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The prevalence of antibody to the viruses of bovine virus diarrhoea, bovine herpes virus 1, rift valley fever, ephemeral fever and bluetongue and to *Leptospira* sp in free-ranging wildlife in Zimbabwe

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SUMMARY

The prevalence of antibody to the viruses of bovine virus diarrhoea (BVD), bovine herpes virus type1 (BHV1), rift valley fever (RVF), bovine ephemeral fever (BEF) and bluetongue (BT) and to *Leptospira* sp. was determined in wildlife populations in Zimbabwe. Evidence of infection with BVD virus was found in 14 of the 16 species examined but was greatest in eland *Taurotragus oryx*, nyala *Tragelaphus angasi* and bushbuck *Tragelaphus scriptus*. Persistent infection with BVD virus was found in 1 of 303 antibody-free eland but not in the smaller sample of 102 antibody-free buffalo *Syncerus caffer*. Antibody to BHV1 was widespread, being found in 10 of 16 species with the highest prevalence being in buffalo and eland. Antibody to RVF was most prevalent in black rhino *Diceros bicornis* and white rhino *Ceratotherium simum*, buffalo and waterbuck *Kobus ellipsiprymnus*. Both BEF and BT were widespread in all the species examined. Evidence of infection with *Leptospira* sp. was found in 7 species. Infections were due to up to 3 of 8 different serovars.

INTRODUCTION

Wildlife conservation policies in Zimbabwe are based on the concept of sustainable utilization and this has resulted in the rapid development of game ranching in the commercial farming sector. Since 1989, some 20000 wild herbivores have been translocated from those areas of the country where they abound to other parts from which they had been largely eliminated in the past. Most of these animals have come from ranches in the southern lowveld and Matabeleland and have been moved north or eastwards respectively into the highveld farming areas. There have also been translocations from the wildlife areas of the Zambezi valley and from the National Parks (Fig. 1).

In conservation terms, these translocations will have a very positive effect on the re-distribution of species and on their long term survival. The subsequent growth of these populations will be influenced by a number of factors which include the suitability of their new environment and the impact of parasites and disease. An understanding of the susceptibility of wildlife species to infectious agents and whether or not these cause disease will assist in formulating management practices especially in small game parks.

The prevalence of brucellosis has already been determined [1]. This report gives the results of a serological survey of other infections in wildlife populations in Zimbabwe. It describes the prevalence of bovine virus diarrhoea (BVD), bovine herpes virus 1 (IBR), bovine ephemeral fever (BEF), rift valley fever (RVF) and bluetongue (BT). In addition samples from a number of different species were screened for antibodies to 8 leptospira serovars.

METHODS

Collection of sera

Sera were collected between 1989 and 1995 mainly from animals captured for translocation though some

