

## The distribution of African swine fever virus isolated from *Ornithodoros moubata* in Zambia

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### SUMMARY

African swine fever (ASF) has been reported in the Eastern Province of Zambia since 1912 and is now considered to be enzootic there. A survey of the distribution of ASF virus in Zambia was carried out by virus isolation from *Ornithodoros moubata* ticks collected from animal burrows in National Parks and Game Management Areas in northern, eastern, central and southern Zambia. ASF virus was isolated from ticks in all areas examined. The prevalence of infection in *O. moubata* was between 0.4% in South Luangwa National Park and 5.1% in Livingstone Game Park and mean infectious virus titres ranged from  $10^{3.4}$  HAD<sub>50</sub>/tick in Kakumbe Game Management Area to  $10^{5.9}$  HAD<sub>50</sub>/tick in Chunga and Nalusanga Game Management Areas. The prevalence of infection in adult ticks was between 4.7% and 5.3% in all areas examined except Sumbu National Park and Livingstone Game Park, where the prevalence was 15.1% and 13.2% respectively in adult ticks. The ratio of infected females to males for all the infected adult ticks in all areas of Zambia was 3.2:1.

### INTRODUCTION

The most important objective of livestock development in Zambia is to increase animal production to meet the growing demand for animal protein. Pigs and pig products are the source of approximately 7% of the country's total meat supply and Zambia has been self-sufficient in the production of pork since 1975. Commercial pig production is located chiefly near the main consumer centres, namely along the line of rail from the capital Lusaka to the Copperbelt, with additional producers in the Southern Province (Livingstone), and the north of the Central Province (Mkushi). The number of sows in these commercial units is estimated to be about 5200. In addition, there are over 147 000 indigenous pigs in the country, kept under village conditions. Almost 90% of these pigs are in two Provinces; one quarter of the total being in the Southern Province and almost two-thirds in the Eastern Province (Fig. 2).

In addition to problems of husbandry, nutrition and parasitism, the existence of African swine fever (ASF) is one of the major factors limiting swine production in the Eastern Province of Zambia. The disease was first reported in Kenya as early as 1909 (Montgomery, 1921) and since then outbreaks have been reported from almost all the countries in Africa which lie on or south of the Equator (Neitz, 1963; Wilkinson, 1981).

ASF was first reported in Zambia in 1912 and 1914 in the vicinity of Fort Jameson, now Chipata, in the Eastern Province as an outbreak in pigs with clinical signs which were different from European or classical swine fever. Subsequently there have been repeated reports of its occurrence in the same area, where it is now considered to be endemic. Laboratory confirmation has been made on only five occasions between 1974 and 1983 (Table 1). There are no reports of any disease resembling ASF from any other parts of Zambia.

The role of wart hogs (*Phacochoerus aethiopicus*) as carriers of ASF virus has been demonstrated in several countries in Africa, including Tanzania (Plowright, Parker & Peirce, 1969) and northern Botswana (Simpson & Drager, 1979). Since it proved difficult to demonstrate direct virus transmission from infected wart hogs to either healthy wart hogs or domestic pigs, it was suggested that biting arthropods might be involved in virus transmission and ASF virus was isolated from soft ticks (*Ornithodoros moubata*) from wart hog burrows in East Africa in 1969 (Plowright, Parker & Peirce, 1969). It has since been shown that this species of tick is a reservoir and vector of ASF virus in both East and South Africa (Plowright, 1977; Pini, 1977; Thomson *et al.* 1983).

The first report of the association between *O. moubata* and wart hogs is based on observations of Lloyd (1915) in the Luangwa Valley, Zambia and the tick was later shown to be present in other areas of Zambia (Keirans, 1985). An investigation to determine the possible role of *O. moubata* in the persistence of ASF in the enzootic area of Zambia and also the distribution of the virus in the country was carried out by collecting ticks from animal burrows in four separate areas of Zambia and examining them for the presence of ASF virus.

## MATERIALS AND METHODS

### *Location of collecting sites*

The wart hog is widespread throughout Zambia (Fig. 1), with the exception of the extreme northwest, and is most common in and around National Parks, where it prefers tree and bush savannah and open perennial grassland (Ansell, 1978). The animal often sleeps in holes of the antbear (*Orycteropus afer*). Consequently, tick collections were carried out from animal burrows in four National Parks (NP) and/or surrounding Game Management Areas (GMA) in different regions of the country (Fig. 2). Ecological details of the study areas are given in Table 2.

The general ecology of each tick collecting site was recorded including signs of wart hog use or other inhabitants, particulars of the surroundings, such as situation on an abandoned anthill or in flat ground, type of vegetation and soil (Table 3).

