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SEASONAL VARIATION AND AGE-RELATED CORRELATES OF BUGGY CREEK VIRUS (TOGAVIRIDAE) INFECTION IN NESTLING HOUSE SPARROWS

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ABSTRACT: Wild birds are rarely found with active arbovirus infections, and relatively little is known about the patterns of viremia they exhibit under field conditions or how infection varies with date, bird age, or other factors that potentially affect transmission dynamics. Buggy Creek virus (BCRV; *Togaviridae*, *Alphavirus*) is an arbovirus associated with colonially nesting Cliff Swallows (*Petrochelidon pyrrhonota*) and transmitted by its vector, the hematophagous swallow bug (*Oeciacus vicarius*), an ectoparasite of the Cliff Swallow. Introduced House Sparrows (*Passer domesticus*) that have occupied swallow nests at colony sites in peridomestic settings are also exposed to BCRV when fed upon by swallow bugs. We used data from 882 nestling House Sparrows in western Nebraska from 2006 to 2008 to examine seasonal variation and age-related correlates of virus infection in the field. Over 17% of nestling House Sparrows had active infections. Prevalence was higher in 2007 than in 2008 when birds from all colony sites were analyzed, but there was no significant difference between years for sites sampled in both seasons. Buggy Creek virus prevalence was similar in early and late summer, with a peak in midsummer, coinciding with the greatest swallow bug abundance. Nestlings 10 days of age and younger were most commonly infected, and the likelihood of BCRV infection declined for older nestlings. Average viremia titers also declined with age (but did not vary with date) and were high enough at all nestling ages to likely infect blood-feeding arthropods (swallow bugs). Length of viremia for nestlings in the field was ≥4 days, in agreement with an earlier study of BCRV. Nestling birds offer many advantages for field studies of arbovirus amplification and transmission.

Key words: Alphavirus, arbovirus, Buggy Creek virus, House Sparrow, invasive species, *Passer domesticus*, virus ecology, virus transmission.

INTRODUCTION

Wild birds are important amplifying hosts for many arboviruses (arthropod-borne viruses), with considerable research attention directed toward determining what species are most important in natural transmission cycles (McLean and Bowen, 1980; Reisen et al., 2000; Howard et al., 2004). Because active virus infections are detected infrequently in most birds in the field (Brown and O'Brien, 2011), emphasis has often been on screening wild-caught birds for antibodies or experimentally infecting birds in the laboratory. The consequence is that we know relatively little about patterns of natural viremia in birds in the wild and how infection varies with time, bird age, or other factors that potentially affect arbovirus transmission dynamics. Some studies suggest that the typical duration of viremia may be different for birds in the wild versus in the laboratory (Scott et al., 1984) and that immune function varies with time of the summer (Kinnard and Westneat, 2009), thus affecting birds' susceptibility to arbovirus infection. If, for example, viremias in birds in the field are shorter in duration, perhaps because a virus is apt to more rapidly kill wild birds, experimental infection studies on captive birds may overestimate the potential for virus amplification.

The relatively few studies that have reported data sufficient for exploring ecologic correlates associated with arbovirus infection in birds in the field have largely involved nestling birds. Nestlings offer several advantages for studying natural patterns of arbovirus infection and thus the potential for transmission:

1) large numbers, especially of colonially nesting species (Buescher et al., 1959; Scott et al., 1984; O'Brien et al., 2011), can be located easily and predictably; 2) immunologically naïve nestlings are readily susceptible to infection even in areas where an arbovirus is enzootic and many of the adults have antibodies to the virus (Holden et al., 1973a); 3) nestlings, especially younger ones, may be more likely than adult birds to be fed on by insect vectors because the nestlings have not developed defensive behaviors to thwart biting by arthropods (Blackmore and Dow, 1958; Scott et al., 1990); and 4) nestling birds may not have as well-developed immune systems as adults (Apanius, 1998; Klasing and Leschinsky, 1999; Palacios et al., 2009) and therefore may exhibit longer-lasting or higher-titer viremias, facilitating detection and increased virus transmission to uninfected arthropod vectors.

