

RABIES PREVALENCE IN MIGRATORY TREE-BATS IN ALBERTA AND THE INFLUENCE OF ROOSTING ECOLOGY AND SAMPLING METHOD ON REPORTED PREVALENCE OF RABIES IN BATS

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ABSTRACT: The migratory tree-roosting hoary bat (*Lasiurus cinereus*) and silver-haired bat (*Lasionycteris noctivagans*) are among the bat species with the highest reported prevalence of rabies in North America. However, bats submitted for rabies testing typically have been those that have come in contact with humans or pets. Given the roosting ecology of *L. cinereus* and *L. noctivagans*, contact with healthy individuals of these species is expected to be rare, with a bias in contact and submission of infected individuals and thus an overestimation of rabies prevalence. We tested 121 *L. cinereus* and 96 *L. noctivagans* specimens, collected during mortality surveys at wind energy facilities in Southern Alberta, Canada in 2007 and 2008, for rabies. None of the *L. cinereus* (0%) and one *L. noctivagans* (1%) tested positive for rabies. Prevalence of rabies was significantly lower than previously reported estimates, passive and active, for *L. cinereus* and *L. noctivagans*. In a review of the literature including multiple bat species, we found a significant difference in estimates of rabies prevalence based on passive versus active surveillance testing. Furthermore, roosting ecology influenced estimates of rabies prevalence, with significantly higher prevalence among passive surveillance submissions of nonsynanthropic species compared to synanthropic species, a trend not evident in active surveillance reports. We conclude that rabies prevalence in randomly collected *L. cinereus* and *L. noctivagans* is low and comparable to active surveillance estimates from other species ($\leq 1\%$), and that roosting ecology influences estimates of rabies prevalence among bats submitted to public health laboratories in North America.

Key words: Chiroptera, epidemiology, hoary bat, *Lasionycteris noctivagans*, *Lasiurus cinereus*, rabies surveillance, silver-haired bat, turbine-related fatalities.

INTRODUCTION

Rabies is an acute progressive viral encephalomyelitis that affects mammals on all continents except Antarctica (Nel and Markotter, 2007). The first reports of rabies in North America occurred in the early 18th century in association with a high number of wild dogs (Baer, 1994). Although there is no cure for rabies (Takayama, 2008), increased education and improved diagnostics (Woldehiwet, 2005), combined with safe and effective vaccines, have resulted in a dramatic reduction in the annual number of human rabies cases and effective control of rabies among domestic animals and wildlife in North America (Smith, 1996). However, bats are natural reservoirs of rabies viruses (Kuzmin and Rupprecht, 2007), and historic and contemporary spillover events present a formidable challenge to rabies control efforts (Leslie et al.,

2006; Blanton et al., 2009). Old World bats are reservoirs for 10 of the 11 *lyssavirus* genotypes, with the exception being Mokola virus (Kuzmin and Rupprecht, 2007). New World bats are reservoirs of rabies virus (RABV, genotype 1) with evidence of species-specific variants (Smith et al., 1995), which suggests that enzootic foci in bats are maintained by intraspecific transmission, although a recent study highlights significant cross-species transmission of rabies among some species of North American bats (Streicker et al., 2010). Many RABV variants have bat-related origins (Smith et al., 1995; Davis et al., 2006) and following improved vaccination regulations and the elimination of dog rabies in North America (Velasco-Villa et al., 2008), bat-associated variants of the virus are the foremost source of rabies in reported human cases in North America (Messenger et al., 2002; Blanton et al., 2009).

Rabies virus was initially discovered in a fruit bat (*Artibeus lituratus*) in Brazil (Haupt and Rehaag, 1921) and later from several species of phyllostomid bats in Trinidad (Pawan, 1936), but has been reported in insectivorous bats in North America since 1953 (Venters et al., 1954). Subsequently, increased awareness and surveillance revealed rabies infection in the majority of insectivorous bat species throughout the USA and Canada (Constantine, 1979). Given the nocturnal and elusive nature of bats, the disease is difficult to study in natural populations and many gaps remain in understanding the epizootiology of rabies in bats. Estimates of rabies prevalence in North American bats have been highly variable among species and across years within species (e.g., Pybus, 1986; Mondul et al., 2003). Fluctuations in rates may be an indication of the frequency of human contact with infected individuals, rather than the prevalence of the virus (Trimarchi, 1978; Baer and Smith, 1991; Rupprecht et al., 1995). The majority of bats submitted for testing are those that come in contact with humans or pets, which is a peculiar occurrence for healthy (i.e., normally behaving) bats. This may result in a bias in sampling, with rabid bats more likely to exhibit atypical behaviors, and thus more likely to be submitted for testing (Pool and Hacker, 1982; Pybus, 1986). In addition, a dilution effect, or negative correlation between the number of individuals submitted for testing and the proportion found to be infected has been previously suggested (Pybus, 1986).

