Onderstepoort J. vet Res., 56, 131–134 (1989)

EXCRETION OF ALCELAPHINE HERPESVIRUS-1 BY CAPTIVE AND FREE-LIVING WILDEBEEST *(CONNOCHAETES TAURINUS)*

B. J. H. BARNARD[(1)], R. G. BENGIS[(2)], MONICA D. GRIESSEL[(1)] and V. DE VOS[(3)]

ABSTRACT

BARNARD, B. J. H., BENGIS, R. G., GRIESSEL, MONICA D. & DE VOS, V., 1989. Excretion of alcelaphine herpesvirus-1 by captive and free-living wildebeest *(Connochaetes taurinus)*. *Onderstepoort Journal of Veterinary Research*, 56, 131–134 (1989).

Excretion of alcelaphine herpesvirus-1 (AHV-1) is for all practical purposes limited to wildebeest calves under the age of 4 months. Sixty-one per cent of calves 1–2 months of age excreted virus with a mean titre of 9,8 × 10^4 cytopathic-forming foci/mℓ in their ocular fluid. The incidence declined sharply to less than 2 % in wildebeest older than 6 months. No difference in age-related excretion of virus could be detected between free-living and captive wildebeest and no virus could be isolated from free-living pregnant wildebeest cows or from captive cows and their calves during the first 4 weeks after birth. The occurrence of wildebeest-derived malignant catarrhal fever (WD MCF) during spring, when wildebeest do not excrete virus, is a strong indication of the existence of an alternative host or an intermediate host capable of biological transfer of AHV-1.

INTRODUCTION

The epizootology and the seasonal occurrence of wildebeest-derived malignant catarrhal fever (WD MCF) is based on the work of Plowright (1964) and on results obtained in another investigation involving 12 wildebeest calves (Mushi, Karstad & Jesset 1980). Infection in cattle usually occurs where cattle live in close association with wildebeest, and in east Africa the disease is seen in April–July, when wildebeest calves are 3–5 months old. In South Africa 2 peaks are encountered, the higher incidence being in September–November, when wildebeest calves are 9–11 months old (Barnard, 1984; Barnard & Van de Pypekamp, 1988; Barnard, Van de Pypekamp & Griessel, 1989). Furthermore, the disease occurs when fences and even distances of up to several hundred metres, separate cattle and wildebeest. These observations prompted an investigation of the excretion pattern of AHV-1 by wildebeest.

In this paper the results are described of such an investigation carried out on captive and free-living wildebeest of different age-groups.

MATERIALS AND METHODS

Wildebeest

Two groups of wildebeest in captivity and 123 free-living wildebeest in the Kruger National Park (KNP) were used in this investigation.

Fourteen wildebeest calves 3,5–4,5 months of age from a herd associated with the occurrence of WD MCF in cattle (Barnard *et al.*, 1989) were captured in March 1987 and hand-reared in a small camp on the farm. They were sampled at monthly intervals until they were 9,5–10,5 months old. In December 1987, 7 wildebest cows, due to calve within a few weeks, were captured in the KNP and kept in an enclosure at Skukuza. Specimens were collected from the cows and their calves within the 1st week of birth and at weekly intervals thereafter for the next 4 weeks. Two cows aborted and 2 calves died during this period.

(1) Veterinary Research Institute, Onderstepoort 0110

(2) P.O. Box 12, Skukuza 1350

(3) Private Bag X402, Skukuza 1350

Received 30 January 1989—Editor

Specimens from free-living wildebeest were collected in widely separated localities in the KNP. Specimens were obtained from calves 2–10 months old, from adults 18 months and older and from 20 pregnant cows 1 month before the beginning of the calving season. All the wildebeest were immobilized with etorphine hydrochloride[1] (M99) and xylazine hydrochloride[2] (Rompun) for the collection of specimens.

