Isolation of African swine fever virus from ticks of the *Ornithodoros moubata* complex (Ixodoidea: Argasidae) collected within the African swine fever enzootic area of Malawi

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SUMMARY

Ticks of the *Ornithodoros moubata* complex were collected from domestic pig sties and dwelling houses, and from a warthog habitat, and tested for the presence of African swine fever (ASF) virus. Collections were made in 9 of the 24 districts of Malawi, these being primarily the districts in which *O. moubata* is most numerous. ASF virus was isolated from ticks collected in both domestic pig sties and houses in certain villages in Mchinji district where ASF outbreaks had recently occurred. Mchinji district is in the centre of a large ASF enzootic area which stretches into other districts of Malawi and also into Zambia and Mozambique. The high titre of virus in some of the ticks demonstrates that *O. moubata* can act as a virus reservoir and potential vector of disease in the field situation in Malawi.

INTRODUCTION

It has long been known that wild pigs such as warthogs (*Phacochoerus aethiopicus*) and possibly bush pigs (*Potamochoerus porcus*) are potential carriers of African swine fever (ASF) in Africa, although they do not show signs of disease, and they have been considered to be the natural hosts and primary reservoirs of the virus (Montgomery, 1921). ASF virus was first recovered from naturally infected warthogs in South Africa (Steyn, 1932) and later in Kenya (Hammond & DeTray, 1955) and has also been found in bush pigs in South Africa (Thomas & Kolbe, 1942). Establishing how the virus could be transmitted from wild to domestic pigs was recognized as a major problem since direct contact between them is rare, and attempts to demonstrate transfer of virus by contact from 'carrier' warthogs to domestic pigs usually failed (DeTray, 1963; Scott, 1965; Heuschele & Coggins, 1969; Plowright, Parker & Peirce, 1969*b*).

The discovery that the argasid tick *Ornithodoros moubata*, collected from warthog burrows in East Africa contained ASF virus (Plowright, Parker & Peirce, 1969*a*) was a major step forward. Following this it was established that ASF virus replicates in the ticks, that naturally infected ticks can transmit the

disease to domestic pigs under experimental conditions, and that both transovarial and sexual transmission of virus are possible in ticks (Plowright, Parker & Peirce, 1969b; Plowright, Perry & Peirce, 1970; Plowright et al. 1970; Plowright, Perry & Greig, 1974). It was therefore proposed that, once a tick became infected by feeding on a viraemic vertebrate it could maintain virus for long periods, possibly for life, without further exposure to a viraemic host. The involvement of ticks of the *Ornithodoros moubata* complex as an efficient virus vector provided an explanation of how virus could be transferred from wild to domestic pigs.

Ticks of the *Ornithodoros moubata* complex have many potential hosts beside warthogs, including man and many domestic animals, of which Hoogstraal (1956) suggests the domestic pig to be the most important. It seemed likely that ticks associated with domestic pigs in Africa may be more important in the epizootiology of ASF in domestic pigs than those associated with warthogs. However, no studies on the isolation of ASF virus from *O. moubata* collected from domestic pig premises in Africa had been done prior to this study.

Initial investigations in Malawi showed *O. moubata* to be present in domestic pig pens (kholas) in several districts with a more widespread distribution in dwelling houses. It is particularly numerous in pig kholas in several areas where ASF is enzootic, and may play a role in the epizootiology of ASF in the Central Region of Malawi (Haresnape & Mamu, 1986). The purpose of this study was to assess the importance of ticks associated with domestic animals in acting as reservoirs of ASF virus and vectors of disease.

MATERIALS AND METHODS

Collection of ticks

Extensive collections of *O. moubata* from domestic pig kholas and houses were made in many parts of Malawi both within the ASF enzootic area and elsewhere (Figs. 1 and 2). One collection was made in Liwonde National Park from a culvert frequented by warthogs. Collections were made by staff of the Central Veterinary Laboratory (CVL), Lilongwe, and by Veterinary Assistants stationed in rural areas, as described previously (Haresnape & Mamu, 1986). Ticks were packed in plastic tubes, keeping those from different pig kholas and houses separate, and the tubes were packed in sealed plastic bags and despatched at ambient temperature to the Pirbright Laboratory, Institute for Animal Health, UK.

