Host and viral ecology determine bat rabies seasonality and maintenance


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Rabies is an acute viral infection that is typically fatal. Most rabies modeling has focused on disease dynamics and control within terrestrial mammals (e.g., raccoons and foxes). As such, rabies in bats has been largely neglected until recently. Because bats have been implicated as natural reservoirs for several emerging zoonotic viruses, including SARS-like corona viruses, henipaviruses, and lyssaviruses, understanding how pathogens are maintained within a population becomes vital. Unfortunately, little is known about maintenance mechanisms for any pathogen in bat populations. We present a mathematical model parameterized with unique data from an extensive study of rabies in a Colorado population of big brown bats (Eptesicus fuscus) to elucidate general maintenance mechanisms. We propose that life history patterns of many species of temperate-zone bats, coupled with sufficiently long incubation periods, allows for rabies virus maintenance. Seasonal variability in bat mortality rates, specifically low mortality during hibernation, allows long-term bat population viability. Within viable bat populations, sufficiently long incubation periods allow enough infected individuals to enter hibernation and survive until the following year, and hence avoid an epizootic fadeout of rabies virus. We hypothesize that the slowing effects of hibernation on the following year, and hence avoid an epizootic fadeout of rabies virus. We present a mathematical model for BRV parameterized from these newly available empirical and experimental data to explore mechanisms of BRV maintenance and seasonality. Specifically, we focus on how seasonal bat mortality, virus incubation period, and bat reproduction could affect BRV maintenance and seasonality. Initially, we describe the essential biology for big brown bats and BRV in a bat population studied for 5 y in Colorado that is used to develop a mathematical model representing BRV dynamics. Thereafter, we compare model predictions to empirical data and examine the impact of changes in the key factors (seasonal bat mortality, virus incubation periods, and bat reproduction) to bat population and viral maintenance. Last, we use sensitivity analysis on all of the model parameters (Table 1) to identify key biological features of the host and pathogen that facilitate pathogen maintenance in this wildlife reservoir.

Bat Rabies Virus and Bat Biology. In almost all mammals, rabies is typically a fatal disease once clinical signs manifest. The disease is caused by viruses in the genus Lyssavirus, and is mainly transmitted through bites via infected saliva. Bats can experience either a lethal or nonlethal rabies virus infection. A lethal infection consists of typical disease progression where the virus replicates and migrates through neural tissue to the central nervous system; for some, virus presents in saliva facilitating transmission, and clinical signs manifest, including death. In a nonlethal infection, bats exposed to rabies virus do not develop clinical signs and do not become ill or infectious; rather, they develop virus-neutralizing antibodies and become immune (13–15). As such, the most parsimonious explanation for the identification of virus-neutralizing antibodies in bats is an abortive infection, not recovery from illness (16).

Big brown bats, an insectivorous colonial species, are wide ranging and common in North America. For several reasons, their ecology and rabies biology support the idea that local disease dynamics are most influential to BRV maintenance within


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PNAS Early Edition | 1 of 6
populations of this species. First, rabies virus variants are typically species-specific (17). Second, there are no persistent multihost dynamics for BRV in bats (17, 18), which seems to be a requirement for rabies maintenance in East African wildlife (19, 20). Third, big brown bats have high fidelity to roosting areas within (21) and across seasons and years (22, 23). Thus, we can assume that we are capturing the dynamics of the same population through time. Fourth, big brown bats do not migrate on a continental or broad regional scale (24, 25). Thus, migration and long-distance pathogen translocation can generally be dismissed as a major contributing factor to BRV disease dynamics in big brown bats (19, 26). Thus, we can consider rabies in big brown bats in Colorado to approximate a closed system.

### Epizootically Distinct Periods

Based on contact patterns, disease progression, and big brown bat ecology, we define three epi-

<table>
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<th>Parameter</th>
<th>Description</th>
<th>Default (Ref.)</th>
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<td>Transmission rate*</td>
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<td>ϕ</td>
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<td>Immunity rate* 5</td>
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<td>1.05 (33)</td>
<td>1.02–1.07</td>
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</table>