Rabies testing requires euthanasia of the animal (Woldehiwet, 2005), and it is often difficult to capture sufficient numbers of animals to estimate prevalence accurately. This is especially true with migratory tree-roosting bats, which often occur alone or in small colonies and are difficult to capture while roosting due to the inaccessibility of their roosts (i.e., high among leaves or deep within trees). In addition, hoary bats (*Lasiurus cinereus*)

and silver-haired bats (*Lasionycteris noctivagans*) are migratory, resulting in a broad and variable geographic range, and are high, fast flyers, making capture of free-flying individuals difficult. Few studies have estimated rabies prevalence among random samples of migratory tree-roosting bats, and the most recent reports are from the 1960s (Constantine, 1966, 1967a).

With the rapid development of wind energy facilities in North America (American Wind Energy Association [AWEA], 2010; Canadian Wind Energy Association, 2010 [CWEA]), large numbers of bat fatalities have been reported at some facilities (Dürr and Bach, 2004; Johnson, 2005; Arnett et al., 2008). In North America, these fatalities primarily involve migratory, tree-roosting bats (Johnson, 2005; Arnett et al., 2008; Baerwald and Barclay, 2009). At wind energy sites in Southern Alberta, *L. cinereus* and *L. noctivagans* comprise the majority (>90%) of fatalities (Baerwald et al., 2009). Although there is great concern over the ecologic impact of these fatalities, the situation provides the impetus and the means to test a large number of randomly selected individuals for rabies in species that are difficult to capture.

Our objective was to estimate and compare rabies prevalence in *L. cinereus* and *L. noctivagans* obtained from carcasses collected at wind energy facilities in Southern Alberta to prevalence estimates reported in the literature. We hypothesized that reported rabies prevalence estimates for these two species from passive surveillance would be higher than estimates from randomly collected individuals in the wild. We also surveyed the literature to test the hypothesis that prevalence of infection is generally higher in passive versus active collections of bats, and draw conclusions about sampling method and roosting ecology as confounding factors in determining accurate estimates of rabies prevalence in wild populations of bats. The roosting ecology of bat

species often is overlooked when reporting and interpreting rabies prevalence and we predicted that nonsynanthropic species have lower submission rates and higher reported rabies rates than bat species that frequently roost in proximity to humans.

MATERIALS AND METHODS

Sample collection

We collected bat carcasses during mortality surveys conducted at three wind energy facilities in 2007 and 2008. All three facilities were in mixed agricultural and native grasslands within 20 km of Pincher Creek, Alberta, Canada (49°29'N, 113°56'W). We conducted carcass searches at two sites containing a total of 41 turbines from 15 July through 30 September 2007 and 2008. We searched all turbines once a week, but in 2007 we also searched a subset of 10 turbines daily (Baerwald and Barclay, 2009). When a bat carcass was discovered, we determined species, sex, and age (subadult or adult based on degree of wing-joint ossification; Anthony, 1988), as well as the approximate date of death based on condition of the carcass (Baerwald et al., 2008). Additional carcasses came from mortality surveys conducted by an ecologic assessment team on a neighboring site in summer 2008.

Detection of rabies virus antigen

We submitted a subsample of 217 bat carcasses to the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA for rabies diagnosis. We tested brain stem impressions for presence of RABV antigen by the direct fluorescent antibody (DFA) test (Dean et al., 1996), with the use of fluorescein isothiocyanate (FITC) -labeled monoclonal antibody conjugate (Fujirebio Diagnostics, Inc., Malvern, Pennsylvania, USA).

