NEUTRALISING ANTIBODIES TO PARAINFLUENZA 3 VIRUS IN AFRICAN WILDLIFE, WITH SPECIAL REFERENCE TO THE CAPE BUFFALO (Syncerus caffer)

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NEUTRALISING ANTIBODIES TO PARAINFLUENZA 3 VIRUS IN AFRICAN WILDLIFE, WITH SPECIAL REFERENCE TO THE CAPE BUFFALO *(Syncerus caffer)*

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Abstract: As part of a study to assess the prevalence of common viral agents in African wildlife, nearly 3,300 sera from 44 different wild species, from eight African countries, have been examined for neutralising antibodies to parainfluenza 3 (PI_3) virus. Antibody was demonstrated in 20 of the 44 species examined, including seven species not previously reported as sero-positive. Sera were collected between 1963 and 1977 and results indicated that infection has been widespread for a considerable time.

The high prevalence of antibody, and the range of titres, to PI_3 virus found in free-living populations of buffalo suggest that this species is particularly important as a reservoir of infection in the wild.

INTRODUCTION

Parainfluenza 3 virus has a wide host range and serological evidence and isolations from widely separated areas indicate that infection is worldwide.[24] In South Africa, Erasmus *et al.*,[6] using a human strain of PI_3 virus, showed infection to be widespread in domestic animals and also demonstrated the presence of antibody in some species of wild animals. Similarly, serologic evidence of PI_3 infection has been reported in cattle and small numbers of some species of wild animals in Central Africa.[16,19] Infection has also been shown to be widespread in cattle in East Africa[13,14,18] and Kalter *et al.*[12] have demonstrated antibody in three of five wild animal species examined.

This paper presents the results of serum neutralisation (SN) tests, against the T_1 strain of PI_3 virus, of nearly 3,300 sera collected from 44 species of wildlife in a number of African territories. Particular reference is made to the African buffalo for which successive samples, taken from a single population over a number of years, were available.

MATERIALS AND METHODS

Virus and reference antiserum

The virus used in SN tests was the T_1 strain of PI_3 virus obtained as a freeze-dried preparation from Dr. P.H. Lamont of the Central Veterinary Laboratory (CVL) at Weybridge, England. It was used at the tenth calf kidney passage. Aliquots of virus for test were stored at -70 C without the addition of serum or preservatives.

The reference antiserum, also obtained from the CVL, had been prepared in cattle against the SF_4 strain of PI_3 virus.

Test sera

Sera had been collected from free-living wild animals during the course of epizootiological studies of foot-and-mouth disease in a number of African territories between 1963 and 1977. Sera were stored at -20 C.

Serum neutralisation tests

SN tests were carried out on pre-formed monolayers of secondary bovine testes (BT) cells in tissue culture grade flat-bottomed microtitre plates. These

monolayers were propagated in a growth medium consisting of 50% Eagle's medium (Glasgow modification) and 50% Hanks' balanced salt solution containing 0.5% lactalbumin hydrolysate and 0.01% yeast extract. Phenol red indicator (0.001%) and antibiotics were included and 10% foetal calf serum (FCS) was added. Serum neutralisation tests were carried out in a maintenance medium similar to the above but with the addition of only 2% FCS. The FCS had previously been screened for the absence of antibodies to PI_3 virus.

Sera for test were diluted 1 in 2 in maintenance medium and heated at 56 C for 30 min. Twenty-five μl amounts of each serum were then used to prepare a 2-fold dilution series in microtitre transfer plates, using 25 μl diluting loops. Pre-titrated virus, diluted in maintenance medium to contain an estimated 100 $TCID_{50}$ per 25 μl, was added to each well. Following incubation at 37 C for one h, the serum/virus mixtures were transferred to the pre-formed BT monolayers. The monolayers had previously been washed with phosphate buffered saline and 50 μl of maintenance medium had been added to each well. The plates were then sealed with pressure-sensitive tape and incubated at 37 C for 3 days. Included in each test was a reference antiserum of known titre, a negative serum and a virus titration from which the actual amount of virus used in each test was calculated.

