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THE EPIDEMIOLOGY OF AFRICAN SWINE FEVER: THE ROLE OF FREE-LIVING HOSTS IN AFRICA

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ABSTRACT

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The known distribution of African swine fever (ASF) virus in Africa is reviewed in relation to the distributions of its free-living hosts as are the infection rates of these species in different localities in southern Africa. Mechanisms by which ASF virus is maintained in its sylvatic state and ways in which the infection may enter domestic pig populations are discussed.

INTRODUCTION

In its sylvatic and presumably historic habitat, African swine fever (ASF) virus is confined to Africa south of the Sahara where it is associated with free-living suids and their associated argasid ticks.

Montgomery (1921) first observed that this virus was capable of infecting domestic pigs, in which it produced an acutely lethal infection. Once infected, domestic pigs readily transmit the disease to other pigs both directly and indirectly, virus being excreted in urine and faeces as well as in oral, nasal, pharyngeal, ocular and genital secretions (Greig & Plowright, 1970).

In most instances in Africa, ASF has either been effectively controlled or is self-limiting because of the high mortality rate. It is acknowledged, however, that in some African countries the disease has become endemic in the domestic pig population where the mortality rate has decreased and a higher proportion of chronic and inapparent infections prevail (Wardley, Andrade, Black, De Castro Portugal, Enjuanes, Hess, Mebus, Ordas, Rutili, Sanchez Vizcaino, Vigario, Wilkinson, Moura Nunes & Thomson, 1983; Haresnape, 1984). It is not clear whether this has been due to attenuation of the virus strains by repeated pig-to-pig transmissions or by perpetuation of virus strains which were inherently of reduced virulence (Wardley *et al.*, 1983). Whatever the case, this situation enabled the spread of ASF virus via pig products to countries removed from Africa, initially the Iberian Peninsula in 1957/60 and thence to other parts of Europe, the Caribbean and Brazil (Wardley *et al.*, 1983).

Because of its potentially devastating effect on pig populations, its relatively recent spread from its historic habitat and the lack of an effective vaccine, ASF has aroused considerable international interest. Understandably, since only approximately 1,2 % of the world's pigs are located in Africa, most investigations at present are not concerned with the African situation, but are aimed at preventing the importation of ASF as an exotic disease of pigs or its elimination from swine herds in which it has become endemic. However, because ASF has an inhibitory effect on the expansion of the pig industry in Africa and since Africa will, for the forseeable future, remain a potential source of the infection for the rest of the world, an understanding of the epidemiology of ASF in its natural habitat constitutes an important aspect of international control. This paper will therefore concentrate on the relationship between ASF virus and its reservoir hosts in Africa, particularly in southern Africa.

The free-living hosts of ASF virus

Three African wild suids have been shown to become naturally infected with ASF virus, viz., warthog *(Phacochoerus aethiopicus)*, bushpigs *(Potamochoerus* spp.) and, in one recorded instance only, the giant forest hog *(Hylochoerus meinertzhageni)* (Montgomery, 1921; Heuschele & Coggins, 1965). Whether other vertebrate species are involved in the epidemiology of this infection is a moot point. The report by Cox (1963) that ASF virus had been isolated from hippopotamus, porcupine and hyaena has not been substantiated (Stone & Heuschele, 1965; Table 1), but this aspect has not been adequately examined. In particular, some reptiles, e.g., the black mamba and the Egyptian cobra which are likely to come into contact with infected argasid ticks and which have distributions in South Africa similar to that of endemic ASF (Stuckenberg, 1969), might be fruitfully investigated.

The virus has also been identified in argasid ticks associated with suids, i.e. *Ornithodoros erraticus* (in Europe only) and the *O. moubata/porcinus* complex *sensu* Walton (Sanchez Botija, 1963; Plowright, Parker & Peirce, 1969b). Because at least 3 *Ornithodoros* spp., which do not normally parasitize suids and which are prevalent in the Americas, have been reported as being potential vectors of ASF virus (Groocock, Hess & Gladney, 1980; Gibbs & Butler, 1984), it is prudent to assume that all members of the genus have this ability until the contrary is proved.

Limited attempts at identifying possible alternative arthropod hosts in other genera have so far proved unsuccessful (Plowright, 1977; Table 2).

The distribution of identified free-living hosts of ASF virus in relation to recorded ASF in domestic pigs in African countries

While warthog or bushpigs, and usually both, are found in all the countries in Africa where ASF in domestic pigs has been diagnosed, the giant forest hog does not occur in some regions where ASF in domestic pigs is relatively common, e.g. southern Africa (FAO-WHO-OIE, 1981; Ansell, 1971; Kingdon, 1979; Table 3). Thus the latter species cannot play a universally important role in the epidemiology of ASF in Africa.

Although the distributions of both warthog and bushpigs (Ansell, 1971; Kingdon, 1979) roughly coincide with areas where ASF virus has been identified (Table 3), it is widely believed that warthog are more important in the maintenance of this virus because of their generally greater numbers, more even distribution and higher infection rates (Mansveld, 1963; Neitz, 1963; De Tray, 1963). Certainly in the RSA the location of the ASF endemic control area (Control of Animal Diseases by the Division of Veterinary Services) follows the distribution of warthog more closely than that of bushpigs (Thomas & Kolbe, 1942; Fig. 1). It must be acknowledged, however, that in comparison with warthog little is known about bushpigs in relation to ASF largely because of the secretive nocturnal habits and the thick vegetation of their habitat.

