# EXPERIMENTAL INFECTION OF WILDEBEEST WITH THE HERPESVIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS/INFECTIOUS PUSTULAR VULVOVAGINITIS

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# EXPERIMENTAL INFECTION OF WILDEBEEST WITH THE HERPESVIRUS OF INFECTIOUS BOVINE RHINOTRACHEITIS/INFECTIOUS PUSTULAR VULVOVAGINITIS

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*Abstract:* Intravaginal inoculation of a wildebeest *(Connochaetes taurinus)* with a wildebeest strain of the infectious bovine rhinotracheitis/infectious pustular vulvovaginitis herpesvirus induced only mild vulvovaginitis. The same virus did not produce any disease in another wildebeest exposed intranasally. A wildebeest bull which was inoculated by preputial instillation developed mild posthitis. The virus was reisolated only from the sites of inoculation. A carrier state was initiated in a wildebeest inoculated only once, intravaginally. The presence of this virus in the various secretions is a potential source for venereal transmission.

## INTRODUCTION

Vulvovaginitis in wildebeest is caused by a herpesvirus which appears to be the same as that which causes infectious bovine rhinotracheitis/infectious pustular vulvovaginitis (IBR/IPV) otherwise known as bovine herpesvirus 1.[2,4] Pustular vulvovaginitis erupted in 13/13 parous female wildebeest after being inoculated with the corticosteroid, betamethasone.[4,5,9] In all these cases, IBR virus was isolated from vaginal swabs but the respiratory form of disease was not observed.

Since IBR and IPV viruses are identical in many respects,[2,7] the ability of the genital wildebeest IBR virus to cause the respiratory form of disease in wildebeest was investigated. Also the effect of the virus on the genitalia of a male wildebeest was studied.

## MATERIALS AND METHODS

### Animals

An adult wildebeest bull was captured on the Athi-Kapiti plains of Kenya during the calving season, April to June, 1978, and put in a paddock at Muguga. The bull was acquired for breeding purposes and was allowed to graze freely with a group of 6 captive adult female and two subadult female wildebeest 2.5 and 1.5 years old, born in captivity.

To facilitate handling, the wildebeest were injected with a mixture of 0.15 mg/kg fentanyl [1] and 0.15 mg/kg xylazine [2] or 0.4 mg/kg of xylazine alone, via projectile syringes. The bull and two subadult wildebeest were devoid of virus neutralizing (VN) antibodies to IBR/IPV virus prior to inoculation with an isolate of wildebeest IBR virus.[9] All the wildebeest received about $10^5$ $TCID_{50}$ of the virus, the bull by preputial instillation, the 2.5-year-old female by vaginal instillation and the 1.5-year-old female by intranasal spray via an atomizer. [3] The wildebeest were clinically examined and morning rectal temperatures recorded.

### Sampling

Vaginal, nasal and preputial secretions and uncoagulated blood were

[1] Janssen Pharmaceutica, Beerse, Belgium.

[2] Bayer, Leverkussen, W. Germany.

[3] The DeVilbiss Co., Somerset, Pennsylvania, USA.

collected as previously described.[4,9] The specimens were inoculated into confluent secondary roller tube calf thyroid (B.Th.) cell cultures prepared as described by Karstad *et al.*[4] The cell cultures were examined for the development of cytopathic effects (c.p.e.) for 14 days.

The wildebeest sera were heat-inactivated and tested for virus neutralising antibodies as previously described.[4]

### Treatment with corticosteroid

The 2.5-year-old wildebeest which had been infected intravaginally was inoculated with a corticosteroid, betamethasone [d] in an attempt to reactivate the infection 6 weeks after the lesions from the primary infection had healed and 8 weeks after the first inoculation. The wildebeest was injected with 0.2 mg/kg betamethasone for 6 consecutive days, also 5 mg/kg oxytetracycline daily to prevent secondary bacterial infections. The wildebeest was clinically examined daily and samples collected for virus isolation.

