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PREVALENCE OF NEUTRALIZING ANTIBODIES TO BOVID HERPESVIRUS 2 IN AFRICAN WILDLIFE

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Abstract: A total of 3,470 sera, collected between 1963 and 1980 from 45 different species of wildlife in nine African countries, was examined for virus neutralizing (VN) antibodies to bovid herpesvirus 2. Antibodies were demonstrated in 20 species including 15 Bovidae, two Suidae, hippopotamus *(Hippopotamus amphibius)*, giraffe *(Giraffa camelopardalis)* and a green monkey *(Cercopithecus aethiops)*; 11 of these species had not been previously recorded as sero-positive. Although the significance of neutralizing antibodies in the absence of virus isolation remains in doubt, results suggest that infection is widespread in wildlife. The highest VN titres were recorded in waterbuck *(Kobus ellipsiprymnus* and *K. defassa)*, reedbuck *(Redunca arundinum)* and buffalo *(Syncerus caffer)*. Infection appears to be continuous in free-living populations of buffalo and antibodies are present in the majority of animals by the age of 2 yr.

INTRODUCTION

In 1957, Alexander et al. reported the isolation of pox and herpes-like viruses from cattle during outbreaks of lumpy skin disease in South Africa. Both virus types are associated with disease and Weiss (1963) has emphasized the difficulty in distinguishing clinically between true lumpy skin disease caused by the pox virus, prototype Neethling, and pseudo lumpy skin disease caused by bovid herpesvirus 2 (BHV2), prototype Allerton. Virus isolations of BHV2 have also been made from cattle in Kenya (MacOwan, 1961), Ruanda-Urundi (Huygelen et al., 1960) and Zambia (Hedger et al., 1977). These isolations together with the neutralizing antibodies reported in sera from cattle in Kenya (Martin and Gwynne, 1968), East Africa (Plowright and Jessett, 1971), Zambia (Hedger et al., 1977) and Nigeria (Nawathe et al., 1978) indicate that infection in Africa is widespread.

A strain of BHV2 was isolated from buffalo *(Syncerus caffer)* during severe disease in Tanzania (Schiemann et al., 1971) but was considered to be coincidental and not related to the high mortality recorded in the buffalo. Neutralizing antibodies to this strain of BHV2 have been reported by Plowright and Jessett (1971) in a number of other species of wildlife in East Africa.

This paper presents a serological investigation of the prevalence of neutralizing antibodies to BHV2 in 3,470 sera collected from 45 different species of free-living wild animals from nine countries in southern, eastern and central Africa.

MATERIALS AND METHODS

Virus and reference serum

Neutralization tests were carried out using the bovine herpes mammillitis TV strain of BHV2 (Rweyemamu and Johnson, 1967). The reference serum used was prepared in rabbits against the homologous virus.

Test serum samples

Serum samples had been collected from free-living wild animals during the course of epidemiological studies of foot-and-mouth disease and from various cull-

ing operations in a number of African territories since 1963. Sera were stored at -20 C and, prior to use, thawed and heat inactivated at 56 C for 30 min.

Virus neutralization tests

Virus neutralization (VN) tests were carried out in flat-bottomed, tissue culture grade, microtitre plates using methods essentially similar to those described by Hedger and Hamblin (1978). The growth medium used throughout the test was Eagle's/High Sugar Low Salt 50/50 V/V supplemented with 4.0% heat inactivated fetal calf serum previously screened for the absence of antibodies against BHV2. After incubation of the serum/virus mixture for 2 h at 37 C, 50 μl of a suspension of secondary calf testis cells at a concentration of 5×10^5 cells per ml was added to each well.

Controls in each test included a reference antiserum of known titre, a negative serum, cell and medium controls and a virus titration from which the actual amount of virus used in the test was calculated. Plates were sealed and read microscopically after 3 days incubation at 37 C. Only wells showing no evidence of cytopathic effect were considered to be protected by the serum. Virus neutralization titres were expressed as the reciprocal of the final dilution of serum present in the serum virus mixtures at the 50% end point estimated according to the method of Kärber (1931).

Samples for virus isolation

Esophageal/pharyngeal (E/P) samples were collected in esophageal cups and handled as previously described (Hedger, 1968). Nasal, conjunctival, preputial and vaginal samples were taken on cotton wool swabs. Immediately after addition of transport medium, all samples were placed on solid carbon dioxide.

