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ARBOVIRUS INFECTION IS A MAJOR DETERMINANT OF FITNESS IN HOUSE SPARROWS (*PASSER DOMESTICUS*) THAT INVADE CLIFF SWALLOW (*PETROCHELIDON PYRRHONOTA*) COLONIES

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Abstract.—Wild birds play a key role in the amplification and transmission of many of the arthropod-borne viruses (arboviruses). Determining the extent to which birds are affected by these viruses is critical in predicting the pathogens' spread or maintenance in vertebrate host populations. Little is known about how arboviruses affect amplifying hosts' fitness, especially in cases where these viruses infect nestling birds. Buggy Creek virus (BCRV; Togaviridae: *Alphavirus*) is an RNA arbovirus transmitted by the Swallow Bug (*Oeciacus vicarius*) to Cliff Swallows (*Petrochelidon pyrrhonota*) and House Sparrows (*Passer domesticus*) that have recently occupied Cliff Swallow nesting colonies. We examined fitness, as measured by fledging success, of House Sparrows occupying Cliff Swallow nesting colonies in western Nebraska. Most nestlings naturally infected by BCRV at 4–6 days of age died, and even older nestlings, when infected, showed fledging success of <40%. Whether ≥1 nestling in a brood was infected with BCRV was the most important determinant of nest success in this population. Colony sites with high BCRV prevalence had lower rates of nest success, and surviving broods at those sites were smaller. Although most infected nestling House Sparrows eventually die, they survive long enough to infect Swallow Bugs, and in this way they play a critical role in BCRV transmission. Frequent emigration of unexposed House Sparrows into these colonies and their renesting throughout the summer leads to these sites being perpetual foci for BCRV epizootics. Few other arboviruses are known to have such extreme effects on the fitness of their vertebrate hosts. *Received 8 March 2012, accepted 4 June 2012.*

Key words: Buggy Creek virus, fledging success, House Sparrow, invasive species, virus ecology, wildlife disease.

La Infección por Arbovirus es un Factor Determinante de la Aptitud de Individuos de *Passer domesticus* que Invaden las Colonias de *Petrochelidon pyrrhonota*

Resumen.—Las aves silvestres juegan un papel importante en la amplificación y transmisión de muchos de los virus transmitidos por artrópodos (arbovirus). Determinar el grado al que las aves se ven afectadas por estos virus es crítico para predecir la propagación o el mantenimiento de los patógenos en las poblaciones de vertebrados hospederos. Se sabe muy poco sobre cómo los arbovirus afectan la aptitud de los hospederos, especialmente en casos en los que estos virus infectan aves anidantes. El virus Buggy Creek (BCRV; Togaviridae: *Alphavirus*) es un arbovirus de RNA transmitido por el insecto *Oeciacus vicarius* a las golondrinas *Petrochelidon pyrrhonota* y a los gorriones *Passer domesticus* que hayan ocupado recientemente las colonias de anidación de las golondrinas. Examinamos la aptitud, medida como la proporción de pichones que llegan a dejar el nido, de individuos de *P. domesticus* que ocupan las colonias de anidación de *P. pyrrhonota* en el occidente de Nebraska. Muchos de los pichones de entre 4 y 6 días de edad infectados naturalmente por BCRV murieron, e incluso pichones mayores mostraron una reducción de más del 40% en el éxito de emplumamiento al ser infectados. El determinante más importante del éxito de anidación en esta población fue tener más de un pichón de una camada infectado con BCRV. Las colonias con alta prevalencia de BCRV tuvieron menores tasas de éxito de anidación y las camadas que sobrevivieron en esos sitios fueron más pequeñas. Aunque la mayoría de los individuos de *P. domesticus* infectados eventualmente mueren, éstos sobreviven lo suficiente como para infectar a los vectores, de modo que juegan un papel crítico en la transmisión del BCRV. La migración frecuente de gorriones no expuestos al virus hacia estas colonias y su reanidación en el transcurso del verano conducen a que estos sitios sean focos perpetuos de las epizootias de BCRV. Se conocen pocos arbovirus con efectos tan extremos sobre la aptitud de sus hospederos vertebrados.

Wild birds are known to be amplifying hosts for many of the arthropod-borne viruses, including ones such as West Nile virus and western equine encephalomyelitis virus that directly affect wildlife or human populations (Brown and O'Brien 2011). Upon being bitten by an infected arthropod, birds of some species are capable of replicating the virus in their blood to a level sufficient to infect additional blood-feeding arthropods and thereby sustain transmission cycles in natural populations (Yuill 1986, Morris

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1988, Scott 1988, Reisen and Monath 1989, Calisher 1994). Understanding transmission is important if we are to predict where virus epizootics or epidemics may occur, and considerable work has been done to identify which avian species are susceptible to the different arboviruses and potentially involved in their transmission (Reeves 1990, Reisen et al. 2003, Howard et al. 2004, McLean 2006).

