EXPERIMENTAL INFECTION OF WARTHOG (*PHACOCHOERUS AETHIOPICUS*) WITH AFRICAN SWINE FEVER VIRUS

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ABSTRACT

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Although there were no obvious signs of illness following experimental infection of young warthog with African swine fever virus, the animals developed viraemias between $10^{2,4}$ and $10^{3,6}$ $HD_{50}/m\ell$ within the first week of infection, and virus concentrations in a number of lymphatic tissues attained high levels ($\geqslant 10^6$ HD_{50}/g). Unlike in blood, and to some extent in the spleen, virus titres in lymph nodes did not decline appreciably during the 33-day observation period, since at the end of the period lymphatic tissues from 2 warthog were still infectious for domestic pigs to which these tissues were fed.

Résumé

INFECTION EXPÉRIMENTALE DU PHACOCHÈRE (PHACOCHOERUS AETHIOPICUS) AVEC LE VIRUS DE LA FIÈVRE PORCINE AFRICAINE

Bien qu'aucun signe évident de maladie ne fut apparent à la suite de l'infection expérimentale de jeunes phacochères avec le virus de la fièvre porcine africaine, les animaux développèrent des virémies entre $10^{2,4}$ et $10^{3,6}$ $HD_{50}/m\ell$ dans la première semaine d'infection et les concentrations de virus dans plusieurs tissus lymphatiques atteignirent des niveaux élevés ($\geqslant 10^6$ HD_{50}/g). Contrairement au sang et, dans une certaine mesure, dans la rate, les titres de virus dans les nodules lymphatiques ne déclinèrent pas de manière appréciable pendant la période d'observation de 33 jours étant donné qu'à la fin de la période les tissus lymphatiques de deux phacochères étaient encore infectieux pour le porc domestique alimenté de ces tissus.

INTRODUCTION

African swine fever (ASF) is probably the most serious disease confronting the pig-producing areas of the world. Within the last 20 years it has spread from its historic habitat in Africa to countries in Southern Europe, the Caribbean and South America, in some of which it has become enzootic in the domestic pig population.

The means by which ASF virus is able to leave its sylvatic hosts and infect domestic pigs, which are unusual hosts, are not clearly understood. For this reason the measures used to prevent ASF infection of domestic pigs in Africa do not have a sound basis.

In Africa, 2 free-living hosts are known to harbour the infection, namely, wild suidae, particularly the warthog (De Tray, 1963), and the eyeless tampan, *Ornithodorus porcinus porcinus, sensu* Walton (1964) which inhabits warthog burrows (Plowright, Parker & Pierce, 1969a). Both warthog and tampans have been suggested as the usual source of infection for domestic pigs, but there is uncertainty in this regard. Plowright, Parker & Pierce (1969b) pointed out that the available evidence was against warthogs being the source of infection for domestic pigs, whether by direct contract or by pigs ingesting infected warthog tissues, and that it was probably via the bites of infected tampans that domestic pigs acquire the infection in Africa. However, it is apparent from a subsequent publication (Plowright, 1977) that outbreaks of ASF in domestic pigs may occur in the absence of the tampan.

Free-living warthog with a viraemia sufficient to infect tampans engorging on them have not been encountered (Plowright, 1977). Which suggest that warthog are an unlikely source of virus for tampans. Thus although tampans are capable of transmitting the infection in the process of feeding (Plowright *et al.* 1969a), uninfected tampans are apparently incapable of acquiring the infection by the same process.

The experimental infection of warthog described here was carried out in an effort to shed more light on the role of warthog in the epizootiology of ASF. Although experimental infection has been carried out previously (Montgomery, 1921; Walker, 1933; De Tray, 1957), laboratory techniques available then did not allow detailed observations on the host's immune status or the level of viral replication in different tissues.

MATERIALS AND METHODS

Animals

Warthog: Eighteen warthog, approximately 3 months old, were captured in the Hluhluwe Game Reserve (Northern Natal), where neither tampans nor evidence of ASF infection in warthog had been found (Thomson & Lewis, 1979, to be published). They were housed in groups of 3 or 4 for a month prior to the transference of 11 of them to an isolation block where they were housed together in one large pen. Three animals died prior to the commencement of the experiment and, although the cause of death was not established, no ASF virus could be isolated from any of the tissue suspensions inoculated into blood leucocyte cultures or antibody detected in serum. Another 4 animals were killed and examined for virus infection prior to their being transferred to the isolation block, without virus being detected in their tissues or antibody in their serum. The animals were maintained on pig meal and fresh lucerne *ad lib.* and $0,5\ell$ each of cow's milk per day. Because the warthog remained extremely wild, they could not be handled regularly without the danger of injuring them. For this reason daily body temperatures and regular bleedings were not obtained.

