Antibodies to Bluetongue Viruses in Animals Imported into United States Zoological Gardens

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ABSTRACT

Three hundred forty-five serum samples from 30 zoological animal species which had been imported into the United States were examined retrospectively for the presence of antibody to bluetongue viruses. Ninety eight (28.4%) were positive for antibody to bluetongue group antigen by the bluetongue agar gel immunodiffusion test. Bluetongue antibodies, most of which were against serotypes exotic to the United States, were detected in 13 animal species from Africa not previously reported to be infected by bluetongue virus. The lack of virus neutralizing antibody to any of the 20 known bluetongue virus types in four of the 28 positive serums studied may indicate the existence of new bluetongue virus serotypes, cross reactions with other orbiviruses or a more rapid decline of neutralizing than precipitating antibody. The possibility of recrudescence of bluetongue virus infection from some inapparently infected zoological animals and existence of a known bluetongue vector (*Culicoides variipennis*) in the United States would suggest that further assessment of bluetongue in zoological animals be made.

RÉSUMÉ

Cet article présente les résultats de la recherche rétrospective d'anticorps à l'endroit du virus de la fièvre catarrhale du mouton, dans 345 échantillons de sérum prélevés chez des individus de 30 espèces d'animaux de jardin zoologique. La réaction de précipitation en milieu gélifié révéla que 98 de ces échantillons, i.e. 28,4%, possédaient de tels anticorps. La plupart de ceux qu'on décela dans le sérum d'individus de 13 espèces importées d'Afrique et auparavant reconnues comme exemptes de fièvre catarrhale, se rapportaient à des sérotypes exotiques pour les États-Unis. L'absence d'anticorps neutralisants à l'endroit de l'un ou l'autre des 20 sérotypes connus, dans quatre des 28 échantillons éprouvés à cette fin, semble indiquer l'existence de nouveaux sérotypes du virus, celle de réactions croisées avec d'autres orbivirus, ou un déclin plus rapide des anticorps neutralisants que des précipitines. La possibilité d'une recrudescence de l'infection par le virus de la fièvre catarrhale du mouton, à partir de certains animaux de zoo apparemment non infectés, et la présence, aux États-Unis, d'un vecteur connu du virus, à savoir *Culicoides variipennis*, devraient nous inciter à vérifier de façon plus étroite l'évolution de la fièvre catarrhale du mouton, chez les animaux de zoo.

INTRODUCTION

Bluetongue, a disease caused by orbiviruses, was first described in Africa in 1876 (10). Bluetongue viruses which mainly affect members of the family Bovidae may infect animals from other families in the order Artiodactyla such as camels (26), deer (18, 29, 31) and elk (14). Bluetongue is widespread on the continent of Africa and is considered clinically important primarily in sheep (27). Bluetongue virus (BTV) or antibody has been detected in 25 varied African animal species. The wide range of species indicates the enzootic nature, extensive host range and very large wildlife reservoir of bluetongue in Africa (Table I).

At least 14 animal species in North America, some of which were exotic zoological animals, have shown evidence of infection by BTV (Table II). As in Africa, the impressive number of North American wildlife species represents a large potential reservoir for bluetongue viruses.

This study was done retrospectively on serums from zoological animals which had been imported into the United States to determine if animals serologically positive to BTV were entering the country.

MATERIALS AND METHODS

SPECIES EXAMINED

Serums from thirty species of zoological animals from Kenya and Southwest Africa were examined in the study. Serums were collected during importation quarantine and were stored in a repository at -20°C until used. The 345 animals were candidates for importation to the United States following

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prescribed testing and quarantine procedures. Essentially all of the animals tested were found acceptable for importation before the present work was performed and made their ways to zoological gardens in the United States. Taxonomic classification (33) of the animals in relation to common name is listed in Table III.

