

A study of African swine fever virus infected ticks (*Ornithodoros moubata*) collected from three villages in the ASF enzootic area of Malawi following an outbreak of the disease in domestic pigs

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SUMMARY

A detailed study was made in 1983–5 in three villages in Mchinji district in the African swine fever (ASF) enzootic area of Malawi, following an outbreak of ASF which affected all three villages.

Ticks of the *Ornithodoros moubata* complex were collected from both pig sties and houses shortly after the outbreak, and approximately 24% contained ASF virus. The proportion of ticks infected did not differ significantly in the three villages, or more surprisingly in different types of premises, and was equivalent in all stages of ticks. The proportion infected decreased with the passage of time, but infected ticks were still present in all three villages 8 months after the outbreak, some with high titres of virus.

The proportion of seropositive pigs in the three villages approached 100% following the outbreak, with many apparently healthy pigs being seropositive. It is suggested that Malawian isolates of ASF virus may be less virulent in African than European breeds of domestic pig.

INTRODUCTION

This investigation followed an African swine fever (ASF) outbreak amongst village pigs in the south-west part of Mchinji district in the Central Region of Malawi (Fig. 1). This area is at the centre of a large enzootic area covering about 8000 km² of the Central Region, extending westwards into the Eastern Province of Zambia and southwards into Tete Province of Moçambique (Haresnape, Lungu & Mamu, 1987). Mchinji district was described as being infected with swine fever, presumably ASF, as long ago as 1923 (Turnbull, 1933) and was first described as an endemic area in 1931 (Turnbull, 1932). Heavy losses occurred as a result of the disease in Mchinji district in 1948 (Stuchbery, 1949), and further outbreaks have been reported on many subsequent occasions. More recently outbreaks of ASF

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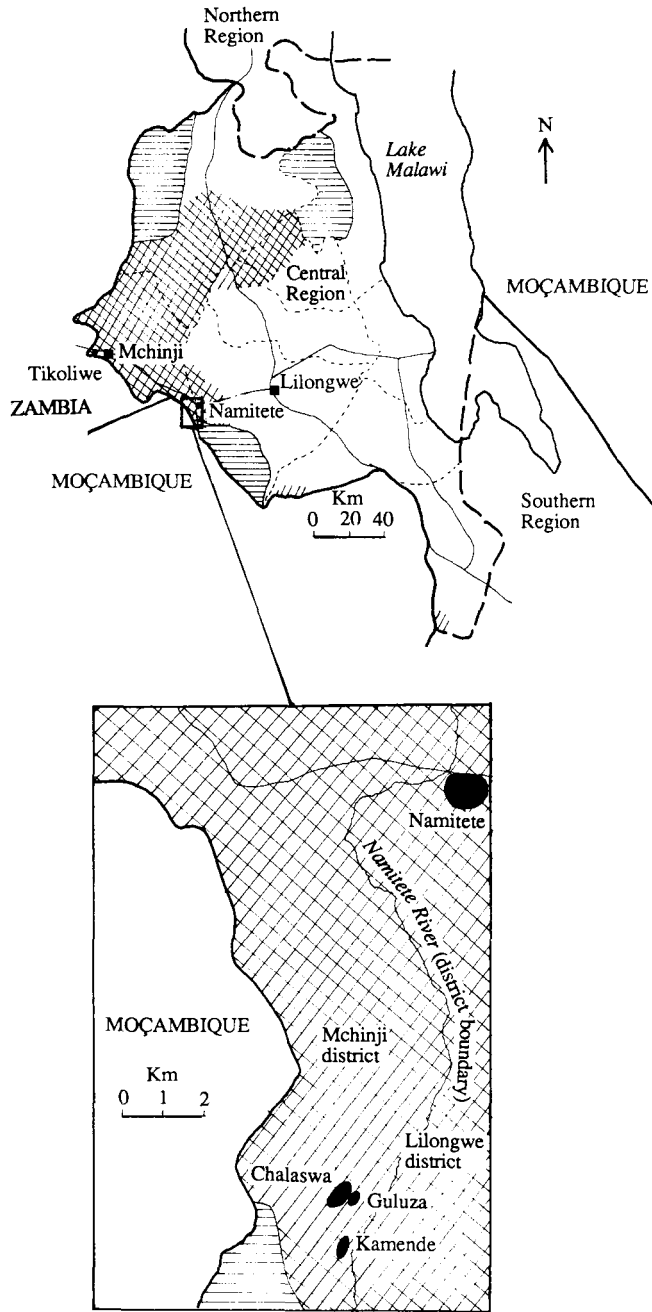


