Serological study of pigs for antibody against African swine fever virus in two areas of southern Malawi


Summary: A serological survey was conducted in July 1991 on domestic pigs in two areas of southern Malawi which were severely affected by the African swine fever (ASF) epizootic in 1989-1991. Sixty-six of the 216 owners questioned reported having witnessed ASF in their pigs. Forty-seven owners had pigs with antibodies against ASF virus, and the overall prevalence of pigs with anti-ASF virus antibodies was found to be 12.4%, in 445 pigs sampled in 35 villages. Spread of ASF was thought to occur principally through the slaughter and sale of infected animals, and due to the free-ranging of pigs. Permanent penning of pigs significantly reduced the attack rate ($\chi^2 = 7.59, P < 0.01, 1 \text{ df}$) in pig pens in Thyolo, an area where permanent penning of pigs was widely practised. Feeding of kitchen scraps did not appear to have been an important means of virus spread. Ornithodoros ticks were found in only 1 of the 35 villages. Although virus was not isolated from 203 pooled sera from pigs in the Mulanje district or from collected ticks, the seroconversion of a small proportion of pigs born after the last reported date of ASF occurrence suggests that the virus had continued to circulate to a limited extent in this area.

KEYWORDS: African swine fever – Carrier state – Malawi – Serological survey – Ticks.

INTRODUCTION

In 1989, a severe outbreak of African swine fever (ASF) was reported in the southern region of Malawi, which is estimated as having killed 30,000 of 35,000 pigs in the five affected districts within 10 months (2). A survey conducted in 1990 (1) reported that over 1,000 pigs had survived the ASF infection and that carriage of virus in a proportion of these animals could therefore constitute a potential reservoir of infection.
This paper presents the results of a serological survey and farmer questionnaire survey conducted in two districts in the southern region (Fig. 1) to determine the prevalence of pigs recovered from ASF, and to provide information on the effect of the ASF outbreak on smallholder pig-keeping. The Mulanje and Thyolo districts were previously identified as being severely affected by the outbreak. Pig husbandry was believed to differ slightly between the areas. A total of 35 villages known to have been affected by the outbreak were chosen, and all 216 pig owners in these villages were questioned to provide detailed background information. Blood samples were taken from all pigs over three months old wherever possible. A total of 445 sera were collected and analysed for antibodies against ASF virus (ASFV) by indirect enzyme-linked immunosorbent assay (ELISA).

The presence or absence of the soft tick Ornithodoros moubata, which can act as a biological vector of ASFV, was investigated in each village to determine the distribution of this tick in the affected area of the Mulanje and Thyolo districts.

MATERIALS AND METHODS

Collection of questionnaire data

Interviews were conducted with all the current pig owners in the 35 selected villages which had been affected by ASF during the 1989-1990 outbreak. Many people had stopped keeping pigs after the outbreak and were therefore not interviewed. A total of 216 pig owners who kept 529 pigs were questioned. The aim was to determine the history of ASF in pigs kept by the owner, and to obtain information about husbandry and trade in pigs. Owners were questioned for evidence of free-range husbandry of pigs and the feeding of waste from food preparation. Owners were also shown an Ornithodoros tick and asked if such ticks were present in their village.

Collection of sera

Blood samples were taken from all pigs over three months of age, except when heavily pregnant or recently farrowed, or when the owner could not be located. A total of 445 sera were collected for analysis. Sera were frozen and transported on ice to the Central Veterinary Laboratory in Lilongwe, for analysis by indirect ELISA using a modification of the method described by Sanchez Vizcaño et al. (7).