Partial amplification and sequencing of the nucleoprotein gene

Following detection of RABV antigen by DFA, we extracted total RNA from brain tissue with the use of TRIzol (Invitrogen, Carlsbad, California, USA), according to the manufacturer's instructions. We produced complementary DNA by RT-PCR, with the use of primers 304 sense and 1066 antisense, as described previously (Smith, 2002). We amplified a partial (264-nt) fragment of the RABV nucleoprotein gene (SAD B19 position 1157 to 1420; Conzelmann et al., 1990; De Mattos et al., 1999; Velasco-Villa et al., 2005),

which we then sequenced on an ABI 3730 DNA capillary sequence analyzer.

Viral isolation and titration

We grew monolayer cultures of mouse neuroblastoma (MNA) cells at 37 C in Eagle's minimum essential medium (Gibco Laboratories Life Technologies, Inc., Grand Island, New York, USA). We prepared a 20% brain suspension in phosphate-buffered saline (PBS), supplemented with 2% fetal calf serum, and clarified by centrifugation (500XG) for 15 min. We inoculated the virus supernatant (100 μ l) into suspensions containing (2×10^6) MNA cells plated in 96-well plates at serial 10-fold dilutions and incubated at 37 C at 0.5% CO₂ and 90% humidity for 40 hr. Following incubation, we fixed cell monolayers in 80% acetone at -20 C for 1 hr and visualized RABV antigen by DFA. We carried out all titrations in triplicate and calculated median tissue culture infective dose (TCID₅₀) as described by Reed and Muench (1938).

Literature review

We searched both peer-reviewed and government reports for reported rabies prevalence in bats in North America. We compiled rabies rates for passive surveillance from public health records spanning the past 56 yr and obtained many active-surveillance estimates from a review by Constantine (1967a). For statistical comparisons, we only considered reports that identified bats to species and included the total number of individuals of each species tested. Although misidentification of bat species is possible, the large sample size used in our analysis should make any statistical effects negligible. To compare our estimates of rabies prevalence among *L. cinereus* and *L. noctivagans*, we used unpublished data from the CDC as well as surveillance reports published in the United States and Canada for these two species (Table 1).

Statistical analyses

We used chi-square or Fisher's exact tests to compare estimates of rabies prevalence from this study with estimates from the literature statistically. For the larger comparative analysis of rabies prevalence, we used data from all sources that reported rabies prevalence by species and included the number of submissions. Despite trying several data transformations, prevalence data could not be normalized. We used a generalized linear model to investigate the effect of sampling method (passive versus active surveillance), and roost-

TABLE 1. Rabies prevalence estimates used in our analyses. Bat species are listed by common name. Rabies data shown are summed for each species from the sources listed. Letters denote the use of sources for passive- or active-surveillance numbers (a and b, respectively) and roosting ecology (c).

