J. Hyg., Camb. (1980), 85, 175
Printed in Great Britain

The demonstration of cell-free malignant catarrhal fever herpesvirus in wildebeest nasal secretions

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(*Received* 15 *March* 1979)

SUMMARY

Malignant catarrhal fever (MCF) herpesvirus was isolated from the nasal secretions of 4/11 young wildebeest calves. In two cases virus was demonstrated in filtered secretions. The presence of cell-free MCF virus would suggest that the virus can be transferred from wildebeest to wildebeest and into cattle in nasal secretions.

INTRODUCTION

Although malignant catarrhal fever (MCF) has been associated with the close herding of cattle with wildebeest (*Connochaetes taurinus*) the exact mode of transfer of infection from wildebeest to cattle remains unknown (Mettam, 1923; Henning, 1956; Plowright, 1965, 1968). Transmission by close contact of wildebeest calves and cattle has been demonstrated and is a prerequisite for the transfer of infectivity from wildebeest to cattle (Mettam, 1923; Plowright, 1964, 1965). This observation coupled with the seasonal nature of the disease led some workers to suggest that arthropod transmission was responsible for the spread of MCF from wildebeest to cattle, but Plowright (1965) advanced good reasons against this hypothesis. Attempts to transmit MCF by placing lice picked from wildebeest on cattle were unsuccessful (Mettam, 1923). Rweyemamu *et al.* (1974) isolated MCF virus from the nasal secretions of wildebeest under the stresses of confinement and betamethasone treatment. This led them to suggest that MCF was disseminated among wildebeest and probably spread to cattle by the virus shed in wildebeest nasal secretions. Transmission of a strictly cell-associated virus in this way would seem unlikely. We have therefore studied the infectivity of wildebeest calf nasal secretions before and after millipore membrane filtration.

MATERIALS AND METHODS

Animals

Wildebeest calves were captured on the Athi-Kapiti plains during the calving season of February to April 1978. They were about 1 week old, for the umbilical

* Members of the United Kingdom Ministry of Overseas Development, Scheme R3253.

0022-1724/80/8 0022-1980 $01.00 © 1980 Cambridge University Press

https://doi.org/10.1017/S0022172400063208 Published online by Cambridge University Press

cord had not yet fallen off and the central temporary incisors had just broken through the gum (Plowright, 1964). On arrival at the laboratory the calves were bottle-fed on cow's milk, usually in four or five meals of 300 ml per day. The loose box was heated by an infra-red bulb.

Cell cultures

Calf thyroid (BTh) monolayers were prepared as described by Plowright & Ferris (1961), but were grown in Eagle's minimum essential medium (MEM, Wellcome Ltd) supplemented with 10% ox serum and antibiotics. Usually the cells were used after one passage in culture.

Collection of specimens

Blood samples of 14 ml each were collected into universal bottles containing 7 ml EDTA (0·015 g/ml). Nasal swabs were collected into 3 ml of phosphate buffered saline containing 0·1 % bovine albumin (BAPBS).

Specimens were collected soon after capture and subsequently on alternate days and inoculated into confluent secondary BTh test-tube cultures. One nasal swab sample from each animal was inoculated after filtration through 450 nm millipore membrane filters (Millipore). The cell cultures were examined for the development of cytopathic effects (CPE) over a period of 21 days.

Fluorescent antibody staining

Cell cultures which developed CPE were detached with a mixture of 0·02% versene and 0·25% trypsin and inoculated into test-tubes containing a flying cover-slip. When CPE appeared the cover-slip was washed in PBS and fixed in cold acetone for 10 min. The cover-slip was stained with fluorescein-conjugated MCF hyperimmune ox serum for 30 min. The cover-slip was thoroughly washed in PBS and mounted on glass slides using 9:1 glycerol:PBS and examined using a u.v. microscope.

RESULTS

Management of wildebeest calves

It was relatively easy to adapt the calves to bottle feeding. In the field wildebeest calves stay with their mothers all the time and are suckled at short intervals. In an attempt to simulate the natural condition, small quantities of milk were given 4–5 times a day. Wildebeest milk has twice the amount of carbohydrates, protein and fat as cow's milk (Paling, Karstad & Grootenhuis, 1978) and the wildebeest calves were soon showing general unthriftness.

The majority of the calves died during the second week of captivity (Table 1). Some deaths were probably due to chilling, for 6/11 calves died when there was an electricity supply failure lasting for more than 12 h.

No clinical signs suggestive of MCF were observed in any of the wildebeest calves, and no lesions typical of MCF were encountered at the post-mortem examination.

