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An investigation into natural resistance to African swine fever in domestic pigs from an endemic area in southern Africa

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Summary

A population of domestic pigs in northern Mozambique with increased resistance to the pathogenic effects of African swine fever (ASF) virus was identified by the high prevalence of circulating antibodies to ASF virus. An attempt was made to establish whether the resistance in this population was heritable. Some of these pigs were acquired and transported to a quarantine facility and allowed to breed naturally. Offspring of the resistant pigs were transferred to a high security facility where they were challenged with two ASF viruses, one of which was isolated from one of the Mozambican pigs and the other a genetically closely-related virus from Madagascar. All but one of the 105 offspring challenged developed acute ASF and died. It therefore appears that the resistance demonstrated by these pigs is not inherited by their offspring, or could not be expressed under the conditions of the experiment. The question remains therefore as to the mechanism whereby pigs in the population from which the experimental pigs were derived co-existed with virulent ASF viruses.

Keywords

African swine fever – Domestic pigs – Genetic resistance – Mozambique.

Introduction

African swine fever (ASF) is a viral haemorrhagic fever of domestic pigs that results in up to 100% mortality (18). The disease is the most serious constraint for pig production in most sub-Saharan African countries (1, 2, 4, 5, 6, 7, 13, 14, 15). Lack of an effective vaccine renders control of the disease difficult. The virus was historically maintained in a sylvatic cycle that involves warthogs (*Phacochoerus* spp.) and probably to a lesser extent other wild suids in Africa and argasid ticks of the *Ornithodoros moubata* complex (10, 18, 23). Where the sylvatic cycle of infection persists, the disease can be controlled by preventing contact between domestic pigs and wild suids and their ticks (18). However, in parts of Africa the disease has become endemic in populations of domestic pigs that show increased resistance to the pathogenic effects of the disease, with a high proportion of healthy pigs having

antibodies to ASF virus. This was first reported in the Mchinje District of Malawi by Haresnape *et al*. (8, 9). Evidence of increased resistance to ASF has also been reported in Angola (12, 13) and in eastern Zambia (27).

In 1998, a serological survey of village pigs in the Angonia District of the Tete Province of Mozambique, close to the Malawi border, indicated that almost 40% of 54 healthy pigs sampled had antibodies to ASF virus (S. Swanepoel & G. Thomson, unpublished report, 1998).

In order to determine whether the offspring of these pigs would show similar resistance under laboratory conditions and, if so, to attempt to identify genetic markers that would permit selection of pigs with innate resistance to the pathogenic effects of ASF, twenty sows and five boars were purchased from pig owners in the Angonia District and quarantined at an experimental station in Ulongwe, the district capital of Angonia. An outbreak of ASF occurred immediately after their arrival, resulting in the death of eight of the pigs with typical signs and lesions of ASF. Studies on samples taken from the pigs that died indicated that two genetically different viruses were involved. Five naive (i.e. serologically negative at the time of the outbreak) pigs, three adults and two juveniles, and a juvenile of unknown status, survived the outbreak without at any time showing any sign of illness, as did all ten pigs that were serologically positive when purchased. All of the surviving pigs were serologically positive at the second bleed six weeks after entry into quarantine.

The pigs that survived the required quarantine period in the area of origin, together with a boar purchased during the quarantine period after the loss of four of the original five males, were imported to a quarantine facility at the Onderstepoort Veterinary Institute (OVI), South Africa.

Two ASF viruses were used in five different experiments to challenge 105 offspring derived from the imported herd. One of the viruses was isolated from the group of purchased pigs and the other, which was shown by partial deoxyribonucleic acid (DNA) sequencing to be closely related to the first isolate, came from Madagascar. Only one of the offspring survived challenge and it was therefore concluded that the group of purchased pigs had levels of resistance no greater than those found in commercial pig breeds.

Keeping the pigs under experimental conditions offered an opportunity to study the persistence of the antibodies to ASF virus, the presence and persistence of maternal antibodies, the virological status of piglets farrowed by serologically positive sows, the carrier status of the serologically positive pigs and their response to challenge infection.

Materials and methods

The experimental protocol used in this study was approved by the Animal Ethics Committee of the OVI.

Breeding herd

Twenty-five domestic pigs (five boars and twenty sows), ranging in age from approximately three months to adults, were purchased from five villages in the Angonia district of the Tete Province, Mozambique, in November 1998 (Table I). Seven of the pigs that had been bled and marked in July 1998 were available for purchase. The pigs were of an unimproved type, black-skinned and long-haired, with a long thin snout and a distinct nuchal crest (Fig. 1). The pigs were quarantined in a stable at the Estação Zootécnica e Agronómica (EZA) at Ulongwe for three months under veterinary supervision while the tests required for import into South Africa were carried out. Within the first three weeks of quarantine, eight of the pigs died with typical signs and lesions of ASF, reducing the herd to two boars and fifteen sows. Another boar died subsequently of causes unrelated to the outbreak. An additional boar was therefore acquired in late January and quarantined with the other pigs. After all the conditions for importation had been satisfied, they were transported by air to South Africa and quarantined in a specially modified facility at the OVI. Nine of the adult sows farrowed shortly after arrival at OVI. Challenge experiments were carried out on piglets from these litters and subsequent litters bred at OVI. Litters were identified three days after birth by ear notches. Ear tags with individual numbers were applied at weaning.