Common name (species)	Passive submissions			Active submissions			Prevalence (%)	Roosting ecology synanthropic	Sources ^a		
	Submitted	Positive	Prevalence (%)	Submitted	Positive	Prevalence (%)			a	b	c
Allen's big-eared bat (<i>Idionycteris phyllotis</i>)	1	0	0.00	—	—	—	—	No	40		3,18,1
Big brown bat (<i>Eptesicus fuscus</i>)	28,905	1695	5.86	1,146	26	2.27	2.27	Yes	47,4,50,55,48,45,44,8,11,43,37	45,48,50,5,16,19,20,23,28,58,59,62,71,61	3,60,35,41,1
Big free-tailed bat (<i>Myotis macrotis</i>)	14	3	21.43	31	0	0.00	0.00	No	40	15	3,39,1
Brazilian free-tailed bat (<i>Tadarida brasiliensis</i>)	975	235	24.10	5,363	31	0.58	0.58	Yes	47,43,40	61,49,13,54,71	3,70,1
California leaf-nosed bat (<i>Macrotus californicus</i>)	4	0	0.00	—	—	—	—	Yes	40		3,1
California myotis (<i>Myotis californicus</i>)	483	21	4.35	21	0	0.00	0.00	Yes	44,40	23,5,20,71,	3,53,46,1
Cave myotis (<i>Myotis velifer</i>)	2	0	0.00	285	2	0.70	0.70	No	40	20,17,71	3,26,1
Eastern pipistrelle (<i>Perinyotis subflavus</i>)	168	25	14.88	374	2	0.53	0.53	No	47,4,1,43,40	61,49,19,62,71	3,27,60,1
Eastern red bat (<i>Lasiurus borealis</i>)	2,405	257	10.69	282	4	1.42	1.42	No	47,4,55,8,11,43,40,37	61,23,49,14,16	3,51,60,41,1
Eastern small-footed bat (<i>Myotis leibii</i>)	44	0	0.00	—	—	—	—	Yes	4,50,40		3,6
Evening bat (<i>Nycticeius humeralis</i>)	901	25	2.77	244	0	0.00	0.00	Yes	47,8,43,40	61,49	3,65,60
Fringed myotis (<i>Myotis thysanodes</i>)	4	0	0.00	21	0	0.00	0.00	Yes	40	23,20,71	3,42,1
Gray bat (<i>Myotis grisescens</i>)	3	0	0.00	281	1	0.36	0.36	No	47	49	3,21
Hoary bat (<i>Lasiurus cinereus</i>)	1,095	259	23.65	182	2	1.10	1.10	No	47,4,50,55,48,44,8,11,43,40,37,10	23,49,14,16,56	3,52,60,41,1
Indiana bat (<i>Myotis sodalis</i>)	3	0	0.00	—	—	—	—	No	40		3,57
Keen's myotis (<i>Myotis keenii</i>)	1,562	32	2.05	—	—	—	—	No	47,4,55,44,8,11,40		25

TABLE 1. Continued.

Common name (species)	Passive submissions			Active submissions			Roosting ecology synanthropic	Sources ^a			
	Submitted	Positive	Prevalence (%)	Submitted	Positive	Prevalence (%)		Sources ^a			
								a	b	c	
Lesser long-nosed bat (<i>Leptonycteris curasoae</i>)	15	0	0.00	-	-	-	No	40			1,12
Little brown bat (<i>Myotis lucifugus</i>)	10,050	142	1.41	2,235	22	0.98	Yes	4,50,55,48,45,44, 8,11,40,37	23,5,19,62,28,22, 59,48,45		3,24,60,41,1
Long-legged myotis (<i>Myotis volans</i>)	27	3	11.11	23	0	0.00	Yes	50,44,40	23,20		3,63,60,41,1
Mastiff bat (<i>Eumops perotis</i>)	5	0	0.00	-	-	-	Yes	40			3,7,1,
Mexican long-tongued bat (<i>Choeronycteris mexicana</i>)	14	0	0.00	-	-	-	No	40			3,67,1
Northern long-eared bat (<i>Myotis septentrionalis</i>)	7	0	0.00	-	-	-	No	50,37			9,1
Northern yellow bat (<i>Lasiurus intermedius</i>)	40	10	25.00	717	20	2.79	No	43,40	61,49		3,68
Pallid bat (<i>Antrozous pallidus</i>)	102	21	20.59	52	0	0.00	No	44,40	23,71		29,60,41,1
Pocketed free-tailed bat (<i>Nyctinomops femorosaccus</i>)	53	7	13.21	-	-	-	No	40			3,32,1,
Rafinesque's big-eared bat (<i>Corynorhinus rafinesquii</i>)	5	0	0.00	-	-	-	No	47,43			31
Seminole bat (<i>Lasiurus seminolus</i>)	150	18	12.00	846	6	0.71	No	47,43,40	61,49		3,69
Silver-haired bat (<i>Lasiurus teris noctivagans</i>)	2,474	199	8.04	105	1	0.95	No	47,4,50,55,48,45,44, 8,11,43,40,37,10	23,19,16,56		3,34,60,41,1
Southeastern myotis (<i>Myotis austroriparius</i>)	9	1	11.11	2,127	1	0.05	Yes	47,43	61,49		3,30
Southern yellow bat (<i>Lasiurus ega</i>)	32	7	21.88	-	-	-	No	40			3,36
Southwestern myotis (<i>Myotis auricularis</i>)	9	0	0.00	-	-	-	Yes	40			3,64,1

TABLE 1. Continued.