## RESULTS

### Clinical and Virological observations

The wildebeest inoculated intranasally did not show any clinical abnormalities over a period of 2 weeks. Mild pustular vulvovaginitis was observed in the 2.5-year-old wildebeest inoculated by vaginal instillation. A few pustules appeared on the vulvar mucosa by day 7 post infection (p.i.) and these had completely healed by day 14 p.i. The lesions were accompanied by mucopurulent vaginal discharge but neither pyrexia nor any other signs of systemic disturbance were observed.

The wildebeest bull remained devoid of VN antibodies after staying for 3 months with female wildebeest which were known to be IBR virus carriers.[4,9] After exposure to the IBR/IPV virus by preputial instillation, a serous preputial discharge appeared on the 3rd day p.i. Later the preputial discharge became mucoid and copious. Finally the preputial discharge again became serous by day 14 p.i. The preputial orifice edges were inflamed from day 4 p.i. and 1-2 mm pustules were visible. These became shallow erosions on the edges of the preputial orifice. They had healed leaving white scars about 2 mm in diameter by day 14 p.i. Unfortuately the penis could not be examined, as it was never possible to expose it, not even during drug immobilization. Mild pyrexia seemed to be present from the 3rd day p.i. but there were no signs suggestive of upper respiratory tract infection. Possibly the increases in body temperature were caused by excitement and activity during drug immobilization.

The virus could be recovered only from the sites of inoculation (Tables 1, 2 and 3). Specimens which were collected before inoculation of the wildebeest failed to yield virus. In all cases, virus replication was rapid and high titres were attained on the second or third day of infection. The highest titre of recovered virus was from a nasal swab taken from the intranasally-infected animal, although no clinical abnormalities were observed. Virus shedding in the nasal secretions of this animal ended abruptly, without generalization of infection. When this animal died 7 weeks p.i. from severe *Dictyocaulus viviparus* infection, IBR/IPV virus could not be isolated from 10% tissue suspensions of spleen, kidney, liver, tonsil, pharyngeal lymph node, turbinate, trachea, lung and vaginal mucosae inoculated into B.Th. cell cultures.

### Antibody response

Low levels of virus neutralising antibodies were detected first on the 14th

[d] Glaxo Laboratories Ltd., Greenford, England.

TABLE 1. Virus isolation from wildebeest infected intranasally.

| Specimen | Days Post Infection | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 7 | 11 | 14 |
| Buffy Coat | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Nasal Swab | 0.0 | 4.2* | 4.2 | 7.0 | 0.0 | 0.0 |
| Vaginal Swab | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

*$\log_{10}$ $TCID_{50}$/ml

TABLE 2. Virus isolated from wildebeest infected intravaginally.

| Specimen | Days Post Infection | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 2 | 4 | 7 | 11 | 14 |
| Buffy Coat | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Nasal Swab | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Vaginal Swab | 0.0 | 5.2* | 5.2 | 5.4 | 2.2 | 0.0 |

*$\log_{10}$ $TCID_{50}$/ml

day p.i. in all the infected wildebeest (Table 4). Higher levels of VN antibodies had been attained by the 8th week p.i. except for the bull which had become seronegative. The wildebeest inoculated intranasally showed a greater antibody response than the one inoculated intravaginally. The antibody levels were not altered by the corticosteroid inoculations.

### Reactivation of latent infection

IBR virus was recovered from the vagina of the intravaginally infected wildebeest on the 6th day of betamethasone administration. Unfortunately this animal died from self-inflicted trauma and stress on the seventh day from the start of betamethasone administration. Up to this time, no lesions had been observed. Also IBR virus was not isolated from 10% tissue suspensions of vulva, cervix and spleen collected at necropsy and inoculated into B.Th. cell cultures.