So far as the *O. moubata/porcinus* complex is concerned, the striking feature is that, whereas in southern, east and central Africa, suid-associated ticks are present

TABLE 1 Mammals other than suids examined for antibody* to ASF virus in ASF endemic areas in the RSA

Species	Locality	No. tested	No. positive
Hippopotamus	Kruger National Park	108	0
Buffalo	Kruger National Park	98	0
Rhinoceros (black)	Kruger National Park	14	0
Blue wildebeest	Kruger National Park	19	0
Hyaena (spotted)**	Kruger National Park	25	0
Cheetah	Kruger National Park	2	0
Leopard**	Kruger National Park	4	0
Lion	Kruger National Park	20	0
Baboon	Kruger National Park	24	0
Porcupine**	Northern Transvaal	4	0
Jackal** (black-backed)	Northern Transvaal	5	0
Jackal (black-backed)	Mkuze Game Reserve	2	0
Ant-bear**	South West Africa	5	0

* using immunoelectroosmophoresis

** animals which lie up in situations where *O. moubata/porcinus* complex ticks could be encountered

TABLE 2 Arthropods other than *Ornithodoros* sp. examined for the presence of ASF virus

Species	Locality	No. examined	Result
Auchmeromyia sp. flies	Northern Transvaal*	15	—
Auchmeromyia sp. larvae	Northern Transvaal*	98	—
Auchmeromyia sp. pupae	Northern Transvaal*	10	—
Lice	Northern Transvaal*	417	—
Phlebotomines	Northern Transvaal*	868	—
Culicoides spp.	Northern Transvaal*	367	—
Simulium sp.	Northern Transvaal*	87	—
Fleas	Northern Transvaal*	319	—
Auchmeromyia sp. larvae	Mkuze Game Reserve	40	—

* All arthropods were captured in or near warthog burrows known to be inhabited by infected warthog

— no virus isolated

in almost all areas where ASF is known to occur (Hoogstraal, 1956; Plowright, 1977; Thomson, Gainaru, Lewis, Biggs, Nevill, Van der Pyperkamp, Gerber, Esterhuysen, Bengis, Bezuidenhout & Condy, 1983; Table 3), they have not been recorded from some countries in West Africa where ASF has been diagnosed, i.e. Benin, Cameroon and the Central African Republic (Leeson, 1953; Hoogstraal, 1956; Neitz, 1963; FAO-WHO-OIE, 1981; African swine fever Newsletter No. 24, 1982). Human-associated members of this complex are also not generally found in West Africa (Hoogstraal, 1956). It is possible therefore that the epidemiology of ASF in West Africa differs significantly from that in the rest of the continent.

Unfortunately the systematics of the *O. moubata/porcinus* complex are complicated and not well understood. It possibly comprises 4 species (Walton, 1964; Walton, 1979) which commonly parasitize human beings, warthogs or domestic pigs (Hoogstraal, 1956). It is not known whether those associated with domestic pigs differ from the warthog-associated species. However, Haresnape (1984) has reported the isolation of ASF virus from *Ornithodoros* sp. obtained from domestic pig pens in Malawi so that it is probable that they are capable of transmitting ASF as do warthog-associated ticks (Plowright, 1977).

The relevance to ASF of the so-called small variety of *O. erraticus* both in Kenya and Uganda and in north and north-west Africa (Heisch & Guggisberg, 1952, Heisch, 1952; Hoogstraal, 1956) is not known, but it is unlikely that they play any significant role since these ticks usually parasitize small rodents.

Infection of warthog with ASF virus

Vertical transmission of ASF virus between warthogs is unlikely. No virus was isolated from the uterine contents (including 52 foetuses) of 17 sows, 10 of which were infected with virus at the time of sampling. Negative results were also obtained from the mammary tissues of 5 lactating sows (Plowright, Parker & Peirce, 1969a; Plowright, 1981). In addition, ASF virus has been isolated from only a few neonatal warthogs (Table 5) where infection rates in older animals in the same localities are high (Table 4).

Horizontal transmission between warthog has never been demonstrated, and, as Table 6 indicates, we were unable to effect transmission between experimentally infected 4-month-old warthog, captured in an ASF-free area (Hluhluwe Game Reserve—Table 4), and either similar uninoculated individuals or susceptible pigs housed with the infected animals.

Because in southern Africa there is a close association between the presence of *O. moubata/porcinus* and the occurrence of ASF virus in warthog populations (Table 4; Fig. 1), it is concluded that infection of warthog is effected by argasid ticks which have been shown to transmit the infection readily while feeding, virus being present in saliva, coxal fluid and guanine (malpighian excrement) (Plowright, 1977). Ticks containing large quantities of virus ($>10^5$ HD_{50}) could also be crushed against skin abrasions or ingested, and thus set up infection (Montgomery, 1921; Plowright, 1977). Horak (personal communication, 1982) has observed argasid tick remains in the intestinal contents of warthog.

It should be borne in mind, however, that in both north-central Kenya (Nanyuki) and western Uganda a high proportion of warthogs become infected with ASF virus despite the fact that at Nanyuki no argasid ticks could be found in warthog burrows (Peirce, 1974) and in western Uganda infection rates in ticks are extremely low (Plowright, 1977).