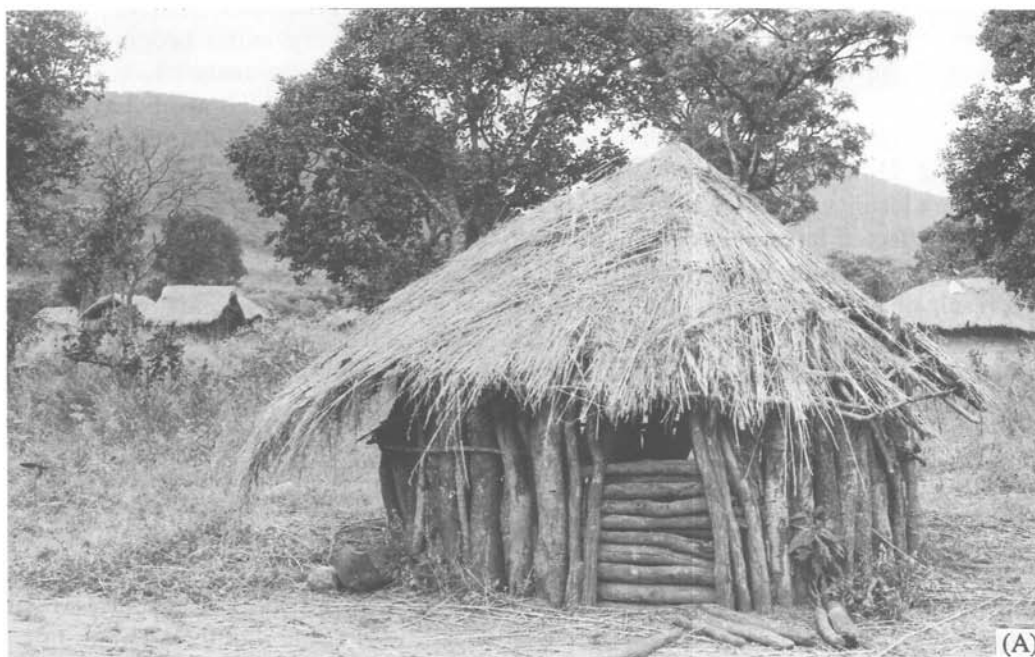
Fig. 1. Location of Chalaswa, Guluza and Kamende within the ASF enzootic area in the Central Region of Malawi. —, International boundary; - - - -, Regional boundary; ·····, District boundary; —, main road; ≡, National Park, Game or Forest Reserve; ×××, ASF enzootic area (Haresnape, Lungu & Mamu, 1985); ///, Probable extension of ASF enzootic area (Haresnape, Lungu & Mamu, 1987); ■, District centre; ●, Village.

have been reported in the district every year since 1982, but generally with mortality substantially less than 100%, and only a very small proportion of reported outbreaks have been confirmed by laboratory tests. Laboratory confirmation was obtained for only 2 of the 19 outbreaks which are believed to have occurred in the district between 1981 and 1986 (Haresnape, 1984; Haresnape, Lungu & Mamu, 1985; Haresnape, Lungu & Mamu, 1987). One of these was in Bongera locality in 1983 where at least 130 pigs died, but estimates of the numbers of pigs affected have always been difficult to obtain. Since many more outbreaks probably go unreported, it is likely that several thousand village pigs have died as a result of ASF in the enzootic area of Malawi over the last few years, and many more in epizootics outside the enzootic area which are characterized by a mortality approaching 100% (Haresnape, Lungu & Mamu, 1985).

A serological survey undertaken between 1981 and 1984 included 10 localities in Mchinji district, and seropositive pigs were identified in all of them. Between 21 and 74% of the pigs were seropositive and highest proportion was in the south-west part of the district. Mortality was estimated from serological data to be between 47 and 83% for one locality in the district and between 77 and 92% for another (Haresnape, Lungu & Mamu, 1985).

It has been suggested that most outbreaks of ASF outside the enzootic area probably start following introduction of infected meat from an affected area, but that some outbreaks within the enzootic area may represent new incursions of virus from a natural reservoir (Haresnape, Lungu & Mamu, 1985). The soft tick, *Ornithodoros moubata*, which is known to be a reservoir of ASF virus and vector of the disease (Plowright, Parker & Pierce, 1969; Thomson *et al.* 1983), is common both in houses and pig pens (kholas) in the enzootic area, particularly in Mchinji district (Haresnape & Mamu, 1986). Collections of these ticks were started in 1982, and examined for the presence of ASF virus. Up to November 1983, 1262 ticks collected from pig kholas in eight villages in Mchinji district were tested, and only three were infected. None of the 977 ticks collected from houses in the same villages were infected. The three infected ticks were from kholas in three different villages in the west part of Mchinji district and there had been suspected cases of ASF in all three just prior to the time of collection (Haresnape, Wilkinson & Mellor, 1988). The overall prevalence of infection in the 364 ticks from these three kholas was 0.8%.

One batch of 297 ticks, of which none was infected, was collected in July 1983 from pig kholas in Chalaswa, a village in the south-west of Mchinji district which suffered an ASF outbreak 4 months later. The cause of the outbreak is not known but it may have started following a bite from an infected tick, or been introduced by an infected pig or pig meat. The work presented here describes an investigation of the prevalence of ASF virus infection in ticks collected in Chalaswa and two adjacent villages following this outbreak, the virus titres in infected ticks and serological examination of the pigs which survived the outbreak.