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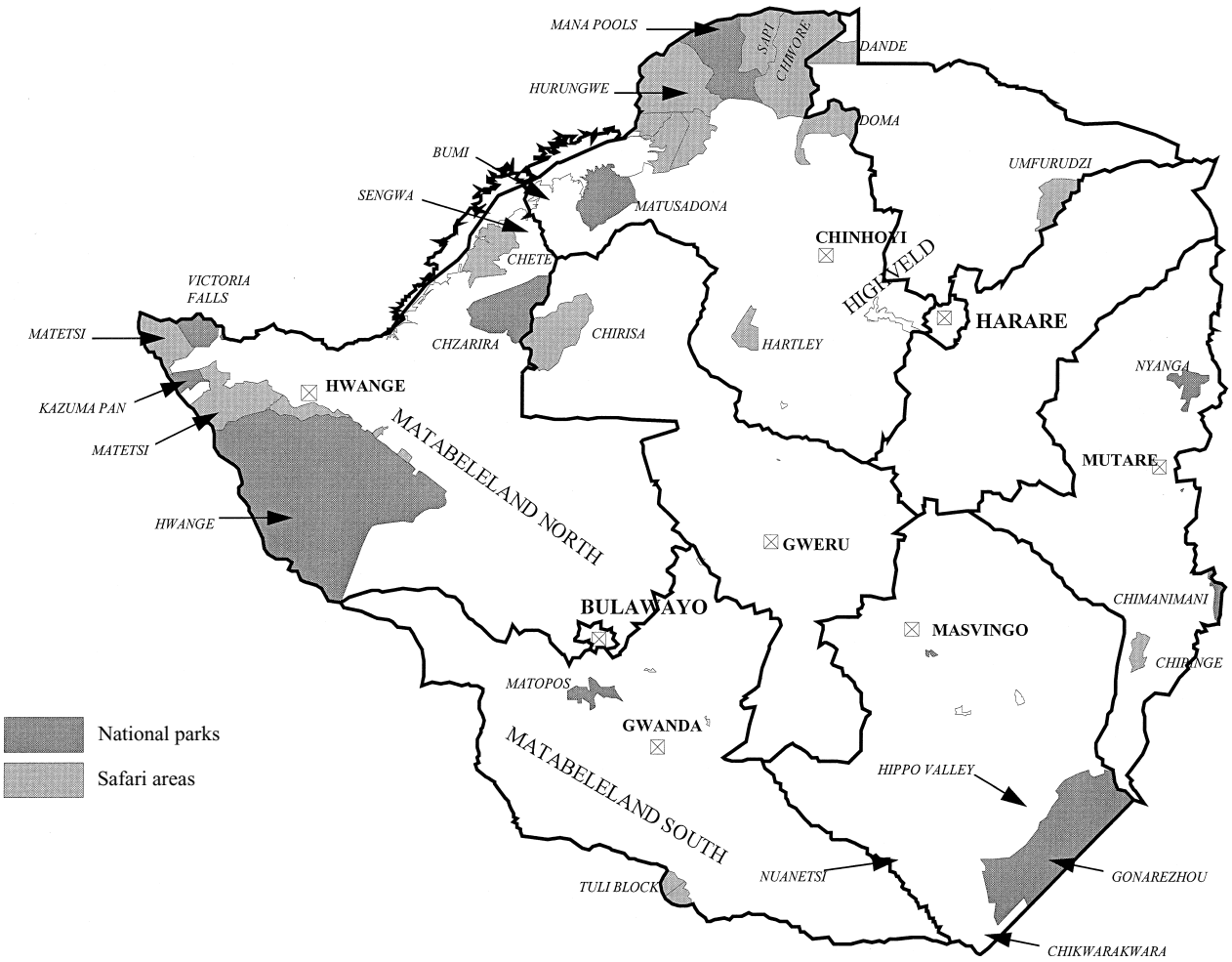

Fig. 1. National Parks and Safari areas of Zimbabwe.

were also collected during culling operations. Blood samples were collected within 1–2 weeks of capture, following either chemical immobilization or physical capture and stored at 4 °C. Serum was either removed in the field or following return to the laboratory and stored frozen at −20 °C.

Serological assays

Bovine virus diarrhoea

Antibodies against BVD for all species except elephant, rhino and warthog were detected using the indirect ELISA as described by Howard and colleagues [2]. The antigen used was prepared from the Oregon C24 strain and bound antibody was detected with an anti-bovine conjugate. Sera were tested at a single dilution of 1/50 and those giving an optical density (450 nm) of twice the mean of the negative control serum value (P/N > 2·0) were considered positive. The elephant, rhino and warthog sera were assayed in a neutralization test using secondary calf kidney cells previously shown to be free of BVD virus and ten percent BVD antibody-free foetal calf serum. Positive and negative bovine sera were kindly provided by C. Hamblin, Pirbright Laboratory, Institute for Animal Health, U.K.

Blood samples collected into EDTA from a proportion of animals testing negative for antibody to BVD were screened for the presence of BVD virus using the capture ELISA described by Drew, Frost and Edwards [3]. The buffy coat cells from at least 10 ml of blood were collected and residual red cells lysed by the addition of buffered 0·84% ammonium chloride, pH 7·4. Following washing with PBS, the cells were transfered to Eppendorf tubes and lysed by the addition of 1% Triton X-100. The nuclei were spun down and the cytosol screened for BVD antigen in a trapping ELISA using the monoclonal antibody (Mab) WB112 as the capture antibody and Mab

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WB103b as the second antibody (both Mabs kindly supplied by T. Drew, S. Edwards, Central Veterinary Laboratory, Weybridge, UK). In addition buffy coat cells were inoculated on to secondary calf kidney cell monolayers previously shown to be free of BVD virus and grown in equal volumes of Glasgow modified Eagle's MEM and lactalbumin hydrolysate/yeast extract in Hanks basic salt solution containing 10% BVD antibody-free foetal calf serum (GMEM/LYH). All monolayers were subcultured once. This was done 6–7 days after inoculation. Half the cells were washed, lysed and tested in the antigen detection ELISA while the other were suspended in GMEM/LYH. After a further 6–7 days the cells from these cultures were tested as above.