Serological status of the breeding herd at the start of the experiment

Ten of the 25 pigs initially purchased had antibodies to ASF virus. All ten survived the outbreaks in the quarantine facility. The remaining 6 surviving pigs had antibodies when tested in January 1999 (Table I). The additional boar, purchased that month and allocated the number 1, was serologically negative.

Virological status of the breeding herd at the start of the experiment

Blood samples were taken from all but one of the 25 pigs the day after they entered quarantine at Ulongwe in November 1998. ASF virus was isolated using bone marrow cultures from the blood of one of the adult sows (number 32). The sow showed no visible signs of disease, and was one of the animals with antibodies during the survey in July 1998. In addition, ASF virus was detected by polymerase chain reaction (PCR) in the blood of an adult boar (number 31) from the same village, but it could not

Table I
Results of blood tests for African swine fever virus (ASFV) and antibodies to ASFV in pigs imported from Mozambique

Date of purchase of pigs: all pigs, except Number 1, were purchased on 18 and 19 November 1998; date of purchase of Number 1: 23 January 1999; date of transport to Onderstepoort Veterinary Institute, South Africa: 24 February 1999

Pig	Dates of PCR for ASFV			Dates at which pigs were tested for antibodies						
number	Nov. 98	Jan. 99	Mar. 99	Jul. 98	Nov. 98	Jan. 99	Mar. 99	Nov. 99	Jun. 00	Jan. 01
1♂J	N/S	N/S	NEG	N/S	N/S	N/S [a]	NEG	NEG	N/S	N/S
4♀A	NEG	POS	NEG	POS	POS	POS	POS	POS	POS	N/S
7♀A	NEG	POS	NEG	POS	POS	POS	POS	POS	N/S	N/S
16♀J	NEG	POS	NEG	NEG	NEG	POS	POS	POS	POS	N/S
28♀A	NEG	POS	NEG	NEG	NEG	POS	POS	N/S	POS	N/S
29♀A	NEG	NEG	NEG	POS	POS	POS	POS	N/S	POS	N/S
31♂A	POS	NEG	NEG	NEG	POS	POS	POS	POS	N/S	POS
32♀A	POS	NEG	NEG	POS	POS	POS	POS	POS	POS	N/S
61♀J	NEG	NEG	NEG	N/S	POS	POS	POS	POS	N/S	N/S
65♀A	NEG	NEG	NEG	N/S	POS	POS	POS	N/S	POS	N/S
67♀A	NEG	POS	NEG	N/S	NEG	POS	POS	N/S	POS	NEG
68♀A	NEG	POS	NEG	N/S	POS	POS	POS		Died Sep. 99 (POS)	
71♀A	NEG	NEG	NEG	N/S	POS	POS	POS	N/S	POS	N/S
72♀J	N/S	POS	POS	N/S	N/S [b]	POS	POS	POS	N/S	N/S
73♀A	NEG	POS	NEG	N/S	POS	POS	POS	POS	N/S	POS
74♀A	NEG	NEG	NEG	N/S	NEG	POS	POS	N/S	POS	N/S
77♀J	NEG	POS	NEG	N/S	NEG	POS	POS	POS	N/S	POS
60♀J	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
62♀A	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
63♀J	NEG	N/S	N.S	N/S	NEG		Died Dec. 98			
64♀J	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
66♂A	NEG	N/S	N/S	N/S	NEG		Died Dec. 99			
69♂J	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
70♀A	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
75♂J	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			
76♂J	NEG	N/S	N/S	N/S	NEG		Died Dec. 98			

A: adult
J: juvenile
N/S: not tested
PCR: polymerase chain reaction
a) acquired January 1999 after second bleed
b) not able to obtain a sample

be cultured. Subsequently, blood samples were taken from the sixteen pigs remaining in quarantine in January 1999. The ASF virus was detected by PCR in nine of the pigs, but could not be cultured; no virus was detected in the blood of pigs 31 and 32.

Viruses

In addition to the blood samples described above, organ samples were taken from the eight pigs that died with signs and lesions indicative of ASF and examined for ASF virus. Because the samples were preserved in formol-glycerosaline, the only preservative available at the quarantine station, culture was not possible, but virus was detected in all the samples by PCR.

The viruses detected in three of the blood samples and in the organs were sequenced using PCR technology that targets VP72, as described below. The remaining positive blood samples taken in January did not contain enough material for genetic characterisation. Results indicated the presence of two distinct genotypes of ASF virus. The virus isolated from the blood of sow 32 and the virus detected in boar 31 and four of the eight dead pigs belonged to one genotype, while the virus detected in the blood of one of the pigs (a gilt, number 77) in January and the remaining four dead pigs belonged to a different, unrelated genotype. The detailed study on the co-circulation of the two ASF viruses and its implications has been published elsewhere.