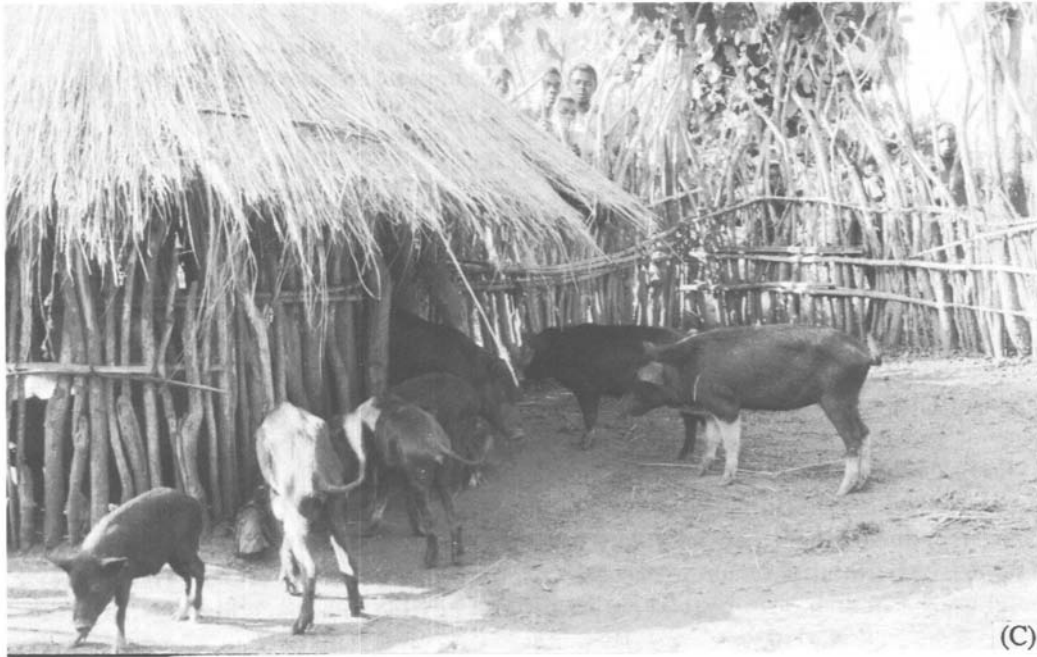


Fig. 2. Typical pig kholas in Mehinji district. (A) Small khola. (B), (C) Kholas with outer fence. The close proximity of house and pig khola is most clearly seen in (B).

MATERIALS AND METHODS

Collection of ticks

O. moubata were collected from both domestic pig kholas (Fig. 2) and houses in the villages Chalaswa, Guluza and Kamende, by staff of the Central Veterinary Laboratory (CVL), Lilongwe. Chalaswa and Guluza are contiguous, and Kamende is situated approximately 1 km to the south (Fig. 1). Ticks from different premises were collected separately and the owner and type of premises (khola or house), and their relative location in the village noted. The first collection was made in November 1983 approximately 3 weeks after the reported ASF outbreak in the area, and a further six collections were made over the following 12 months. A final collection was made in September 1985, nearly 2 years after the outbreak. Ticks were packed in plastic tubes which were put in sealed plastic bags and despatched by air to the Pirbright Laboratory of the Institute for Animal Health, UK within a few days of collection.

Collection of interview data

All pig-owners in Chalaswa, Guluza and Kamende were interviewed in November 1983 and asked how many pigs they owned, whether any had died, and if so the numbers which died, with dates, clinical signs and duration of illness. Those deaths which seemed likely to be the result of ASF were identified and a presumptive diagnosis was based on an assessment of clinical details given by the pig-owner (Haresnape, Lungu & Mamu, 1985). Numbers of surviving pigs in each

khola were noted, and these were visited regularly over the following 9 months, any sickness noted, and changes in number of pigs in each khola as a result of death or other reasons were recorded. No detailed interviews were conducted after August 1984.

Serology

Blood samples were taken from the ear vein from apparently healthy pigs at intervals throughout the study. Pigs were not individually tagged so some pigs may have been sampled on more than one occasion. Sera were tested at CVL, Lilongwe using the ELISA test described previously (Haresnape, Lungu & Mamu, 1985). Antibody titres were measured using an adaptation of this test, and titres expressed as \log_{10} of the highest serum dilution at which a clear reaction was seen in the ELISA test.

Assay of ASF virus in ticks

Adult females, adult males, large nymphae (N4, N5) and small nymphae (N1, N2, N3) in each batch of ticks were counted, and assayed either individually or in pools. Individual ticks or pools were ground up in 2 ml of diluent (phosphate-buffered saline containing 1% ox serum and antibiotics), or 5 ml diluent for the larger pools. For the November 1983 collection, maximum pool sizes of 3 for adult females, 5 for adult males, 19 for large nymphae and 22 for small nymphae were chosen. Adults from later collections were assayed individually, and maximum pool sizes of 6 for large nymphae and 13 for small nymphae were used. Virus was assayed by haemadsorption (HAD) in pig bone marrow cultures at Pirbright (Haresnape, Wilkinson & Mellor, 1988). Titres were expressed as \log_{10} 50% haemadsorbing doses (HAD₅₀) per tick or per pool.

Estimation of proportion of ticks infected (p)

The proportion (p) of ticks infected with ASF virus was estimated using the formula

$$N = (1 - p)^s,$$

where N = proportion of pools negative, s = pool size. The proportion infected (p) was estimated for each value of s , and a mean value of p , weighted in favour of low pool size, was estimated for different stages of tick in each village and each type of premises.

The 95% confidence interval (CI) was calculated as recommended by Gardner & Altman (1986) using the normal approximation to the binomial distribution

$$p = \pm 1.96 \sqrt{\frac{p(1-p)}{n}},$$

where p = estimated proportion of ticks infected, n = number of ticks.