BLUETONGUE AGAR GEL IMMUNODIFFUSION TEST (BT AGID)

The procedure followed was that described by Pearson *et al* (22), with the exception that the test was read after three days of incubation and 0.6% agarose was used. Antigen[1] was placed in the central well with the test and reagent serums[1] in alternate peripheral wells. Tests were read and scored as follows: Negative — the test serum caused no bending of the control line, very weak positive — the test serum produced a noticeable bend of the control line, weak positive — the test serum produced a dramatic bend of the control line, strong positive — the test serum caused for-

TABLE I. Species of African Animals with Documented Evidence of Bluetongue Infection

Species	Evidence of infection[a]	Reference Number
antelope, sable	S	26
blesbuck	V	20
buffalo, African	S	9
camel	S	7, 20
cattle, African	S	5
eland	S	5, 32
gazelle, Grants	S	5
gazelle, Thomson's	S	5, 26
gazelle, mountain	V	3
gemsbok	S	26, 27
goat	S	7, 27
hartebeeste	S	5, 26
impala	S	5, 26, 32
kongoni	S	32
kudu	S	20
lechwe	S	26, 27
oribi	S	7
oryx	S	7
reedbuck	S	5, 32
sheep, domestic	S	7, 26
springbok	S	27
topi	S	32
tsesseby	S	26, 27
waterbuck	S	5
wildebeeste	S	5, 27, 32

[a]S = serological evidence, V = virus isolated

TABLE II. Species of Animals in North America with Documented Evidence of Bluetongue Infection

Species	Evidence of infection[a]	Reference
antelope, pronghorn	V,S	13, 30
bison, American	S	14
cattle, American	V	8
deer, mule	S	17
deer, white-tailed	V,S	27, 31
elk, North American	S,V	14, 19
impala	S	14
kudu, greater	V,S	13, 14
muntjac, Reeve's	V	13
reindeer	S	14
sheep, bighorn	V	25
sheep, domestic	V	18
situtunga	S	14

[a]S = serological evidence, V = virus isolated

TABLE III. Common and Scientific Names (33) of Species Examined for Antibody to Bluetongue Viruses

Common Name	Family	Genus	Species
antelope, harnessed	Bovidae	*Tragelaphus*	*scriptus*
antelope, Hunter's	Bovidae	*Beatragus*	*hunteri*
antelope, Nyala	Bovidae	*Tragelaphus*	*angasi*
antelope, pronghorn	Bovidae	*Antilocarpo*	*americana*
antelope, sable	Bovidae	*Hippotragus*	*niger*
Bison, American	Bovidae	*Bison*	*bison*
blesbok	Bovidae	*Damaliscus*	*albifrons*
bongo	Bovidae	*Boocercus*	*eurycerus*
buffalo, African	Bovidae	*Syncercus*	*caffer*
camel, African	Camelidae	*Camelus*	*dromadarius*
cattle, African	Bovidae	*Bos*	*indicus*
cattle, American	Bovidae	*Bos*	*taurus*
deer, mule	Cervidae	*Odocorileus*	*hemionus*
deer, white-tailed	Cervidae	*Odocorileus*	*virginianus*
dik dik	Bovidae	*Madoqua*	*phillipsi*
duiker, black-fronted	Bovidae	*Cephalophus*	*niger*
duiker, Jentinki	Bovidae	*Cephalophus*	*jentinki*
duiker, Maxwell	Bovidae	*Cephalophus*	*maxwelli*
duiker, red-flanked	Bovidae	*Cephalophus*	*rifilatum*
duiker, yellow-backed	Bovidae	*Cephalophus*	*sylvicultor*
duiker, zebra	Bovidae	*Cephalophus*	*zebra*
eland	Bovidae	*Taurotragus*	*oryx*
elephant, African	Elephantidae	*Laxdonta*	*africana*
elk, North American	Cervidae	*Cervus*	*canadensis*
gazelle, Grant's	Bovidae	*Gazella*	*grantii*
gazelle, mountain	Bovidae	*Gazella*	*gazella*
gazelle, Thomson's	Bovidae	*Gazella*	*thomsonii*
gemsbok	Bovidae	*Oryx*	*gazella*
gerenuk	Bovidae	*Litocranius*	*walleri*
giraffe, Angolan	Giraffidae	*Giraffa*	*camelopardalis*
giraffe, reticulated	Giraffidae	*Giraffa*	*reticulata*
gnu, brindled (wildebeeste)	Bovidae	*Connochaetes*	*taurinus*
gnu, white-tailed	Bovidae	*Connochaetes*	*gnou*
hartebeeste	Bovidae	*Beatragus*	*hunteri*
impala	Bovidae	*Aepyceros*	*melampus*
klipspringer	Bovidae	*Oreotragus*	*oreotragus*
kudu, greater	Bovidae	*Tragelaphus*	*strepsiceros*
kudu, lesser	Bovidae	*Tragelaphus*	*imberbis*
lechwe	Bovidae	*Kobus*	*leche*
muntjac, Reeve's	Bovidae	*Muntiacus*	*reeves*
oribi	Bovidae	*Ourebia*	*ourebia*
oryx	Bovidae	*Oryx*	*cervila*
reedbuck	Bovidae	*Redunca*	*fulvorfula*
reindeer	Cervidae	*Rangifer*	*tarandus*
sheep, bighorn	Bovidae	*Ovis*	*canadensis*
sheep, domestic	Bovidae	*Ovis*	*aries*
situtunga	Bovidae	*Tragelaphus*	*spekii*
springbok	Bovidae	*Antidorcas*	*marsupialis*
suni	Bovidae	*Nesotragus*	*moschatus*
tsesseby	Bovidae	*Damaliscus*	*lunatus*
topi	Bovidae	*Damaliscus*	*korrigum*
waterbuck	Bovidae	*Kobus*	*ellipsiprymnus*