Comparative genetic studies indicated that the second virus, which was not available as an isolate, was identical

Fig. 1
Domestic pig from the Angonia District, Tete Province, Mozambique, November 1998

to an outbreak virus from Madagascar based on VP72, and this identity was confirmed using a CVR-PCR (central variable region polymerase chain reaction) (3). It was therefore concluded that the Madagascan isolate could be regarded as a genetically similar virus and it was thus used in the challenge experiments.

The two viruses were prepared for use in the challenge experiments as follows:

– The first, designated MOZ/1/98, was isolated from blood of sow 32 taken in November 1998. Haemadsorption was detected on the swine macrophage cultures after two passages. It was multiplied by inoculation into susceptible pigs and re-isolation from their tissues after they died of ASF.

– The second virus, designated MAD/1/98, was isolated from clinical specimens from pigs that died in outbreaks of ASF in Madagascar in 1998.

Both of the viruses proved highly virulent when inoculated into commercial Landrace cross pigs. Virus was stored at –86°C as 10% spleen and lymph node homogenates prepared using wash buffer in 1, 5, 10, and 20 ml aliquots.

Laboratory techniques

Virus isolation

One gram of swine tissue material was homogenised in 10 ml of wash buffer with sterile sand using a sterilised pestle and mortar. The homogenates were centrifuged at 900 g for 10 min. The supernatants were diluted 1:10, 1:100 and 1:1000 in wash buffer.

Primary porcine macrophage cultures were prepared from heparinised swine blood as previously described (11); 1 ml of heparinised blood was frozen at –86°C for 1 h, thawed and then diluted in 9 ml of wash buffer. Fifty microlitres of each dilution of the homogenate were placed in a row of multiple wells of a 96-well plate containing porcine peripheral mononuclear cell cultures. A positive control (ASF virus with known titre) and a negative control (wash buffer) were added to separate rows on each plate. The samples were monitored for the attachment of large numbers of pig erythrocytes to the surface of infected cells (haemadsorption) and/or a reduction in the number of adherent cells (cytopathic effect) from 24 h up to six days post-inoculation.

Polymerase chain reaction

The presence of ASF-viral DNA was detected by a PCR that targets a central region of VP72, and which is described in detail in Chapter 2.1.12. of the OIE (World Organisation for Animal Health) *Manual of Standards for Diagnostic Tests and Vaccines* (16). Genotyping was performed using a PCR that targets the C-terminal end of VP72, as described by Bastos *et al.* (2).

Indirect enzyme-linked immunosorbent assay

Antibodies to ASF virus were detected by an indirect enzyme-linked immunosorbent assay (ELISA) performed as follows: cytoplasmic soluble antigen was prepared from ASF virus (Zaire strain) as previously described (17). A negative control antigen was similarly prepared from uninfected cells. Microtitre plates were coated with an optimal dilution of both positive and control antigen in 0.05M carbonate buffer overnight at 4°C. Plates were washed with phosphate-buffered saline/0.05% Tween 20 and stored at –20°C until used. A constant dilution of 1:20 of the test sera, as well as appropriate positive and negative control sera, were added to pre-coated plates and incubated for 1 h at 37°C. Protein A-peroxidase conjugate was added to washed plates and incubated for 1 h at 37°C, whereafter substrate/chromogen (H_2O_2/OPD) was added. The reaction was allowed to develop for 15 min. after which it was stopped with 1.25M H_2SO_4 and read at 492 nm in a Multiskan. Positive/negative cut-off points were determined by subtracting the values obtained for the negative antigen from those obtained from the positive antigen and comparing these values with those obtained with negative and positive control sera. Values at least double those obtained for the negative control serum were considered positive.

Challenge experiments

The challenge experiments were carried out in the stables of a high security facility of the OVI maintained under negative air pressure with 21 air changes per minute.

Before the pigs entered the experimental facility, blood samples in heparin and hairs including the roots were taken from each animal. These samples were stored for

genetic investigation in the event of the offspring proving resistant to challenge with ASF virus.

Pigs were allowed to settle in for two to seven days until the fighting stopped and then they were challenged with 10^4HAD$_{50}$ (50% haemadsorbing units per g tissue or per ml blood). This dose level was chosen because it is known that one tissue culture infectious dose is capable of resulting in infection by needle inoculation (18): this quantity was used to be sure infection would occur. For oral challenge, 10^5 was used because it is known that 10^4 is approximately the threshold for oral infection of pigs (24).

Since it was assumed that infection by contact with sick pigs would be the usual method of transmission under natural conditions, the first two experiments relied upon contact infection of pigs subsequent to needle inoculation of one or two pigs. In subsequent experiments all the pigs were inoculated simultaneously on Day 0, to expose all the pigs to an equal dose of virus at the same time. Because pigs would most likely be infected via the oro-nasal route under natural conditions, it was decided to use one or more of these routes rather than needle infection. The only attempt at oral infection by mixing the inoculum with feed failed (Experiment 2), and was not repeated. Intranasal infection failed in only one of two groups on the first attempt (Experiment 3), but was highly effective in the other group and in experiments 4 and 5, probably owing to improved technique.

Pigs were observed for clinical signs, but were mostly not monitored individually in order to avoid excessive handling. For the same reason, temperatures were not monitored except during the initial period of the first experiment. After all the pigs in the first experiment failed to survive, everything possible was done to reduce stress during the experimental period in the hope that this would maximise the chance of survival.