Yet despite extensive field surveys on seroprevalence in different species and many laboratory experiments on how certain birds respond to being inoculated with viruses, we still know relatively little about how arbovirus infection affects birds in the wild. Surveys of antibody presence in live birds do not tell us how many birds died of infection before being sampled, and experimental virus inoculations in the laboratory do not always mimic field infection because birds in the wild may be under different stresses that can affect how they respond to virus infection (Wobeser 2008, Brown and O'Brien 2011). These problems are difficult to surmount for many arboviruses, which occur unpredictably in space and time and are often detected at low frequency in both vertebrate hosts and arthropod vectors. Furthermore, most birds cannot be monitored when initially infected in the field to determine whether they live, die, or exhibit impaired reproductive performance. Only when a viral pathogen has dramatic, catastrophic effects on a host (such as West Nile virus on corvids; Yaremych et al. 2004, Caffrey et al. 2005) can we know how an arbovirus affects host fitness.

If we are to better understand how pathogens and microparasites are maintained or spread in a natural population, we need information on the extent to which avian hosts that are infected die and, therefore, how much population-wide immunity develops. And even if mortality is extensive, do infected vertebrate host individuals live long enough to infect additional arthropods? On the other hand, which individuals exhibit little response to infection and, thus, do not contribute to arbovirus transmission? Like other pathogens and parasites, arboviruses may affect wild birds in many different ways, depending on the time of year, the host's breeding stage, the extent of the host's energetic stress, or other phenotypic factors (Wobeser 2008).

Nestling birds offer many advantages for studying the effects of arboviruses on vertebrate hosts (O'Brien and Brown 2012). They tend to occur in locally high density at certain times of the year (e.g., summer), especially among colonial species. Once nests are found, nestlings are predictable in space, and this facilitates monitoring for virus, which can commence upon hatching and continue until fledging (or until the bird dies). However, despite these advantages, surprisingly little is known about how arboviruses affect nestling birds, in what contexts arbovirus infection represents a significant fitness cost to certain species, or the role nestlings play in arbovirus transmission in general (Unnasch et al. 2006, Burkett-Cadena et al. 2010, O'Brien et al. 2010b, Brown and O'Brien 2011).

Here, we explore how Buggy Creek virus (BCRV; Togaviridae, *Alphavirus*), an arbovirus with antigenic similarities to the widely distributed western equine encephalomyelitis virus (Hayes et al. 1977, Calisher et al. 1980), affects vertebrate host fitness in the wild. The host is the House Sparrow (*Passer domesticus*), an invasive species that has come into contact with BCRV upon this bird's invasion of Cliff Swallow (*Petrochelidon pyrrhonota*) colonies and its occupancy of Cliff Swallow nests. Buggy Creek virus historically was associated only with Cliff Swallows as its vertebrate host and the ectoparasitic Swallow Bug (Hemiptera: Cimicidae: *Oeciacus vicarius*) as its arthropod vector. Cliff Swallows rarely even show evidence of infection by BCRV, but House Sparrows in Cliff Swallow colonies are frequently infected by BCRV (Scott et al. 1984; O'Brien and Brown 2011, 2012; O'Brien et al. 2011). Here, we examine the extent to which BCRV affects fitness, as measured by nesting success, of House Sparrows occupying Cliff Swallow colonies, and use these results to increase our understanding of the factors that maintain BCRV epizootics in certain spatial foci. By focusing on nestling birds that could be monitored from hatching to fledging in the field, we were able to quantify the effect of this arbovirus on its vertebrate host more precisely than in most field studies of similar pathogens. We also evaluate the contribution of the invasive House Sparrow to annual transmission of this endemic arbovirus in its relatively specialized Cliff Swallow–Swallow Bug environment.

Methods

Study organisms and study area.—House Sparrows were introduced repeatedly into North America beginning in the 1850s (Lowther and Cink 2006). They are semicolonial, often forming aggregations of 2 to 20 nests in close proximity and remain at or near breeding sites year round (Anderson 2006). House Sparrows are multibrooded, with nesting in our study area beginning in late April and ending in early August, and with peak egg-laying periods in mid-May, late June, and late July.

Occupation of Cliff Swallow nests by House Sparrows is common throughout much of North America (Brown and Brown 1995). The first House Sparrows likely began to use 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 Cliff Swallows to form colonies near humans and, thus, brought Cliff Swallows into close proximity to House Sparrows. House Sparrows evict Cliff Swallows from their mud nests or occupy abandoned nests in colonies where Cliff Swallows are either present or absent.