We take advantage of a high prevalence of arbovirus infection in nestling House Sparrows (*Passer domesticus*) to examine how virus infection varies within a summer transmission season and between years, how infection prevalence and extent of viremia is related to age of nestlings and date of sampling within the summer, and the estimated duration of viremia for birds in the wild. Buggy Creek virus (BCRV; *Togaviridae*; *Alphavirus*) and the similar Fort Morgan virus (FMV) commonly infect nestling House Sparrows and can cause serious pathology in nestlings (Hayes et al., 1977; Scott et al., 1984; O'Brien et al., 2010a). The principal vector for BCRV is the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*) that transmits the virus to House Sparrows when blood feeding. Swallow bugs are ectoparasites of Cliff Swallows (*Petrochelidon pyrrhonota*) and encounter House Sparrows whenever sparrows nest in abandoned Cliff Swallow nests. Although Cliff Swallows are also exposed to BCRV, they rarely exhibit detectable viremia and show no pathologic effects (O'Brien et al., 2011), and thus they are not suitable for studying the dynamics of BCRV infection in vertebrate hosts. Our analyses are designed to help better understand seasonal transmission dynamics of BCRV, its maintenance in the swallow colony site environment, and its potential effects on House Sparrows occupying these nesting sites.

MATERIALS AND METHODS

Study organisms

House Sparrows were introduced repeatedly into North America beginning in the 1850s (Lowther and Cink, 2006) and are now widely dispersed and found mainly in peridomestic settings. Sparrows are semicolonial, often forming aggregations of 2–20 nests. They are sedentary, remaining at or near breeding sites year round (Anderson, 2006). House Sparrows are multibrooded; nesting in our study area extends from late April to late July, with peak egg laying mid-May, late June, and late July. Mean (±SE) clutch size for sparrows at latitudes similar to that of our study area is 4.6–4.8 (±0.8) eggs, and nestlings fledge at 14–17 days of age (Anderson, 2006).

Cliff Swallows are highly colonial, migratory passerines that breed across much of western North America (Brown and Brown, 1995). They build gourd-shaped mud nests on the sides of cliff faces, inside road culverts, and under bridges. Nests can be closely spaced (often contiguous), and colonies may contain up to 6,000 active swallow nests. The mud nests persist from year to year and are frequently reused by Cliff Swallows for multiple seasons (Brown and Brown, 1996). Swallows arrive in our study area in early to mid-May and typically raise a single brood, with most nestlings fledging by mid-July. Individual colonies are highly synchronous and are quickly vacated by swallows after the nestlings fledge.

House Sparrows likely first began occupying Cliff Swallow colonies in our study area after the construction of the interstate highway system in the late 1960s, which provided substrates (bridges, culverts) for colonies near humans and brought swallows into contact with House Sparrows. Sparrows evict Cliff Swallows from their mud nests or occupy abandoned swallow nests.

The swallow bug is a hematophagous nest-based ectoparasite primarily of the Cliff Swallow. Numbers in Cliff Swallow colonies

can be up to 2,600 bugs per swallow nest (Brown and Brown, 1996) and 2,400 per House Sparrow nest (V. O'Brien, unpubl. data). Swallow bugs live primarily in or near the mud nests and blood feed on birds mostly at night.

Buggy Creek virus is a single-stranded, positive-sense RNA alphavirus (Hopla et al., 1993). Fort Morgan virus (Calisher et al., 1980), also found in swallow bugs, is a strain of BCRV (Pfeffer et al., 2006; Padhi et al., 2008); thus, FMV and BCRV are synonymous. Stone Lakes virus, recently isolated from California, is probably also a strain of BCRV (Brault et al., 2009). Buggy Creek virus is ecologically distinct from other alphaviruses in that its vector is the swallow bug, rather than a mosquito (Rush et al., 1980, 1981). Prevalence of BCRV in swallow bugs averages ~25% of bug pools over the whole study area and across different years (Brown et al., 2001, 2007; Moore et al., 2007).