Analysis of sera

ELISA plates were coated with 50 µl per well of 1:200 dilution ASFV protein 73 (VP73; supplied by the Institute for Animal Health [IAH] in Pirbright, United Kingdom) in 0.05M carbonate/bicarbonate buffer (pH 9.6) and kept at 4°C overnight. After washing, 25 µl of phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBST) and 2% (w/v) dried skim milk powder were added to each well. Following this, 25 µl of each test serum were diluted 1:15 in PBST and added to each test well, to give a final dilution of 1:30. Control positive and negative sera (four wells of each), at a final concentration of 1:30, were added to appropriate wells and incubated at 37°C for 1 h. After washing in one-fifth strength PBS (pH 7.4), 50 µl per well of horseradish peroxidase-conjugated protein A at 1:3,000 dilution in PBST containing 1% (w/v) dried skim milk powder was added, and the plates were incubated at 37°C for 1 h. Ortho-phenylenediamine hydrochloride (OPD), at a final concentration of 0.04% (w/v), was produced using 30 mg OPD tablets in 75 ml of distilled water. After final washes of the
ELISA plate, 50 µl of this preparation were then added to each well. After 10 min, the reaction was stopped by the addition of 50 µl of 1 M H₂SO₄ to each well. All washing stages consisted of five washes with PBS diluted 1:4 in distilled water.

Each serum was tested in at least duplicate. Control sera used in each plate were pools of sera from indigenous Malawi pigs which were known to be positive or negative for anti-ASFV antibody.

**Collection and analysis of ticks**

Ticks collected from *kholas* (pig houses) were packed in plastic tubes, kept at ambient temperature and sent to the IAH in Pirbright within three weeks of collection. Primary cultures of pig bone marrow (PBM) cells were used for the detection of infectious virus, using the method described by Malmquist and Hay (6). Homogenates of ticks which had been pooled in groups as nymphs, larvae or adults were centrifuged at 700 ml and the supernatant assayed for virus by the inoculation of 0.33 ml of 1:10 and 1:100 dilutions of inoculum per PBM tube, with three tubes per dilution. Tubes were examined daily for haemadsorption or cytopathic effect for fourteen days post-inoculation.

**Virus isolation from pooled sera**

A total of 203 sera collected from the Mulanje area were transported on ice to the IAH. Sera were pooled by village, and PBM cell cultures were used for the detection of infectious virus.

**Fig. 1**

Location within southern Malawi of the villages investigated for evidence of antibodies to African swine fever in pigs
RESULTS

Losses due to African swine fever

Thirty-one percent (66/216) of pig owners reported losses due to ASF. Loss of 100% of pigs through direct ASF mortality and slaughter of sick pigs at the time of the outbreak was reported by 57% and 48% of pig owners in Thyolo and Mulanje, respectively. Losses of 50% or less were reported by only 6% of pig owners in both areas.

Death and slaughter were the causes of the loss of 79% and 83% of pigs in affected *kholas* in Thyolo and Mulanje, respectively (Table I). A higher percentage of pigs were slaughtered in affected *kholas* in Mulanje (35%) than in Thyolo (8%). This difference is further emphasised by the fact that pig meat was sold during the outbreak by 48% (15/31) of owners of affected pigs in Mulanje, compared to only 14% of owners of affected animals in Thyolo.

<table>
<thead>
<tr>
<th>Area</th>
<th>No. (%) of pigs</th>
<th>Dead</th>
<th>Slaughtered</th>
<th>Recovered</th>
<th>Apparently unaffected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyolo</td>
<td>144 (71)</td>
<td>17</td>
<td>2 (1)</td>
<td>41 (20)</td>
<td>204 (100)</td>
<td></td>
</tr>
<tr>
<td>Mulanje</td>
<td>69 (48)</td>
<td>50</td>
<td>2 (1)</td>
<td>23 (16)</td>
<td>144 (100)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>213 (61)</td>
<td>67</td>
<td>4 (1)</td>
<td>64 (18)</td>
<td>348 (100)</td>
<td></td>
</tr>
</tbody>
</table>

When questioned further, villagers in Mulanje said that the sale of affected meat was commonplace in local markets during the outbreak, and that the price of pig meat had dropped from K2.5/kg to as little as K0.2/kg (K1 = US$0.3 [1990 rate]).

Of the 19% of pigs in affected *kholas* reported as alive after the outbreak, only four (1% of total) were reported to have recovered from clinical disease.

