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Source: Journal of Wildlife Diseases, 12(1) : 2-5

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-12.1.2

THE PREVALENCE OF ANTIBODY TO INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS IN SOME GAME ANIMALS OF EAST AFRICA

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Abstract: Antibody to infectious bovine rhinotracheitis virus was present in the sera of 10 of 25 species of game animals from Kenya, Tanzania and Uganda during 1960-1973. Prevalence of antibody varied considerably between, but not within, species.

INTRODUCTION

Investigations into the occurrence of antibody to infectious bovine rhinotracheitis virus (IBRV) in Tanzanian wildlife have been conducted.[9,10] There have been no published reports on the prevalence of antibody in the game animals of Kenya and Uganda. This paper records the results of a serological survey which includes sera obtained from each of the three East African countries.

MATERIALS AND METHODS

Game Sera

Serum samples have been collected and stored at the East African Veterinary Research Organization, and have been maintained at —20 C. A total of 2,228 samples were tested from 25 species of animals. Prior to use the sera were thawed and heat inactivated at 56 C for 30 min.

Cell Cultures

Established monolayer tube cultures of primary bovine kidney cells (BK), 7-8 days old, were used following techniques already described.[7] A 5% ox serum supplement, derived from steers which had been previously shown to possess no antibody to IBRV was added to the medium. Cultures were incubated at 37 C ± 0.5 C and rolled at 8 revolutions per hour.

Virus Propagation and Assay

All the serum-virus neutralization tests were carried out using the Oxford strain of IBRV. This virus was obtained from Dr. J. H. Darbyshire of the Central Veterinary Laboratory, Weybridge, Surrey, U.K. The freeze-dried product was reconstituted with deionised water and inoculated onto BK cell monolayers in 590 cc prescription bottles. Each bottle contained 50 ml of growth medium to which 0.5 ml of the virus, at a titre of $10^{7.2}$ TCD_{50} per ml, was added. Maximal cytopathic effect (CPE) occurred 2 days after inoculation. The tissue culture fluids from this stock virus were then clarified by centrifugation, and stored in aliquots at —70 C as the 30th passage. When required the virus was thawed rapidly at 37 C, and titrated in 1.0 $\log_{10}$ steps to $10^{-9.0}$, using as diluent phosphate-buffered saline containing 0.1% bovine plasma albumen (BAPBS). Each of the dilutions was inoculated into five BK culture tubes, at a dose of 0.2 ml per tube, and readings were taken on the third and sixth days after inoculation. The titre of the stock virus was $10^{7.3}$ TCD_{50} per ml estimated by the method of Spearman-Kärber.[1]

Immune Serum

IBRV antiserum was prepared by immunizing a steer which had been previously screened and shown to have no

[1] Members of the United Kingdom Overseas Development Administration Research Scheme R2843.

antibody to this virus. IBRV, at a titre of $10^{7.3}$ TCD_{50} per ml, was mixed with an equal volume of Freund's Incomplete Adjuvant and a steer was inoculated intramuscularly with 2.0 ml of this mixture. Seven days later the inoculation procedure was repeated. After a further 7 days a final inoculation of 2.0 ml of virus, without adjuvant, was given intravenously. Twenty-one days after the last inoculation the animal was bled and stock serum prepared. The mean titre of this serum after 10 titrations in neutralization was $10^{1.6}$ $\log_{10}$ SN_{50}.

Serum-virus Neutralization Tests

After inactivation at 56 C for 30 min all the sera were screened by mixing an aliquot of undiluted serum with a similar volume of IBRV, the latter being diluted to give approximately $10^{2.0}$ TCD_{50} per 0.1 ml. Following storage at 4 C overnight, the mixtures were inoculated into three BK culture tubes, at a dose of 0.2 ml per tube. Sera showing complete inhibition of CPE in one or more tubes were regarded as evidence of exposure to IBRV infection.

Titrations of stock virus alone, the immune serum, and the serum added to the medium were set up with each batch of tests. The immune serum was titrated using the variable serum-constant virus technique, with the results indicated above. The serum in the medium previously had been shown to contain no antibody to IBRV, but aliquots of this serum were added to aliquots of virus dilutions to show that non-specific inhibition of virus did not occur.

To determine the range of antibody level occurring in the different species, quantitative titrations of a suitable range were carried out using 4-fold dilutions of serum in BAPBS. All sera which had shown protection in only one or two tubes in the screening test were titrated. A proportion of the sera from each species showing complete protection of all three screening tubes in a large number of their sera were also titrated. Serum titres, expressed as the reciprocal of the $\log_{10}$ SN_{50} end point, were calculated where possible. Where protection was only afforded in one or two tubes by undiluted sera, they were recorded as "trace."

RESULTS

Table 1 shows the total number of samples screened and the percentage in which antibodies were found. Table 2 gives the results obtained from titrations of positive sera.

Of the reedbuck and waterbuck sera, 4/14 and 14/29, respectively, proved to be toxic when used undiluted in the screening test. Titration of three of the toxic waterbuck sera showed a mean titre of 4.12 $\log_{10}$ SN_{50}.

The distribution of antibody amongst the various animal species in the three countries is shown in Table 3. The percentage of positive samples between species varied considerably, but generally, animals from each country showed a similar rate of infection. Samples were collected from 1962 onwards, except for wildebeest sera collected in 1960. Positive antibody reactions were observed in each case from the earliest dates on which samples were collected.

DISCUSSION

Of the 25 species of wildlife tested, 10 were shown to have antibody to IBRV. Where no samples were available, or the sampling rate was low, it is possible that those species not adequately tested in some countries would also show antibody.