Table 1. *History of African swine fever outbreaks in Zambia*

Year	Location	Remarks
1912	Fort Jameson*	First report of 'Swine Fever' in district.
1914	Fort Jameson	Large outbreak. Report of clinical signs different from European swine fever.
1921	Kachebera	40 pigs died; 16 pigs killed.
1932		Swine fever included in list of scheduled diseases.
1933	No outbreaks reported	Previous heavy losses in Fort Jameson (Chipata) district attributed to ASF by Le Roux (V.R.O.).
1935	Petauke	Widespread outbreak in native-owned pigs with high mortality.
1936	Fort Jameson (Kawasa division)	Recrudescence in European-owned pigs early in year. Disease active in native-owned pigs in area.
1937-39	No outbreaks reported	
1944-51	No mention in Annual Reports	
1952	E. Province	Outbreak suspected - not confirmed.
1953	E. Province	Outbreak suspected. (Confirmed in 1954 by virus isolation in Kenya - DeTray, 1963).
1956	Fort Jameson	Outbreaks on 2 farms. 100% mortality on 1 farm. Disease endemic in native pigs which may have some immunity.
1957-64	No mention in Annual Reports	
1965	E. Province	6 cases diagnosed from pathological specimens.
1966	E. Province	2 small outbreaks in remote area. No spread reported.
1967		Scattered outbreaks of unconfirmed ASF.
1968	Msekera, near Chipata	1 unconfirmed outbreak. Reports of total depopulation of village pigs due to ASF. Disease reported as endemic.
1969-70	Chassa, Katete District.	1 outbreak.
1971	Chassa, Katete District.	1 outbreak killed all pigs at secondary school and in some nearby villages. Reports of previous outbreak in same area in 1969.
1972-73	No outbreaks reported.	
1974	Katete District (St Francis Mission)	14/15 pigs died in isolated units. (Laboratory confirmation in Lilongwe, Malawi.)
1975	Petauke	1 outbreak confirmed in laboratory in Lilongwe, Malawi.
1976	Chipata	Suspected outbreak, hundreds of deaths.
1977	Chipata district	Suspected outbreak in February.
	Petauke district	Laboratory confirmation of outbreak in March.
1978	Petauke	Laboratory confirmed outbreak.
	Katete - Chadiza	Suspected outbreaks. Involvement of wild pigs and wart hogs suspected.
1979	Petauke - Lundazi	3 confirmed and 1 suspected outbreak.
1980	Chadiza - Katete - Petauke	3 suspected outbreaks (no laboratory confirmation).
1982	Chipata	Outbreak.
1983	Chipata	1 laboratory confirmed outbreak. 200 pigs died (mortality rate 77%).

\* Now Chipata

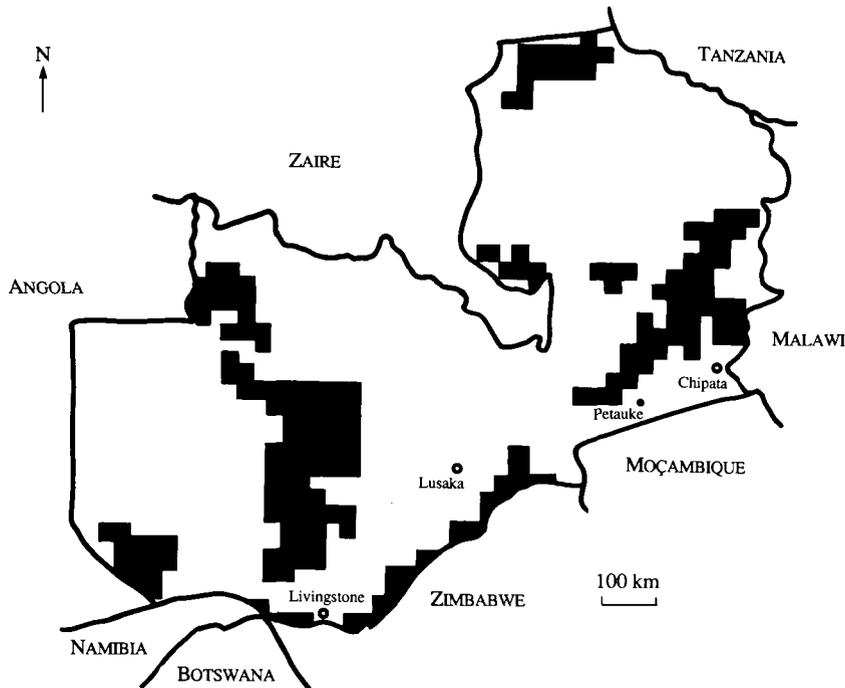


Fig. 1. The distribution of warthogs in Zambia (redrawn from Ansell, 1978).

#### *Collection of O. moubata from burrows*

After checking for other inhabitants, soil was collected with a shovel, at first from the floor and ceiling of the entrance and then as deep as possible from the interior, including cracks and crevices. The material was sieved and spread out on white cloth in the sun. Ticks were collected with forceps and transferred into universal bottles with a fine wire mesh in the screw cap, and transported to the Central Veterinary Research Institute, Lusaka. The identity of *Ornithodoros* ticks was confirmed microscopically and specimens were separated into the various nymphal stages and adult males and females. Collections were then forwarded by airfreight to Pirbright in the UK for virus isolation.

#### *Virus isolation in tissue culture*

Adult ticks were examined individually and the nymphal stages were first pooled; N1 and N2–N3 were pooled in groups of 10–20, N3–N4 were pooled in groups of 5–10 and N4–N5 were tested in groups of 5. Individual ticks or pools were ground up in 2 ml of diluent (PBS containing 1% ox serum and antibiotics) per adult or small group of nymphs and the larger pools were ground up 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  $-70^{\circ}\text{C}$ .

