A serological survey of rinderpest antibody in wildlife and sheep and goats in Northern Tanzania

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SUMMARY

An extensive serological survey for rinderpest antibody in wildlife, principally buffalo (*Syncerus caffer*), and sheep and goats has been undertaken in the previously endemic region of Northern Tanzania to determine whether or not the virus has continued to cycle in susceptible species since the last occurrence of overt disease in 1982. The results show that infection but not disease has occurred at least until 1987 in buffalo in parts of the Serengeti National Park but not in the other game areas of Tanzania where samples were taken. Sero-positive sheep and goats were widely distributed and have been found in 10 of the 14 districts sampled but there have been no reports of disease. These findings bring into question the possibility of eradicating the disease from Africa and continuous annual monitoring of this and other similar ecological zones will be required.

INTRODUCTION

Outbreaks of rinderpest used to occur annually in cattle and wildlife in northern Tanzania and southern Kenya up until 1962. Its apparent disappearance has been supported by a lack of any serological evidence of the disease since 1965/66 [1–3]. The last recorded clinical outbreak of rinderpest in the area occurred in cattle in Loliondo to the east of the Serengeti National Park in 1967 but a serological survey [4], found no evidence of transmission to wildlife particularly wildebeest. However, in 1982 the disease was diagnosed on clinical and pathological grounds in buffalo (*Syncerus caffer*) and cattle at several widely separated localities. Deaths in cattle occurred in the districts of Mwanga and Same to the east of Mount Kilimanjaro adjacent to the Kenya–Tanzania border, in Handeni district in Tanga region and in Kiteto district in the Masai steppeland to the south of Arusha (Fig. 1.) The disease also resulted in the deaths of about 200 buffalo in the Kleins Camp area in the north of the Serengeti National Park (Fig. 2) with others being reported in the Ngorongoro Conservation area. In addition, deaths of lesser kudu (*Tragelaphus imberbis*) were reported in Mwanga, Same and Kiteto districts, of

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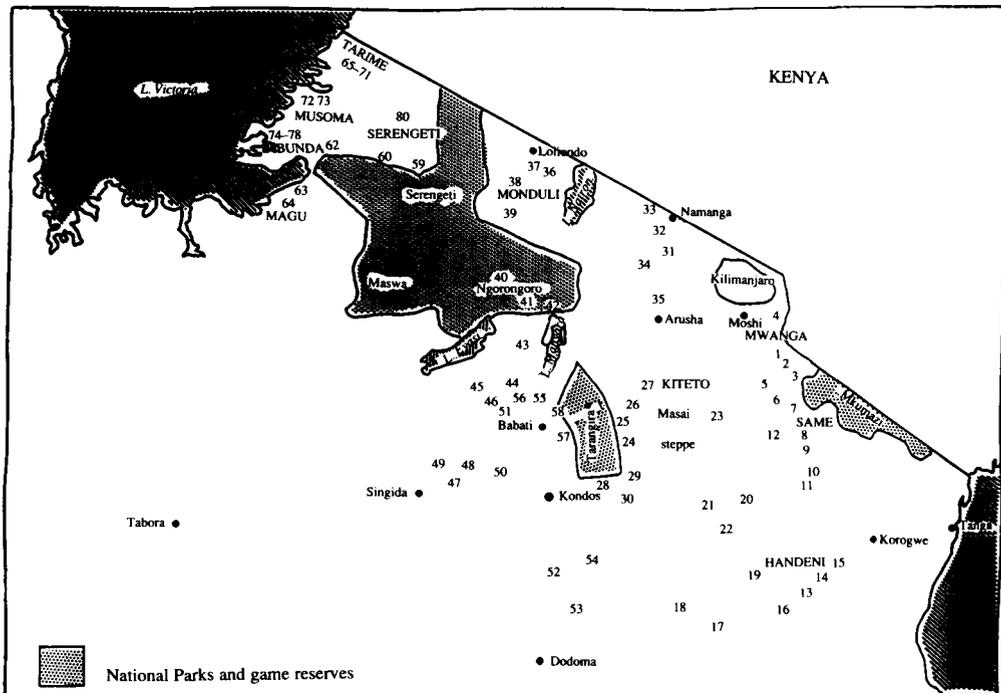
Fig. 1. The places designated by numbers where samples were collected from sheep and goats.

eland (*Taurotragus oryx*) in Ngorongoro and of warthog (*Phacochoerus aethiopicus*) in Handeni district (J Nyange, personal communication). The suspected presence of rinderpest in 1982 was confirmed by retrospective serology in cattle (WP Taylor, personal communication) and buffalo (*Syncerus caffer*) (L Karstad, and PB Rossiter, personal communication).