Study site

Our study sites are in a 60×200-km area along the North and South Platte rivers in western Nebraska, USA, that is centered at the Cedar Point Biological Station (41°13′N, 101°39′W), in Keith County. The study area also includes portions of Lincoln, Garden, Duel, and Morrill counties (Brown and Brown, 1996). Each year we monitor approximately 170 Cliff Swallow colony sites that are occupied to varying degrees by Cliff Swallows and/or House Sparrows. In the summers of 2006–2008, we studied House Sparrows at swallow colonies in concrete culverts beneath highways or railroads and on the sides of bridges.

Field sampling

In 2006, we blood sampled House Sparrow nestlings for virus on two occasions from one colony site in Morrill County. Nestling age in 2006 was estimated using criteria in Weaver (1942), based principally on extent of feather development. In 2007, we systematically blood sampled nestlings from 21 colony sites throughout the study area. House Sparrow nests were examined for eggs using a dental mirror and flashlight. All nests that were initiated at each colony site throughout the summer were monitored in 2007. Nests containing eggs were numbered and visited every 2–4 days to determine hatching date and nestling age. In 2008, we blood sampled nestlings from 16 colony sites throughout the study area and used our prior experience with known-age nestlings to determine the birds' age at the time of sampling.

Nestlings were 4–17 days old when sampled, with all birds bled once or twice during the nesting period by brachial (in 2006) or jugular (in 2007–08) venipuncture with a 29-gauge insulin syringe. Upon collection, 0.1 ml of blood was placed in 0.4 ml of BA-1 virus diluent (Moore et al., 2007). Sampled nestlings were banded with US Geological Survey bands and returned to the nest. Samples were stored on wet ice in the field, returned to the laboratory, clarified by centrifugation, supernatant removed, and stored at −70 C.

Laboratory analyses

Viral RNA was extracted from bird sera by adding 25 µl of thawed serum in BA-1 diluent to 100 µl of a guanidine thiocyanate–based lysis buffer (O'Brien et al., 2008). After the addition of 400 µl of 100% ethanol, RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, Valencia, California, USA), following the manufacturer's protocol (Moore et al., 2007). A positive BCRV control (derived from swallow bugs) was included in each extraction, and negative controls were placed between every five samples. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on samples using the OneStep RT-PCR Kit (Qiagen), following the manufacturer's protocol. We used BCRV-specific primers that yielded a 208-bp fragment from the E2 region of the viral genome (Moore et al. 2007). Electrophoresis of product (6.5 µl) on a 4% Nusieve/agarose gel identified positive samples, using a BCRV-positive control on each gel.

Samples that were initially BCRV positive by RT-PCR were subjected to plaque assay on Vero cells as described by Huyvaert et al. (2008), with the exception of using yeast-extract lactalbumin instead of M-199 growth media. Viremia titers were determined by serial dilution. Samples that did not confirm by exhibiting plaque formation on Vero cells were subjected to re-extraction and RT-PCR to confirm presence of viral RNA in the sample. Titers were expressed in $\log_{10}$ plaque-forming units (PFU).

A House Sparrow blood sample was considered BCRV positive if either it was RT-PCR positive on initial screening and confirmed by plaque assay, or it was RT-PCR positive on initial screening, negative by plaque assay, and positive by RT-PCR on second screening. Some birds were sampled multiple times during the nestling period. For analyzing prevalence by date or age, a bird found

positive on first sampling was considered positive only for that date and age and was not used for analyses after that time (i.e., it was only counted once because it could presumably be infected only once during its nestling period). However, because birds without virus may become infected later in the nestling period, individuals that were initially negative were also used in calculating prevalence when sampled subsequently (i.e., they were counted twice if sampled twice). In analyses of how virus prevalence varied across the summer in 2007, we established arbitrary 2-wk intervals from 21 May through 12 August; these intervals were chosen so that the majority of colony sites were repeatedly sampled within each interval.

Statistical analyses

Chi-square tests were used to analyze how virus prevalence differed among years and among sampling intervals within years. The effects of nestling age on virus prevalence and the level of viremia were evaluated with Spearman correlations. We used multiple regression to separate the effects of age, date, and their potential interaction on the level of viremia.