Specimens

Blood, nasal mucus and ocular fluid were collected for virus isolation. Blood was collected in vacutainers containing EDTA. Nasal mucus was collected with self-made gauze swabs. A piece of gauze was attached to a twisted stainless steel wire, 25 cm in length, and placed in a silicone tube. Tubes were individually wrapped in paper and heat sterilized. One mucus specimen was collected from each wildebeest by introducing the swab as deeply as possible into the nasal cavity without injuring the turbinate mucosa.

Initially, ocular fluid was collected with cotton-wool swabs capable of absorbing up to 0,1 mℓ of fluid. Later, disposable pipette tips attached to rubber bulbs were used for the collection of ocular fluid from both eyes. The tips were calibrated to collect 10 μℓ.

Directly after sampling the swabs were thoroughly rinsed and the fluid squeezed out into bottles containing 2 mℓ of cell culture medium. Within 1 h–8 h of collection the ocular fluid was mixed with 2 mℓ of cell culture medium and kept at below 20 °C until processed within 1 h–8 h of collection.

Blood was also collected in vacutainers without anticoagulant. The blood was allowed to clot, and the serum was decanted and stored at −20 °C. All the serum specimens were tested simultaneously for the presence of virus neutralizing antibody (VNA).

Cell culture

Foetal lamb kidney (FLK) cells, cultivated in Eagle's medium containing 5 % irradiated bovine serum, benzyl penicillin (400 iu/mℓ) streptomycin sulphate (200 μg/mℓ), vancomycin (0,5 mg/mℓ) and

[1] R & C Pharmaceuticals (Pty) Ltd

[2] Bayer

TABLE 1 Isolation of AHV-1 from captive and free-living wildebeest of different ages

Group	Isolations made in								
	January	February	March	April	May	June	July–October	November	Percentage
Just calved	0/7	—	—	—	—	—	—	—	0
Calves in captivity	0/5	—	—	4/14	3/14	1/10	0/10	—	15,0
Free-living calves	—	13/20	3/12	—	2/13	1/10	0/14	—	27,5
Free-living adults	—	0/4	0/8	—	0/7	1/10	0/6	—	1,8
Pregnant cows	—	—	—	—	—	—	—	0/20	

amphotericin B (2,5 μg/mℓ), were used for virus isolation. This medium was also used as transport medium for specimens.

Virus isolation

The processing of specimens and inoculation of cell cultures were done in temporary facilities erected on the farm and in the KNP.

The erythrocytes of 1 mℓ of blood were osmotically lysed and the leucocytes separated by centrifugation for 5 min at approximately 3 000 rpm in a small bench-type centrifuge. The leucocyte fractions, ocular fluid and nasal mucus in transport medium were co-cultured with FLK cells in 25 mℓ plastic flasks[3]. On the following day the cultures were transported to the Veterinary Research Institute, Onderstepoort for further incubation at 33 °C–35 °C.

The cultures were examined daily for 18 days for the appearance of cytopathic foci (CPF) typical of AHV-1. Three days after the first appearance of CPF they were counted to determine the virus titre.

Virus identification

Isolated virus were identified by the cytopathic (CP) changes produced, by pathogenicity for cattle and rabbits and by virus neutralization.

Virus neutralization

A microtitre test (Muschi & Plowright, 1979) was used to determine VNA in serum and for virus identification. The WC11 isolate of AHV-1 (Plowright, 1964) was used as antigen in the test and for the production of positive serum in rabbits.

Results

The volumes of ocular fluid and nasal mucus absorbed by cotton-wool swabs were too inconsistent to be used in determining the titre of virus accurately. The use of calibrated pipette tips for the collection of ocular fluid enabled us to obtain more accurate results. The nasal mucus of young wildebeest calves is initially serous but changes to an almost dry tacky mucus in animals over 4 months of age.

Fungal contamination of the ocular fluid and nasal mucus cultures was frequently encountered. However, as most fungi were not cytopathic and did not adhere to the cells, the cultures could be cleaned temporarily for observation and counting of CPF.

