Antibodies against some viruses of domestic animals in southern African wild animals

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ABSTRACT

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Twenty-four species of South African wild animals were tested for the presence of antibodies against the viruses of 16 common diseases of domestic animals. Positive results were obtained for African horsesickness, equine encephalosis, equid herpes virus-1, infectious bovine rhinotracheitis, Allerton disease (*Herpes mammillitis*), lumpy skin disease, parainfluenza, encephalomyocarditis, bluetongue, Wesselsbron disease, bovine ephemeral fever, and Akabane disease complex. No antibodies could be demonstrated against the viruses of equine influenza, equine infectious anaemia, equine viral arteritis and Rift Valley fever. The negative results substantiate observations that the latter diseases, with the exception of equine viral arteritis, are absent in South Africa. The number of animal species found positive for a specific virus, ranged from 0–16. No antibodies were found in crocodiles and warthogs, whereas antibodies against Wesselsbron and bovid herpes virus-1 were present in 16 species. Antibodies against viruses of horses were found almost exclusively in zebras and, although elephants reacted to African horsesickness, no neutralizing antibodies against it could be demonstrated in their sera. Zebras were also found to be positive for Wesselsbron and Akabane, which are usually regarded as viruses of ruminants. Antibodies against most viruses were encountered in all vegetation zones in South Africa but, as a rule, most viruses were more prevalent in the high-rainfall zone in KwaZulu-Natal.

Keywords: Antibodies, domestic animals, epidemiology, viruses, wild animals

INTRODUCTION

The presence of antibodies in serum usually indicates prior infection and consequently the potential for involvement in the epidemiology of the disease. The role of some wild animals in South Africa as reservoirs and/or amplifiers of viruses such as foot-and-mouth disease (Bengis, Thomson, Hedger, De Vos & Pini 1986; Hedger 1976), African swine fever (Thomson 1985) and rabies (Barnard 1979; Barnard & Hassel 1981) are reasonably well known. On the other hand, the susceptibility and involvement of a large number of indigenous wild animals is largely undetermined. In South Africa, the expansion of the game industry has resulted in the large-scale translocation of game and the reintroduction of these animals into areas where certain species had disappeared. It was therefore deemed necessary to investigate the possibility that game is involved in the epidemiology of some of the most common viral diseases of livestock in South Africa (Coetzer, Thomson & Tustin 1994).

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MATERIALS AND METHODS

Serum samples

Almost all serum samples of free-living wild animals (Table 1) were collected from 1993–1995. A small number were collected before or after this period.

Collections were made in South Africa in the major vegetation zones consisting of the semi-desert, Cape shrubland, grassland, woodland and forest transition (Fig. 1). Samples from ostriches were collected from semi-domesticated and free-living birds. For comparison, serum samples of 13 donkeys, kindly supplied by G.J. Venter, Onderstepoort Veterinary Institute, were included in the tests for African horsesickness (AHS). Buffalo serum from the Kruger National Park (KNP) became available in 1996 and was tested for parainfluenza-3 (PI3) only, while ten serum samples of Cape mountain zebra from Cradock and six of Burchell's zebra from Cape Town also became available in 1996.

In most instances, the samples were collected by state veterinarians, private practitioners and staff of nature conservation organizations. An assortment of samples collected in Botswana were kindly supplied by Dr S. Osofsky (U.S. Agency for International Development Bureau for Global Programs, Field Support and Research SA-18, Washington, D.C. 20523–1812,USA). On arrival, the samples were clarified by centrifugation, divided into volumes of 1 mℓ, catalogued and stored at –20°C until testing.

Rainfall

The annual average rainfall (Fig. 1) for an area, over a period of 20 years, was calculated from data obtained from the South African Weather Bureau. Data from at least three weather stations in close proximity to the main collection sites in a particular zone were used.

Serological tests

Standard serological tests in use for routine diagnostic work were employed. An enzyme-linked immunosorbent assay (ELISA) (Williams 1987) with minor modifications was used to test for antibodies against the viruses of AHS, equine encephalosis (EE), equine influenza (EI), equid herpes-1 (EHV-1), Rift Valley fever (RVF), bovid herpes-1 (BHV-1) and parainfluenza-3 (PI3). Neutralizing antibodies against equine viral arteritis (EVA) were measured with the micro-neutralization (MN) method of Morailon & Morailon (1978), with minor modifications. The method of Barnard (1993) was used for bovid herpes virus-2 (BHV-2), lumpy skin disease (LSD), encephalomyocarditis (EMC), AHS, malignant catarrhal fever (MCF) and Wesselsbron disease (WSL). Briefly, two-fold dilutions of inactivated serum in 96-well microtitre plates (Nunc Denmark) were used to neutralize 30–100 plaque-forming units of virus per well. Agar-gel immuno-diffusion (AGID) (Blackburn & Swanepoel 1988) was used for bluetongue (BT), equine infectious anaemia (EIA), and a micro-adaptation of the complement-fixation (CF) test (Bradstreet & Taylor 1962) was used for bovine ephemeral fever (BEF) and the Akabane (AKA) group of viruses.

Interpretation of results

The test results are shown as positive or negative and the cut-off points were based on many years' experience with tests performed on the sera of domestic

TABLE 1 Wild animals from which samples were collected

Family/subfamily	Genus and species	Common names
Elephantidae	*Loxodonta africana*	African elephant
Rhinocerotidae	*Ceratotherium simum*	White rhinoceros
	Diceros bicornis	Black rhinoceros
Equidae	*Equus burchellii*	Burchell's zebra
	E. zebra	Cape mountain zebra
Suidae	*Phacochoerus aethiopicus*	Warthog
Giraffidae	*Giraffa camelopardalis*	Giraffe
Bovidae		
Acelaphinae	*Connochaetes gnou*	Black wildebeest
	C. taurinus	Blue wildebeest
	Alcelaphus buselaphus	Red hartebeest
	Damaliscus dorcas phillipsi	Blesbok
	D. lunatus	Tsessebe
Antilopinae	*Antidorcas marsupialis*	Springbok
Aepycerotinae	*Aepyceros melampus*	Impala
Hippotraginae	*Hippotragus niger*	Sable antelope
	Oryx gazella	Gemsbok
Bovinae	*Syncerus caffer*	African buffalo
	Tragelaphus strepsiceros	Kudu
	T. angasii	Nyala
	T. scriptus	Bushbuck
	Taurotragus oryx	Eland
Reduncinae	*Kobus ellipsiprymnus*	Waterbuck
Crocodilidae	*Crocodilus nilotica*	Crocodile
Struthionidae	*Struthio camelus*	Ostrich

animals. In most cases, antibody titres higher than ten were regarded as positive. In the case of EVA, MCF and LSD titres, higher than four was judged to be positive. To validate the results, representative samples were retested and, where applicable, group-specific test results were validated by MN.

Significance of results

Where available, at least six samples of a specific species per locality were tested and evaluation was based on test results of at least eight samples, obtained from two or more locations. In most instances, the number of samples tested was significantly greater. For the calculation of prevalence in vegetational zones, only results of species that reacted positively were used.

In all tables, the results are shown as the number of positive samples over the number tested.

RESULTS

The numbers of tsessebe, sable antelope, duiker, nyala and waterbuck were too small for the drawing of any meaningful conclusions. Warthogs and crocodiles yielded no antibodies against any of the viruses tested for. Results on sera from these species were therefore not evaluated or used for comparative purposes.