Collection of interview data

Details of any cases of ASF in the areas where collections were made were obtained from interviews with pig owners. Some pig owners in areas where ticks were found in houses but not in pig kholas were also interviewed. Where laboratory confirmation of ASF had not been obtained deaths from ASF were assessed on the basis of the interview data as described previously (Haresnape, 1984; Haresnape, Lungu & Mamu, 1985).

Virus isolation in tissue culture

Adult ticks were examined individually or in pools of 2–6. The nymphal stages were pooled in groups of up to 22. Individual ticks or small pools were ground up
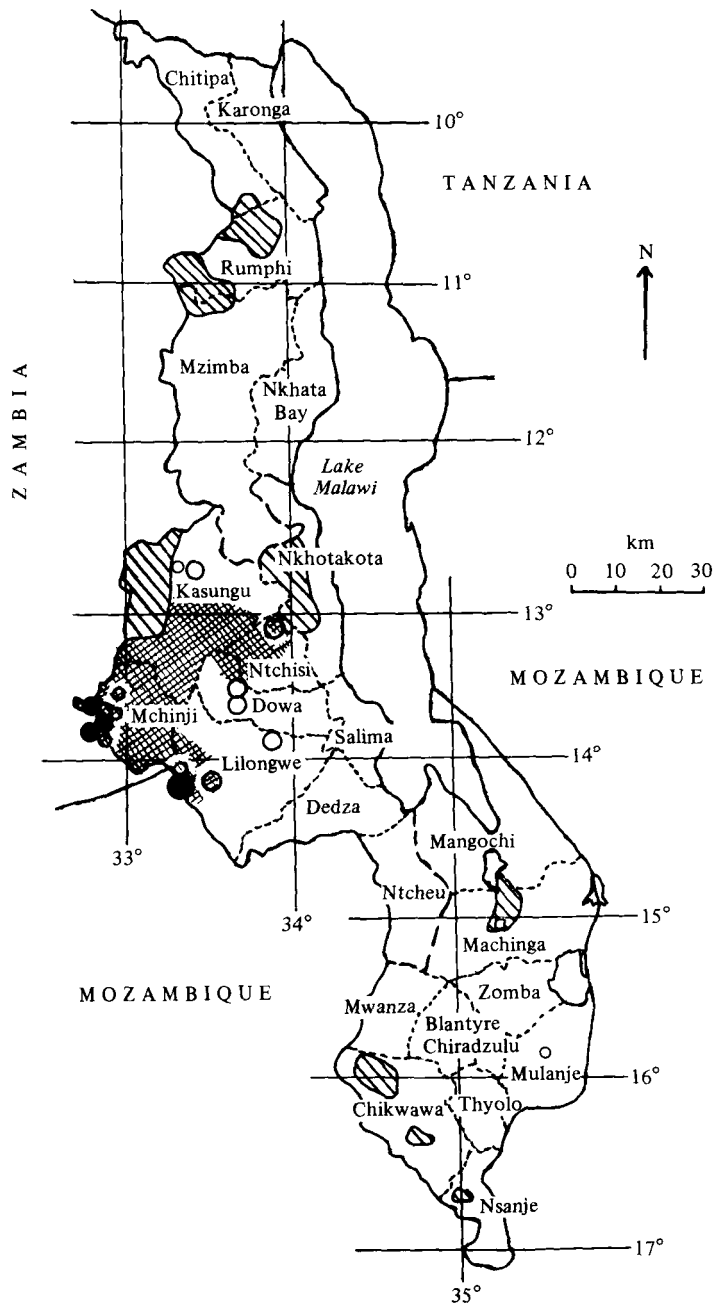
Fig. 1. Sites where ticks were collected from pig kholas and warthog habitats and tested for presence of ASF virus. Number of ticks