Bovine herpes virus 1

An indirect ELISA was also used to detect antibodies to IBR. The antigen was prepared from the Oxford strain of the virus. Sera were tested at a single dilution of 1/100 and samples giving a P/N ratio of 2·0 or greater were considered positive. Positive and negative bovine control sera were kindly supplied by C. Hamblin, Pirbright Laboratory, Institute for Animal Health, U.K.

All elephant and rhino sera were tested in neutralization tests using secondary calf kidney cells.

Rift Valley fever

Antibody to RVF virus was detected in a haemaglutination inhibition (HAI) test [4, 5] and confirmed in an indirect ELISA [6]. The antigen for the HAI test was kindly supplied by the Veterinary Research Institute, Onderstepoort. Briefly, unheated serum samples were diluted 1/10 in 0·85% NaCl and then extracted first with cold acetone and then with 50% goose red blood cells (rbcs) to remove non-specific inhibition and haemagglutinins respectively. Serial dilutions of the extracted sera were made in microtitre plates and reacted with diluted acetone/sucrose extracted mouse brain antigen (obtained from the Veterinary Research Institute, Onderstepoort, South Africa). Serum and antigen were diluted in borate buffered saline, pH 9·0 containing 0·4% w/v bovine serum albumin (BABS). Goose rbcs diluted to 8% in BABS adjusted to either pH 5·75, 6·0 and 6·2 as predetermined by titration of the antigen at these pH levels. Incubation of each stage took place at room temperature. Positive control serum was obtained from a convalescent bovine.

The antigen for the ELISA was prepared by growing the virus in BHK21 cells. The cells were harvested after 3–4 days when CPE was evident and washed before lysing with 1% Triton X-100 and one freeze-thaw cycle.

The lysate was clarified and stored at −80 °C.

Bovine ephemeral fever

Antibody to BEF was detected in a micro-neutralization test using BHK21 cells. Positive bovine control serum was kindly supplied by C. Hamblin, Pirbright Laboratory, Institute for Animal Health, U.K.

Bluetongue

Antibody to BT was detected in a competitive ELISA as described by Asfar and colleagues, [7]. The Mab and control sera were kindly supplied by J. Anderson, Pirbright Laboratory, Institute for Animal Health, UK. The rhino and elephant sera were also screened in the ELISA.

Leptospirosis

Sera were screened in the microscopic agglutination microtitre test [8] using 8 different serovars (hardjo, hebdomadis, mini, autumnalis, ballum, interogans, pomona, batavi).

RESULTS

Bovine virus diarrhoea

The prevalence of sero-positive wild herbivores is shown in Figure 2. Infection with BVD virus was detected in 14 of the 16 species tested with the highest prevalence found in nyala *Tragelaphus angasi* – 75%; eland *Taurotragus oryx* – 46% and bushbuck *Tragelaphus scriptus* – 41%. There were also significant numbers of positive animals found in the Lichtenstein's hartebeest *Alcelaphus lichtensteini* – 20%; kudu *Tragelaphus strepsiceros* – 14·5%; reedbuck *Redunca arundinum* – 11%; buffalo *Syncerus caffer* – 10% and sable *Hippotragus niger* – 10%. Lower prevalences were found in impala *Aepyceros melampus*; wildebeest *Connochaetes taurinus*; waterbuck *Kobus ellipsiprymnus*; tsessebe *Damaliscus lunatus*; giraffe *Giraffa camelopardalis* and possibly black rhino *Diceros bicornis*.

