Behavioral Ecology and Sociobiology (2021) 75:106
https://doi.org/10.1007/s00265-021-03040-1

ORIGINAL ARTICLE

Group-size effects on virus prevalence depend on the presence of an invasive species

Amy T. Moore[1] · **Valerie A. O'Brien**[1,2] · **Charles R. Brown**[1]

Received: 11 April 2021 / Revised: 17 May 2021 / Accepted: 26 May 2021

Abstract

The extent to which host group size affects the hosts' exposure to parasites and pathogens has been explored by behavioral ecologists for almost 50 years, and we know that host and parasite taxa, mobility of host and parasite, and the extent of spatial structure within groups all affect the group-size relationship. Here we examine how the prevalence of an arthropod-borne viral pathogen changes with host group size in a host-parasite system recently invaded by an introduced species that also serves as a host for the virus. Infection by the alphavirus, Buggy Creek virus (BCRV), in swallow bugs (*Cimex vicarius*) increased with colony size of the cliff swallow (*Petrochelidon pyrrhonota*; the bugs' principal host) in the absence of invasive house sparrows (*Passer domesticus*) but decreased with swallow colony size in the presence of sparrows. The sparrow-adapted lineage A of BCRV declined to near extinction in the largest cliff swallow colonies, regardless of sparrow presence. The results may reflect BCRV's divergence into a lineage (B) that amplifies mostly in bugs and thus is transmitted more effectively in large cliff swallow colonies that maintain high numbers of the blood-feeding bugs, whereas the other lineage (A) is adapted to house sparrows and does not require large numbers of bugs and cliff swallows for effective transmission and persistence. The results show that an alternative host can modify the group-size consequences for the original host and illustrate another complexity in analyzing the costs and benefits of coloniality whenever invasive species are present.

Significance statement

Predicting the spread of viral pathogens can be important in assessing threats to wildlife or humans, and the size of a host's social group is regarded as a potentially important determinant of pathogen exposure. Invasive species can sometimes profoundly alter disease dynamics when they enter a host-parasite system and serve as alternative hosts, thereby either increasing or decreasing the original host's exposure to pathogens. For an arthropod-borne virus that is vectored by a blood-feeding bug that parasitizes a colonially breeding bird, we found that virus infection in bugs increased with host (bird) group size when an invasive bird species was absent but decreased with host group size when the invasive was present. The study indicates that the presence of alternative hosts is another variable to consider in trying to predict how pathogen exposure is affected by host group size.

Keywords Arbovirus · Buggy Creek virus · *Cimex vicarius* · Cliff swallow · Disease ecology · House sparrow

This article is a contribution to the Topical Collection Sociality and Disease - Guest Editors: Rebeca Rosengaus, James Traniello, and Theo Bakker

Communicated by T. C. M. Bakker

✉ Charles R. Brown
charles-brown@utulsa.edu

[1] Department of Biological Sciences, University of Tulsa, 800 S. Tucker Dr, Tulsa, OK 74104, USA

[2] Present Address: School of Science and Mathematics, Tulsa Community College−Metro Campus, 909 S. Boston Ave, Tulsa, OK 74119, USA

Introduction

A fundamental question in behavioral ecology is to what extent host group size affects the prevalence and abundance of parasites or pathogens within a group. Early predictions (Alexander 1971, 1974) that a larger social group should enhance transmission of parasites among group members were initially supported in a variety of animals (Hoogland and Sherman 1976; Hoogland 1979; Brown and Brown 1986; Møller 1987; Moore et al. 1988; Rubenstein and Hohmann 1989; Davies et al. 1991; Ezenwa 2004), and later meta-analyses confirmed this result

Published online: 05 July 2021

for at least certain kinds of parasites and for certain measures of parasitism (e.g., prevalence, abundance; Poulin 1991; Côté and Poulin 1995; Nunn and Heymann 2005; Bordes et al. 2007; Rifkin et al. 2012; Patterson and Ruckstuhl 2013). More recent work, however, has revealed that a positive effect of group size on parasitism is far from a universal pattern, with some species (especially mammals) showing no (or a negative) effect of host group size (Viljoen et al. 2011; Leclaire and Faulkner 2014; Nunn et al. 2015). Some studies now suggest that within-group social structure (e.g., frequency of interactions with particular individuals) may be a better predictor of parasite risk (Craft 2015; Briard and Ezenwa 2021; Lucatelli et al. 2021) than group size per se. We also know that "contagious" parasites (those directly transmitted among hosts by physical contact) are more likely to show positive effects of host group size than mobile parasites (that do not require hosts to be in close proximity to transmit), which may even decrease in larger groups due to encounter-dilution effects (Mooring and Hart 1992; Côté and Poulin 1995; Buck and Lutterschmidt 2017; Samson et al. 2019). Studies of group-size effects have often focused on how macroparasites (e.g., fleas, ticks, flies, bugs, helminths) vary with host group size.

Host population size is also critical in sustaining microparasite (e.g., virus) transmission (Dietz 1988; Anderson and May 1992; de Jong et al. 1995; Keeling and Grenfell 1997; Jeong and McCallum 2021), with the persistence of contagious pathogens such as viruses often depending specifically on host density (Swinton et al. 1998; Packer et al. 1999), which often varies directly with group size (Brown and Brown 1996; Altizer et al. 2003). Mitigating the spread of directly transmitted viruses has usually consisted of reducing host density to reduce the likelihood of close contact between hosts, methods that have worked to control disease incidence in both animal and human populations (Dynes et al. 2019; Masters et al. 2020; Khataee et al. 2021; Stockmaier et al. 2021).

How host group size affects transmission is still relatively poorly understood for one group of viruses, the arthropod-borne viruses (arboviruses; Rifkin et al. 2012). These viruses typically require a mobile arthropod (e.g., tick or fly) to transmit the virus via blood-feeding to a vertebrate amplifying host. The extent to which the arthropod vector itself transmits via close contact among hosts could determine whether hosts in groups are more or less likely to suffer virus infection. While some arthropod vectors (e.g., mosquitoes) are known to be attracted to larger groups of hosts (Davies et al. 1991; Brown and Sethi 2002; Robert et al., 2003; Nunn and Heymann 2005; Kent et al. 2009), in others the per-capita rate of hosts being bitten declines in larger groups through phenomena such as the encounter-dilution effect (Mooring and Hart 1992). Thus, for arboviruses, group size of both the invertebrate vectors and the vertebrate hosts potentially determines virus prevalence in the system.

Buggy Creek virus (BCRV) is an alphavirus in the western equine encephalomyelitis virus (WEEV) complex (Calisher et al. 1980, 1988; Hopla et al. 1993) that is vectored by a cimicid swallow bug (Hemiptera: Cimicidae: *Cimex vicarius*) and infects colonially nesting cliff swallows (*Petrochelidon pyrrhonota*) and invasive house sparrows (*Passer domesticus*) that occupy cliff swallow nests. A short-term study suggested that swallow bug vectors were more likely to be infected with BCRV in larger cliff swallow colonies (Brown et al. 2001), and other work showed that house sparrow nestlings were more likely to be infected in larger sparrow colonies and when sparrow nests were closer together (O'Brien and Brown 2011). Depending on their competence as amplifying hosts, invasive species such as house sparrows can disrupt natural disease dynamics whenever they serve as alternative hosts for a pathogen, where they can either increase the likelihood of native species becoming infected through parasite "spillback" (Kelly et al. 2009) or decrease virus transmission to native hosts through the "dilution effect" (Ostfeld and Keesing 2000; Keesing et al. 2006; Johnson and Thieltges 2010). Thus, evaluating the effect of group size on pathogen prevalence may require accounting for the presence of invasive species and how they influence host–pathogen dynamics. With the proliferation of introductions of invasive species around the globe, understanding the suite of their effects on host-parasite systems is critical (Telfer and Bown 2012; Westby et al. 2019).

In this study, we revisit the effect of host group size on the prevalence of BCRV, with the work differing in two major ways from the earlier (Brown et al. 2001) report: (1) here we use 11 years of data (as opposed to 3), which provide a large enough sample size to do more sophisticated statistical analysis, and (2) we address explicitly the role of invasive house sparrows in producing any group-size effects. We confine our analysis here to the prevalence of BCRV in swallow bug vectors and investigate how cliff swallow colony size, presence of sparrows, and other co-variates (e.g., year, date, site usage, bug age, bug behavior) potentially affect BCRV infection in bugs. Although other work has examined correlates of BCRV infection among bugs in the fall, winter, and spring when no cliff swallows are present (Brown et al. 2010b), this paper addresses effects of group size only for the summer season, while swallows are breeding at their colonies. Because the number of swallow bugs in a colony varies directly with the number of cliff swallows in the colony (Rannala 1995; Brown and Brown 1996), our results apply generally to the effects of group size in both the bug vectors and their swallow hosts.