Buggy Creek virus is a single-stranded, positive-sense RNA alphavirus (Hopla et al. 1993) that is ecologically distinct from other alphaviruses (e.g., western equine encephalomyelitis virus, eastern equine encephalitis virus) in that its only known vector is the Swallow Bug, rather than mosquitoes (Rush et al. 1980, 1981; Hopla et al. 1993; Brown et al. 2008). The Swallow Bug is a hematophagous nest-based ectoparasite primarily of the Cliff Swallow, but Swallow Bugs also parasitize House Sparrows nesting in Cliff Swallow nests and, thus, transmit BCRV to House Sparrows. Density of bugs in Cliff Swallow colonies can be quite high, with as many as 2,600 bugs per Cliff Swallow nest (Brown and Brown 1996) and 2,400 per House Sparrow nest (V. O'Brien unpubl. data). Bugs feed on birds, mostly at night, and cluster on the outside of active nests during the day after blood feeding.

Our study area is a 60 × 200 km area largely contiguous with the North and South Platte rivers in western Nebraska and is centered at the Cedar Point Biological Station (41°12.591′N, 101°38.969′W) in Keith County. It also includes portions of Lincoln, Garden, Duel, and Morrill counties. Each year, we monitor ~170 Cliff Swallow colony sites, which are occupied to varying degrees by only Cliff Swallows, Cliff Swallows and House Sparrows

together, or only House Sparrows. The study area is described in detail by Brown and Brown (1996). We studied House Sparrows at colonies located throughout the study area in concrete culverts beneath highways or railroads and on the sides of bridges.

Field sampling.—In May–July 2007, we systematically blood-sampled nestling House Sparrows from 16 colony sites throughout the study area. House Sparrow nests were examined for the presence of eggs or nestlings using a dental mirror and flashlight to see inside the nests. Nests that contained eggs were numbered and visited every 2–4 days to determine hatching date and nestling age. Whenever feasible, we blood-sampled all nestlings in a nest for BCRV. Nestlings were between 4 and 17 days of age when sampled. All birds were bled either once or twice during the nestling period by jugular 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). Nestlings were banded with federal 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 until screened for virus. In the summers of 2008–2009, we repeatedly mist netted adult House Sparrows at the same set of colony sites to look for banded birds from 2007 and 2008 that had recruited into the breeding population. Nestlings screened for virus in 2008 (n = 136) were used only in measuring recruitment, because we sampled only one or two nests per colony that year.

For each colony site where we blood-sampled House Sparrows, we measured the distance (m) between active House Sparrow nests and between active House Sparrow and active Cliff Swallow nests within the colony. Distances between colonies were measured using a global positioning system handheld unit (Garmin International, Olathe, Kansas).

We recorded House Sparrow fledging success by noting whether ≥1 nestling was found alive within a nest at ≥12 days of age. House Sparrows fledge between 14 and 17 days of age (Anderson 2006), but we used the 12-day criterion because checking nests after nestlings were older than 12 days often led to premature fledging and, thus, an inability to specify which nestlings had survived. Nestling age in the analyses here represented the oldest age reached when known to be BCRV-negative, or the age at when it was first detected as positive for BCRV. Some nestlings sampled for virus were not followed until fledging, which means that sample sizes differ among analyses, depending on whether knowing nest success was necessary.

Laboratory analyses.—Viral RNA was extracted from bird sera by first adding 25 μL of thawed sera 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 to the sera, RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, Valencia, California), following the manufacturer's protocol. A positive BCRV control (BCRV isolates from Swallow Bugs) was included in each extraction, and negative controls were placed between every 5 samples. Reverse-transcription 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-base pair (bp) fragment from the E2 region of the viral genome, as described in Moore et al. (2007). Product (6.5 μL) was electrophoresed on a 4% Nusieve–agarose gel to identify any positive samples, using at least one BCRV-positive control on each gel and a 100-bp ladder. See Moore et al. (2007) for additional details on the RT-PCR methods.

Samples that were initially BCRV-positive by RT-PCR were subjected to plaque assay on Vero cells at the Centers for Disease Control (Fort Collins, Colorado), as described in Huyvaert et al. (2008), with the exception of using yeast-extract lactalbumin instead of M-199 growth media. 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. A blood sample was considered BCRV-positive if either (1) it was RT-PCR-positive on initial screening and confirmed by plaque assay or (2) it was RT-PCR-positive on initial screening, negative by plaque assay, and positive by RT-PCR on second screening. Some birds were sampled on multiple days during the nestling period; in analyzing prevalence, a bird that tested positive upon first sampling was considered positive for the rest of the nestling period, whereas individuals that were initially negative were also used in calculating prevalence when sampled subsequently (because a negative status can change with time).

Statistical analyses.—For colony-based analyses of fledging success in relation to BCRV prevalence, we used the percentage of nests that were BCRV-positive (defined as ≥1 BCRV-positive nestling in the nest at any time) in a colony as the measure of BCRV prevalence at the site over the course of the summer. Fledging success was the percentage of total nesting attempts in the colony in which ≥1 nestling survived to 4 days of age that ultimately produced ≥1 nestling alive at day 12. Every nest in a colony that had ≥1 nestling alive at 4 days was sampled for BCRV; those failing before nestlings were old enough (4 days) to screen for virus were not included. All nests in each colony were collapsed into a single data point describing colony-wide fledging success or virus prevalence. Because nests with one BCRV-positive nestling were more likely to have additional positive nestlings, and less likely to have negative nestlings, than nests drawn at random (O'Brien and Brown 2011), infection among the nestlings within a nest was not independent. This required for most analyses that we use the nest as our unit of analysis.