Common name (species)	Passive submissions			Active submissions			Roosting ecology synanthropic	Sources ^a		
	Submitted	Positive	Prevalence (%)	Submitted	Positive	Prevalence (%)		a	b	c
Spotted bat (<i>Eudermia maculatum</i>)	1	1	100.00	—	—	—	40	—	—	3,66,60,41,1
Townsend's big-eared bat (<i>Corynorhinus townsendii</i>)	33	3	9.09	51	2	3.92	No	44,40	23,20,16,71	3,33,60,41,1
Waterhouse's leaf-nosed bat (<i>Macrotus waterhousii</i>)	—	—	—	234	1	0.43	No	—	20,16	2,3
Western long-eared myotis (<i>Myotis evotis</i>)	255	25	9.80	28	0	0.00	No	44,40	23,5,20,	3,60,38,41,1
Western pipistrelle (<i>Parastrellus hesperus</i>)	193	41	21.24	70	0	0.00	No	40	23,71	3,1
Yuma myotis (<i>Myotis yumanensis</i>)	274	5	1.82	61	0	0.00	Yes	44,40	23,5,71	3,60,41,1
Total	50,317	3,035	6.03	14,779	121	0.82				

^a 1 = Adams (2003), 2 = Anderson (1969), 3 = Barbour and Davis (1969), 4 = Beauguard (1969), 5 = Bell et al. (1957), 6 = Best and Jennings (1997), 7 = Best et al. (1996), 8 = Burnett (1989), 9 = Caceres and Barclay (2000), 10 = CDC (unpubl. data), 11 = Childs et al. (1994), 12 = Cole and Wilson (2006), 13 = Constantine et al. (1968), 14 = Constantine (1959), 15 = Constantine (1961), 16 = Constantine (1967a), 17 = Constantine (1967b), 18 = Czaplewski (1983), 19 = Daniels et al. (1960), 20 = Dean et al. (1960), 21 = Decher and Choate (1995), 22 = Dorward et al. (1977), 23 = Enright et al. (1955), 24 = Fenton and Barclay (1980), 25 = Fitch and Shump (1979), 26 = Fitch et al. (1981), 27 = Fujita and Kunz (1984), 28 = Girard et al. (1965), 29 = Hermanson and O'Shea (1983), 30 = Jones and Manning (1989), 31 = Jones (1977), 32 = Kumirai and Knox Jones (1990), 33 = Kunz and Martin (1982), 34 = Kunz (1982a), 35 = Kurta and Baker (1990), 36 = Kurta and Lehr (1995), 37 = Liesener et al. (2006), 38 = Manning and Knox Jones (1989), 39 = Milner et al. (1990), 40 = Mondul et al. (2003), 41 = Nagorsen and Brigham (1993), 42 = O'Farrell and Studier (1980), 43 = Parker et al. (1999), 44 = Prins and Lowen (1988), 45 = Pybus (1986), 46 = Racey and Entwistle (2000), 47 = Richardson et al. (1966), 48 = Rosatte (1985), 49 = Schneider et al. (1957), 50 = Schowalter (1980), 51 = Shump and Shump (1982a), 52 = Shump and Shump (1982b), 53 = Simpson (1993), 54 = Steece and Altenbach (1989), 55 = Steece et al. (1982), 56 = this study, 57 = Thomson (1982), 58 = Tjalma and Wentworth (1957), 59 = Trimarchi and Debbie (1977), 60 = van Zyll de Jong (1985), 61 = Venters et al. (1954), 62 = Verts and Barr (1961), 63 = Warner and Czaplewski (1984), 64 = Warner (1982), 65 = Watkins (1972), 66 = Watkins (1977), 67 = Webster and Knox Jones (1985), 68 = Webster et al. (1980), 69 = Wilkins (1987), 70 = Wilkins (1989), 71 = Yancey et al. (1997).

ing ecology (synanthropic versus nonsynanthropic) on rabies prevalence, with the number of specimens tested as a covariate to control for sample size. We considered passive surveillance to be testing of bats submitted by the public, and active surveillance to be testing of randomly surveyed populations. Based on published species accounts (Table 1), we considered synanthropic bat species to be those known to commonly roost in human structures, such as occupied buildings and bridges, and nonsynanthropic bat species to be those that primarily roost in natural structures, such as trees, caves, and rock crevices. We analyzed the data as a negative binomial distribution and removed nonsignificant interactions. In addition, we used a chi-square test to compare the number of submitted and positive individuals by roosting ecology to determine if nonsynanthropic bats comprised a disproportionate number of positive cases. Prevalence estimates represent pooled data for each species. We conducted all statistical analyses with the use of SAS v9.0 (SAS Institute, Cary, North Carolina, USA).