Antibodies against viruses of horses

No antibodies against the viruses of EI, EIA and EVA, could be detected in any of several species, including 80 zebra samples from 11 localities in the five vegetation zones (Table 2). ELISA antibodies against AHS virus were found in the sera of 48 of 112 zebras and in 31 of 47 elephants (Table 2). However, no significant levels of neutralizing antibodies could be demonstrated in elephant sera. ELISA antibodies against EE virus were found in 28 of 117 zebras and

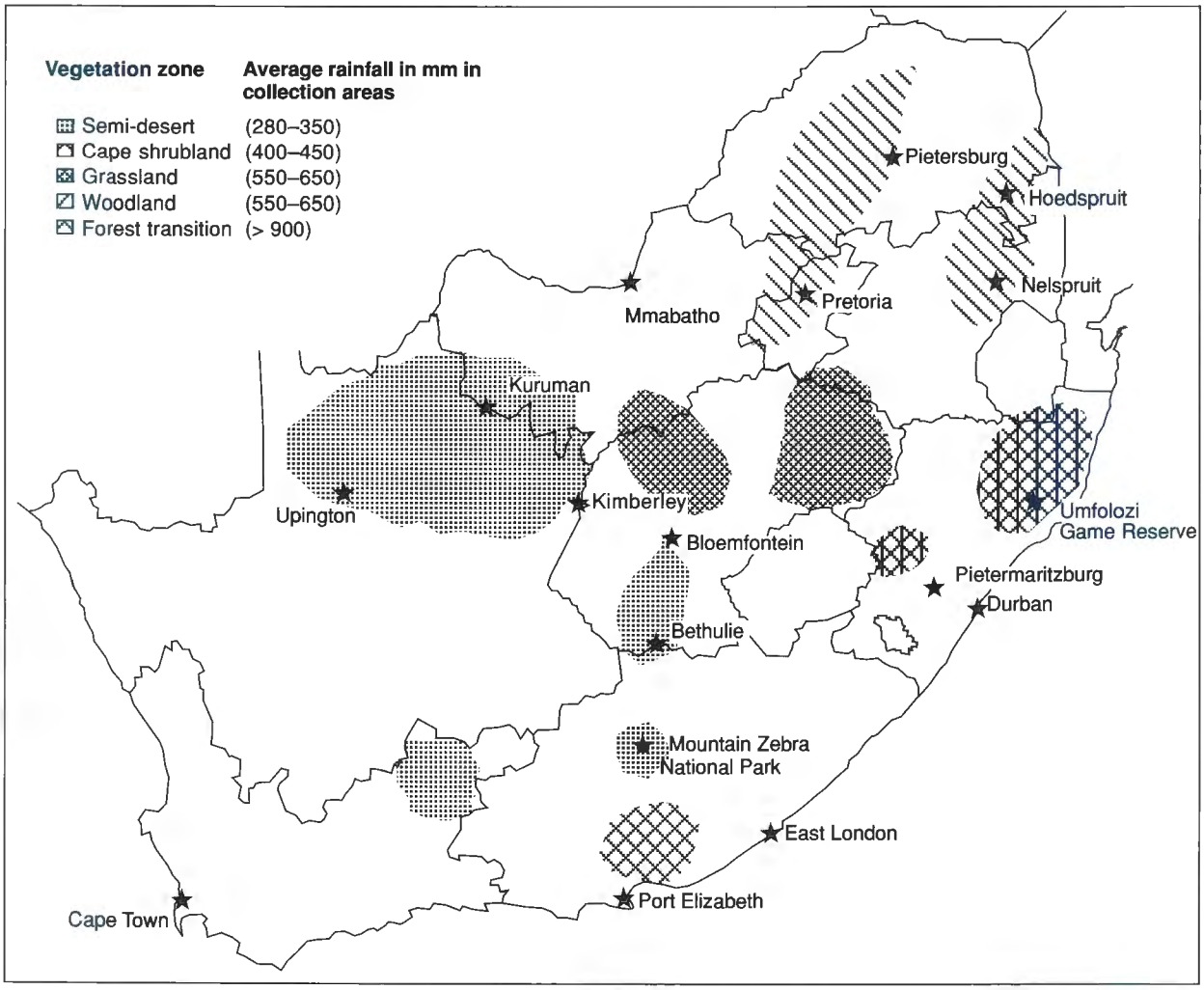


FIG. 1 Vegetation zones and collection areas in the Republic of South Africa

in four of 49 elephants. Of 11 game species tested for ELISA antibodies against EHV-1, 50 of 56 zebras reacted positively. However, no antibodies against this virus could be detected in the sera of the ten other species.

The results (Fig. 2) show the prevalence of AHS, EE and EHV-1 in Kenya, South Africa and Botswana. The geographic distribution of AHS declines from almost 100% in Botswana and the north-eastern part of South Africa to a virtual absence in the central part and a total absence in the southern part. On the other hand, EE virus with a prevalence of 1–50% and EHV-1 with a prevalence of almost 100% were present in all localities sampled in these zones. Two EE-virus-positive zebras from the Mountain Zebra Park in the semi-desert were found in six samples collected in 1982, while ten samples collected in 1996 were all negative for both EE and AHS.

The prevalence of type-specific antibodies against AHS virus in zebras and donkeys within seven localities in South Africa and in Botswana is shown in Table 3. Neutralizing antibodies against all nine serotypes were found in almost all zebras from areas with a high prevalence of ELISA antibodies, while in the semi-desert zone antibodies against three or less serotypes were present in samples from Kuruman, Bethulie and Cradock. Serotype nine, which only rarely produces disease in SA, was the only type found in a donkey in the Eastern Cape Province.

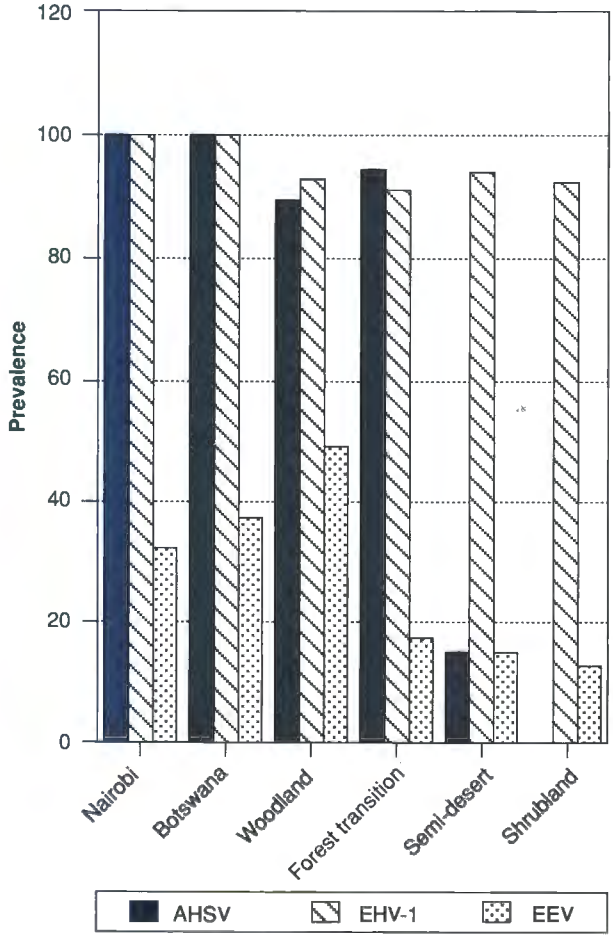


FIG. 2 The prevalence of antibodies against the viruses of African horsesickness, equine encephalosis and equid herpes virus-1 in Botswana, Kenya and four vegetation zones in South Africa

Antibodies against viruses of cattle and sheep

The number of species that had antibodies (Tables 4, 5 and 6) varied from none for RFV to 16 for WSL. Antibodies against viruses of cattle and sheep were not restricted to antelope. Antibodies against a number of viruses were also found in the two rhinoceros species and in elephants, while zebras tested positive for WSL and AKA, and ostriches for WSL.

