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NON-BITE TRANSMISSION OF RABIES IN KUDU *(TRAGELAPHUS STREPSICEROS)*

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ABSTRACT

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The titres of rabies virus in the saliva of kudu are higher than those of the salivary glands. The high titres are an indication of active excretion and multiplication in tissues other than the salivary glands. Two out of 4 kudu died of rabies after experimental infection by the instillation of infected saliva onto their buccal and nasal mucosae. Mice and 2 cattle resisted a similar exposure. Kudu also developed antibodies against rabies after instillation of HEP Flury virus onto their nasal and buccal mucosae. Cattle did not react when they were treated in the same way. These results suggest a high susceptibility of kudu to rabies when the virus is applied to their mucous membranes.

INTRODUCTION

The transmission of rabies following non-bite exposure is well documented (Afshar, 1979). The most important non-bite route of infection appears to be the respiratory system (Hronovsky & Benda, 1969; Murphy, 1977). Factors playing a role in infection are the dose of the virus (Hronovsky & Benda, 1969) and the virus strain (Bell, 1975). Following respiratory infection, the virus reaches the central nervous system via the olfactory mucosa and the *fila olfactoria* after replication in the nasal mucosa (Murphy, 1977). Studies in hamsters have shown that the sensory organs in the oral cavity, such as taste buds in the tongue, are sites of virus proliferation after oral administration of the virus.

In an outbreak of rabies in kudu *(Tragelaphus strepsiceros)* in South West Africa/Namibia circumstantial evidence suggested a horizontal spread from kudu to kudu via a non-bite route (Barnard & Hassel, 1981). The purpose of this study was to determine the possibility of non-bite transmission of rabies in kudu.

MATERIALS AND METHODS

Virus titration

The brains and salivary glands collected from kudu that had died of rabies were preserved in 50% aqueous glycerine. The saliva was preserved in Eagle's medium containing 5% bovine serum, 500 international units of penicillin and 500 micrograms of streptomycin (EMA) per millilitre. The specimens were submitted to the Veterinary Research Institute, Onderstepoort, for virus titration in 3-week-old mice. The time between the collection of the specimens and titration varied from 4–16 days. Specimens were kept at room temperature during most of this period.

Groups of 6 mice were injected intracerebrally (ic) with 0,03 mℓ of tenfold dilutions of organ suspensions in EMA. The mice were observed for 28 days and the presence of rabies in mice that died was confirmed by the fluorescent antibody test for rabies (FATR).

Infectivity of saliva

Kudu, captured and kept in isolation, were fed on grass hay, lucerne and antelope cubes. After several months in captivity 4 kudu were artificially exposed to infection. Saliva of a kudu with rabies was instilled into the nasal and buccal cavities of 4 kudu and 2 oxen. Their mucous membranes appeared to be intact. The experimental animals were observed daily. Specimens of experimentally-infected kudu that died of rabies were collected and tested to confirm the diagnosis.

Infectivity of HEP Flury virus

Three kudu, kept in isolation, and 3 cattle, kept at Onderstepoort, were exposed in the same way to the HEP Flury virus grown in BHK 21 cell cultures. The freeze-dried virus was reconstituted with 1 mℓ of EMA and applied to the buccal and nasal mucosae of the experimental animals. Each animal received approximately 2×10^6 mouse LD 50 of virus.

Three other kudu and 3 cattle were exposed to HEP Flury virus added to their drinking water after they had been starved for 24 h. Each animal was then supplied with 2 litres of drinking water that contained 3×10^6 mouse LD 50 of HEP Flury virus. This procedure was repeated after 30 days in the case of the cattle only.

The experimental animals exposed to the HEP Flury virus were bled 30, 60 and 90 days after exposure. The serum thus obtained was tested for the presence of rabies virus-neutralizing antibodies. The test was done according to the constant virus serum dilution technique. The CVS strain of rabies, diluted to contain 100–300 mouse LD 50/0,03 mℓ, was used as antigen.

Susceptibility of mice

Groups of 3-week-old mice were staved for 24 h and then allowed to consume as much infected kudu brain as possible in a period of 2–4 hours. They were then supplied with mouse cubes and a 1 in 50 suspension of infected brain in their drinking water. It was estimated that each mouse consumed 0,2 to 1,0 g of infected brain. They were observed for 28 days. Brains of mice that died were examined with the FATR.

RESULTS

The period between the collection and titration of specimens varied from 4–16 days (Table 1). The titre of virus in the brains of kudu varied from 10^4–10^7 mouse LD 50/mℓ. In 3 out of the 3 cases titrated the tires of virus in the saliva were $10^{0,7}$–$10^{2,9}$ mouse LD 50/mℓ higher than those in the salivary glands. The titres of virus in the blood of case 570/80 was 10^1 mouse LD 50/mℓ.

TABLE 1 Rabies virus titres in kudu that died of natural rabies

Case No.	Time[1] (days)	Brain	Salivary glands	Saliva	Blood
1253/79	4	6,5[2]	N[3]	N	N
77/80	6	7,0	N	N	N
94/80	9	4,8	N	N	N
100/80	10	4,8	N	N	N
448/80	16	4,0	4,4	N	N
350/80	16	4,1	3,5	N	N
570/80	4	6,1	3,5	6,4	1,0
660/80	5	N	3,8	4,5	N
662/80	5	N	2,8	4,0	N

[1] Time from collection to titration
[2] Expressed as log 10 mouse LD 50/mℓ
[3] N=Not available for determination

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Two out of 4 kudu (Table 2), infected experimentally by the instillation of infected saliva into the buccal and nasal cavities, died of rabies 21 and 33 days after exposure respectively. Rabies virus was isolated from their brains and salivary glands. Both oxen infected with saliva survived for more than 15 months.