Virus isolation attempts were made on primary bovine thyroid cells and secondary calf kidney cells in roller tubes.

RESULTS

General survey

A total of 3,470 serum samples from 45 different species of free-living wild animals was tested for neutralizing antibodies to BHV2. Table 1 shows the zoological classification (Morris, 1965) and the numbers of sera from each species tested and includes the percentage and range of VN titres recorded in each of the sero-positive species. Disregarding those species where only small numbers of sera were available, the prevalence of antibodies was greatest in buffalo, waterbuck and reedbuck. The highest antibody titres were also recorded in these three species. Neutralizing antibodies were demonstrated in a further 12 species of Bovidae, two species of Suidae, hippopotamus, giraffe and a green monkey.

The geographic distribution of the species sampled together with the results for individual countries are detailed in Table 2.

Buffalo studies

In Botswana a population of free-living buffalo was sampled over a number of years between 1965 and 1976 (Table 3). Virus neutralizing antibodies were present in a high proportion of the animals in all age groups sampled each year, suggesting a continuous infection within the population for at least 12 yr. Seven hundred and ninety three of these buffalo had been captured and released after sampling between 1972 and 1976. The age of individual animals was estimated. Figure 1 shows the percentage of animals with antibodies and the geometric mean VN titre in each age group. Twenty-five of these buffalo were recaptured and sampled on successive occasions. Although 4 to 5-fold fluctuations in neutralizing antibody titre were recorded in individual buffalo from year to year, at least 16-fold increases in titre were recorded in sera from three young animals aged 2 yr, 6 mo and 11 mo respectively when first sampled.

TABLE 1. The prevalence of neutralizing antibodies to BHV2 in various species of wild animals in Africa.

	Species	Total sampled	Percentage positive	Range of VN reciprocal titres
Buffalo	*Syncerus caffer*	1,428	88	4-708
Nyala	*Tragelaphus angasi*	1		
Bushbuck	*Tragelaphus scriptus*	37	3	8
Greater kudu	*Tragelaphus strepsiceros*	178	26	4-64
Eland	*Taurotragus oryx*	57	44	6-64
Waterbuck	*Kobus ellipsiprymnus*	20	85	6-2048
Defassa waterbuck	*Kobus defassa*	10	90	22-1024
Lechwe	*Kobus leche*	95		
Puku	*Kobus vardoni*	16		
Kob	*Kobus kob*	3		
Reedbuck	*Redunca arundinum*	11	82	11-708
Sable antelope	*Hippotragus niger*	29	52	4-90
Roan antelope	*Hippotragus equinus*	14	57	4-22
Oryx	*Oryx gazella*	16	13	8-11
Topi	*Damaliscus korrigum*	24	4	64
Tsessebe	*Damaliscus lunatus*	56	20	4-16
Blesbok	*Damaliscus dorcas*	4		
Hartebeeste	*Alcelaphus buselaphus*	11	36	11-45
Blue wildebeeste	*Connochaetes taurinus*	143	6	4-64
Impala	*Aepyceros melampus*	337	4	4-11
Grant's gazelle	*Gazella granti*	3		
Springbok	*Antidorcas marsupialis*	53	2	64
Klipspringer	*Oreotragus oreotragus*	1		
Oribi	*Ourebia ourebia*	3		
Steinbok	*Raphicerus campestris*	8		
Grysbok	*Raphicerus melanotis*	5		
Duiker	*Sylvicapra grimmia*	37		
Hippopotamus	*Hippopotamus amphibius*	70	21	4-90
Elephant	*Loxodonta africana*	351		
Giraffe	*Giraffa camelopardalis*	31	68	4-45
Bush pig	*Potamochoerus porcus*	16		
Wart hog	*Phacochoerus aethiopicus*	305	1	4-45
Giant forest hog	*Hylochoerus meinertzhageni*	2	100	8-11

TABLE 1. (continued)

	Species	Total sampled	Percentage positive	Range of VN reciprocal titres
Common zebra	*Equus burchelli*	39		
Green monkey	*Cercopithecus aethiops*	1	100	22
Miscellaneous species [a]		55		
Total		3,375		

[a] Lion (*Panthera leo*), 3; hyaena (*Crocuta crocuta*), 5; wild dog (*Lycaon pictus*), 2; wild cat (*Felis libyca*), 1; civet (*Viverra civetta*), 2; cape hare (*Lepus capensis*), 10; Serval (*Felis serval*), 1; spring hare (*Pedetes capensis*), 16; porcupine (*Hystrix* sp.), 1; baboon (*Papio* sp.), 13; vulture (*Pseudogyps* sp.), 1.