The source of the 1982 outbreak has never been determined but inevitably there has been much debate both in relation to outbreaks in the past [2] and following the 1982 outbreak on the possibility for reservoirs of infection in the very large biomass of susceptible animals in northern Tanzania. Both wildlife and domestic small ruminants, which are not vaccinated, can act as indicator species for the presence of virus and therefore a comprehensive serological survey for rinderpest antibody was carried out between 1986 and 1989 to try and clarify the role of these species in the maintenance of the virus.

METHODS

Wildlife

The game areas and National Parks where wildlife samples have been collected are shown in Fig. 3 and the details of the location and species are given in Table 1. The majority of samples were collected by chemical immobilization. Sampling was targeted mainly at buffalo as they are amongst the most susceptible of species, occur in large numbers and their gregarious habits favour the transmission of infection. They are also widely distributed and in this study samples were
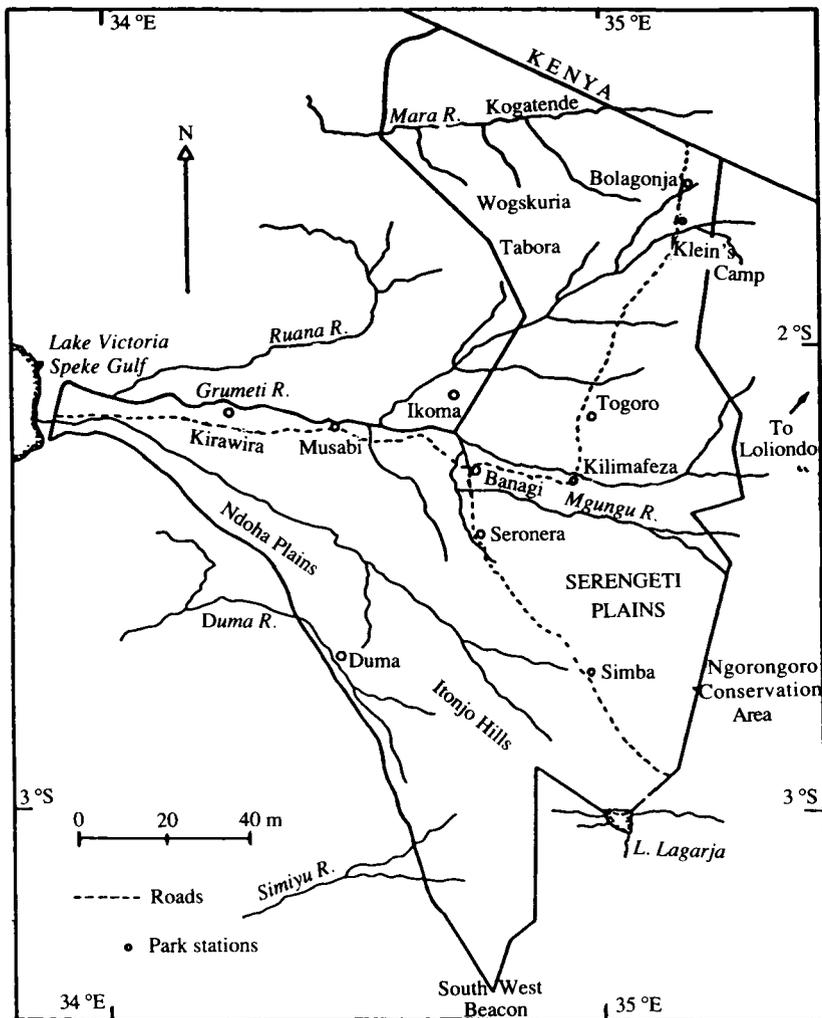
Fig. 2. The Serengeti National Park.

collected from buffalo in the Serengeti, Manyara and Tarangire National Parks as well as the Ngorongoro Conservation area in northern Tanzania, in Katavi National Park in the west, in Ruaha and Mikumi National Parks in central Tanzania and in the Selous game reserve in the south-east (Fig. 3).