The presence of infection in the monolayers was demonstrated by haemadsorption with guinea pig red blood cells, using the techniques described by Fuccillo *et al.*[7] for parainfluenza 1 virus. The absence of haemadsorption was taken as indicative of virus neutralisation.

Neutralisation titres were expressed as the reciprocal of the final dilution of serum present in the serum/virus mixtures inhibiting haemadsorption at the 50% end point, estimated according to the method of Kärber.[15] For the purpose of this survey, titres $\geq$1 in 4 were accepted as positive.

RESULTS

A total of 3,288 serum samples from 44 different species of free-living wild animals were tested. Table 1 shows the numbers of each species tested, their zoological classification,[17] the percentage of sera with antibody and the range of SN titres recorded in each of the 20 sero-positive species. Antibody titres were most consistently found in sera of buffalo, lechwe, waterbuck, sable antelope, topi and tsessebe, although high titres were also recorded in several other species where the prevalence of antibody was lower - for example in warthog and springbok.

The geographic and species distribution of the animals tested is shown in Table 2. Neutralising titres were demonstrated in all the territories included in this survey except one, Kenya, from which only elephant sera were tested.

Buffalo

The high proportion of sero-positive buffalo and the range of antibody titres are similar to those reported by several authors in cattle in Africa.[6,13,19,22]

Figure 1 shows the age distribution of antibody to PI_3 virus in 757 sera collected from a free-living buffalo population in north-western Botswana between 1972 and 1976. These animals had been captured during foot-and-mouth disease investigations[8] and released immediately after sampling. At the time of capture, all animals were examined clinically and their ages estimated with reasonable accuracy, particularly in the younger age groups. A number of individual buffalo were recaptured and sampled on successive occasions. The results of SN tests on sera from these animals (Table 3) show that circulating antibody may persist for a considerable period and illustrate the fluctuations in titre which may occur from year to year; for instance,

TABLE 1. The prevalence of neutralising antibody to PI_3 virus in various species of wild animals in Africa.

Species				Total positive/ total sampled	% positive	Range of SN titres
BOVIDAE	BOVINI	BUFFALO	*SYNCERUS CAFFER*	1279/1424	89.9	4-2048
	STREPSICEROTINI	NYALA	*TRAGELAPHUS ANGASI*	0/1		
		BUSH BUCK	*TRAGELAPHUS SCRIPTUS*	0/37		
		KUDU	*TRAGELAPHUS STREPSICEROS*	12/171	7.0	6-22
		ELAND	*TAUROTRAGUS ORYX*	9/59	15.3	8-128
	REDUNCINI	WATERBUCK	*KOBUS ELLIPSIPRYMNUS*	13/27	48.1	45-256
		LECHWE	*KOBUS LECHE*	42/93	45.2	4-64
		PUKU	*KOBUS VARDONI*	0/16		
		KOB	*KOBUS KOB*	1/3	33.0	11
		REEDBUCK	*REDUNCA ARUNDINUM*	6/10	60.0	11-512
	HIPPOTRAGINI	SABLE ANTELOPE	*HIPPOTRAGUS NIGER*	12/28	42.9	4-64
		ROAN ANTELOPE	*HIPPOTRAGUS EQUINAS*	2/14	14.3	16-22
		ORYX	*ORYX GAZELLA*	8/10	80.0	6-355
	ALCELAPHINI	TOPI	*DAMALISCUS KORRIGUM*	11/24	45.8	4-32
		TSESSEBE	*DAMALISCUS LUNATUS*	35/52	67.3	4-128
		BLESBOK	*DAMALISCUS DORCAS*	0/3		

TABLE 1. (continued)

