variance was carried out with the titre values as response or dependent variables while the six organs in tables IV and VIII and the two virus isolates were used as the explanatory variables or factors. The effect of the possible organ/virus isolates interaction was also tested. The statistical package GLIM was used for the analysis.

The results showed that the variation in the mean virus titres in organs was not significantly affected by the virus isolates used to infect the pigs ($p > 0.01$), implying there was no significant interaction between the organs and the virus isolates. Also, the overall mean virus titres in organs from pigs infected with the two virus isolates did not differ significantly ($p > 0.01$). However, the mean virus titres varied significantly from one organ to the other ($p < 0.01$).

Table VII

Viremia in pigs infected with the CAM/88 isolate of ASFV ($log_{10}HAD_{50}$ /ml)

Pig No.	Days post inoculation										
	3	6	10	16	20	23	27	30	34	41	45
RM25	9.2	7.5	6.2	dead							
RM26	9.2	6.5	7.0	dead							
RM27	7.5	6.2	6.0	dead							
RM28	7.2	6.2	7.0	dead							
RM29	6.2	7.5	7.0	dead							
RM30	9.2	7.5	7.0	dead							
RM31	9.2	7.2	7.0	5.2	3.5	3.5	dead				
RM32	9.2	8.2	6.0	dead							
RM33	9.2	7.2	6.2	dead							
RM34	9.2	7.2	5.5	4.5	5.2	5.0	4.2	3.5	NVD	NVD	NVD
Mean	8.5	7.1	6.5	4.8	4.3	4.2					

NVD: no virus detected (< 1.0 HAD_{50} /ml)

Table VIII

Virus titre in tissues of pigs collected during necropsy at various times after infection with the CAM/88 isolate of ASFV

Pig No.	DPI	DPP	Virus titre (log_{10} HAD_{50} /gm)					
			Spleen	Kidney	Lung	Tonsil	Gastro-hepatic L. N.	Mandibular L. N.
RM 25	12	8	5.25	5.0	7.0	4.0	6.75	6.75
RM 25**	12	7	6.75	3.75	6.75	4.25	6.75	4.25
RM 27	12	8	6.75	4.25	6.25	3.25	6.75	4.0
RM 28	12	7	4.24	5.25	5.75	2.0	6.0	5.0
RM 29	14	10	6.0	6.0	7.0	5.0	6.0	5.0
RM 31**	24	21	3.25	1.0	4.0	1.0	2.25	1.0
RM 30	16	9	NT	NT	NT	NT	NT	NT
RM 32	16	9	NT	NT	NT	NT	NT	NT
RM 33	16	9	NT	NT	NT	NT	NT	NT

NT: not tested
** Animals killed *in extremis*

■ DISCUSSION

The results of this study have shown that the clinical signs, gross lesions and virus titres in tissues of pigs infected with the CAM/86 and CAM/88 ASFV isolates were very similar despite the differences observed between the genomes of these isolates.

All ten pigs in each group became infected and the first clinical sign was fever of 40°C or more that was observed at 3-6 DPI. Other signs that became evident after 7 DPI were essentially the same in both groups of pigs and included inappetence, posterior incoordination, diarrhea, lameness and prostration. Eight pigs (80%) infected with the CAM/86 virus isolate died and 9 pigs (90%) infected with the CAM/88 isolate also died of the infection. No significant difference ($p > 0.01$) was observed between the duration of illness before death produced by the two virus isolates. This period ranged between 6-16 days (median 10 days) in the pigs infected with the CAM/86 isolate and 7-21 days (median 8 days) in the pigs infected with the CAM/88 virus isolate.

The gross lesions in pigs from both groups were similar and they included excess fluid in the thoracic and abdominal cavities, varying degrees of hemorrhages in the kidney and visceral lymph nodes. The gastro-hepatic lymph nodes were the most severely affected in both groups of pigs and they had a deep red color resembling a blood clot. The spleens in both groups of pigs were generally normal with no change in color and consistency except for two cases in the pigs infected with the CAM/86 isolate and two others in the group infected with the CAM/88 isolate, which showed slight splenomegaly and a dark coloration.

Hemorrhages in the ventricle of the heart were commonly observed in both groups of pigs. Two pigs in each group showed hemorrhages in the gall bladder and small and large intestines. The liver appeared normal in all pigs in both groups except one infected with CAM/88 virus isolate, which had fibrin around the liver.