Samples were collected in the Serengeti in 1986, 1987, 1988 and 1989. Samples were only collected from animals over 12 months of age and to begin with included adults in order to obtain confirmatory evidence of the 1982 outbreak of rinderpest. In 1988 and 1989 animals of between 1 and 3 years of age were mainly sampled in order to obtain information of the current epidemiological situation. Buffalo were only sampled once in the other game areas. The four southern game areas constituted a negative control population as rinderpest has not been recorded south of the central railway line from Dar es Salaam to Kigoma for more than 30
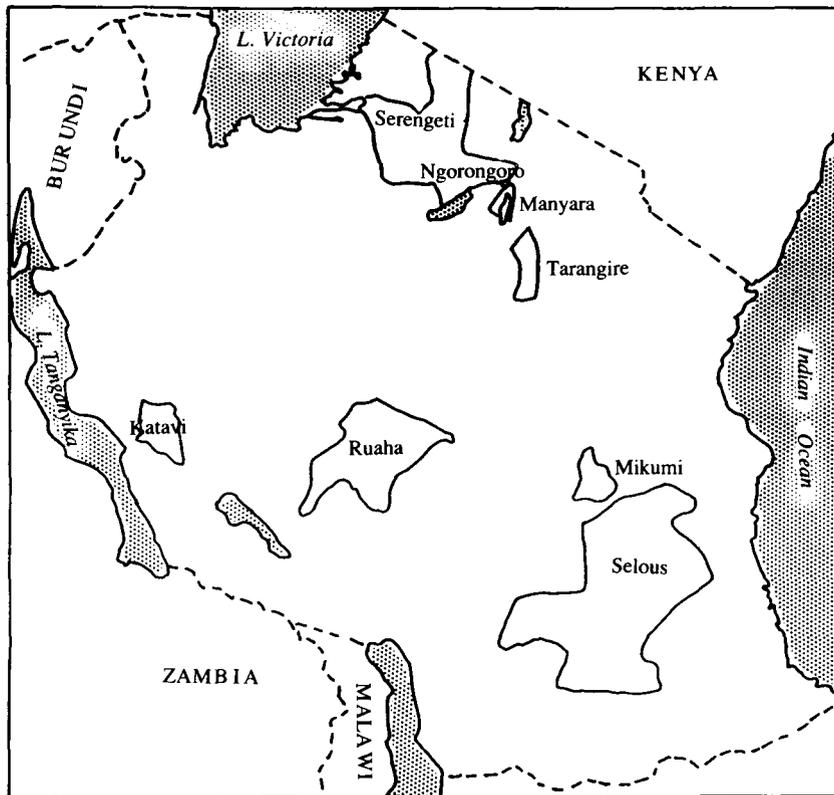
Fig. 3. The game areas and National Parks where samples were collected.

years. A serological survey carried out in cattle in 1985 [5] did not provide any evidence for the presence of rinderpest infection in the southern part of the country.

Chemical immobilization. Blood samples were collected following chemical immobilization. We found that in practice it was necessary to limit the variation in the dosages of the various drug combinations used as much as possible. The details of the drug combinations and dosages used throughout this survey are given in Table 2. Drugs were delivered using Dist-Inject (Peter Ott Ltd, PO Box 16, CH-4007, Basel, Switzerland) or Daystate (Daystate Ltd, Newcastle Street, Stone, Staffs, ST15 8JU, UK) equipment.

Ageing. Buffalo were aged according to dentition, horn shape and development and body size based on personal experience. We considered that the 1st pair of permanent incisor teeth erupted at 1 year 9 months and were fully erupted by $2\frac{1}{4}$ years. The 2nd pair were fully erupted by $2\frac{1}{2}$ years, the 3rd pair by 3 years and the last pair by $3\frac{1}{2}$ years. Sinclair [6] gave 33 months, 42 months, 4 years and 5 years respectively for ages of eruption of the permanent incisors. Our interpretation therefore gave ages younger than Sinclair's would have done. The other species sampled were aged on size alone.

