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Authors: SCHIEMANN, B., and GWAMAKA, B.

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PATHOGENICITY FOR A BUFFALO (Syncerus caffer) OF ALLERTON-TYPE HERPES VIRUS ISOLATED FROM A TANZANIAN BUFFALO

B. SCHIEMANN, and B. GWAMAKA

Serengeti Research Institute, P.O. Box 3134, Arusha, Tanzania

M. KALUNDA*

East African Veterinary Research Organization, Muguga P.O. Kabete, Kenya, East Africa

Abstract: A buffalo (Syncerus caffer) was inoculated intravenously and intradermally with a high dose of Allerton-type herpes virus isolated from a sick buffalo during an cutbreak of a disease in buffaloes in Serengeti National Park, Tanzania, in 1969. The animal reacted severely and generalized skin nodules as well as oral and nasal lesions were observed. Viraemia was detected and the virus was isolated from swabs and also from ticks which were fed on the animal during the viraemic phase. The buffalo produced neutralizing antibody and recovered after a long illness. It would therefore appear that under certain conditions the Allerton-type herpes virus can be of high pathogenicity for buffaloes.

INTRODUCTION

A severe outbreak of disease was reported in buffaloes (Syncerus caffer) in the Serengeti National Park, Tanzania in December 1969 by Schiemann, Plowright and Jessett." A virus, isolated from a tongue ulcer in one of the sick animals, was found to be similar to the prototype strain of Allerton herpes virus.8 It was designated buffalo Allerton-type (BA).⁸ Susceptible cattle infected experimentally with varying doses of this agent and employing different routes of inoculation reacted mildly.5 It was therefore decided to infect a susceptible buffalo with the same virus and to compare the results with those obtained previously.

This paper describes the clinical and virological observations which were made on a single captive buffalo which was successfully infected by inoculation of BA virus.

MATERIALS AND METHODS

Virus strain

The BA strain[®] was used after it had been passaged twice in primary calf testis monolayers and three times in monolayers of calf kidney cells.

Experimental buffalo

The captive male buffalo was over 3 years old but its serum was found to be free from neutralizing antibody to BA virus. During the experiment its rectal temeprature was recorded every morning and any rise to or above 38.9 C was considered abnormal; a clinical examination was conducted daily.

Route of infection

The buffalo was inoculated intravenously with 10 ml of infected culture fluid or $10^{n,0}$ TCD₅₀ of the virus. In addition, 4

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^{*}Requests for reprints should be addressed to the third author at E.A.V.R.O., Muguga, P.O. Kabete, Kenya, East Africa.

inoculations of 0.5 ml of culture fluid were made by the intradermal route on the lateral aspect of the neck.

Ticks

Clean nymphs and adults of the hard tick, *Rhipicephalus appendiculatus*, were placed on the buffalo on the first day postinoculation (pi). These ticks were collected in small batches and were processed within 3 days for virus recovery. During any delay period, they were stored at 4 C.

Collection and treatment of samples

The method of collecting and treating samples, the cell culture methods and

neutralization tests were as previously described.⁵ Ticks were processed for virus recovery in a manner similar to that for skin biopsies, i.e. they were treated in TenBroeck grinders to produce a 10% suspension in culture maintenance medium.

RESULTS

Clinical Observations

i. Pyrexia:

A rise of temperature was first observed on the 3rd day after inoculation. It attained 40 C on the 5th day and did not fall below this until the 18th day pi (Table 1).

TABLE 1. Temperature, Virus Excretions and Production of Neutralizing Antibody in Buffalo.

	Morning	Titre* of virus in			Titre** of	
Day Post- inoculation	temperature C	Buffy coat	Oral swab	Nasal swab	virus in ticks	N.A.
1	38.6	Nil	Nil	Nil	N.T.	Nil
2	38.9	Nil	Nil	Nil	N.T.	Nil
3	39.0	Nil	Nil	Trace	N.T.	Nil
4	39.8	Nil	Trace	1.0	3.3	Nil
5	40.0	Nil	1.0	1.5	3.8	Nil
6	40.7	Trace	1.8	2.0	cytotoxic	Nil
7	40.8	Trace	2.9	1.8	4.7	Nil
8	40.9	Trace	2.6	3.0	cytotoxic	Nil
9	40.8	Nil	2.6	4.0	3.3	Nil
10	40.4	Nil	Trace	1.8	3.7	0.6
11	40.2	Nil	Nil	Nil	3.2	1.1
12	40.2	Nil	Nil	Nil	1.8	1.4
13	40.1	Nil	Nil	Nil	Trace	1.8
14	40.2	Nil	Nil	Nil	Nil	1.7
15	40.1	Nil	Nil	Nil	Nil	1.8
16	40.0	Nil	Nil	Nil	Nil	1.8
17	40.2	Nil	Nil	Nil	Nil	1.8
18	39.8	Nil	Nil	Nil	Nil	1.8
19	39.8	Nil	Nil	Nil	Nil	1.9
20	39.8	Nil	Nil	Nil	Nil	1.8
21	39.7	Nil	Nil	Nil	Nil	1.8
30	38.8	Nil	Nil	Nil	Nil	2.0