Full necropsies were performed on all the pigs that died after challenge. Presence of the challenge virus in blood, lymphoid tissues and other organs was confirmed by PCR.

A control group of Large White × Landrace pigs, a race known to be highly susceptible to the pathogenic effects of ASF, was used only in the first experiment. Subsequent experiments were confined to the offspring of the Mozambique pigs, since they were apparently highly susceptible to ASF. This reduced the number of animals exposed to the infection and thus the amount of suffering induced.

In the first experiment, antibody levels were measured before the experiment in the Mozambique pigs to ensure the absence of maternal antibodies, and at death in the pigs that were infected by contact in both groups. In subsequent experiments, antibody levels were measured before challenge only if the pigs were young enough to have maternal antibodies, and were not measured again at death. Confirmation of infection with ASF virus was achieved by detecting the challenge virus using PCR.

Monitoring of the herd

With the exception of occasional stillborn piglets that were already autolysed and/or trampled when discovered, full necropsies were performed on all pigs that died or were euthanased in the breeding unit. When obtainable, lymphoid tissue samples and heart blood from piglets that died were examined for the presence of ASF virus and antibodies. When appropriate, samples were submitted for bacteriological and histopathological examination to determine the cause of death.

Results

Challenge experiments

With two exceptions, all challenged and in-contact pigs either developed clinical signs of acute ASF and died, or died of peracute ASF without developing clinical signs. Post-mortem lesions were consistent with ASF, and challenge virus was recovered from all pigs sampled. The results of the challenge experiments are summarised in Table II. Detailed results of experiments 1 and 5 are presented separately in Tables III and IV.

Experiment 1

The objective of this experiment was to determine whether the offspring of sows with proven resistance to the pathogenic effects of natural infection with an ASF virus, and no longer protected by maternal antibodies, would demonstrate a better rate of survival than modern cross-bred pigs when challenged with ASF virus.

Ten male pigs aged six to seven months derived from litters conceived during the quarantine period in Mozambique, and without maternal antibodies to ASF virus, and ten male pigs of similar size, aged four months, derived from Large White/Landrace crosses were transferred to the isolation facility on the same day. The ten Mozambique-derived piglets were from sows 7, 28, 29, 67, 68, and 74. Their paternity was uncertain, as there were two adult males in the quarantine stable at the time of conception.

One pig in each group was needle-infected in a gluteal muscle with 1 ml of virus suspension containing 10^4HAD$_{50}$ MOZ/1/98 virus. The pigs were monitored for clinical signs of ASF, but temperature-taking was stopped on Day 9 as indicated above. At death, blood samples were taken from all the pigs except those that were needle-infected and the sera were tested for antibodies to ASF virus by indirect

Table II
Summary of results of challenge with African swine fever virus of offspring of pigs that had survived natural infection

Challenge	Number of pigs	Virus	Route	Age of pigs (months)	Number with antibodies at start	Days to death [a]	Number survived
Experiment 1	1	MOZ/1/98	I/m	4 [b]	0	6	0
	9	MOZ/1/98	Contact	4	0	13-22 (15.2)	0
	1	MOZ/1/98	I/m	7	0	6	0
	9	MOZ/1/98	Contact	7	0	12-25 (15.7)	0
Experiment 2	4	MOZ/1/98	I/m	3	2	7-9 (8)	0
	16	MOZ/1/98	Contact	3	6	12-31 (19.5)	0
Experiment 3	11	MOZ/1/98	I/n	6	(0)	7-13 (9.2)	0
	12	MAD/1/98	I/m	6	(0)	7-15 (9.9)	0
Experiment 4	9	MOZ/1/98	I/n	15	(0)	8-16 (10.5)	0
Experiment 5	26	MOZ/1/98	I/n	4	7	7-17 (10.8)	0
	27	MAD/1/98	I/n	4	5	9-25 (14.8)	1
Experiment 6	1	Both	I/m	Adult	1	N/A	1

I/m: intramuscular inoculation I/n: intranasal inoculation
N/A: not applicable
a) range and mean
b) European breed pigs

Table III
Results of contact challenge of two groups of pigs, with African swine fever virus MOZ/1/98 (Experiment 1)

Pig number	Antibody status before challenge	Time of onset of clinical signs (days)	Time to death (days)	Antibody status at death
Group 1: Large White × Landrace				
4*	N/S	5	6	N/S
5	N/S	–	13	NEG
6	N/S	13	13	NEG
8	N/S	13	13	NEG
9	N/S	13	13	NEG
7	N/S	–	14	NEG
2	N/S	–	15	NEG
10	N/S	–	15	NEG
3	N/S	19	19	NEG
1	N/S	–	22	NEG
Group 2: Mozambique (first generation)				
50*	NEG	–	6	N/S
17	NEG	–	12	NEG
6	NEG	13	13	NEG
21	NEG	13	13	NEG
43	NEG	–	13	NEG
26	NEG	–	16	NEG
30	NEG	13	16	NEG
44	NEG	–	17	POS
47	NEG	–	17	NEG
49	NEG	–	25	NEG

N/S = not tested
* pigs exposed to infection by needle inoculation

ELISA. The results are presented in Table III. Of the two pigs inoculated on Day 0, the Large White × Landrace pig developed clinical signs of fever and lethargy on Day 5 and died on Day 6, while the Mozambique pig died on Day 6 without developing clinical signs. The remaining pigs in both groups died between Day 12 and Day 25. Only one pig, number 44 in the Mozambique group, developed an antibody level high enough to be regarded as positive.