Sheep and goats

The places from which samples were collected are shown in Fig. 1 and detailed

Table 1. *Numbers of blood samples collected from different species in each wildlife area according to year of collection*

		No. of samples and year of collection				
Area	Species	1986	1987	1988	1989	Total
1. Serengeti						
(a) North	Buffalo	10	33	2	32	77
	Wildebeest	—	10	21	—	31
	Topi	—	2	—	—	2
(b) West	Buffalo	10	37	—	76	123
	Wildebeest	—	15	—	—	15
	Topi	—	1	—	—	1
	Warthog	—	—	—	8	8
(c) South-west	Buffalo	10	15	8	—	33
	Wildebeest	—	17	21	—	38
	Topi	—	6	3	—	9
	Kongoni	—	1	3	—	4
	Warthog	—	5	3	—	8
2. Tarangire	Buffalo	—	19	—	—	19
3. Ngoronogoro	Buffalo	—	20	—	—	20
	Wildebeest	—	4	—	—	4
4. Manyara	Buffalo	—	15	—	—	15
5. Katavi	Buffalo	—	—	30	—	30
6. Ruaha	Buffalo	—	—	51	—	51
7. Mikumi	Buffalo	—	—	16	—	16
8. Selous	Buffalo	—	—	19	—	19

Table 2. *Drug combinations and dosages used in chemical immobilization*

Species	Age	Immobilon*	Rompun†	Vetalar‡	Stresnil§
Buffalo (*Syncerus caffer*)	All ages	2 ml	2 ml	—	—
Wildebeest (*Connochaetes taurinus*)	Yearlings	1·2 ml	1·3 ml	—	—
Topi (*Damaliscus korrigum*)	Adult	1·6 ml	1·4 ml	—	—
Hartebeest (*Alcephalus cokii*)	Adult	1.8 ml	1.5 ml	—	—
Warthog (*Phacochoerus aethiopicus*)	Subadult	—	—	2 ml	2 ml

* Imobilon L.A. (C-Vet), ethorphine HCl 2·45 mg, acepromazine maleate 10 mg per ml.
† Rompun (Bayer), xylazine 20 mg per ml.
‡ Vetalar (Parke, Davis), ketamine HCl 100 mg per ml.
§ Stresnil (Janssen), azaperone 40 mg per ml.

in Table 6. Samples from localities 1–58 were collected in 1988 and the remainder in 1989. Additional confirmatory samples were collected in 1989 from locations 24, 27 and 28 in Kiteto district and from location 32 in Monduli district. All the samples were taken from animals of less than $3\frac{1}{2}$ years of age.

Collection of blood samples. Blood samples were collected aseptically into vacutainers (Becton Dickinson) and stored at 4 °C for up to 4 days. Serum was removed using sterile pipettes and stored in sterile plastic tubes (Sarstedt) at −20 °C.

Table 3. *The proportion of samples collected from buffalo of different ages in three
areas of the Serengeti National Park with rinderpest antibody*

Area		Year of birth								Total
		< 1981	1982	1983	1984	1985	1986	1987	1988	
North	(a)	1/1	4/4	1/1	4/7	1/13	1/37	0/8	0/3	12/74
	(b)	100%	100%	100%	57%	8%	2·7%	nil	nil	16.2%
	(c)	2·55	1·5	1·8	1·5	1·05	—	—	—	
West Musabi	(a)	2/2	—	2/2	2/7	6/22	6/36	2/16	—	20/85
	(b)	100%	—	100%	29%	27%	17%	12·5%	—	23·5%
	(c)	1·2	—	0·75	1·3	1·1	1·1	1·5	—	—
Kirawira and Ndabaka plains	(a)	—	—	—	—	1/3	1/19	1/11	0/1	3/34
	(b)	—	—	—	—	33%	5%	9%	nil	8·8%
	(c)	—	—	—	—	1·05	1·05	1·35	—	—
Seronera Hemol Moru	(a)	4/4	3/3	2/2	1/6	0/8	1/12	2/3		
	(b)	100%	100%	100%	17%	nil	8%	67%		
Kopjies	(c)	2·0	1·1	1·35	0·6	—	1·0	1·0		

(a) Proportion of animals with antibody.
(b) Percentage of animals with antibody.
(c) Mean titre ($\log_{10}$) of the positives.

Antibody assay. Each wildlife serum was assayed for neutralizing antibody to rinderpest virus in both a micro-neutralization/ELISA test [7] and a tube neutralization test using either secondary calf kidney cells or the Madin Derby bovine kidney (MDBK) cell line. The virus strain used in all the tests was the attenuated RBOK strain at a concentration of between 30-300 $TCID_{50}$ per test. The sheep and goat samples were inactivated at 56 °C for 30 min and then screened in a tube neutralization test at a dilution of 1/10 using two tubes per dilution. All positive sera were then retested at dilutions of 1/10 and 1/30 to confirm the result and where necessary retested to obtain final end-point titres.