from pig kholas tested for presence of ASF virus (○, 10–100; ◐, 100–1000; ◯, more than 1000). Filled in circles represent sites where one or more infected ticks were found. Number of ticks from warthog habitats tested for presence of ASF virus (□, more than 1000). — — —, Regional boundary; ------, District boundary; ◪, National Park or Game Reserve; ⌗, ASF enzootic area.
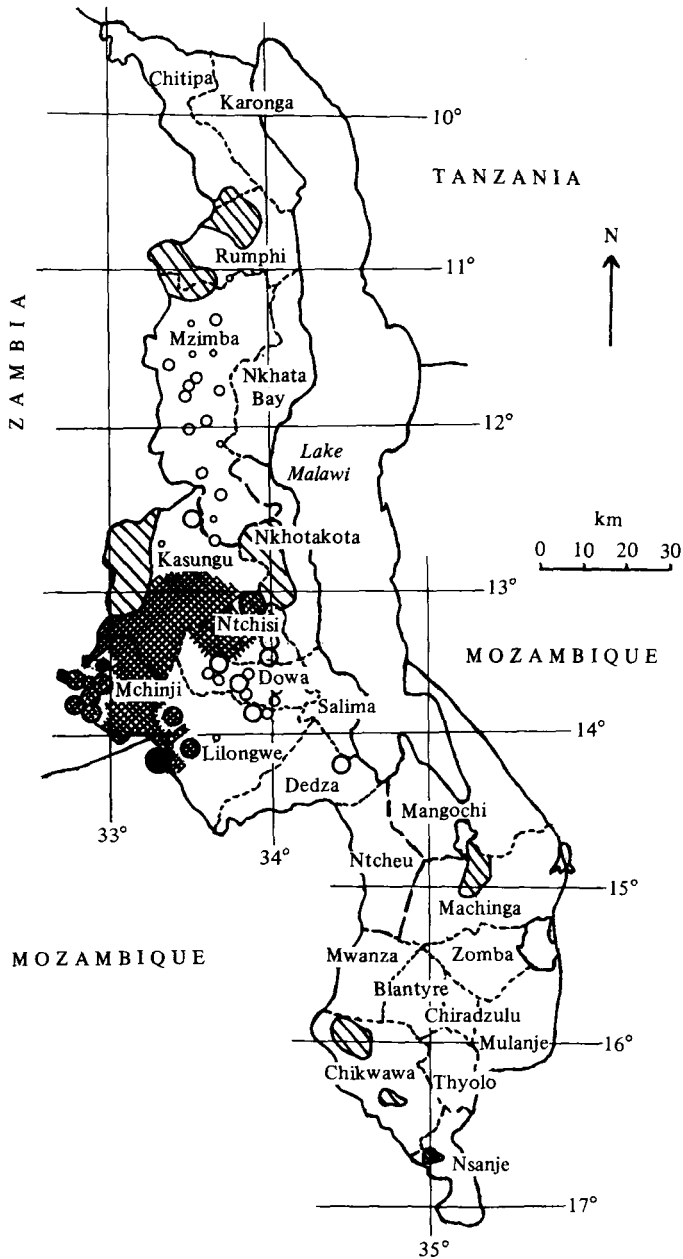
Fig. 2. Sites where ticks were collected from houses and tested for presence of ASF virus. Number of ticks from houses tested for presence of ASF virus ○, less than 10; ○, 10–100; ○, 100–1000; ○, more than 1000). Filled in circles represent sites where one or more infected ticks were found. ⸺, Regional boundary; ------, District boundary; ◪, National Park or Game Reserve; ⌗, ASF enzootic area.