Effect of management on losses due to African swine fever

All owners provided their pigs with a pen or pig house (*kholas*). This took various forms but was commonly constructed from wood or mud bricks with a thatched roof. The permanent penning of pigs varies between the Thyolo and Mulanje areas, as illustrated in Table II. In Thyolo, 87% of pig owners said they did not allow their pigs to range free at any time in the year. A significant difference ($\chi^2 = 7.59, P < 0.01, 1$ df) was found between the frequency of ASF outbreaks reported in Thyolo by pig owners who allowed their pigs to range free at some time, and that reported by owners who did not. Those who allowed pigs to range free reported ASF with a higher frequency than those who permanently penned their pigs.

In contrast, only 48% of pig owners in Mulanje permanently penned their animals, and therefore more pigs were allowed to range free at some point in the year, usually to scavenge for food in the dry season. Unlike the situation in Thyolo, permanent penning in Mulanje did not significantly affect the frequency of ASF outbreaks reported by pig owners.
TABLE II
Reported occurrence of African swine fever (ASF) in free-ranging and permanently-penned pigs in two areas of southern Malawi

<table>
<thead>
<tr>
<th>Area</th>
<th>No. of villages</th>
<th>No. of pig owners reporting ASF occurrence</th>
<th>Permanent penning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Some free ranging ASF</td>
<td>No ASF</td>
</tr>
<tr>
<td>Thyolo</td>
<td>19</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Mulanje</td>
<td>16</td>
<td>18</td>
<td>42</td>
</tr>
</tbody>
</table>

The most commonly-reported feedstuffs for penned pigs were maize bran and sweet potato leaves. Kitchen scraps were used as an occasional food source in Thyolo and Mulanje by 60% and 48% of pig owners, respectively. There were no statistically-significant relationships between the feeding of kitchen scraps and the frequency of ASF in pigs in either area.

Distribution of Ornithodoros ticks

Ornithodoros ticks were found in only one of the 35 villages in the two areas studied, namely Nalikata in Mulanje district. In this village, ticks were found in two kholas belonging to one owner: one of the two mud brick kholas was heavily infested, while only a few ticks were found in the other khola 20 metres away. Other kholas in Nalikata village were examined thoroughly but no more ticks were found.

Approximately 400 ticks were collected from the heavily-infested khola, but virus was not isolated from these ticks in PBM cultures.

Serological survey in Thyolo and Mulanje

The overall prevalence of antibodies against ASFV in the 445 pigs sampled in the Thyolo and Mulanje areas was 12.4% (55/445), as shown in Figure 2. No significant difference ($\chi^2 = 2.44, P > 0.05, 1 \text{ df}$) in the proportion of seropositive pigs was found between the two areas.

![Figure 2](image)

Proportion of pigs with serum antibodies against African swine fever virus in two areas of southern Malawi
Pigs with antibodies against ASFV were found in 10/19 (53%) and 11/16 (69%) of the sampled villages in Thyolo and Mulanje, respectively. Therefore, approximately 40% of the villages affected by the ASF outbreak did not contain any pigs with anti-ASFV antibody at the time of the July 1991 survey. In villages which did contain pigs with anti-ASFV antibody, between 4% and 27% of pigs were seropositive in villages in Thyolo compared to between 4% and 67% in villages in Mulanje.

The population structures of pigs with antibodies against ASFV in Thyolo and Mulanje are shown in Figure 3. The highest proportion of seropositive pigs was in the 18- to 23-month age group, but in both areas a percentage of pigs in all age groups showed antibodies against ASFV. From the questionnaire, the last reported occurrence of ASF was in October 1990 in Thyolo, and in February 1991 in Mulanje. At the time of sampling, however, seven seropositive pigs in Mulanje were in the three- to five-month age range. These animals must therefore have been born after the last reported outbreak in each area.

![Age-related profile of pigs with serum antibodies against African swine fever virus in southern Malawi in July 1991](image)

Of the 20 pigs reported to have remained alive after ASF had occurred in affected kholas, seven were positive for anti-ASFV antibody. Six of these were said to have shown no clinical signs, and one was reported to have recovered from the disease.