After being inoculated with virus, the warthog were observed daily for loss of appetite and signs of illness. Pairs were killed at various intervals after infection, namely, after 3, 5, 7 (1 animal only), 11, 18 and 33 days. The animals were killed by exsanguination, after being anaesthetized with Trilene[1], and the tissues listed in Table 1 were collected for virus and antibody titration. Tissues not used for virus isolation or histopathological examination were used for feeding pigs (Table 2).

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TABLE 1 The sequential titres of ASF virus in tissues of experimentally infected warthog

Days after infection	3		5		7		11		18	33	
Warthog No.	1	2	3	4	5	6	7	8	9	10	11
Blood	3,7*	2,8	3,6	2,4	3,6	2,6	2,4	0,8	—	—	—
Lung	3,6	4,2	2,8	6,0	1,6	5,6	2,1	2,2	3,6	—	—
Liver	NR	3,2	3,0	3,4	—	—	+	+	—	—	—
Kidney	+	+	—	+	NR	—	2,8	2,6	—	—	—
Spleen	5,2	5,2	5,4	6,0	4,4	2,2	3,6	2,4	2,2	2,2	2,8
Tonsil	4,0	—	4,6	—	NR	—	4,4	—	3,0	—	—
Mandibular, parotid, medial and lateral pharyngeal[1]	4,6	4,0	6,0	5,0	—	—	5,6	4,8	5,2	4,8	4,8
Dorsal superficial cervical[1] (prescapular)	3,0	NR	4,2	3,4	+	—	4,8	4,8	4,4	4,8	3,8
Caudal deep cervical[1]	4,4	NR	NR	3,0	NR	3,2	5,4	3,2	3,0	—	2,4
Hepatic (portal)[1]	2,8	2,6	5,8	5,5	3,6	5,2	6,2	5,6	5,0	6,2	2,2
Mesenteric[1]	+	—	+	+	+	—	—	—	—	—	—
Medial iliac[1]	4,2	4,4	5,0	+	+	5,8	4,8	—	4,4	3,2	3,2
Superficial inguinal[1]	NR	4,8	5,2	5,3	5,2	4,4	5,2	5,7	6,4	4,2	3,6
Serum antibody titre	—	—	—	—	16**	8	16	32	8	8	16

NR No result
[1] lymph node
* HD_{50}/g (solid tissue) or mℓ (blood)
** Reciprocal of the highest serum dilution giving a positive result
+ Virus detected but in quantities too low to titrate
— No virus isolated or no antibody detected

TABLE 2 The infectivity of minced warthog tissues fed to domestic pigs

Pig No.	Organs	Origin[1] (warthog No.)	Mass (g)	Total infectivity[2]	Infectivity per pig[2]	Mortality rate
878 & 879	Liver	1–7	607	5,9	7,7	2/2
	Lung	1–7	373	8,0		
	Kidney	1–7	124	3,9		
Totals			1 104	8,0		
880 & 881	Spleen	1–7	87	8,1	7,8	1/2
	Mandibular, parotid, medial & lateral pharyngeal[3]	1–7	10	6,3		
	Mesenteric[3]	1–7	6	+[4]		
	Superficial inguinal[3]	1–7	7	5,9		
	Tonsil	1–7	1	—		
Totals			111	8,1		
874 & 875	Liver	10 & 11	341	—	5,1	1/2
	Lung	10 & 11	120	—		
	Kidney	10 & 11	62	—		
	Spleen	10 & 11	26	2,9		
	Mandibular, parotid, medial & lateral pharyngeal[3]	10 & 11	4	5,3		
	Superficial inguinal[3]	10 & 11	3	4,3		
	Mesenteric[3]	10 & 11	5	—		
	Dorsal superficial cervical (prescapular)[3]	10 & 11	1	3,8		
Totals			562	5,4		

[1] See Table 1
[2] Log_{10} HD_{50}
[3] Lymph node
[4] Infectivity present but concentration too low to titrate
— No infectivity present

Domestic pigs: Six 30 kg cross-bred Landrace pigs were used in the "feeding experiment". They were maintained within an isolation block, singly or in pairs, in pens raised from the floor. Different groups were housed in different rooms to prevent cross-infection.

Cell cultures

The blood leucocyte (BC) cultures used have been described previously (Thomson, Gainaru & Van Dellen, 1979).

Virus

The 5th passage in BC cultures of the CV strain of ASF (Thomson et al., 1979) was used to inoculate all 11 warthog. Each animal received $10^{4,0}$ HD_{50} in a 0,5 mℓ inoculum administered intramuscularly into the rump.

