

## Excretion of Rabies Virus in the Saliva of Dogs

Makonnen Fekadu,\* John H. Shaddock,  
and George M. Baer

*From the Viral Zoonoses Branch, Virology Division,  
Center for Infectious Diseases, Centers for Disease Control,  
Lawrenceville, Georgia*

Thirty-nine dogs were injected intramuscularly with either an Ethiopian strain or a Mexican strain of rabies virus. The excretion of rabies virus in the saliva was studied before and during illness. Nine of 17 dogs that died after injection with the Ethiopian strain had virus in the submaxillary glands. Four of these dogs excreted virus in the saliva up to 13 days before signs of disease were observed. Sixteen of 22 dogs that died after injection with the Mexican strain had virus in the submaxillary glands. Eight of these dogs also excreted virus in the saliva up to seven days before signs of disease were observed. These findings indicate that rabid dogs may excrete virus in their saliva much earlier than previously reported.

The transmission of rabies from animals to humans by animal bites depends on the entry of infected saliva into the fresh wound. The level of virus in the saliva seems to vary among the different species involved in the rabies cycle [1-6].

The most important health question concerning the excretion of rabies virus is the precise time that excretion in the saliva begins. In most instances the virus appears to be excreted in the saliva after the onset of clinical signs of disease. Early investigators reported isolating rabies virus from experimentally infected dogs as early as seven days before signs of disease appeared [7]. In a recent study, however, rabies virus was detected in the saliva of experimentally infected dogs just three days before signs of disease were observed [3]. In other animals, especially wild animals, the virus may be detectable even earlier than that reported in dogs: five days in skunks, one to two days in foxes, and 12 days in insectivorous bats [8-10].

The excretion of rabies virus in the saliva of dogs infected with one of two "street" strains of

rabies virus in relation to the onset of illness is reported here.

### Materials and Methods

Two strains of rabies virus were used: one strain was isolated from the saliva of an apparently healthy dog in Ethiopia [11, 12] and the other from the salivary glands of a dog from an area in Mexico in which rabies is endemic. Virus suspensions consisting of 20% salivary glands from dogs that had been experimentally infected with one of the strains of rabies virus were prepared by homogenization with a diluent of 0.75% bovine albumin in phosphate-buffered saline [13]. Before use the suspension of stock virus was titrated by inoculating the suspension into weanling mice intracerebrally (five three-week-old mice were used for each dilution). Two groups of 24 beagles (age, one to two years) were injected in the right masseter with 1 ml of salivary gland suspension from either the Ethiopian or the Mexican strain of rabies virus (virus titer,  $10^{1.7}$ - $10^{2.8}$  mouse intracerebral LD<sub>50</sub>) (tables 1 and 2). All of the dogs were individually caged throughout the study and were observed twice a day after they were inoculated.

**Specimen collection.** Saliva specimens were collected from the study dogs three times a week. Cotton swabs were immersed in 1 ml of phosphate-buffered saline, the excess diluent was expressed against the wall of the tube, and the anterior surface of the tongue and the cheek mucosa was swabbed for 15-20 sec. The used swab was again immersed in the diluent, the excess fluid was expressed, and the swab was discarded.

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Please address requests for reprints to Dr. Makonnen Fekadu, Centers for Disease Control, Lawrenceville Facility, P.O. Box 363, Lawrenceville, Georgia 30246.

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**Table 1.** Titers of rabies virus in the submaxillary glands (SG) of dogs inoculated im with an Ethiopian strain of rabies virus.

Inoculum (no. dead/ no. inoculated), dog no.	Incubation period (days)	Morbidity period (days)	Virus titer/g of SG tissue	
			Right SG	Left SG
5.8 (4/5)				
E-48	10	1	-	-
E-OB	10	1	-	-
E-40	10	*	-	-
E-OA	9	2	3.2	2.2
4.8 (4/5)				
E-44	13	3	-	-
E-49	11	1	-	-
E-11	11	4	-	-
E-41	21	4	-	2.1
3.8 (3/5)				
E-50	16	*	-	-
E-158	16	*	-	-
E-42	29	1	3.5	6.3
2.8 (4/4)				
E-4	24	*	<1	<1
E-135	16	7	4.9	4.5
E-3	23	4	4.3	4.6
E-19	27	2	5.3	4.5
1.8 (2/5)				
E-32	30	6	5.3	6.3
E-28	41	2	<1	3.0

NOTE. The inoculum consisted of 20% salivary glands from dogs that had been experimentally infected with an Ethiopian strain of rabies virus. The suspension was homogenized with a diluent of 0.75% bovine albumin in phosphate-buffered saline. The inoculum was then injected intracerebrally into weanling mice and titrated. The inoculum and the titer of rabies virus/g of SG tissue are expressed as the  $\log_{10}$  of the mouse intracerebral  $LD_{50}$ . Negative virus titer = (-).

\* The dog was found dead; no signs of disease were observed.