**Virus isolation**

ASFV was not isolated from pooled sera in Mulanje. This suggests that there was no active disease present in these villages at the time of sampling. In contrast, there is no indication that pigs were not virus carriers, as viraemias are considered infrequent and of short duration. In addition, the sensitivity of virus isolation from pooled sera may have been reduced by the presence of antibody against ASFV in some of the sera.
DISCUSSION

The difference in pig husbandry between the two areas of Malawi appears to have had a significant effect on the epidemiology and control of the disease in these two areas. A significantly higher frequency of ASF was reported by the small number (13%) of owners in the Thyolo area who allowed their pigs to range free. In the Mulanje area, however, the majority (57%) of owners allowed pigs to range free, and no significant reduction in ASF incidence was reported by owners practising permanent penning in this area. The higher percentage of owners slaughtering and selling meat from affected kholas may have been the result of greater difficulties in implementing control measures in the Mulanje area. The neighbouring Phalombe area was not included in the area where restrictions were placed on the slaughter and sale of pig meat until the outbreak was well established (A. Souter, personal communication, 1991), and may thus have acted as an outlet for infected meat.

The results of the serological survey show that 12.4% of pigs present in the sampled villages in July 1991 had experienced ASFV infection, and a proportion of these would be expected to have carried ASFV for at least five months after infection if the carriage of virus in Malawian village pigs is similar to that in European breeds (11). No significant difference was found in prevalence of anti-ASFV antibodies between pigs sampled in Thyolo and Mulanje.

The true significance of the presence of anti-ASFV antibody in pigs in the population in southern Malawi is not fully understood. Experimental work suggests that recovered pigs only occasionally experience recurrences of fever and viraemia (9, 11). Stress-induced viraemia has been demonstrated, with difficulty, in pigs with antibodies against ASFV (11), but transmission of ASF through viral excretion by carriers has not been demonstrated. It is possible that ASFV persists in a cell-associated latent form, which is not recovered by standard virus isolation procedures, but which is capable of being reactivated, resulting in the reappearance of infectious virus. Theoretically, the viraemias which have been recorded in carriers were high enough for infection and transmission by ticks (10). It can therefore be suggested that the role of the carrier could be important where ticks are present as vectors and reservoirs of the virus.

In areas where ticks are extremely uncommon, such as Thyolo and Mulanje, the carrier may be a potential hazard in a number of ways. It has been shown that if animals are slaughtered within six months of infection, virus can be isolated from lymph nodes in sufficient quantities to infect susceptible pigs per os (11). Virus has not been isolated later than six months, except in the transient episodes of viraemia mentioned above. Carriers which become viraemic appear normal and will pass routine meat inspection (5). These pigs, like other viraemic animals, could present an important source and means of virus dissemination if slaughtered and sold. The frequency of viraemic carriers under field conditions requires further investigation, however, especially in the typical stressful conditions of poor nutrition, ectoparasite infestation and intercurrent disease which regularly occur under traditional pig husbandry in Malawi.

Outbreaks of ASF in the southern region of Malawi have been reported sporadically since 1935, and the disease appears to occur in epidemic form. Field data show that there were periods between these epidemics when clinical cases were not reported. This differs from the situation experienced in the central region of Malawi; field and laboratory data collected from the central region indicate that virus has been circulating within parts of that region since 1981 (3), in an area which has been considered enzootic
for ASF since 1923 (8). One striking difference between the Thyolo and Mulanje localities and the enzootic area of the central region is the presence of Ornithodoros ticks. Thus, although the most likely means of spread of virus during an outbreak is the slaughter of pigs and sale of infected pig meat, some credence may be given to the suggestion of Haresnape et al. (4) that ticks play an important role as a reservoir of virus and vector of the disease in parts of the central region.