RESULTS

The ASF outbreak of November 1983

When the three villages Chalaswa, Guluza and Kamende were visited on 28 November 1983, no sick pigs could be found and those which had died had all been

Table 1. Deaths from ASF in Chalaswa, Guluza and Kamende in November 1983

	Affected kholas		Unaffected kholas	
	Number of kholas	Number of dead pigs Total pigs	Number of kholas	Number of dead pigs Total pigs
Chalaswa	8	36/45	7	0/42
Guluza	2	14/14	2	0/16
Kamende	2	8/9	4	0/19
Total	12	58/68 (85%)	13	0/77

disposed of. Interviews with pig owners revealed that some pigs had died in each of the three villages at the beginning of November after showing symptoms characteristic of ASF. These were generally described as sudden onset of fever, weakness, vomiting, loss of appetite, loss of balance or weakness in the hindquarters and refusal to walk. The duration of illness reported varied from 1 day to 2 weeks. Descriptions of post-mortem findings were difficult to obtain, but three owners reported that internal organs were unusually dark in colour. Laboratory confirmation was not obtained as no samples were submitted to the laboratory. A total of 58 pigs from 12 kholas were reported to have died in the outbreak (Table 1). In seven kholas all the pigs died and a total of ten pigs were still alive in the other five affected kholas at the end of December. Sera collected from five of the survivors were all antibody-positive. The presumptive diagnosis of ASF was based on owners descriptions of the disease together with this serological evidence.

Tick collection of November 1983

A total of 1445 ticks were collected from the area on 28 and 29 November 1983, these being 813, 354 and 278 from Chalaswa, Guluza and Kamende respectively. The majority were from pig kholas, but the collection included 255 ticks from houses.

Of the 13 kholas from which ticks were collected, 9 had lost pigs during the ASF outbreak 3 weeks earlier and 4 were apparently unaffected. No ticks could be found in the other 3 of the 12 affected kholas (Table 1). Virus was isolated from ticks collected from all 9 affected kholas and 3 of the 4 apparently unaffected kholas. Infected ticks were also present in 7 of the 8 houses sampled.

Estimated prevalence of infection in November 1983

The calculation of the proportion of ticks infected (p) showed that an estimated 0.24 of all the ticks collected in November 1983 were infected with virus (95% confidence interval 0.22–0.26, Fig. 3D). The p values of 0.23, 0.24 and 0.23 estimated for Chalaswa, Guluza and Kamende are not significantly different from one another (95% CIs 0.20–0.26, 0.19–0.28 and 0.18–0.28 respectively, Fig. 3A), nor are the p values of 0.21 (95% CI 0.16–0.26) for adult females, 0.22 (95% CI 0.19–0.26) for adult males and 0.24 (95% CI 0.21–0.27) for nymphae (Fig. 3C). Surprisingly, the p value of 0.31 (95% CI 0.25–0.37) estimated for ticks collected from houses was considerably higher than that of 0.23 (95% CI 0.20–0.25)

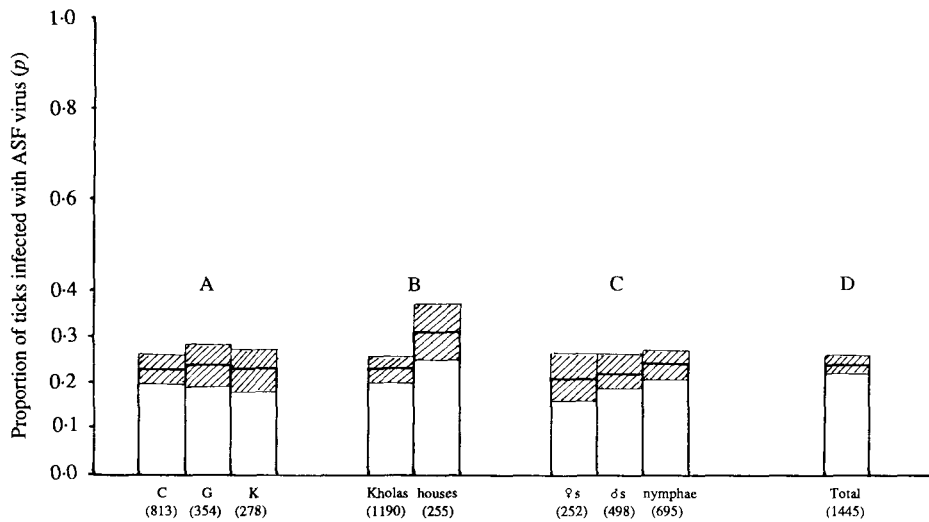


Fig. 3. Comparison of the proportions of ticks infected with ASF virus in November 1983. (A) Ticks collected from Chalaswa (C), Guluza (G) and Kamende (K). (B) Ticks collected from pig kholas and houses. (C) Adult female ticks, adult male ticks and nymphae. (D) Total. Figures in parentheses refer to numbers of ticks tested. Shaded area shows 95% confidence interval.

estimated for ticks from pig kholas (Fig. 2B), but this difference is of only borderline significance at the 5% level.

