LETTERS

Journal of Wildlife Diseases, 48(2), 2012, pp. 508–511

Extended Incubation Period of Rabies Virus in a Captive Big Brown Bat (*Eptesicus fuscus*)

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ABSTRACT: Rabies virus incubation in bats is typically less than 180 days, yet longer incubation periods have been described. We report a 267-day incubation in a big brown bat (*Eptesicus fuscus*) exposed to rabies virus before entering our captive colony.

The incubation of rabies virus in various domestic species has been well studied, providing the medical and veterinary community with recommendations for a 10-day observation period and a 6-mo quarantine for suspect dogs, cats, and ferrets (NASPHV, 2011). The incubation period in wildlife, such as bats, has not been as extensively researched. In zoological parks and research settings, bats may be routinely held from 30 days to 6 mo before their introduction to a public setting or use in research. Because incubation periods in bats are highly variable, the Compendium of Animal Rabies Prevention and Control recommends a 6-mo quarantine for all wild-caught mammals (Trimarchi, 1978; OIE, 2010).

On 30 January 2009, a colony of big brown bats (*Eptesicus fuscus*) was established at the Wadsworth Center, New York State Department of Health. The colony included adult, mixed-gender bats housed in groups of five. All bats were provided water and gut-loaded mealworms ad libitum. Bats were examined daily and weighed twice a week. On 24 October 2009, 267 days after entry into the captive colony, two adult female bats were observed fighting in their cage. Bat 2 was seen biting the face of bat 1 and immediately after, bat 1 attacked the feet of bat 2. The bats were removed and examined; puncture marks were clearly identified on both bats. Bat 1 appeared distressed and was moved to a separate cage, whereas bat 2 seemed calm and was placed back in the original cage. Rabies was suspected on the basis of the behavior of bat 1 and an oral swab was collected. Rabies was not suspected in bat 2, and an oral swab was not collected. The oral swab was placed in 500 µl of cell growth medium (Eagles Minimum Essential Media supplemented with 10% fetal bovine serum, 2.0 mM glutamate, and 100 IU penicillin G, 50 µg streptomycin, and 2.5 mg amphotericin B per milliliter) for virus isolation and reverse transcriptase polymerase chain reaction (RT-PCR). The sample was stored at −80 C. Two days after the sample was collected, 200 µl was inoculated into neuroblastoma cell culture for virus isolation, as previously described (Rudd and Trimarchi, 1987). RNA was extracted from the oral swab using 200 µl of sample added to Trizol LS reagent and processed per manufacturer's recommendations (Invitrogen, Carlsbad, California, USA). The cDNA was generated from extracted RNA as described in the Quanta qScript™ cDNA Synthesis Kit (Quanta BioSciences, Gaithersburg, Maryland, USA). A 400–base-pair region of the rabies virus N gene was amplified using the QIAGEN HotStarTaq DNA Polymerase PCR manufacturer's protocol (Qiagen, Germantown, Maryland, USA) using primers 21G (5′-ATGTAACACCCCTACAATG-3′) and 390 (5′-CTTGTCAACTCCATACC-3′ (Shankar et al., 2004).

Over the next 6 days, bats 1 and 2 continued to eat and drink normally and maintained steady weight. Although bat 1

TABLE 1. Incubation times for rabies virus in naturally infected, wild-caught bats.

Study	Year	Species (*n*)	Incubation (days)[a]	Duration of clinical signs (days)[b]	Virus present in oral swabs (day)[c]	Anti-rabies viral-neutralizing antibodies (VNA)
Davis, unpubl. data	2011	*Myotis lucifigus* (1)	85[d]	6	Yes (87)[f]	ND[h]
Davis et al., 2011	2009	*Eptesicus fusucs* (1)	267[d]	7	Yes (267)[f,g]	≤0.125 IU
Davis et al., in press	2004	*Eptesicus fusucs* (2)	132	1	No	≤0.125 IU[i]
			190[e]	1	No	≤0.125 IU[i]
Davis et al., 2005	2002	*Eptesicus fusucs* (1)	135	4	ND[h]	ND
Shankar et al., 2004	2001	*Eptesicus fusucs* (2)	28	4	ND	ND
			44[e]	2	Yes (36&44)[f]	280 IU
Moore and Raymond, 1970	1970	*Eptesicus fusucs* (1)	209	4	Yes (209–213)[g]	ND

[a] Days until clinical signs were first apparent.