RESULTS

During the 2-yr study, 993 bat carcasses of five species were recovered at our study sites (Baerwald, 2008). We deemed 121 *L. cinereus* and 96 *L. noctivagans* carcasses to be in suitable condition for rabies testing. Rabies testing yielded a single positive result in a subadult *L. noctivagans* and was typed as a silver-haired bat variant (SHBRV). The titer of the silver-haired bat RABV isolate was $10^{3.25}$ TCID₅₀/ml. Rabies prevalence in our sample of *L. cinereus* was 0% (0 of 121), and 1% (1 of 96) in *L. noctivagans*. For both species, prevalence was lower ($\chi^2_1 = 26.77$, $P < 0.0001$ and $\chi^2_1 = 4.83$, $P = 0.0280$ for *L. cinereus* and *L. noctivagans*, respectively) than the combined estimates of prevalence reported from passive surveillance (23.7%, $n = 1,095$ and 8.0%, $n = 2,474$ for *L. cinereus* and *L. noctivagans*, respectively). However, the prevalence estimates in this study did not differ (Fisher's exact test, $P = 0.1160$ and $P = 0.9151$ for *L. cinereus* and *L. noctivagans*, respectively) from previous estimates for these two species (3%, $n = 61$ and 0%, $n = 9$ for

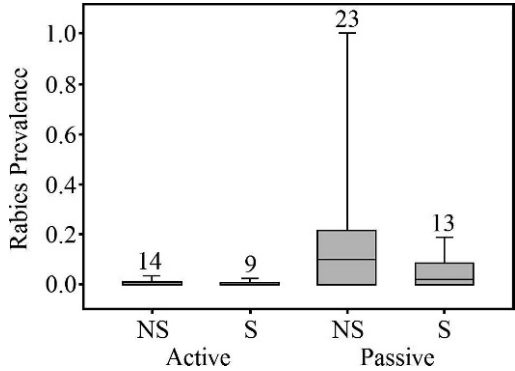


FIGURE 1. Box plot of rabies prevalence among bats with different roosting ecology and sampling method. Sample size, the number of species in each group, is indicated above each box. NS = nonsynanthropic bat species, S = synanthropic bat species.

L. cinereus and *L. noctivagans*, respectively) based on active-surveillance studies (Table 1).

The model assessing the influence of sampling method and roosting ecology on reported rabies prevalence explained a significant amount of the variation in the number of rabies positive bats ($\chi^2_5 = 41.61$, $P < 0.0001$). Rabies prevalence estimates based on passive surveillance (6.0%, $n = 50,317$) were higher than estimates from active surveillance (0.8%, $n = 14,779$) across all bat species ($\chi^2_1 = 8.85$, $P = 0.0029$). Nonsynanthropic bat species had higher rabies prevalence (7.8%, $n = 12,165$) than synanthropic species (4.2%, $n = 52,931$; $\chi^2_1 = 5.50$, $P = 0.0191$). Although the interaction between roosting ecology and sampling method was not significant ($\chi^2_1 = 0.18$, $P = 0.6734$), nonsynanthropic passively surveyed bats showed a trend toward higher, more variable rabies prevalence estimates ($13.6 \pm 4.4\%$, $n = 23$) compared to other categoric levels of the interaction term (Fig. 1). Roosting ecology also had a significant effect on the number of submissions ($\chi^2_1 = 12.67$, $P = 0.0004$), with nonsynanthropic bat species being underrepresented (18.7%, $n = 12,165$) in the total number of submissions for rabies testing ($n = 65,096$), but comprising a disproportionately high proportion (30.1%, $n = 949$)

of the total positive cases ($n=3,156$; $\chi^2_1=156.72$, $P<0.0001$).