Only kudu reacted after exposure to the HEP Flury virus of tissue culture origin by the production of virus-neutralizing antibodies in 4 out of the 6 kudu exposed (Table 3). None of the mice fed on infected kudu brain developed signs of rabies during an observation period of 28 days (Table 4).

TABLE 2 Reaction of kudu and cattle to experimental exposure to rabies-infected saliva

Animal	Outcome	FATR[1]	Virus isolation
Kudu 1	Survived	•	
Kudu 2	Survived	•	Saliva negative Day 33
Kudu 3	Died Day 33	+	Brain & salivary gland
Kudu 4	Died Day 21	+	Brain & salivary gland
Cattle 1	Survived	•	
Cattle 2	Survived	•	

[1] FATR=Fluorescent antibody for rabies

TABLE 3 Serological response of kudu and cattle following oral and nasal exposure to HEP Flury virus

Animal	Route of exposure	Virus-neutralizing antibody titres on		
		Day 30	Day 60	Day 90
Kudu				
1	2×10^6 MLD50	<1:4	1:4	<1:4
2		<1:4	1:12	1:12
3	Mucosol surface	1:12	1:4	1:4
Cattle				
1		<1:4	<1:4	<1:4
2		<1:4	<1:4	<1:4
3		<1:4	<1:4	<1:4
Kudu				
4	3×10^6 MLD50	<1:4	1:6	<1:4
5		<1:4	<1:4	<1:4
6		<1:4	<1:4	<1:4
Cattle				
4[1]	Drinking water	<1:4	<1:4	<1:4
5		<1:4	<1:4	<1:4
6		<1:4	<1:4	<1:4

[1] Cattle 4, 5 and 6 re-exposed after 30 days

TABLE 4 Susceptibility of mice to oral administration of rabies virus-infected kudu brain

Virus isolate	Virus titre	Pathogenicity
1253/79	6,5[1]	0/12[2]
1016/79	4,0	0/12
1106/79	5,5	0/12
79/80	7,0	0/32
94/80	4,8	0/16

[1] Expressed as log 10 mouse LD 50/mℓ
[2] Ratio of deaths to number of mice exposed

DISCUSSION

The titres of rabies virus obtained in this study are possibly not a true indication of the actual titres, since the specimens were exposed to room temperature during the period form collection to titration. The period also varied from 4–16 days. Nevertheless, the results shown in Table 1 clearly illustrate the high concentration of virus in the saliva of the kudu that died of rabies.

The titres of rabies virus in the saliva of dogs can vary from a trace to 10^5 mouse LD 50/mℓ (Vaughn, Gerhardt & Newell, 1965). In most dogs titres between 10^2 and 10^3 mouse LD 50/mℓ are found. The concentration of virus in the saliva of only a small percentage of experimentally-infected foxes was higher than $10^{5,5}$ mouse LD 50/mℓ (Sikes, 1962). In skunks, the mean titre of virus in the saliva was usually equal to or lower than the titre of virus in their salivary glands (Parker & Wilsnack, 1966).

In the 3 kudu tested the titres of virus in the saliva were higher than those in the salivary glands (Table 1). In kudu 570/80 it was $10^{2,9}$ mouse LD 50/mℓ higher than that in the salivary glands. The high titre of virus in the saliva is an indication of active excretion of virus. It possibly also indicates replication of virus in tissues other than salivary glands. The high titres of virus in the saliva and the grooming habits of kudu provided ample opportunity for virus transmission from kudu to kudu (Barnard & Hassel, 1981).

The titres of virus (Hronovsky & Benda, 1969) and the virus strain (Bell, 1975) play an important role in non-bite transmission of rabies. In this study it was shown that, in some instances at least, the susceptibility of the species involved is also of importance. Attempts to infect 2 cattle (Table 1) with infected saliva or mice (Table 4) with infected brain, failed, whereas 2 out of 4 kudu were readily infected with infected saliva. They died 21 and 33 days after oral and nasal instillation. Kudu were also readily infected with HEP Flury virus. Four out of the 6 kudu exposed reacted by the production of virus-neutralizing antibodies (Table 3), whereas none of 6 oxen developed antibodies to rabies virus after oral and nasal exposure with the HEP Flury strain. These results indicate that kudu are highly susceptible to oral and/or nasal infection.

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REFERENCES

AFSHAR, A., 1979. A review of non-bite transmission of rabies virus infection. *British Veterinary Journal*, 135, 142–148.

BARNARD, B. J. H. & HASSEL, A. H., 1981. Rabies in kudus *(Tragelaphus strepsiceros)* in South West Africa/Namibia. *Journal of South African Veterinary Association*, 52, 309–314.

BELL, J. F., 1975. Latency and abortive rabies. *In:* The natural history of rabies. G. M. BAER (ed.), New York: Academic Press.

HRONOVSKY, V. & BENDA R., 1969. Experimental instillation infection of laboratory rodents with rabies virus. *Acta Virologica* (Prague), 13, 193–197.

MURPHY, F. A., 1977. Rabies pathogenesis. Brief review. *Archives of Virology*, 54, 279–297.

PARKER, R. L. & WILSNACK, R. E., 1966. Pathogenesis of skunk rabies virus: Quantitation in skunks and foxes. *American Journal of Veterinary Research*, 27, 33–38.

SIKES, R. K., 1962. Pathogenesis of rabies in wildlife. Comparative effect of varying doses of rabies virus inoculated into foxes and skunks. *American Journal of Veterinary Research*, 23, 1041–1047.

VAUGHN, J. B., GERHARDT, PHYLLIS & NEWELL, K. W., 1965. Excretion of street rabies virus in the saliva of dogs. *Journal of the American Medical Association*, 193, 363–368.

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