Prevalence of antibodies against herpes viruses of cattle in wild animals

Antibodies against BHV-1 (Fig. 3B) were found mostly in ruminants. The only other species that had antibodies against BHV-1 was white rhinoceros. The highest prevalence, 80% for BHV-1, was found in kudu (eight of ten), followed by blue wildebeest (six of 12) and buffalo (26 of 59). No antibodies against BHV-1 could be found in 79 springbok, 20 eland and 31 impala. Antibodies against BHV-2 (Fig. 3A), on the other hand, occurred in all ruminants and in both species of rhinoceros as well as in elephants and giraffes.

Antibodies against both BHV-1 and BHV-2 (Fig. 4C) were found in all vegetation zones. The highest prevalence for BHV-2 (63%) was in the forest transition, and the lowest (14%) in the shrubland, while the lowest prevalence for BHV-1 was in the semi-desert. Both viruses were more or less equally prevalent (32–35%) in the woodland and grassland.

The results for AHV-1 (Table 4) consist of tests done over the period 1981–1995. The highest prevalence of antibodies was found in blue wildebeest (89%) and black wildebeest (81%). The levels of antibodies (not shown) in blue wildebeest were notably higher than in black wildebeest, and the prevalence of antibodies in other species ranged from 7% in blesbok to 50% in both sable and gemsbok.

Prevalence of antibodies against LSD, PI3 and EMC

Lumpy skin disease, with a prevalence of 10–27% (Fig. 3D), occurred in both wildebeest species, and in eland, springbok and impala. The prevalence in the different zones (Fig. 4D) of animals that had antibodies, varied from 17% in the grassland to 33% in the forest transition.

Antibodies against PI3 (Fig. 3C) occurred in low prevalences, except in black wildebeest (24%) and buffalo (62%).

TABLE 2 Antibodies against viruses of horses in sera of wild animals in South Africa

Species	Antibodies against the viruses of					
	AHS	EE	EH	EVA	EI	EIA
Burchell's zebra	48/96	26/101	50/56	0/80	0/80	0/80
Mountain zebra	0/16	2/16	5/6	–[a]	–	–
Elephant	31/74[b]	4/49	0/26	–	0/10	–
Buffalo	0/49	0/39	0/12	–	–	–
Blesbok	0/12	–	–	–	–	–
Impala	0/12	–	–	–	–	–
Gemsbok	0/8	0/8	–	–	–	–
Kudu	0/8	0/8	–	–	–	–
Springbok	0/12	0/22	0/8	–	–	–
White rhinoceros	0/66	0/20	0/17	0/16	0/20	0/20
Black rhinoceros	0/36	0/24	0/24	0/10	0/10	–
Blue wildebeest	0/8	–	0/8	–	–	–
Black wildebeest	0/8	–	0/8	–	–	–
Giraffe	0/25	0/12	0/12	0/8	0/8	0/8
Warthog	0/12	0/12	–	–	–	–
Crocodile	0/23	0/23	0/12	–	–	–
Ostrich	0/40	0/40	0/6	–	–	–

[a] Not tested [b] No neutralizing antibodies could be demonstrated in elephant sera

TABLE 3 Type-specific antibodies against African horsesickness virus in serum of zebras and donkeys from nine areas in southern Africa

Origin (area) of serum samples	Species	Number of animals tested	Number of animals with antibodies against AHS serotype									Number of tests (posiive/tested)	Percentage positive
			1	2	3	4	5	6	7	8	9		
Botswana	Zebra	6	5	6	5	6	5	6	5	6	5	49/54	91
South Africa													
Woodland													
Hoedspruit	Zebra	6	5	6	3	6	5	6	6	6	6	49/54	91
	Donkey	5	4	5	5	5	5	5	5	5	5	44/45	98
Pretoria	Zebra	3	2	1	0	2	0	1	0	3	0	9/27	33
	Donkey	2	1	0	2	1	0	2	0	2	0	8/18	44
Forest transition													
Umfulozi	Zebra	6	6	5	4	4	4	5	5	6	4	43/54	80
Semi-desert													
Kuruman	Zebra	2	1	0	0	2	0	0	0	0	0	3/18	17
	Donkey	2	1	1	0	2	0	0	0	0	0	4/18	22
Bethulie	Zebra	3	2	0	0	0	1	1	0	0	0	4/27	15
	Donkey	2	0	1	0	0	1	1	0	0	0	3/18	17
Cradock	Zebra	6	0	0	0	0	0	0	0	0	0	0/54	0
	Donkey	2	0	0	0	0	0	0	0	0	1	1/18	2
Cape scrubland													
Cape Town	Zebra	6	0	0	0	0	0	0	0	0	0	0/54	0

The geographic distribution of antibodies against PI3 is shown in Fig. 4D. The prevalence in buffalo ranged from 25% in the shrubland to 86% in the southern part of the Kruger National Park in the woodland.

Tests to detect antibodies against EMC were limited to animals in the Northern Province and elephant samples from Botswana (Table 5). All elephants tested negative, but six of 68 samples from hartebeest, impala and giraffe from the woodland reacted positively with low antibody titres ranging from 4–32.

Prevalence of antibodies against insect-transmitted viruses of cattle and sheep

No antibodies against RVF were found in any of 500 samples representing 24 species from several localities in all areas in South Africa. On the other hand,

TABLE 4 Antibodies against herpes viruses of cattle and sheep in sera of African wild animals

Species	Viruses and percentage sera positive					
	BHV-1	%	BHV-2	%	AHV-1	%
Black wildebeest	3/14	21	3/20	15	29/36	81
Blue wildebeest	6/12	50	7/13	54	74/83	89
Red hartebeest	1/29	3	6/27	22	8/23	35
Blesbok	10/30	33	2/11	18	2/30	7
Tsessebe	0/1		0/1		5/10	50
Springbok	0/79		9/32	28	0/15	
Impala	0/31		7/25	28	0/8	
Sable antelope	0/1		0/1		3/6	50
Gemsbok	1/14	7	4/19	21	1/6	17
Duiker	0/1		0/1		–[a]	
Buffalo	26/59	44	14/41	34	0/20	
Kudu	8/10	80	4/24	17	0/27	
Nyala	0/2		0/2		0/8	
Bushbuck	3/8	38	2/8	25	0/10	
Eland	0/20		10/18	56	0/19	
Waterbuck	0/6		1/2		0/21	
African elephant	0/54		15/26	58	0/7	
White rhinoceros	2/34	6	8/14	57	0/9	
Black rhinoceros	0/23		10/19	53	0/18	
Warthog	0/12		0/12		0/12	
Giraffe	0/23		10/19	53	0/12	
Ostrich	0/12		0/18		–	
Crocodile	0/12		0/12		–	
Zebra	0/20		0/24		0/17	