[b] Clinical signs in these bats, such as conjunctivitis and decreased or increased activity while maintaining average weight and food and water consumption, were compatible with rabies but commonly seen in noninfected bats. Bats were euthanized when obvious clinical signs of rabies were apparent (i.e., anorexia, ataxia, aggression, unusual vocalization, or positive oral swab PCR).

[c] Day that oral swab was first positive during the incubation period.

[d] Number of days after the bat was removed from a hibernaculum.

[e] Fighting among cage mates was reported and was potentially the result of intracolony transmission.

[f] Oral swabs confirmed positive by PCR.

[g] Oral swabs confirmed positive by virus isolation.

[h] ND = not described.

[i] Based on serum samples taken when introduced into captive colony.

was more vocal when disturbed, no other suggestive clinical signs of rabies were noted. Seven days after bat 1 had been moved to a new cage, it became ataxic and anorexic. When hand-feeding was attempted, the bat became vocal and struggled to move away. The bat continued to decline and was euthanized 6 hr later. Serum was collected and tested for rabies virus neutralizing antibodies (VNA), as previously described (Trimarchi et al., 1996). Serum was also collected from the four cage mates the following week.

Immediately after euthanasia, brain tissue was removed and tested for rabies virus antigens via the direct fluorescent antibody test, using the U.S. National Standard Protocol (www.cdc.gov/rabies/pdf/RabiesDFASPv2.pdf). Rabies virus antigens stained brightly and were detected throughout the brain and in every field examined. Rabies virus was isolated from the oral swab that had been taken the day the bats were observed fighting and were separated, 267 days after the bats were brought into the captive colony and 7 days before euthanasia. Sequence analysis revealed the bat was infected with an *Eptesicus fuscus* rabies virus variant (GenBank 1423734). Serum was obtained from all bats upon entry; all were negative for VNA. Bat 1 did not seroconvert as indicated by its terminal titer of $\leq$0.125 IU. No rabies VNA was detected in the four surviving cage mates. All four cage mates, including bat 2, remained healthy for at least 1 yr after this case. The lack of VNA in bat 1 was not surprising. Although rabies virus–infected animals often seroconvert in the terminal phase of disease, many remain antibody-negative throughout the incubation and clinical phase of disease (Jackson et al, 2008).

The incubation period for the bat described in this report was at least 8 mo and 25 days. This bat was captured from a hibernaculum on 30 January 2009. Thus, it is possible that the bat was infected during the previous fall, before entry into hibernation. The bat continued to eat and drink and did not exhibit signs of abnormal behavior for 7 days after infectious rabies virus was isolated from saliva. The clinical period was acute, and the bat declined rapidly after the onset of clinical signs compatible with rabies virus infection.

Studies have demonstrated infectious rabies virus in saliva in experimentally inoculated animals. Bell et al. (1969) and Moreno and Baer (1980) reported the presence of infectious rabies virus in bat saliva within 24 to 216 hr of developing clinical signs.

In previous experimental studies of infected bats, the incubation time ranged from 7 to 140 days but was typically less than 30 days (Sétien et al., 1998; Jackson et al., 2008). In the few documented cases of naturally occurring rabies in wild bats, the incubation period ranged from 21 to more than 200 days after capture (Table 1). Despite the unpredictable incubation time observed in naturally acquired rabies virus infections, all bats that shed virus developed clinical signs of rabies virus infection. These observations, and the evidence described in this paper, underscore the concerns associated with quarantine and subsequent transportation of bats.

This work was supported by NIH grant 5R21AI081388-02 to M. Schnell at Thomas Jefferson University. We thank colleagues at the Wadsworth Center for helpful discussions and the Wadsworth Center Molecular Genetics Core. Animal studies were performed in compliance with the Wadsworth Center Institution Animal Care and Use Committee.

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Submitted for publication 8 May 2011.
Accepted 30 September 2011.