Interview data

No evidence of any further deaths from ASF could be found when the three villages were revisited in December 1983 or on any of the subsequent visits. Only 1 of the 12 affected kholas had been restocked by the time the last detailed interviews were made in August 1984. Most farmers who lost all their pigs responded by turning their attentions away from pig keeping but those who had some pigs which survived waited to build up stocks by breeding from the survivors.

Serology

Some sera had been collected from Chalaswa before the ASF outbreak and more were collected from all three villages at intervals afterwards. Only 1 of the 12 sera collected in July 1983, 4 months before the outbreak, was antibody-positive. A few weeks after the outbreak, 5 of the 7 sera tested were positive. The two seronegative pigs were from affected kholas but presumably had either not yet become infected, or had not yet responded by producing sufficient antibodies to detect. Both were antibody-positive when sampled again 2 months later. Sera were collected on three subsequent occasions in 1984, from both affected and unaffected kholas, and a high proportion (81–100%) were antibody-positive on each occasion (Table 2). Most pigs had therefore been infected with virus although they had apparently not shown any obvious signs of disease. Thirty-two seropositive pigs from apparently unaffected kholas were present throughout the study period. Antibody-positive

Table 2. Percentage of seropositive pigs before and after the ASF outbreak of November 1983 in Chalaswa, Guluza and Kamende

Date	Sera positive (%)
	Number tested
July 1983	1/12 (11)
November 1983	5/7 (71)
January 1984	14/14 (100)
March–April 1984	27/33 (81)
August 1984	34/34 (100)
April 1986	6/12 (50)

pigs may be protected against disease although not against reinfection (Wilkinson, 1984). One further collection of 12 sera was made in the area 2 years later, and only 6 (50%) were antibody-positive. The 6 seronegative pigs were all young animals born since the ASF outbreak, although 4 of them were from kholas in which virus-infected ticks had been demonstrated.

Some sera were titrated, and antibody titres remained high throughout the study period. Titres of \log_{10} 3.1, \log_{10} 3.4, \log_{10} 4.0, \log_{10} 4.0 and \log_{10} 4.3 were measured in 5 pigs in November 1983, \log_{10} 3.4 and \log_{10} 3.4 in 2 pigs in January 1984, \log_{10} 3.1, \log_{10} 3.4, \log_{10} 3.4 and \log_{10} 4.0 in 4 pigs in March–April 1984 and \log_{10} 3.1, \log_{10} 3.1, \log_{10} 3.1 and \log_{10} 4.9 in 4 pigs in August 1984.

Subsequent tick collections

Ticks collected from both kholas and houses after the initial collection of November 1983 totalled 4272 (210, 565, 419, 307, 801 and 832 in January, March, April, July, August and November 1984 respectively, and 1138 in September 1985). The January 1984 collection was smaller than the others because it followed an attempt by Ministry of Health officials to reduce tick infestation by spraying with acaricide. Where possible the same premises were sampled on each occasion (Tables 3 and 4). Seven kholas in which pigs had died of ASF were sampled regularly and six of these contained infected ticks in March 1984. Two still contained infected ticks in July, 8 months after the ASF outbreak, and both of these (C3 and K1, Table 3) housed apparently healthy survivors. In the case of C3, sera collected in January 1984 from each of the four survivors were positive. Three of these were bled again in August 1984 and were still seropositive. No serum was collected from the survivors in khola K1. Three of the kholas in which pigs had died remained empty following the outbreak but ticks were collected regularly and two (C1 and C2) contained infected ticks 5 months after they last housed any pigs.

Five unaffected kholas were sampled between 2 and 5 months after the ASF outbreak and infected ticks were found on at least one occasion in each of them (C6, C7, G3, K3 and K4, Table 3). Three still housed infected ticks in April 1984, 5 months after the outbreak, and all housed apparently healthy pigs. The owner of one unaffected khola in Kamende (K3) sold his pigs and later restocked but the pigs remained healthy despite the presence of infected ticks in the khola. The newly introduced young pigs were seropositive when sampled shortly after their

Table 3. *Presence of ticks infected with ASF virus in kholas*

Premises	Number of ticks† from which ASF virus was isolated/Number tested							
	Nov. 83	Jan. 84	Mar. 84	Apr. 84	July 84	Aug. 84	Nov. 84	Sep. 85
Affected kholas								
C1	6/67	1/20*	1/53*	1/26*	0/23*	0/20*	0/3	0/30
C2	11/132*	2/12*	4/118*	1/38*	0/10*	0/22*	0/15	0/33
C3	18/98	2/6	4/35	0/21	1/44	0/19	0/108	0/26
C4	17/152	1/7	NC	NC	NC	0/31	0/20	0/55
C5	14/83	NC	NC	NC	NC	0/19	NC	0/36
G1	7/56*	0/5*	0/31*	0/38*	0/20*	0/21*	0/76	0/15
G2	9/70	2/12	1/7	1/7*	0/8*	0/20*	0/63	NC
K1	6/34	0/3	1/61	0/30	1/33	0/29	NC	0/30
K2	9/130	0/9	1/19	0/23	0/16	0/20	0/74	0/33
Subtotal	97/822	8/74	12/324	3/183	2/154	0/201	0/359	0/258
Unaffected kholas								
C6	21/142	1/6	0/16	0/54	0/16	0/19	0/40	0/36
C7	NC	NC	0/15	1/32	0/3	0/19	0/7	0/80
C8	NC	NC	NC	NC	NC	0/33	0/49	0/36
C9	NC	NC	NC	NC	NC	0/74	0/63	0/28
G3	14/129	NC	2/34	1/14	0/24	0/19	0/69	NC
K3	7/79	4/27*	2/43	1/12	0/20	0/40	0/5	NC
K4	0/18	0/7*	NC	NC	NC	0/19*	0/12	0/64
Subtotal	42/368	5/40	4/108	3/12	0/63	0/223	0/245	0/244
Total	139/1190	13/114	16/432	6/295	2/217	0/424	0/604	0/502

Letters C, G and K refer to Chalaswa, Guluza & Kamende.