[a] Not tested

TABLE 5 Antibodies against lumpy skin disease (LSD), parainfluenza 3 (PI3) and encephalomyocarditis (EMC) virus in the sera of wild animals in South Africa

Species	Virus				
	PI3	%	LSD	%	EMC
Black wildebeest	5/21	24	3/31	10	–[a]
Blue wildebeest	1/15	7	4/15	27	–
Red hartebeest	0/31		0/29		2/8
Blesbok	0/12		0/21		–
Tsessebe	0/2		0/2		–
Springbok	2/58	3	12/53	23	–
Impala	0/26		5/25	20	2/19
Sable antelope	0/2		0/2		–
Gemsbok	1/18	6	0/20		–
Duiker	0/1		0/1		–
Buffalo	48/77	62	0/15		–
Kudu	0/12		0/12		0/10
Nyala	0/17		0/2		–
Bushbuck	0/8		0/8		–
Eland	3/20	15	1/15	7	
Waterbuck	0/6		0/9		–
African elephant	0/43		0/4		0/21
White rhinoceros	0/17		0/6		–
Black rhinoceros	0/15		0/6		–
Warthog	0/12		0/12		–
Giraffe	0/6		0/21		2/8
Ostrich	0/20		0/6		–
Crocodile	0/12		0/6		–
Burchells' zebra	0/46		0/13		–

[a] Not tested

antibodies against BT, WSL, BEF and AKA, were found in several ruminants (Table 6). In addition, antibodies against BT were also present in the sera of elephants. On the other hand, high prevalences of antibodies against WSL occurred in rhinoceros, zebras and ostriches.

The prevalences of antibodies against BT (Fig. 3F) in blue and black wildebeest were 72 and 44%, respectively. The prevalences in gemsbok, red hartebeest, eland, springbok and impala were lower than 27% and no antibodies were found in 13 kudu samples. Giraffes and elephants with prevalences of, respectively, 24 and 91%, were the only non-ruminants found positive.

Antibodies against WSL (Fig. 3E) were found in all ruminants as well as in white rhinoceros, black rhinoceros, giraffes, zebras and ostriches. Twenty-eight impala tested negative for WSL.

Antibodies against AKA were present in both ruminants and non-ruminants (Fig. 3H), while antibodies against BEF (Fig. 3G) were found only in ruminants.

Geographic distribution of wild animals with antibodies against insect-transmitted diseases of domestic animals

The geographic distributions of animals with antibodies against BT and WSL (Fig. 4A) are very much the same. The highest prevalence occurred in the forest-transition, and the lowest in the semi-desert and woodland areas. A high prevalence of animals that had antibodies against AKA (Fig. 4B) was found in the forest transition, and positively tested animals were found in all other zones. The prevalence in the grassland was only 4%. The geographic distribution of BEF (Fig. 4B) shows a high prevalence in the grassland and in the forest transition.

DISCUSSION

Absence of antibodies against a particular virus may be ascribed to several factors. In the case of insect-transmitted viruses, absence of antibodies in a specific species in the presence of virus and competent vectors is most probably an indication of the insusceptibility of the species. A high prevalence of antibodies against a specific virus usually indicates susceptibility. The role of a species in the epidemiology of disease cannot be based solely on the prevalence of antibodies, because a low prevalence may not reflect its potential role, as it may be ascribed to inadequate exposure as a result of a low population density of susceptible animals and/or insufficient transmission as a result of a small vector population. Animals such as springbok, red hartebeest and gemsbok, which prefer semi-desert conditions, are therefore less likely to be exposed to vectors that thrive in low-lying, moist areas. On the other hand, animals preferring this type of habitat are usually more exposed to vectors and may have a high prevalence of antibodies. Prevalence of antibodies may also be influenced by climatic conditions. In dry conditions, the numbers of *Culicoides*, the vector for AHS and BT, and of mosquitoes that transmit RVF and WSL, are usually low. For a number of years preceding and during the sampling period, the rainfall in South Africa was well below average. Therefore the results obtained in this investigation may not reveal the actual susceptibility of individual species.

High prevalences of antibodies against viruses transmitted by contact seem more likely in species that are gregarious and form large herds. Therefore wildebeest, eland and buffalo, should they be susceptible, are expected to have high prevalences of antibodies against such viruses.

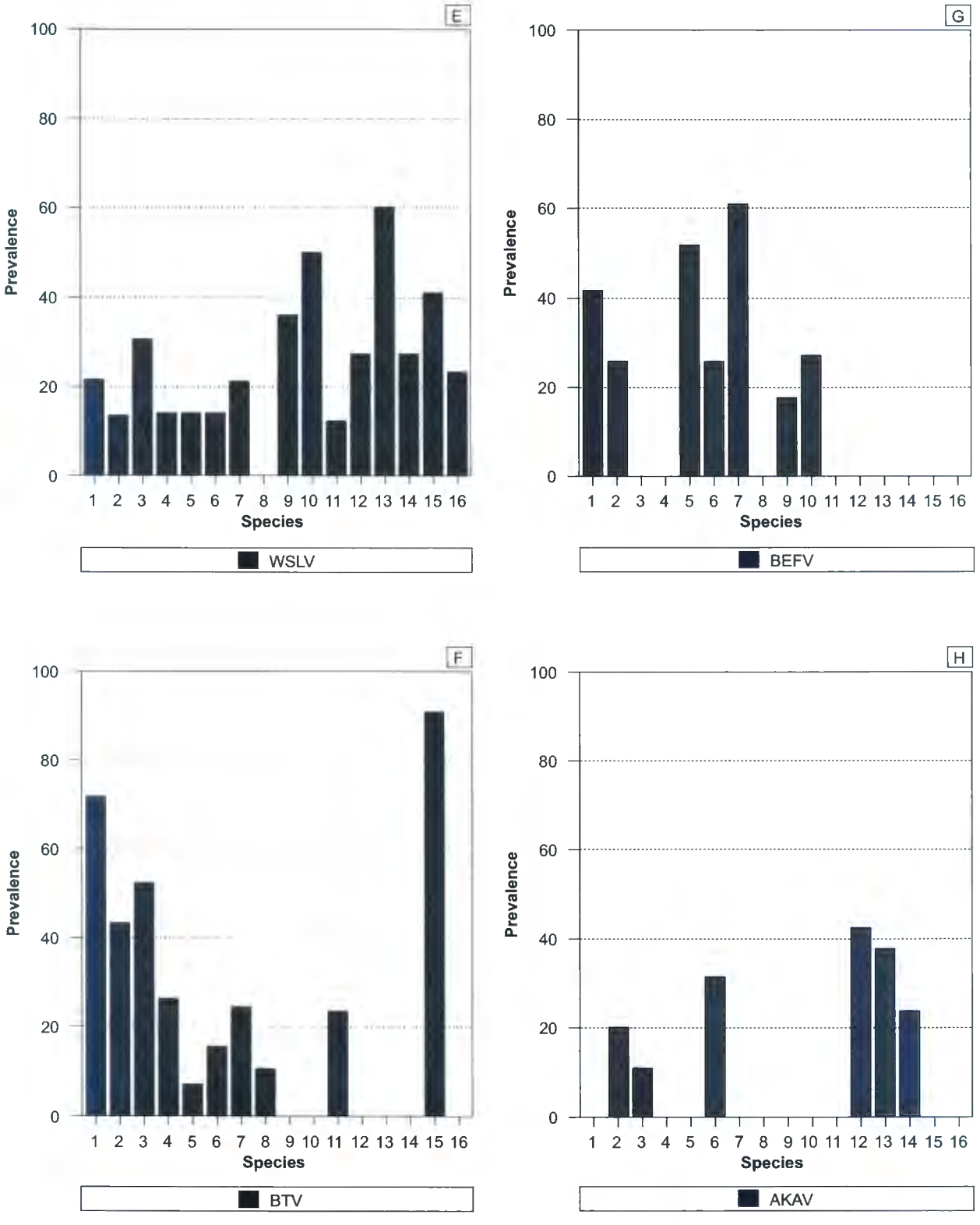
In this investigation, the overall prevalences of antibodies against the different viruses ranged from none for EI, EVA, EIA and RVF, to a high of almost 100% for EHV-1, BT in elephants from Botswana and AHS in zebras from the north-eastern parts of South Africa and Botswana.