Experiment 2

The objective of the experiment was to determine whether the presence of maternal antibodies provided protection.

Twenty piglets aged three months from two litters derived from the Mozambique sows 29 and 67, sired by boar 31 were mixed and divided into two groups of ten each. Each group contained piglets from both litters. Eight piglets had maternal antibodies to ASF virus.

Two piglets in Group 1 were inoculated intramuscularly with 1 ml $10^4 HAD_{50}$ MOZ/1/98 virus. In Group 2, two piglets were force-fed with 1 ml $10^5 HAD_{50}$ MOZ/1/98 virus mixed with feed. One month later, after no illness was observed in Group 2 piglets, the two piglets previously force-fed received 1 ml each of the suspension containing $10^4 HAD_{50}$ MOZ/1/98 virus by intramuscular injection on Day 27, which became Day 0 for the purposes of recording time to death of the pigs in Group 2.

All the pigs died of acute to peracute ASF. There was no apparent difference between the two litters, or the presence or absence of maternal antibodies. The two needle-infected pigs in Group 1, both from sow 67, one each with positive and negative pre-inoculation antibody levels, developed

clinical signs on Day 6 and Day 7 and died on Day 8 and Day 9 respectively. Both inoculated pigs in Group 2, one from sow 67, serologically negative, and one from sow 29, serologically positive, developed clinical signs on the fourth day after inoculation and died on Day 7 and Day 8, respectively. There was no difference in time to death between contact-infected pigs with and without maternal antibodies.

Experiment 3

The objective of the experiment was to compare the effect of the two challenge viruses.

Twenty-three piglets aged approximately six months that resulted from mating the serologically negative boar 1 with serologically positive sows 16, 32 and 61 were divided into two groups of eleven and twelve piglets, respectively. All the pigs in Group 1 were infected intranasally with 1 ml 10^4HAD_{50} MOZ/1/98 virus and all the pigs in Group 2 with 1 ml 10^4HAD_{50} MAD/1/98 virus. Intranasal infection in the second group apparently failed to induce infection, and the pigs were all injected intramuscularly one month later with 1 ml 10^4HAD_{50} MAD 1/98 virus.

All the pigs died of acute or peracute ASF (Table II). Two of the pigs in Group 2 had comparatively elevated levels of antibodies to ASF on Day 21. These pigs did not survive the second inoculation, administered on Day 23, any better than the rest of the group, all of which died between Day 7 and Day 15. The pigs in Group 1 all died between Day 7 and Day 13.

Experiment 4

The objective of the experiment was to determine whether offspring from a sire and a dam believed to be closely related would be more resistant. Nine male pigs aged approximately fifteen months were divided into two groups and all were infected intranasally with 1 ml 10^4HAD_{50} MOZ/1/98 virus. The pigs were the result of mating that took place while all the pigs were quarantined together at OVI. It was presumed that the serologically positive boar 31 was the sire, as the other was very young at the time and was dominated by the older boar. The groups included pigs from sow 32, the source of MOZ/1/98, as well as sows 16 and 61. Since sow 32 and boar 31 originated from the same village, it was possible that their offspring were inbred.

The pigs all died of acute or peracute ASF between Day 8 and Day 16 after inoculation (Table II). The two longest surviving pigs were not from the litter that was believed to be inbred and therefore expected to survive best.

Experiment 5

In order to perform a crossover experiment to determine whether there was a difference in resistance to challenge between the offspring of boar 1 and boar 31, and whether there was a difference in response to the two viruses between litters, nine sows were bred naturally during a four-week period in order to produce as many piglets as possible. Fifty-three four-month-old piglets from seven sows and both boars (sows 7, 71, 74 + boar 1; sows 32, 65, 72, 77 + boar 31) were divided into two groups of 26 and 27 piglets, respectively. Piglets from all litters were represented in each of the groups. The 26 piglets in Group 1 were infected intranasally with 1 ml 10^4HAD_{50} MOZ/1/98 virus and the 27 piglets in Group 2 were infected intranasally with 1 ml 10^4HAD_{50} MAD/1/98 virus.

Of the 53 piglets used in the experiment, only one survived (Tables II and IV). The pig, a four-month-old male without antibodies, derived from sow 74 and boar 1, survived intranasal challenge with MAD/1/98 virus. It was in close contact throughout the experiment with the other 26 pigs in the group, all of which died of acute ASF. It subsequently survived challenge with MOZ/1/98 virus. After the first challenge it developed mild clinical signs of fever and inappetence, but had recovered completely on Day 25 and was serologically positive for antibodies to ASF virus. The pig grew well to adulthood. Subsequent inoculations with both viruses did not result in clinical signs.