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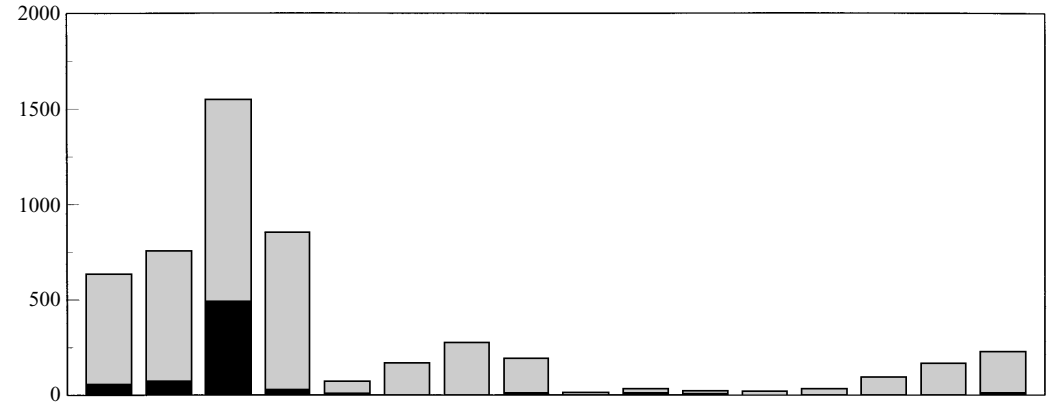

	buff	sabl	elan	impa	kudu	wild	gira	wate	hart	bush	nyala	reed	wart	whit	blac	tses
pos	57	68	490	27	9	4	13	8	2	9	9	2	0	0	1	12
total	577	686	1059	825	62	164	261	183	10	12	12	18	33	94	167	215

Fig. 2. Prevalence of antibody to bovine virus diarrhoea virus.

Table 1. *Age distribution of animals positive for BVD antibody*

	Percent BVD antibody positive		
Species	Juvenile	Subadult	Adult
Nyala	Nil	Nil	77
Eland	49	27	59
Bushbuck	nil	8	18
Buffalo	11	5	7
Waterbuck	Nil	11	12
Reedbuck	ND	ND	13
Sable	8	7	12
Giraffe	14	8	2
Tsessebe	Nil	Nil	13
Kudu	20	27	34
Wildebeest	Nil	Nil	4

The only nyala from Zimbabwe that were tested were all from one area along the Bubi river in southern Matabeleland. In contrast, a group of 40 nyala captured in the south of Malawi were all negative.

The eland were mainly from the south-east lowveld (Fig. 1) where they range widely throughout this cattle ranching area. The bushbuck tested were also from the south-east. Positive buffalo were found in all the wildlife areas of the country (Fig. 1). Positive kudu were only found in the south-east while positive impala were found both in the south-east and south west of the country. Positives of the other species were found in all the localities where samples were collected. The one positive black rhino was at Chipinge in the eastern highlands though this animal was originally translocated from the Zambezi valley where it could have acquired the infection.

The age distribution of positive animals of each species is shown in Table 1.

The survey for persistently infected (PI) eland resulted in the consistent isolation of a non-cytopathic virus from only 1 of 303 animals that were antibody negative. This animal was a subadult female. Virus was also isolated from one other eland, an adult female, but on only 1 occasion.

A total of 102 antibody-free buffalo were all negative for virus. These were from widely distributed places namely: the Hwange National Park in the north-west, a safari area to the south of Hwange National Park, the Sengwa safari area on the shores of Lake Kariba and the Gona-re-zhou National Park in the south-east. Blood from 19 giraffe and 12 sable, all from the south-east lowveld, were also negative for BVD virus.

Bovine herpes virus 1

Antibody to BHV1 was found in 10 of the 16 species examined (Fig. 3). The highest prevalence was in eland and buffalo (30%). Sable, impala, kudu and wildebeest all had 12–14% positives. There was much lower prevalence in giraffe, bushbuck, nyala and tsessebe. The positives were distributed throughout