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Table 1. *Wildebeest calves examined, and MCF virus isolations*

Calf no.	Description	Time in captivity (days)	Cause of death	Sample	Day virus isolated
M180	Captured	12	Hypothermia	BC	11
181	Captured	5	Pneumonitis	NSF	1
182	Captured	15	Hypothermia	—	—
183	Captured	14	Hypothermia	NSW	8
184	Captured	29	Pneumonitis	NSW	26
				NSF	29
				NSW	29
185	Captured	10	Hypothermia	—	—
186	Captured	2	Pneumonitis	—	—
188	Captured	10	Hypothermia	—	—
189	Born at Muguga	13	Enteritis	BC	8
190	Born at Muguga	10	Hypothermia	—	—
191	Born at Muguga	10	Pneumonitis	NSW	4

BC, buffy coat; NSF, filtered nasal secretions; NSW, unclarified nasal secretions.

MCF virus isolations from wildebeest calves

The CPE was the first criterion for identifying a viral agent as that of the herpesvirus of MCF. Small stellate syncytia developed and detached leaving a 'clearing'. The CPE did not spread readily (Plowright, Ferris & Scott, 1960). Confirmation was by demonstration of fluorescence in the cells involved in the syncytia when a cover-slip was stained with fluorescein-conjugated MCF ox antiserum (Wibberley, 1976).

MCF virus was recovered from the buffy coat and nasal secretions of some wildebeest calves (Table 1). Most of the virus recoveries were made during the second week of captivity and only a few days before death. Probably the stress of confinement and change in diet caused increased release of virus.

Viraemia was demonstrated in only 2/11 wildebeest calves. In each of these calves, viraemia was demonstrated during the second week of captivity (Table 1), and 1 day before death from hypothermia for calf M180. Generally the infectivity was low, for only 1/5 cell culture tubes were affected and the CPE appeared 11 days after inoculation.

MCF virus in nasal secretions

Virus capable of inducing typical MCF CPE in calf thyroid cells was recovered from nasal secretions of 4/11 wildebeest calves (Table 1). More wildebeest calves shed virus in their nasal secretions than showed viraemia and virus could be isolated from the time of capture up to 4 weeks of captivity. In one instance MCF virus was isolated from the unclarified nasal secretions of a 4-day-old wildebeest calf. This would suggest that the calf was born with active infection, probably acquired *in utero*.

At no time was virus isolated from both blood and nasal secretions of the same calf. Also most virus recoveries were made on a single occasion except for calf M184 which shed virus on days 26 and 29.

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Viral infectivity was low, for only 1/5 cell culture tubes were affected and CPE appeared only after 15 days of incubation. But in calf M184 CPE was detected as early as the 6th day of incubation and 3/5 cell culture tubes showed CPE.

On several occasions unclarified nasal secretions were titrated in BTh tube cultures but CPE was only present in tubes inoculated with the undiluted sample, indicating that the infectivity was quite low, about 10 TCD 50/ml.

MCF virus was demonstrated in filtered nasal secretions on two occasions only. The first instance was from a calf sampled 1 day after capture (M181). In the second calf (M184) infectivity was detected in 3/5 tubes on day 6 which was earlier than that from unfiltered nasal secretions. In this calf MCF virus was recovered from unclarified and filtered nasal secretions but not from blood.

DISCUSSION

Week-old wildebeest calves were captured and raised artificially, but considerable difficulties were experienced in the rearing, as the calves did not thrive on the unnatural regimen.

Viraemia was demonstrated in only 2/11 wildebeest calves. This value is lower than the finding by Plowright (1965) that one third of wildebeest calves were viraemic during the first 12 weeks of life. The fact that only one calf was observed for as long as 4 weeks could account for the low virus recovery rate.

MCF virus was isolated from the nasal secretions of 4/11 wildebeest calves. Infectivity was demonstrable in both the unclarified and in filtered nasal secretions from one calf. This is the first report of an unequivocal demonstration of cell-free MCF virus in wildebeest nasal secretions. Rweyemamu *et al.* (1974) isolated MCF virus from the supernatant of a sample of lightly centrifuged wildebeest nasal secretions, but the infectivity could have been due to the deposited cells being resuspended. In our cases all the cellular elements were removed by filtration through 450 nm millipore membrane filter, thus any viral infectivity in the filtrate must have been extracellular.

The presence of cell-free MCF virus in wildebeest calf nasal secretions indicates that the upper respiratory tract cou' ' probably be the portal of exit of this virus, as suggested by Rweyemamu *et al.* (1974). The stability of this cell-free virus has not been studied but it is likely that it could be more easily transferred than viable cells from one animal to another. However, the ability of cell-free wildebeest nasal secretions to produce MCF in cattle has still to be demonstrated.

MCF virus was demonstrated in only 6/11 wildebeest calves and only in low titres. The low rate of MCF virus shedding by wildebeest calves could account for the low morbidity rate of bovine MCF.

This was a cooperative project of the Government of Kenya, supported in part by the Canadian International Development Agency, with the International Development Research Centre as executing agency. This paper is published with the permission of the Director, Veterinary Research Department, Kenya Agricultural Research Institute, Kenya, and the Director of Veterinary Services, Kenya.

https://doi.org/10.1017/S0022172400063208 Published online by Cambridge University Press

We wish to acknowledge the able technical assistance of Messrs D. Waweru, J. Mumira and H. Agili.

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