A white male piglet used for virus multiplication unexpectedly survived as well. It was purchased from a nearby commercial pig farm and infected by contact with a cohort injected with 0.4 ml of untitrated MAD/1/98 virus extract. It developed early clinical signs of ASF (elevated temperature and inappetence) but recovered after a day. That it had been infected was demonstrated by the presence of antibodies against ASF virus ten days after infection. Subsequent challenge by intranasal and intramuscular routes with 10^4HAD_{50} of both challenge viruses failed to produce clinical signs of ASF. Recovery was evidently complete and the pig grew to adulthood without any signs of illness other than periodic lameness that resulted from being overweight and kept on a hard floor, and was alleviated by the provision of soft bedding.

Both of these pigs were euthanased as healthy adults because it was not possible to release them from the high security laboratory owing to facility regulations relating to foot and mouth disease. No lesions suggestive of ASF were present in either pig at necropsy, and no virus was recovered from their tissues.

Experiment 6

To confirm that a serologically positive sow remained resistant, an imported, serologically positive sow (number 4), which had never bred, was transferred to the high security facility and challenged intranasally with 1 ml 10^4HAD_{50} MAD/1/98 virus and fourteen days later with 1 ml 10^4HAD_{50} MOZ/1/98 virus. Fourteen days later

Table IV
Results of challenge of two genetically similar groups of piglets, derived from resistant sows, with African swine fever viruses MOZ/1/98 and MAD/1/98 respectively (Experiment 5)

Group 1: inoculated intranasally with 1 ml 10^4HAD(TCID)$_{50}$ MOZ/1/98			Group 2: inoculated intranasally with 1 ml 10^4HAD(TCID)$_{50}$ MAD/1/98		
Pig number	Antibody status pre-challenge	Number of days until death	Pig number	Antibody status pre-challenge	Number of days until death
X40	NEG	7	X27	NEG	9
X6	NEG	8	X4	NEG	10
X13	POS	8	X25	NEG	10
X19	NEG	8	X35	NEG	11
X22	NEG	9	X12	NEG	11
X16	NEG	9	X50	NEG	12
X51	NEG	9	X30	NEG	12
X15	NEG	9	X11	NEG	13
X47	NEG	9	X8	NEG	13
X33	NEG	10	X39	NEG	13
X2	NEG	10	X53	POS	13
X1	NEG	10	X26	POS	14
X52	POS	10	X45	POS	14
X32	POS	11	X31	NEG	14
X48	NEG	11	X41	POS	14
X3	POS	11	X36	NEG	15
X28	POS	11	X37	NEG	15
X42	NEG	11	X21	NEG	15
X14	NEG	12	X20	NEG	15
X5	NEG	12	X44	POS	16
X29	NEG	13	X24	NEG	17
X38	NEG	13	X23	NEG	18
X7	POS	14	X10	NEG	21
X43	NEG	15	X9	NEG	22
X18	NEG	16	X34	NEG	23
X46	POS	17	X17	NEG	25
		X49	NEG	Survived	

the sow was euthanased and a full necropsy was performed. No clinical signs of ASF were observed at any time, and no lesions of ASF were present at necropsy. No virus was recovered from her tissues. It is unlikely that infection was unsuccessful for technical reasons because all but one of the other intranasal challenges were successful.

Comparison between the two viruses used for challenge

There was no apparent difference in virulence between the two viruses used for challenge. In Experiment 3, deaths of pigs inoculated with the MAD/1/98 virus reached a peak earlier than the group inoculated with MOZ/1/98, but this trend was reversed in Experiment 5.

Monitoring of the herd

Necropsies were performed on 21 piglets that had been farrowed by imported sows during the first year of the project and that died from unknown causes. Blood, spleen and lymphoid tissues of 20 that were tested were negative on bone marrow culture for ASF virus. All the piglets that had suckled before death and yielded blood samples (n = 10) were positive for antibodies to ASF virus. All but five of the deaths were within the first week of life; most occurred among piglets that were born weak and could be ascribed to exposure or hypoglycaemia, and in one case to trauma resulting from being overlain by the sow. The sows were excellent mothers, and 130 of the approximately 159 piglets farrowed to December 1999 were weaned. (The exact number of piglets born is not known because a litter

was eaten by the boars at birth during the first quarantine period when the facility did not permit adequate separation.) Four older piglets died of other causes, namely a severe case of dermatitis caused by *Staphylococcus hyicus* in a three-week-old piglet, dilated cardiomyopathy caused by a congenital septal defect resulting in heart failure in another three-week-old piglet, *Escherichia coli* septicaemia in a three-month-old piglet, and interstitial pneumonia of unknown origin in a four-month-old weaner.

Three adult pigs, apart from the sow used in Experiment 6, were euthanased during the course of the project. The two sows (67 and 68) were both severely lame owing to osteoarthrosis, and the older boar (31) had severe prostatitic hyperplasia causing urinary tract obstruction. Necropsy and histopathological examination of multiple tissues did not reveal any abnormalities or lesions attributable to ASF. The presence of viral DNA in various samples from sow 68 (spleen, lung, liver, kidney, and hepatogastric, mesenteric, and submandibular lymph nodes) was indicated by a weak positive reaction on PCR, but no virus could be cultured in spite of multiple passages. The PCR detected no virus in any of the multiple tissues examined from the other two animals.