Virus specific CPF became visible 5–8 days after inoculation, were clearly distinguishable within the next 2 days and could be counted 7–10 days after inoculation of the flasks. Secondary foci were rare and developed only after prolonged incubation. The

[3] Nunc

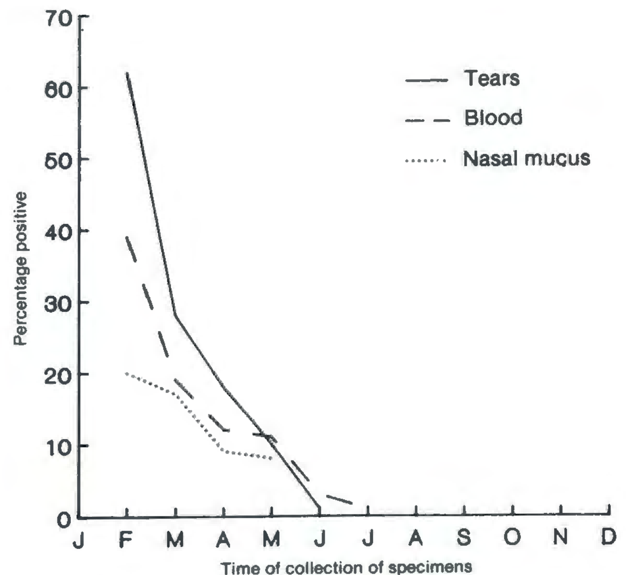

FIG. 2 Virus neutralizing antibody titre in captive and free-living wildebeest in the Republic of South Africa

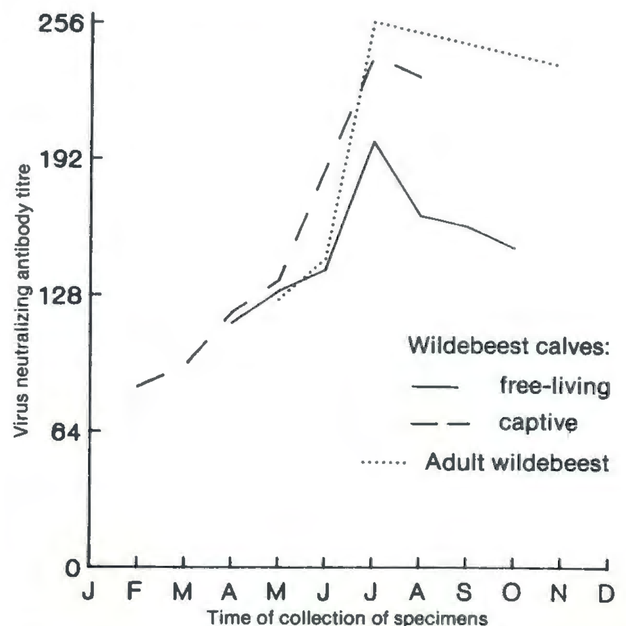

FIG. 1 Monthly incidence of excretion of AHV-1 by free-living and captive wildebeest in the Republic of South Africa

smallness of the volume of ocular fluid collected did not allow us for the detection of low titres of virus.

The highest incidence of wildebeest harbouring virus (65 %) occurred in February in free-living calves 1–2 months old (Fig. 1). In this group, 61 % excreted virus in their ocular fluid and 23 % in nasal

TABLE 2 Titre of AHV-1 in blood, ocular fluid and nasal mucus of captive and free-living wildebeest of different ages