RESULTS

Wildlife

The details of the total number of samples collected are given in Table 1. The majority of samples collected were from buffalo and the results from each game area are examined separately. A total of 233 samples from buffalo were collected in the Serengeti National Park between 1986 and 1989. These were collected from the north close to the Kenya border, in the western corridor and in the Seronera area in the centre of the park (Fig. 2) and the results obtained are given in Table 3. During the 1982 outbreak mortality in buffalo occurred in the north in the Kleins Camp area. All those animals born either prior to the outbreak or in the year after were found to be positive for neutralizing antibody with high titres

Table 4. *The proportion of samples collected from buffalo of different ages in Tarangire National Park in 1987 that were positive for rinderpest antibody*

			Year of birth			
	< 1981	1982	1983	1984	1985	1986
(a)	3/5	1/1	—	1/1	0/7	0/5
(b)	60%	100%	—	100%	nil	nil
(c)	1·7	1·8	—	0.75	—	—

(a) Proportion of animals positive for rinderpest antibody.
(b) Percentage of animals positive for rinderpest antibody.
(c) Mean titre ($\log_{10}$) of positives.

Table 5. *The proportion of buffalo of different ages in the Ngorongoro Conservation area and in Manyara National Park that were positive for rinderpest antibody*

			Year of birth			
	< 1982	1983	1984	1985	1986	1987
Ngorongoro	1/1	—	—	0/5	0/10	0/3
Manyara	0/1	—	0/7	0/1	0/6	—

when sampled not less than 4 years later. The samples from younger animals provided evidence for the persistence of the virus in this area at least until 1986 when 2·7% of samples were positive but with the mean titre of the positives ($\log_{10}$ 1·5) much lower than at the time of the outbreak. The 11 animals born in 1987 and 1988 were all negative, at the time the samples were collected in either 1988 or 1989.

At Musabi in the western corridor antibody was found in all animals alive at the time of the outbreak. The titres in these older animals were not as high as found in this age group in the north. No deaths were reported in this area in 1982. Antibody has been detected in animals born each year up to 1987 the youngest age group sampled. One of the two antibody-positive buffalo born in 1987 and sampled when 20 months old in 1989 had antibody levels of $\log_{10}$ 1·65. The samples collected further west at Kirawira and on the Ndabaka plains were also positive each year up to 1987, with the one positive animal born in 1987 and sampled when $2\frac{1}{4}$ years old in 1989 having a titre of $\log_{10}$ 1·35.

The results from buffalo sampled in the Seronera and Moru Kopjies area are similar with all animals alive at the time of the outbreak being positive for antibody. The four animals born before 1981 had a mean titre of $\log_{10}$ 2·0. Antibody positive individuals were found in groups born up to 1987 but individual titres were lower.

A total of 94 samples from wildebeest were also collected from the same three areas of the park. All were found to be negative as were the small number of other species sampled (Table 1).

The overall results from Serengeti indicate that infection was more widespread around the time of the outbreak in 1982 than the observed mortality suggested. The virus had infected buffalo until at least 1987 in the west and around Seronera.

Table 6. *Details of the places where samples were collected from sheep and goats,*
the numbers of samples collected and the distribution of the positive samples

District	Numbers given to places sampled*	Total samples	Places where positives found	Number of each species positive	Mean titre (log_{10}) of positive (range)
Mwanga	1–12	650	nil		
Handeni	13–16	340	nil		
Kiteto	17–30	1616	20, 21, 23, 24, 27, 28, 30	13 sheep 17 goats	1·29 (1·0–1·8)
Monduli	31–35	383	32	1 goat 1 sheep	1.23 (1·0–1·45)
Ngorongoro	36–40	480	37, 39	3 goats	1·4 (1·25–1·5)
Mbulu	41–46	300	45	1 sheep	1·15
Hanang	47–51	243	nil		
Kondoa	52–54	260	nil		
Babati	55–58	254	55, 56	1 sheep 3 goats	1·3 (1·15–1·5)
Serengeti	59, 60, 80	210	59, 60, 80	3 sheep 3 goats	1·18 (1·15–1·45)
Bunda	61–62 74–79	1477	61, 62, 75, 78, 79.	12 goats	1·13 (1·0–1·6)
Magu	63–64	343	63, 64	2 sheep 2 goats	1·23 (1.15–1.45)
Tarime	65–71	612	65, 66, 67, 68, 69, 70, 71	3 sheep 24 goats	1·18 (1·0–2·1)
Musoma	72–73	128	72	16 goats	1·02 (1·0–1·15)

* See Figure 1.