*Per capita per day.
†See SI Methods.
‡Where $\dot{\phi} = rK$, and $r$ is the intrinsic growth rate, which for our model is $r = (\alpha₂ + \alpha₃) / 2 - (\muₗₐ + \muₜₐ) / 2$.
§Main transmission season.
¶Early transmission season.
⁴Hibernation season.
⁵Per season per capita.
zoologically distinct periods in each year: main transmission, hibernation, and early transmission. In Colorado, big brown bats exhibit seasonal variation in aggregation that is hypothesized to result in seasonal variation of rabies virus transmission. In the spring and summer months (approximately early June to mid-September), females form maternity colonies of tens to a few hundred individuals, often in buildings near humans (27). This period can be described as the main transmission period, and represents optimal climate conditions for birth and growth of young big brown bats as well as optimal conditions for rabies virus transmission among bats, more than likely because of bat abundance and temperatures for viral activity. In maternity roosts, bats are in close contact, allowing for maximum pathogen transmission. By mid-September, bats in Colorado begin moving to hibernacula, where they preferentially hibernate in rock crevices at higher elevations (25). By mid-October, all bats have left maternity roosts until the following spring. Thus, in autumn and winter, big brown bats (adult males, adult females, and juveniles) enter the second period, hibernation (21), which can last up to 6 mo (28). In early spring, after bats arouse from hibernation, they enter the third period, an early transmission period where they maintain irregular use of daily torpor as females form maternity colonies close to humans (29). Recent research has quantified the annual cycle of big brown bats in Colorado (30).

Key factors affecting disease dynamics vary across the three ecological periods and include seasonal bat mortality, the virus incubation period, and bat reproduction.

**Seasonal mortality.** Using mark-recapture techniques from previous research (22, 23), we demonstrated that mortality rates in big brown bats vary across the different seasons (Table 1). Mortality is lower during the hibernation period and higher during the summer.

**Rabies incubation period and facultative heterothermy.** Incubation periods typically range from 2 to 25 wk but, rarely, can be greater than a year (31). Interestingly, like most temperate zone bats, big brown bats are facultative heterotherms: their body temperatures maintain constant internal temperatures during warm season conditions and approach ambient temperatures during hibernation and torpor. During torpor, the metabolic rate also reduces to low levels correlating to ambient temperatures (32, 33). Experimental research (34, 35) suggests that cooler temperatures slow viral development rates in bats. Thus, facultative heterothermy causes seasonal variation in rabies incubation periods across the different seasons.

**Annual birth pulse.** In Colorado, big brown bats give birth once annually (late June); the modal litter size is 1.0 (mean 1.1) (36), and adult female survival is high (22). Although passively acquired antibodies may transiently protect some young bats, there is no known vertical transmission of rabies virus within big brown bats, so the birth pulse supplies immunologically naïve individuals into the population each year.

**Results**

**Validation.** Our model quantifies the number of individuals in different disease classes (susceptible, exposed, infectious, and resistant) through time. We validated the model by comparing model output to empirical data in four ways. First, we considered how well the model predicted bat population size. As a part of the 5-y study on BRV in Colorado, researchers inspected 406 buildings of the ~65,000 addresses in the city limits of Fort Collins. Of the buildings inspected, 0.5–0.7% had a maternity colony at the time of the inspection. The observed roosts pre-birth had a geometric mean size of 47 bats (30). Therefore, multiplying the number of addresses by the proportion with maternity colonies generates a crude estimate of ~15,000–20,000 bats in maternity roosts (65,000 × 0.005 × 47 = 15,275). The model generates bat populations (~20,000) in the range of this estimate.

Second, we considered how well the model predicted the number of infectious individuals. Similar studies on Brazilian free-tailed bats (Tadarida brasiliensis mexicana) found rabies viral RNA in 0–2.5% of their salivary swabs (10). The model generates ~1% of the total population (~200 bats) as infectious during the transmission season.

Third, we considered the timing of BRV incidence by comparing model output of the number of infectious big brown bats each year to a time series of positive cases of rabid bats in Colorado from passive surveillance data from the Centers for Disease Control and Prevention (Fig. 2). The timing of the peak number of cases predicted by the model corresponds with observed peak period in public health data for BRV, which demonstrates that our model describes the seasonal patterns well. The number of infectious bats generated by the model is larger than those in the empirical data. This is to be expected, because the passive surveillance data reflect only the number of infectious cases that are found and reported, whereas the model predicts the total number of infectious bats in the population.

Last, using data from the Colorado Department of Public Health and Environment, we compared model predictions in the timing of incidence in different age classes to rabies-positive samples. The comparison showed that adult female bats are infectious earlier in the year, whereas infectious juveniles appear later in summer. Our model qualitatively reflects that female adults precede juveniles as the dominant group of infectious individuals. Thus, several model predictions qualitatively and quantitatively fit independent, empirical data on big brown bats and bat rabies dynamics (population size, size of infected class, seasonal peak of BRV, and peak of infection across age classes).

**Model Analysis and Sensitivity Analysis.** Behavior of the seasonal deterministic model for parameter values surrounding the default parameter set demonstrates three general outcomes: (i) the bat population persists but BRV is not maintained; (ii) neither the bat population nor BRV are maintained; or (iii) the bat population and BRV are maintained. These three quasi-equilibria are consistent with analytical exploration of a simple SEIR model (susceptible, exposed, infected, resistant) representing the transmission season (11). Model behavior for the default parameters provides model output where both the bat population and BRV are in the population persist.