in 2 ml of diluent (PBS containing 1 % ox serum and antibiotics) and larger pools in 5 ml of diluent. Suspensions were clarified by centrifugation for 5 min at 1000 rpm and the supernatant medium was either assayed immediately or stored at -70 °C.

Primary cultures of pig bone marrow (PBM) cells were used for the detection and titration of infectious virus. Cultures were prepared as described previously (Wilkinson *et al.* 1977) in tubes containing 10^7 cells in 1·5 ml medium which were incubated in stationary racks at 37 °C and used after 3–4 days. Tick suspensions were assayed to determine whether or not they contained infectious virus by the inoculation of 0·33 ml into each of three tubes of PBM cells, which were examined daily for 6 days for haemadsorption. Positive ticks or tick pools were then titrated using 10-fold dilutions in diluent and three culture tubes per dilution, each tube being inoculated with 0·33 ml. Cultures were examined daily for haemadsorption and discarded if positive. Five days after inoculation 0·2 ml of a 1 % suspension of pig erythrocytes was added to each culture and final readings were made on day 6. Titres were expressed as 50 % haemadsorbing doses (HAD_{50}) per tick or per pool.

Virulence tests

Cross-bred Large White × Landrace pigs were infected with the original suspension of tick (isolate TIK/82), with virus isolated at Pirbright from pig bone marrow cells infected with tick suspension (LIL 18/2, LIL 20/1, LIL 31/20), with tissues from a pig inoculated with tick suspension (KON/83), or with virus isolated at CVL, Lilongwe from a pig which died of ASF (BONGERA/83). Between two and four pigs were used for each isolate, and were infected by the inoculation of between $10^{0·6}$ and $10^{3·6}$ HAD_{50} of ASF virus by the intramuscular route or by keeping them in contact with pigs infected by inoculation and observed until death. Blood samples were taken at intervals and viraemia measured using pig bone marrow cells.

RESULTS

Virus isolation from ticks

A total of 17405 ticks consisting of 8335 from pig kholas, 7670 from houses and 1400 from a single warthog habitat, were collected in Malawi and tested at Pirbright for the presence of ASF virus. Of these 7183 three were from pig kholas and 5146 were from houses within the ASF enzootic area (Fig. 1). Virus assays were carried out on a total of 5581 ticks from pig kholas and 3034 ticks from houses in Mchinji district, which is within the enzootic area. Virus was found in 181 pools from the pig kholas and 48 pools from the houses. No virus was isolated from ticks collected in pig kholas or houses in any other district or from the ticks from the culvert used by warthog in Liwonde National Park in Machinga district (Tables 1 and 2, Figs. 1 and 2).

Enzootic area

(1) *Mchinji district*

(*a*) *Chalaswa area*. The vast majority of infected ticks were collected from three closely associated villages, Chalaswa, Guluza and Kamende, in the south-eastern

Table 1. *O. moubata collection sites within the ASF enzootic area and results of ASF virus isolation tests*

| | | | | No. of pools positive | |
| | | | | Total no. of ticks tested | |
District	Locality	Map square	Date collected	Pig khola	House
Ntchisi	Malomo	1333B2	Nov. 84–Sep. 85	0/565	0/1348
	Khuuwi	1333B4	Nov. 1984	-—	0/9
			Total	0/565	0/1357
Lilongwe	Mkoko	1333C4	Jun. 83–Dec. 84	-—	0/153
	Kamandauna	1433A2	Apr. 1983	—	0/3
	Chilinda	1433B1	Jan–Jun. 84	0/1037	0/599
			Total	0/1037	0/755
Mchinji	Molosiyo	1332D2	Mar. 82, Nov. 83	0/23	0/117
	Ndawambe	1332D2	Mar. 1982	1/323	—
			May. 83, Nov. 83	0/83	0/314
	Sitolo	1332D2	Mar. 82, Nov. 83	0/84	0/51
	Mkanda	1332D2	Feb. 85, May. 85	0/38	0/45
	Tikoliwe	1332D4	Apr. 1982	1/33	—
			May 83–May 84	0/81	0/178
	Kondoole II	1332D4	June 1983	1/8	0/189
			Nov. 83–May 84	0/462	0/63
	Kondoole I	1332D4	Apr.–May 84	0/304	0/47
	Menyani	1332D4	May 82–Oct. 83	0/37	0/109
	Chamani	1433A2	June 1983	0/30	0/3
	Chalaswa	1433A2	July 1983	0/297	—
			Nov. 1983	141/1190	31/255
			Jan. 1984	13/114	10/96
			Mar. 1984	16/432	5/133
			Apr. 1984	6/295	1/124
			July 1984	2/217	1/89
			Aug. 1984	0/424	0/377
			Nov. 1984	0/604	0/228
			Sep. 1985	0/502	0/616
			Total	181/5581	48/3034
			Find Total	181/7183	48/5146

part of Mchinji district close to the Mozambique border, hereafter referred to as Chalaswa (Table 1, Figs. 1 and 2).