RESULTS

In the summers of 2006–08, we collected 1,043 blood samples from 882 nestling House Sparrows in 255 nests (9 nests in 2006, 196 in 2007, and 50 in 2008). Overall BCRV prevalence in nestling House Sparrows by RT-PCR or plaque assay across all years was 17.5%. In 2007–08, 90 of 246 nests (36.6%) in the study area contained at least one BCRV-positive House Sparrow nestling.

Among-year differences

Yearly differences in BCRV prevalence in House Sparrows were apparent when using nests from all colony sites sampled in the study area in 2007 and 2008 (Fig. 1), with significantly higher prevalence in 2007. Because of potential site-related differences in prevalence, we also compared BCRV prevalence in 2007 versus 2008 only for sites that we sampled in both years. For these colonies, there was no significant yearly difference

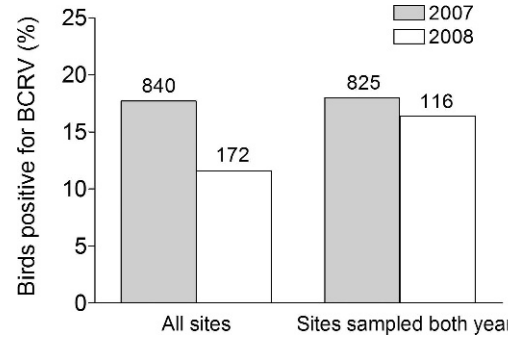

FIGURE 1. Between-year comparison of Buggy Creek virus prevalence in House Sparrow nestlings (percentage of birds positive) in western Nebraska, 2007–08, using all colonies sampled ($n=19$ colonies) and only colonies sampled in both years ($n=12$). Number of birds sampled shown above each bar. Prevalence in nestlings was significantly different between years for all colonies ($\chi^2_1=3.83$, $P=0.05$), but there was no difference between years for nestlings in colonies that were sampled in both years ($\chi^2_1=0.17$, $P=0.68$).

(Fig. 1). Furthermore, for the one colony site sampled in all 3 yr, there was no significant difference in BCRV prevalence among the nestlings in 2006 (46%, $n=37$), 2007 (26%, $n=50$), and 2008 (20%, $n=5$; $\chi^2_2=4.23$, $P=0.12$).

Date-related effects within a season

Buggy Creek virus prevalence in nestling House Sparrows differed significantly with 2-wk date interval across the 2007 nesting season (Fig. 2). There was a spike in prevalence in late June, but no evidence of a systematic increase or decrease in prevalence across the summer (Fig. 2). Mean age of nestlings sampled and BCRV prevalence were not correlated for these 2-wk intervals ($r_s=-0.31$, $P=0.54$, $n=6$), so it is unlikely that these results (Fig. 2) reflect changing nestling age distributions across the summer.

Effects of nestling age

Prevalence of infection in House Sparrows was highest in younger nestlings and declined significantly as bird age increased, with the decline in prevalence

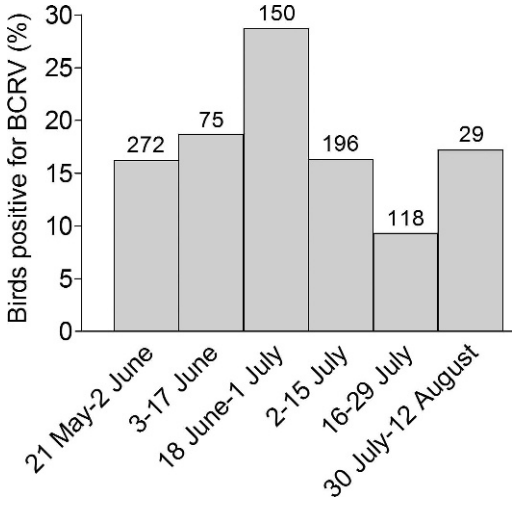

FIGURE 2. Prevalence of Buggy Creek virus in House Sparrow nestlings (percentage of birds positive) by 2-wk intervals in summer 2007. Prevalence varied significantly among the intervals ($\chi^2_5=18.8$, $P=0.002$). Number of birds sampled shown above each bar.

most obvious for birds older than 10 days (Fig. 3).