Maximum virus titres in blood were observed between 3-6 DPI for pigs infected with the CAM/86 virus isolate and ranged between $10^{8.5}$-$10^{9.2}$ HAD_{50} /ml (mean $10^{8.5}$ HAD_{50} /ml) and for pigs infected with the CAM/88 virus isolate, maximum viremia was observed between 3-6 DPI. The viremia became undetectable after 30 DPI and 45 DPI in two pigs, which survived infection with the CAM/86 virus isolate, and 34 DPI in another, which survived infection with the CAM/88 isolate. The maximum virus titre in the organs was observed in the spleen for the pigs infected with the

CAM/86 virus isolate and they ranged between $10^{4.3}$-$10^{7.3}$ HAD_{50} /gm and in the lung for the pigs infected with the CAM/88 isolate and they ranged from $10^{4.0}$ to $10^{7.0}$ HAD_{50} /ml.

A significant difference was observed ($p < 0.01$) in mean virus titres between organs infected with the CAM/88 virus isolate with the highest titres being recorded in the lungs and none was observed between the means of virus titres in different organs infected with the CAM/86 ASFV isolate ($p > 0.01$). A pairwise comparison of virus titres in similar organs of infected pigs from both groups showed that there was no significant difference ($p > 0.01$) between virus titres in organs of pigs infected with the CAM/86 and CAM/88 ASFV isolates. Another analysis of variance was carried out using virus titre in tissues of pigs with virus isolates as an additional explanatory factor. This showed that the virus titres varied significantly with the organs infected ($p < 0.01$) and not with the virus isolates used to infect the pigs ($p > 0.01$). It is, therefore, clear from these results that the CAM/86 and CAM/88 ASFV isolates are very similar in relation to the clinical signs and lesions they produce in infected pigs despite the variations observed in their genomes.

Extensive bleeding into the subscapular region was observed in two pigs from each of the infected groups of pigs which was presumed to be due to a failure in the clotting mechanism when the animals were bled two days before they died. The causes of these hemorrhagic lesions in ASF infections have been investigated by a number of different workers.

Edwards *et al*. (6, 7) and Edwards and Dodds (5) suggested that the coagulation defects observed in ASF infections were probably due to the formation of immune complexes early in the course of the infection which induced platelet aggregation and thrombopenia. Neser *et al*. (18) and Neser and Kotze (19) observed degenerative changes in thrombocytes and platelets in pigs infected with ASF and these included enlargement, irregular shapes and fragmentation. These changes were associated with prolonged bleeding and thrombin-clotting time, impaired clot retraction and platelet aggregation. Anderson (2) reported that the hemorrhagic diathesis in pigs infected with ASF was primarily a result of the endothelial damage by the virus, leading to release of prostaglandin E2, platelet aggregation and release of the pro-aggregatory prostaglandin, thromboxane A2. The PGE2 also promoted vascular dilation and permeability resulting in hemolytic anemia especially in acute ASF.

Ekue *et al*. (9) recorded similar lesions and clinical signs with those described in this study when they infected pigs with the CAM/82 virus isolate although the mortality rate was lower (33%). Another similar study was carried out by Wilkinson *et al*. (23) by infecting pigs with the Malta/78 virus isolate. The infected animals developed a fever (> 40°C) which lasted up to 14 days with maximum viremia ranging between 10^{7}-10^{9} HAD_{50} /ml which fell to undetectable levels between 35-55 days after the onset of fever. Mortality rates varied in their different experiments from 0-100% and clinical signs varied from only fever and anorexia to severe respiratory distress, prostration and death. At the onset of generalized infection high titres of virus ranging from $10^{5.3}$ to $10^{8.8}$ HAD_{50} per ml or per gram were found in all organs and tissues examined. The results obtained in the present study were also similar to those observed in pigs infected with ASFV isolates from Brazil (Brazil/78) and the Dominican Republic (DR/78) (15). These isolates from the Western hemisphere were characterized by mild clinical signs and lesions but with lower mortality rates (44% for the Brazil/78; 30% for the DR/78). Viremia fell to below detectable levels in pigs infected with the Brazilian isolate between 24-38 DPI and 18-30 DPI in pigs infected with the Dominican Republic virus isolate. It could therefore be concluded that the two virus isolates used in this study produced a similar response when inoculated into domestic pigs as those observed with the CAM/82, the European isolates and those from the Western hemisphere. Generally, when pigs recover from acute disease caused by these less virulent ASF virus isolates (including those used in this study) and become clinically normal, virus is not detected in the circulation for later than 8 weeks after infection, but persists in tissues for up to 6 months. Waste meat from such pigs may contain sufficient virus to cause oral infection for up to 12-15 weeks (24). The reason for this viral clearance from the blood stream is not known. However, virus titres in tissues also decrease and persist for up to 6 months in tonsils and lymph nodes (24).