Virus titrations

Ten per cent organ/tissue suspensions were prepared by grinding a quantity of tissue with a pestle and mortar, using the appropriate quantity of modified Eagle's (Glasgow) medium. Standing the suspension for an hour at room temperature allowed the larger debris to settle and the supernatant was than used. For blood, ten-fold dilutions were prepared from specimens collected from the anterior vena cava into "Venoject" tubes containing EDTA([1]). Tube cultures

([1]) Jintan Terumo Co. Ltd. Tokyo

were inoculated with 0,2 mℓ quantities of serial ten-fold dilutions of 10% tissue suspension or whole blood made in Eagle's medium. Titres were expressed per gram of organ or mℓ of blood.

Antibody determinations

Antibody titres were obtained, using a modification (Thomson *et al.*, 1979) of the immunoelectroosmophoresis test developed by Pan, De Boer & Hess (1972).

Feeding of warthog tissues to domestic pigs

Organs (Table 2) which were not required for virus titration or histopathological examination were used for feeding to domestic pigs. The organs were mass-measured and minced with a kitchen mincer and then mixed with an equal volume of pig meal. The mixture was then fed to pigs from which food had been withheld for the previous 24 hours.

RESULTS

Clinical

At no time did the feed intake of the warthog diminish and they remained apparently healthy throughout the duration of the experiment.

Pathological

Post-mortem examinations revealed no macroscopic lesions apart from some congestion and haemorrhage of the medial illiac lymph nodes. However, these and the microscopic lesions encountered will form the basis of a further communication.

Virological

As demonstrated in Table 1, viraemia ($10^{2,8}$ and $10^{3,7}$ HD$_{50}$/mℓ) occurred within 3 days of infection and, although levels declined after 11 days, the viraemia persisted in at least one warthog until 18 days following infection. By 33 days no detectable viraemia was present in either warthog tested (Table 1).

Although the warthog sampled within 18 days of infection contained appreciable quantities of virus in their lungs, that is, between $10^{1,6}$ and $10^{6,0}$ HD$_{50}$/g, only 2 had comparable levels in their kidneys, while titratable quantities of virus in liver were only observed up to 5 days after infection (Table 1).

Within 3 days of infection both warthog tested had titres of $10^{5,2}$ HD$_{50}$/g in their spleens, a level which was higher than in any lymph node tested at that time. With one exception titres in the spleens of warthog killed more than 7 days after infection were less than $10^{3,0}$ HD$_{50}$/g, that is, the titres appeared to decline after a week (Table 1).

Apart from mesenteric lymph node and tonsil, all the other lymphatic tissues tested, with a few inexplicable exceptions, contained between $10^{2,0}$ and > $10^{6,0}$ HD$_{50}$/g. While mesenteric lymph nodes never contained appreciable quantities of virus, tonsilar tissue was variable in this respect, that is, 4/10 tested had titres > $10^{3,0}$; in the rest no virus were detectable. Titres in lymph nodes were not appreciably lower 33 days after infection than they were after 3–5 days; for example, warthog 10 hepatic (portal) lymph node contained $10^{6,2}$ HD$_{50}$/g (Table 1).

Serological

None of the 4 warthog killed within 5 days of infection contained detectable antibody, but thereafter all had titres between 1/8 and 1/32 (Table 1).

Feeding of warthog tissues to domestic pigs

Minced lung, liver and kidney of warthog 1–7 were fed to 2 pigs, while similarly treated lymphatic tissues from the same warthog were fed to 2 further pigs. Tissues from the 2 warthog killed 33 days after infection (Nos. 10 and 11, Table 1) were also minced and fed to 2 pigs (Table 2). At least 1 pig in each of these groups died of acute ASF (i.e., within 9 days of eating the warthog tissues) indicating that the tissues were infectious for domestic pigs for at least 1 month after experimental infection. Although pigs 878, 879, 880 and 881 ingested high concentrations of virus ($10^{7,7}$ to $10^{7,8}$ HD$_{50}$ each), the other 2 pigs consumed only $10^{5,1}$ HD$_{50}$ each. This was sufficient, however, to produce lethal infection in one of them (Table 2).

DISCUSSION

None of the 11 inoculated warthog showed any obvious ill effects from infection with the virulent strain (Thomson *et al.*, 1979) of ASF used. It is unfortunate that body temperatures could not be obtained as Montgomery (1921) recorded pyrexic responses in 2 experimentally infected warthog. It is doubtful, however, whether the mortality in free-living warthog reported by him was due to ASF.

Nevertheless, it is clear that considerable viral replication occurred following experimental infection, since virus titres in lymphatic tissues exceeded 10^{6} HD$_{50}$/g on a number of occasions (Table 1). This is still, however, 2–3 log$_{10}$ units lower than virus titres encountered in domestic pigs dying of ASF infection (Greig & Plowright, 1970; Thomson *et al.*, 1979).