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

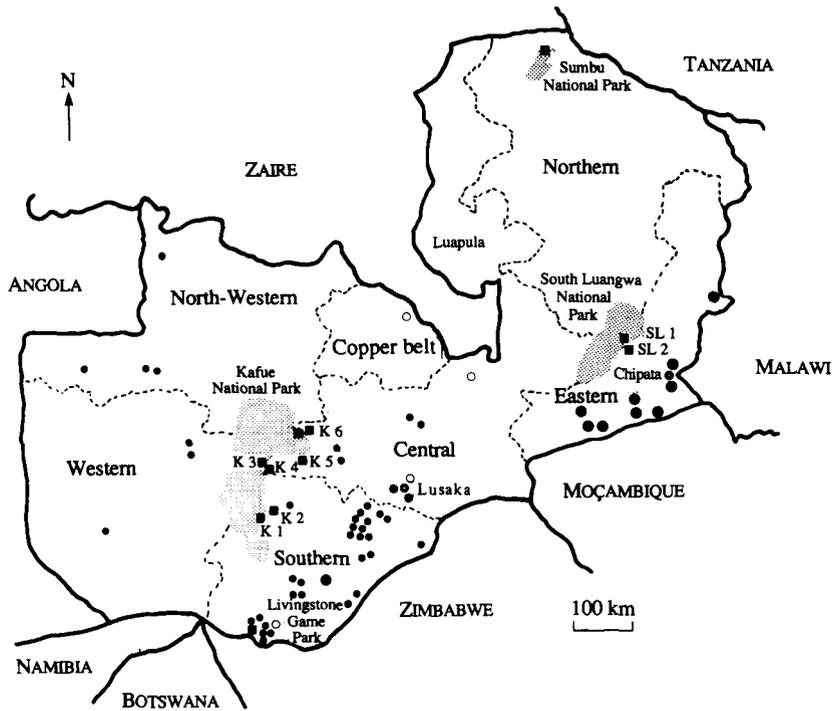


Fig. 2. *Ornithodoros moubata* collection sites and the distribution of commercial and indigenous pigs in Zambia. ■, Tick collection sites – details are given in Table 1. Pig husbandry sites, with approximate numbers at each: ○, Commercial pigs (Sows) 501–1000; ●, Indigenous pigs 501–1000; ●, Indigenous pigs 5001–10000.

The population of indigenous pigs in each Province in 1982 was:

Eastern	96264 (65.4%)	Western	3166 (2.2%)
Southern	36025 (24.5%)	Lusaka	1595 (1.1%)
Central	4297 (2.9%)	Northern	1174 (0.8%)
North-Western	4234 (2.9%)	Luapula	343 (0.2%)
Total	147 098		

(Data from Department of Veterinary and Tsetse Control, Lusaka, 1982.)

incubated in stationary racks at 37 °C and used after 3–4 days. Tick suspensions were first 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<sub>50</sub>) per tick or per pool.

Table 2. Details of study areas in *Zambian National Parks*

National Park	Province	Area (km <sup>2</sup> )	Elevation (m)	Mean annual rainfall (mm)	Mean annual temperature (°C)	Soil	Vegetation
South Luangwa	East	9050	500–1000	800–900	22.5–25	Alkaline mopane, river clays and alluvium	Miombo woodland (Brachystegia), miombo scrub and mopane woodland.
Kafue	N. Western/ Central/Southern	22400	970–1470	800–900	20–22.5	Sandy to medium-textured, strongly leached	Miombo woodland, open grassland dambos
Sumbu	Northern	2020	800–1250	1200	22.5–25	Sandveldt	Thickets and miombo woodland
Livingstone Game Park in Mosi-oa-Tunya NP	Southern	10	880	600–700	20–22.5	Alkaline mopane soils and leached Kalahari soils	Mopane woodland

Table 3. The predominant characteristics of the burrows examined in the National Parks, the number of burrows containing *O. moubata* and the number of burrows from which *O. moubata* infected with ASF virus were recovered

National Park*	Date of collection	No. holes with ticks/No. holes examined	No. holes with infected ticks/No. examined	Predominant characteristics of burrows		
				Situation	Soil type	Vegetation
Kafue (K1-6)						
Kafue NP (Ngoma) (K1)	Dec. 1981	5/5	1/5	On termite hills	Not recorded	Woodland
Namwala GMA (K2)	Dec. 1981	1/11	1/1	Below ground level	Not recorded	Open woodland grassland dambo
Kafue NP (Chunga) (K3)	June 1982	12/23	0/3	Below ground level	Fine sand	Miombo woodland
Chunga GMA (K4)	June 1982	8/10	1/2	Below ground level	Dry, coarse loamy	Miombo woodland
Nalusanga GMA (K5)	June 1982	8/13	2/2	Below ground level	Dry, loamy	Woodland
Kafue NP (Kabulushi) (K6)	Dec. 1983	7/8	2/7	On termite hills	Sandy	Woodland, dambo
South Luangwa (SL1 and 2)						
South Luangwa NP (Mfue) (SL1)	Nov. 1981	7/15	0/4	Below ground level	Coarse, grey gravel	Mopane woodland
Kakumbe GMA (SL2)	Nov. 1981	3/8	2/2	Below ground level	Coarse, grey gravel	Mopane woodland
South Luangwa NP (Mfue) (SL1)	July 1982	3/13	3/3	Below ground level	Gravel and red sand	Mopane woodland
Kakumbe GMA (SL2)	July 1982	5/11	3/5	Below ground level	Coarse grey gravel and red sand	Mopane woodland
Mosi-oo-Tunya						
Livingstone Game Park						
	June 1982	6/9	3/6	On termite hills	Dry, grey gravel	Mopane woodland
	Aug. 1983	11/16	6/11	On termite hills	Dry, grey gravel	Mopane woodland
Sumbu						
Sumbu NP	June 1983	22/35	3/10	On flat ground	Dry, sandy	Woodland