We constructed *a priori* models to investigate factors that may have had an effect on the likelihood of nest success (≥1 nestling in the nest reaching 12 days of age). We used logistic regression to determine maximum likelihood estimates for each candidate model. The outcome was 0 (nest failed) or 1 (nest succeeded). Predictor variables were hatching date (HATCH), brood size at sampling (BROOD), linear distance from the nearest active House Sparrow nest (NNH), linear distance from the nearest active Cliff Swallow nest (NNC), and a binary variable (PN) indicating whether the nest contained ≥1 nestling positive for BCRV, coded as 0 (BCRV-negative nest) or 1 (BCRV-positive nest). Because of the range of the spatial data and distance-related outliers, potential predictors that used nearest-neighbor distance as a metric (NNH, NNC) were rank-transformed in SAS prior to logistic regression. To test for multicollinearity in predictor variables, we calculated the variance inflation factor for each continuous predictor using SAS (Proc REG with options VIF TOL; SAS Institute 2004). Models showing overdispersion (Hosmer-Lemeshow test, $\chi^2 > df$) were not considered in further analysis. We used Akaike's information criterion corrected for small sample size (AIC_c) to determine the best fitting of our remaining

candidate models. The AIC minimizes loss of information in the data by relating the maximum likelihood to the number of parameters in the model (Burnham and Anderson 2002). Weight of evidence for each model was determined by normalizing relative likelihood values generated by AIC_c using computed Akaike weights (w_i) for all candidate models. We included only models with an Akaike weight within 10% of the highest weight in our confidence set of models (Royall 1997) and used these models with their weights to compute model-averaged parameter estimates for each predictor variable (Burnham and Anderson 2002).

We interpreted effect size and direction in individual predictors using the values of model-averaged partial regression coefficients (β) and their respective 95% confidence intervals (CI) and log-odds ratios (e^{β}). We examined the shape of the predicted probabilities of the continuous variable (NNC) showing a likelihood of an effect on the response variable (95% CI did not include zero) by holding all other parameters constant at their mean and varying the focal parameter using the formula:

$$P_i = \frac{\text{Exp}\left(\beta_1 X_1 + \beta_2 X_2 + \ldots \beta_k X_k + \beta_0\right)}{1 + \text{Exp}\left(\beta_1 X_1 + \beta_2 X_2 + \ldots \beta_k X_k + \beta_0\right)}$$

Results

We had data on infection status and survival to fledging for 334 nestling House Sparrows from 101 nests at 13 colonies. When considered irrespective of the fate of their nestmates, nestling House Sparrows identified as first infected between 4 and 6 days of age were significantly less likely to survive to fledge than similarly aged counterparts that were not infected, and the same result held for birds at older ages (Fig. 1). Birds infected at older ages (7–11 days) had a significantly higher likelihood of fledging than those infected at younger ages (Fig. 1), although <40% of infected nestlings at the older ages were known to survive to day 12 and, presumably, to fledge.

Overall House Sparrow reproductive success at a given colony was strongly associated with overall BCRV prevalence at that site (Fig. 2). For 9 colonies where we had ≥5 nests with information on virus prevalence and fledging success, nesting success (≥1 nestling surviving to day 12) declined significantly as BCRV prevalence at the site increased (Fig. 2).

There were five models in the confidence set when we considered the likelihood that a nest produced ≥1 nestling to fledging age, and none singly had substantial weight of support (Table 1). The top two ranked models were of equal weight (w_i = 0.265 and 0.263; Table 1), and the fifth-ranked model was only 2× less supported. This demonstrates model uncertainty within the confidence set of models. Models without an effect of brood size at sampling (BROOD) or without ≥1 BCRV-positive nestling in the nest (PN) had little to no weight of support in the model set (Table 1).