Three of the 5 experimental warthog developed a viraemia > 10^{3} HD$_{50}$/mℓ within one week of infection, whereafter the titres decreased, and by 33 days after infection viraemia could not be demonstrated in either of the remaining 2 aminals tested. On the other hand, investigations carried out on free-living warthog, including young animals removed from burrows, revealed that, although high virus titres occurred in lymph nodes, virus concentrations in the blood in every case but one were low, namely, < $10^{1,0}$ HD$_{50}$/mℓ (Heuschele & Coggins, 1969; Plowright *et al.*, 1969b; Plowright, 1977). Plowright (1977) calculated that blood levels less than 10^{3} HD$_{50}$/mℓ would be insufficient to infect tampans engorging on it, so it is logical to conclude that warthog are "end hosts", since there is as yet no evidence of lateral or vertical transmission between warthog (Plowright *et al.*, 1969b).

There are 2 possible reasons why the present results differ from those obtained from the field investigations mentioned above. Firstly, the free-living animals may have been sampled too long after primary infection. This possibility is supported by our finding that, although spleen titres were high in recently infected warthog (Table 1), in the field investigations the spleen was a poor source of virus. The other less likely possibility is that maternally-derived antibody depresses the level of virus in blood and spleen of young warthog in enzootic areas. How such a mechanism would operate is not clear, since neutralizing antibody to ASF has never been conclusively demonstrated (De Boer, Pan & Hess, 1972). Furthermore, final bleed sera from warthogs 5 and 8 (Table 1) failed to show neutralizing activity against homologous virus (results not shown). On the other hand, the warthog used in this investigation were 4 months old, which is older than the age at which most warthog

become infected (Plowright et al., 1969b). It is possible therefore that new-born animals would show even higher viraemias due to immunological immaturity.

Although the virus titres in lymph nodes (Table 1) were generally higher (1–2 $\log_{10}$ units) than those found in field investigations (Heuschele & Coggins, 1969; Plowright et al., 1969b), the persistence of virus in lymph nodes (with the marked exception of the mesenterics) at undiminished levels for a month is consistent with the finding of Plowright (1977) that the incidence of virus recovery from warthog lymph nodes decreased gradually over a period of years. However, Plowright et al., (1969b) were unable to infect domestic pigs by feeding them on homogenized warthog lymph node containing $10^{3.7}$ to $10^{6.1}$ HD_{50} of ASF virus. They therefore drew the conclusion that ingestion of warthog tissue by domestic pigs was not likely to be a mechanism whereby domestic pigs acquire the infection, as suggested by Montgomery (1921), Hammond & De Tray (1955) and Heuschele, Stone & Coggins (1965), and as is commonly believed to be the case by veterinarians and farmers in southern Africa. As can be seen from Table 2, however, one of the 2 pigs (No. 874) fed warthog tissues containing $10^{5.1}$ HD_{50} of virus developed acute ASF and died 9 days after being fed the minced tissue. Three of the 4 pigs fed between $10^{7.7}$ and $10^{7.8}$ HD_{50} of virus also contracted acute ASF and died 7 or 8 days later. This therefore demonstrates that ingestion of warthog tissue by domestic swine is indeed a potential method of ASF virus transmission.

Nevertheless, it must be conceded that the ingestion of warthog tissue is probably not the usual mechanism of transmission to domestic pigs, because it is likely that the quantity of virus fed to pigs 874 and 875 (Table 2) approaches the minimum oral infective dose (Maurer et al., 1954, quoted by Heuschele, 1967) and that these levels have not been found in the lymph nodes of adult free-living warthog (Heuschele & Coggins, 1969; Thomson & Gainaru, 1979, unpublished results), which are usually the source of offal fed to pigs.

One of the dilemmas with regard to the epizootiology of ASF is how the infection is maintained in tampan populations where infection rates are usually less than 1% and certainly no more than 5% (Pini, 1977; Plowright, 1977; Thomson & Gainaru, unpublished results). If warthog do not provide a source of infection, it is difficult to understand how sexual and transovarial infection (Plowright, 1977), which are inefficient mechanisms, ensure the survival of the virus. That these mechanisms are inefficient is demonstrated by the low infection rates in free-living tampan populations as indicated above. If, however, as seems likely from these results, a proportion of warthog develop a transient viraemia sufficient to be infectious for tampans feeding on them, it would explain not only how the virus survives but also why infection rates in tampans never reach a high level. Since susceptible warthog would only be present in any number during the farrowing season, it is reasonable to assume that this is the time (October to December in southern Africa) when exchange of infection between tampans and warthog occurs. How nearly 100% of susceptible warthog become infected

in this short time is not clear, but there are 3 possibilities: (a) even with these low infection rates the numbers of tampans present in warthog burrows ensure infection, (b) at the time of farrowing there is a "burst" of viral activity in the tampan population resulting from the ingestion of viraemic blood and (c) either contact transmission between young warthog or mechanical transmission effected by biting arthropods (tampans, Auchmeromyia larvae, biting-flies, etc.) ensures infection of young warthog.

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