Wildebeest AHV-1 cytopathic-forming foci/mℓ isolated from:						
Age and number	Captive wildebeest			Free-living wildebeest		
	Blood	Ocular fluid	Nasal mucus	Blood	Ocular fluid	Nasal mucus
1–2 months						
1	.	.	.	4×10^{1}	$3{,}7 \times 10^{5}$	+ [1]
2	.	.	.	– [2]	$1{,}3 \times 10^{5}$	+
3	.	.	.	–	$9{,}7 \times 10^{4}$	–
4	.	.	.	–	$5{,}8 \times 10^{4}$	contam.
5	.	.	–	3×10^{2}	$2{,}8 \times 10^{3}$	contam.
6	.	.	–	–	$3{,}1 \times 10^{4}$	$1{,}5 \times 10^{2}$
7	.	.	.	–	$3{,}0 \times 10^{2}$	–
8	.	.	.	$4{,}1 \times 10^{4}$	–	–
9	.	.	.	6×10^{2}	–	–
Mean				$1{,}1 \times 10^{3}$	$9{,}8 \times 10^{4}$	$1{,}5 \times 10^{2}$
3–4 months						
1	–	6×10^{1}	–			
2	8×10^{1}	$1{,}6 \times 10^{2}$	–			
3	$8{,}2 \times 10^{2}$	–	–			
4	–	–	$6{,}4 \times 10$			
5	–	13×10^{2}	–			
6	+	+	–			
7	.	.	.	5×10^{1}	contam.	4×10^{1}
8	.	.	.	3×10^{2}	contam.	3×10^{1}
9	.	.	.	–	–	$1{,}4 \times 10^{3}$
Mean	$4{,}5 \times 10^{2}$	$1{,}2 \times 10^{2}$		$1{,}8 \times 10^{2}$		$6{,}0 \times 10^{2}$

[1] + = Positive but titre not determined
[2] – = Negative

mucus. Viraemia was present in 37 %. The incidence of virus in ocular fluids dropped to less than 20 % in calves 3 and 4 months old and to less than 2 % in wildebeest over the age of 6 months.

Virus was isolated from calves (Table 1) but not from adult wildebeest, nor from 20 near-term cows and 7 cows kept in captivity and sampled within 1 week of calving or abortion. Virus was not detected in their calves.

No obvious difference in virus titre or virus excretion was seen in captive and in free-living wildebeest calves of the same age. The average titre of virus in the ocular fluid of free-living 1–2 month-old calves ($9{,}8 \times 10^{4}$ CPF/mℓ) was much higher than the titre in blood ($1{,}1 \times 10^{3}$) or in nasal mucus ($1{,}5 \times 10^{2}$) (Table 2).

Virus isolates from wildebeest in the KNP and from the farm had similar reactions in the cell cultures. They killed rabbits and cattle within the expected time and no difference could be detected in the neutralization test.

The serological response, as measured by the micro-neutralization test for VNA, was very similar in wildebeest calves in captivity and in free-living wildebeest (Fig. 2). In both groups, the VNA titre increased with age and reached a peak in July when calves were 6–7 months old, after which it decreased slightly. A similar response occurred in adult wildebeest; the virus titres increased during winter and decreased slightly in spring.

Discussion

Alcelaphine herpes virus-1 was isolated from wildebeest associated with an outbreak of WD MCF in cattle as well as from several herds of free-living wildebeest in the KNP. These had had no contact with cattle for many generations. Thus the presence of cattle is not important for the perpetuation and maintenance of AHV-1 in wildebeest. The different isolates appeared to be similar in their ability to infect rabbits and cattle, their cytopathogenicity in cell culture and their reaction in virus neutralization tests.

Under experimental conditions, cattle contracted WD MCF when they were housed in close contact with viraemic wildebeest calves, but adult wildebeest with discontinuous low-level viraemia were unable to infect cattle (Plowright, 1964). Under natural conditions in east Africa, cattle contracted the disease when wildebeest calves were 3–5 months old (Plowright, 1964) and the source of infection is presumed to be virus shed in ocular and nasal secretions over a short period of time (Mushi *et al.*, 1980). In an investigation involving 12 wildebeest calves under the age of 3 months, the authors established that 50 % of animals excreted virus with a mean titre of $10^{2,25}$ TCID50/mℓ in ocular and $10^{2,5}$ TCID50/mℓ in nasal excretions.