Methods

Study organisms

BCRV was first isolated in 1980 from swallow bugs collected at a cliff swallow colony along Buggy Creek in Grady County, west central Oklahoma (Loye and Hopla 1983; Hopla et al.

1993). It is a strain of Fort Morgan virus (Padhi et al. 2008), which is also associated with cliff swallows and swallow bugs (Hayes et al. 1977; Calisher et al. 1980, 1988; Scott et al. 1984). BCRV has been isolated only from swallow bugs, cliff swallows, and house sparrows, and this virus is unique among its relatives (e.g., WEEV, Highlands J virus, eastern equine encephalitis virus) in not being vectored by mosquitoes (Calisher et al. 1980; Rush et al. 1980; Allison et al. 2015). There are two BCRV lineages (designated A and B) circulating in our Nebraska study area, with these differing from each other by about 6% at the nucleotide level (Pfeffer et al. 2006). The lineages have diverged relatively recently, and lineage A is more associated with (and more likely to amplify in) house sparrows than is lineage B, which transmits mostly among swallow bugs (Padhi et al. 2008, 2011; Brown et al. 2009a).

Swallow bugs are nest-based parasites that overwinter in cliff swallow nests or in the cracks and crevices of the nesting substrate near the nests. They are hematophagous, feeding on the birds mostly at night, and they travel on the adult birds relatively rarely (Loye 1985; George 1987; Brown and Brown 1996, 2004). Infestations can reach 2600 bugs per nest, and the bugs affect many aspects of cliff swallow life history (Brown and Brown 1986, 1992, 1996, 2002; Chapman and George 1991; Loye and Carroll 1991; Brown et al. 2021). They are long-lived ectoparasites that begin to reproduce as soon as they feed in the spring. Eggs are laid in several clutches that hatch over variable lengths of time, ranging from 3–5 days (Loye 1985) to 12–20 days (Myers 1928). Nymphs undergo five instars in about a 10-week period before maturing, and they feed on birds' blood at each instar stage. Because swallow bugs are confined to cliff swallow nests and colony substrates and rarely travel on the adult birds, they only have access to hosts when cliff swallows (or house sparrows) occupy a colony site or reuse existing nests. The birds do not use all of the colony sites in a given year (Brown and Brown 1996; Brown et al. 2013), and bugs are adapted to withstanding long periods of host absence, in some cases persisting at a site not used by cliff swallows for up to 4 consecutive years (Smith and Eads 1978; Loye 1985; Loye and Carroll 1991; Rannala 1995). Bugs also take blood meals from house sparrows and barn swallows (*Hirundo rustica*) that occupy nests in some cliff swallow colonies (Scott et al. 1984; Kopachena et al. 2007; O'Brien et al. 2011).

Cliff swallows (Fig. 1a) are highly colonial passerines that breed commonly in much of North America (Brown et al. 2020). They build gourd-shaped mud nests and attach them to the vertical faces of cliff walls, rock outcrops, or artificial sites such as the eaves of buildings or bridges. Their nests tend to be stacked closely together, often sharing walls. Cliff swallows are migratory, wintering in southern South America, and have a relatively short breeding season in North America. They begin to arrive at our study site in late April or early May, and most depart by late July. Individual colonies are highly synchronous and are quickly vacated by swallows after the nestlings fledge. Nestlings are in the nest for about 26 days before fledging (Brown et al. 2020).

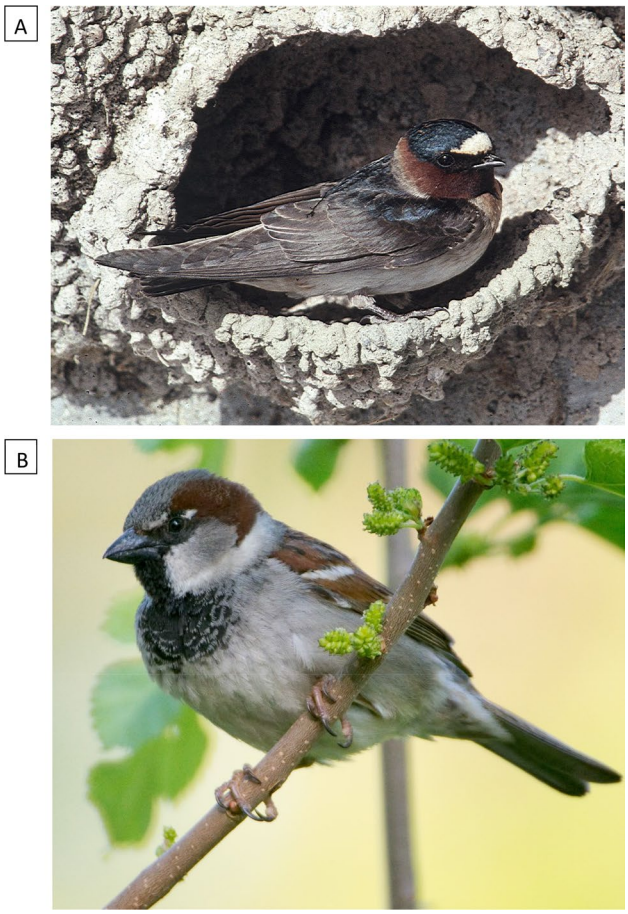

Fig. 1 The native cliff swallow (**a**) and the invasive house sparrow (**b**)

House sparrows (Fig. 1b) were introduced repeatedly into North America beginning in the 1850s (Lowther and Cink 2020) and are now found mainly in peri-domestic settings. Sparrows are both solitary and semi-colonial, sometimes forming aggregations of 2–20 nests in close proximity. They are sedentary, remaining at or near breeding sites year-round (Anderson 2006). House sparrows are multi-brooded, with nesting in our study area beginning in late April and ending in August; peak egg laying periods are in mid-May, late June, and late July. New broods are started soon after earlier ones fail or fledge. Nestlings fledge at 14–17 days of age (Anderson 2006). 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 brought the swallows into close proximity to house sparrows. Sparrows evict cliff swallows from their mud nests or occupy abandoned nests in colonies where cliff swallows are either present or absent

(Brown and Brown 1996). House sparrows in our study area are most commonly found at cliff swallow colonies within 1 km of human-associated activity.

Study site

Our study site is centered at the Cedar Point Biological Station (41.2097° N, 101.6480° W) near Ogallala, in Keith County, along the North and South Platte Rivers, and also includes portions of Deuel, Garden, Lincoln, and Morrill counties, western Nebraska, USA. We have studied cliff swallows there since 1982 (Brown et al. 2021). Approximately 220 physical sites where colonies of cliff swallows nest are in our 200 × 60 km study area, and about a third of these sites are not used in a given year. Annual colony size varies widely; in our study area, it ranges from 1 to 6000 nests, with the mean (± SD) being 404 nests (± 631; $n = 3277$ colonies). Each colony site tends to be separated from the next nearest by 1–10 km but in a few cases by ≥ 20 km. The study site is described in detail by Brown and Brown (1996) and Brown et al. (2013).

Field methods

We collected swallow bugs for virus isolation in two ways. At inactive colony sites where no cliff swallows were nesting that year, or at active sites after nestlings had fledged, we removed nests in their entirety from the substrate and bagged them individually in plastic bags. The nests were broken apart in pans and bugs removed individually with forceps. In 1 year only (1998), we used Berlese funnels to harvest bugs from chunks of nests (Brown et al. 2001). At active colony sites, nest collecting was done only for nests where birds had fledged within 1–2 weeks prior to collection.

The other collection method used a wire brush to sweep bugs off the outsides of nests into a wide-mouthed jar, which did not involve collecting the nest. This technique allowed us to sample bugs at active nests still containing eggs or nestlings. Swallow bugs on the outsides of nests typically were either clustered on the inner rim of the tubular entrance of the nest or distributed in lower density across the bottom, the sides of the nest, and on the front below the entrance. Bugs swept into jars were placed into transparent plastic bags and later extracted from pans with forceps.