### Distribution of antibodies in wildebeest

During the course of this study sera from wildebeest of different ages and sexes, shot or captured in Kajiado District, Kenya, were tested for the presence of VN antibodies. Seven of 11 wildebeest calves which were less than 3 months old possessed circulating VN antibodies. Antibodies were also demonstrated in calves which were less than 2 weeks old and one calf which was separated from the dam before suckling was found to be seronegative. Very few yearling wildebeest had circulating VN antibodies (Table 5). In the adult wildebeest

TABLE 3. Virus isolation from wildebeest bull.

| Specimen | Days Post Infection | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 14 | 23 |
| Buffy Coat | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Nasal Swab | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Preputial Swab | 0.0 | 1.0* | 1.2 | 4.8 | 2.6 | 3.2 | 2.4 | 0.6 | 2.4 | Trace | 0.0 |

*$\log_{10}$ $TCID_{50}$/ml

TABLE 4. Virus neutralising antibodies in wildebeest.

| Animal No. | Route of Inoculation | Days Post Infection | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 7 | 11 | 14 | 8 wks |
| 1 | Intravaginal | 0.0 | 0.0 | 0.0 | 0.5* | 1.0 |
| 87 | Intranasal | 0.0 | 0.0 | 0.0 | 0.7 | 1.8 |
| 99 | Preputial instillation | 0.0 | 0.0 | 0.0 | Trace | 0.0 |

*$\log_{10}$ $VN_{50}$

population, two thirds of the cows had VN antibodies, and 50% of the bulls were seropositive (Table 5).

## DISCUSSION

Intravaginal inoculation of a wildebeest with the wildebeest strain of IBR/IPV virus induced only mild vulvovaginitis. The lesions were quite similar to those induced in bovine heifers inoculated with the same virus.[4,9] Apparently, a single exposure was capable of initiating the carrier state for virus was isolated following corticosteroid injections.

This virus did not produce any disease in a wildebeest exposed intranasally, but the virus multiplied very well in the upper respiratory tract. In this, our experience differs from that of others who found that bovine genital isolates of IBR/IPV virus caused mild respiratory disease in experimentally infected cattle.[6,7,10] Possibly the wildebeest IBR/IPV virus has become so well adapted to the genital tract that it has lost its ability to produce the respiratory form of disease.

Although posthitis has not been observed in the few wildebeest bulls examined in the wild, the IBR virus induced a mild posthitis in a wildebeest bull infected by preputial instillation. Although the virus multiplied readily in the prepuce, the bull was not ill and so could easily transfer the virus to females during breeding, further supporting the probability of a venereal mode of transmission of this virus.[9]

The studies on the distribution of antibodies in free-ranging wildebeest throw some light on the epizootiology of this infection although only few animals were tested. Most calves acquire colostrum-derived antibodies during early life but this immunity wanes and yearling wildebeest are seronegative, as reported in our previous communication.[9] The majority of the adult animals have been infected, as in some other wildlife species.[3] Although the exact mode of transmission is unknown, the infection could be spread readily during breeding and the virus could be reactivated by the stresses of calving and oestrus. The lower prevalence of antibodies in wildebeest bulls could be due

TABLE 5. Distribution of antibodies to IBR/IPV virus in free-ranging wildebeest.

| | No. wildebeest with VN antibodies | Total tested | % wildebeest with VN antibodies |
|---|---|---|---|
| Subadult Male | 2 | 8 | 25 |
| Subadult Female | 3 | 17 | 18 |
| Adult Male | 21 | 45 | 47 |
| Adult Female | 31 | 46 | 67 |

to the fact that the wildebeest is a territorial animal. One dominant bull may copulate with many wildebeest cows and subordinate bulls do not succeed in establishing territories, roaming instead in bachelor herds.[1,11]

The ability of the IBR/IPV virus to replicate in the prepuce of a wildebeest bull suggests that the infection is transmitted by coitus as is the case with the reproductive form of IBR/IPV virus infection in cattle.[2]

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