Absence of antibodies against EI, EVA and EIA

Eighty zebras, from all localities, tested negative for antibodies against EI, which first entered South Africa in 1986 (B.J. Erasmus, Onderstepoort Veterinary Institute, unpublished data 1986). However, the extensive use of vaccine against the disease has stabilized the situation and it is now believed that the country is free of EI. The absence of antibodies against EI virus in zebras, despite their susceptibility to experimental infection (B.J. Erasmus, Onderstepoort Veterinary Institute, unpublished data 1986), indicates that the virus has not become established among zebras of which the population dynamics, characterized by foaling in every month of the year, is ideal for the development of an endemic situation.

The absence of antibodies against EIA in all species tested, was to be expected, as it is generally accepted that South Africa is free from this disease (Verwoerd & Tustin 1994).

Until recently, equine viral arteritis in South Africa was believed to be limited to a small Lippizaner population (B.J. Erasmus, Onderstepoort Veterinary Institute, unpublished observations 1994). However, serological evidence (Paweska & Barnard 1993) and the isolation of EVA virus from donkeys (Paweska 1994) indicate a widespread occurrence of the infection in donkeys. In a recent investigation, no antibodies against EVA could be demonstrated in the sera of zebras from the KNP (Barnard & Paweska 1993). In the present investigation, a similar result was obtained with sera from different zebra populations from



Key to Fig. 3

1 Blue wildebeest
2 Black wildebeest
3 Buffalo
4 Red hartebeest
5 Eland
6 Springbok
7 Blesbok
8 Impala
9 Kudu
10 Bushbuck
11 Giraffe
12 White rhinoceros
13 Black rhinoceros
14 Zebra
15 Elephant
16 Ostrich

FIG. 3(A–D) The prevelance of antibodies against viruses of domestic ruminants in wild animals in South Africa and Botswana

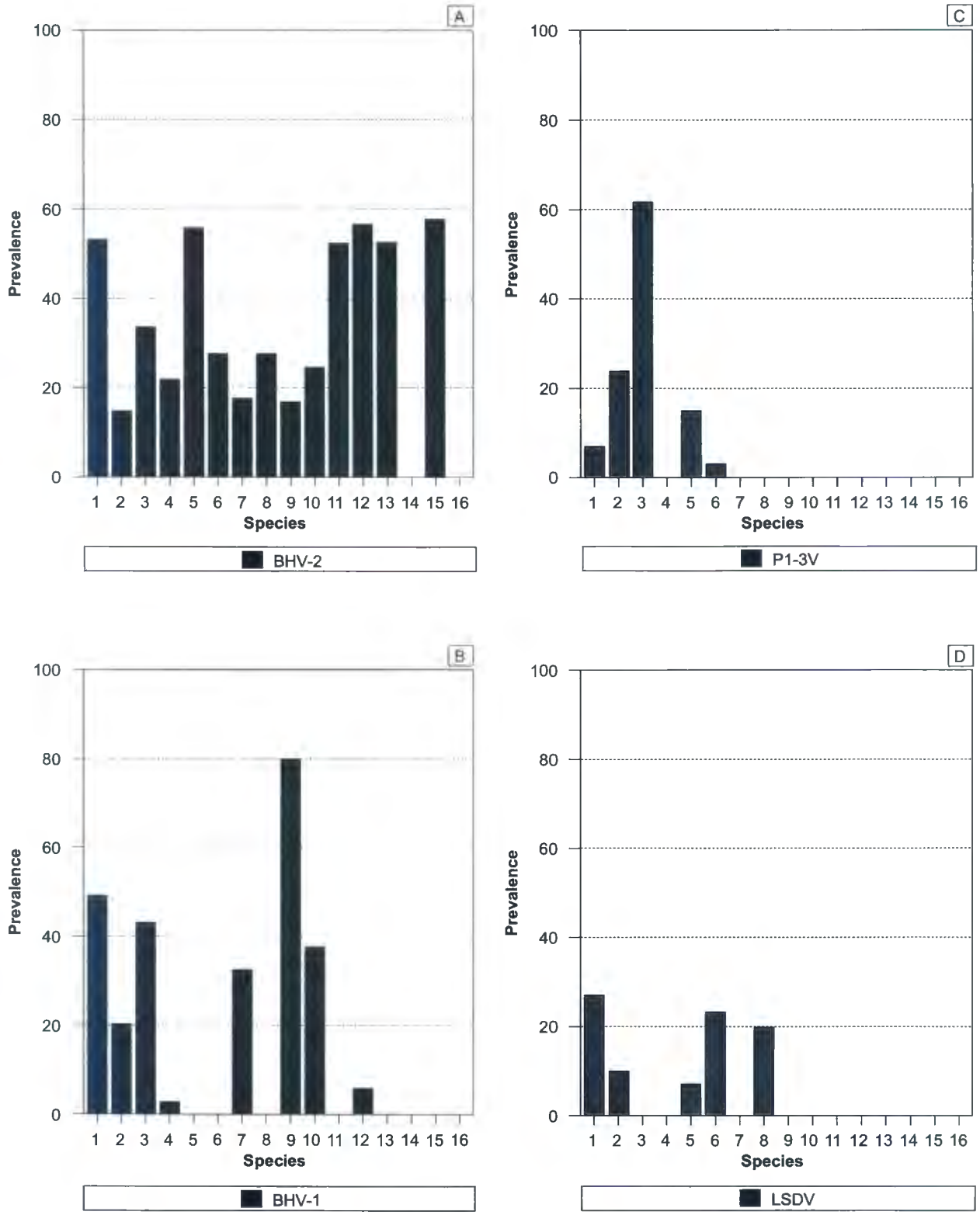


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FIG. 3(E–H) The prevelance of antibodies against viruses of domestic ruminants in wild animals in South Africa and Botswana

TABLE 6 Prevalence in wild animals of antibodies against insect-transmitted viruses of domestic ruminants in South Africa