We attempted to randomly sample nests from all parts of a colony, but portions of colonies were sometimes inaccessible due to high water underneath nests. We usually collected at least 1000 bugs per site and typically sampled 10–30 nests per site (except at small colonies < 10 nests in size), depending on the level of infestation. In some cases, bugs from multiple nests were combined, and we did not record which specific nest each bug came from within a colony. This was deemed justified given bugs' mobility and their frequent movement between adjacent nests (Brown et al. 2001; VAO and CRB, unpubl. data). Most colony sites were sampled once (on one date) during each summer, but when an inactive site later became active that season, samples were taken on a second date.

Live swallow bugs were sorted into pools of 100 for virus screening and immediately frozen at − 70 °C. Pooling arthropods for testing has been standard in arbovirology for at least 60 years and is still widely used, mostly because of the impracticality of screening each individual among the large numbers of arthropods typically available for collection (Chiang and Reeves 1962; Chisenhall et al. 2008; Maya-Delgado et al. 2020; Tang et al. 2020). While sorting bugs into pools, we noted whether a pool consisted exclusively of adult bugs, instars, or a mixture. Adults and instars can be identified by the instars' smaller size and greater head to thorax ratio (Usinger 1966), although late instars (e.g., the 5th) can be difficult to separate from adults. In some cases, we could not get 100 bugs per pool, so pool size was used as a co-variate in analyses.

Pools were also categorized by the bugs' behavior at the time of collecting. Bugs clustered at the nest entrances were designated as *clustered*, with clustering typically confined to nests that had been inactive that season prior to collecting. Other categories were *outside*, for those swept from the exteriors of active nests, and *inside*, for those harvested from nests that were removed in their entirety. We also categorized the colony site and nest as active (cliff swallows present that summer) or inactive (no swallows nesting there that summer). Throughout, whenever we refer to an active or inactive colony site or nest, it is in reference to the presence or absence of cliff swallows, respectively. Only bugs collected between May and July (the cliff swallow's breeding season) were used in these analyses, and date was designated as 1 = 1 May, 32 = 1 June, 62 = 1 July, etc.

Cliff swallow colony size was defined as the maximum number of nests at a site to have contained one or more eggs. Active swallow nests were counted at some sites by periodically checking the nest contents with a dental mirror and flashlight, whereas the swallow colony size at other sites was estimated by counting the total number of nests in sections of the colony known to be active. Full details on these methods of determining colony sizes are given in Brown and Brown (1996) and Brown et al. (2013). Whether house sparrows were present was noted at each colony site we sampled for BCRV; if at least one nest contained sparrows, the colony was categorized as sparrows present for that season. We recorded actual sparrow colony sizes (number of active nests) at a subset of 37 sites in 2006–2008, where we checked each house sparrow nest for eggs or nestlings throughout the season. These sites were mostly ones where we focused on

measuring BCRV prevalence in house sparrow nestlings but not in swallow bugs.

Colony site refers to the physical location where a group of birds nest, while colony denotes collectively the individual birds breeding there that year. In determining the proportion of prior years that a colony site had been active (one or more active cliff swallow nests), we used all years for which we had data, which for most sites was > 20 years. However, if we had discovered the site within the past 3 years, knew nothing of its usage history prior to then, and thus the number of prior years was < 4, we did not use that colony site in analyzing prior site use. The 4-year threshold for inclusion was arbitrary.

Laboratory methods

Bug pools were triturated by mortar and pestle and suspended in 1.0 ml of BA-1, a growth medium containing antibiotics and 20% fetal bovine serum. The homogenates were centrifuged and subsequently stored at − 70 ˚C. In 1998–2003, virus screening was done exclusively by plaque assay, in which 100 µl of the supernatant was added in duplicate to a monolayer of Vero cells in a six-well cell culture plate, incubated for 1 h at 37.8 ˚C in 5% CO_2, and overlaid with 3-ml 0.5% agarose in M-199 medium supplemented with 350 mg/l sodium bicarbonate, 29.2 mg/l L-glutamine, and antibiotics and returned to the incubator. A second overlay containing 0.004% neutral red dye was added after 2 days' incubation for plaque visualization. Plaques were scored daily for 5 days, with the titer expressed as plaque-forming units (PFU) per 0.1 ml. Any pool with PFU ≥ 0.5 was considered positive by plaque assay.

In 2004–2008, we used reverse transcription polymerase chain reaction (RT-PCR) to initially screen all pools and identify negatives. RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen) following the manufacturer's protocol, modified by increasing the amount of buffer AVE (water) to yield 100 µl total RNA per sample. A negative control was placed between every five samples during extraction and maintained in the same position for RT-PCR. A positive BCRV control was also included in each extraction and RT-PCR (Moore et al. 2007).

RT-PCR was performed using the OneStep RT-PCR Kit (Qiagen) following the manufacturer's protocol. Thermocycler conditions and gel electrophoresis are described in detail in Moore et al. (2007). We used BCRV-specific primer sequences forward 5'-TAAGTTTGTTGGTCGAGAGCA GTATC-3' and reverse 5'-ACACTCATAGGTAACAGTTTT TCCAGAC-3', which yielded a 208-bp fragment from the E2 part of the viral genome. The E2 in alphaviruses codes for a receptor-binding envelope protein and is considered the main target of the host's immune response (Weger-Lucavelli et al. 2015) .

Samples from 2004–2008 initially positive by RT-PCR were also subjected to plaque assay. A pool was considered positive for BCRV if it showed positive both by the initial RT-PCR and by plaque assay. For 2004–2008 samples showing no plaques on Vero cells, we re-extracted RNA from the remaining homogenate and performed RT-PCR again (with the same primers). A pool with no plaque development was considered BCRV positive if it tested positive twice by RT-PCR. Non-cytopathic BCRV tended to occur mostly at inactive colony sites where no swallows or sparrows were present (Brown et al. 2010a). Because RT-PCR is more sensitive than plaque assay in detecting virus, screening method was used as a co-variate in statistical analyses.

To assign virus lineage, viral RNA was extracted from 100 µl of the infectious pre-cleared supernatant of a Vero cell passage or from the bug homogenates using the Qiagen Qiamp Mini Viral RNA extraction kit (Qiagen, Hameln, Germany). Five microliters of the eluted RNA suspension was subjected to RT-PCR to amplify the entire 1269 bp of the E2 gene, using a protocol modified from Pfeffer et al. (2006). The amplicon DNA was subjected to cycle sequencing using the ABI Prism BigDye Terminator Cycle Sequencing Kit, version 3.1 (Applied Biosystems, Foster City, CA). Sequencing primers and other details are given in Pfeffer et al. (2006) and Brown et al. (2008).

Sequences were aligned against the corresponding region in a BCRV reference sequence from 1981 (strain 81V1822, GenBank AF339474) and fragments combined for a given isolate using SeqMan 6.1 (DNAStar, Lasergene) to obtain a contiguous nucleotide sequence for each sample. All sequences generated from this study are deposited in GenBank (accession numbers EU483667 – EU484043). Only a randomly chosen subset of BCRV isolates was assigned to lineage, although sequencing was successful mostly for ones grown on Vero cells, presumably because those samples had relatively high concentrations of BCRV RNA.

Each BCRV isolate is actually a sample of multiple and potentially variable virus particles within the host (Domingo 1998; Pfeffer et al. 2006); in our study an isolate from a given sample represents the dominant genotype present. In three cases where an isolate had evidence of a sequence polymorphism, indicating the presence of multiple viral genotypes, it was excluded from analysis.

Fully blinded methods were not possible in this study, as vials of bugs were labelled in the field with the colony site designation, and the colony site was obviously known when we were collecting bugs or designating information about the site or bug collections. However, laboratory procedures were done while relatively unfamiliar with the colony size at each site in that year.

Statistical methods

We used mixed models to explore whether group size (cliff swallow colony size) and other co-variates potentially predicted the likelihood of a swallow bug pool being positive for BCRV. Because the dependent variable (BCRV yes/no) was categorical, we used Proc GLIMMIX in SAS (SAS Institute 2004). Independent variables were cliff swallow colony size (number of active nests), sparrow presence (yes/no), colony site status (active/inactive), nest status (active/inactive), behavior of bugs collected (clustering/outside/inside), date within the season as a continuous effect, year (1998–2008 as a continuous effect), age of bugs in the pool (adult/instar/mixed), screening method (plaque assay/RT-PCR), number of bugs per pool, and proportion of past years a colony site was occupied by cliff swallows (logit-transformed). Because past-year histories were unknown for some sampled sites, analyses of the proportion of past years a site was active could only use a subset of the samples, and these analyses were kept separate. For sites inactive in a given year, we used the past year's colony size, with sites also unused the past year receiving a 0. We began with a global model containing all independent variables and then removed each sequentially based on the highest p-value in each step. For those removed, the p value is shown for the step at which it was removed from the model. The final model contained only variables with $p \leq 0.157$ (Vergouw et al. 2010). Biologically plausible but non-significant interactions were removed in preliminary analyses and not considered further. When an interaction was retained, its main effects were also included but not interpreted.