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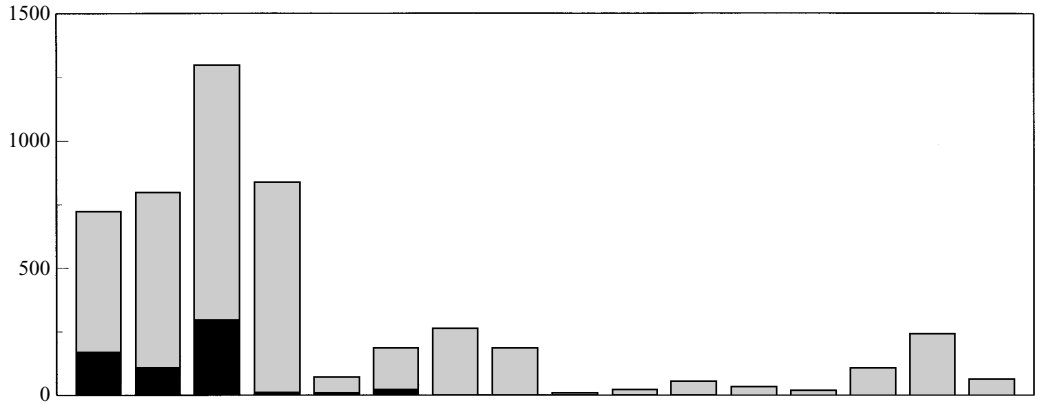
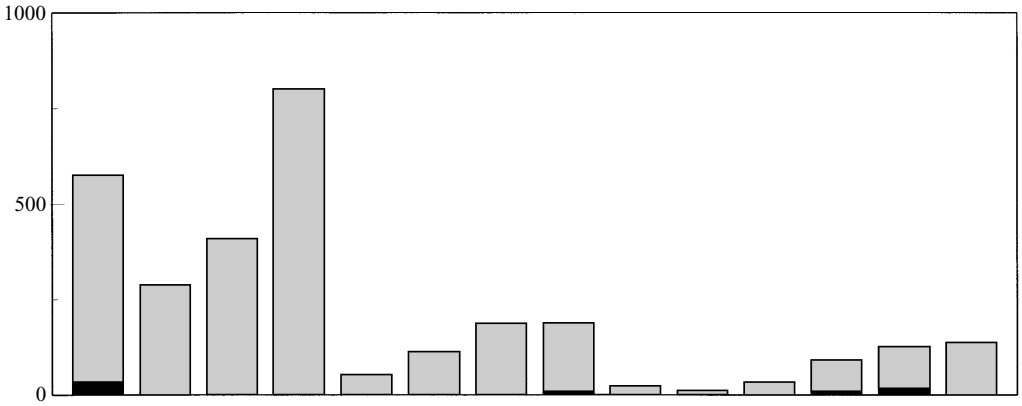


	buff	sabl	elan	impa	kudu	wild	gira	wate	hart	bush	nyala	wart	whit	blac	tses	eleph
pos	168	97	294	10	8	21	3	0	0	1	1	0	0	0	1	0
total	553	701	1004	828	63	164	258	183	8	22	55	33	19	106	240	61

Fig. 3. Prevalence of antibody to bovine herpes virus 1 virus.



	buff	sabl	elan	impa	kudu	wild	gira	wate	bush	nyala	wart	whit	blac	tses
pos	34	1	0	1	0	0	0	8	0	0	0	7	16	0
total	541	286	409	801	52	112	186	179	23	12	33	84	110	136

Fig. 4. Prevalence of antibody to Rift Valley fever virus.

the sampling points. All the waterbuck, Lichtenstein's hartebeest and warthog *Phacochoerus aethiopicus* were negative as were white rhino *Ceratotherium simum*, black rhino and elephant *Loxodonta africana*.

Rift Valley fever

Antibody to RVF virus was most prevalent in black rhino, white rhino, buffalo and waterbuck (Fig. 4). These infections had occurred at all the sampling points for the two species. A very low prevalence of antibody was detected in impala and sable. Positive black and white rhino were found in Hwange National Park in the north-west of the country. In addition, four positive black rhino were found in Chizerira National Park which overlooks Lake Kariba and one was found at Chipinge in the south-east.

Bovine ephemeral fever

Only 8 species were tested for antibody to BEF (Fig. 5). All but the waterbuck showed evidence of infection