* Log₁₀ TCD₅₀/swab

** Log_{10} TCD₅₀/g

 $N.A. = Titre of neutralizing antibody log_{10} SN_{50}$

N.T. = Not tested

ii. Oral and nasal lesions:

The oral mucosa became moderately congested on the 5th day pi. Necrosis was observed on the lower gum on the next day, followed by small erosions, 2 to 4 mm in diameter. These erosions increased in number and size, reaching a mean of 10 mm diameter 3 to 5 days later; they were seen on the lips, gums and tongue and were accompanied by excessive salivation, drooling of saliva, grinding of teeth and a foetid smell.

Severe congestion, necrosis and raised greyish nodules, 2 to 7 mm in diameter, were also observed near the nares at the same time as lesions appeared in the mouth. These changes were associated with copious nasal discharges which were at first serous and later mucopurulent; they resulted in severe dyspnoea and foulsmelling exhalations. Healing of the erosions and ulcers was almost complete 7 to 17 days after their appearance.

iii. Eye lesions:

Severe congestion of the conjunctiva, swelling, necrosis and erosions of the eye-

lids were first seen on the 7th day pi. Corneal opacity as well as severe photophobia were noticed a few days later. Profuse ocular discharges were observed and the animal became blind for nearly 3 weeks until the opacity of the cornea disappeared.

iv. Skin lesions:

Raised, round and painful nodules, diameter 0.5 to 2 cm, appeared at the sites of intradermal inoculations on the 5th day pi. They increased in size to about 7 to 8 cm, fused and became very painful 2 to 4 days later. On the 7th day pi multiple nodules of the same size appeared on the neck, at the base of the tail and on the medial aspects of the limbs. The whole body surface showed numerous nodules after a further 3 to 4 days fig. 1). They caused the buffalo to rub against stationary objects, resulting in bleeding of superficial lesions; extensive necrosis of those around the anus and in the axilla and groin was associated with a foetid smell which attracted many flies. The scrotum was severely inflamed, enlarged and painful.



FIG. 1. Buffalo showing generalized skin lesions.

The limb joints were swollen and painful and extensive necrosis occurred at the coronary band and between the claws, followed by bleeding and suppuration. The animal became very weak, anorectic and recumbent between the 14th and 20th days pi. Treatment with antibiotics, to control secondary infection, was started on the 30th day pi because of extensive necrosis of skin lesions and a persistent elevated temperature. Rapid recovery was made thereafter and the buffalo began to eat again 10 days later. The animal showed a progressive fall in hemoglobulin and red cell count in the course of the disease; leukopenia was also detected.

Virus Recovery

A minimal viraemia was detected in cell cultures between the 6th and 8th days and BA virus was recovered from oral, nasal and skin lesion swabs between the 3rd and 10th days pi. Virus titres were highest in nasal swabs. This agent was also isolated from pools of ticks, both nymphs and adults, collected between the 4th and 13th days pi. The titre of virus in the pools of ticks was nearly always higher than that of any swabs obtained on the same day (Table 1).

Serological Reaction

Neutralizing antibody was first detected in serum samples on the 10th day after infection. It increased to a plateau 3 days later and maintained this titre up to the last sample (Table 1).

DISCUSSION

Experimental transmission of BA virus to a susceptible buffalo elicited severe disease similar to that observed in buffaloes in a natural outbreak.[®] Oral and nasal erosions as well as generalized skin nodules developed and the animal had a severe generalized reaction. The skin lesions were similar to those reported by other workers using different strains of Allerton-type virus.^{1,2,8,4,6,7}

Cattle and the buffalo were similar with regard to the duration of virus recovery and the neutralizing antibody response. The main difference was in the clinical reaction of these two species. In our previous experiments, cattle given an equal or higher dose of the same virus did not become very sick although some of them developed oral and nasal lesions as well as skin nodules confined to the head and neck.³

Schiemann *et al.*^o and Plowright and Jessett^s had previously suggested that BA virus was probably not the primary cause of a natural outbreak of disease in buffaloes because of the extremely high incidence of neutralizing antibody to this agent in this species and because of the lack of reports of disease. The present experiment showed that in some circumstances, Allerton-type herpes virus can be highly pathogenic for buffaloes.

Virus isolation from pools of ticks fed on a buffalo during the viraemic phase supports a previous suggestion that transmission is commonly by a vector.⁸ The ticks which were observed in large numbers on sick buffaloes in Tanzania in 1969 could well have played a role in the transmission of the infection.

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