TABLE 2. The distribution of neutralizing antibodies to BHV2 in wildlife from various African countries.

Species	Botswana	Zimbabwe	S. Africa	S.W. Africa	Zambia	Tchad	Kenya	Tanzania	Uganda
Buffalo	805/837 [a]	224/282	63/116	2/3	86/93	54/57			27/40
Nyala		0/1							
Bushbuck	1/9	0/17			0/10				0/1
Greater kudu	9/28	29/122	3/5	3/12	3/11				
Eland		24/50	0/1		0/4	1/2			
Waterbuck		5/8			6/6				6/6
Defassa waterbuck						9/9		0/1	
Lechwe	0/27		0/2		0/66				
Puku					0/16				
Kob						0/3			
Reedbuck		6/8				1/1			2/2
Sable antelope	15/22	0/7							
Roan antelope		2/2		0/1	5/9	1/2			
Oryx	0/4			0/9				2/3	
Topi						1/1			0/23
Tsessebe	8/40	3/15						0/1	
Blesbok				0/4					
Hartebeeste						4/11			
Blue wildebeeste	4/47	3/45	2/33	0/13	0/5				
Impala	3/77	1/122	10/88	0/7	0/16		0/27		
Grant's gazelle								0/3	
Springbok	1/8		0/3	0/42					

TABLE 2. (continued)

Klipspringer		0/1										
Steinbok	0/1	0/4			0/2		0/1					
Oribi			0/1					0/2				
Grysbok		0/4					0/1					
Duiker	0/2	0/28					0/5	0/1		0/1		
Hippopotamus		1/2	0/9				14/58	0/1				
Elephant		0/40	0/76				0/124	0/4	0/107			
Giraffe		4/5	15/15		0/6		1/1			0/2		1/2
Bush pig		0/14			0/1							
Wart hog	0/28	3/212	0/3				0/6	1/56				
Giant forest hog								2/2				
Common zebra		0/33	0/1				0/5					
Green monkey							1/1					
Miscellaneous species[b]	0/20	0/11					0/21	0/3				

[a]Total positive/sampled.

[b]Hyaena, 5; wild dog, 2; wild cat, 1; civet, 2; serval, 1; spring hare, 16; cape hare, 10; baboon, 13; porcupine, 1; vulture, 1; lion, 3.

During various game control operations, six gravid female buffalo were shot and serum taken from both dams and fetuses. Although five dams had antibodies to BHV2, antibodies were not detected in the sera from their fetuses, which varied from between 5 and 7 mo to almost full term. It is believed, therefore, that the antibodies demonstrated in young buffalo calves during the first few months of life are colostrally derived. The decline of neutralizing antibodies to BHV2 in young buffalo calves was demonstrated in a group of eight captured when approximately 3 mo old. Following capture, the calves were held in isolation and sampled at monthly intervals up to 12 mo of age. Antibodies were detected in sera from all the calves and these persisted up to 7 mo of age.

No clinical signs of pseudo lumpy skin disease or mammillitis were observed in any of the captured buffalo throughout the study. In the latter part of the study, several hundred attempts were made to isolate herpesvirus from E/P samples, feces and nasal, conjunctival, preputial and vaginal swabs, without success.

DISCUSSION

These studies indicated that infection with BHV2 was prevalent and widespread in African wildlife. The presence of antibodies was confirmed in all nine wild species previously reported as seropositive in East Africa (Plowright and Jessett, 1971). In addition, antibodies were demonstrated in sera from a further 11 wild species, viz kudu, reedbuck, sable antelope, roan antelope, topi, tsessebe, hartebeeste, springbok, warthog, giant forest hog and green monkey.

Virus neutralizing antibody titres in sera from buffalo, waterbuck and reedbuck were generally higher than those detected previously in cattle. The water-dependence of these three wild species, which often favor swampy areas, suggests the possibility of insect-borne

TABLE 3. The prevalence of VN antibody to BHV2 in a free-living population of buffalo in Botswana.