NC, Not collected.

* No pigs living in khola at time of collection.

† Number of tick pools in the Nov. 83 collection.

introduction to the khola, but whether or not they were seronegative beforehand is not known.

Eight out of nine houses sampled within 3 months of the ASF outbreak contained infected ticks, and one infected tick was found in a house (G5, Table 4) in July 1984, 8 months after the last reported deaths from ASF.

Subsequent estimates of prevalence of infection

The estimated percentage of ticks infected with ASF virus was 24% in November 1983 and this had fallen to 11.0% by January 1984 and to 1.0% in July 1984, 8 months after the ASF outbreak (Fig. 4). None of the ticks collected in August or November 1984 or in September 1985 was infected. The proportion of ticks infected was as high in unaffected kholas as in affected kholas (Table 3). Moreover no significant differences were observed between the *p* values in ticks from kholas and houses or between adult females, adult males and nymphae in the collections after November 1983.

Infectious virus titres in infected ticks

Examination of the virus titres in infected ticks showed that these ranged from $\log_{10} 0.5$ to $\log_{10} 4.8$ per pool, (pool mean $\log_{10} 2.1$) in the November 1983 collection.

Table 4. Presence of ticks infected with ASF virus in houses

Premises	Number of ticks* from which ASF virus was isolated/Number tested							
	Nov. 83	Jan. 84	Mar. 84	Apr. 84	July 84	Aug. 84	Nov. 84	Sep. 85
Houses								
C10	0/6	NC	0/5	0/22	0/9	0/21	0/28	0/32
C11	2/24	NC	1/6	0/32	0/6	0/32	0/28	0/19
C12	5/68	0/25	NC	NC	NC	0/23	0/10	0/22
C13	2/25	NC	1/33	1/17	0/2	NC	0/54	0/175
C14	1/16	4/32	2/46	0/25	0/18	0/20	NC	0/27
C15	NC	NC	NC	NC	NC	0/79	0/14	0/47
C16	NC	NC	NC	NC	NC	0/20	0/27	0/37
G4	7/32	NC	NC	NC	0/3	NC	NC	0/150
G5	9/66	NC	1/28	0/12	1/26	0/17	NC	NC
G6	NC	5/35	NC	NC	0/17	NC	NC	0/27
K5	5/18	1/4	0/12	0/16	0/3	0/38	0/35	0/28
K6	NC	NC	0/3	NC	NC	0/26	0/14	NC
K7	NC	NC	NC	NC	NC	0/82	NC	NC
K8	NC	NC	NC	NC	NC	0/19	0/15	0/24
K9	NC	NC	NC	NC	0/5	NC	0/3	0/28
Total	31/255	10/96	5/133	1/124	1/89	0/377	0/228	0/616

Letters C, G and K refer to Chalaswa, Guluza and Kamende.

NC, Not collected.

* Number of ticks pools in the Nov. 83 collection.

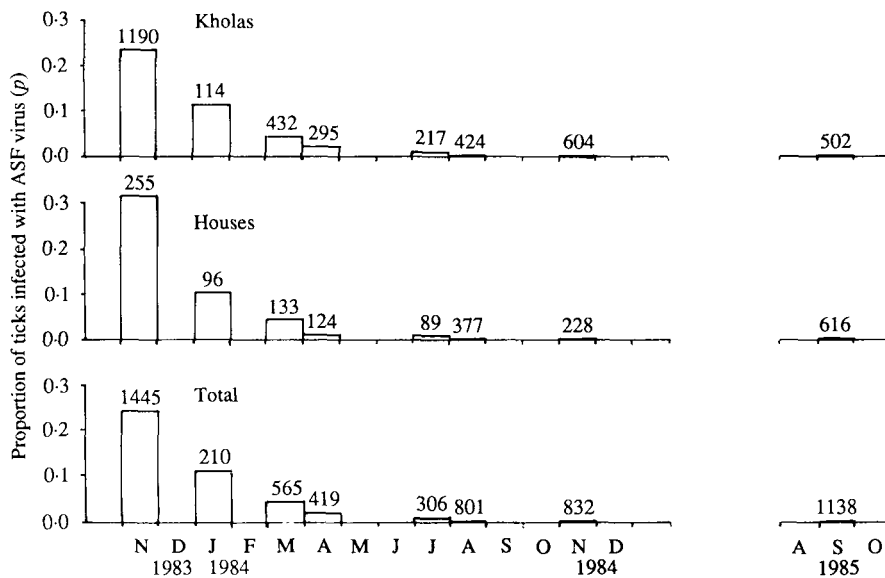


Fig. 4. The proportion of ticks infected with ASF virus in houses and kholas following the ASF outbreak in November 1983. Figures on histogram refer to number of ticks tested.