\* K1-6 and SL1-2 are the numbers of the collection areas shown in Fig. 2.

## RESULTS

*Sumbu NP*

Most holes in this area were at the edge of a large dambo, which is an area of impeded drainage, and although 22/35 holes contained ticks, none of them was heavily infested (Table 3). ASF virus was isolated from 10/481 ticks (2.1%) found in 3 out of 10 holes examined (Table 4), of which 8 were adults and 2 were large nymphs. The proportion of adult ticks infected was 15.1% and of nymphs was 0.47%. One of the 3 holes in which infected ticks were found was a group of 3 culverts under a road which had recently been inhabited by wart hog. The mean virus titre of the infected ticks was  $10^{4.4}$  HAD<sub>50</sub>/tick with a range of  $10^{2.1}$ – $10^{6.3}$  HAD<sub>50</sub>/tick.

*South Luangwa NP and Kakumbe GMA*

Two locations were visited in 1981 and 1982: Mfue in the South Luangwa NP (SL1) and the nearby Kakumbe GMA (SL2) which lies outside the Park boundary (Fig. 2 and Table 3). In both these areas, less than half the burrows examined contained *O. moubata* but, with the exception of the Mfue area in 1981 where there were no infected ticks, ASF virus was isolated from ticks in 8 of the 10 burrows examined in Mfue in 1982 and Kakumbe in 1981 and 1982 (Table 5). The total number of ticks tested for ASF virus in the 8 burrows was 1254 of which 17 were positive (1.4%). Thirteen were adult ticks and 4 were nymphs.

In South Luangwa NP 4.7% of the adult ticks and 0.07% of the nymphs were infected and in Kakumbe GMA the proportion of adult ticks infected was 5.3% while 0.55% of the nymphal ticks were infected.

The proportion of infected ticks in the three collections in which virus was successfully isolated was very similar; the lowest was 1.1% in South Luangwa in 1982 and the highest was 1.9% in Kakumbe GMA in 1981. The mean titre of infectious virus in ticks from Kakumbe GMA was  $10^{3.4}$  HAD<sub>50</sub>/tick ( $10^{1.7}$ – $10^{4.7}$  HAD<sub>50</sub>/tick) in 1981 and  $10^{4.8}$  HAD<sub>50</sub>/tick ( $10^{2.3}$ – $10^{5.9}$  HAD<sub>50</sub>/tick) in 1982 and in Mfue in 1982 the mean virus titre/tick was  $10^{4.5}$  HAD<sub>50</sub> ( $10^{0.6}$ – $10^{5.9}$ ).

Extensive examination of five pig houses in two villages near Chipata failed to reveal the presence of *O. moubata* and the local people claimed that they had not seen ticks on their pigs. Pig owners were able to recognize *Argas* spp present in chicken huts but did not recognize specimens of *O. moubata* which were shown to them. No wart hogs were reported in any of the villages visited in the area. Bush pigs, however, were present in the area and, although they normally had no contact with the villages, they came to feed in the maize fields nearby in the rainy season in February and March. In addition, no ticks were found in 10 traditional domestic pig houses in two villages near Petauke which is approximately 150 km south west of Chipata (Fig. 1).

*Kafue NP and Namwala, Chunga and Nalusanga GMAs*

Six locations were visited and ticks collected from animal burrows in 1981–83 (K1–6) (Fig. 2 and Table 3). Three of these were in the National Park (K1, K3 and K6) and three were in the Game Management Areas at Namwala (K2), Chunga (K4) and Nalusanga (K5). The proportion of burrows examined which contained

Table 4. Isolation of ASF virus from *O. moubata* recovered from animal burrows in Sumbu NP and Livingstone Game Park

Collection site	Year	No. ticks infected/No. examined (% ticks infected)										Infectious virus titre/tick (log <sub>10</sub> HAD <sub>50</sub> )		
		Adults										Total	Mean	Range
		Female	Male	N4-N5	N3-N4	N2-N3	N1-N2							
Livingstone Game Park	1982	2/18 (11.1)	4/45 (8.9)	3/115 (2.6)	0/99 (0)	0/101 (0)	—	9/378 (2.4)					4.2	2.1-5.9
	1983	24/87 (27.6)	11/160 (6.9)	0/111 (0)	4/163 (2.5)	10/201 (5.0)	0/33 (0)	49/755 (6.5)					5.5	0.6-8.1
Sumbu NP	1983	5/24 (20.8)	3/29 (10.3)	2/65 (3.1)	0/66 (0)	0/215 (0)	0/82 (0)	10/481 (2.1)					4.4	2.1-6.3