Alternative model formulations were considered, including no pathogen transmission or viral activity during the early transmission submodel, and no pathogen transmission with slower viral activity (SI Methods). However, overall dynamics did not differ qualitatively from those reported here. Model validation and sensitivity analysis results are also qualitatively invariant across these different model structures.

Sensitivity analysis on the stochastic implementation of the model demonstrated parameter thresholds for BRV maintenance and bat population persistence. Sensitivity analysis suggested BRV extinction is affected strongly by three parameters (Fig. 3 and Figs. S1 and S2): (i) proportion of individuals infected that become infectious, or rather, the case fatality rate (μf); (ii) the natural mortality rate of juveniles during the transmission season (μj); and (iii) the incubation period (μi). Bat population extinction is largely insensitive to all parameters (μ < 0.6; Fig. 3), except the natural mortality rate of juveniles during the transmission season (μj = 3.3) and the proportion of individuals infected that become infectious (μi = 2.8).

**Discussion**

This study of rabies within a major reservoir in North America illustrates the general importance of seasonal mechanisms in pathogen maintenance within wildlife systems. Our mathematical
model of bat rabies dynamics, calibrated and validated using available empirical and experimental data, illustrates that seasonality in bat biology has a complex interaction with BRV dynamics, host persistence, and pathogen/host coexistence. The model predictions based on direct parameter estimates compare well to independent data from the field study, suggesting that the model provides a good description of the biology of this system.

The juvenile mortality rate and viral incubation period in the main transmission season, along with the case fatality rate, were the most important parameters driving bat population and pathogen dynamics. These parameters affect viral maintenance in two different ways: (i) through effects on bat population viability and (ii) through effects on viral maintenance within a viable bat population. Bat population viability is a necessary but not a sufficient condition for viral maintenance. Bat population persistence creates an envelope of opportunity for viral maintenance, because if host populations are not present, then neither are their directly transmitted pathogens. Juvenile mortality in the main transmission season exemplifies how viral maintenance is delimited by bat population viability (Fig. 4A). As the juvenile mortality rate increases past a threshold, bat population viability in the system, and therefore viral maintenance, drastically declines (Fig. 4A). Also, big brown bat populations in Colorado experience substantial differences in mortality rates across a year (Table 1), and this variation plays a unique role in maintaining viable bat populations. To more fully consider the importance of seasonal variation of bat mortality, we explored model dynamics without hibernation seasons. This nonseasonal model exhibited consistent bat population crashes, demonstrating the importance of the moderating effect of lower mortality rates during hibernation. Decreased mortality during winter allows bat populations to avoid extinction; mortality rates during the transmission season, particularly among juveniles, are too high in the absence of winter hibernation for population viability of big brown bats. Hibernation is a mechanism to avoid seasonally harsh climates and a reprieve from high mortality during spring and summer months, with optimal climate and high bat activity resulting in greater exposure to a variety of mortality factors.

Our model suggests that the incubation period does not affect population viability but has a strong impact on viral maintenance (Figs. 3 and 4B). The fewer incubating or infectious bats in the population, the more prone the virus is to epizootic fadeout. The incubation period determines how quickly exposed individuals, and ultimately infectious individuals, enter the population after transmission. Shorter incubation periods generate fewer exposed individuals at any given time because exposed bats progress to infectiousness quickly, thereby leaving this class. More importantly in our model, such individuals follow an epizootic curve during the transmission season with the least number of exposed individuals remaining in the population late in the summer (Fig. S3). Thus, the shorter the incubation period the more prone the...
population is to a rabies epizootic fadeout before the hibernation season occurs (Fig. 4B).

The interaction of incubation period with the hibernation season and annual birth pulse generates a complex dynamic. The longer the incubation period, the more likely infected bats will survive long enough to enter hibernation and provide infectious contacts in the subsequent main transmission season (Fig. S3). The combination of long incubation periods and the metabolic effect of cold temperatures that suppress viral activity during the hibernation season combine to make a temporal maintenance reservoir, preserving rabies virus until the birth pulse provides a new supply of immunologically naïve bats.

The case fatality rate adds even greater complexity (Fig. 4C) because it affects bat population viability and viral maintenance independently. At lower levels, an insufficient number of infectious individuals are created, minimizing chains of transmission in the number of susceptible bats, and ultimately viral maintenance. At the midrange to higher values, bat population viability becomes increasingly uncertain as more bats succumb to rabies. At high case fatality rates, both the population and viral maintenance are diminished simultaneously. For viral maintenance, there exists a restricted range of case fatality rates that allow both host and pathogen to persist.