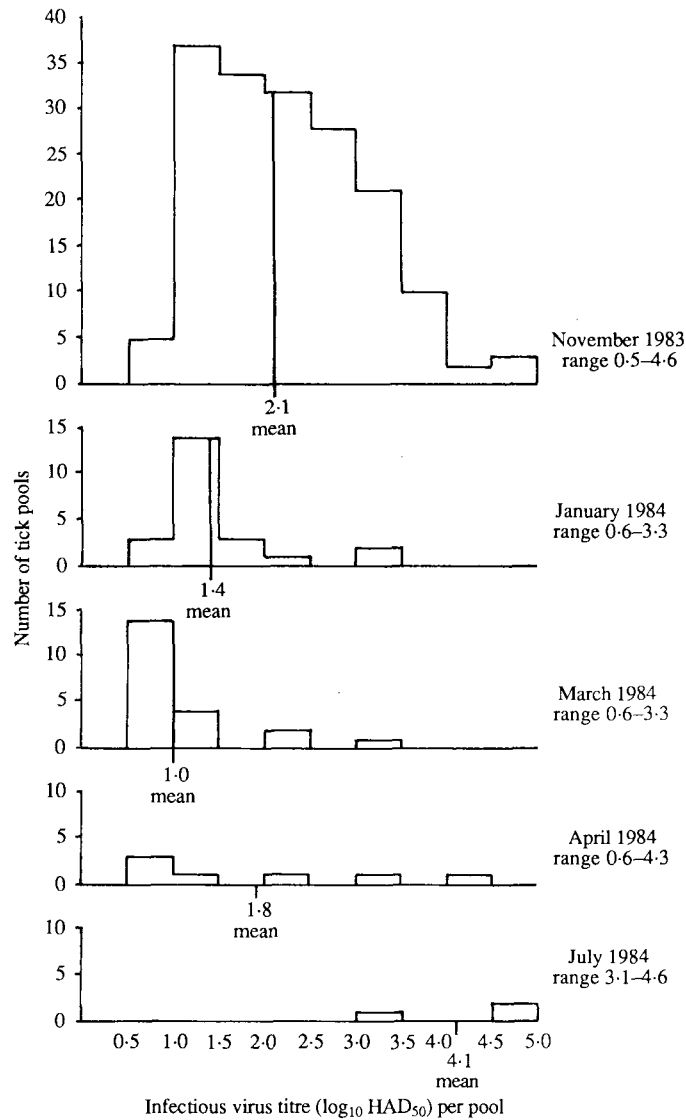


Fig. 5. Distribution of infectious virus titres in infected tick pools collected in November 1983, January 1984, March 1984, April 1984 and July 1984.

For pools of adult females the titres ranged from \log_{10} 1.0 to \log_{10} 4.8 per pool (pool mean \log_{10} 2.6), for adult males titres ranged from \log_{10} 0.8 to \log_{10} 3.7 (pool mean \log_{10} 2.1) and for nymphae titres ranged from \log_{10} 0.5 to \log_{10} 4.3 (pool mean \log_{10} 1.9). Five pools had titres greater than \log_{10} 4.0, four of these being pools of adult females and one a pool of nymphae.

Later collections had a much lower proportion of ticks infected (Fig. 4), and mean virus titres were also lower in January and March 1984 (Fig. 5). Many of the infected ticks in these collections had titres of less than \log_{10} 1.0, although a few individuals in each collection had titres of \log_{10} 3.0 or more. In the July 1984 collection all three of the infected ticks had high titres (\log_{10} 4.6, \log_{10} 3.1 and

\log_{10} 4.6, mean \log_{10} 4.1, Fig. 5), indicating that virus replication may have taken place in a few individuals.

DISCUSSION

The widespread occurrence of *O. moubata* in the ASF enzootic area of Malawi has already been reported (Haresnape & Mamu, 1986) but its significance as a reservoir of virus or vector of disease has not been elucidated. In the present study, an estimated 24% of the tick population in the three villages in south-west Mchinji district in November 1983 was infected with virus following an ASF outbreak. Almost all premises sampled 3 weeks after the outbreak contained infected ticks. The only two premises from which none of the ticks collected on this occasion were infected were those from which the numbers collected were very small (18 or fewer), and infected ticks were found in one of these on a subsequent visit when a larger collection was made. Infected ticks were widespread throughout each of the villages at this time and were present in affected and unaffected kholas and also in both kholas and houses, which indicates that the ticks are highly mobile and that the ticks in kholas and houses comprise a single population within each village.

It is perhaps surprising that infected ticks were found in apparently unaffected kholas, but there are several possible explanations. Firstly, the infected ticks may have come from another khola and been collected before biting the pigs. Secondly, the pigs may already have been seropositive and immune, so did not get the disease when bitten by an infected tick. Thirdly, the pigs may have had a subclinical infection which can produce viraemia sufficient to infect ticks although no disease is observed (P. J. Wilkinson, personal communication).

