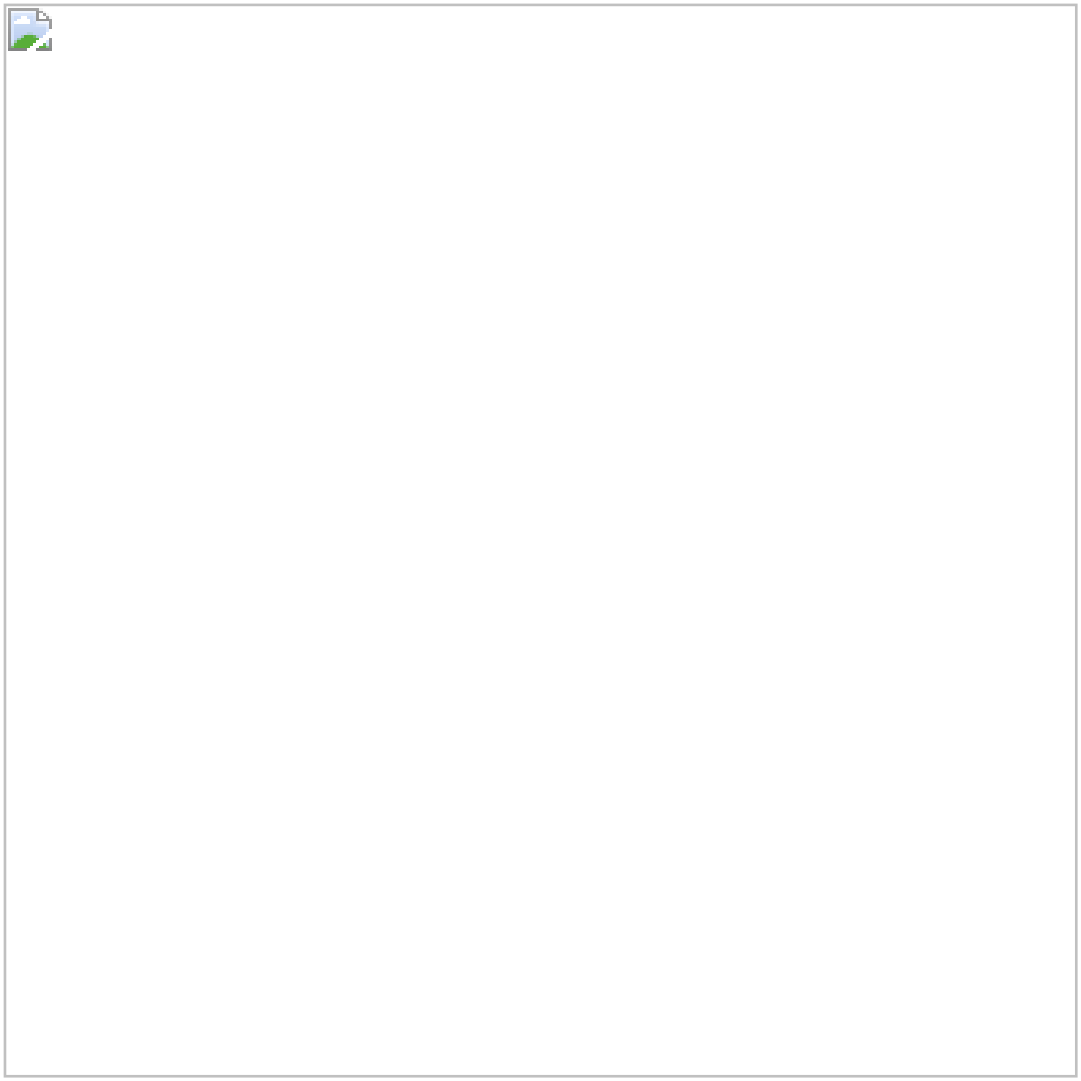

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VULVOVAGINITIS IN WILDEBEEST CAUSED BY THE VIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS

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Abstract: During studies on bovine malignant catarrhal fever, 8 recently captured female wildebeest (*Connochaetes taurinus*) were injected daily for 1 week with the corticosteroid betamethasone. All developed pustular vulvovaginitis 7 to 9 days after the first injection. Infectious bovine rhinotracheitis virus (IBRV) was isolated from vaginal swabs from seven animals. Serum neutralizing antibody to IBRV was present in seven animals before injection of the corticosteroid, and the titres increased during convalescence. It is presumed that the wildebeest were latent carriers of IBRV in genital tissue. One of the IBRV isolates produced mild vulvovaginitis in a domestic heifer inoculated by the vaginal route.