Both in SWA/Namibia and the Kruger National Park some warthogs acquire infection with ASF virus within a week or 2 of birth (Table 5), while in east Africa, Plowright *et al.*, (1969a) found that at Kirawira (Tanzania) warthog had all acquired infection by 3 months of age.

It is probable that infection of young animals occurs in the face of maternally-acquired antibody, since some

TABLE 3 The occurrence of ASF and the known free-living hosts associated with it in African countries

Country	Suids				*Ornithodoros* spp.	
	ASF recorded in domestic pigs	Warthog	Bushpigs	Giant forest hog	*moubatal porcinus* complex	*erraticus*
Mauritania		+				+
Western Sahara						+
Morocco						+
Algeria						+
Tunisia						
Libya						+
Egypt						+
Sudan		+	+		+[1]	
Ethiopia		+	+	+	+	
Djibouti		+			+	
Somalia		+	+		+	
Kenya	+	+*	+*	+*	+[1]*	+
Uganda		+*	+	+	+[1]*	+
Tanzania	+	+*	+	+	+[1]*	
Malawi	+	+	+		+[1]*	
Zambia	+	+	+		+[1]*	
Ruanda	+	+	+	+	+[1]	
Burundi	+	+	+	+	+[1]	
Zaire	+	+	+	+	+[1]	
Congo	+		+	+	+	
Chad		+			+	
Niger		+			+	
Upper Volta		+				+
Mali		+				+
Senegal	+	+	+			+
Gambia		+	+			
Guinea (Bissau)	+	+	+			
Guinea		+	+			
Sierra Leone			+		+[1]	
Liberia			+			
Ivory Coast		+	+			
Ghana		+	+		+	
Togo		+	+			
Benin	+	+	+			
Nigeria		+	+			
Cameroon	+	+	+	+	+	
Equatorial Guinea			+	+		
Gabon			+	+	+	
Central African Republic	+	+	+	+		
Mozambique	+	+	+		+[1]	
Angola	+	+	+		+[1]	
Zimbabwe	+	+*	+		+[1]	
Botswana		+*	+		+[1]	
Lesotho						
Swaziland			+			
South Africa	+	+*	+*		+[1]*	
SWA/Namibia	+	+*	+		+[1]*	

\+ Denotes presence of that host in the country concerned

[1] Denotes presence of suid-associated *Ornithodoros* sp.

* Denotes countries in which ASF virus has been proved present in that species

This table was compiled using the following references: Hoogstraal, 1956; Ansell, 1971; Kingdon, 1979; FAO-WHO-OIE, 1984; Neitz, 1963; Morel, 1980; Leeson, 1953; Plowright, 1977; Thomson *et al.*, 1983; Simpson & Drager, 1979; Walton, 1964; Haresnape, 1984; Wilkinson (personal communication, 1982); FAO, 1982; Mansveld, 1963; De Tray, 1963; Smithers (personal communication, 1981).

animals with relatively high viraemias also had high antibody levels (Table 5). This is possibly due to the absence of neutralizing antibody to ASF virus in warthog sera (Thomson, Gainaru & Van Dellen, 1980). Whether repeated infection with antigenic variants occurs is unknown (see below), but the absence of viraemias in older warthog (Heuschele & Coggins, 1969; Plowright, 1977; Thomson & Gainaru, unpublished data, 1981) comparable with those of neonates (Table 5), suggests that this is either not the case or that effective immunological cross-reactivity exists between such variants.

With the remarkable exception of the Mkuze Game Reserve, all large warthog populations where ASF virus is present have high rates of infection (i.e. >80 %); the frequency of antibody to the virus in sera of sampled individuals is evidence of this (Plowright, 1977; Table 4).

The only large warthog population conclusively shown to be free of ASF virus is in the Hluhluwe/Umfolozi Game Reserve complex in northern Natal, although other smaller populations in southern Africa also appear to be free (Table 4). Nigerian warthogs are also probably free of the infection although only limited numbers of animals from 2 localities were examined (Taylor, Best & Couquhoun, 1977).

In experimental infections of young, previously uninfected warthog, Thomson *et al.* (1980) showed that, despite the absence of any obvious ill effect, the virus is capable of producing a generalized infection with high virus titres occurring in lymph nodes and spleen ($10^{6.2}$ and $10^{6.0}$ HD_{50}/g, respectively), the former being the more persistent. Viraemias $\geq 10^{2.0}$ HD_{50}/mℓ were present for the first 11 days following infection only and were no longer detectable by the 33rd day. These results complement the observations of Heuschele & Coggins (1969) and Plowright (personal communication, 1981) on naturally infected warthog in which lymph nodes were consistently shown to contain the highest virus titres. The generally low virus concentrations found in blood and spleen reported by these workers suggest that the animals were