Year	Number of animals	Percentage positive	Reciprocal VN titre Geometric mean	Range
1965	3	100	50	22-90
1968	5	100	32	16-90
1970	36	100	32	11-128
1971	20	85	64	16-512
1972	204	89	64	6-355
1973	204	98	40	4-355
1974	189	95	32	6-708
1976	196	98	40	6-512

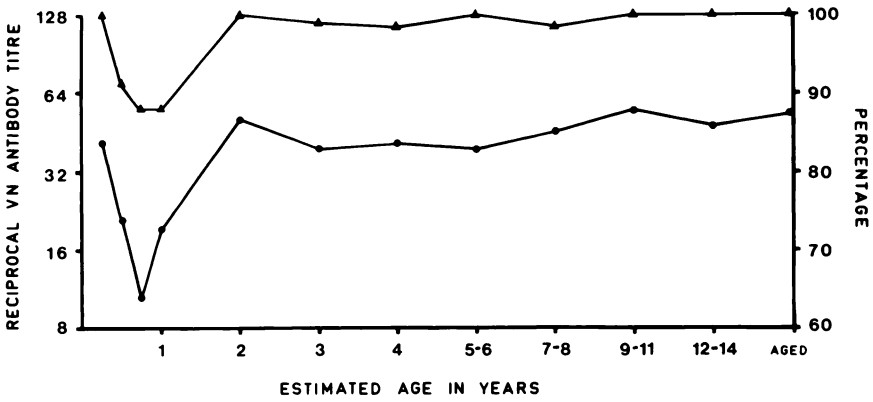

FIGURE 1: The distribution and prevalence of neutralizing antibody to BHV2 in sera of 793 buffalo captured from a free-living population in Botswana between 1972 and 1976. ▲——▲, percentage of animals with antibody in each age group. ●——●, geometric mean VN titre.

infection as a likely means of transmission. Sometimes these species share the same watering points as domestic stock and they may therefore be important as maintenance hosts of BHV2. The isolation of an Allerton type virus from a sick buffalo calf in Tanzania has already been reported (Schiemann et al., 1971).

Antibodies, generally of lower titre, were also prevalent in kudu, eland, sable and roan antelope, tsessebe, hartebeeste, hippopotamus and giraffe. There was a low prevalence in bushbuck, topi, wildebeeste, impala, springbok, oryx and warthog. The role of these species in the maintenance of BHV2, however, must remain speculative. Plowright and Jessett (1971) have suggested that some species may not be susceptible to infection with BHV2, particularly when a low proportion of the animals are recorded sero-positive. Serological cross reactions are known to occur between herpes simplex virus types 1 and 2 and BHV2 (Sterz et al., 1974) and it is possible that the neutralizing antibodies demonstrated in some of these individual animals are a reflection of cross-reactions with other herpes viruses not

yet identified but which are antigenically similar to BHV2.

The significance of low VN titres in some species could be regarded as doubtful. However, in buffalo calves, after the waning of maternal immunity, no antibodies were detected after 7 mo of age. Low titres could therefore be significant.

The high proportion and widespread distribution of VN antibodies in sera collected from buffalo over several years suggested that infection with BHV2 has been present in African buffalo for a very long time. Sera from three buffalo sampled on successive years in Botswana showed a significant rise (at least 16-fold) in antibody titre, indicating that infection was continuous within the population. Latent infection with BHV2, previously demonstrated experimentally in cattle (Martin and Scott, 1979), sheep and goats (Westbury, 1981), may also occur in buffalo and could explain the fluctuations in VN titre recorded in individual animals sampled on successive occasions. Such fluctuations emphasize that estimation of the time of infection cannot be based on antibody titre alone.

The absence of antibodies in fetuses from dams with VN antibodies indicates that the subsequent immunity demonstrated in young calves is colostrally acquired. This immunity persists for approximately 5-6 mo. The study in Botswana shows that between 6 mo and 1 yr of age there is a marked reduction in the percentage of animals with antibodies and up to 10% of the buffalo of this age become susceptible. By the age of 2 yr, the majority of buffalo appear to have experienced infection with BHV2.

The failure to isolate BHV2 from E/P samples, feces and swabs may have been due in part to the absence of overt disease at the time of sampling and in part to the presence of foot-and-mouth disease virus in a high proportion of the samples. This latter virus grows quickly, rapidly destroying the tissue culture cells, thus obscuring the presence of other slower growing viruses. The results presented here, however, suggest that further observations of wild animals and more attempts to isolate BHV2 from them might well be rewarding and help in the understanding of the epidemiology of the disease.

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