Table 5. Isolation of ASF virus from *O. moubata* recovered from animal burrows in South Luangwa NP and Kakumbe Area GMA

Collection site	Year	No. ticks infected/No. examined (% ticks infected)										Infectious virus titre/tick (log <sub>10</sub> HAD <sub>50</sub> )		
		Adults										Total	Mean	Range
		Female	Male	N4-N5	N3-N4	N2-N3	N1-N2							
South Luangwa NP Mfue (SL1)	1981	0 adults/10		0/72 (0)	0/97 (0)	0/415 (0)	0/340 (0)	0/934 (0)					(0)	—
	1982	3/31 (9.7)	2/65 (3.1)	1/99 (1.0)	0/231 (0)	0/106 (0)	0/26 (0)	6/558 (1.1)					4.5	0.6-5.9
Kakumbe GMA (SL2)	1981	6 adults/55 (10.9)		0/82 (0)	0/57 (0)	0/113 (0)	0/5 (0)	6/312 (1.9)					3.4	1.7-4.7
	1982	2/38 (5.3)	0/58 (0)	2/65 (3.1)	0/37 (0)	1/118 (0.9)	0/68 (0)	5/384 (1.3)					4.8	2.3-5.9

Table 6. Isolation of ASF virus from *O. moubata* recovered from animal burrows in Kafue NP and associated GMAs

Collection site	Year	No. ticks infected/No. examined (% ticks infected)					Infectious virus titre/tick ( $\log_{10}$ HAD <sub>50</sub> )			
		Adults		N1-N2	N2-N3	N3-N4	N4-N5	Total	Range	
Female	Male	N1-N2	N2-N3							N3-N4
Kafue NP Ngoma (K 1)	1981	8 adults/177 (4.5)	—	1/68 (1.5)	0/55 (0)	0/405 (0)	0/14 (0)	9/719 (1.3)	4.5	3.0-6.7
Namwala GMA (K 2)	1981	—	—	0/3 (0)	—	1/8 (12.5)	—	1/11 (9.1)	3.7	—
Kafue NP Chunga (K 3)	1982	—	0/1 (0)	0/18 (0)	0/5 (0)	0/17 (0)	—	0/41 (0)	—	—
Chunga GMA (K 4)	1982	3/28 (10.7)	0/48 (0)	0/17 (0)	0/38 (0)	0/114 (0)	0/54 (0)	3/299 (1.0)	5.9	5.9-6.0
Nalusanga GMA (K 5)	1982	2/28 (7.1)	2/15 (13.3)	1/30 (3.3)	0/26 (0)	—	0/30 (0)	5/129 (3.9)	5.9	5.4-6.9
Kafue NP Kabulushi (K 6)	1983	2/19 (22.2)	0/26 (0)	1/71 (2.1)	0/57 (0)	0/46 (0)	0/58 (0)	3/277 (1.1)	4.2	3.1-5.3

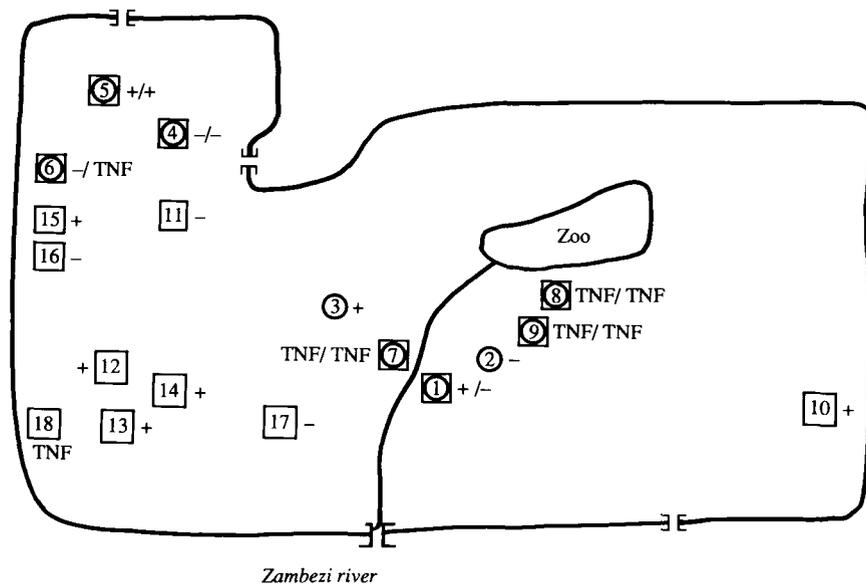


Fig. 3. Location of animal burrows in Livingstone Game Park examined in 1982 and 1983, showing those in which *Ornithodoros moubata* infected with African swine fever were present. Dimensions of Park: approx 4.5 km  $\times$  2.2 km. ○, burrows examined in June 1982; □, burrows examined in August 1983; +, *O. moubata* infected with ASF virus present; -, Uninfected *O. moubata* present; TNF, Ticks not found.