To account for non-independence of observations from the same colony or colony site (and potential pseudo-replication in the data), we used two random intercept variables: *colony site*, coded the same for each site across years, to account for potential spatial dependence of samples at a colony site's physical location in different years, and *colony-site-by-year*, coded the same for all samples at a colony site in the same year but different between years, to account for potential temporal dependence among samples at a single colony site within a year. Attempts to use year as a categorical random effect led to non-convergence of the model and thus were abandoned. For calculating predicted values (and SEs) from mixed models, we used Proc PLM in SAS that derived these estimates from the final model (one with non-significant variables removed) that also included the relevant random effects.

We estimated the site-level repeatability of BCRV infection (measured as the proportion of positive samples among all samples tested at that colony site that summer) across years using Proc GLM in SAS, with proportion positive as the dependent variable and colony site as the independent predictor. The output from that model was used to calculate the intraclass correlation coefficient (r_I) using the methods of Zar (1999). The number of years for which we had BCRV prevalence data at a site in this analysis ranged from 2 to 10. The estimated intraclass correlation coefficients were adjusted for the number of measurements (i.e., years) being > 2 for some colony sites using the methods of Lessells and Boag (1987).

Results

Across all colonies and years, the overall proportion of BCRV positives was 0.236 ($n = 5291$ swallow bug pools) at active colony sites and 0.194 ($n = 882$ pools) at inactive sites. The proportion of positives at active sites with house sparrows was 0.287 ($n = 1690$ pools) and at active sites without sparrows, 0.213 ($n = 3601$ pools). Inactive sites with sparrows had a proportion positive of 0.278 ($n = 90$ pools) and inactive sites without sparrows, 0.184 ($n = 792$ pools). Across all colonies and years, the proportion of positives that was lineage A was 0.429 ($n = 340$).

BCRV prevalence

At active cliff swallow colony sites, the likelihood of a bug pool being positive for BCRV was influenced by a cliff swallow colony size*presence of sparrows interaction, date within the season when sampled, age of the bugs in the pool, and number of bugs in the pool (Table 1). The interaction revealed opposite effects of group size on virus prevalence depending on whether sparrows were present at a site: BCRV prevalence in bugs increased with swallow colony size in the absence of sparrows but decreased with swallow colony size in their presence (Fig. 2). BCRV was more likely in pools of bugs consisting of adults (0.311, $n = 1806$) than in mixed age (0.211, $n = 1793$) or instar (0.182, $n = 1688$) pools (Table 1). The positive regression coefficient for date (Table 1) showed that BCRV prevalence in bug pools increased during the summer. Even though plaque assay produced a lower prevalence of BCRV in pools (0.159, $n = 1502$) than did RT-PCR (0.267, $n = 3789$), the method used was not significant in explaining prevalence when accounting for other variables (Table 1). Although the proportion of positives at active sites varied from 0.0842 ($n = 404$) in 2001 to 0.316 ($n = 1006$) in 2006, over the years 1998–2008, there was no significant linear effect of year (Table 1). BCRV prevalence did not vary significantly among bugs clustering at the nest entrance (0.334, $n = 110$), on the outside of the nest (0.221, $n = 3898$), or inside the collected nest (0.262, $n = 1064$; Table 1). Whether the nest itself was active or inactive within an active colony also had no significant effect on BCRV prevalence (Table 1). Both random-intercept variables were significant (Table 1), indicating some dependence among bug

Table 1 Fixed-effect and random-effect predictors of whether a swallow bug pool was positive for BCRV at active cliff swallow colonies ($n = 5287$ pools)

Fixed effect[1]	β	SE	$F_{1,5105}$	p	
Colony size[2]	−0.000670	0.000334	0.82	0.37	
Date	0.01168	0.005055	5.34	0.021	
Bug age	0.6062	0.1458	37.85	<0.0001	
Sparrow presence	−0.5792	0.3293	3.09	0.079	
No. bugs in pool	0.2022	0.003351	36.38	<0.0001	
Colony size * Sparrow presence	0.000952	0.000418	2.28	0.023	
Year	0.02380	0.03530	0.45	0.50	
Bug behavior	0.03837	0.1907	0.72	0.49	
Assay method	-0.5143	0.06873	1.37	0.24	
Nest status	0.1989	0.2384	0.70	0.40	
Random effect	Estimated variance component	SE	Levels	z	p
Colony site	0.6136	0.2974	52	2.06	0.020
Colony-site-by-year	0.9599	0.1856	178	5.17	<0.0001

[1]Variables retained in the final model are shown in bold; remaining are shown for the step at which they were removed

[2]Number of cliff swallow nests in the current season

pools collected within a season at each colony and among those from the same site in different years. Repeating the same analysis (Table 1) with the subset of sites where we knew the proportion of past years the site was active (cliff swallows present) yielded no significant effect of proportion years used (β = 0.0685, SE = 0.532, $t = 0.13$, $p = 0.90$).

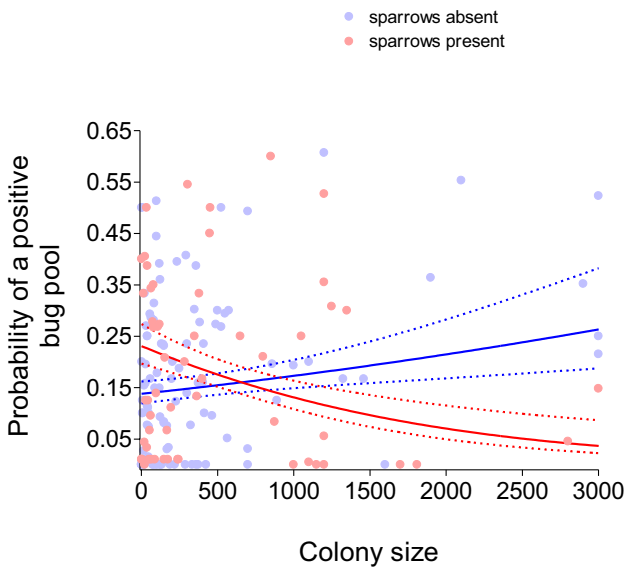

Fig. 2 Probability that a swallow bug pool was positive for BCRV (either lineage) in relation to cliff swallow colony size in the absence (blue) and presence (red) of house sparrows at active swallow colony sites. Predicted values (solid lines) with SE (dotted lines) come from a model with a swallow colony size*presence of sparrows interaction (Table 1). Circles indicate actual proportion of pools positive for each colony size and overlap in some cases; 10 outliers (y > 0.65) are not shown for reasons of scale but were included in analyses

At inactive cliff swallow colony sites, there was a similar but statistically weaker swallow colony size*presence of sparrows interaction (Table 2). The direction of the group-size effect (measured as the swallow colony size the preceding year) in the presence versus absence of sparrows (Fig. 3) was the same as that at active sites (Fig. 2). Screening method was a significant predictor of BCRV infection at inactive sites, with plaque assay yielding a proportion positive of 0.0621 ($n = 306$ pools) and RT-PCR, 0.264 ($n = 576$ pools), likely because there was little cytopathic BCRV at sites without cliff swallows. The number of bugs in a pool also increased the likelihood of detecting BCRV, but we found no significant effects of date within the season, year, or bug behavior at inactive sites (Table 2). Models with bug age would not converge, presumably because most bugs at inactive sites were scored as adults. The random intercept variable colony-site-by-year was significant (Table 2), indicating some dependence among bug pools collected from the same inactive site within the season, but models with the random effect of colony site would not converge. The proportion of past years the site had been used by cliff swallows was not a significant predictor of BCRV infection for inactive sites (β = −0.744, SE = 0.617, $t = -1.21$, $p = 0.23$).

The repeatability of the proportion of BCRV positive pools across years at a colony site was relatively low but significant at active sites that had house sparrows present ($r_I = 0.222$, $p = 0.038$) and for those that had no sparrows ($r_I = 0.257$, $p = 0.002$).