		HARTEBEEST	*ALCELAPHUS BUSELAPHUS*	4/8	50.0	6-22
		WILDEBEEST	*CONNOCHAETES TAURINUS*	27/112	24.1	4-64
	ANTILOPINI	IMPALA	*AEPYCEROS MELAMPUS*	12/264	4.5	4-45
		SPRINGBOK	*ANTIDORCAS MARSUPIALIS*	12/43	27.9	4-178
	NEOTRAGINI	KLIPSPRINGER	*OREOTRAGUS OREOTRAGUS*	0/1		
		ORIBI	*OUREBIA OUREBIA*	1/3	33.0	16
		STEINBOK	*RAPHICERUS CAMPESTRIS*	0/7		
		GRYSBOK	*RAPHICERUS MELANOTIS*	0/5		
		DUIKER	*SYLVICAPRA GRIMMIA*	0/35		
HIPPOPOTAMIDAE		HIPPOPOTAMUS	*HIPPOPOTAMUS AMPHIBIUS*	0/69		
ELEPHANTIDAE		ELEPHANT	*LOXODONTA AFRICANA*	0/346		
GIRAFFIDAE		GIRAFFE	*GIRAFFA CAMELOPARDALIS*	0/14		
SUIDAE		BUSH PIG	*POTAMOCHOERUS PORCUS*	2/16	12.5	11-22
		WART HOG	*PHACOCHOERUS AETHIOPICUS*	32/300	10.7	4-178
		GIANT FOREST HOG	*HYLOCHOERUS MEINERTZHAGENI*	0/1		

(continued)

TABLE 1. (continued)

EQUIDAE	ZEBRA	*EQUUS BURCHELLI*	0/38		
FELIDAE	LION	*PANTHERA LEO*	1/3	33.0	16
		*MISCELLANEOUS SPECIES**	0/51		
		TOTAL	3288		

*HYAENA (Crocuta crocuta) 5; WILD DOG (Lycaon pictus) 2; WILD CAT (Felis libyca) 1; CIVET (Viverra civetta) 2; SERVAL (Felis serval) 1; SPRING HARE (Pedetes capensis) 16; CAPE HARE (Lepus capensis) 10; BABOON (Papio sp.) 11; MONKEY (Cercopithecus aethiops) 1; PORCUPINE (Hystrix sp.) 1; VULTURE (Pseudogyps sp.) 1.

TABLE 2. The distribution of neutralising antibody to PI_3 in various African territories.

Species	Botswana	Rhodesia	S. Africa	S.W. Africa	Zambia	Tchad	Uganda	Kenya
Buffalo	822/837	266/332	39/59	3/3	87/94	35/54	27/40	
Nyala		0/1						
Bush Buck	0/9	0/16			0/9		0/1	
Kudu	0/6	1/124	1/4	2/12	8/25			
Eland		9/52	0/1		0/4	0/2		
Waterbuck		1/7	0/1		0/6	6/7	6/6	
Lechwe	8/19				34/74			
Puku					0/6			
Kob						1/3		
Reedbuck		3/7				1/1	2/2	
Sable Antelope	11/22	1/6						
Roan Antelope		1/3		1/1	0/8	0/2		

TABLE 2. (continued)

Oryx	0/1			8/9				
Topi						0/1	11/23	
Tsessebe	28/38	4/14						
Blesbok				0/3				
Hartebeest						4/8		
Wildebeest	21/36	2/48	1/10	1/13	2/5			
Impala	5/47	0/128	1/52	0/7	5/30			
Springbok			0/3	12/40				
Klipspringer		0/1						
Oribi			1/1			0/2		
Steinbok		0/4		0/2	0/1			
Grysbok		0/4			0/1			
Duiker	0/2	0/28			0/5			
Hippopotamus		0/2	0/9		0/58			
Elephant		0/40	0/71		0/123	0/4		0/108
Giraffe		0/5		0/6	0/1		0/2	
Bush pig		1/14		0/1		1/1		
Wart Hog	0/21	25/210	2/3		2/11	3/55		
Giant Forest Hog						0/1		
Zebra		0/32	0/1		0/5			
Lion					1/2	0/1		
Miscellaneous Species*	0/20	0/11			0/18	0/2		
Totals	1058	1096	215	97	497	143	74	108

*HYAENA 5; WILD DOG 2; WILD CAT 1; CIVET 2; SERVAL 1; SPRING HARE 16; CAPE HARE 10; BABOON 11; MONKEY 1; PORCUPINE 1; VULTURE 1.