In the present investigation, excretion of virus was for all practical purposes limited to wildebeest calves under the age of 4 months. The maximum incidence of excretion and viraemia occurred in calves 1–2 months old. After this the incindence declined sharply to less than 2 % in wildebeest older than 6 months. Sixty-one per cent of the 1–2 months old calves shed virus in their ocular fluid and 23 % in the nasal mucus with a mean titre of $9{,}8 \times 10^{4}$ CPFU/mℓ and $1{,}5 \times 10^{2}$ CPFU/mℓ respectively. This result favours the hypothesis that virus shed in ocular secretions of wildebeest calves is the source of infection of cattle and is the reason for the seasonal occurrence of the disease. However, in South Africa the highest incidence of WD MCF in cattle is in September–November, when wildebeest calves are 9–10 months old and no longer excrete significant amounts of virus. The occurrence of WD MCF in the

absence of a known source of virus in wildebeest during this period is indicative of the existence of an alternative host or an intermediate host capable of biological transfer of the virus.

Stress may play a role in the recrudescence in latently infected wildebeest (Rweyemamu, Karstad, Mushi, Otema, Jesset, Rowe, Drevemo & Grotenhuis, 1974). Plowright (1964) isolated AHV-1 from 3 wildebeest cows in the last month of pregnancy but not from 19 non-pregnant animals. On another occasion (Plowright, 1964) was unable to detect virus in pregnant wildebeest. In the present investigation no viraemia or excretion could be detected in stressed wildebeest, in 20 pregnant cows sampled 1 month before the beginning of the calving season or in 7 near-term cows, captured and kept in an enclosure and sampled within 1 week of calving. Furthermore, virus excretion by 14 wildebeest calves, captured and hand-reared in a small camp, was similar to that of free-living wildebeest, and the titre of virus in their ocular and nasal secretions was similar to that of free-living calves of the same age.

The above findings indicate that recrudescence of AHV-1 virus in wildebeest is rare and probably occurs only under conditions of exceptional stress. Consequently, it is unlikely that the recrudescence of virus plays an important role in the infection of cattle under natural conditions.

The antibody response in captive and free-living wildebeest calves was very similar. The antibody titres increased till the calves were 6–7 months old, and then declined slightly. The increase in the mean antibody titre of adult wildebeest came as a surprise, but is probably due to reinfection with virus excreted by the calves.

REFERENCES

BARNARD, B. J. H., 1984. Transmitters and potential transmitters of malignant catarrhal fever: Proceedings, XIII World Congress on Diseases in cattle. Durban, South Africa, September 1984.

BARNARD, B. J. H. & VAN DE PYPEKAMP, H. J., 1988. Wildebeest-derived malignant catarrhal fever: Unusual epidemiology in South Africa. *Onderstepoort Journal of Veterinary Research*, 55, 69–71.

BARNARD, B. J. H. VAN DE PYPEKAMP, H. E. & GRIESSEL, MONICA D., 1989. Epidemiology of wildebeest-derived malignant catarrhal fever in an outbreak in the North-western Transvaal: Indications of an intermediate host. *Onderstepoort Journal of Veterinary Research*, 56, 135–139

MUSHI, E. Z. & PLOWRIGHT, W., 1979. A microtitre technique for the assay of malignant catarrhal fever virus and neutralizing antibody. *Research in Veterinary Science*, 27, 230–232.

MUSHI, E. Z., KARSTAD, L. & JESSET, D. M., 1980. Isolation of bovine malignant catarrhal fever virus from ocular and nasal secretions of wildebeest calves. *Research in Veterinary Science*, 29, 161–171.

PLOWRIGHT, W., 1964. Studies on bovine malignant catarrhal fever of cattle. D.V.Sc. Thesis, University of Pretoria, South Africa.

RWEYEMAMU, M. M., KARSTAD, L., MUSHI, E. Z., OTEMA, J. C., JESSET, D. M., ROWE, L., DREVEMO, S. & GROOTENHUIS, J., 1974. Malignant catarrhal fever virus in nasal secretions of wildebeest: a probable mechanism for virus transmission. *Journal of Wildlife Diseases*, 10, 478–487.

Printed by the Government Printer, Private Bag X85, Pretoria, 0001