[1]National Veterinary Services Laboratory, Ames, Iowa 50010.

mation of a line of identity with the control line.

VIRUSES

Bluetongue virus serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 14, 15, 16, 18, and 19 were obtained from Dr. B.J. Erasmus, Veterinary Research Institute, Onderstepoort, South Africa. Serotypes 10, 11, 13 and 17 were obtained from Dr. T.L. Barber, Arthropod-Borne Animal Disease Research Laboratory, USDA, Denver, Colorado. Serotype 20 was obtained from Dr. R.E. Shope, Yale Arbovirus Research Unit, New Haven, Connecticut.

BLUETONGUE ANTISERUM

Serotype specific mouse ascitic fluid was prepared (9) and was used as a positive control in the virus neutralization test.

VIRUS NEUTRALIZATION TEST

The virus neutralization (VN) test was a modification (9) of that described by Parker *et al* (21). Virus suspensions were titered in 96 well microtiter plates to find the 50% tissue culture infective dose ($TCID_{50}$), and volumes were adjusted with Eagles' minimum essential medium (MEM) plus 0.1 BSA to give 100% cytopathic effect at four days. The cell culture used in the test was the Mengeling Vaughn porcine kidney (MVPK) cell line which was cloned (6) and found to be highly susceptible to BTV (4). The virus dose varied from 200-500 $TCID_{50}$ per 0.1 mL, of virus suspension. Equal volumes, 0.1 mL, of virus suspension and serum dilution (1:25 to give a final dilution of 1:50) were dispensed into 96 well microtiter plates and with serum controls, tested in quintuplicate. The serum virus mixtures were incubated at 37°C for one hour and then overnight at 4°C. Approximately 5 x 10^4 MVPK cells in 0.05 mL of Eagles' MEM containing 25% horse serum, 1000 units of penicillin, 1000 mcg of streptomycin and 100 units of mycostatin per mL were added to each well and the plates incubated and observed for complete cytopathic effect at four days when they were stained with 1% crystal violet in 10% formalin. Protection was considered complete by lack of macroscopic signs of cytopathic effect in at least three out of five wells. Partial protection was judged to have occurred if part of the cell monolayers were intact in three out of five wells.

RESULTS

BLUETONGUE AGAR GEL IMMUNODIFFUSION TEST

Three hundred forty-five serums from 30 species of zoological animals were examined for antibody to bluetongue group antigen (BTGA) using the BT AGID test. Animals from 24 of the 30 species were found to have precipitating antibody to BTGA (Table IV). Antibody was detected in seven of 12 impalas, ten of 12 Hunter's antelopes, four of 13 bongos, five of eight red-flanked duikers, ten of 31 gerenuks, five of 14 gemsboks and ten of 40 lesser kudus. Positive samples were obtained from the small number tested from other species.