These villages were affected by an ASF outbreak early in November 1983 in which 58 pigs died and the estimated mortality in infected pigs was 77–92% (Haresnape, Lungu & Mamu, 1985). Tick collections were made before the outbreak, 3 weeks following the outbreak, and at intervals afterwards. Those collected in November 1983 were assayed in pools of between 1 and 22 ticks, and 141 out of 1190 pools of ticks from pig kholas and 31 out of 255 pools of ticks from houses contained virus (Table 1). The actual number of infected ticks was undoubtedly much larger than this but was not ascertained exactly. Of the 11 kholas in which ASF-infected ticks were found, 7 had suffered losses from the disease. The proportion of infected ticks from houses in Chalaswa was not significantly different from that in pig kholas at any time during the study. The overall proportion of adult females, adult males, larger nymphae and smaller

Table 2. O. moubata *collection sites outside the ASF enzootic area and results of ASF virus isolation tests*

District	Locality	Map square	Date collected	No. positive/No. tested		
				Pig khola	House	Warthog habitat
Mzimba	Luzi	1033D4	Sep. 1983	—	0/17	—
	Various*	1133	Sep.–Nov. 85	—	0/270	—
	Various*	1233	Oct. 1983	—	0/197	—
Kasungu	Various*	1233	Aug. 83–May 85	0/152	0/300	—
Ntchisi	Chintembwe	1333B4	Apr.–May 83	—	0/727	—
Dowa	Mponela*	1333D	Sep. 83–Aug. 85	0/692	0/481	—
	Chimangamasa*	1334C	Aug. 83, Nov. 85	—	0/34	—
Lilongwe	Jumpha*	1333D4	Aug. 83, Aug. 85	0/241	0/364	—
	Chinsapo	1433B1	Oct. 1983	—	0/5	—
Dedza	Nyama	1434A2	June–Aug. 83	—	0/129	—
Machinga	Liwonde	1535A2	July 1985	—	—	0/1200
			Nov. 1985	—	—	0/200
Mulanje	Mpasa	1535D3	May 1985	0/67	—	—
			Total	0/1152	0/2524	0/1400

* Collections made over a wide area covering several localities (see Figs. 1 and 2).

nymphae infected were not significantly different. The proportion of infected ticks gradually decreased following the outbreak and virus-infected ticks were still present in July 1984, 8 months after the outbreak. The virus titres in the three single infected ticks collected on this occasion were high ($10^{4.6}$, $10^{4.6}$, $10^{3.1}$ HAD$_{50}$ per tick) although no deaths from ASF had occurred in Chalaswa after November 1983. ASF isolates LIL 18/2, LIL 20/1 and LIL 31/20 were made in pig bone marrow cultures from pools of 3 adult females, 4 adult males and 5 adult males respectively, collected from pig kholas in Chalaswa in November 1983.

(b) *Ndawambe*. Ndawambe village is in the western part of Mchinji district about 1 km from the Mozambique border. Three hundred and twenty three ticks were collected from 11 pig kholas in March 1982, and ASF virus was isolated from a single adult tick in a batch of 82 adults and 29 nymphae collected in one khola containing 20 pigs. The titre of virus in the tick was not measured. There had been suspected cases of ASF in the village in January and February 1982, just prior to the time of collection, but these 20 pigs were apparently unaffected. More cases of ASF were reported between October 1982 and January 1983 and a further collection of ticks including 67 from this khola, was made in May 1983 but all were negative.

(c) *Tikoliwe*. Tikoliwe village is also in western Mchinji district close to the Mozambique border. Thirty-three ticks were collected from two pigs kholas on 29 April 1982, and ASF virus was isolated from one adult female of 31 ticks from one khola. The titre of virus in this tick was $10^{6.4}$ HAD$_{50}$ (isolate TIK/82). There had been an outbreak of ASF in March 1982 just prior to the time of collection but the four pigs in the khola were apparently unaffected. Further collections of ticks made in May 1983, November 1983 and May 1984 were not infected with virus (Table 1).

(d) *Kondoole*. Kondoole consists of two villages, Kondoole I and Kondoole II.

situated 0·5 km apart between Ndawambe and Tikoliwe. Both villages had been affected by ASF in early June 1983 and 10 out of 15 pigs died of the disease in two kholas in Kondoole II. Eight ticks were collected from one khola in Kondoole II on 10 June 1983, and ASF virus was isolated from one N4/N5 nymph. The virus titre in the tick was not measured but an isolate was made following passage in a pig (KON/83). The five surviving pigs were still alive and apparently healthy in November 1983. The estimated percentage mortality in infected pigs in Kondoole II was thus 67 %. In November 1983 a further 383 ticks were collected from five kholas in Kondoole II, including 186 from a khola in which pigs had died, but no virus was isolated from any of them. Further losses from ASF occurred in both Kondoole I and Kondoole II in March and April 1984, but no virus was isolated from a further 383 ticks collected in April and May from 12 kholas which included 4 of those affected by ASF.