Table 2 shows that sera from 8 of the 10 species of animals possessing antibody showed mean titre levels between 0.83 and 1.18. The two exceptions to this were the reedbuck and waterbuck sera which gave mean titres of 3.32 and 4.08 respectively. Titration of three waterbuck sera, which were toxic when used undiluted, gave a mean titre of 4.12. These two species showed high serum titres compared with sera obtained from other animals but it seemed possible that toxicity may have had a bearing on the unusually high level of antibody shown.

TABLE 1. Neutralising Antibody to Infectious Bovine Rhinotracheitis Virus in East African Game Animals.

Species	Number Tested	Number Positive	Percentage Positive	Date Collected
Buffalo—(*Syncerus caffer*)	140	89	64	1963-71
Bushbuck—(*Tragelaphus scriptus*)	8	0	—	1970
Bushpig—(*Potamochoerus porcus*)	1	0	—	1969
Dik-dik—(*Madoqua kirki*)	2	0	—	1965 & 1969
Duiker—(*Sylvicapra grimmia*)	1	0	—	1970
Eland—(*Taurotragus oryx*)	80	34	43	1962-72
Elephant—(*Loxodonta africana*)	49	0	—	1965-70
Giraffe—(*Giraffa camelopardalis*)	43	0	—	1966-71
Grants gazelle—(*Gazella granti*)	104	0	—	1963-71
Hippopotamus—(*Hippopotamus amphibius*)	188	181	96	1963
Hyena—(*Crocuta crocuta*)	3	0	—	1966 & 1970
Hyrax—(*Procavia capensis*)	1	0	—	1970
Impala—(*Aepyceros melampus*)	268	9	3	1963-73
Kob—(*Kobus (Adenota) kob*)	22	5	23	1967-70
Kongoni—(*Alcelaphus buselaphus cokii*)	128	0	—	1967-72
Lion—(*Panthera leo*)	1	0	—	1963
Oryx—(*Oryx beisa callotis*)	5	0	—	1970
Reedbuck—(*Redunca redunca*)	10	4	40	1970
Rhinoceros—(*Diceros bicornis*)	2	0	—	1970
Thomsons gazelle—(*Gazella thomsonii*)	242	49	20	1965-71
Topi—(*Damaliscus korrigum*)	63	7	11	1969-73
Warthog—(*Phacochoerus aethiopicus*)	11	0	—	1965-71
Waterbuck—(*Kobus ellipsiprymnus* or *Kobus defassa*)	15	6	40	1963-70
Wildebeest—(*Connochaetes taurinus*)	727	234	31	1960-73
Zebra—(*Equus burchelli*)	114	0	—	1965-72

TABLE 2. The Titre of Neutralizing Antibody to Infectious Bovine Rhinotracheitis Virus.

Species	Number Titrated	Mean* Titre	Standard Deviation	Range
Buffalo	49	1.11	0.37	0.48-1.68
Eland	28	0.93	0.38	0.48-1.74
Hippopotamus	16	1.18	0.25	0.72-1.68
Impala	4	1.07	0.50	0.60-1.56
Thomsons gazelle	27	0.65	0.19	0.48-1.20
Reedbuck	4	3.32	0.40	2.76-3.69
Waterbuck	5	4.08	0.11	3.96-4.20
Wildebeest	36	0.83	0.43	0.36-1.80

N.B. Titre estimations in Kob and Topi could not be made due to lack of serum.

* Expressed as the reciprocal of the Log_{10} SN_{50} end-point.

TABLE 3. Neutralizing Antibody to Infectious Bovine Rhinotracheitis Virus by Countries.

SPECIES	KENYA		UGANDA		TANZANIA	
	No. pos./ No. tested	Percentage positive	No. pos./ No. tested	Percentage positive	No. pos./ No. tested	Percentage positive
Buffalo	17/31	55	57/78	73	15/31	48
Eland	17/57	30	—	—	17/23	74
Hippopotamus	0/1	—	181/188	97	—	—
Impala	4/149	3	—	—	5/119	4
Kob	0/1	—	—	—	5/21	24
Reedbuck	0/5	—	4/5	—	—	—
Thomsons gazelle	33/194	17	0/2	—	13/46	28
Topi	6/48	13	1/14	7	0/1	—
Waterbuck	4/13	31	2/2	—	—	—
Wildebeest	170/488	35	1/1	—	63/238	27

Antibody of IBRV was demonstrated amongst the positive groups of animals for each year between 1960 and 1973. This indicated the existence of infection for the past 14 years in the species examined.

IBRV antibody has been demonstrated in Zambian hippopotami and buffalo.[3] The disease has also been reported in Central Africa[8] and in South Africa.[6] Our results confirm the evidence of a widespread dissemination of this virus.

In Kenya, a serological survey of cattle showed that antibody to IBRV was present in 62% of adult animals,[2] whilst the virus recently has been isolated from wildebeest captured on the Athi-Kapiti plains in the southern part of the country.[4] Virus isolations have been made from Tanzanian cattle,[5] the virus being spread by donor bulls from an artificial insemination centre. In this country cattle have also been shown to have antibody to IBRV.[9,10]

These reports, and the fact that there is considerable intermingling of the wildlife and domestic animal populations in East Africa, would suggest that an infectious disease is to be found in both.

Acknowledgements

We are indebted to workers in the field who have collected sera over the years, although it is not possible to name them individually. We also wish to thank members of the staff of the Division of Virus Diseases for their technical assistance, particularly Mr. H. Agili who prepared the tissue cultures. This paper is published with the approval of the Director, EAVRO.

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Received for publication 10 September 1975