Although parallels can be drawn between outbreaks in the past and now, there has been an enormous increase in the mobility and density of the human population, and thereby also in trade in pig products. Movement of infected pig meat, particularly through roadside sales, is probably the most important method of dissemination of the virus and a serious problem in confinement of infected areas. Thus, although carriers of ASFV in Thyolo and Mulanje are a potential source of virus, their importance has to be considered in terms of the current ASF situation in Malawi as a whole. Spread of ASF from the central region along the national highway presents a serious threat to the long-term productivity of pig-keeping in the south, as the outbreak which started in 1989 – after spread from the central region – persisted for at least sixteen months (2). A relatively low rate of introduction of virus from the enzootic area could therefore result in continuous presence of ASFV in pigs in the southern region.

ACKNOWLEDGEMENTS

The authors would like to thank the Chief Veterinary Officer in Malawi, Dr J. Msiska, for his assistance, and the Overseas Development Administration of the Government of the United Kingdom for financial support of the visit of E.C. Allaway. The invaluable assistance of Mr K. Sezamanja is gratefully acknowledged, and field veterinary staff and villagers are thanked for their co-operation.

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Résumé : Une enquête sérologique a été effectuée en juillet 1991 sur des porcs domestiques dans deux régions du sud du Malawi, particulièrement touchées par une épidémie de peste porcine africaine entre 1989 et 1991. Sur 216 éleveurs interrogés, 66 ont signalé la présence de la maladie dans leur élevage. Quarante-sept d'entre eux avaient des porcs qui possédaient des anticorps vis-à-vis du virus de la peste porcine africaine ; la prévalence totale d'animaux ayant ce type d'anticorps était de 12,4 % sur 445 porcs ayant fait l'objet de prélèvements dans 35 villages. La propagation de la maladie a été attribuée essentiellement à l'abattage et à la vente d'animaux infectés ainsi qu'au type d'élevage (porcins en liberté dans les villages). L'élevage permanent en porcherie a considérablement réduit le taux d'incidence ($\chi^2 = 7,59; P < 0,01; 1$ddl) à Thyolo, région où ce type d'élevage est largement répandu. L'alimentation des porcs à base de déchets de cuisine ne semble pas avoir joué un rôle majeur dans la propagation du virus. Des tiques du genre Ornithodoros ont été trouvées seulement dans un village sur 35. Bien que le virus n'ait pas été isolé dans les 203 mélanges de sérum provenant de porcs du district de Mulanje ni chez les tiques, l'apparition
d'anticorps chez quelques rares porcs parmi ceux qui sont nés après le dernier cas déclaré de peste porcine africaine montre que le virus a continué à circuler dans cette région, mais faiblement.


Resumen: En julio de 1991 se llevó a cabo una encuesta basada en exámenes serológicos de cerdos domésticos procedentes de dos regiones del sur de Malawi que habían resultado muy afectadas por la epizootia de peste porcina africana (PPA) de 1989-1991. Sesenta y seis de los 216 propietarios interrogados refirieron haber observado la enfermedad en sus cerdos. Cuarenta y siete propietarios poseían cerdos con anticuerpos contra el virus de la PPA. A partir de una muestra de 445 ejemplares, procedentes de 35 pueblos, la prevalencia total de cerdos con anticuerpos contra el virus de la PPA se estimó en un 12,4%. El sacrificio y la venta de cerdos infectados, junto al tipo de cría sin corrales fueron considerados los mecanismos básicos de propagación de la PPA. En Thyolo, zona en la que es práctica común la estabulación permanente de los cerdos, la tasa de ataque de la enfermedad era significativamente menor ($X^2 = 7,59$; $P < 0,01$; 1 df). La alimentación a base de basura de cocina no parecía haber constituido un mecanismo importante de transmisión del virus. Sólo en uno de los 35 pueblos se encontraron garrapatas de la especie Ornithodoros. A pesar de que el virus no se aisló ni en las 203 mezclas de suero de cerdos provenientes del distrito de Mulanje ni en las garrapatas que pudieron recogerse, la seroconversión observada en algunos de los animales nacidos tras el último caso de PPA registrado parece indicar que el virus seguía circulando en una forma limitada en esa región.


REFERENCES