Table 2 Fixed-effect and random-effect predictors of whether a swallow bug pool was positive for BCRV at inactive cliff swallow colonies ($n = 881$ pools)

Fixed effect[1]	β	SE	$F_{1,843}$	p	
Past size[2]	−0.00532	0.003286	1.71	0.19	
Assay method	−1.0891	0.4435	6.03	0.014	
Sparrow presence	−1.3103	0.8423	2.42	0.12	
No. bugs in pool	0.02014	0.009148	4.85	0.028	
Colony size * Sparrow presence	0.006285	0.003371	3.48	0.063	
Year	−0.04477	0.1951	0.05	0.82	
Bug behavior	0.3394	0.3017	1.27	0.26	
Date	0.006119	0.07875	0.60	0.44	
Random effect	Estimated variance component	SE	Levels	z	p
Colony-site-by-year	0.4673	0.2521	37	1.85	0.032

[1]Variables retained in the final model are shown in bold; remaining are shown for the step at which they were removed

[2]Number of cliff swallow nests in the previous season

Lineages and group size

Whether a BCRV positive sample was lineage A or B was predicted only by cliff swallow colony size, nest status (active or inactive), and the presence of house sparrows, and we found no significant swallow colony size*presence of sparrows interaction (Table 3). The likelihood of a positive being lineage A (as opposed to B) declined with cliff swallow colony size (Fig. 4). The proportion of lineage A in bugs from active nests was 0.441 ($n = 324$) versus 0.188 ($n = 16$) from inactive nests within active colonies. The proportion of lineage A at colonies with house sparrows was 0.564 ($n = 165$), compared with 0.303 ($n = 175$) at colonies without house sparrows. There was no significant effect of date within the season, year, number of bugs in a pool, bug age, or bug behavior on whether an isolate was lineage A (Table 3). The random intercept variable of colony site was significant, indicating some dependence among years at the same site in lineage designation, but the colony-site-by-year random effect was not significant (Table 3). Too few samples at inactive colony sites yielded enough virus from plaque assay to allow sequencing for lineage.

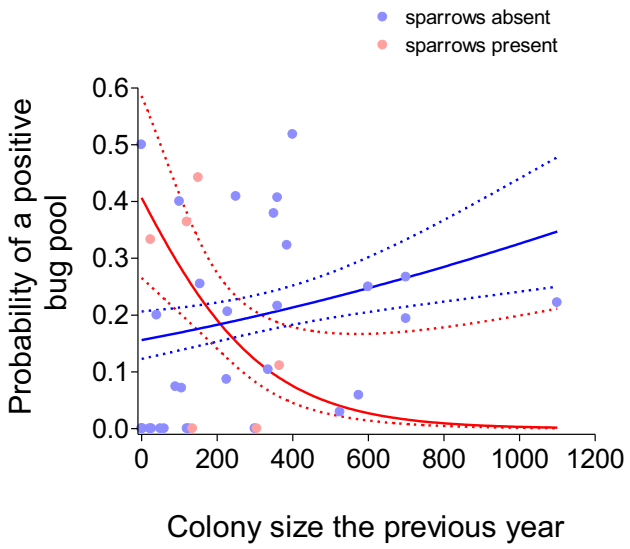

Fig. 3 Probability that a swallow bug pool was positive for BCRV (either lineage) in relation to cliff swallow colony size the previous year in the absence (blue) and presence (red) of house sparrows at inactive swallow colony sites. Predicted values (solid lines) with SE (dotted lines) come from a model with a swallow colony size*presence of sparrows interaction (Table 2). Circles indicate actual proportion of pools positive for each colony size and overlap in some cases

Swallow vs sparrow colony sizes

The proportion of house sparrow nests among those of both species combined declined significantly with the total nests present in a colony ($\beta = -0.00040$, SE = 0.000171, $t = -2.34$, $p = 0.036$; Fig. 5). The random effect of colony site was not significant (estimate = 0.05109, SE = 0.04112, $z = 1.24$, $p = 0.11$), indicating limited temporal dependence in the proportion of house sparrows present at a site across years.

Discussion

The most surprising result of this study was how the presence of invasive house sparrows at a site affected the relationship between BCRV infection in swallow bug vectors and cliff swallow colony size. After accounting for the effect of other co-variates, BCRV infection increased with swallow colony size in the absence of sparrows but decreased in the sparrows' presence. We are unaware of any other study of

Table 3 Fixed-effect and random-effect predictors of whether a BCRV isolate was lineage A at active cliff swallow colonies ($n = 340$ isolates)

Fixed effect[1]	β	SE	$F_{1,266}$	p	
Colony size[2]	−0.00186	0.000908	4.19	0.042	
Nest status	2.5064	1.2559	3.98	0.047	
Sparrow presence	−2.0039	0.9653	4.31	0.039	
No. bugs in pool	−0.00322	0.02072	0.02	0.88	
Colony size * Sparrow presence	0.002814	0.002157	1.70	0.19	
Year	0.004820	0.1644	0.00	0.98	
Bug behavior	−0.7604	1.0407	0.47	0.62	
Bug age	−0.4841	0.8307	0.17	0.85	
Date	0.009681	0.02208	0.19	0.66	
Random effect	Estimated variance component	SE	Levels	z	p
Colony site	5.1605	2.0734	52	2.49	0.006
Colony-site-by-year	0.8546	0.6708	178	1.27	0.10

[1]Variables retained in the final model are shown in bold; remaining are shown for the step at which they were removed

[2]Number of cliff swallow nests in the current season

virus prevalence and host group size that illustrates such an opposing effect of an invasive species. This pattern is likely at least partly attributable to the colony-size effect we also documented for the two respective BCRV lineages, in which the sparrow-adapted A lineage declined with colony size. That we found no statistical interaction between sparrow presence and colony size for the lineage analysis could partly reflect a relatively small sample of isolates identified to lineage ($n = 340$), at least as compared with our prevalence data ($n = 5291$ and 888 for active and inactive sites, respectively).

House sparrows are a frequent alternative host for swallow bugs, especially at colony sites without cliff swallows in a given year. Swallow bugs take blood meals from house sparrows in the absence of cliff swallows, but when both species are present bugs prefer cliff swallows (O'Brien et al. 2011). Nestling sparrows are more likely to be infected by BCRV than are nestling cliff swallows, and infected sparrows are more likely to die, especially at younger nestling ages (O'Brien et al. 2010, 2011). The consequence is that house sparrows are more effective amplifying hosts for the virus, making them more likely to infect bugs that feed on them and leading to virus "spillback" (sensu Kelly et al. 2009) that increases cliff swallows' exposure to BCRV at mixed colonies. At the same time, the bugs' preference to feed on cliff swallows may direct infection away from house sparrows, with cliff

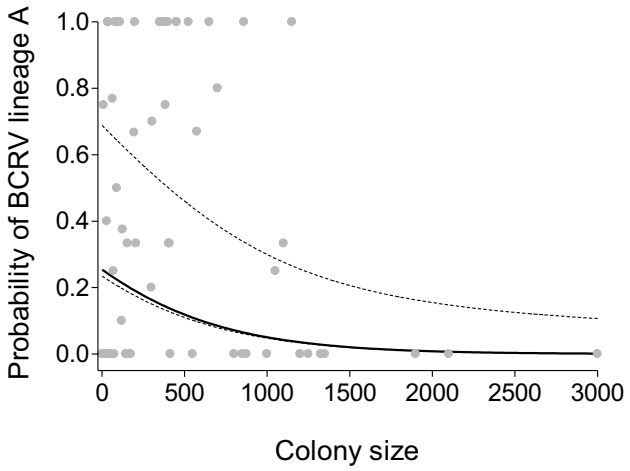

Fig. 4 Probability that a BCRV-positive swallow bug pool was lineage A in relation to cliff swallow colony size at active swallow colony sites. Predicted values (solid lines) with SE (dotted lines) come from the model in Table 3. Circles indicate actual proportion of lineage A among isolates sequenced for each colony size and overlap in some cases

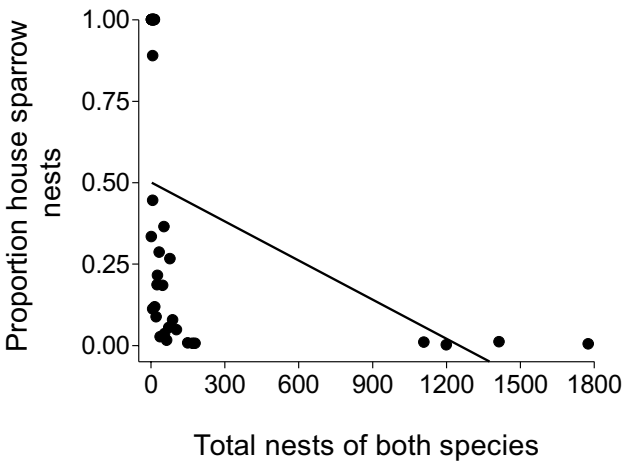

Fig. 5 The proportion of nests that were house sparrows in relation to the total nests of both sparrows and cliff swallows in a colony. Line shows best-fit least-squares regression

swallows thus conferring a dilution effect (sensu Ostfeld and Keesing 2000) for house sparrows (O'Brien et al. 2011).