Of House Sparrow samples that were BCRV positive by RT-PCR, 72.7% ($n=183$) exhibited plaque formation on Vero cells (≥ 1.7 $\log_{10}$ PFU/ml). No samples from birds older than 13 days plaqued. Mean ($\pm$SE) titer for all samples

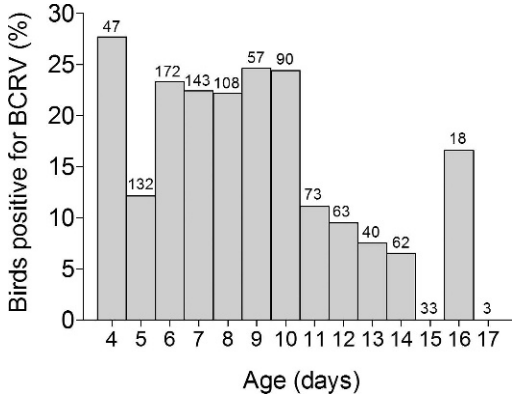

FIGURE 3. Prevalence of Buggy Creek virus in House Sparrow nestlings (percentage of birds positive) by age (days) at time of sampling. Prevalence declined significantly as bird age increased ($r_s=-0.75$, $P=0.002$, $n=14$). Number of birds sampled shown above each bar.

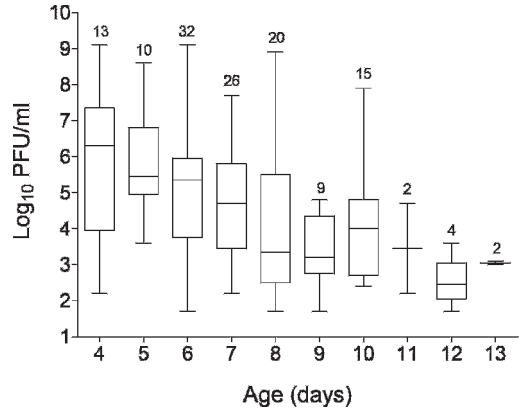

FIGURE 4. Median (horizontal line), 25th–75th percentiles (box), and range (lowest to highest values, vertical line) of Buggy Creek virus viremia titers by age (days) for field-sampled House Sparrow nestlings, 2007–08. Mean titer declined significantly as bird age increased ($r_s=-0.89$, $P<0.001$, $n=10$), as did maximum titer ($r_s=-0.88$, $P=0.001$). Only samples with detectable plaques (≥ 1.7 $\log_{10}$ plaque-forming units [PFU]/ml) were included; sample size given above each bar. One outlier, titer = 14.1 $\log_{10}$ PFU/ml, not shown.

positive by plaque assay ($n=133$) was 4.6 (± 0.20) $\log_{10}$ PFU/ml. Five nestlings, aged 4–8 days, had titers of 8.6–9.1 $\log_{10}$ PFU/ml. One 8-day-old nestling titer was 14.1 $\log_{10}$ PFU/ml. Titers declined significantly with nestling age (Fig. 4). Nestlings of all ages (except 12 days) had median titers ≥ 3.0 $\log_{10}$ PFU/ml (Fig. 4). Although we did not know the precise time of first virus exposure for these individuals (Fig. 4), each bird regardless of age had to have been infected within 4–5 days prior to being sampled.

Date did not affect viremia titer. Only age was significantly associated with titer ($F_{1,130}=5.54$, $P=0.020$); both date ($F_{1,130}=0.11$, $P=0.74$) and a date$\times$age interaction ($F_{1,130}=0.27$, $P=0.60$) were nonsignificant ($n=133$ birds).