Species	Virus and percentage positive								
	BT	%	WSL	%	RVF	BEF	%	AKA	%
Black wildebeest	7/16	44	2/14		0/6	5/19	26	3/15	20
Blue wildebeest	13/18	72	5/23	22	0/16	5/12	42	0/10	–[a]
Red hartebeest	8/30	27	9/66	14	0/28	0/24		0/20	–
Blesbok	5/20	25	5/24	21	0/10	14/23	61	0/25	–
Tsessebe	1/1		0/1		0/1	0/1		0/1	–
Springbok	12/75	16	9/66	14	0/84	10/38	26	4/13	31
Impala	3/27	11	0/28		0/28	0/28		0/28	–
Sable antelope	0/1		0/1		0/1	–		–	–
Gemsbok	1/15		2/18	11	0/15	0/15		0/16	–
Duiker	0/1		0/1		0/1	–		–	–
Buffalo	33/62	53	18/59	31	0/71	0/46		5/47	11
Kudu	0/13		5/14	36	0/15	2/11	18	0/4	–
Nyala	0/2		0/2		0/2	–		–	–
Bushbuck	0/8		4/8	50	0/8	3/11	27	–	–
Eland	1/15	7	3/22	14	0/15	11/21	52	0/25	–
Waterbuck	0/2		0/2		0/2	3/4		0/2	–
African elephant	21/23	91	9/22	41	0/40	–		–	–
White rhinoceros	0/20		26/97	27	0/35	0/10		8/19	42
Black rhinoceros	0/20		15/25	60	0/25	0/10		3/9	33
Warthog	0/12		0/12		0/6	0/6		–	–
Giraffe	6/25	24	2/17	12	0/9	0/15		0/15	–
Ostrich	0/25		9/40	23	0/39	0/6		0/15	–
Crocodile	0/10		0/12		0/20	–		–	–
Burchell's zebra	0/40		26/97	27	0/22	0/35		3/13	23

[a] Not tested

all regions in the country. Horses that are susceptible to experimental infection with the donkey isolate of EVA virus, appear to be relatively resistant (Paweska, Aitchison, Chirnside & Barnard 1996), and although the susceptibility of zebras has not yet been established, the possibility that they may be susceptible cannot be ignored, as situations where donkeys and zebras are kept in close proximity or even permitted to inter-breed, do occur on rare occasions. This should be avoided at all cost so as to prevent infection of zebra populations. Indigenous wild animals are known for their resistance to endemic viruses, but their susceptibility to exotic viruses is largely unknown, and infection of the zebra population may have a detrimental effect.

Presence of antibodies against viruses of horses in wild animals

The presence of antibodies against AHS virus in zebras in Kenya (Davies & Otieno 1977) and in zebras from the KNP (Erasmus, Young, Pieterse & Boshoff 1978; Barnard 1993), has been reported. Likewise, the presence of antibodies against EE virus (Erasmus, Boshoff & Pieterse 1978) as well as EE and EH virus (Barnard & Paweska 1993) has been reported in zebras from the KNP. The present study confirms the previous reports and provides further information on the geographic occurrence of these viruses.

Zebra populations from the most important vegetation zones in South Africa were positive for EH virus, with a prevalence of almost 100%, and for EE virus, with a prevalence ranging from 13–50%. The prevalence of antibodies against EE virus in all zones is in contrast to the restricted distribution of antibodies against AHS virus. The prevalence of antibodies against AHS declines from almost 100% in Botswana and the north-eastern part of South Africa to a total absence in the southernmost parts of South Africa. This observation is in line with the decreasing occurrence of AHS in horses towards the southern part of the country (G. Gerdes, Onderstepoort Veterinary Institute, unpublished data 1996). In addition, this distribution closely correlates with the distribution of AHS-positive donkeys. The decline of prevalences of antibodies in zebras and donkeys and the restricted occurrence of AHS, constitute strong evidence that there is no effective virus reservoir in the southern part of the country, nor is there a continuous circulation of AHS virus. Rare cases of AHS encountered in some years in the central and southern part of the country, usually coincided with above-average rainfall, and this results in an abundance of vectors and often follows the introduction of infected horses and/or infected *Culicoides* from the endemic AHS area. The similarity in distribution of antibodies against AHS virus in zebras and donkeys suggests that these two species are equally susceptible to infection with the virus. Reasons for the absence of a circulation cycle in these areas as opposed to that in the KNP (Barnard 1993) are considered to be the absence of large zebra populations and sufficient numbers

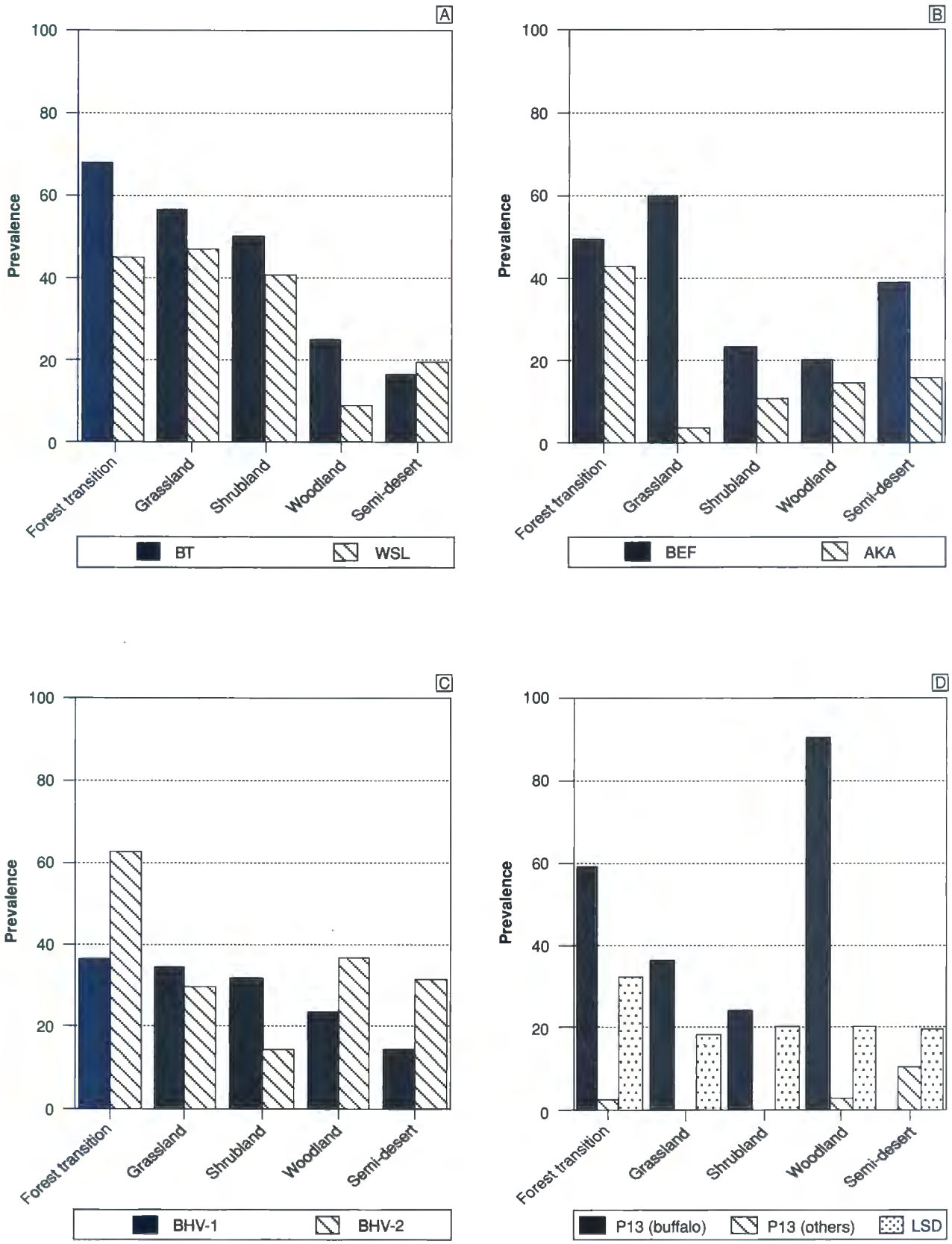

FIG. 4 The geographic distribution of wild animals with antibodies against viruses of domestic ruminants

(Jupp, McIntosh & Nevill 1980) of competent *Culicoides* vectors in most of the semi-desert regions in the southern part of the country.