The increase in BCRV infection with group size in the absence of house sparrows (Figs. 2, 3) is consistent with observed patterns in other contagious, less mobile parasites, or pathogens (Côté and Poulin 1995; Rifkin et al. 2012). As cliff swallow colony size increases, nest density also increases (Brown and Brown 1996), and other work showed BCRV more likely to transmit between sparrow nestlings in nests close together on the substrate (O'Brien and Brown 2011). Marking experiments with swallow bugs show that they frequently crawl between nests (Rannala 1995; VAO and CRB, unpubl. data), and thus infected bugs are more likely to reach another suitable nest nearby if the cliff swallow colony is large and dense. This could be one mechanism to increase infection prevalence among bugs in larger colonies.

Another explanation for the increase in BCRV with colony size in the absence of sparrows could be that lineage B, the dominant lineage in large colonies (Fig. 4), seems to be a more bug-associated variant of BCRV. The increased numbers of bugs in the larger cliff swallow colonies (Brown and Brown 1996) has probably selected for bug-to-bug transmission via both horizontal and vertical pathways (Brown et al. 2009b). If lineage B's bug-to-bug transmission requires a larger critical community size to sustain itself, it more likely achieves that in larger colonies where there are both more cliff swallows and (more importantly) more of their bug parasites. Some evidence indicates that lineage B is increasing overall in the Great Plains and lineage A decreasing (Padhi et al. 2008), perhaps reflecting BCRV's adaptation to an increasing cliff swallow population and a declining house sparrow population since 1966 (Sauer et al. 2017).

In contrast, BCRV infection in bugs declined with swallow colony size when house sparrows were present (Figs. 2, 3). This pattern would be expected if lineage A is adapting to be a mostly bird-associated virus that specializes on house sparrows owing to their competence as amplifying hosts (Brown et al. 2009a). As the proportion of house sparrows declines in larger colonies (Fig. 5), there are fewer relative opportunities for lineage A at such sites, leading to a reduction of lineage A (Fig. 4). The presence of house sparrows at a given site may be driving lineage A to outcompete lineage B locally, resulting in less lineage B circulating among bugs collected from cliff swallow nests at sites with sparrows. This could explain the decline in overall BCRV prevalence with group size when sparrows are present (Figs. 2, 3). Higher virus prevalence at smaller colonies in the presence of sparrows also may reflect lineage A continuing to infect bugs at cliff swallow nests through spillback, given that there are relatively more sparrows compared with cliff swallows at small colonies (Fig. 5).

We had earlier suggested that one reason virus infection increases in larger cliff swallow colonies is because sites with larger colonies are more likely to be perennially occupied by cliff swallows and thus BCRV has a longer time to accumulate there without periodic annual extinctions (Brown et al. 2001). That infection prevalence showed some degree of repeatability between years at a site supports this interpretation. In addition, because dispersing bugs are less likely to be infected (Moore and Brown 2014), colony sites without virus are slow to be (re)infected by immigrant bugs. However, perhaps surprisingly, our results here show that past history of colony site use by cliff swallows is unimportant: the proportion of past years a site was used had no significant effect on BCRV prevalence when controlling for other co-variates. On the other hand, we found the same general effect of the past year's cliff swallow colony size on BCRV prevalence at inactive sites (Fig. 3), indicating there is at least a year-long legacy of colony size in predicting virus at a site. We also found no linear effect of year over the 11 years of our study, suggesting that there have been no short-term temporal changes in BCRV prevalence that could account for the recent reduction in the cost of swallow bug parasitism to cliff swallows (Brown et al. 2021).

The group-size patterns reported here suggest that exposure of cliff swallows to BCRV and the effects of colony size depend on whether house sparrows occupy a given colony site. While sparrows can be destructive and usurp swallow nests, destroying eggs and nestlings in the process (Brown and Brown 1996; Leasure et al. 2010), sparrow presence may also reduce transmission of BCRV to cliff swallows despite bugs' preferring cliff swallows as hosts. That cliff swallows do not amplify BCRV well (O'Brien et al. 2011) could have selected for other means of transmission, such as bug-to-bug (lineage B) or a switch to house sparrows (lineage A). The near extinction of lineage A in the largest cliff swallow colonies (Fig. 4) would clearly suggest that lineage A is not dependent on cliff swallows or their bugs for amplification. Lineage A being more common at sites with house sparrows also is consistent with its rapid switch to the recently invasive sparrow. This example is a case of an invasive species disrupting the dynamics of an endemic pathogen and altering the potential fitness payoffs associated with group size. The presence of house sparrows is an additional ecological complexity to account for when analyzing the parasite-related costs and benefits of colonial breeding in cliff swallows.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-021-03040-1.

Acknowledgements We thank our principal BCRV collaborators without whom we would know much less about this virus and some of whom were responsible for generating these data: Mary B. Brown, Eric Edwards, Jerome E. Foster, Kathryn Huyvaert, Nicholas Komar, Carol

Meteyer, Abinash Padhi, Martin Pfeffer, William Reisen, Stephanie Strickler, Ginger Young, and others who assisted in the field and/or laboratory. Nicholas Komar directed the plaque assays, and Jerome Foster and Abinash Padhi did the lineage assignments. We thank the Oren Clary family, Duane Dunwoody, Dave and Deb Knight, and Loren Soper for access to land. Field work was done at the University of Nebraska-Lincoln's Cedar Point Biological Station. Kenton Miller allowed use of his house sparrow photograph in Fig. 1b. Two reviewers provided helpful comments on the manuscript.

Authors' contributions All authors designed the study and collected field data. ATM did the laboratory analyses. CRB did the statistical analyses and wrote the first draft of the manuscript. All authors reviewed the manuscript and contributed to the final version.

Funding Financial support came primarily from the National Institutes of Health (AI057569) and a series of grants from the National Science Foundation (most recently, DEB-1930803).

Data availability Data associated with this paper is available in Supplementary Information.

Declarations

Ethical approval Aspects of this work involving vertebrate animals followed all applicable international, national, and institutional guidelines and were approved by a series of Institutional Animal Care and Use Committees of the University of Tulsa.

Conflict of interest The authors declare no conflicts of interest.

References

Alexander RD (1971) The search for an evolutionary philosophy of man. Proc R Soc Vic 84:99–120

Alexander RD (1974) The evolution of social behavior. Annu Rev Ecol Syst 5:325–383

Allison AB, Stallknecht DE, Holmes EC (2015) Evolutionary genetics and vector adaptation of recombinant viruses of the western equine encephalitis antigenic complex provides new insights into alphavirus diversity and host switching. Virology 474:154–162

Altizer S, Nunn CL, Thrall PH et al (2003) Social organization and parasite risk in mammals: integrating theory and empirical studies. Annu Rev Ecol Syst 34:517–547

Anderson RM, May RH (1992) Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford

Anderson TR (2006) Biology of the ubiquitous house sparrow. Oxford University Press, New York

Bordes F, Blumstein DT, Morand S (2007) Rodent sociality and parasite diversity. Biol Lett 3:692–694

Briard L, Ezenwa VO (2021) Parasitism and host social behaviour: a meta-analysis of insights derived from social network analysis. Anim Behav 172:171–182

Brown CR, Brown MB (1986) Ectoparasitism as a cost of coloniality in cliff swallows (*Hirundo pyrrhonota*). Ecology 67:1206–1218

Brown CR, Brown MB (1992) Ectoparasitism as a cause of natal dispersal in cliff swallows. Ecology 73:1718–1723

Brown CR, Brown MB (1996) Coloniality in the cliff swallow: the effect of group size on social behavior. University of Chicago Press, Chicago

Brown CR, Brown MB (2002) Ectoparasites cause increased bilateral asymmetry of naturally selected traits in a colonial bird. J Evol Biol 15:1067–1075