This research increases our understanding of disease dynamics in wildlife populations and specifically within a significant disease reservoir. Because BRV shares properties with other emerging pathogens associated with bats, our validated model can be re-parameterized or modified to predict dynamics of newly emerging diseases. This model will be particularly important when few data are available for validation of a novel host/pathogen system, where it likely will be unclear which ecological aspects of the system are most important in driving host/pathogen dynamics. Finally, because so few data exist for pathogens associated with bats, using sensitivity analyses on modified models can help direct field and laboratory work to collect data with the highest impact for understanding maintenance mechanisms of bat-borne pathogens.

**Methods**

**Model.** We developed a model of BRV dynamics in big brown bats by coupling submodels that reflect the distinct epizootic periods or “seasons” of BRV based on big brown bat ecology and the biology of BRV (main transmission, hibernation, and early transmission). Parameters are described in Table 1 and the parameterization section below. The seasonal BRV model we propose is characterized as follows.

**Main transmission season submodel (June 1—September 21; 112 d).** Because big brown bats can exhibit nonlethal and lethal rabies virus infections, the submodel for the main transmission season described in Swinton et al. (1) follows females and juveniles in susceptible (S), exposed (Ea and E), resistant (R), and infectious (I) disease classes based on progression of rabies in bats. Bats in the exposed disease class are those that have been exposed to, infected with, and incubating the rabies virus. Bats that are infectious die quickly from the disease (Table 1), so natural mortality is ignored for infectious individuals. Density-dependent population regulation is a generally observed feature of wildlife populations (3), so we included it in the main transmission season submodel where it would be most readily apparent in a growing population that is aggregated for breeding. The j subscript indicates age classes: juvenile, 1- to 2-year-old adults females, 2- to 5-year-old adult females.

\[
\frac{dS_j}{dt} = -\beta_j S_j I_j - \mu_j S_j - \phi N S_j \\
\frac{dE_{aj}}{dt} = (1 - \rho)I_j S_j - (\sigma_{aj} + \mu_j + \phi N)E_{aj} \\
\frac{dE_a}{dt} = \rho I_j S_j - (\sigma_a + \mu_a + \phi N)E_a \\
\frac{dR_j}{dt} = \sigma_{aj}E_{aj} - \mu_j R_j - \phi N R_j \\
\frac{dI_j}{dt} = \sigma_a E_a - \phi N I_j \\
\]

For simplicity in our model, we approximate the birth pulse at the beginning of the main transmission season in a single day, where births are added to the susceptible class. Because adult male big brown bats primarily roost solitarily during the summer (21), we advance all male females in each age class to the next highest age class, and half of the juvenile age class (females) advances into the main transmission season model to reflect the return of females to roost sites in spring. Following the birth pulse, the main transmission model is implemented.

**Hibernation submodel (September 22—April 5; 187 d).** During hibernation, disease progression is likely suspended because of cold temperatures and metabolic effects associated with torpor (34, 35). Infectious bats die quickly from the disease (Table 1), and more than likely do not make it into hibernation. Thus, the hibernation submodel describes primarily overwinter mortality for each of the disease classes.

\[
\frac{dS_j}{dt} = -\nu_j S_j \\
\frac{dE_{aj}}{dt} = -\nu_j E_{aj} \\
\frac{dE_a}{dt} = -\nu_a E_a \\
\frac{dR_j}{dt} = -\nu_j R_j \\
\frac{dI_j}{dt} = -\nu_I I_j \\
\]

**Early transmission season submodel (April 6—May 31; 55 d).** In spring, BRV likely progresses within individuals; however, the intermittent use of daily torpor during this period will be more pronounced because of variable and lower temperatures. Therefore, our model assumes BRV progresses within individuals...
vials at a slower rate. Because early transmission occurs before the birth pulse (see below), bat densities will be lowest, which reduces the need for including density-dependent mortality during this season.

\[
\frac{dS}{dt} = -\mu_S S_i,
\]

\[
\frac{dE}{dt} = -\sigma_S E_i - \mu_E E_i,
\]

\[
\frac{dE_i}{dt} = -\sigma_E E_i - \mu_E E_i,
\]

\[
\frac{dI}{dt} = \sigma_E E_i - \mu_I I_i,
\]

\[
\frac{dR}{dt} = \mu_I R_i - \nu_I I_i.
\]

Parameterization. The model has been parameterized using literature and field data (Table 1). Unique to this study, we determined parameter space generally, but focused on the range of our estimated parameters to determine dynamical outcomes (i.e., host persistence and viral maintenance, disease-free host persistence, and host population extinction) of the model. Following this numerical stability analysis, we determined how sensitive model output (probability of pathogen extinction and probability of bat population extinction) was to changes in all model parameters (Table 1) using techniques described by Webb et al. (37). In our study, the output variable was the percent of simulations (100 simulations per parameter combination) that maintained bat populations for 100 (viral) dynamics.

Field sampling of bats was approved by the Institutional Animal Care and Use Committees of Colorado State University and the US Geological Survey. Use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US government.

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