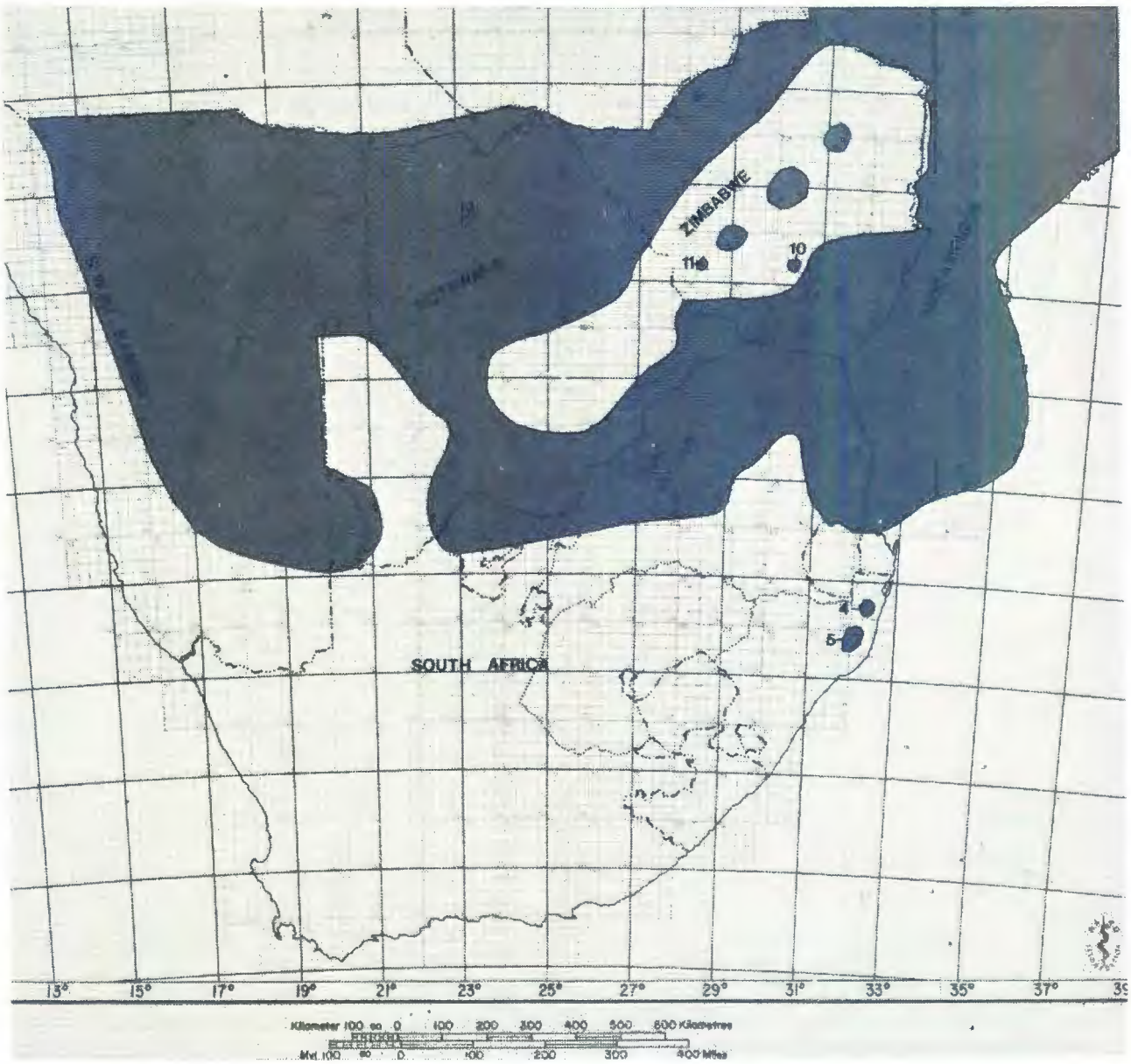

FIG. 1 Map of the southern African region showing localities from which warthog sera were examined for antibody to ASF virus. For results see Table 4.

The shaded area gives the approximate distribution of warthog (after Joubert & Mostert, 1975; Smithers, 1971; Smithers & Lobao Tello, 1976; Smithers & Wilson, 1979.)

Districts from which warthog were sampled in S.W.A./Namibia were: Tsumeb, Grootfontein, Outjo, Otjiwarongo, Omaruru, Okahandja, Gobabis, Windhoek, Rehoboth, Bushmanland & Hereroland East.

sampled some time after initial infection. In general, free-living warthog encountered outside burrows (i.e. older animals) have a low incidence of viraemia and, if present, the virus levels are untitratably low (De Kock, Robinson & Keppel, 1940; Neitz, 1963; Plowright *et al.*, 1969a; Thomson & Gainaru, unpublished data, 1981). Conversely, the relatively high variaemias detected in some neonatal warthogs (Table 5) are consistent with acute infection (Thomson *et al.*, 1980).

Infection of argasid ticks by ASF virus

In a remarkable series of papers, Plowright and his colleagues demonstrated that ASF virus can be maintained in warthog-associated argasid ticks [stated by Walton, (1977) as probably being *O. porcinus*] by transtadial, transovarial and sexual (male to female, but usually not vice versa) transmission mechanisms and that the virus, despite reaching levels $>10^{5.0}$ HD_{50}/tick, has no appreciable effect on the longevity or fecundity of the ticks (Plowright *et al.*,1969a & b; Plowright, Perry, Peirce & Parker, 1970; Plowright, Perry & Peirce, 1970; Plowright, Perry & Greig, 1974). It was also shown that, in the laboratory at least, these mechanisms can be very efficient, e.g. 55–80 % success for transovarial infection and 88 % for male to female transmission (Plowright, 1977). On the other hand, some ticks inexplicably "lost" the infection (Plowright, 1977).