INTRODUCTION

Herpesviruses have often been found to persist as latent infections in carrier animals. Following the report by Sheffy and Davies[13] of reactivation of IBRV infection in cattle by injection of corticosteroids, it was decided to try this method to increase nasal shedding of malignant catarrhal fever virus (MCFV) in carrier wildebeest.[12]

MATERIALS AND METHODS

Animals

Eight adult female wildebeest captured in September 1973 on the Athi-Kapiti plains in Kenya, were housed 1, 2, 2 and 3 in pens, out of contact with other wild or domestic ruminants. The capture and quarantine of these animals has been described elsewhere.[12]

Treatment with corticosteroid

Under sedation with 60-80 mg xylazine, all animals were injected intramuscularly daily for 7 consecutive days with 40 mg betamethasone.

Sampling

Vaginal secretions for virus isolation attempts were obtained from one animal on day 7, and from most of the others on days 9 and 16 or 17 using the method outlined by Rweyemamu et al.[12]

Blood with EDTA as an anticoagulant was taken before the first injection of betamethasone (day 0) and 3, 7, 9, 11, 14, and 16 or 17 days thereafter, for measurement of haemoglobin, packed cell volume, and numbers of leukocytes and erythrocytes.

Each animal was injected intramuscularly with 4 million i.u. of procaine penicillin G and 5 g of dihydrostreptomycin at each time of handling.

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Cell cultures

Primary calf kidney (BK) and calf thyroid (BTh) monolayers were used. BK roller cultures were prepared as described by Plowright et al[9] and the method of preparation of BTh cultures has been outlined elsewhere.[12]

Virus isolation and identification

Swabs of vaginal secretion were placed into screw-capped vials containing 2 ml of HSLS medium[7] containing 200 i.u./ml penicillin, 200 mg/ml dihydrostreptomycin and 50 units/ml nystatin (Mycostatin, Squibb). These specimens were held on ice until inoculated, within 24 hours of collection, in 0.2ml volumes onto four or five replicate tube monolayers of secondary BTh cultures per specimen. Incubation was on roller drums at 37C.

The isolates were tested for sensitivity to ether by the method of Andrews and Horstmann.[1]

The virus isolates were tested for neutralization by bovine antisera specific for the viruses of IBR (Oxford strain) and MCF. Approximately one hundred 50% tissue culture infective doses ($TCID_{50}$) of the viruses were exposed to antisera at concentrations at least 10 times the 50% serum neutralization titre (SN_{50}) with the homologous virus. Paired sera from all the wildebeest were simultaneously tested for neutralization of the Oxford strain of IBRV. Serum dilutions of 10, $10^{-0.6}$, $10^{-1.2}$ and $10^{-1.8}$ were mixed with an equal volume of virus containing approximately $10^{2.0}$ $TCID_{50}$/0.1 ml. The serum-virus mixtures were shaken and held at 4 C for 18 hours; then 0.2 ml volumes were inoculated into five tubes of BK cells for each serum dilution. Titrations of IBRV antiserum and of virus alone served as controls.

Experimental inoculations

One heifer was inoculated intravenously with 5 ml of cell culture medium containing $10^{6.5}$ $TCID_{50}$ IBRV isolated from one of the wildebeest, and a second heifer was given this dose by intravaginal instillation. Two other heifers served as contact controls. Body temperatures were recorded daily and vaginal swabs were taken every 2nd day.

RESULTS

Clinical observations

One day after the last injection of betamethasone, yellowish, raised plaques were noticed on the vulva and vagina of one wildebeest. Two days later this pustular vulvovaginitis was present in all the animals, the lesions being focal, about 5 mm in diameter, yellowish and slightly raised; early lesions in the vagina were flat and white, sometimes with narrow red borders. Vaginal secretion was increased to a watery mucoid discharge containing whitish flecks. The first animal to be affected showed inappetence, but the remainder ate and drank normally. One animal had slight diarrhoea.

The first animal affected died 7 days after the last injection of betamethasone, presumably due to the stress of capture, confinement and corticosteroid injection. Lesions in the remaining animals persisted for 7-8 days after their first appearance. Healing was uncomplicated.

Histopathology

Histological examination of the vulva and vagina of the animal that died showed abnormality of epithelial cells, ranging from hypertrophy, with indistinct eosinophilic intranuclear inclusion bodies, to necrosis and leukocyte (mainly neutrophil) infiltration of the underlying tissue.

Virus isolation and identification

Isolation of a virus serologically identical to IBRV was made from seven of the eight wildebeest (Table 1). All isolates caused a cytopathic effect (CPE) in 48-72 hours on primary inoculation of BTh cells with vaginal swab material. Destruction of the cell sheet was complete within 6 days. The virus grew

equally well on BK cells. CPE, as seen in cover-slip preparations stained with haematoxylin and eosin, was at first focal with shrinkage of cells and development of scattered, small syncytia containing nuclei with pale, eosinophilic, large inclusions. Severely affected cells became hyperchromic, rounded and shrunken, and eventually floated free in the medium. All virus isolates appeared to be identical in CPE.