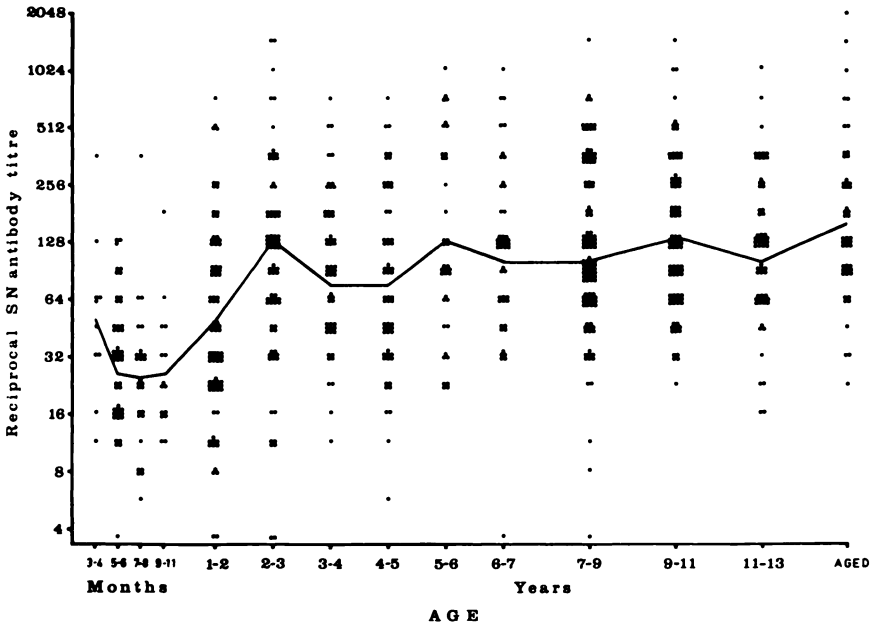

FIGURE 1. The distribution and mean SN titres in a free-living buffalo population in North-Western Botswana 1972-1976.

in animal number 103 a 16-fold rise in titre was recorded between 1974 and 1976. During various game control operations a small number of gravid female buffalo were shot and serum was taken from both dams and foetuses. Although SN antibody was detected in seven of the eight dams, no antibody was detected in their foetuses.

In a joint study with Dr. J. C. Condy in Rhodesia, a number of buffalo calves captured at approximately three months of age were held in isolation for a period of one year (Condy and Hedger, in press). Sera were taken at regular intervals and tested. The results in Table 4 show that maternal antibody may persist from five to seven months after birth.

Nasal swabs were taken from a number of captured buffalo during 1974 and 1976, but no PI_3 virus was isolated. During sampling, no clinical disease was reported or observed in any of the animals sampled, their herds of origin or animals with which they were in contact.

DISCUSSION

Infection with PI_3 virus has been shown to be widespread, with a world-wide distribution in both man and domestic animals, but relatively small numbers of wild species have been previously studied. The demonstration of antibody in sera from 20 of 44 wild species collected from seven different African countries between 1963 and 1977 shows that PI_3 virus is widespread also in wild animals in Africa and has been present in them for a considerable time. Our results, using the SN test, confirm those of Erasmus *et al.*[6] working in South Africa and of other workers[12,19] who, using the haemagglutination inhibition (HI) test, have examined small numbers of sera from wild animals in Africa. In addition, antibody was demonstrated in

TABLE 3. Reciprocal SN antibody titres to PI_3 virus in resampled free-living buffalo.

YEAR:	1972		1973			1974			1976
Animal No.	Age*	Titre	Animal No.	Age	Titre	Animal No.	Age	Titre	Titre
43	10y	708			355			128	
45	2y	128			128				
151	5y	708			355				
35	7y	355			64				
127	7y	45			32				355
48	2y	45			90				
144	10y	256						178	
105	2y	64						355	
			179	10y	355			355	
			158	6y	64			32	
			101	3y	178			355	
			114	3y	128			128	
			99	8y	128			90	
			150	7y	128				128
						25	7y	178	64
						31	12y	90	45
						36	15y	128	128
						74	9m	32	128
						77	5-6m	32	≤3
						84	11y	178	45
						103	11m	22	355
						115	3½y	256	178
						171	15y	90	708
						166	6-7y	32	256
						133	7y	90	355

*Estimated age

TABLE 4. The presistence of maternal antibody in buffalo calves.