TABLE IV. Species of African Animals Tested which had Antibody to Bluetongue Group Antigen

Common Name	No. Positive	No. Negative
antelope, harnessed	1	4
antelope, Hunter's	10	2
antelope, Nyala	3	5
blesbok	4	15
bongo	4	9
dik dik	3	13
duiker, black-fronted	3	14
duiker, red-flanked	1	4
duiker, Maxwell's	4	8
duiker, yellow-backed	5	3
duiker, zebra	3	4
eland	4	11
gazelle, Thomson's	1	1
gemsbok	5	9
gerenuk	10	21
giraffe, reticulated	5	12
gnu, brindled	4	3
gnu, white-tailed	3	1
gnu[a]	1	2
hartebeeste	2	3
impala	7	5
klipspringer	1	17
kudu, lesser	10	30
kudu[a]	2	7
oryx	1	1
springbok	1	3
Total 24 species	98	207

[a]Species not specified

It was noted that approximately an equal distribution of very weak, weak and strong positive reactions on the BT AGID test occurred among the positive sera (32 very weak, 34 weak and 32 strong).

Antibody against BTGA was not detected in the samples from six species of zoological animals (one Jentinki duiker, three African elephants, seven giraffes, four greater kudus, 19 sunis and six waterbucks).

The percentage of animals positive for antibody to BTGA from Kenya was 26.9% (64 of 238 tested) and from Southwest Africa 31.8% (34 of 107 tested).

Data on sex of 290 animals was available and indicated that 20.5% of the males (15 of 73) and 31.3% of the females (68 of 217) were positive for antibody on the BT AGID test. The higher rate of infection in females was significant at the p 0.08 level (X^2 test).

Serums were examined from animals which had been imported from the year 1974 through 1978. The number of animals imported varied from 40 to 90 per year and the positive animals from 20 to 31.1%. The lowest percentage positive occurred during 1976 when the smallest number of animals was imported.

VIRUS NEUTRALIZATION

Virus neutralization tests were performed on 28 BT AGID strong positive serums from 15 species of zoological animals. Complete virus neutralization was detected against all bluetongue virus serotypes except serotypes 7, 10, 17, 18 and 20 (Table V). Partial neutralization was shown against the latter serotypes by some of the serums.

Of the 28 serums, 18 had antibody against serotype 12, nine against serotype 1, six against serotypes 2 and 3, and five against serotype 8 (Tables V and VI). Eighteen of the 28 serums neutralized between two and seven serotypes of BTV. Four serums did not neutralize any of the 20 bluetongue virus serotypes. The number of serums tested was too small to ascertain if antibody against specific BTV serotypes was more pre-

valent in Kenya or in Southwest Africa (Table VI).

DISCUSSION

Serological studies on 345 serums from 30 species of zoological animals for importation into the United States revealed that 13 species of animals had antibodies to BTV in addition to several species previously reported. The newly reported species included the following: harnessed antelope, Nyala antelope, Hunter's antelope, dik dik, black-fronted duiker, red-flanked duiker, Maxwell's duiker, yellow-backed duiker, zebra duiker, brindled gnu, white-tailed gnu, klipspringer and recticulated giraffe. This is apparently the first report of bluetongue antibody being detected in a member of the family Giraffidae. The high prevalence of bluetongue antibody in zoological animal species is not surprising since bluetongue is enzootic in Africa. The fact that bluetongue antibody had not previously been detected in these 13 species is probably the result of infrequent sampling.

TABLE V. Results of Virus Neutralization Tests with Bluetongue Viruses on 28 Serums from African Zoological Animals having Strong Reactions on the Bluetongue Agar Gel Immunodiffusion test

Virus Type	Number Positive	% Positive
1	9	32
2	6	21
3	6	21
4	3	11
5	4	14
6	1	4
7	0	0
8	5	18
9	3	11
10	0	0
11	1	4
12	18	64
13	3	11
14	2	7
15	2	7
16	2	11
17	0	0
18	0	0
19	4	14
20	0	0

Simpson (26) reported that the positive reactions on the AGID tests from wild animals were not as strong as those detected from domestic animals. Comparing results of tests for antibody to BTV of zoological serums and of serums from livestock we regularly detect about the same distribution of very weak, weak, and strong positive reactions (J.A. House, unpublished data; Plum Island Animal Disease Center).

