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

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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 6mo quarantine for suspect dogs, cats, and ferrets (NASPHV 2011 Compendium). The duration of 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 6mo, prior to their introduction to a public setting or use in research. Because incubation periods in bats appear to be highly variable, the Compendium of Animal Rabies Prevention and Control recommends a 6mo 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. An oral swab was collected from bat 1, but not bat 2, since rabies was suspected based on the behavior of bat 1. The oral swab was placed in 500 ul of cell growth media (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 ml) for virus isolation and reverse transcriptase-polymerase chain reaction (RT-PCR). The sample was stored at −80C. Two days after the sample was collected, 200ul was inoculated into neuroblastoma cell culture for virus isolation, as previously described (Rudd and Trimarchi, 1987). RNA was extracted from the oral swab using 200ul of sample added to Trizol LS reagent and processed per manufacturer's recommendations (Invitrogen, Carlsbad, California). The cDNA was generated from extracted RNA as described in the Quanta qScript ™ cDNA Synthesis Kit (Quanta BioSciences, Gaithersburg, Maryland). 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) using primers 21G (5'-

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ATGTAACACCCCTACAATG-3') and 390 (5'-CTTGTCAACTCCATACC -3' (Shankar et al., 2004).

Over the next six days, bats 1 and 2 continued to eat and drink normally and maintained a steady weight. Although bat 1 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 6hr 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 following euthanasia, brain tissue was removed and tested for the presence of rabies virus antigens via the direct fluorescent antibody test (DFA), using the U.S. National Standard Protocol, as previously described (www.cdc.gov/rabies/pdf/RabiesDFASPv2.pdf). Rabies virus antigens stained brightly, 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 to be fighting and were separated, 267 days after the bats were brought into the captive colony and seven days prior to euthanasia. Sequence analysis revealed the bat was infected with an *Eptesicus fuscus* rabies virus variant (Genbank accession no. 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 <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 one year after the report of 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 seronegative throughout the incubation and clinical phase of disease (Jackson et al, 2008).

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

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

Rabies incubation periods in bats are highly variable. In previous experimental studies of infected bats, the incubation time ranged from 7 days to 140 days but was typically less than 30d (Sétin et al., 1998; Jackson et al., 2008). In a few documented cases of naturally occurring rabies in wild bats, the incubation period ranged from 21 days after capture to more than one year. 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.

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Table 1

Incubation times in naturally infected wild caught bats.

Study	Year	Species (no.)	Incubation in days [a]	Duration of clinical signs in days [b]	Virus present in oral swabs [c]	Anti-rabies viral neutralizing antibodies (VNA)
Davis, A	2011	*Myotis lucifigus* (1)	85[d]	6	Y (87)[f]	ND
Davis et al, 2011	2009	*Eptesicus fusucs* (1)	269[d]	7	Y (267)[f,g]	<0.125 IU
Davis et al, in press (2011)	2004	*Eptesicus fusucs* (2)	132 190[e]	1 1	N N	<0.125 IU[h] <0.125 IU[h]
(Davis et al, 2005)	2002	*Eptesicus fusucs* (1)	135	4	ND	ND
Shankar et al (2004)	2001	*Eptesicus fusucs* (2)	28 44[e]	4 2	ND Y (36&44)[f]	ND 280 IU
Trimarchi Personal communication	1978	*Eptesicus fusucs*(1)	365[e]	*	ND	ND
Moore and Raymond (1970)	1970	*Eptesicus fusucs* (1)	209	4	Y (209–213)[g]	ND

[a] Number denotes day clinical signs were first apparent.

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

[c] (Number) denotes when the day oral swabs were first positive during the incubation period

[d] Denotes the number of days after the bat removed from a hibernacula.

[e] Fighting among cage mates was reported and are potentially the result of intra colony transmission

[f] Oral swabs were confirmed positive via PCR

[g] Oral swabs were confirmed positive via virus isolation

[h] Based on serum samples taken when introduced into the captive colony

* Not described