Three predictor variables had model-averaged parameter estimates with a 95% CI that did not include zero (Table 2). The odds of ≥1 nestling House Sparrow in a nest reaching 12 days of age (fledging) if any nestling in that nest was BCRV-positive (PN) were 67% lower (e^{β} = 0.334) than if all nestlings in the nest were BCRV-negative. A large brood size at sampling (BROOD) predicted fledging success, with the odds of a successful nest being 1.9× higher

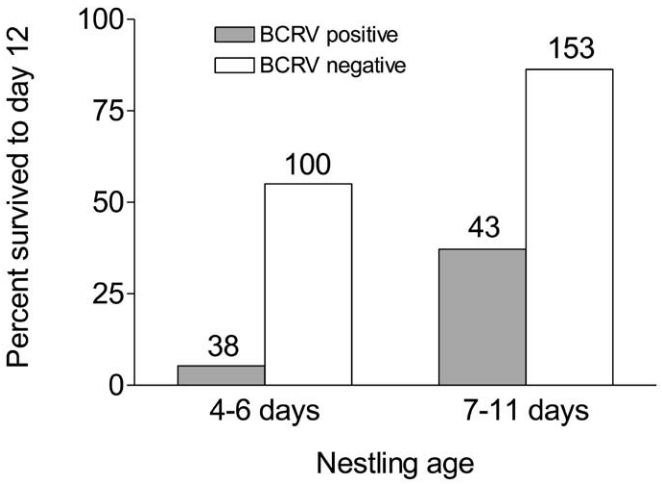

Fig. 1. Percentage of total nestling House Sparrows that survived to fledge (present at 12 days or beyond) in relation to whether (and at what age) they were infected with Buggy Creek virus (BCRV) at Cliff Swallow colony sites in southwestern Nebraska in 2007. Infected (BCRV-positive) birds are indicated by hatched bars, and uninfected (BCRV-negative) birds by clear bars. Sample sizes (number of nestlings) are shown above bars. For birds 4–6 days old, infected birds had significantly lower fledging success than uninfected birds (χ^2 = 28.1, df = 1, P < 0.0001), as did birds 7–11 days old (χ^2 = 43.7, df = 1, P < 0.0001). Infected birds 4–6 days old had lower fledging success than infected birds 7–11 days old (χ^2 = 11.9, df = 1, P = 0.001).

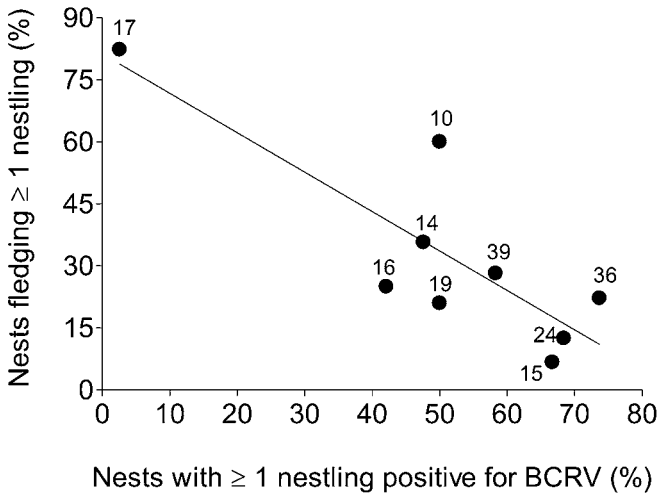

Fig. 2. Overall House Sparrow nest success (as measured by the percentage of nests fledging ≥1 nestling) in relation to Buggy Creek virus (BCRV) prevalence at the site (as measured by the percentage of House Sparrow nests with ≥1 nestling positive for virus) at Cliff Swallow colony sites in southwestern Nebraska in 2007. Sample sizes (number of nests) for each colony are shown by dots. Line indicates best-fit least-squares regression. Colony fledging success declined as BCRV prevalence at the site increased (r = –0.84, P = 0.005, n = 9 colonies).

Table 1. Model selection results for logistic regression on House Sparrow fledging success from nests in Nebraska in 2007 (n = 101 nests). A nest was considered successful if ≥1 nestling reached 12 days of age. Global model included all predictor variables. Predictor variables are defined in the text.

Model	k	AIC_c	ΔAIC_c	–2LL	w_i	Model description
PN, DATE, BROOD, NNH	5	101.083	0.000	90.451	0.265	No effect of NNC
PN, DATE, BROOD	3	101.103	0.020	92.451	0.263	No effect of NNC or NNH
Global	6	101.947	0.864	89.451	0.172	Full model
PN, DATE, BROOD, NNC	5	102.106	1.023	91.451	0.159	No effect of NNH
PN, BROOD, NNH, NNC	5	102.451	1.368	91.451	0.134	No date effect
PN, DATE, NNH, NNC	5	108.616	7.533	97.451	0.006	No effect of brood size
DATE, BROOD, NNH, NNC	5	117.636	16.553	107.451	0.000	No effect of BCRV infection
Null	1	134.749	33.646	132.451	0.000	Intercept-only

(e^{β} = 1.91) for each additional bird found in the nest at sampling. The predicted probability that a brood of 1 reached 12 days of age was extremely low (<25%).

Because brood size at sampling could reflect BCRV infection and associated mortality in very young nestling House Sparrows prior to their first being sampled, we compared observed brood sizes for nests in two colonies with low BCRV prevalence (5.0%, n = 40 nestlings; and 0.5%, n = 186 nestlings) with nests in all other colonies sampled (mean overall BCRV prevalence = 17.9% ± SE 3.6, n = 614 nestlings). There was a significant difference in the percentage of brood-size distributions between the low-virus colonies and the high-virus colonies that was most pronounced at the brood-size extremes (1 and 6 nestlings; Fig. 3).