Infection rates in field collections of eyeless tampans made in East and southern Africa were both low and variable—0,17–1,35 % and 0,06–2,6 % respectively (Plowright, 1977; Pini, 1977; Thomson *et al.* 1983). It is probable that the real infection rates of tampans in southern Africa reported by Thomson *et al.* (1983) were lower than are indicated by the above figures since, of the 77 isolates obtained from 22 416 ticks, only 12 were from tampans containing titratable quantities of virus. It was therefore speculated that minimal quantities of virus in ticks do not reflect replication of the virus in the arth-

TABLE 4 Results of serological examinations performed on warthog sera from different geographical locations in southern Africa and the presence or absence of *Ornithodoros* sp. in those areas

Locality (No. [1])		Country	Sera positive/No. tested (%)		Ticks found (+) or not (−) (No. burrows positive/No. examined)
			IEOP	ELISA	
Kruger National Park	(1)	South Africa	$^{32}/_{34}$ (94)	$^{68}/_{73}$ (93)	$+(^{10}/_{18})$
N.W. Transvaal north of latitude 25°S	(2)	South Africa	$^{29}/_{32}$ (91)	$^{47}/_{51}$ (92)	$+(^{16}/_{36})$
N.W. Transvaal south of latitude 25°S	(3)	South Africa	$^{1}/_{24}$ (4)	$^{0}/_{16}$ (0)	$-(^{0}/_{44})$
Mkuzi Game Reserve	(4)	South Africa	$^{5}/_{206}$ (2)	$^{11}/_{260}$ (4)	$+(^{13}/_{40})$
Hluhluwe/Umfolozi Game Reserve Complex	(5)	South Africa	$^{0}/_{297}$ (0)	$^{0}/_{297}$ (0)	−[2]
Nylsvley Nature Reserve	(6)	South Africa	$^{0}/_{7}$ (0)	$^{1}/_{4}$ (25)	$-(^{0}/_{15})$
All areas where warthog occur (see Fig. 1)	(7)	SWA/Namibia	$^{178}/_{192}$ (93)	ND	+[2]
Buffalo Range	(8)	Zimbabwe	$^{34}/_{43}$ (79)	$^{38}/_{40}$ (95)	+[2]
Sebungwe	(9)	Zimbabwe	$^{15}/_{15}$ (100)	$^{13}/_{14}$ (93)	+[2]
Kyle National Park	(10)	Zimbabwe	$^{0}/_{6}$ (0)	$^{0}/_{5}$ (0)	$-(^{10}/_{20})$
Matopos National Park	(11)	Zimbabwe	$^{0}/_{6}$ (0)	ND	$-(^{0}/_{15})$
Maun Game Park	(12)	Botswana	$^{16}/_{30}$ (53)	$^{25}/_{30}$ (83)	+[2]

[1] See Fig. 1
[2] Details of burrows examined not recorded

IEOP—Immunoelectroosmophoresis
ELISA—Enzyme-linked immunosorbent assay

TABLE 5 Summary of viraemias due to ASF virus detected in neonatal* warthog in 4 different localities

Country	Locality	Date	No. litters positive**	No. warthog positive**	Viraemias detected[1]			No. warthog with detectable antibody[2]
			No. tested	No. tested	1,0–2,0	2,1–3,0	3,1–4,0	
SWA	Windhoek	Dec., 1980	$^{0}/_{3}$ or 4	$^{0}/_{10}$	—	—	—	$^{8}/_{10}$
SWA	Otjiwarongo	Dec., 1980	$^{3}/_{4}$	$^{5}/_{9}$	2	2	1	$^{9}/_{9}$
SWA	Okahandja	Dec., 1980	$^{1}/_{1}$	$^{1}/_{1}$	—	1	—	$^{1}/_{1}$
SWA	Windhoek, Okahandja	Dec., 1981	$^{0}/_{5}$ or 6	$^{0}/_{14}$	—	—	—	NT
RSA	Kruger National Park (Pafuri)	Dec., 1982	$^{2}/_{11}$	$^{2}/_{25}$	—	1	1	$^{14}/_{23}$
RSA	Kruger National Park (Malelane Rd.)	Dec., 1982	$^{0}/_{1}$	$^{0}/_{6}$	—	—	—	$^{6}/_{6}$
			$^{6}/_{25,26}$ or 27	$^{8}/_{65}$ (12%)	2	4	2	$^{38}/_{49}$

* ±4 weeks of age (Cumming, 1975)
**i.e., with viraemias >$10HD_{50}/m\ell$
[1] Log $_{10}HD_{50}/m\ell$
[2] Tested by immunoelectroosmophoresis
NT Not tested

ropod, but merely persistent virus concentrations below the infection threshold ingested with warthog blood (Thomson *et al.*, 1983).

The marked discrepancy between the infection rates reported by Pini (1977) and Thomson *et al.* (1983) may be due to differences present at the times of sampling since the procedures employed for virus isolation were identical. This would imply a seasonal or some other cyclic phenomenon. In one area (Rooibokkraal, N.E. Transvaal) ticks were collected every 2 months for 16 months without any detectable change in infection rate (Thomson, Gainaru & Van der Pyperkamp, unpublished data, 1980).