There was not a great difference in the percentage of antibody positive animals originating from Kenya or Southwest Africa. This result might be expected since conditions in these areas are suitable for effective transmission of BTV (5, 26, 27, 32).

A difference in the percentage positive on the BT AGID test in males and females (20.5 and 31.3% respectively) was significant at the p 0.08 level. This trend may have been due to the reported higher antibody production by females (12, 24). No literature could be

TABLE VI. Occurrence of Virus Neutralizing Antibody to 20 Bluetongue Serotypes in Zoological Animals for Importation from Africa

Serum No.	Species	Origin	1	2	3	4	5	6[a]	8	9	11	12	13	14	15	16	19
1	antelope, Hunter's	Kenya	[b]				+										
2	bongo	Kenya	+		+						+	+	+			+	
3	duiker, black-fronted	Kenya										+					
4	duiker, black-fronted	Kenya															
5	duiker, red flanked	Kenya							+			+					+
6	duiker, yellow-backed	Kenya		+								+					
7	duiker, yellow-backed	Kenya	+						+								
8	duiker, yellow-backed	Kenya					+			+		+				+	
9	duiker, zebra	Kenya															
10	gerenuk	Kenya			+												
11	kudu, lesser	Kenya	+	+					+	+		+		+			
12	oryx	Kenya	+		+	+			+			+	+				
13	blesbok	S.W. Africa		+								+					
14	blesbok	S.W. Africa				+					+						
15	blesbok	S.W. Africa	+									+					
16	blesbok	S.W. Africa			+												
17	eland	S.W. Africa		+													
18	eland	S.W. Africa		+					+			+			+		+
19	gemsbok	S.W. Africa	+									+			+		
20	gemsbok	S.W. Africa				+	+										+
21	gemsbok	S.W. Africa		+								+					
22	gemsbok	S.W. Africa	+									+					
23	gnu, white-tailed	S.W. Africa	+		+			+				+	+			+	+
24	gnu, white-tailed	S.W. Africa	+		+					+		+					
25	hartebeeste	S.W. Africa															
26	hartebeeste	S.W. Africa					+					+		+			
27	impala	S.W. Africa															
28	impala	S.W. Africa										+					

[a]Antibody was not detected against serotypes 7, 10, 17, 18 and 20

[b]A blank indicates that complete neutralization was not detected at a 1:50 final serum dilution

found indicating that *Culicoides* preferably feed on females.

Only six species were found free of antibody to BTGA (BT AGID test). Small numbers were tested except for 19 sunis; perhaps the sunis are not attractive to the vector of BTV in Africa or they may live in an area where the vector is in low concentration.

Twenty-eight serums strongly positive on the BT AGID test were examined using the virus neutralization test employing 20 bluetongue serotypes. It is noteworthy that serotype 12 (64% positive) and 1 (32% positive) as well as serotypes 2, 3, and 8 (21, 21 and 18% positive respectively) appear to have a high prevalence among the species examined. Possible explanations are that these serotypes were prevalent in areas of origin of the animals tested or that a high degree of cross reactivity occurred when these zoological animal species were infected with BTV or other orbiviruses. The greater majority of neutralizing antibody detected was against serotypes considered exotic to the United States. The VN test using serum at a final dilution of 1:50 has not detected antibody in experimental animals free of BTV infection (C.M. Groocock, unpublished data; Plum Island Animal Disease Center). Data is not available on serums from zoological animals known to be free of BTV infection so the neutralizing activity of such serums may only be assumed to be neutralizing antibody. No VN antibody (1:50 final serum dilution) was found in the 28 serums against five bluetongue viruses (serotypes 7, 10, 17, 18 and 20). Serotype 17 has been described only on the North American continent (1) and type 20 only in Australia (28). Lack of antibody to these five serotypes may be due to the small sample size.

Four serums had no type specific neutralizing antibodies detectable at 1:50 dilution to any of the 20 bluetongue serotypes but were strongly positive on the BT AGID test which detects antibody to BTGA. There are three possible reasons: 1) the animals may have developed a strong reaction on the AGID test as a result of infection with a new bluetongue serotype, 2) they represent a strong cross reaction with other orbiviruses or, 3) the BT AGID antibody (against BTGA) persisted at a higher level than type specific virus neutralizing antibody (23).