All of the virus isolates were identified as IBRV by neutralization with antiserum to the Oxford strain of IBRV. There was no neutralization with antiserum to MCFV. Also, sera from seven of the eight animals neutralized the Oxford strain of IBRV before the first injection of betamethasone, and in all but one, antibody titres increased after the development of vulvovaginitis (Table 2).

Haematology

Leukocyte counts increased markedly during the course of betamethasone injections, but decreased rapidly after the injections stopped (Table 1)—at about the time of appearance of clinical vulvovaginitis. Accidentally poor staining of blood smears made accurate differential leukocyte counts impossible, but it was apparent that the increase in total count could be attributed to an increase in neutrophils. Erythrocyte counts and packed cell volumes did not change significantly.

Experimental infection

Five days after exposure to IBRV, the heifer inoculated by intravaginal instillation developed vaginal lesions similar to

TABLE 1. Isolation of infectious bovine rhinotracheitis virus (IBRV) and leukocyte counts (in thousands/mm^3) of eight wildebeest injected on 7 consecutive days with betamethasone.

Animal No.	Days: 0	3	7	9	11	14	16	17
1	5.6	8.7	10.1	8.2*	9.7	12.8	6.1	
	= = = = = = = = = = =							
2	7.7	11.4	11.5	11.1*	9.4	3.5	3.7*	
	= = = = = = = = = = =							
3	5.4	9.8	23.5	18.9*	16.4	9.7	8.3*	
	= = = = = = = = = = =							
4	5.2	20.4	14.8	10.4*	9.6	2.7		8.3
	= = = = = = = = = = =							
5	5.2	20.8	14.4	10.0	9.2	5.4		4.5
	= = = = = = = = = = =							
6	4.8	13.4	17.2	5.4*	3.5	6.9		3.8
	= = = = = = = = = = =							
7	4.6	13.9	12.4	16.6*	10.2*	3.2	dead	
	= = = = = = = = = = =							
8	4.4	7.0	14.4*	12.4*	11.1	7.1		2.7
	= = = = = = = = = = =							
Mean	5.4	12.7	14.8	11.6	9.9	6.4	6.0	4.8

* IBRV isolated from vaginal secretions: Vaginal secretions were swabbed on days 7, 9, 11, 16, and 17.

= = = = = Bars indicate the seven-day course of betamethasone injections.

TABLE 2. Neutralizing Antibody to the Virus of Infectious Bovine Rhinotracheitis in Captive Wildebeest with Vulvovaginitis.

Animal Number	Neutralizing Titre* Before Onset of Vulvovaginitis	Neutralizing Titre* 8-9 days after Onset of Vulvovaginitis	IBRV** Isolation
1	0.60	1.08	+
2	0.36	0.48	+
3	0.00	0.96	+
4	1.20	1.65	+
5	0.96	1.56	—
6	0.60	1.56	+
7	0.84	0.60	+
8	1.20	1.68	+

* Neutralizing Titre as $-\text{Log}_{10}\ SN_{50}$

** + = IBR virus isolated.

— = No virus isolated.

those in the wildebeest. The lesions persisted for 8 days. IBRV was reisolated from vaginal secretions 2, 4 and 7 days after intravaginal inoculation. None of the other heifers showed clinical evidence of disease. Sera of the four heifers did not contain IBRV antibody before experimental challenge. Three weeks later the animal exposed intravaginally still had no significant level of neutralizing antibody but the serum of the heifer inoculated intravenously neutralized $10^{1.4}$ $TCID_{50}$ IBRV. The two controls remained serologically negative.

DISCUSSION

Detection of IBRV neutralizing antibody in the sera of seven of eight recently captured wildebeest, and development in all of them of vulvovaginitis associated with a virus serologically identical to IBRV when they were subjected to a series of corticosteroid injections, indicates that IBRV can be carried by wildebeest in the absence of clinical disease. Whether IBRV infection can spread from wildebeest to cattle is a matter for speculation.

Previous observation [5,13] of the activation of latent herpesviruses by natural or artificial stress are supported by the observations reported here. The occurrence of the genital form of IBRV infection, as an entity distinct from the upper respiratory form, is in keeping with observations in cattle.[6,16] Absence of IBRV antibody in some cattle after confirmed infection with IBRV is in agreement with observations of other investigators.[6]

Although there are several reports of serological evidence of IBRV infection in wild ruminants,[2,3,4,8] we have not found reports of the isolation of IBRV from a naturally infected wild animal, nor of the implication of IBRV as a cause of disease in wildlife The only published report of the probable occurrence of IBRV in wildebeest is that of Rweyemamu,[11] who found neutralizing antibody to IBRV in the sera of two of 56 wildebeest in Masailand, Tanzania, an area contiguous with that where the animals described here were captured.

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