We documented 10 nestlings in 2007 that were virus positive on first sampling and virus negative on second sampling; of these, eight were ≥ 12 days old on second sampling. This indicates that BCRV can be cleared by the time birds fledge. Duration of viremia was ≥ 4 days in two nestlings

that were BCRV positive on both first and second sampling. One was sampled at 6 days old and resampled 4 days later, and the other was 7 days old at first sampling and was resampled 5 days later. Virus titers at first sampling for the two nestlings were 4.1 and 4.9 $\log_{10}$ PFU/ml, respectively, and at second sampling both were confirmed positive by RT-PCR but did not form plaques in Vero-cell assays.

DISCUSSION

One objective of our work was to determine to what extent prevalence of BCRV infection in nestling House Sparrows varied from year to year. Nestling birds in general, because of the availability of large numbers (especially in colonial species) and the fact that they are all of local origin, can provide useful data on yearly variation in arbovirus prevalence in a given area. Our finding of higher prevalence of BCRV among nestling House Sparrows in 2007 than in 2008 when we analyzed all colony sites sampled each year may have reflected sampling of several high-virus sites in 2007 only (or low-virus sites in 2008 only), given that there was no significant difference when comparing only sites sampled both seasons. This illustrates the necessity of consistent sampling of the same sites among years for meaningful longitudinal monitoring.

The transmission peak in late June for BCRV coincides with the time that swallow bugs are most numerous in colonies across the study area (Brown and Brown, 2004), suggesting that prevalence of virus in House Sparrows in part reflects the number of bugs potentially feeding at a given time. The late-June peak may also reflect early-season amplification and transmission of BCRV by the infected early nestlings, with the high viremia titers in these nestlings leading to an increase in the number of infected bugs in May and June. Infection tends to occur in spatial "hot spots" within a colony; when nestlings in one nest are infected, nearby nests are more likely to also have infected occupants (O'Brien and Brown, 2011).

Buggy Creek virus transmission to House Sparrows did not increase in July or August after most Cliff Swallows had fledged and departed from the colony sites. An increase in infection of sparrows might be expected then, given that the presence of swallows (e.g., at mixed-species sites) lowers BCRV prevalence in nestling House Sparrows, perhaps because swallows are more attractive than sparrows to blood-feeding bugs (O'Brien et al., 2011). Lowered BCRV prevalence in sparrows after late June may largely reflect decreasing activity of the bugs. Bugs are adapted to the Cliff Swallow's highly seasonal breeding phenology; many bugs become dormant as early as mid-July as Cliff Swallows vacate the study area (C. Brown, pers. obs.). Perhaps bugs have not had sparrows available as alternative hosts for a long enough time to evolve a lengthened reproductive season in response. However, even without a late-summer increase in BCRV transmission among House Sparrows, over 17% of nestlings were still infected with BCRV in August, matching the average prevalence across the entire season. This demonstrates that bugs and virus can be active much later in the summer at House Sparrow sites than would be the case at Cliff Swallow–only colonies in our study area.

We found a decline in new BCRV infections as House Sparrow nestlings became older. In contrast, for western equine encephalomyelitis virus (WEEV), Holden et al. (1973b) found infection prevalence highest in 9–12-day-old nestling House Sparrows, which in this species would be birds approaching fledging (Anderson, 2006; Lowther and Cink, 2006). This result might be expected for a virus such as WEEV that is transmitted by a mosquito, as some studies show a mosquito feeding preference for older

nestlings (Scott et al., 1990; Griffing et al., 2007; O'Brien et al., 2010b). The decline in BCRV prevalence with age, even when using relatively sensitive RT-PCR to detect viral RNA, may reflect 1) decreasing rates of biting by swallow bugs as nestling House Sparrows become feathered and start to preen, a pattern seen in Cliff Swallows (Brown and Brown, 1995; C. Brown, pers. obs.), or 2) a developing immune system that is increasingly like that of the adults as nestlings get older. Like other alphaviruses, BCRV seems to have little effect on adult House Sparrows, with experimental infection studies showing that infected adults have either low-level viremia of short duration or none at all (Huyvaert et al., 2008; Brault et al., 2009) and field screening of adults showing the same result (O'Brien et al. 2011). The highest virus prevalence among the youngest nestlings also confirms work suggesting limited transfer of maternal antibodies to arboviruses in House Sparrows (Holden et al., 1973a; Nemeth et al., 2008). Our results indicate that most of the mortality and pathology attributable to BCRV (O'Brien et al., 2010a) occurs among House Sparrow nestlings 10 days or younger, perhaps because virus replicates to such a high degree in nestlings of those ages.