The importation of exotic animals into the United States brings with it the potential of introducing exotic types of bluetongue virus which may be a threat to the cattle, sheep and goat industries in this country. The relative danger of this threat is very difficult to assess. Some species such as the North American elk are able to be infected and show recrudescence of BTV when stressed (19). Cattle, which have been shown to yield virus up to 402 days after infection (15) by routine sampling and up to 1,783 days after challenge when sampled "biologically" by *Culicoides variipennis* feeding (16), may be the significant bluetongue viral reservoir under most conditions. However, other species such as the mountain gazelle may have a viremia for up to a month but in such species the virus may not recrudesce under stress (2). It is impossible to predict which species of animal sill show recrudescense of the virus. An additional factor of great concern is that bites of *Culicoides variipennis* to zoological animals may stimulate a "showering" of virus (16) as occurs with Buttonwillow virus (11). Zoological animals in open parks are readily exposed to vectors and offer a potential source of exotic BTV.

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REFERENCES

1. **BARBER, T.L.** Temporal appearance, geographic distribution and species of origin of bluetongue virus serotypes in the United States. Am. J. vet. Res. 40: 1654-1656. 1979.
2. **BARZILAI, E.** and **A. TADMOR.** Experimental infection of the mountain gazelle (*Gazella gazella*) with bluetongue virus. Refuah vet. 29: 45-50. 1972.
3. **BARZILAI, E., A. TADMOR** and **A. SHIMSHONY.** Natural bluetongue infection in the mountain gazelle (*Gazella gazella*). Refuah vet. 28: 93-97. 1971.
4. **CAMPBELL, C.H., T.L. BARBER** and **J.M. JOCHIM.** Antigenic relationship of Ibaraki, bluetongue and epizootic hemorrhagic disease virus. Vet. Microbiol. 3: 15-22. 1978.
5. **DAVIS, F.G.** and **A.R. WALKER.** The distribution in Kenya of bluetongue virus and antibody and the *Culicoides* vector. J. Hyg., Camb. 72: 265-272. 1974.
6. **DINKA, S.K., L.M. SWANEY** and **J.W. McVICAR.** Selection of a stable clone of the MVPK-1 fetal porcine kidney cell line for assays of foot-and-mouth disease virus. Can. J. Microbiol. 23: 295-299. 1977.
7. **EISA, M., A.E. KARRAR** and **A.H. ABA ELRAHIM.** Incidence of bluetongue virus precipitating antibodies in sera of some domestic animals in the Sudan. J. Hyg., Camb. 83: 539-545. 1979.
8. **FOSTER, N.M.** Bluetongue virus characterization. *In* Proc. a. Meet. U.S. Anim. Hlth Ass. 79: 350-366. 1975.
9. **GROOCOCK, G.M.** and **C.H. CAMPBELL.** Isolation of an exotic serotype of bluetongue virus from imported cattle in quarantine. Can. J. comp. Med. 46: 160-164. 1982.
10. **HAIG, D.A.** Report of the cattle and sheep diseases commission. *In* Proc. Int. Vet. Cong. Madrid, Spain. 16: 215-225. 1959.
11. **HARDY, J.L., R.N. LYNESS** and **W.A. RUSH.** Experimental vector and wildlife host ranges of Buttonwillow virus in Kern County, California. Am. J. trop. Med. Hyg 21: 100-109. 1972.
12. **HERLYN, D., V. SCHULZ** and **J. GROPP.** Der einfluss von anabolida auf die immunanantwort des kalbes. Fortschr. Tierphysiol. Tierernahrung. 6: 66-75. 1976.
13. **HOFF, G.L., L.A. GRINER** and **D.P. TRAINER.** Bluetongue virus in exotic ruminants. J. Am. vet. med. Ass. 163: 565-567. 1973.
14. **HOURRIGAN, J.L.** and **A.L. LINGSPORN.** Epizootiology of bluetongue: The situation in the United States of America. Aust. vet. J. 51: 203-208. 1975.
15. **LUEDKE, A.J., M.M JOCHIM** and **R.H. JONES.** Bluetongue in cattle: Effects of vector transmitted bluetongue virus on calves previously infected in utero. Am. J. vet. Res. 38: 1697-1700. 1977.
16. **LUEDKE, A.J., R.H. JONES** and **T.E. WALTON.** Overwintering mechanism for bluetongue virus: Biological recovery of latent virus from a bovine by bites of *Culicoides variipennis*. J. trop. Med. Hyg. 26: 313-325. 1977.