Animal Number	Age in Months										
	2½	3½	5	6	6½	7	8	9	12½	13	13½
2	NS+	16*	11	6	11	≤3	≤3	≤3	≤3	≤3	≤3
3	16	11	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3	≤3
4	45	NS									
5	45	16	6	NS	≤3	≤3	≤3	≤3	≤3	≤3	≤3
6	32	32	16	6	≤3	≤3	≤3	≤3	≤3	≤3	≤3
7	32	22	NS								
8	128	90	32	16	11	11	≤3	≤3	≤3	≤3	≤3
9	NS	22	NS								

+NO SAMPLE
*Reciprocal SN antibody titre

seven species which have not previously been reported as sero-positive. These were lechwe, tsessebe, springbok, oribi, warthog, bush pig and one lion in which a titre of 1 in 16 was recorded. Nineteen of the twenty sero-positive species belong to the families Bovidae or Suidae.

In some species only small numbers of sera were available and the absence of antibody in these should not necessarily be taken as lack of susceptibility to PI_3 virus. It is curious, however, that no positive results were recorded in the 346 elephant and 69 hippopotamus sera examined, although previous workers[6,12] had reported sero-positives in these two species, using the HI test.

In North America, serological evidence of PI_3 virus infection in some free-living wild species - for example, pronghorn antelope *(Antilocapra americana)*[23] and bighorn sheep *(Ovis canadensis)*[11] - has been followed by isolations of the virus.[23,11] However, the significance of SN titres in the absence of confirmation of infection by virus isolation, especially in species which have not been extensively studied, is of course unknown. It is known, however, that the parainfluenza viruses 1, 2 and 3, mumps and Newcastle disease virus, while antigenically distinct from each other, all show some cross-reaction either in the neutralisation or in the complement fixation test with at least one other member of the group.[1] It is possible, therefore, that some of the low titres to PI_3 virus might be due to a cross relationship with other virus infections as yet unrecognized. However, since titres ≥ 1 in 4 have been accepted as significant in cattle,[3] similar titres have been accepted as indicative of infection in this survey. Nevertheless, in the majority of the sero-positive species very much higher titres have also been recorded. In some species (warthog, for example), although the prevalence of antibody was low, high titres were recorded in individual animals.

The high percentage of sero-positive buffalo suggests that this species is particularly important as a reservoir of infection in the wild. The extended study in north-western Botswana showed little variation in prevalence of antibody from year to year. The sequence of events in infection in a free-living population of buffalo is probably: almost 100% of animals have experienced infection prior to calving and the newborn calf consequently enjoys a transferred maternal immunity. As in cattle,[4] maternally derived antibody persists for 5 to 7 months and the calf becomes infected in the first year or two of life. The persistence of antibody following infection is not known, although fluctuations in titre were observed in animals sampled on successive occasions. Existing titres in some adult animals - for example, Nos.

127, 171 and 166 in Table 3 - showed 8 to 10-fold increases over two or more years. This suggests that reinfection probably occurs when the level of antibody is no longer protective. Previous workers have considered the threshold of immunity in cattle to be equivalent to HI titres of ≤ 1 in 32 and ≤ 1 in 40 respectively[5,21] and in humans to be equivalent to an SN titre of ≤ 1 in 32.[2] Our results suggest that the threshold of immunity in buffalo may be similar.

Despite repeated attempts to isolate virus from random nasal swabs taken from captured buffalo, no isolation was made. In spite of the high prevalence of antibody, there may be a relatively short time during which virus may be isolated from an animal. Although PI_3 has been associated with active respiratory disease in cattle[20] and sheep,[10] there was no evidence of clinical disease in any of the wild species at the time of handling and sampling.

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