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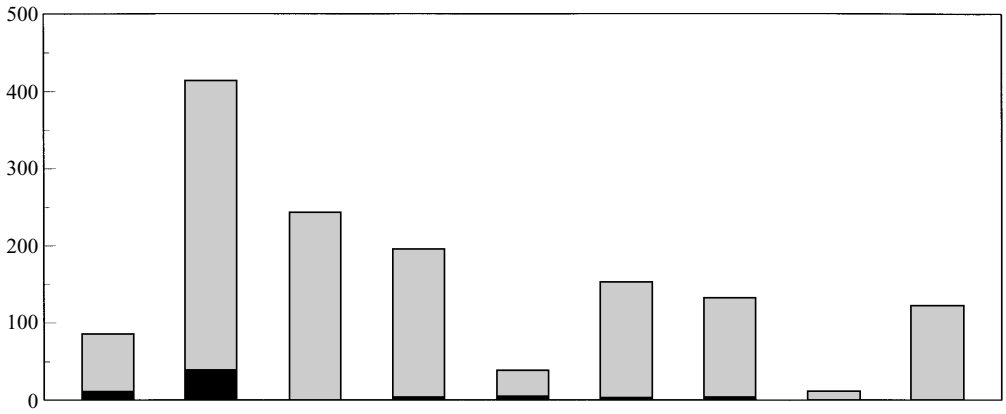
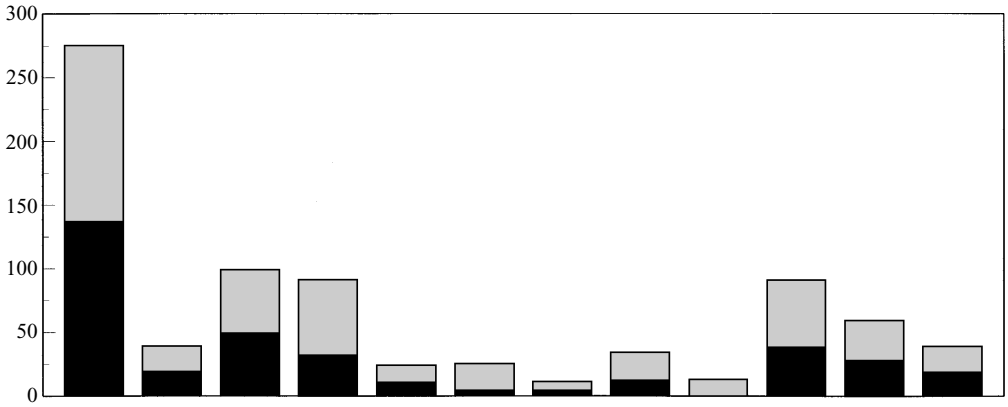


	buff	sabl	elan	impa	kudu	wild	gira	wate	tses
pos	11	38	16	3	4	2	3	0	2
total	75	376	227	192	34	151	129	11	120

Fig. 5. Prevalence of antibody to bovine ephemeral fever virus.



	buff	sabl	elan	impa	kudu	wate	reed	whit	blac	eleph	wild	tses
pos	137	19	49	32	11	4	4	12	0	38	28	19
neg	138	20	50	59	13	21	7	22	13	53	31	20

Fig. 6. Prevalence of virus to bluetongue virus.

with the highest prevalence being in buffalo, kudu and sable. Neutralizing titres ranged from $\log_{10}$ 1·15–$>$1·75 but were mostly $\log_{10}$ 1·15. The higher titres were found in the sable (data not shown).

Bluetongue

Antibody to bluetongue virus was widespread (Fig. 6) with almost half the buffalo, sable, eland, kudu, wildebeest and tsessebe tested being positive for bluetongue antibody. The only species that was negative was the black rhino.

Leptospirosis

A low prevalence of infection with leptospira was found in buffalo, eland, kudu, wildebeest, zebra *Equus burchelli* and both white and black rhino (Table 2).

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Table 2. *Proportion of animals from each species with antibody to one or more leptospira serovars*

Species	Number tested	Number positive (serovar)							
		Hardjo	Hebdomadis	Mini	Autumnalis	Ballum	Interogans	Pomona	Batavia
Buffalo	347	9	5	7					
Sable	28								
Eland	74	3	4		1				
Impala	182								
Kudu	38								
Wildebeest	54				9				
Giraffe	20								
Waterbuck	28								
Hartebeest	2								
Bushbuck	2								
Reedbuck	9								
Oribi	1								
Tsessebe	20								
Zebra	11				1	1			
Lion	1								
Elephant	1								
W. rhino	72						1		
B. rhino	102						1	3	1

Antibodies to 3 of 8 serovars were found in buffalo, eland and black rhino. Antibodies to two serovars were found in zebra and to 1 serovar in white rhino and wildebeest. Sable, impala, kudu, giraffe, waterbuck, bushbuck, Lichtenstein's hartebeest, oribi *Ourebia ourebi*, reedbuck, tsessebe, lion *Panthera leo* and elephant were negative.

DISCUSSION

A previous serological survey [9] for BVD infections in wildlife populations in southern and eastern Africa gave no details of the precise origin of the samples. The survey reported here was intended to provide a more comprehensive picture of the occurrence and possible significance of BVD infections in Zimbabwean wildlife. BVD virus infection was found to be widespread with a particularly high prevalence in eland. The way in which BVD infection is spread in eland populations is of interest. It has been shown that in cattle herds [10] a high prevalence of antibody positive animals correlate with the presence of PI animals. BVD virus is spread most readily by close contact with PI animals and in this study 1 of 303 eland was found to be persistently infected. Spread of the virus from these PI animals may be assisted by mechanical spread by biting flies [11] particularly during the rainy season. PI animals may therefore be important in the spread of BVD in eland populations in Zimbabwe.