The threshold of infection of ingested virus for 2 East African tick/virus combinations was between $10^{0.9}$ and $10^{2.1}$ HD_{50} and for the 3rd combination, between $10^{3.7}$ and $10^{4.3}$ HD_{50} (Plowright *et al.*, 1970a). Individual variability precluded the calculation of 50 % infective doses for ticks but it was estimated that virus levels tenfold to a hundredfold greater than the infection threshold would achieve this (Plowright, 1977). Plowright further calculated that for warthog blood to be infective for tampans, viraemias would have to reach 10^3 to 10^4 $HD_{50}/m\ell$, but these levels were never observed in the several hundred warthog examined in east Africa. He therefore concluded that warthog blood was an unlikely source of infection for ticks (Plowright, 1977).

The experiments quoted above do not give a breakdown of the relationship between virus dose and infection for different age and sex classes. This is important, because, for example, adult female ticks from our laboratory colony ingest, on the average, 0,2 mℓ of blood per feed (some >0,4 mℓ) while, on the same basis adult males and large nymphae take 0,03 mℓ (Thomson, unpublished data, 1983). If one assumes that the thresholds for the first 2 tick/virus combinations above ($10^{0.9}$–$10^{2.1}$ HD_{50}) apply to adult female ticks, then viraemias between $10^{1.6}$ and $10^{2.7}$ $HD_{50}/m\ell$ would be infective. As can be seen from Table 5, such titres have been found in 6 out of 65 free-living warthog less than 3 weeks of age which were recovered from burrows.

Further evidence that viraemic warthog actually provide a source of infection for ticks in the field is the fact that infection rates in ticks increase with increasing size and age, the implication being that the likelihood of infection increases with the volume of blood meal and number of feeds (Plowright, 1977; Thomson *et al.*, 1983). This, and not merely sexual transmission, may explain why infection rates in adult female ticks are higher than those in adult males (Thomson *et al.*, 1983).

TABLE 6 The failure of 4 warthog (3–4 months old) artificially infected with ASF virus to transmit the infection to unnoculated warthogs and piglets in contact with them

	Species	Virus inoculation[1]	Viraemia[2]	Clinically observable reaction[3]	Antibody titre[4]			Clinical reaction to challenge infection[2] 30 d.p.i.[7]
					Before infection	10 days after infection	30 days after infection	
Group 1*	WH[5]	+	$^3/_3$	—	—	64	16	—
	WH	+	$^3/_3$	—	—	64	ND	—
	WH	—[6]	$^0/_3$	—	—	—	—	—
Group 2**	WH	+	$^3/_3$	—	—	8	16	—
	WH	+	$^3/_3$	—	—	32	16	—
	WH	—	$^0/_3$	—	—	—	—	—
	Pig	—	$^0/_3$	—	—	—	—	Died of ASF
	Pig	—	$^0/_3$	—	—	—	—	Died of ASF

[1] Subcutaneous inoculation of CV strain ($10^{4.2}$ HD_{50})

[2] Viraemias were determined on 3 occasions between 3 and 17 days after infection; $^3/_3$ denotes viraemia detected on 3 occasions

[3] Refers to inappetence or body temperature appreciably greater than those of the control warthog

[4] Determined by immunoelectroosmophoresis (reciprocal of the end-point dilution)

[5] WH Warthog

[6] Uninoculated or negative result

[7] d.p.i. Days after infection of the warthog

Group 1: Housed in the open air in a small pen provided with an infra-red heated sleeping box (0,5 m^3)

**Group 2:* Housed in a 36,5 m^3 room undergoing 20 air changes per hour and containing an identical sleeping box

TABLE 7 Infection rates of laboratory-raised *Ornithodoros* sp. fed on ASF virus-containing blood

Stage of development	Source of viraemic blood	Titre of virus in blood[1]	Estimated virus intake[2]	No. ticks infected[3] No. fed (%)	Virus strain designation
2–3 Stage NY	Pig/M	3,6	NEA	$^0/_5$	CV
3–4 Stage NY	Pig/M	3,6	2,3	$^0/_{10}$	CV
Female	Pig/M	3,6	3,1	$^0/_3$	CV
Male	Pig/M	3,6	2,4	$^0/_5$	CV
Female	Pig/M	5,2	4,2	$^0/_{16}$	CV
Male	Pig/M	5,4	4,0	$^0/_{11}$	CV
Female	Pig/M	5,4	4,7	$^0/_{16}$	CV
4–5 Stage NY	Pig/LF	4,1	2,9	$^1/_2$ (50)	CV
4–5 Stage NY	Pig/LF	4,1	3,0–3,7	$^5/_7$ (71)	CV
4–5 Stage NY	Pig/LF	6,3	5,1–5,9	$^4/_9$(44)	CV
4–5 Stage NY	Pig/LF	7,2	4,8–5,0*	$^0/_6$	CV
4–5 Stage NY	Pig/LF	7,2	5,1–6,0*	$^2/_{30}$ (7)	CV
4–5 Stage NY	Warthog/LF	2,2	1,5	$^0/_{67}$	$114a_2$
4–5 Stage NY	Warthog/LF	2,4	1,7	$^0/_{35}$	$114a_2$
4–5 Stage NY	Warthog/LF	2,9	2,2	$^0/_{34}$	$114a_2$

[1] Log_{10} $HD_{50}/m\ell$

[2] Log_{10} HD_{50} estimated on the basis of mean mass gain immediately after feeding (Thomson, unpublished data, 1983)

[3] Ticks were incubated for 30 days at 30 °C and 90 % relative humidity before checking for virus content

* Interrupted feeding on viraemic pig (to limit virus ingestion) followed by feeding to repletion on an uninfected pig 24 hours later

NY nymphae

M fed through a membrane on defibrinated blood to which virus cultured *in vitro* had been added

LF fed on live viraemic pig or warthog

NEA no estimate available

The fact that the tampan gut is a predilection site for viral replication (Greig, 1972) suggests that the oral route of infection in ticks is likely to operate in nature.