Infection is probably still present but further annual sampling will be required to confirm this.

The other three game areas in the north of the country where samples were collected were sampled only once in 1987. The results are given in Tables 4 and 5. In Tarangire National Park buffalo born in 1982 or earlier had high antibody titres. The one animal born in 1984 was positive but had a much lower titre. The 12 animals born in 1985 and 1986 were negative. In Ngorongoro the one old animal sampled was positive with a titre of log_{10} 1·3. The 15 buffalo sampled in Manyara were all negative.

As expected the 116 buffalo sampled in the four game areas in the west and south of the country in 1988 were all negative, confirming the absence of infection in wildlife south of the central railway line.

Sheep and goats

A total of 7296 samples collected throughout northern Tanzania have been assayed. The places where animals positive for rinderpest antibody have been found are listed in Table 6 and their location illustrated in Fig. 1. Samples were collected in 14 administrative districts and positive animals have been detected in

10 of them. The highest proportions of positive samples were found in Musoma (12·5%), Tarime (4·4%), Serengeti (2·9%), Kiteto (1·9%), and Babati (1·6%).

The highest proportion of positives was found in the north-west of the region with most positives being found in Musoma district. In this district mainly goats were sampled – in fact all the positive animals were goats from two herds in Butiama. All had antibody levels of $\log_{10} 1·0$–$1·15$. In contrast, in Tarime district on the border with Kenya, positive animals were found at every place where samples were taken and three of the positive goats had significantly high titres of $\log_{10} 1·45$, $\log_{10} 1·75$ and $\log_{10} 2·1$ respectively. Antibody titres of this order were also found in individuals in Serengeti, Bunda and Magu districts in this region.

Sero-positive animals were widely distributed in Kiteto district to the east of the Tarangire National Park and 6 of 30 positive animals had antibody titres of $\log_{10} 1·6$ or greater.

DISCUSSION

This study gives the results of a comprehensive survey undertaken to determine whether or not rinderpest virus has continued to cycle subclinically in the previously endemic region of northern Tanzania following the re-occurrence of the disease in 1982. The survey was conducted in both wildlife species and sheep and goats. Cattle were not included because most have been vaccinated during the last 4 years and emergency vaccinations were carried out in 1982–4 in the regions where the 1982 outbreak occurred.

As far as the wildlife are concerned, a significant number of samples were collected only from buffalo and wildebeest. The survey in buffalo is the most comprehensive yet undertaken in this species in this area. In the 1982 outbreak deaths in buffalo attributed to rinderpest occurred in the north of the Serengeti National Park and in the Ngorongoro Conservation area. Our results confirm this with a high proportion of the animals born before or at this time being positive for rinderpest antibody. The disease was, however, more widespread than the observed occurrence of mortality suggested as seropositive animals of this age group have also been found in Tarangire National Park though not in the Manyara National Park. Samples from buffalo have not yet been collected to the east of Mount Kilimanjaro in the vicinity of the Mkomazi game reserve where deaths in cattle were reported in 1982. The persistence of rinderpest infection since that time has only been confirmed in the Serengeti National Park but the proportion of buffalo sampled and shown to be positive has decreased progressively each year. Similarly the neutralizing antibody titre of the positive animals has also decreased. In the Serengeti, buffalo have been exposed to rinderpest virus in the north, in the western corridor and in the central Seronera area (Fig. 2) since 1982 but by 1987 seropositive animals were found only in the western corridor and around Seronera. However, only a small proportion of the total buffalo population has been sampled and it would be necessary to take annual samples for several more years before one could be certain that the virus is confined to the two parts of the Park. In the western corridor seropositive animals born in 1985, 1986 and 1987 have been found in the Musabi plains area each year and further west in the Kirawira and Ndabaka plains area in 1987. As buffalo do not range more than a

radius of some 30 kilometres these two populations can be considered to be distinct as far as transmission of infection is concerned. The system of ageing that we have used predicts the animals to be younger than that of Sinclair [6] but this does not alter the interpretation of the results in any way and makes it certain that none of the positives can possibly be due to maternally-derived antibody because all were definitely over 12 months of age.