The presence of antibodies against EE virus, with a prevalence of 13–50% in zebras from all vegetation zones, is in agreement with the suggestion (Anonymous 1990) that the virus is endemic in most parts of South Africa and Botswana and, as in the case of AHS, it has been suggested that the virus is probably transmitted by midges. This supposition is based on observations that horses stabled overnight are less inclined to become infected than those that are not stabled. However, the geographic prevalence of EE virus is quite different from that of AHS, which decreases progressively to the southern part of the country. It is unlikely that his difference is attributable to the effect of the vaccination of horses against AHS, as the prevalence of antibodies against EE virus is significantly lower than that of AHS in areas where the prevalence of antibodies to AHS is almost 100%. This distribution suggests an epidemiological pattern and vectors unlike that of AHS.

Elephants were the only other species with ELISA antibodies against the viruses of horses in their serum, but the levels of antibodies against AHS were low and it could not be confirmed by AGID and MN tests. This observation is in agreement with a previous report (Barnard, Bengis, Keet & Dekker 1994) and is an indication that the sera of elephants react non-specifically in the ELISA. A similar observation was made with complement fixation (Erasmus, Young, Pieterse & Boshoff 1978). The positive results obtained for EE virus can probably be ascribed to the same phenomenon, but needs further investigation. In contrast, the levels of ELISA antibodies against BT in elephant sera were significantly higher and could be confirmed with AGID tests.

Antibodies against viruses of ruminants

No antibodies against RVF virus were detected. However, the presence of RVF-virus antibodies in the serum of blue wildebeest, red hartebeest and waterbuck (Davies 1975), and the susceptibility of African buffalo (Daubney & Hudson 1932; Davies & Karstad 1981) have been described. In South Africa, the indigenous forests of KwaZulu-Natal were previously regarded as endemic RVF areas (Smithburn, Haddow & Gillet 1949; Smithburn, Haddow & Lumsden 1948; Kokernot, Heymann, Muspratt & Wolstenholme 1957), and in 1981 RVF virus was isolated from mosquitoes from KwaZulu-Natal (Jupp, McIntosh & Thompson 1983). However, epidemics of RVF in South Africa occur in cycles of a few to more than 20 years, the last of which was from 1974–1976 (Barnard & Botha 1977). In the present survey, no antibodies against this virus could be detected in sera of 500 wild animals representing 24 species, including 93 samples from the forest transition in KwaZulu-Natal. The freedom from RVF in South Africa for 20 years and the absence of antibodies in wild animals are indications that South Africa has become free from RVF. Furthermore, it casts some doubt on the belief that RVF in South Africa can maintain itself among indigenous wildlife for long inter-epidemic periods.

Antibodies against BHV-1 have, in addition to their demonstration in cattle, also been demonstrated in African buffalo, eland, wildebeest, impala and waterbuck (Hedger & Hamblin 1978a; Karstad 1978; Gibbs & Rweyemamu 1977; Rweyemamu 1974; Rampton & Jesset 1976). Furthermore, the ability of BHV-1 to produce disease in wild animals was demonstrated by the isolation of the virus from a wildebeest with vulvovaginitis (Karstad, Jesset, Otema & Drevemo 1974). A recent finding of herpes viruses specific to wildlife species, but serologically indistinguishable from BHV-1, makes interpretation of serological surveys difficult (Pastoret, Thiry, Brochier, Schwers, Thomas & Du Buisson 1988). In the present investigation, a high prevalence of BHV-1 ELISA antibodies, confirmed by neutralization tests, was found in blue wildebeest, black wildebeest, blesbok, buffalo and bushbuck. A noteworthy result is the absence of antibodies in two of the most abundant species, springbok (none of 79) and impala (none of 31), in which a low prevalence of 1% was reported previously (Rampton & Jesset 1976). It is not known to what extent the results obtained can be ascribed to climatic conditions in the woodland and semi-desert zones where the virus has a low prevalence, or to the insusceptibility of these species. The latter consideration seems to be the most likely one. It is also clear that the prevalence of antibodies in species in dry areas is remarkably lower than in the other areas.

Previous reports on the presence of antibodies against BHV-2 (Plowright & Jesset 1971; Schiemann, Gwamaka & Kalunda 1972) in wild animals, showed that buffalo, eland, giraffe, impala, gemsbok, bushbuck, and waterbuck are susceptible. Similar results were obtained in this study, and antibodies were found in 16 of 21 species. This wide range of a diverse group of animal species with antibodies against BHV-2 demonstrates the versatile nature of this virus. In this investigation, no correlation could be detected between the positive results against BHV-1 and BHV-2 or AHV-1, indicating that cross-reactions between these viruses did not play a role in the high prevalences of antibodies against them.

The presence of antibodies to AHV-1, exclusively in species of the subfamilies Alcelaphinae and Hippotraginae, is in agreement with results of previous reports (Plowright 1967; Heuschele, Swansen & Fletcher 1982) and, although only wildebeest are associated with classic disease, other members of these two families cannot be excluded as potential

reservoirs of the virus. However, lower levels of antibodies and a lower prevalence are indications that they probably do not play a significant role in the epidemiology of MCF.

LSD has not been reported to cause natural infections in wildlife in southern Africa, but antibodies against the virus have been demonstrated in African buffalo in Kenya (Davies 1982). However, in a previous investigation, buffalo were found to be resistant to experimental infection when the susceptibility of giraffes and impala was demonstrated (Young, Basson & Weiss 1970). In the present investigation, antibodies against LSD were found in blue wildebeest, black wildebeest, springbok, impala and in one of 15 eland. Animals tested positive were more or less equally distributed in all areas. However, no antibodies could be found in 21 giraffes from different vegetational zones, or in 15 buffalo from the forest transition where the highest prevalence of LSD antibodies was recorded.

Antibodies against PI3 have been demonstrated in a number of South African wild animals (Erasmus, Boshoff & Pieterse 1967; Hedger & Hamblin 1978b), and the virus has been isolated from buffalo (Hamblin, Hedger & Condy 1980). Generally speaking, antibodies against viruses affecting the respiratory track of cattle are more or less equally prevalent in several game species. This, however, is not the case with PI3, for which positive results were found predominantly in buffalo. Even among buffalo there was a marked difference in the prevalences in the different game parks. It is generally accepted that dissemination of respiratory infections is more effective among animals in close contact and one may therefore speculate that the reason for the high prevalence in the KNP is the result of the presence of significantly more buffalo in larger herds than in other parks. The highest prevalence (86%) occurred in buffalo suffering from bovine tuberculosis (Bengis, Kriek, Keet, Raath, De Vos & Huchzermeyer 1996). The interaction between PI3 and tuberculosis in game is unknown, but it is known that PI3 does predispose lung tissue to bacterial invasion, mainly as a result of its effect on alveolar macrophages (Brown & Ananaba 1988), which play an important role in the elimination of bacteria, including mycobacteria (Dannenberg 1989). The relationship between PI3 virus (and perhaps other respiratory viruses) and tuberculosis among buffalo in the KNP, warrants further investigation.