In view of the finding that neonatal warthogs develop viraemias which are potentially capable of overcoming the infection threshold of *Ornithodoros moubata/porcinus* complex ticks, we have attempted to conclusively demonstrate this possibility. A laboratory colony of *Ornithodoros* sp., collected from warthog burrows in the Northern Transvaal some years ago (no other details are available), and 2 ASF viruses which also originated from the N. Transvaal (1 from pigs and the other isolated from wathog-associated *Ornithodoros* sp.), were used.

Initially ticks were fed on viraemic pigs in the early stages of infection (2–3 days after virus inoculation). Sometimes feeding was interrupted (Table 7). This showed that, while ticks could be infected with between $10^{2.9}$ and $10^{6.0}$ HD_{50}, the proportion that became infected did not increase with increasing virus doses (Table 7).

When the same virus strain grown in blood leucocyte cultures was diluted in defibrinated blood from uninoculated pigs and fed through silicone/parafilm membranes (Davis, Butler, Roberts, Reinert & Kline, 1983, as adapted by Randall & Nevill, personal communication, 1983), none of the 66 ticks so fed became infected, despite the fact that at least 46 (70 %) ingested $>10^3$ HD_{50} (Table 7).

None of the 136 ticks fed on young viraemic ($\leqslant 10^{2.2}$ $HD_{50}/m\ell$) warthog inoculated with a tick isolate 114a become infected (Table 7).