The results of serological examination of the adults (Table I) indicate that antibodies can persist for at least two years without re-exposure. Three of the pigs from which samples were taken in January 2001 were still positive. However, the blood sample taken at necropsy from sow number 67 in the same month was negative for antibodies to ASF, suggesting that persistence of antibodies is not always lifelong.

Maternal antibodies were found in all piglets tested before three months of age, except those that died without suckling. Maternal antibodies apparently waned after three months, and most piglets of five months and older were negative. Two out of a sample of five six-month-old piglets from the first litters were positive, suggesting that antibodies may have persisted a little longer in these litters, but all the piglets were negative at 7.5 months.

Discussion

Although both the serology of pigs in the Angonia District of Tete Province, Mozambique, and the outcome of the outbreak in the quarantine facility soon after the Mozambican pigs were purchased indicated that about 40% of the pigs in their natural system survived infection with ASF virus, experimental infection of the offspring of these pigs with homologous virus resulted in close to 100% mortality. This is no different to the expected survival rate in fully susceptible pigs. Similar results were obtained by Vasco (26), who conducted an investigation with pigs of improved breed that survived natural outbreaks of ASF in Portugal. However, Vasco found that survival improved markedly in litters derived from back crossing pigs that survived experimental infection to their sires or dams. This potential was not investigated during the present experiment. It is likely that there is usually some inbreeding in village pig populations, as a few boars are generally shared amongst producers. However, presumptively inbred offspring derived from the serologically positive boar and sow from the same village all died when challenged with ASF virus (Experiment 4). These results suggest that resistance is not highly heritable, and may be due to some factor that was not replicated in the experimental situation.

The objective of the project was to determine whether the resistance observed in the Angonia population was heritable, and provision was not made for investigating the mechanism for resistance should it prove not to be heritable. However, because it appears that a mechanism other than inherent and heritable resistance to ASF occurs in the pig population of Angonia it is obviously important to establish its nature. Alternative explanations for resistance include the following:

– pigs are protected from death caused by virulent viruses by prior exposure in the field to antigenically similar viruses of reduced virulence

– exposure for the first time to infection when protected by maternal antibodies may result in resistance (21, 22)

– exposure to infection in the field is likely to be by the oro-pharyngeal route and initial exposure to small quantities of infectivity may result in a sub-lethal infection that confers immunity to subsequent challenge with larger amounts of infectivity.

There is no evidence that viruses of reduced virulence are present in the area from which the pigs originated, although the possibility was not investigated specifically. Both viruses identified in tissues of the pigs imported from Mozambique proved highly virulent. Examination of blood and organ samples using PCR technology did not reveal DNA of any other virus. However, previous exposure to other viruses cannot be ruled out, because evidence from this study supports earlier studies (25, 28) that virus can be isolated from the tissues of recovered pigs for a relatively short time only.

In areas where ASF is endemic in domestic pigs, exposure of piglets to infection with ASF virus while still protected by maternal antibodies is likely, as local information indicates that outbreaks occur at frequent intervals. Because the objective was to investigate possible genetic resistance, the first challenge was carried out on piglets that no longer had maternal antibodies. When all of those pigs died, it was decided that the possibility of protection by maternal antibodies should be included in the

investigation. Unfortunately the persistence of maternal antibodies was greatly reduced in subsequent litters. It was therefore not possible to conduct challenge experiments while levels were likely to be high, because transferring the piglets to the experimental facility immediately after weaning would have been highly stressful and likely to result in deaths unrelated to challenge with the virus. Piglets with detectable circulating antibody levels in the second and fifth experiments were clearly not protected, as they died at the same rate as serologically negative cohorts, but the levels of maternally-derived antibodies present may have been too low to afford effective protection. The only pig that survived challenge (Experiment 5) was serologically negative when infected.

Antibodies induced by infection with ASF virus may be expected to persist for at least two years without re-infection. One of the imported pigs that died had no antibodies at necropsy two years after importation (Table I, sow number 67), but the boar that died a month later still had antibodies. A study in Spain reported a mean half-life of 1.8 years for antibodies in pigs that recovered from ASF (19). The outbreak at EZA demonstrated that exposure to ASF virus while protected by maternal antibodies is not a prerequisite for later resistance. Five pigs, including a young piglet (sow 77) and two sub-adults, which had no antibodies at the first bleed, survived the outbreak and were serologically and in some cases PCR positive at the second bleed, indicating that they had not escaped infection (Table I). However, the contribution of passive protection to the higher than expected survival rate should be further investigated, preferably under natural conditions.

Immunity conferred by sub-lethal infection may be responsible for the survival of a proportion of the pigs under their normal husbandry conditions. It was hoped that the effects of limited exposure could be investigated experimentally, but in practical terms the facility used did not have the physical space or personnel to guarantee the necessary separation.