In this study a non-cytopathic virus was also isolated from another subadult eland but on one occasion only, indicative of an acute infection. The high prevalence of antibody positive eland indicates that most infections are infections of the neonatal or post natal animal and, as in cattle, these are not likely to result in disease.

Experiments are presently being conducted in eland to examine the effect of infection in early pregnancy in order to be able to attempt to assess the significance of BVD infections in this species.

A high proportion of nyala and bushbuck were found to be seropositive but the numbers sampled were small. In buffalo herds where the prevalence of seropositive animals was much lower no PI animals were found in the 102 buffalo tested. The only other virus isolates from game animals have been those reported by Plowright [12] from a captured giraffe and a sick buffalo. Both these viruses were cytopathic. A survey in Namibia [13] showed that BVD infection was most prevalent in giraffe (38%) and kudu (67%) which contrasts with the Zimbabwean situation where only 14% of kudu and 5% of giraffe were positive.

Infection with BHV-1 virus was found to be widespread and affected many species with the highest prevalence being in buffalo and eland. A similar

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situation was found in East Africa [14] where 10 of 25 species had antibody with buffalo, eland, Thomson's gazelle *Gazella thomsonii*, kob *Kobus kob* and blue wildebeest being most commonly infected. In Tanzania [15], waterbuck and warthog were also found to be positive in contrast to those tested in this survey. Hippopotomi in both Zambia and Uganda [16] had high seroprevalences. In this study no evidence of infection was found in either white or black rhino or in elephant. Some of the nyala were positive in contrast to a group of 40 nyala from south Malawi which were all negative.

RVF antibodies were found mainly in both species of rhino, buffalo and waterbuck but also in impala and sable. A previous study of buffalo and antelope [17] in the north-west safari areas of Zimbabwe found little evidence of infection. In Kenya no antibody was found in buffalo although a low prevalence was found in some antelope species [18]. It is probable that wild herbivores also play a role, similar to domestic herbivores, in infecting *Aedes* mosquitoes particularly during epidemic periods [17]. The possible significance of RVF can be judged from the results of a controlled experimental infection [18] in which a single buffalo calf showed fever and malaise and 4 of 5 others showed a transient viraemia. One of 2 pregnant females aborted. In South Africa abortions occurred in springbok *Antidorcas marsupialis* and blesbok *Damaliscus dorcas phillipsi* during a suspected outbreak of RVF in cattle [17].

While neutralizing antibody to BEF virus was found it is uncertain whether these were specific for BEF virus or were due to infection with a related lyssavirus. However, low antibody titres to BEF were found in buffalo, sable, eland, impala, kudu, wildebeest, giraffe and tsessebe but not in waterbuck. In Kenya, Davies [19] recorded antibodies to BEF in buffalo, wildebeest, kongoni *Alcelaphus cokei* and waterbuck *Kobus deffassa* and *K. ellipsiprymnus*. In Tanzania [20] antibodies were also found in topi *Damaliscus korrigum* and impala. Unpublished observations cited in [20] have reported antibodies in lechwe *Kobus lechwe*, elephant, warthog, hippopotamus *Hippopotamus amphibius*, oryx *Oryx gazella* and Grant's gazelle *Gazella granti*. As with other studies [20, 21] a very high prevalence of antibodies to BT was found in all species except the back rhino. The results for BEF and BT indicate the likely significance of wildlife as important reservoirs of virus for both of these arthropod-borne viruses.

The only bacterial infection tested for in this study was leptospirosis. Evidence of infection was found in buffalo, eland, wildebeest, zebra and both species of rhino to one or more of eight serovars. Studies in northern Natal and in the Kruger National Park in South Africa [22, 23] found evidence of leptospira infection in nyala (serovar *tarrasovi*), black rhino (serovar *mini*/*hardjo*, *copenhagen*/*pomona*/*tarrasovi*), reedbuck (serovar *mini*), bushbuck (serovar *mini*), black wildebeest *Connochaetes gnou* (serovar *mini*) and buffalo (serovars *tarrasovi* and *hardjo*).

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