The estimated proportion (p) of ticks in houses infected with ASF virus may be higher than the actual value because only houses belonging to pig-owners were sampled in this study. The finding that the estimated value of p for ticks in houses in November 1983 was higher than that for ticks in pig kholas may simply be a result of this, together with possible sampling error because of the smaller numbers of ticks collected from houses, since the difference between the two estimates was only of borderline significance. However, it is still surprising that the proportion of infected ticks in houses should be as high as that in pig kholas so soon after the ASF outbreak.

O. moubata habitually bite while their human or animal hosts are asleep at night, and hide in cracks in the walls of houses or kholas during the day. They are thus relatively mobile creatures, leaving their hosts regularly to find hiding places. Kholas and houses are often situated within a few yards of each other (Fig. 2B) and it has been suggested (Haresnape & Mamu, 1986) that movement of ticks from one premises to another is assisted by the custom of spreading blankets over animal kholas or other constructions to air. An infected tick might be moved to new premises in this way.

The proportion of infected ticks declined rapidly from 0.24 in November 1983 to 0.11 after 2 months, and to 0.04 after 4 months. This was probably primarily due to the gradual increase in numbers of new generations of uninfected ticks but may also be partly attributable to loss of virus from infected ticks.

The majority of infected ticks contained low titres of virus and some of these may have had virus temporarily in the alimentary canal rather than being actively infected. However, titres of 4.6 HAD₅₀ or more were noted in three pools of adult females in the November 1983 collection made 3 weeks after the ASF outbreak. It is interesting to note that a tick collected from Tikoliwe in March 1982, approximately 1 month after an ASF outbreak there, which had an extremely high titre of 6.4 HAD₅₀, was also an adult female and it is suggested that infected ticks may have transmitted ASF virus to the pigs in both Tikoliwe and Chalaswa (Haresnape, Wilkinson & Mellor, 1988). In general, titres decreased with the passage of time but it appears that virus multiplied to high titres in a small proportion of infected ticks including adult females in the Chalaswa area in November 1983 and in Tikoliwe in March 1982, and also in the three nymphae identified in this study in July 1984, 8 months after the ASF outbreak. It is unlikely that the higher titres observed in these ticks was residual virus from recently ingested blood meals since it was so long after the last reported cases of ASF, and because the time between collection in Malawi and assay at Pirbright would have resulted in considerable reduction in the amount of infectious virus in the ticks (Plowright *et al.* 1970). Thus *O. moubata* may become a reservoir of virus and initiate a new ASF outbreak by transmitting virus to a susceptible pig during feeding.

It seems surprising that all the pigs in the three villages remained healthy after November 1983 despite the presence of large numbers of infected ticks. The disease was not reported in other kholas after this time although infected ticks were found in all three villages as long as 8 months after the last known deaths from ASF. The virus isolated from the ticks was highly virulent when tested in susceptible European pigs at Pirbright, UK (Haresnape, Wilkinson & Mellor, 1988) and there are several possible explanations for this. Firstly, there may be a real difference in the pathogenicity of Malawi virus isolates in European and indigenous Malawi village pigs. Secondly, since ASF is enzootic in the area, the pigs may have recovered from a previous infection and been immune rather than fully susceptible animals. Thirdly the numbers of infected ticks present may have been so low that the probability of transmission of virus was extremely low, particularly after the application of acaricide less than 2 months after the outbreak. Although acaricides are not very effective against large populations of *O. moubata* in buildings because the numerous small nymphae hiding deep in cracks in infested premises are likely to remain unaffected, the number of ticks was temporarily reduced. Fourthly, since ticks with virus titres ≤ 4 HAD₅₀ do not excrete infectious virus during feeding whereas those with titres > 4 HAD₅₀ do excrete virus in both salivary and coxal fluids (P. J. Wilkinson & P. S. Mellor, unpublished observations), it is possible to speculate that the ticks, although infected, did not inoculate virus when feeding, or inoculated only small amounts of virus which produced seroconversion but did not cause noticeable disease.

Surviving pigs probably play an important role in the epizootiology of ASF in this region. Recovered European pigs with circulating antibodies do not develop clinical disease if challenged with related (homologous) strains of European isolates of ASF virus, but challenge virus may replicate and be transmitted to other pigs by direct contact, shedding of blood or by infecting ticks (Wilkinson,

1984). If such pigs are used for breeding, although there is no experimental evidence that the stress of pregnancy causes virus reactivation in recovered sows or that virus can cross the placenta (Schlafer, McVicar & Mebus, 1984), newborn piglets may be passively protected by antibodies in colostrum which do not prevent infection but reduce the clinical course, duration and titres of viraemia after challenge (Schlafer, Mebus & McVicar, 1984). Since recovered sows are frequently used for breeding in Malawi, their piglets may be seropositive, and produce only mild clinical disease when exposed to challenge virus.

Seropositive pigs were present throughout the study period but the proportion rose following the outbreak and the 34 pigs sampled in August 1984 were all seropositive. Since the proportion of seropositive pigs in Chalaswa before November 1983 was low (estimated at 11%), it seems likely that most of those sampled in August had become seropositive since the ASF outbreak 9 months earlier even though they had appeared healthy throughout the period of investigation. They may have had a subclinical infection, or a chronic or subacute form in which signs of disease were not noticed. Nevertheless, the fact that pigs in Malawi recover whereas European pigs have a high mortality rate following infection with the same virus indicates that the isolates of ASF circulating in Malawi are less virulent in African domestic pigs than in European breeds.

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