DISCUSSION

As predicted, our estimates of rabies prevalence were lower than those reported for *L. cinereus* and *L. noctivagans* from passive surveillance. This supports our hypothesis that most previously reported prevalence estimates for these two species are overestimates due to a bias in sampling. Although it has been suggested that this sampling bias is due to low submission numbers (Pybus, 1986), our data indicate that biases are also due to sampling method, an idea that has been widely discussed (Pool and Hacker, 1982; Constantine, 1988; Baer, 1991; Baer and Smith, 1991; Rupprecht et al., 1995) but not statistically tested. The percent of rabies-suspect bats submitted to CDC that are positive for rabies is typically 9–10% (Blanton et al., 2006, 2007, 2008, 2009; Constantine, 2009), but bats actively sampled from their natural habitats consistently show markedly lower rabies prevalence (Table 1). Constantine (1967a) reported rabies prevalence estimates from surveys in the United States that randomly tested individuals of several bat species, including several *Myotis* species and a small number of migratory, tree-roosting species. Prevalence estimates were typically <1%, relatively low compared to passive surveillance sampling. Indeed, our rabies prevalence estimates were low and consistent with those reported in other studies based on randomly surveyed *L. cinereus* and *L. noctivagans*.

Bats have adapted to roost in a wide variety of structures. Some species prefer natural roosts such as trees, caves, and rock crevices, whereas others have adapted to roost in anthropogenic structures, such as buildings and bridges (Kunz, 1982b). The result is increased exposure of some bat species to humans. Sampling for rabies testing has largely been dependent on bats coming in contact with

humans, an occurrence largely governed by the roosting behaviors of different bat species. Considerably higher rabies prevalence estimates are consistently reported for nonsynanthropic species, such as *L. cinereus* (20.1%), when compared to synanthropic species, such as *Eptesicus fuscus* (5.7%). Our results support the idea that high rabies prevalence estimates in some bat species are partly due to a bias in sampling resulting from the difference in roosting ecology. Species that tend to roost synanthropically in large colonies likely encounter the public more frequently than nonsynanthropic, tree-, cave-, or crevice-roosting species (Pool and Hacker, 1982), resulting in the submission not only of a higher total number of individuals, but also of a larger proportion of healthy individuals (Schowalter, 1980). Conversely, solitary species that typically do not roost in proximity to humans are less likely to encounter the public unless sick, resulting in the submission of fewer individuals and the predisposed submission of infected individuals.

Maintenance of RABV in natural populations of bats is likely influenced by the ecology and sociality of reservoir species. Given the small colony sizes or solitary lifestyle of most nonsynanthropic tree-roosting bats, such as the lasiurines, there may be differences in transmission dynamics when compared to more gregarious species, due to less opportunity for intracolony transfer between individuals. In large colonies, bats roost in close proximity to one another, increasing the opportunity for a rabies-infected individual to bite and infect conspecifics. Fission–fusion societies, in which individuals move among roosting groups, have been described in several bat species (Willis and Brigham, 2004; Garroway and Broders, 2007; Metheny et al., 2008) and may provide a mechanism for transmission between roosts in colonial species. In addition, multiple bat species have been found roosting together (Schowalter, 1980; Constantine, 2009), and geographic

range overlap has been shown to be an important predictor of cross-species rabies transmission (Streicker et al., 2010). It could be argued that for solitary mammals, such as *L. cinereus*, opportunity for the virus to spread in a population would be limited. However, results from our survey and other surveys of natural populations of bats suggest rabies prevalence around or below 1% across all bat species, regardless of roosting ecology.

It has been suggested that colonial bats tend to show dumb or paralytic symptoms of rabies more often than furious symptoms, with the opposite trend in solitary species (Kuzmin and Rupprecht, 2007). From the perspective of the virus, this would be adaptive; furious symptoms cause the infected individual to seek out other individuals, which may be necessary for the spread of the virus from a solitary host (Constantine, 1967a). There have been accounts of possibly rabid *L. cinereus* attacking and biting other bat species in mid-air (Bishop, 1947; Orr, 1950; Bell, 1980). Conversely, close roosting proximity of colonial species likely allows rabies transmission even in the absence of aggressive symptoms (Constantine, 1988; Kuzmin and Rupprecht, 2007; Constantine, 2009). However, we suggest that the observed difference in clinical signs between solitary and colonial bats may again be influenced by roosting ecology. Solitary individuals showing dumb or paralytic symptoms would most likely expire at their roost and not be encountered by humans, whereas synanthropic individuals are more likely to be encountered and documented. Regardless of the clinical signs of infection, RABV is enzootic across multiple solitary and colonial bat species.