*O. moubata* was very low in Namwala GMA (K2) where only 1 out of 11 burrows examined was infested, but in all the other locations the infestation rate of the burrows was between 50% (K3) and 100% (K1).

ASF virus was isolated from ticks in all areas except the small collection of 41 ticks from the National Park near Chunga (K3) in 1982 (Table 6). During the 3-year period of the survey infected ticks were found in 7/18 (38.9%) of the infested burrows which were examined and virus was isolated from 21 (1.4%) out of a total of 1476 ticks tested. The infection rate of adult ticks was 5.0% and of nymphs was 0.3%. The high proportion of infected ticks (9.1%) in Namwala GMA (K2) represents only 1 infected tick in a group of 11 nymphs from 1 burrow. In the other 4 collections of ticks from which virus was isolated, the proportion of infected ticks was 1.0% in Chunga GMA (K4), 1.1% in Kafue NP at Kabulushi (K6), 1.3% in Kafue NP at Ngoma (K1) and 3.9% in Nalusanga GMA (K5). The mean virus titre per tick in these 4 groups of ticks ranged from  $10^{4.2}$  HAD<sub>50</sub>/tick at Kabulushi to  $10^{5.9}$  HAD<sub>50</sub>/tick in Chunga and Nalusanga GMAs.

#### Livingstone Game Park

Eighteen burrows in this small enclosed Park were examined in 1982 and 1983 (Fig. 3, Table 7), 10 of which were on termite hills in mopane woodland. Six of the 9 burrows examined in 1982 contained ticks and virus was isolated from ticks in 3 of these 6 burrows. In 1983, 16 burrows were examined. Ticks were found in only 3 of the holes in which they were present in 1982 and virus was isolated from the

Table 7. *The distribution of O. moubata infected with ASF virus in animal burrows in Livingstone Game Park examined in 1982 and 1983*

Burrow number	June 1982			August 1983		
	No. ticks infected/ no. tested	% ticks infected	Remarks	No. ticks infected/ no. tested	% ticks infected	Remarks
1	2/37	5.4	Wart hog burrow in use	0/16	0	Recently inhabited
2	0/126	0		Hole not found		
3	1/60	1.7		Hole not found		
4	0/12	0		0/30	0	Recently inhabited
5	6/131	4.6		9/74	12.2	Recently inhabited
6	0/12	0		No ticks found		Not in recent use
7-8-9	No ticks found			No ticks found		Unused
10				1/19	5.3	Recently inhabited
11				0/44	0	Recently inhabited
12				1/58	1.7	Not in recent use
13			(Burrows 10-18 not examined in 1982)	35/294	11.9	Group of 9 holes in use
14				2/30	6.7	Recently inhabited
15			1/76	1.3	Recently inhabited	
16			0/97	0	Not in recent use	
17			0/17	0	Not in recent use	
18			No ticks found		Not in recent use	
Total	9/378	2.4		49/755	6.5	

ticks in only 1 of these burrows. Nine new burrows were investigated and ticks were present in 8 of these, of which 5 contained infected ticks. Virus was isolated from ticks in 6 of the 11 infested burrows examined in 1983. All the burrows, except one, in which infected ticks had been found were in current use by wart hogs.

There was no clear pattern to the distribution of burrows from which ASF virus-infected ticks were recovered (Fig. 3). Burrows containing infected ticks were present in all areas of the Park which had been surveyed. Virus was isolated from only one burrow (No. 5) in both 1982 and 1983 and, although virus was isolated from ticks in burrow 1 in 1982, there were very few ticks present in it in 1983 and these were negative. Ticks were not found at all in burrows 7, 8 and 9 which were being used by wart hogs in 1982, but were unused when re-examined in 1983.

The proportion of ticks infected with virus was high and virus was isolated from 58 (5.1%) of the 1133 ticks examined in 1982 and 1983 (Table 4). The infection rate in the female ticks was 24.8%, in the male ticks it was 7.3% and the overall infection rate of the adult ticks was 13.2%. The population of nymphs had infection rates of 1.0% in 1982 and 2.8% in 1983. Infected nymphs were found in 1983 in burrow 5 (3/42), burrow 14 (2/22) and burrow 13 which had 9 out of 205 infected nymphal ticks. Burrow 13 was a group of 9 holes covering an area of approximately 25 m<sup>2</sup> in which 50% of the females, 15.1% of the males, 3.4% of the N3–N4 nymphs and 8.5% of the N2–N3 nymphs were infected.

Mean virus titres of infected ticks were  $10^{4.2}$  HAD<sub>50</sub>/tick ( $10^{2.1}$ – $10^{5.9}$  HAD<sub>50</sub>/tick) in 1982 and  $10^{5.5}$  HAD<sub>50</sub>/tick ( $10^{0.6}$ – $10^{8.1}$  HAD<sub>50</sub>/tick) in 1983. There was no difference between the mean titres of virus per tick in the females, males and nymphs collected in 1983, the titre was  $10^{5.5}$  HAD<sub>50</sub>/tick in each group.