Brown CR, Brown MB (2004) Empirical measurement of parasite transmission between groups in a colonial bird. Ecology 85:1619–1626

Brown CR, Brown MB, Padhi A, Foster JE, Moore AT, Pfeffer M, Komar N (2008) Host and vector movement affects genetic diversity and spatial structure of Buggy Creek virus (Togaviridae). Mol Ecol 17:2164–2173

Brown CR, Brown MB, Pyle P, Patten MA (2020) Cliff swallow (*Petrochelidon pyrrhonota*), version 1.0. In: Rodewald PG (ed) Birds of the world online. Cornell Lab of Ornithology, Ithaca. https://doi.org/10.2173/bow.cliswa.01

Brown CR, Brown MB, Roche EA (2013) Spatial and temporal unpredictability of colony size in cliff swallows across 30 years. Ecol Monogr 83:511–530

Brown CR, Hannebaum SL, O'Brien VA, Page CE, Rannala B, Roche EA, Wagnon GS, Knutie SA, Moore AT, Brown MB (2021) The cost of ectoparasitism in cliff swallows declines over 35 years. Ecol Monogr 91:e01446

Brown CR, Komar N, Quick SB, Sethi RA, Panella NA, Brown MB, Pfeffer M (2001) Arbovirus infection increases with group size. Proc R Soc Lond B 268:1833–1840

Brown CR, Moore AT, Young GR, Komar N (2010a) Persistence of Buggy Creek virus (Togaviridae, *Alphavirus*) for two years in unfed swallow bugs (Hemiptera: Cimicidae: *Oeciacus vicarius*). J Med Entomol 47:436–441

Brown CR, Moore AT, Young GR, Padhi A, Komar N (2009a) Isolation of Buggy Creek virus (Togaviridae: *Alphavirus*) from field-collected eggs of *Oeciacus vicarius* (Hemiptera: Cimicidae). J Med Entomol 46:375–379

Brown CR, Padhi A, Moore AT, Brown MB, Foster JE, Pfeffer M, O'Brien VA, Komar N (2009b) Ecological divergence of two sympatric lineages of Buggy Creek virus, an arbovirus associated with birds. Ecology 90:3168–3179

Brown CR, Sethi RA (2002) Mosquito abundance is correlated with cliff swallow (*Petrochelidon pyrrhonota*) colony size. J Med Entomol 39:115–120

Brown CR, Strickler SA, Moore AT, Knutie SA, Padhi A, Brown MB, Young GR, O'Brien VA, Foster JE, Komar N (2010b) Winter ecology of Buggy Creek virus (Togaviridae, *Alphavirus*) in the central Great Plains. Vect-Borne Zoonot Dis 10:355–363

Buck JC, Lutterschmidt WI (2017) Parasite abundance decreases with host density: evidence of the encounter-dilution effect for a parasite with a complex life cycle. Hydrobiologia 784:201–210

Calisher CH, Karabatsos N, Lazuick JS, Monath TP, Wolff KL (1988) Reevaluation of the western equine encephalitis antigenic complex of alphaviruses (family Togaviridae) as determined by neutralization tests. Am J Trop Med Hyg 38:447–452

Calisher CH, Monath TP, Muth DJ, Lazuick JS, Trent DW, Francy DB, Kemp GE, Chandler FW (1980) Characterization of Fort Morgan virus, an alphavirus of the western equine encephalitis virus complex in an unusual ecosystem. Am J Trop Med Hyg 29:1428–1440

Chapman BR, George JE (1991) The effects of ectoparasites on cliff swallow growth and survival. In: Loye JE, Zuk M (eds) Bird-parasite interactions: ecology, evolution and behaviour. Oxford University Press, Oxford, pp 69–92

Chiang CL, Reeves WC (1962) Statistical estimation of virus infection rates in mosquito vector populations. Am J Epidemiol 75:377–391

Chisenhall DM, Vitek CJ, Richards SL, Mores CN (2008) A method to increase efficiency in testing pool-collected mosquitoes. J Am Mosquito Control Assoc 24:311–314

Côté IM, Poulin R (1995) Parasitism and group size in social animals: a meta-analysis. Behav Ecol 6:159–165

Craft ME (2015) Infectious disease transmission and contact networks in wildlife and livestock. Phil Trans R Soc B 370:20140107
Davies CR, Ayres JM, Dye C, Deane LM (1991) Malaria infection rate of Amazonian primates increases with body weight and group size. Funct Ecol 5:655–662
de Jong MCM, Diekmann O, Heesterbeck H (1995) How does transmission of infection depend on population size? In: Mollison D (ed) Epidemic models, their structure and relation to data. Cambridge University Press, Cambridge, pp 84–94
Dietz K (1988) Density-dependence in parasite transmission dynamics. Parasitol Today 4:91–97
Domingo E (1998) Quasispecies and the implications for virus persistence and escape. Clin Diagn Virol 10:97–101
Dynes TL, Berry JA, Delaplane KS, Brosi BJ, de Roode JC (2019) Reduced density and visually complex apiaries reduce parasite load and promote honey production and overwintering survival in honey bees. PLoS ONE 14:e0216286
Ezenwa VO (2004) Host social behavior and parasitic infection: a multifactorial approach. Behav Ecol 15:446–454
George JE (1987) Field observations on the life cycle of *Ixodes baergi* and some seasonal and daily activity cycles of *Oeciacus vicarius* (Hemiptera: Cimicidae), *Argas cooleyi* (Acari: Argasidae), and *Ixodes baergi* (Acari: Ixodidae). J Med Entomol 24:683–688
Hayes RO, Francy DB, Lazuick JS, Smith GC, Gibbs EPJ (1977) Role of the cliff swallow bug (*Oeciacus vicarius*) in the natural cycle of a western equine encephalitis-related alphavirus. J Med Entomol 14:257–262
Hoogland JL (1979) Aggression, ectoparasitism, and other possible costs of prairie dog (Sciuridae, *Cynomys* spp.) coloniality. Behaviour 69:1–35
Hoogland JL, Sherman PW (1976) Advantages and disadvantages of bank swallow (*Riparia riparia*) coloniality. Ecol Monogr 46:33–58
Hopla CE, Francy DB, Calisher CH, Lazuick JS (1993) Relationship of cliff swallows, ectoparasites, and an alphavirus in west-central Oklahoma. J Med Entomol 30:267–272
Jeong J, McCallum H (2021) Using stochastic modeling to predict the effect of culling and colony dispersal of bats on zoonotic viral epidemics. Vect Borne Zoo Dis 21:369–377
Johnson PTJ, Thieltges DW (2010) Diversity, decoys and the dilution effect: how ecological communities affect disease risk. J Exp Biol 213:961–970
Keeling MJ, Grenfell BT (1997) Disease extinction and community size: modeling the persistence of measles. Science 275:65–67
Keesing F, Holt RD, Ostfeld RS (2006) Effects of species diversity on disease risk. Ecol Lett 9:485–498
Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM (2009) Parasite spillback: a neglected concept in invasion ecology? Ecology 90:2047–2056
Kent R, Juliusson L, Weissmann M, Evans S, Komar N (2009) Seasonal blood-feeding behavior of *Culex tarsalis* (Diptera: Culicidae) in Weld County, Colorado, 2007. J Med Entomol 46:380–390
Khataee H, Scheuring I, Czirok A, Nuefeld Z (2021) Effects of social distancing on the spreading of COVID-19 inferred from mobile phone data. Sci Rep 11:1661
Kopachena JG, Cochran BL, Nichols TB (2007) The incidence of American swallow bugs (*Oeciacus vicarius*) in barn swallow (*Hirundo rustica*) colonies in northeast Texas. J Vect Ecol 32:280–284
Leasure DR, Kannan R, James DA (2010) House sparrows associated with reduced cliff swallow nesting success. Wilson J Ornithol 122:135–138
Leclaire S, Faulkner CT (2014) Gastrointestinal parasites in relation to host traits and group factors in wild meerkats (*Suricata suricatta*). Parasitology 141:925–933
Lessells CM, Boag PT (1987) Unrepeatable repeatabilities: a common mistake. Auk 104:116–121
Lowther PE, Cink CL (2020) House Sparrow (*Passer domesticus*), version 1.0. In: Billerman SM (ed) Birds of the world online. Cornell Lab of Ornithology. Ithaca. https://doi.org/10.2173/bow.houspa.01
Loye JE (1985) The life history and ecology of the cliff swallow bug, *Oeciacus vicarius* (Hemiptera: Cimicidae). Cahiers ORSTOM Entomol Med Parasitol 23:133–139
Loye JE, Carroll SP (1991) Nest ectoparasite abundance and cliff swallow colony site selection, nestling development, and departure time. In: Loye JE, Zuk M (eds) Bird-parasite interactions: ecology, evolution and behaviour. Oxford University Press, Oxford, pp 222–241
Loye JE, Hopla CE (1983) Ectoparasites and microorganisms associated with the cliff swallow in west-central Oklahoma. Bull Soc Vect Ecol 8:79–84
Lucatelli J, Mariano-Neto E, Japyassú HF (2021) Social interaction, and not group size, predicts parasite burden in mammals. Evol Ecol 35:115–130
Masters NB, Shih SF, Bukoff A, Akel KB, Kobayashi LC, Miller AL, Harapan H, Lu Y, Wagner AL (2020) Social distancing in response to the novel coronavirus (COVID-19) in the United States. PLoS ONE 15:e0239025
Maya-Delgado A, Madder M, Benítez-Ortíz W, Saegerman C, Berkvens D, Ron-Garrido L (2020) Molecular screening of cattle ticks, tick-borne pathogens and amitraz resistance in ticks of Santo Domingo de los Tsáchillas province in Ecuador. Ticks Tick-Borne Dis 11:101492
Møller AP (1987) Advantages and disadvantages of coloniality in the swallow, *Hirundo rustica*. Anim Behav 35:819–832
Moore AT, Brown CR (2014) Dispersing hemipteran vectors have reduced arbovirus prevalence. Biol Lett 10:20140117
Moore AT, Edwards EA, Brown MB, Komar N, Brown CR (2007) Ecological correlates of Buggy Creek virus infection in *Oeciacus vicarius*, southwestern Nebraska, 2004. J Med Entomol 44:42–49
Moore J, Simberloff D, Freehling M (1988) Relationships between bobwhite quail social-group size and intestinal helminth parasitism. Am Nat 131:22–32
Mooring MS, Hart BL (1992) Animal grouping for protection from parasites: selfish herd and encounter-dilution effects. Behaviour 123:173–193
Myers LE (1928) The American swallow bug, *Oeciacus vicarius* Horvath (Hemiptera, Cimicidae). Parasitology 20:159–172
Nunn CL, Heymann EW (2005) Malaria infection and host behavior: a comparative study of Neotropical primates. Behav Ecol Sociobiol 59:30–37
Nunn CL, Jordán F, McCabe CM, Verdolin JL, Fewell JH (2015) Infectious disease and group size: more than just a numbers game. Phil Trans R Soc B 370:2014011
O'Brien VA, Brown CR (2011) Group size and nest spacing affect Buggy Creek virus (Togaviridae: *Alphavirus*) infection in nestling house sparrows. PLoS ONE 6:e25521
O'Brien VA, Meteyer CU, Ip HS, Long RR, Brown CR (2010) Pathology and virus detection in tissues of nestling house sparrows naturally infected with Buggy Creek virus (Togaviridae). J Wildlife Dis 46:23–32
O'Brien VA, Moore AT, Young GR, Komar N, Reisen WK, Brown CR (2011) An enzootic vector-borne virus is amplified at epizootic levels by an invasive avian host. Proc R Soc B 278:239–246
Ostfeld RS, Keesing F (2000) The function of biodiversity in the ecology of vector-borne zoonotic diseases. Can J Zool 78:2061–2078
Packer C, Altizer S, Appel M, Brown E, Martenson J, O'Brien SJ, Roelke-Parker M, Hofmann-Lehmann R, Lutz H (1999) Viruses of the Serengeti: patterns of infection and mortality in African lions. J Anim Ecol 68:1161–1178