Saliva samples were immediately centrifuged at 1,000 g for 15 min and either injected intracerebrally into weanling mice [13] or frozen at  $-70^{\circ}\text{C}$  for future attempts to isolate the virus. All of the inoculated mice were observed daily for at least 30 days.

**Fluorescence microscopy.** Acetone-fixed touch impressions of the brain and spinal cord were stained with fluorescein isothiocyanate-conjugated horse antirabies globulin [14].

**Isolation of rabies virus.** To isolate the virus, we prepared a suspension of 20% infected brain and salivary gland tissues homogenized in phosphate-buffered saline and injected it intracerebrally into weanling mice [13].

## Results

The incubation periods in the rabies virus-inoculated dogs depended on the strain of virus used and the dose of the inoculum (nine to 41 days with

the Ethiopian strain and 12-69 days with the Mexican strain).

Seventeen (70.8%) of 24 dogs inoculated with the Ethiopian strain and 22 (95.7%) of 23 dogs injected with the Mexican strain died of rabies. Rabies virus was demonstrated by fluorescence microscopy and mouse inoculation tests of the brain tissue of all of the dogs that died. Both of the submaxillary glands were tested for the presence of rabies virus: the virus was isolated from the submaxillary glands of nine dogs (53%) inoculated with the Ethiopian strain (table 1) and from 16 dogs (73%) inoculated with the Mexican strain (table 2).

The presence of virus in the salivary glands of dogs inoculated with the Ethiopian strain depended mainly on the dose of the inoculum and the morbidity periods (table 1). A very short morbidity period (less than one day) correlated with a very low amount of or no virus in the salivary glands. The titer of virus in the salivary glands also appeared

**Table 2.** Titers of rabies virus in the submaxillary glands (SG) of dogs inoculated im with a Mexican strain of rabies virus.

Inoculum (no. dead/ no. inoculated), dog no.	Incubation period (days)	Morbidity period (days)	Virus titer/g of SG tissue	
			Right SG	Left SG
5.7 (5/5)				
M-17	12	2	-	-
M-13	15	2	-	-
M-16	14	2	-	-
M-19	12	2	-	2.2
M-15	15	2	2.7	2.2
4.7 (5/5)				
M-20	15	1	-	-
M-18	18	3	-	<1
M-36	14	3	<1	2.1
M-37	16	2	5.2	5.2
M-12	17	1	4.9	6.2
3.7 (5/5)				
M-31	29	5	6.0	6.1
M-11	20	5	4.2	4.9
M-63	21	3	-	-
M-33	22	2	5.9	6.0
M-52	19	2	3.9	4.3
2.7 (4/4)				
M-6	22	2	<1	-
M-26	37	5	<1	2.2
M-8	25	*	7.2	7.3
M-7	22	2	6.2	6.7
1.7 (3/4)				
M-1	36	4	4.3	5.3
M-5	47	0	4.3	6.4
M-4	69	0	-	-

NOTE. The inoculum consisted of 20% salivary glands from dogs that had been experimentally infected with a Mexican strain of rabies virus. The suspension was homogenized with a diluent of 0.75% bovine albumin in phosphate-buffered saline. The inoculum was then injected intracerebrally into weanling mice and titrated. The inoculum and the titer of rabies virus/g of SG tissue are expressed as the  $\log_{10}$  of the mouse intracerebral LD<sub>50</sub>. Negative virus titer = (-).

\* The dog was found dead; no signs of disease were observed.

to depend on the dose of the inoculum and the incubation and morbidity periods. Dogs that had been inoculated with low doses of the Ethiopian strain of rabies virus had longer incubation periods, higher titers of virus in the salivary glands, and higher levels of virus excretion in the saliva than dogs that had been inoculated with higher doses. The presence of virus in the salivary glands of dogs inoculated with the Mexican strain appeared to be less dependent on the dose of the inoculum and the incubation and morbidity periods (table 2).

The relationship between the excretion of rabies virus in the saliva and the onset of illness is shown in figure 1. The dogs excreted rabies virus in the saliva from one to 13 days before signs of disease were observed. Nine (53%) of the 17 dogs that

died of rabies after injection with the Ethiopian strain had virus in the salivary glands; six (66.6%) of nine dogs excreted virus in the saliva. No relationship was noted between the time that virus was excreted in the saliva and the incubation and morbidity periods. There appeared to be little relationship between the excretion of virus in the saliva and the titer of virus in the salivary glands of dogs inoculated with the Ethiopian strain (figure 1 and table 1). The time of virus excretion in the saliva before any clinical signs were observed ranged from 13 days before the onset of illness to three days after signs of disease were detected (dogs nos. E42, E3, E4, E32, E28, and E135) in dogs inoculated with the Ethiopian strain of rabies virus. Dogs nos. E42, E4, E32, and E28 excreted virus in the saliva as early as 13, five, five, and four days,



on salivary gland infection. Although it has been stated that the virus is not likely to be present in the saliva until a few days before an animal actually has clinical rabies [15], rabies virus has been reported in the saliva as long as seven days before signs of disease appeared [7]. A recent study indicated that this period was only three days [3]. It should be emphasized that neither of these two studies involved inoculating the animals with graded doses of rabies virus.