#### DISCUSSION

The survey of the distribution of ASF virus in *O. moubata* in wart hog burrows has shown clearly that virus is present in all four regions of the country investigated – Sumbu NP, South Luangwa NP and associated GMA, Kafue NP and associated GMAs and Livingstone Game Park in the Mosi-oa-Tunya NP. Virus was readily isolated from all areas examined and it is thus somewhat surprising that the disease has only been reported in the Eastern Province of Zambia (Table 1).

The Eastern Province of Zambia contains the largest proportion (65.4%) of the indigenous pig population which is kept under a free-range management system. The epizootiology of the disease in this part of Zambia has not been elucidated but the two main possibilities are that the disease has become enzootic in the local pig population or that each outbreak is a new epizootic occurring by chance following the introduction of virus by infected *O. moubata*. Either or both these possibilities may explain the situation in Eastern Zambia.

It has recently been reported (Haresnape, Lungu & Mamu, 1985) that African swine fever is enzootic in the western part of the Central Region of Malawi, an area which adjoins the enzootic area of Zambia. The disease in the enzootic area of Malawi is caused by strains of ASF virus which do not kill all infected pigs and surviving pigs have been detected by serological surveys in the area. The

husbandry system is very similar to that in Zambia, in which village pigs are allowed to roam free and are confined only at night. Since there are no wart hogs or associated *O. moubata* in this part of Malawi it is considered that ASF is present as an enzootic disease of reduced virulence primarily in the domestic pig population and that most outbreaks start following the introduction of infected pig meat into villages with susceptible pigs. ASF viruses of reduced virulence are, therefore, present and circulating in the domestic pig population of parts of the Central Region of Malawi and it is quite possible that such viruses are also present in the Eastern province of Zambia. *O. moubata* are present in domestic houses and pig pens in some parts of the enzootic area of Malawi (Haresnape & Mamu, 1986) but in the adjoining enzootic area of Zambia no ticks were found in the villages which were investigated and pig owners did not recognize *O. moubata*, although they were familiar with *Argas* spp. in chicken houses. A more thorough investigation will be required to ascertain whether *O. moubata* are present in villages in any parts of the Eastern Province of Zambia.

The introduction of viruses into the domestic pig population from the natural transmission cycle between *O. moubata* and wart hogs may also occur, either as the main cause of ASF outbreaks in this area of Zambia or as an infrequent event in an enzootic situation such as that which is present in the Central Region of Malawi. Such new introductions of ASF virus into domestic pigs can be made by infected wart hogs or infected *O. moubata*.

The transmission of ASF virus directly from infected wart hogs to domestic pigs is unlikely to occur as Thomson (1985) has reported that ASF virus was not transmitted by direct contact from infected 3–4 month old wart hogs to either wart hogs or domestic pigs. The tissues of acutely infected wart hogs are potentially infectious for pigs which may eat them (Thomson, Gainaru & Van Dellen, 1980). In the field, however, only young wart hogs which are a few months old are acutely infected and these are much less likely to be shot by hunters than the older animals, in which virus levels are generally too low to infect domestic pigs by the oral route.

Domestic pigs are more likely to become infected with ASF virus through the bites of infected ticks associated with wart hogs. If there are no wart hogs in the area it is unlikely that there will be many *O. moubata* in other animal burrows as wart hogs are the preferred hosts (Plowright, Parker & Peirce, 1969; Boreham & Geigy, 1976). Contact between domestic pigs and infected ticks is, therefore, unlikely unless wart hogs increase their range for some reason and they may carry *O. moubata* with them when they leave the burrows (Horak & De Vos, 1983; personal communication cited in Thomson, 1985). One possible area of indirect contact with wart hogs and direct contact with infected ticks is in roadside culverts. A survey of culverts in Northern Tanzania (Boreham & Geigy, 1976) revealed that wart hogs frequently used culverts under the road as temporary resting places and many of these were infested with *O. moubata*. None of these ticks were examined for ASF virus, but we did isolate ASF virus from ticks obtained from a similar situation in the Sumbu NP. These culverts often contain rubbish and, especially if they are near towns or villages, may well be entered by wandering domestic pigs which could become infected if fed on by infected ticks. The other possible way by which ticks may be introduced into villages where

domestic pigs are kept is if wart hogs which are shot are brought back with infected ticks on them. The risk associated with this will clearly depend on the number of ticks carried by such an animal and the infection rate in the ticks. Where the number of ticks carried is small and the infection rate low, there would be a low probability of pigs becoming infected, but where both the number of ticks present on a wart hog and the infection rate are high then the probability of infection of domestic pigs becomes much greater.

Once present in domestic pigs, the virus can be transmitted readily between pigs in the absence of *O. moubata* and no ticks were found in the villages examined near Petauke and Chipata. In the enzootic area of Malawi, however, *O. moubata* are present in pig pens and ASF virus has been isolated from them (Haresnape, 1984; Haresnape, Lungu & Mamu, 1985). Hence they may play a role in the epizootiology of ASF in this area. Biting flies may act as quite efficient mechanical vectors of ASF virus and the stable fly, *Stomoxys calcitrans*, has been shown to transmit the disease to pigs up to 24 h after feeding on viraemic animals (Mellor, Kitching & Wilkinson, 1987).