No samples were sent to CVL from pigs in any of the villages in which infected ticks were found, so laboratory confirmation of ASF as the cause of death of pigs in these villages was not obtained, but pig owners variously described symptoms consistent with ASF such as lack of appetite, immobility, weakness, refusal to walk, loss of balance, huddling together, coughing, shivering, diarrhoea and loss of body condition with death following 3–7 days after onset of sickness. Some internal organs, particularly the spleen, were reported to be unusually dark in colour. However, laboratory confirmation was obtained from an ASF outbreak which occurred in September 1983 in Bongera, which is also in Mchinji district, situated approximately halfway between Chalaswa and Ndawambe and about 40 km from each (Haresnape, Lungu & Mamu, 1985). An isolate from this outbreak was made in pig leucocyte cultures at CVL, Lilongwe (BONGERA/ 83).

(2) *Other districts*

Although no virus infected ticks were found in other districts (Table 1), it is interesting to note that ASF outbreaks had occurred in some villages prior to the time of collection. For example ASF outbreaks occurred between August and September 1983 in Chilinda and neighbouring villages in Lilongwe district, and in September 1984 in Malomo in Ntchisi district (Haresnape, Lungu & Mamu, 1985). Substantial collections of ticks from affected pig kholas were made in January, April and June 1984 in the Chilinda area, and in November and December 1984 and January 1985 from Malomo, but none were infected. These parts of Lilongwe and Ntchisi districts are within the ASF enzootic area (Haresnape, Lungu & Mamu, 1985).

Non-enzootic area

Collections of ticks were also made from areas outside the enzootic area in which ASF outbreaks had occurred (Table 2), such as Mwera Hills in Ntchisi district, Dzoole in Dowa district and Chikuse in Lilongwe district, but these collections were all from houses; no ticks were found in pig kholas in these localities despite thorough searches (Haresnape & Mamu, 1986).

Most of the other localities from which the ticks originated were well outside the ASF enzootic area (Table 2), and in parts of Malawi where ASF has never been reported (Mzimba, northern Kasungu, north-eastern Dedza and Machinga districts).

Table 3. *Virulence of ASF virus isolates from the enzootic area of Malawi in European pigs*

Virus isolate	Route of infection (titre of inoculum)*	Pig no.	Onset of fever (days after infection)	Death (days after infection)	Blood	Spleen
			No. of days to:		Virus titre ($\log_{10} HAD_{50}$)	
TIK/82 (tick isolate)	Intramuscular ($10^{3.3}$)	OP 38	3	9	NT	8·3
		OP 39	3	7	8·3	8·0
	Contact	OP 40	9 dpe†	13 dpe	7·5	7·3
BON/83 (pig tissue)	Intramuscular ($10^{3.3}$)	PA 10	4	7	7·3	NT
		PA 11	4	7	7·3	NT
KON/83 (tick isolate)	Intramuscular ($10^{3.6}$)	PA 39	3	7	7·2	8·8
	Contact	PA 40	6 dpe	13 dpe	8·0	8·1
LIL 18/2 (tick isolate)	Intramuscular ($10^{1.6}$)	PA 43	4	9	8·2	8·3
	Contact	PA 44	12 dpe	14 dpe	8·0	8·0
LIL 20/1 (tick isolate)	Intramuscular ($10^{1.8}$)	PB 40	4	7	8·0	9·1
		PB 41	4	8	7·6	7·8
		PB 42	3	8	7·3	9·3
		PB 43	3	7	9·5	9·8
LIL 31/20 (tick isolate)	Intramuscular ($10^{0.6}$)	PA 41	4	9	8·0	7·6
	Contact	PA 42	13 dpe	14 dpe	7·0	8·3

* HAD_{50}.
† dpe, Days post exposure.
NT, not tested.

ASF virus was not isolated from any ticks collected in the non-enzootic areas.