The decline in virus titer as nestlings aged is consistent with the birds developing a more effective immune system with age. Consistent with this conclusion is the finding of even lower viremias in adult sparrows (Huyvaert et al., 2008). Studies of nestling House Sparrows in a Kentucky population suggested reduced immunocompetence later in the summer, as measured by response of T-lymphocytes to phytohemagglutinin challenge (Kinnard and Westneat, 2009). Our finding no statistical effect of date on titer does not support such a change in immune function over the summer, at least as measured in the birds' response to this alphavirus.

Titers in our field study were generally high, with the younger nestlings routinely exceeding 5.0 $\log_{10}$ PFU/ml. Swallow bugs became infected in the laboratory with the Fort Morgan strain of BCRV (Rush et al., 1980) at titers of 4.8–8.0 $\log_{10}$ PFU/ml, but were not tested for a minimum threshold titer. However, 33% of bugs became infected at 4.8, the lowest titer used in the laboratory study. Mean viremia titer for all nestling samples in our study was 4.6 $\log_{10}$ PFU/ml, and 20% of 133 samples were $\geq$6.0 $\log_{10}$ PFU/ml. The viremia titers shown in our study, which came from field-collected samples from nestlings where date of BCRV infection was not precisely known, represent point-in-time observations of viremia and show birds primarily at the onset or in the middle of viremia. Experimental infections with House Sparrows and swallow bugs are needed to demonstrate a typical course of viremia and the minimum titer in nestlings required to infect vectors. However, our field-collected data demonstrate that high-titer viremia in House Sparrows likely occurs often enough to maintain transmission of BCRV to swallow bugs.

In their study of the Fort Morgan strain of BCRV, Scott et al. (1984) found the duration of viremia in nestling House Sparrows to be at least 3–4 days. They had seven nestlings that survived to be bled multiple times and on which they based their estimate of viremia duration. However, even though our sample of birds screened was equivalent in number to that of Scott et al. (1984) and birds were sampled at approximately the same time intervals, we had only two infected nestlings that survived long enough to estimate the duration of viremia. We would not have detected virus on the second bleed for these birds had we used only plaque assay, the methodology of Scott et al. (1984). That second detections in our study were few was likely because most of the younger nestlings that were infected with BCRV (and thus those potentially available to be rebled) died within 2–3 days. Our data and those of Scott et al. (1984) suggest that

nestling House Sparrows in the wild can be viremic with BCRV for up to 4 days, although their capacity to infect swallow bugs by day 4 is doubtful because of the low titers (with viremia in our birds only detectable at day 4 by RT-PCR). Still, this is a longer time than seen in experimental infections of nestling (Scott et al., 1984) and adult House Sparrows (Huyvaert et al., 2008; Brault et al., 2009), and indicates that measured viremias in the field may sometimes differ from those in the laboratory. Viremias of this length may contribute to House Sparrows' competence as hosts for BCRV and be partly responsible for infection of large numbers of swallow bugs and the maintenance of BCRV epizootics at swallow colony sites that contain House Sparrows.

The recurring availability of young House Sparrow nestlings at Cliff Swallow colony sites, the continual immigration of naïve breeding sparrows into these sites (O'Brien, 2009), and the nestlings' high competence as amplifiers of BCRV will likely enable this virus to persist in this ecosystem indefinitely. The consequence is relative predictability in virus prevalence from year to year, both at a given site (Brown et al., 2001) and over the study area as a whole. Unlike the mosquito-associated arboviruses, known to vary widely in temporal occurrence (Reisen et al., 1990, 1996; Day, 2001), BCRV represents a spatiotemporally stable host-pathogen system that offers many opportunities for virologists, evolutionary ecologists, and modelers to study its effects on its hosts and how it has evolved in response to a relatively new and highly competent House Sparrow host (Brown et al., 2009).

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