Antibodies against insect-transmitted viruses of cattle and sheep

Antibodies to WSL were found in all ruminant species, with the exception of impala (none of 28), from which eight or more samples had been tested. The occurrence of antibodies against WSL in ostriches is further evidence of the susceptibility of ostriches to this virus (Allwright, Geyer, Burger, Williams, Gerdes & Barnard 1995). The occurrence of WSL virus in all areas in southern Africa is well documented and the geographic distribution of the aedine mosquitoes associated with WSL indicates a wide distribution in southern and eastern Africa (Kokernot, Szlamp, Levitt & McIntosh 1965; McIntosh 1980; Smithburn, Kokernot, Heymann, Weinbren & Zentkowsky 1959; Swanepoel 1989). According to these authors the virus is most prevalent in the warmer and moister parts of southern Africa, e.g. in the northern part of KwaZulu-Natal, in Mozambique and in parts of Zimbabwe. Similar results were obtained in this investigation, with a notably lower prevalence in the semi-desert than in the high-rainfall forest-transition area in KwaZulu-Natal.

The geographic distribution of BT is very similar to that of WSL. Bluetongue virus has been recognized in South Africa since Merino sheep were introduced into the country in the late eighteenth century, and it is generally accepted that all South African wild ruminants are probably susceptible to infection. It has also been postulated that the primary cycle involves one or more species of African antelope and *Culicoides* midges, and that the role of antelope has been largely supplanted by cattle in areas where cattle have displaced wild animals (Du Toit 1962b). Animal species reported to be susceptible include blesbok (Neitz 1933), buffalo, hartebeest, blue wildebeest, impala, eland, buffalo and reedbuck (Davies & Walker 1972). In the present investigation, antibodies were indeed found in all ruminant species of which sufficient numbers were tested, as well as in giraffes and elephants. Elephants had the highest prevalence (91%) of all species. In contrast to the high prevalence in elephants, no antibodies could be found in 20 samples each of black and white rhinoceros. The prevalence of antibodies in every vegetational zone indicates that the disease can occur in sheep in most parts in the country. A lower incidence could be expected in the semi-desert area.

AKA virus, according to Al-Busaidy, Hamblin & Taylor (1987) who demonstrated the occurrence of neutralizing antibodies in 25 species of African wild animals, is widespread in Africa. Samples used in their survey included serum samples of 14 species from South Africa. Of these seven species, buffalo, kudu, wildebeest, impala, elephant, giraffe and warthog were found positive. In another investigation (Davies & Jesset 1985), positive results were obtained with buffalo, bushbuck, eland, waterbuck, blue wildebeest and impala. However, in the present investigation only three ruminant species, namely springbok, blue wildebeest and buffalo, were found positive. In addition, both species of rhinoceros as well as zebras reacted positively. It therefore appears that AKA virus cannot be regarded as widespread in South Africa, and that it is restricted mainly to the forest-transition

zone where the highest prevalence was recorded. The low prevalence in the grassland, however, indicates that under ideal climatic conditions this virus may spread countrywide and cause serious problems among highly susceptible cattle and sheep.

Antibodies against BEF have been demonstrated in free-living African buffalo, waterbuck, blue wildebeest, and hartebeest (Davies, Shaw & Ochieng 1975), but no disease has been linked to the infection of wildlife. In this investigation, moderate prevalences of antibodies were found in blue wildebeest, black wildebeest, springbok, kudu, bushbuck, and waterbuck, with high prevalences of 52 and 61% in eland and blesbok, respectively. A remarkable characteristic of the occurrence is the geographic distribution of animals with antibodies. The highest prevalence (60%) occurred in the grassland and the lowest (20%) in the woodland. This distribution differs from that of the other insect-transmitted viruses of cattle, of which BT, WSL and AKA were most prevalent in the forest transition and relatively uncommon in the semi-desert.

The similarities in regional prevalences of insect-transmitted diseases and of those transmitted by contact, demonstrate a general trend. As a rule, the highest prevalence was found in the forest transition, which has a significantly higher average rainfall than the semi-desert, in which the lowest prevalence was recorded. The lower prevalence of antibodies against insect-transmitted diseases in the woodland as compared with that in the grassland, which has a similar rainfall, needs some explanation. It is probably related to multiple factors, including those controlling vector abundance, as well as the availability of susceptible hosts to feed on. The woodland area is relatively mountainous with a few fast-flowing rivers and few open-water or moist places. The higher average temperature in this area also results in higher evaporation of soil water. These factors do not favour large populations of mosquito vectors, as they are dependent on open water (Jupp 1994), nor of *Culicoides* midges, as about half of the known *Culicoides* species need a high moisture content to breed (Meiswinkel, Nevill & Venter 1994). The grassland, on the other hand, is relatively level country with slow-flowing streams, open water and moist places that favour the breeding of larger populations of insect vectors. Furthermore, the years preceding and during collection were characterized by a severe drought that affected the entire country, but particularly the northern parts of the country, which include the woodland area. Furthermore, the availability, composition and population density of insect hosts, including livestock, differ in these two areas. The woodland area and particularly the localities from which serum samples were obtained, are predominantly used for game farming. In the grassland, game is often kept in association with livestock that is hand-fed during the winter months, resulting in higher population densities. This, in turn, provides a larger source of animals to feed on and susceptible hosts to maintain the virus.

An interesting aspect of the prevalence of antibodies against the insect-transmitted viruses, is the decreasing occurrence of AHS in a southerly direction, to reach an almost total absence in the semi-desert, while antibodies against the viruses of BT, WSL, AKA, BEF and EE were encountered in every vegetational zone. This clearly indicates the existence of different epidemiological patterns and vectors for different insect-transmitted diseases.

Disease in wild animals

It is a common characteristic of insect-transmitted viral diseases such as AKA, WSL and BT to cause subclinical infections in domestic animals. This may result in abortions and neonatal losses, with the result that veterinarians in South Africa are often confronted with virus-induced congenital problems. No indication could be obtained that these viruses cause disease in free-living wild animals. This is not surprising, as free-living animals cannot be kept under close surveillance in order to detect sporadic losses, particularly not when predators are present. However, the susceptibility of several species, indicated by the presence of antibodies in their serum, may have an application in instances where they are relocated to a habitat where they do not occur naturally and where they could become exposed to viruses that are rare or absent in their original location. For this reason, relocation of species between vegetational zones should be considered carefully and best be avoided.

Under extensive farming conditions, BHV-1 infection in cattle causes a relatively mild disease, as compared with diseases encountered under conditions of stress in feedlots where the extent of virus exposure may be of importance (Burroughs 1967). The same is true for PI3 infection in feedlots (Barnard 1977) where, in association with other pathogens and stress-inducing situations, it can cause serious disease. A similar situation exists in game animals. In translocation operations game is severely stressed and the custom of keeping game in groups in small enclosures for sales, creates ideal conditions for infection with respiratory viruses. Investigations into the causes of deaths in these operations should include a thorough search for viruses.

Worldwide dissemination of viruses

An important aspect of the results obtained in this investigation is the probable consequences on the international movement of wild animals. Species likely to be infected should be free of virus before export so as to prevent worldwide dispersal of the virus.

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