Virulence tests

Five isolates from ticks (KON/83, TIK/82, LIL 18/2, LIL 20/1 and LIL 31/20) and one isolate from a pig (BONGERA/83) were obtained from Mchinji district and were all found to be very virulent in European cross-bred pigs. The incubation period was 3–4 days in all the inoculated pigs which died between 7 and 9 days after infection. The pigs infected by contact had a longer incubation period (9–13 days) and died 13–14 days after exposure (Table 3). All pigs had high titres of virus in the blood and tissues (tonsil, parotid and gastrohepatic lymph nodes, kidney, spleen and heart).

DISCUSSION

ASF virus infected ticks were found in pig kholas in Mchinji district which is in the centre of a large ASF enzootic area. In all villages where virus infected ticks were found, deaths of pigs from ASF had been reported shortly beforehand and in some villages, such as Kondoole and possibly also Ndawambe and Tikoliwe, deaths from ASF also occurred in kholas after infected ticks were collected from them, possibly as the result of a bite from an infected tick.

High titres of ASF virus were found in some ticks notably that from Tikoliwe ($10^{6.4}$ HAD_{50}), collected on 29 April 1982, which compares with the peak virus titre

of $10^{6\cdot3}$ HAD_{50} recorded by Greig (1972) from a tick approximately 100 days after infection. In Chalaswa, the mean titre of virus in infected ticks was at its highest about 8 months after the ASF outbreak in November 1983 which demonstrates that virus had replicated in the ticks in the field situation. A new outbreak of ASF could start following a bite from an infected tick even many months after the last cases of ASF. Thus domestic pig ticks of the *Ornithodoros moubata* complex are important reservoirs of ASF virus and vectors of disease in rural Malawi.

In Chalaswa, it is perhaps surprising that no further deaths from ASF occurred after the loss of 58 pigs in November 1983, despite the presence of large numbers of infected ticks in the kholas and it seems unlikely that the pigs in infected kholas escaped being bitten by them.

The six Malawi isolates of ASF virus from ticks in the enzootic area all produced acute, fatal ASF in European pigs but the mortality rate in ASF outbreaks in indigenous village pigs in Mchinji district is often much lower than 100 % (Haresnape, Lungu & Mamu, 1985). One explanation for this difference is that field isolates are less virulent for indigenous pigs in Malawi than they are for European breeds of pig. Recovered pigs may be resistant to experimental challenge with homologus virus although this virus may replicate without producing clinical signs (Wilkinson, 1984). A similar situation may also occur in the field in Malawi where recovered pigs could become infected under natural conditions with local virus strains which replicate and produce mild or unnoticed clinical signs. The interaction between recovered pigs and infected ticks may thus be important in the epizootiology of ASF in the enzootic area.

The overall infection rate in ticks collected from pig kholas in the Mchinji district was approximately 3 % (Table 1). However, in Chalaswa, 11 % of the ticks collected from pig kholas during the 3 months following the outbreak of ASF were infected and the high infection rate in ticks collected in November 1983 was presumably a result of their having fed on viraemic pigs in early November. By July to August 1984, the infection rate was 0·3 %, which is similar to the overall infection rate elsewhere in Mchinji district.

The estimated tick infection rates were 1 % (1 in 102), 3 % (1 in 31) and 12 % (1 in 8) respectively, in kholas in Ndawambe, Tikoliwe and Kondoole in each of which one infected tick was found, although these cannot be regarded as accurate estimates because of the small sample sizes.

The collections from pig kholas in localities in Ntchisi and Lilongwe districts which are within the ASF enzootic area and had recent cases of ASF (Table 1) may have been too small to detect virus-infected ticks.

It is somewhat surprising that in Chalaswa equally high infection rates were found in ticks collected from houses or from pig kholas. The common practice in Malawi of airing blankets by spreading them over pig kholas could be the explanation for the movement of ticks between pig sties and human dwellings. This seems a more likely route of transfer than on people, animals and poultry, as the ticks are generally only found on their hosts at night (Haresnape & Mamu, 1986). No infected ticks were found in houses elsewhere, even though 3096 ticks from houses within the enzootic area was tested. In most areas where ticks were found in kholas they were easier to find, and therefore presumably more numerous, in houses (Haresnape & Mamu, 1986) and Chalaswa was the only area where they appeared more numerous in kholas.