Padhi A, Moore AT, Brown MB, Foster JE, Pfeffer M, Brown CR (2011) Isolation by distance explains genetic structure of Buggy Creek virus, a bird-associated arbovirus. Evol Ecol 25:403–416

Padhi A, Moore AT, Brown MB, Foster JE, Pfeffer M, Gaines KP, O'Brien VA, Strickler SA, Johnson AE, Brown CR (2008) Phylogeographical structure and evolutionary history of two Buggy Creek virus lineages in the western Great Plains of North America. J Gen Virol 89:2122–2131

Patterson JEH, Ruckstuhl KE (2013) Parasite infection and host group size: a meta-analytical review. Parasitology 140:803–813

Pfeffer M, Foster JE, Edwards EA, Brown MB, Komar N, Brown CR (2006) Phylogenetic analysis of Buggy Creek virus: evidence for multiple clades in the western Great Plains, United States of America. Appl Environ Microbiol 72:6886–6893

Poulin R (1991) Group-living and infestation by ectoparasites in passerines. Condor 93:418–423

Rannala BH (1995) Demography and genetic structure in island populations. PhD dissertation, Yale University, New Haven

Rifkin JL, Nunn CL, Garamszegi LZ (2012) Do animals living in larger groups experience greater parasitism? A meta-analysis. Am Nat 180:70–82

Robert V, MacIntyre K, Keating J, Trape JF, Duchemin JB, Warren M, Beier JC (2003) Malaria transmission in urban sub-Saharan Africa. Am J Trop Med Hyg 68:169–176

Rubenstein DI, Hohmann ME (1989) Parasites and social behavior of island feral horses. Oikos 55:312–320

Rush WA, Francy DB, Smith GC, Cropp CB (1980) Transmission of an arbovirus by a member of the family Cimicidae. Ann Entomol Soc Am 73:315–318

Samson DR, Louden LA, Gerstner K, Wylie S, Lake B, White BJ, Nunn CL, Hunt KD (2019) Chimpanzee (*Pan troglodytes schweinfurthii*) group sleep and pathogen-vector avoidance: experimental support for the encounter-dilution effect. Int J Primatol 40:647–659

SAS Institute (2004) SAS/STAT user's guide, version 9.1. SAS Institute, Cary, NC

Sauer JR, Niven DK, Hines JE, Ziolkowski DJ Jr, Pardieck KL, Fallon JE, Link WA (2017) The North American Breeding Bird Survey, results and analysis 1966 - 2015. Version 2.07.2017. USGS Patuxent Wildlife Research Center, Laurel, MD

Scott TW, Bowen GS, Monath TP (1984) A field study on the effects of Fort Morgan virus, an arbovirus transmitted by swallow bugs, on the reproductive success of cliff swallows and symbiotic house sparrows in Morgan County, Colorado, 1976. Am J Trop Med Hyg 33:981–991

Smith GC, Eads RB (1978) Field observations on the cliff swallow, *Petrochelidon pyrrhonota* (Vieillot), and the swallow bug, *Oeciacus vicarius* Horvath. J Wash Acad Sci 68:23–26

Stockmaier S, Stroeymeyt N, Shattuck EC, Hawley DM, Meyers LA, Bolnick DI (2021) Infectious diseases and social distancing in nature. Science 371:eabc9991

Swinton J, Harwood J, Grenfell BT, Gilligan CA (1998) Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. J Anim Ecol 67:54–68

Tang Z, Yamada H, Kraupa C, Canic S, Busquets N, Talavera S, Jiolle D, Vreysen MJB, Bouyer J, Abd-Alla AMM (2020) High sensitivity of one-step real-time reverse transcription quantitative PCR to detect low virus titers in mosquito pools. Parasite Vector 13:460

Telfer S, Bown K (2012) The effects of invasion on parasite dynamics and communities. Funct Ecol 26:1288–1299

Usinger RL (1966) Monograph of Cimicidae. Thomas Say Foundation, College Park, MD

Vergouw D, Heymans MW, Peat GM, Kuijpers T, Croft PR, de Vet HCW, van der Horst HE, van der Windt DAWM (2010) The search for stable prognostic models in multiple imputed data sets. BMC Med Res Meth 10:81

Viljoen H, Bennett NC, Ueckermann EA, Lutermann H (2011) The role of host traits, season and group size on parasite burdens in a cooperative mammal. PLoS ONE 6:e27003

Weger-Lucavelli J, Aliota MT, Kamlangdee A, Osorio JE (2015) Identifying the role of E2 domains on alphavirus neutralization and protective immune responses. PLoS Negl Trop Dis 9:e0004163

Westby KM, Sweetman BM, Van Horn TR, Biro EG, Medley KA (2019) Invasive species reduces parasite prevalence and neutralizes negative environmental effects on parasitism in a native mosquito. J Anim Ecol 88:1215–1225

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, Upper Saddle River, NJ