Although 84 samples have been collected from wildebeest no seropositive animals have been found. A further 100 samples from wildebeest collected during a cropping exercise in 1986 were tested in an indirect ELISA and also found to be negative. All the other species sampled have similarly been negative though only a small number were tested.

In the rinderpest survey conducted in the Serengeti between 1967–71 by Plowright [2] low levels of antibody (titre $\leqslant \log_{10} 0.75$) were found in 7 of 15 buffalo from the Mbalageti river and no serological evidence of rinderpest infection was found in wildebeest, eland, Thomson's gazelle, Grant's gazelle, Topi, Coke's hartebeest or warthog after 1962. His conclusion from this survey of East African wildlife was that there was no adequate evidence for the persistence of rinderpest infection during this period. Our evidence suggests that rinderpest virus is present now in both the western corridor and central part of the Serengeti National Park. The attack rate is, however, very low with only 1–3 % of animals of between 1–3 years of age that were sampled being positive and it is therefore uncertain whether the virus will be able to persist indefinitely. However, it is possible that the proportion of detected seropositive animals does not reflect the true number of animals that have been exposed to infection because of the low sampling rate. Also humoral antibody following subclinical infection may wane rapidly to levels that are not detected in the neutralization test. Although unlikely, this was shown to occur following an outbreak in wildebeest by Plowright and McCulloch [3] who observed in a serological survey carried out between 1960 and 1963 that antibody titres fell rapidly following an outbreak to levels around $\log_{10} 0.96 \pm 0.513$. Buffalo calve throughout the year and there are always susceptible animals in a herd. Transmission is favoured by the gregarious habits of buffalo which not only form large breeding herds but also have the habit of lying up in close huddles. This should favour transmission to a higher proportion of susceptible animals than our results suggest. It is also not possible at present to rule out a non-wildlife source of infection for buffalo i.e. cattle.

At what point in time overt disease occurs with resulting mortality remains obscure. Plowright [2] pointed out the absence of disease for 17 years from 1965 to 1982. A similarly long interval may occur again from the last clinical outbreak in 1982. The basis for change in pathogenicity is the subject of intense study and may be concerned with changes around the F_0 protein cleavage site of the virus envelope. It has been shown in another paramyxovirus, Newcastle disease virus, that cleavage of the F_0 protein to its subunit F_1 and F_2 proteins is associated with pathogenicity [8].

This study also provides evidence for rinderpest virus infection in small ruminants. All the samples collected were from animals of 3 years or less and up to 12.5 % of animals in one area were found to be positive. The seropositive animals were very widely distributed although loci containing proportionately

larger numbers of positives have been found in Simanjiru in Kiteto district, and in Babati, Musoma and Tarime districts.

The area to the north-west of the Serengeti National Park where positive animals had the highest antibody titres requires regular surveillance. This is a part of the country into which infection could be introduced through trade in livestock. Rinderpest is frequently inapparent in small ruminants though infected animals are able to transmit the disease to cattle [9]. However, no evidence of clinical disease in either small ruminants or cattle has been obtained. In a smaller survey carried out in the Turkana region of Kenya [10] 34% of samples were positive for neutralizing antibody and a further 24% contained trace amounts of antibody with titres ranged between $\log_{10}$ 0·3 and 2·25. This was a far greater prevalence of positives than found in this survey and suggests active infection perhaps with cattle involved as well.

Cattle have been vaccinated in northern Tanzania annually for many years but the intensity of vaccination has been increased considerably since the 1982 outbreak of rinderpest. Between 1985 and 1988 a countrywide cattle vaccination programme was carried out and the immune status of the national herd monitored [5]. It is known therefore, that the possibilities for transmission of infection between wildlife, small ruminants and cattle have been greatly reduced and the chance of cattle acting as indicator hosts of infection largely removed. Consequently the virus must be cycling separately within each population of domestic small ruminants and buffalo. It must be assumed that this will continue unless there is intervention with vaccination of small ruminants. This, however, will not result in eradication of the virus if it continues to cycle in wildlife. Continued annual monitoring of the situation will be necessary before eradication of rinderpest by vaccination becomes a possibility.

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