Previous studies have indicated that fully recovered pigs apparently do not become long-term carriers of the virus (20, 25, 28). Evidence from the present study is similar. None of the pigs bled six weeks after the outbreak in quarantine in Angonia that killed eight pigs yielded viable virus from blood samples, although viral DNA was detected in nine of sixteen pigs. Three adult pigs euthanased for various reasons during the study did not yield viable virus, although one animal killed nine months after the outbreak produced a weak positive reaction when some tissue suspensions were tested by PCR. Furthermore, a young, serologically negative boar that was introduced into the herd at EZA within two months of the outbreak, lived in close confinement with all the other pigs for the first three months in South Africa, and subsequently served all of the sows, spending periods of up to three months in close confinement with them, remained disease free and serologically negative throughout the study. One pig, sow number 32, yielded virus from a blood sample taken in November 1998 upon entry into quarantine. This animal had antibodies in July 1998. Two passages in pigs were required to isolate the virus, suggesting that the level of virus was low. The pig at no time showed any signs of disease, but was one of the few adult sows that did not conceive during the first three weeks in quarantine. Viraemia may have persisted since the infection that resulted in seroconversion (i.e. at least four months). However, it is possible that the pig became viraemic after re-exposure to infection, because although recovered pigs are generally resistant to re-infection with homologous virus, exposure to heterologous viruses may result in infection followed by disease (18).

ASF virus in Angonia is apparently maintained in a cycle that involves domestic pigs only. Warthogs and bush pigs apparently do not occur in the area. A limited search in some pig shelters and showing samples to the people did not reveal the presence of ticks of the *Ornithodoros moubata* complex, which probably play a key role in viral maintenance in the neighbouring Mchinje district of Malawi where a similar cycle in domestic pigs prevails (8, 9). Maintenance of the virus in Angonia probably depends upon continual circulation in a sufficiently large population of pigs. Information obtained from residents in the area indicated that outbreaks occur frequently, although they are rarely reported.

Conclusion

Although 40% of pigs in the Angonia district survived infection with ASF virus, the results of the experiment suggest that this resistance is not genetic. Even with an increased survival rate, the disease remains a scourge in Angonia and many potential producers are discouraged. The effects are obviously even worse when the disease spreads to areas where less than 5% of pigs survive. Some producers in Angonia overcome the problem by permanently confining their fattening pigs (personal observation, 2002). There is no doubt that ASF can be controlled by correct husbandry that prevents access to the virus. However, because the elements of good husbandry may be beyond the reach of poor farmers, it remains desirable to explore the nature of any apparent resistance to ASF and the possibility of applying it in the control of ASF.

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Étude d'une résistance naturelle à la peste porcine africaine chez des porcs domestiques issus d'une zone endémique en Afrique australe

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Résumé

Une forte prévalence d'anticorps circulants du virus de la peste porcine africaine (PPA) dans le nord du Mozambique a permis de découvrir une population de porcs domestiques présentant une résistance accrue aux effets pathogènes du virus. Les auteurs ont tenté de mettre en évidence une transmission éventuelle de cette résistance à la descendance. À cette fin, plusieurs de ces animaux résistants ont été achetés et transportés dans une station de quarantaine pour s'y reproduire naturellement. Leur progéniture a été transférée dans des installations de haute sécurité, où elle a été inoculée avec deux isolats du virus de la PPA. Le premier isolat avait été prélevé sur l'un des porcs mozambicains ; l'autre isolat était constitué d'un virus génétiquement très proche, d'origine malgache. La totalité de la descendance (105 porcs), à l'exception d'un seul animal, a contracté une forme aiguë de la PPA avec une issue fatale. Cette étude semble donc indiquer que la résistance des géniteurs n'a pas été transmise à leur descendance ou qu'elle n'a pu s'exprimer dans les conditions de l'expérience. En outre, elle n'a pas réussi à élucider les mécanismes qui ont permis aux porcs de la population d'origine de vivre en coexistence avec des virus virulents.

Mots-clés

Mozambique – Peste porcine africaine – Porc domestique – Résistance génétique.

■

Investigación sobre la resistencia natural a la peste porcina africana de una piara de cerdos domésticos criados en una zona endémica del sur de África

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Resumen

Debido a la elevada prevalencia de anticuerpos circulantes al virus de la peste porcina africana (PPA), se detectó una población de cerdos domésticos con una mayor resistencia a los efectos patogénicos de ese virus en el norte de Mozambique. Se intentó determinar si la resistencia de la piara era hereditaria. Para ello, se compraron algunos suidos de esa manada y se los trasladó a una instalación de cuarentena adonde se los dejó reproducirse en forma natural. Posteriormente, las crías de esos cerdos fueron transportadas a una instalación de alta seguridad donde se las expuso a dos virus de PPA: el primero había sido aislado en uno de los cerdos de Mozambique y, el otro, que provenía de Madagascar, estaba íntimamente emparentado, desde un punto de vista genético, con el anterior. De las 105 crías, 104 contrajeron peste porcina aguda y murieron. Por consiguiente, se demostró que la resistencia de los cerdos de esa piara, o no era hereditaria, o no pudo expresarse en las condiciones del experimento. No obstante, aún se ignora la naturaleza del mecanismo por el cual la población de origen pudo vivir coexistiendo con virus virulentos de PPA.

Palabras clave

Cerdo doméstico – Mozambique – Peste porcina africana– Resistencia genética.

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