The World Health Organization Expert Committee on Rabies recommends that the biting animal, if healthy at the time of exposure, be observed for a period of 10 days while postexposure treatment of the person is considered [16]. If the biting animal becomes rabid during the 10 days of observation, the Committee recommends that rabies vaccine be administered to the person who has been bitten.

Healthy unvaccinated dogs may intermittently excrete rabies virus in their saliva for long periods [11, 12, 17]. Because of these findings, we have tried to simulate rabies as it occurs under natural conditions. We used two strains of rabies virus: one that had been isolated from the saliva of an apparently healthy dog in Ethiopia [11], and another from the salivary gland of a dog from an area in Mexico in which rabies is endemic. Our study demonstrated that the incubation periods and excretion of virus in the saliva of dogs experimentally infected depended not only on the dose of the virus inoculum but also on the strain of virus used. Dogs bitten by rabid animals may be expected to die of rabies within 21-60 days [15]; the incubation periods in our experiment were generally within this range (nine to 69 days), with some shorter periods. The proportion of infected submaxillary glands in experimentally or naturally infected dogs is 61%-75% [3, 17]. In our experiment, however, the presence of virus in the submaxillary glands was 25%-100%, depending on the strain and the dose of virus inoculum used.

The results of this and other studies [6, 12, 17] indicate that dogs can excrete rabies virus in the saliva for a long time without showing any signs of disease. On the basis of these findings, it may be necessary to observe prospectively rabid dogs for a longer period than that recommended by the World Health Organization Expert Committee on

Rabies. In countries in which rabies is endemic, selective sampling of saliva may also be advisable when the prospectively rabid dog can be identified and observed.

#### References

1. Schaaf, J., Schaal, E. Die Viruslokalisation bei Tollwut. D.T.W. 75:315-323, 1968.
2. Tierkel, E. Rabies in wild animals. *In* International Symposium on Rabies. Vol. 1. Karger, Basel, 1966, p. 245-250.
3. Vaughn, J. B., Gerhardt, P., Paterson, J. C. S. Excretion of street rabies virus in saliva of cats. J.A.M.A. 184: 705-708, 1963.
4. Vaughn, J. B., Gerhardt, P., Newell, K. W. Excretion of street rabies virus in the saliva of dogs. J.A.M.A. 193: 363-368, 1965.
5. Vaughn, J. B. Cat rabies. *In* G. M. Baer [ed.]. The natural history of rabies. Vol. 2. Academic Press, New York, 1975, p. 139-154.
6. Veeraraghavan, N. Studies on the salivary excretion of rabies virus by the dog from Surandi. Annual Report of the Director 1968 and Scientific Report 1969. Pasteur Institute of Southern India, Madras, 1970, p. 66.
7. Jonnesco, D., Teodosio, V. Passage du virus rabique dans les glandes sous-maxillaires chez le chien. Comptes-Rendus Hebdomadaires des Séances du Société de Biologie et Ses Filiales (Paris) 100:897-898, 1929.
8. Baer, G. M., Bales, G. L. Experimental rabies infection in the Mexican freetail bat. J. Infect. Dis. 117:82-90, 1967.
9. Bell, F. J., Moore, G. J., Raymond, G. H. Protracted survival of a rabies-infected insectivorous bat after infective bite. Am. J. Trop. Med. Hyg. 18:61-66, 1969.
10. Sikes, R. K. Pathogenesis of rabies in wildlife. 1. Comparative effect of varying doses of rabies virus inoculated into foxes and skunks. Am. J. Vet. Res. 23:1041-1047, 1962.
11. Fekadu, M. Atypical rabies in dogs in Ethiopia. Ethiop. Med. J. 10:79-86, 1972.
12. Fekadu, M. Asymptomatic non-fatal canine rabies [letter]. Lancet 1:569, 1975.
13. Koprowski, H. The mouse inoculation test. *In* M. M. Kaplan and H. Koprowski [ed.]. Laboratory techniques in rabies. 3rd ed. World Health Organization, Geneva, 1973, p. 85-93.
14. Goldwasser, R. A., Kissling, R. E. Fluorescent antibody staining of street and fixed rabies virus antigens. Proc. Soc. Exp. Biol. Med. 98:219-223, 1958.
15. Habel, K. Rabies prophylaxis in man. Pediatrics 19:923-936, 1957.
16. W.H.O. Expert Committee on Rabies. Prevention of rabies in man. W.H.O. Tech. Rep. Ser. 201:11-14, 1960.
17. Fekadu, M., Shaddock, J. H., Baer, G. M. Intermittent excretion of rabies virus in the saliva of a dog two and six months after it had recovered from experimental rabies. Am. J. Trop. Med. Hyg. 30:1113-1115, 1981.