African Swine Fever: pathogenicity

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

Recording of 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 Cameroon.

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.

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 102.6 HAD50 of the CAM/86 virus and each pig in group 2 was inoculated i.m. with 102.1 HAD50 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.

Viremnia and virus titres in the tissues

Virus was first detected in the blood at 3 DPI and maximum titres ranging between 107.0-109.2 HAD50 /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 104.3HAD50 /gm in one pig which died 15 days after the onset of fever and 107.3 HAD50 /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 gastrohepatic 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 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 107.2-109.2 HAD50 /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 104.0 HAD50 /gm in one pig, which died at 24 DPI and 107.0 HAD50 /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 \((p < 0.01)\).

**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 108.5-109.2 HAD50 /ml \(\text{(mean 108.5 HAD50 /ml)}\) 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 104.3-107.3 HAD50 /gm and in the lung for the pigs infected with the CAM/88 isolate and they ranged from 104.0 to 107.0 HAD50 /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 107-109 HAD50 /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 105.3 to 108.8 HAD50 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.

References