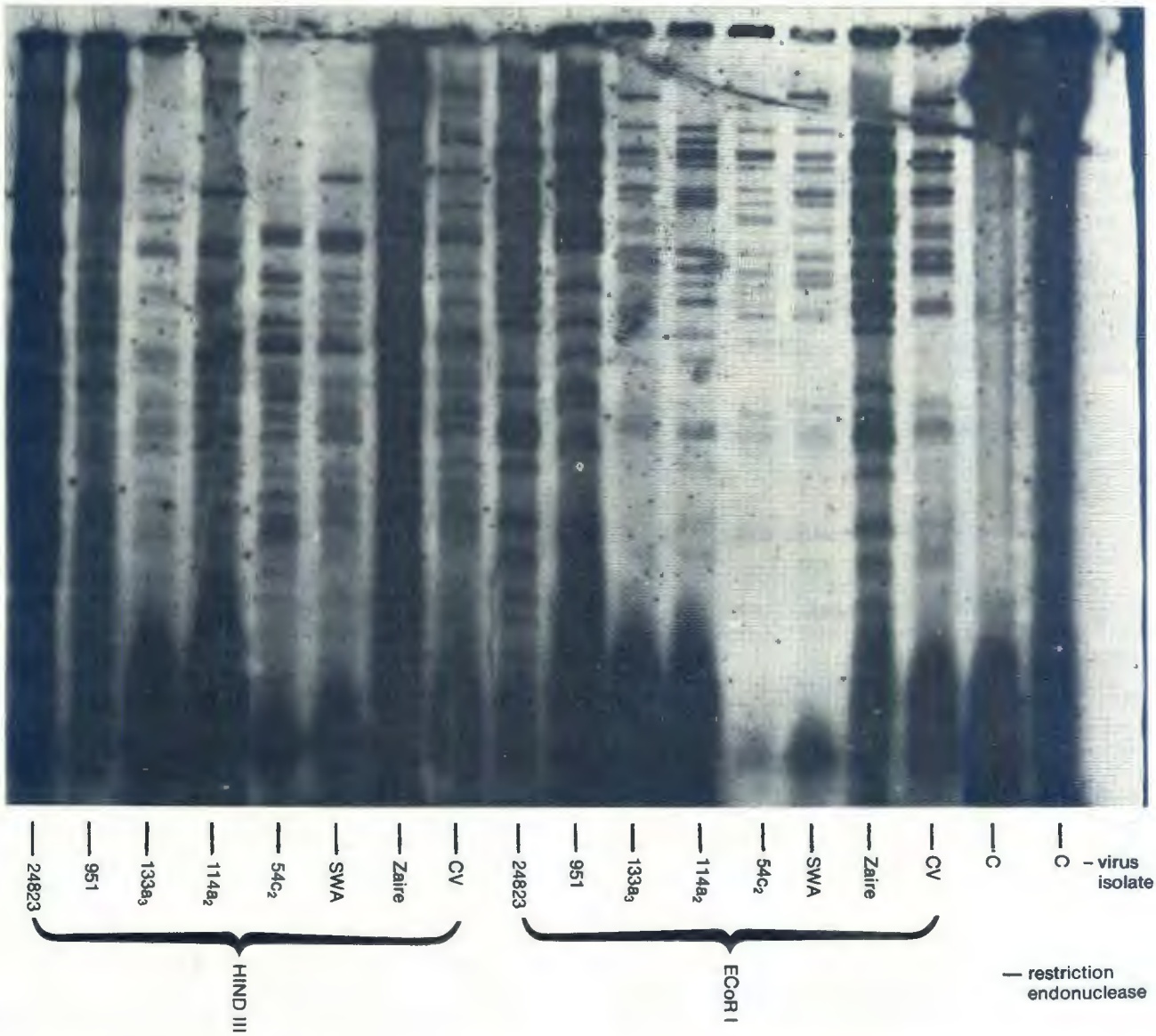

FIG. 2 Photograph of an autoradiograph obtained from an agarose gel in which restriction endonuclease generated fragments of ^{32}P-labelled ASF virus DNA was electrophoresed

These results, taken in conjunction with those of Plowright (1977), indicate that:

(i) The threshold level for tick infection by ingestion of viraemic blood depends on the tick/virus combination, but in 3 out of the 5 combinations used so far the level was $<10^3$ HD_{50}.*

(ii) Apart from infection threshold there are other factors which determine the success or otherwise of infection in ticks, since a proportion of the latter remain refractory to infection even after high virus concentrations are ingested.

(iii) Virus cultivated *in vitro* is less infective for ticks than that present in viraemic pig blood.

The above findings are hardly surprising in view of the complex interactions which exist between, for example, arboviruses and mosquitoes (Hardy, Houk, Kramer & Reeves, 1983). It is clear therefore that the interrelationship between *Ornithodoros* sp. and ASF virus is imprecisely understood. Although 4 potential mechanisms of infection are known for ticks (viz., transtadial, sexual and transovarial as well as ingestion of viraemic blood), it is not known whether they all operate in the field and, if so, to what extent.

Immunogenic variants within the ASF virus population

Little progress has been made regarding the possible existence of immunologically distinct ASF viruses because of the lack of neutralizing antibody in immune sera (Wardley *et al.*, 1983), the virulence of most virus isolates for pigs and the variability experienced in protection experiments. This aspect has been reviewed by Greig (1980) and the weight of evidence suggests that such variants do occur.

The advent of restriction endonucleases for establishing differences between double-stranded DNA molecules has, however, opened another avenue for investigation and European, Caribbean and S. American iso-

* Since the preparation of this manuscript Wilkinson has reported the infection of 20% of female *Ornithodorus* sp. fed on blood containing $10^{2.5}HD_{50}/m\ell$ of virus (Wilkinson, P. J., *Preventive Veterinary Medicine*, 2, 71–82, 1984). This substantiates the probability of these ticks acquiring the infection from viraemic warthog in the field.

lates have been compared using this technique (Wesley & Pan, 1982; Talavera, Almendral, Ley & Vinuela, 1983; Wesley & Pan, 1983). We have also carried out some preliminary comparisons of southern African isolates grown in pig alveolar macrophage cultures labelled with ^{32}P in this way (Fig. 2). All the field isolates were passaged only 3–6 times in order to minimize the alterations in cleavage sites observed by Wesley & Pan (1982) during adaptation of the wild-type virus to cell culture. Using ECoRI and Hind III, major differences were detected among all 6 of the field isolates tested.

Apart from extending the number of isolates and enzymes investigated, it still needs to be established whether the differences observed occur between or within localities and whether the differences reflect important immunological variations.

As stated above, however, titratable viraemias have so far only been found following what must presumably be 1st infection and it would appear unlikely that subsequent infections are followed by the same degree of viral replication.

The source of virus in primary outbreaks of ASF in pigs in Africa

The long-held belief that the source of virus in primary outbreaks of ASF in southern and East Africa is "carrier" wild pigs can, for all practical purposes, be discounted. In only 1 report (De Tray, 1957) was ASF suspected of having been transmitted in this way. The other investigations, including the experiment summarized in Table 6, indicate the contrary (Montgomery, 1921; Heuschele & Coggins, 1969; Plowright *et al.*, 1969a). In addition, although Thomson *et al.* (1981) demonstrated that the tissues of acutely infected warthog are potentially infectious for pigs which may eat them (as postulated by Montgomery, 1921), this is an improbable factor in the field where acutely infected warthog are likely to be young and therefore unsuitable targets for hunters. Domestic pigs are unlikely to encounter such tissues other than through the agency of human beings.

As an alterntive, Plowright *et al.* (1969b) postulated that infected ticks might be transported to the vicinity of domestic pigs either by warthogs themselves or, alternatively, on the carcases of warthogs. Ticks which became dislodged could then remain a potential source of infection for many months. The flaw in this hypothesis was that *Ornithodoros* sp. were believed to travel only occasionally on the bodies of warthog outside burrows (references quoted by Plowright *et al.*, 1969a). This belief, plus the fact that infection rates in ticks are often low (Plowright, 1977; Thomson *et al.*, 1983), suggested that this mechanism was doubtful. Recently, however, Horak & De Vos (personal communication, 1983) recovered 428 *Ornithodoros* sp. nymphae from 32 of 68 warthogs examined in the Kruger National Park, 2 of which carried 97 and 107 ticks, respectively. This adds new credibility to the above theory.

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