The pathogenicity of the two ASFV isolates described in this study was very different from that described for most other African isolates which usually have a short incubation period, severe clinical signs and produce high mortality rates in domestic pigs (14, 16, 17). They also differed in the lesions and clinical signs they produced in infected pigs from those of the Lisbon/57 and Lisbon/60 ASFV isolates that are very much similar to virulent African isolates in the disease produced in domestic pigs. The infection produced by the Lisbon/60 isolate of ASFV in pigs was characterized by severe clinical signs and gross lesions which included reddening of the skin, bloody diarrhea, enlarged, dark and friable spleen, and severe hemorrhages of the visceral lymph nodes and 100% mortality (15).

The antigenic and genomic relationships between the Cameroon ASFV isolates had been investigated and the results from both studies showed that the ASFV isolates from Cameroon are very similar to each other and that they are also very similar to some European isolates (8). Other earlier studies based on restriction enzyme analysis have also shown that the CAM/82 isolate is genetically very similar to the Caribbean ASFV isolates (22). Therefore this study on the pathogenicity of the CAM/86 and CAM/88 virus isolates in pigs has also emphasized the similarities shared between the Cameroon isolates and also between the Cameroon, the European and Caribbean isolates of ASFV.

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REFERENCES

1. ALMENDRAL J.M., BLASCO R., LEY V., BELOSO A. TALAVERA A., VINUELA E., 1984. Restriction site map of African swine fever virus DNA. *Virol*., **133**: 258-270.

2. ANDERSON E.C., 1986. African swine fever: current concepts on its pathogenesis and immunology. *Rev. sci. tech. OIE*, **5**: 477-486.

3. BAROUDY B.M., VENKATESAN S., MOSS B., 1982. Incomplete base-paired flip-flop terminal loops link the two DNA strands of the vaccinia virus genome into one uninterrupted polynucleotide chain. *Cell*, **28**: 315-324.

4. CARRASCOSA J.L., CARAZO J.M., CARRASCOSA A.L., GARCIA N., SANTISTEBAN A., VINUELA E., 1984. General morphology and capsid fine structure of African swine fever virus particles. *Virol*., **132**: 160-172.

5. EDWARDS J.E., DODD W.J., 1985. Platelet and fibrinogen kinetics in healthy and African swine fever-affected swine. ($_{75}$Se) selenomethionine labelling study. *Am. J. Vet. Res*., **46**: 181-184.

6. EDWARDS J.E., DODDS W.J., SLAUSON D.O., 1984. Coagulation changes in African swine fever virus infection. *Am. J. Vet. Res.*, **45**: 2414-2420.

7. EDWARDS J.E., DODDS W.J., SLAUSON D.O., 1985. Megakaryocytic infection and thrombocytopaenia in African swine fever. *Vet. Pathol.*, **22**: 171-176.

8. EKUE N.F., 1989. The epidemiology of African swine fever in Cameroon. Ph.D. Thesis of the Dept of Microbiology, University of Surrey, Guildford, U.K., 231 p.

9. EKUE N.F., WILKINSON P.J., WARDLEY R.C., 1989. Infection of pigs with the Cameroon isolate (CAM/82) of African swine fever virus. *J. Comp. Pathol.*, **100**: 145-154.

10. GERSHLIN P., BERUS K.I., 1974. Characterisation and localisation of naturally occurring cross-links in vaccinia virus DNA. *J. Mol. Biol.*, **88**: 785-796.

11. HESS W.R., 1971. ASF. Virology monographs. In: Von Herausgegeben ed. 1: Handbook of virus research. New York, NY, USA, Springer Verlag, p. 1-33.

12. HESS W.R., 1981. African swine fever: A reassessment. *Adv. Vet. Sci.*, **25**: 39-69.

13. MALMQUIST W.A., HAY D., 1960. Haemadsorption and cytopathic effect produced by African swine fever in swine bone marrow and buffy coat cultures. *Am. J. Vet. Res.*, **21**: 104-108.

14. MAURER F.D., GREISMER R.A., JONES T.C., 1958. The pathology of African swine fever. A comparison with hog cholera. *Am. J. Vet. Res.*, **19**: 517-539.

15. MEBUS C.A., DARDIRI A.H., 1979. Additional characteristics of disease caused by African swine fever virus isolated from Brazil and the Dominican Republic. *Proc. of the US Anim. Health Assoc.*, **83**: 227-239.

16. MONTGOMERY R.E., 1921. On a form of swine fever occurring in British East Africa (Kenya colony). *J. Comp. Pathol.*, **34**: 159-191, 243-262.

17. MOULTON J.E., COGGINS L., 1968. Comparison of acute and chronic lesions in African swine fever. *Cornell Vet.*, **58**: 364-388.