The ASF virus infection rates reported here for naturally infected ticks collected from domestic pigs compare with infection rates in *O. moubata* from warthog burrows of between 0·017 and 1·35% in different localities in East Africa (Plowright, 1977 *a*, *b*) and between 0·06 and 1·4% in South Africa and Namibia (Thomson *et al.* 1983). In ticks collected from warthog burrows the infection rate was higher in adults than in nymphae, but this was not the case in the large numbers of infected ticks found in Chalaswa immediately following the cases of ASF there. If a larger number of persistently infected ticks had been found, the same trend might have been observed.

While warthogs and ticks in warthog burrows must have been the original hosts of ASF virus before domestic pigs were introduced to Africa, they may play only a minor role in the epizootiology in some parts of Africa. *O. moubata*, being a multiple host tick, is ideally suited as a potential vector of ASF virus (Heuschele & Coggins, 1965) and it seems likely that in Malawi ticks from domestic pig premises may be important reservoirs of the virus. Viraemias of 10^7 or 10^8 HAD_{50}/ml are very common in domestic pigs shortly before death from ASF, and these are the likely source of virus for the persistently infected ticks in Malawi. Plowright, Parker & Peirce (1969 *b*) found that the Tengani strain of ASF virus, which originated from Nsanje district in the Southern Region of Malawi (Matson, 1960; Cox & Hess, 1962) caused persistent infection in only about 5% of East African ticks fed on viraemic pigs and this might also be true of other Malawi strains of ASF virus in Malawi ticks. This would then explain the comparatively low numbers of persistently infected ticks, as was found in Chalaswa 8 months after the outbreak of ASF, even though large numbers of ticks were infected immediately after the outbreak of ASF.

In Malawi, ASF is enzootic over an area of approximately 8000 km² in the Central Region, but there is virtually no contact between domestic pigs and warthogs in this area as warthogs are largely confined to the National Parks and Game Reserves (Hough, 1982), where there are no domestic pigs. Although it has been pointed out that the distribution of ASF in Africa as a whole closely resembles the distribution of warthogs (Anon, 1962), this is not the case in Malawi. The warthog habitat in which *O. moubata* were found during this study was in Liwonde National Park in the Southern Region of Malawi, which is well outside the area in which ASF is enzootic for domestic pigs, and none of the ticks collected there were infected, although further south in Lengwe National Park in Chikwawa district, five of six warthog sera tested were seropositive (Haresnape, Lungu & Mamu, 1985).

Domestic pigs come into contact with bush pigs much more frequently, as bush pigs are widely distributed throughout Malawi (Hough, 1982). No evidence was found of ASF infection in 11 bush pigs examined (Haresnape, Lungu & Mamu, 1985) and a brief search for ticks in bush-pig sleeping places in Lilongwe district was unsuccessful. Whether or not they play a role in the epizootiology of ASF in Malawi is therefore not clear.

The findings presented here show that control of ASF will be more difficult than previously envisaged, because of the important role played by ticks feeding on domestic pigs as reservoirs of virus, because these ticks are extremely difficult to eradicate. Acaricides can reduce the tick population but cannot eliminate it entirely as ticks can hide deep in cracks in the wood or mud of kholas and houses

which are inaccessible. Evacuation of infested premises is not likely to be effective as *O. moubata* is extremely resistant to starvation and some ticks of the *O. moubata* complex may survive for 5 years without food (Walton, 1964). Burning infested premises, although possible for pig kholas, would clearly not be acceptable for houses and, since the ticks in houses may be infected and could reinfest new pig kholas, this would not be an effective means of eradication. These ticks occur over a much wider area than the present ASF enzootic area and there is a danger that the enzootic area could become larger in future (Haresnape & Mamu, 1986; Haresnape, Lungu & Mamu, 1987). It is difficult to see any way in which ASF could be eradicated altogether but it is possible that it could be prevented from becoming enzootic over a wider area through imposition of strict control of pig movements from the enzootic area and special efforts to detect the spread of ASF outbreaks in those areas outside the enzootic area where *O. moubata* occur. At particular risk are those parts of Dowa, Lilongwe and Kasungu districts which are at present outside the ASF enzootic area, but where *O. moubata* are common in pig kholas. Burning affected kholas might be advisable in the event of an ASF outbreak in these areas in order to prevent the virus from becoming established in the *O. moubata* population.

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