Although we have assumed that using bats killed by wind turbines provides a random sample and is less biased than using animals submitted for testing, our samples may exclude animals manifesting paralytic symptoms of the disease. In the later stages of the disease, it is possible that infected animals may be unable to fly

and would therefore not be available in the sample obtained from a population migrating through a wind energy facility. Similarly, collection of free-flying bats may not capture similar numbers of sick (e.g., moribund) bats compared to collection of roosting bats; it is much more likely that one would find sick animals unable to fly by the latter method. We also recognize that there may be temporal differences in rabies prevalence when estimates from half a century ago are compared with contemporary estimates. Many early diagnostic reports in the 1950s and 1960s were based on the presence of Negri bodies to confirm RABV, but it is now well recognized that Negri bodies may not always be observed in rabid animals (Jogai et al., 2000). With the increased sensitivity of the fluorescent antibody test for rabies diagnostics, it is possible that estimates of prevalence would be slightly higher than older reports. However, given the extensive collection and temporal representation of reports included in our study, it is unlikely that this is a significant confounding factor in the comparisons or interpretation.

Reports of high numbers of bat fatalities at some wind energy facilities have raised concerns about the ecologic impact on bat populations (Kunz et al., 2007), but little attention has been given to impacts on the surrounding ecosystem. An influx of bat carcasses could be used by scavenging animals as a food source (Klug and Barclay, 2008). Rabies virus remains viable in carrion for some time (Schaefer, 1983), and there may be a risk of disease transmission to mammalian scavengers by way of ingestion of infected tissue (Soave, 1966; Correa-Giron et al., 1970; Ramsden and Johnston, 1975). Live bats have been recovered during mortality surveys (Klug and Baerwald, 2010); if infected with rabies, these individuals have the ability to bite and infect potential scavengers. A number of factors suggest the probability of transmission may be low, including the low rabies prevalence among grounded bats in this study, a low occurrence of

mammalian scavenging, and environmental conditions that favor denaturation of the virus (Klug and Barclay, 2008), in addition to the rarity of reported outbreaks resulting from interspecific spillover of the virus (Winkler, 1975; Krebs et al., 2002; Kuzmin and Rupprecht, 2007). However, typing of the RABV variant is not routinely conducted among rabid animals submitted to public health laboratories, and it is possible that many spillover events between bats and other wildlife remain undetected. Differences in scavenger species, scavenging rate, and environmental conditions between high-fatality wind energy sites may influence the risk of rabies exposure, and further investigation may be necessary.

Estimates of rabies prevalence in the passive surveillance literature are highly variable among years, surveys, and bat species. It has been recognized that passive surveillance estimates are not an accurate reflection of the natural infection dynamics and prevalence of rabies in wild populations of bats, and that small sample size often hinders accurate rabies surveillance (Pybus, 1986). However, other variables contributing to this variation have not been statistically investigated. Much of this variation is likely due to the frequency of public contact with bats, a rare but variable occurrence, and we have shown that roosting ecology and sampling method are important factors. Although we conclude that rabies prevalence in bat populations is lower than is often implied from passive surveillance, the data suggest that bats atypically coming in contact with humans show a higher incidence of rabies infection compared to random samples, which is a public health concern. Therefore, rabies prevalence estimates from publicly submitted samples (passive surveillance) remain appropriate for assessing the risk of human exposure. Knowledge, tolerance, and discretion are needed if humans and bats are to coexist. As bat populations are exposed to novel and possibly dire threats, such as white-nose

syndrome (Blehert et al., 2009; Frick et al., 2010) and turbine-related mortality (Kunz et al., 2007; Arnett et al., 2008), shedding the negative stigma of being highly diseased is necessary for effective conservation of bats and the ecosystem services that they provide.

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