18. NESER J.A., PHILIPS T., THOMPSON G.R., GAINARU M.D., COETZEE T., 1986. African swine fever. I. Morphological changes and virus replication in blood platelets of pigs infected with virulent haemadsorbing and non-haemadsorbing isolates. *Ondersterpoort J. Vet. Res.*, **53**: 133-144.

19. NESER J.A., KOTZE C., 1987. African swine fever. II. Functional disturbances of thrombocytes in pigs infected with virulent haemadsorbing and non-haemadsorbing virus isolates. *Ondersterpoort J. Vet. Res.*, **54**: 147-155.

20. PLOWRIGHT W., PARKER J., STAPLE R.F., 1968. The growth of virulent strain of ASF virus in domestic pigs. *J. Hyg.*, **66**: 117-134.

21. REED L.D., MUENCH H.H., 1938. A simple method of estimating fifty percent end points. *Am. J. Hyg.* **27**: 493-497.

22. WESLEY R.D., TUTHILL A.E., 1984. Genome relatedness among African swine fever field isolates by restriction endonuclease analysis. *Prev. Vet. Med.*, **2**: 53-62.

23. WILKINSON P.J., WARDLEY R.C., WILLIANS S.M., 1981. ASFV (Malta/78) in pigs. *J. Comp. Path.*, **91**: 277-284.

24. WILKINSON P.J., WARDLEY R.C., WILLIANS S.M., 1983. Studies in pigs infected with ASFV (Malta/78). In: African swine fever, Proc. CEO/FAO research Seminar, Sassari, Sardinia, Italia, 23-25 September, 1981. P.J. Wilkinson ed., p. 74-81.

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Resumé

Ekue N.F., Wilkinson P.J. Pouvoir pathogène de deux isolats de virus de la peste porcine africaine chez des porcs domestiques au Cameroun

Une étude a été réalisée pour mettre en évidence les liens pathologiques pouvant exister entre deux types de virus de la peste porcine africaine isolés au Cameroun et identifiés par les codes CAM/88 et CAM/86. Cette étude a permis de montrer que les deux isolats provoquaient les mêmes signes cliniques, des lésions caractéristiques et les mêmes taux d'infestation virale chez les porcs infectés. Les signes cliniques étaient les suivants : fièvre, observée dès 3 à 6 jours après l'inoculation, perte d'appétit, apathie, troubles de la coordination des membres postérieurs, frissons, diarrhée. Les autres symptômes observés étaient une dyspnée et des boiteries. Les lésions les plus courantes chez tous les animaux étaient une congestion pulmonaire, une hémorragie rénale et la présence de ganglions lymphatiques dans les viscères. La comparaison des moyennes des taux d'infestation virale dans les organes des porcs infectés par les deux isolats n'a révélé aucune différence significative ($p > 0.01$). La variation de la moyenne du taux d'infestation virale dans les organes ne dépendait pas du type de virus, il n'y avait donc pas d'interaction significative entre les organes et les isolats ($p > 0.01$). Enfin, la moyenne générale du taux d'infestation virale dans les organes des animaux n'était pas significativement différente ($p > 0.01$) selon le type de virus, mais ces taux variaient significativement d'un organe à l'autre ($p < 0.01$).

Mots-clés : Porcin - Animal domestique - Virus de la peste porcine africaine - Pouvoir pathogène - Cameroun.

Resumen

Ekue N.F., Wilkinson P.J. La patogenicidad de dos grupos de virus de la peste porcina africana aislados en cerdos domésticos en Camerún

Un estudio llevado a cabo con el fin de determinar las relaciones patológicas entre dos grupos genéticos de virus de la PPA, aislados en Camerún, representados por CAM/88 y CAM/86, demostró que ambos grupos muestran signos clínicos, lesiones generales y títulos virales similares en los cerdos infectados. Los signos clínicos incluyen pirexia inicial, observada 3 a 6 días post-inoculación, pérdida del apetito, apatía, incoordinación del tren posterior, temblores y diarrea. Otros síntomas incluyen disnea y cojeras. Las lesiones más frecuentemente observadas en todos los cerdos fueron congestión pulmonar y hemorragia en los riñones y linfonodos viscerales. Una comparación de los promedios de los títulos de virus, en órganos similares de cerdos infectados con los dos aislamientos virales, no mostró diferencia en los títulos virales ($p > 0.01$), lo cual implica que no existe una interacción significativa entre los aislamientos de virus y los órganos. Finalmente, el promedio general de los títulos virales varió significativamente de un órgano a otro ($p < 0.001$).

Palabras clave: Cerdo - Animales domésticos - Virus de la peste porcina africana - Patogenicidad - Camerún.