The distance of an active Cliff Swallow nest from a focal House Sparrow nest (NNC) had a weak negative effect on House Sparrow fledging success, with odds of fledging decreasing by 1.8% (e^{β} = 0.988) for each unit increase in distance from an active Cliff Swallow nest (Fig. 4).

We recaptured 8 House Sparrows in summer 2008 that had been sampled as nestlings for BCRV in 2007, and 2 nestlings from 2008 were recaptured in 2009. None of these birds were viremic when sampled as nestlings, none had siblings with BCRV, and none of the 8 nestlings from 2007 were antibody-positive for BCRV in 2008 (W. K. Reisen pers. comm.), which indicates that they probably had never been exposed to the virus. Four of the recaptured birds were found at their natal colony, 4 more at colonies 0.22–0.71 km from their natal colony, and the other 2 at colonies 6.12 and 16.07 `km, respectively, from their natal colony. Across all ages, only ~9% of infected birds survived to fledge (e.g., Fig. 1), and the distribution of recaptures did not differ significantly from that expected if recaptures were independent of infection status (χ^2 = 0.96, df = 1, P = 0.33).

Table 2. Model-averaged parameter estimates (β), unconditional standard errors (SE), and 95% confidence intervals (CI) for predictors of a House Sparrow nest's fledging success (≥1 nestling reaching 12 days) from logistic regression analysis of nests in Nebraska in 2007. Parameters are defined in the text. CI for parameters shown in bold do not include zero.

			95% CI	
Parameter	β	SE	Upper	Lower
Intercept	–2.0104	1.1594	0.2504	–4.2712
PN	**–1.0962**	**0.2851**	**–0.5403**	**–1.6521**
DATE	0.0187	0.0153	0.0486	–0.0113
BROOD	**0.6464**	**0.2117**	**1.0592**	**0.2337**
NNH	0.0145	0.0112	0.0362	–0.0073
NNC	**–0.0117**	**0.0044**	**–0.0032**	**–0.0202**

Discussion

The most important determinants of fledging success for House Sparrows occupying Cliff Swallow nests in western Nebraska were whether one or more nestlings in a nest were infected with BCRV and, for those infected, at what age were they first exposed to the virus. An age effect may reflect development of a more effective immune system as an individual becomes older (Apanius 1998, Klasing and Leschinsky 1999), given that virus titer levels and overall prevalence declined in older nestlings and adults (O'Brien et al. 2011, O'Brien and Brown 2012). Not unexpectedly, brood size also affected

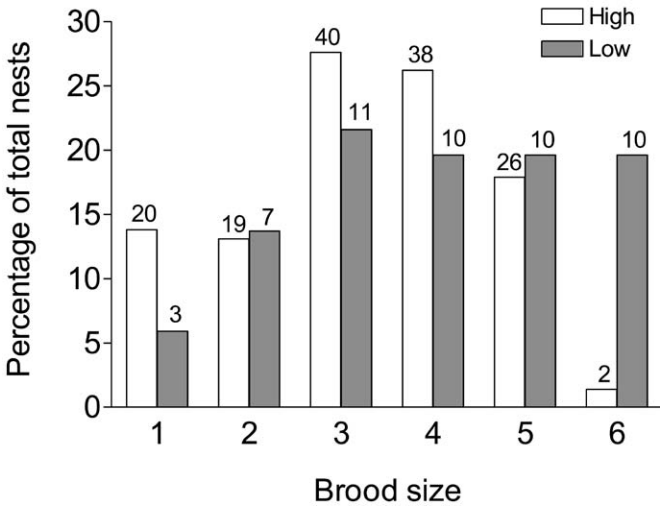

Fig. 3. Percentage of House Sparrow nests of different brood sizes at time of first sampling in colonies with high (clear bars) and low (dark bars) overall prevalence of Buggy Creek virus in nestlings (see text) at Cliff Swallow colony sites in southwestern Nebraska in 2007. Numbers above bars represent number of nests that contained that brood size at first sampling for each group. The brood-size distributions differed significantly between and high- and low-virus sites (χ^2 = 23.8, df = 5, P < 0.0001).