17. **McKERCHER, D.G., B. McGOWAN, J.A. HOWARTH** and **J.K. SAITY.** A preliminary report on the isolation and identification of the bluetongue virus from sheep in California. J. Am. vet. med. Ass. 122: 300-301. 1953.
18. **METCALF, H.E.** Cited as a personal communication by Hourrigan J.L., Klingsport, A.L. *In* Epizootology of bluetongue: the situation in the United States of America. Aust. vet. J. 51: 203-208. 1972.
19. **MURRAY, J.O.** and **D.O. TRAINER.** Bluetongue virus in North American elk. J. Wildl. Dis. 6: 144-148. 1970.
20. **NEITZ, W.O.** The blesbuck (*Damaliscus albifrons*) as a carrier of heartwater and bluetongue. J.S. Afr. vet. med. Ass. 4: 24-26. 1933.
21. **PARKER, J., K.A.J. HERNIMAN, E.P.J. GIBBS** and **R.F. SELLERS.** An experimental inactivated vaccine against bluetongue. Vet. Rec. 96: 284-287. 1975.
22. **PEARSON, J.E.** and **M.M. JOCHIM.** Protocol for the immunodiffusion test for bluetongue. *In* Proc. Am. Ass. Vet. Lab. Diagn., 22nd Meet. pp. 436-471. 1979.
23. **PINI, A., H. OHDER, A.P. WHITLAND** and **L.J. LUND.** Studies on the fluorescent and neutralizing antibodies to bluetongue virus in sheep. Arch. ges. Virus-forsch. 25: 129-136. 1968.
24. **RAVECHE, E.S., J.H. TJIO** and **A.D. STEINBERG.** Genetic studies in NZB mice 3. Induced anti-nucleic acid antibody production. J. Immun. 122: 1454-1460. 1979.
25. **ROBINSON, R.M., T.L. HAILEY, C.W. LIVINGSTON** and **J.W. THOMAS.** Bluetongue in the desert bighorn sheep. J. Wildl. Mgmt 31: 165-168. 1967.
26. **SIMPSON, V.R.** Serological evidence of bluetongue in game animals in Botswana. Trop. Anim. Hlth Prod. 10: 55-60. 1978.
27. **SIMPSON, V.R.** Bluetongue antibody in Botswana's domestic and game animals. Trop. Anim. Hlth Prod. 11: 43-49. 1979.
28. **ST. GEORGE, T.D., H.A. STANDFAST** and **D.H. CYPINSKI.** The isolation of a bluetongue virus from Culicoides collected in the Northern Territory of Australia. Aust. vet. J. 54: 153-154. 1978.
29. **STAIR, E.L., R.M. ROBINSON** and **L.P. JONES.** Spontaneous bluetongue in Texas white-tailed deer. Path. Vet. 5: 164-173. 1968.
30. **TRAINER, D.O.** and **M.M. JOCHIM.** Serological evidence of bluetongue in wild ruminants of North America. Am. J. vet. Res. 30: 2007-2011. 1969.
31. **VOSDINGH, R.A., D.O. TRAINER** and **B.A. EASTERDAY.** Experimental bluetongue disease in white-tailed deer. Can. J. comp. Med. 32: 382-387. 1968.
32. **WALKER, A.R.** and **F.G. DAVIS.** A preliminary survey of the epidemiology of bluetongue in Kenya. J. Hyg., Camb. 69: 47-60. 1971.
33. **WALKER, E.P., F. WARNICK, S.E. HAMLET, K.I. LANGE, M.A. DAVIS, H.E. UIBLE** and **P.A. WRIGHT.** Mammals of the World. Second Edition revised by J.L. Paradiso. Vol. II. Baltimore, Maryland: Johns Hopkins Press. 1960.