Bush pigs present in eastern Zambia have only a seasonal contact with villages, whereas those in Malawi often come into the settlements at night (Haresnape, Lungu & Mamu, 1985), but the role of bush pigs in the epizootiology of ASF still remains equivocal. Although the distribution of both wart hogs and bush pigs roughly coincide with areas where ASF occurs (Thomson, 1985) wart hogs are believed to be more important because of their greater numbers, more even distribution and higher infection rates.

A detailed investigation in the Eastern Province is required to determine whether there are any pigs which have recovered from ASF, and if *O. moubata* are present in any parts of the region before the epizootiology of ASF in this Province can be understood.

Our results have shown quite clearly that ASF virus is present in National Parks and Game Management Areas in areas other than the Eastern Province of Zambia where domestic pigs are also present. Almost one quarter of the indigenous pig population is present in Southern Province, mainly along the line of the railway between Livingstone and Lusaka, and infected ticks are present in Livingstone Game Park in the south and Kafue NP and Namwala GMA in the north-west of this Province. There is no obvious explanation for the absence of disease in domestic pigs in this area in spite of virus being present in the *O. moubata* population. Where pigs are kept under commercial intensive management systems, ASF virus is relatively easy to exclude if there is no contact with any wart hogs brought in from GMAs and the movements of pigs are controlled. The best way to prevent ASF in pigs kept under less restrictive conditions is to adopt the double-fencing system which has been used very successfully elsewhere in Africa. It is possible that the strains of ASF virus in Southern Province are not readily transmitted by *O. moubata* to domestic pigs or that they are of low virulence which can only be determined by transmission and pathogenesis studies. The present survey was limited to two areas of the Province and it is important to establish whether *O. moubata* infected with ASF virus are present in other areas outside the boundaries of the National Park.

Previous investigations of the relationship between ASF virus and *O. moubata*

have been made in East and Southern Africa where it has been shown that both the distribution of virus in tick populations and infection rates are variable (Plowright, Parker & Peirce, 1969; Plowright, 1977; Thomson *et al.* 1983; Thomson, 1985).

In Zambia, *O. moubata* infected with ASF virus were found in all areas investigated. The possibility that some isolates were virus present in the blood meals of recently engorged ticks is unlikely since the titre of virus ingested by ticks feeding on viraemic baby wart hogs would have been low and this virus would have become undetectable in the period between collection and assay of ticks. The overall tick infection rates were 0.78% in South Luangwa NP and Kakumbe GMA, 1.4% in Kafue NP and associated GMAs and 2.1% in Sumbu NP which are very similar to those in the majority of tick populations examined in East Africa, where infection rates were 0.45%–1.35% (Plowright, 1977) and in South Africa where overall infection rates of 0.3%–1.6% were reported (Pini, 1977; Thomson *et al.* 1983). The overall infection rate in Livingstone Game Park, however, was 5.1% which is higher than that previously reported elsewhere in Africa. In contrast to this, we found no *O. moubata* populations which had an overall infection rate as low as those in Queen Elizabeth NP, West Uganda where it was 0.017% (Plowright, Parker & Peirce, 1969) or those in Mkuze Game Reserve in South Africa where the overall infection rate was 0.06% (Thomson *et al.* 1983). The variation in infection rates in different tick populations may not always be due to real differences. Because infection rates increase with increasing size and age of ticks (Plowright, Parker & Peirce, 1969; Thomson *et al.* 1983), the overall infection rate will be determined by the relative proportions of different stages of ticks and will appear very low where the majority of ticks examined were small nymphs and much higher where the population contained a high proportion of adults and large nymphs.

The infection rates of adult ticks in two areas, Kafue (5.0%) and South Luangwa (4.7%) were similar to those reported in East and South Africa (Plowright, Parker & Peirce, 1969; Plowright, 1977; Thomson *et al.* 1983). The high proportion of infected adult ticks (15.1%) in Sumbu NP may not represent the true infection rate since only 53 adult ticks were examined from this area. The tick population in the Livingstone Game Park contained a very high proportion of infected adult ticks (13.2%) and nymphal ticks (2.1%), which suggests that the viruses in this area are well adapted to their host and can infect a high proportion of the ticks which feed on viraemic young wart hogs.

The proportion of infected female ticks was always greater than the infection rate of male ticks and the ratio of infected females to males in our survey varied from 2.0:1 in the Sumbu NP to 4.5:1 in South Luangwa and Kakumbe. The ratio of infected females to males for all the infected adult ticks in all areas of Zambia was 3.2:1, which is very similar to the ratio of 3.3:1 for all the infected adult ticks reported by Thomson *et al.* (1983) in the Republic of South Africa. This ratio was also very similar to the ratio of blood meals ingested by female and male ticks determined by  $^{51}\text{Cr}$  ingestion (P. J. Wilkinson & P. S. Mellor, unpublished observations) and it is, therefore, likely that the infection rates in adult females are higher than those in adult males because the females ingest a larger volume of blood during feeding.

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