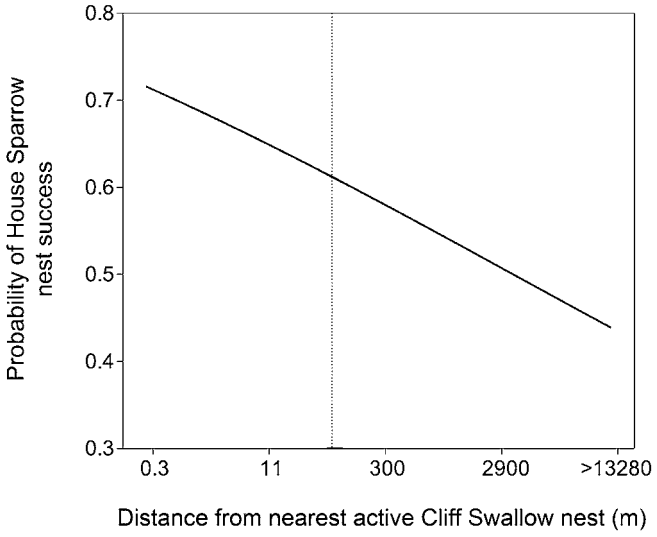

Fig. 4. Predicted probability of a House Sparrow nest being successful (≥1 nestling in the nest at 12 days or beyond) with distance from the nearest active Cliff Swallow nest (NNC) at Cliff Swallow colony sites in southwestern Nebraska in 2007. In some cases, the nearest Cliff Swallow nest was at a different colony site, and the dotted vertical line represents the break between within-colony distances and between-colony distances.

whether some nestlings survived to fledge, but we found that observed brood sizes were influenced strongly by whether a colony site had high virus prevalence. Although reproductive success in many bird species, including House Sparrows, often declines as the season advances (Rowe et al. 1994, Brown and Brown 1999, Kinnard and Westneat 2009), date had no effect in our study, probably because virus infection so heavily affected nestling survival and masked any independent date-related influence. It is also unlikely that the presence of the blood-feeding Swallow Bugs, per se, led to the mortality we documented; nearby nests with uninfected nestling House Sparrows (that survived) typically contained similar numbers of bugs visible on the outside (V. O'Brien and C. Brown unpubl. data). Furthermore, a sample of necropsied nestlings that were found dead or moribund all showed extensive pathology related to virus infection (O'Brien et al. 2010a). Thus, BCRV had a major role in shaping annual reproductive success of House Sparrows in Cliff Swallow-colony habitats; we found no other obvious causes of nest failure. In a similar study of House Sparrows and the Fort Morgan strain of BCRV in eastern Colorado in the 1970s, Scott et al. (1984) also found nestling mortality presumably attributable to virus, but they concluded that the principal source of nest failure was the nests' falling from the substrate. We observed few cases of nest fall in our study, although old Cliff Swallow nests often do fall from the substrates during the off-season (fall, winter, and early spring), when birds are not nesting. No previous studies have shown such a dramatic effect of an arbovirus on avian host fitness during nesting.

Approximately 18% of all nestling House Sparrows in our study area were known to be infected with BCRV (O'Brien et al. 2011), and the results presented here indicate that relatively few fledged. We found no evidence that any nestling that had BCRV survived to recruit into the breeding population, although because so few infected nestlings survived to fledge, we would not expect to recapture many. In another major study of North American arboviruses in nestling birds, Holden et al. (1973b) found that 6.7% of nestling House Sparrows in Texas were infected with arboviruses (principally western equine encephalomyelitis virus). Mortality attributable to virus was not reported (Holden at el. 1973b), but even if all infected nestlings died, House Sparrows in Nebraska still had approximately double the mortality of House Sparrows in Holden et al.'s (1973b) study. The high prevalence of BCRV in House Sparrows and the deleterious effects of this virus may reflect this species' relatively recent exposure to BCRV (O'Brien et al. 2011). Because of the virus's ecological specialization to Swallow Bug vectors and Cliff Swallow nesting colonies, House Sparrows have encountered this pathogen only since they began using old Cliff Swallow nests for breeding, and in our study area this has been within the past 60 years as artificial nesting sites (bridges, culverts) were constructed near towns where House Sparrows occur (O'Brien et al. 2011, Brown et al. 2012).

Although BCRV causes high mortality in nestling House Sparrows, these birds nevertheless appear to be highly suitable amplifying hosts for this virus. Nestlings do not die immediately and often maintain viremias sufficiently high to infect blood-feeding bugs for several days (O'Brien and Brown 2012), which suggests that a viremic nestling can potentially transmit virus to large numbers of Swallow Bugs. The frequent renesting of House Sparrows at the same site, even when their previous nesting attempts failed as a result of BCRV, means that they continually produce susceptible hosts (i.e., nestlings) for most of the summer. The ability of House Sparrows to amplify BCRV so effectively has possibly led to long-term increases in the prevalence of this virus in bugs and increases in virulence to vertebrates at sites that perennially contain both House Sparrows and Cliff Swallows, in contrast to Cliff Swallow-only sites, where no such directional trends are apparent (Brown et al. 2012). The House Sparrow's effectiveness as an amplifying host may be leading to evolutionary changes in the virus, in which BCRV is diverging into two distinct lineages, one dependent on House Sparrows for transmission and the other (in Cliff Swallow-only colonies) primarily a bug virus (Brown et al. 2009). Some evidence indicates that BCRV infection rates in House Sparrows in the western Great Plains have increased over the past 30 years (Brown et al. 2012).

With BCRV being the predominant source of nestling mortality for House Sparrows occupying Cliff Swallow colonies, could this lead to selective sweeps of the sparrow population (*sensu* de Groot et al. 2002) that will eventually result in these birds' becoming resistant to this virus? Cliff Swallows have been exposed to BCRV for a much longer time and show little pathological response to this virus and a poor ability to amplify it (O'Brien et al. 2011). Why House Sparrows and Cliff Swallows react so differently to this virus is unclear. It might reflect the Cliff Swallow's longer coevolutionary history with BCRV (allowing for the evolution of resistance), higher levels of herd immunity in Cliff Swallows, or inherent differences in immunological response between the native Cliff Swallow and the invasive House Sparrow. For example, invasive species, because they have been selected for high reproductive rates, may invest less in their immune systems and may be more susceptible to disease in new environments (Lee and Klasing 2004). On the other hand, highly social species such as the Cliff Swallow often show enhanced immunological response to

parasites and pathogens (Møller et al. 2001), presumably because they typically encounter heavy parasite loads. Currently, work is underway to explore differences between House Sparrows and Cliff Swallows in their immune responses to BCRV.

Whether House Sparrows evolve resistance or herd immunity to BCRV will depend on the extent to which House Sparrows in Cliff Swallow colonies represent closed populations. Our data indicate that turnover by breeders at these colony sites is high: we banded 181 breeding adults in our study colonies in summer 2008, but found that only 8.6% of the 81 adults caught in these colonies in 2009 were recaptures from the previous summer. This is lower philopatry than seen in other House Sparrow populations not using Cliff Swallow colonies (Anderson 2006, D. Mock pers. comm.). High turnover may be caused in part by higher nest-failure rates (because of BCRV) that lead to greater dispersal out of Cliff Swallow colonies by failed House Sparrow breeders, given that breeding dispersal depends heavily on nesting success in most bird species (Greenwood and Harvey 1982, Pärt and Gustafsson 1989, Brown and Brown 1996, Clobert et al. 2001). As failed breeders vacate these sites, openings exist for transient House Sparrows, probably reared at other sorts of nest sites and never exposed to BCRV, to move in. Breeding-bird mortality associated with BCRV is unlikely to explain the high turnover, because BCRV has little apparent effect on adult House Sparrows (Huyvaert et al. 2008, Brault et al. 2009, O'Brien et al. 2011).

If House Sparrows reared in Cliff Swallow nests showed a preference for such nesting sites as adults (and ones reared elsewhere also preferred their natal nest types), relatively closed populations within Cliff Swallow colonies might result, which could lead to the evolution of resistance if some infected nestlings survive. However, one study showed that House Sparrows do not imprint on their natal nest type (Cink 1976), and therefore it is unlikely that nest-type preference leads to any sort of population structure in this species. Furthermore, even if infected birds of any age survive and develop BCRV antibodies, it is unlikely that they pass maternal antibodies to BCRV to their offspring, given that House Sparrows have not shown effective maternal transmission of antibodies to other arboviruses (Holden et al. 1973a, Nemeth et al. 2008). Thus, frequent turnover of breeders, lack of imprinting on nesting sites, and limited maternal transmission of antibodies all suggest that Cliff Swallow colonies will remain perpetually active foci for BCRV infection in House Sparrows. Few other arboviruses are known to be this predictable in their spatial or temporal occurrence.

Some evidence indicates that when House Sparrows occupy nests in active Cliff Swallow colonies, the presence of Cliff Swallows acts to "dilute" the likelihood of House Sparrows being infected by BCRV (*sensu* Hess and Hayes 1970, Schmidt and Ostfeld 2001), probably because Swallow Bugs prefer to feed on Cliff Swallows when both species are present (O'Brien et al. 2011). The analyses reported here further support a dilution effect. The closer a House Sparrow nest was to an active Cliff Swallow nest, the more likely the House Sparrow nest was to contain birds that successfully fledged. This may be because fewer bugs fed on House Sparrows when a Cliff Swallow nest was nearby and, thus, BCRV was less likely to be transmitted to the House Sparrows. House Sparrows occupying abandoned Cliff Swallow colonies likely have lower fitness than their counterparts nesting with Cliff Swallows; this could perhaps contribute to House Sparrows' gradual disappearance from sites that are perennially unused by Cliff Swallows (without Cliff Swallows to rebuild the mud nests periodically, nest fall also contributes to localized House Sparrow extinction at a site). The recent exposure of House Sparrows to BCRV provides a natural experiment to observe whether they adapt to the virus at both ecological and physiological levels and to what extent the presence of Cliff Swallows may influence that adaptation through the dilution effect. That this virus is such a major determinant of host fitness in House Sparrows suggests that the potential effect of arboviruses on other nesting birds should not be overlooked.

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