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No Evidence for Spring Re-introduction of an Arbovirus by Cliff Swallows

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ABSTRACT.—We sampled 100 Cliff Swallows (*Petrochelidon pyrrhonota*), just after arrival in Nebraska breeding areas, to ascertain if migrating birds re-introduce Buggy Creek virus (BCRV; Togaviridae) to north-temperate localities in spring. Most birds sampled were previously banded and were known to have used parasite-free nesting colonies in past summers and/or were seronegative to BCRV; thus, they were unlikely to have been previously exposed to the virus in their breeding areas. None of the birds had evidence of viral RNA in blood, as measured by RT-PCR. These results are consistent with other studies that have shown little evidence that migratory birds re-introduce arboviruses to temperate localities between years. *Received 14 February 2008. Accepted 5 May 2008.*

Whether arthropod-borne viruses (arboviruses) are re-introduced in spring by migratory birds in temperate latitudes is a major question in the study of bird-associated viruses (Reeves 1974, Scott and Weaver 1989, Crans et al. 1994). Arboviruses are rarely found in over-wintering insect vectors such as mosquitoes (Rosen 1987, Reeves 1990, Day 2001), and the conventional wisdom is that infected birds from the tropics—that are fed upon by insect vectors (e.g., mosquitoes) after arrival in breeding areas—may provide a mechanism for annual recurrence of virus in temperate latitudes of central and northern North America (Cilnis et al. 1996, Unnasch et al. 2006). Empirical evidence for this scenario is limited, however, and consists mostly of a few records of birds (bound for unknown destinations) with eastern equine encephalomyelitis virus when captured in spring after crossing the Gulf of Mexico (Calisher et al. 1971). Demonstrating virus re-introduction requires sampling birds upon their arrival at breeding sites sufficiently early in the nesting season that re-infection by local vectors can be excluded if positive birds are found. No studies have systematically surveyed newly arrived migratory birds for arboviruses.

Buggy Creek virus (Togaviridae, *Alphavirus*) is an unusual arbovirus vectored primarily by the swallow bug (Hemiptera: Cimicidae: *Oeciacus vicarius*), an ectoparasite of the

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colonially nesting Cliff Swallow (*Petrochelidon pyrrhonota*). Vertebrate hosts for Buggy Creek virus (BCRV) are Cliff Swallows and House Sparrows (*Passer domesticus*) that occupy nests in swallow colonies (Hayes et al. 1977, Scott et al. 1984). A related alphavirus, Fort Morgan virus, is a strain of BCRV (Pfeffer et al. 2006). BCRV, although not documented to affect humans, is phylogenetically and serologically related to western equine encephalomyelitis virus (WEEV) (Calisher et al. 1988, Powers et al. 2001), and WEEV affects both people and livestock (Reisen and Monath 1989). Birds have been suggested to move WEEV between North and South America (Weaver et al. 1997).

We sampled Cliff Swallows for virus immediately after the birds' arrival at breeding sites in southwestern Nebraska, USA as part of our efforts to understand the population dynamics of BCRV and its association with Cliff Swallows (Brown et al. 2001; Moore et al. 2007; Brown et al. 2007, 2008). Our objective was to examine whether these birds were infected with BCRV upon their return and could potentially re-introduce the virus to their breeding areas.

Cliff Swallows breed throughout much of North America, nesting in large colonies underneath cliff overhangs and bridges, and winter in southern Brazil, Uruguay, and northern Argentina (Brown and Brown 1995). BCRV occurs annually in our Nebraska study area and is commonly isolated from the insect vectors (Brown et al. 2001, 2007; Moore et al. 2007). Its predictable annual occurrence suggests the virus either over-winters in swallow bugs and/or in resident House Sparrows, or is re-introduced each season by Cliff Swallows when they return from their winter range in South America.

METHODS

Long-term work in our study area (in Keith, Garden, Lincoln, and Morrill counties, Nebraska) indicates the first Cliff Swallows appear on about 18 April each year with numbers slowly increasing during the following 10 days (Brown and Brown 1996: 443). The first arrivals tend to concentrate at the same 2–3 colony sites in the study area (C. R. Brown, pers. obs.). We mist-netted Cliff Swallows between 23 and 29 April 2006 and 2007 at two colony sites (41° 15′ N, 101° 37′ W; 41° 13′ N, 101° 37′ W) that contained most birds present in the study area at that time. The early sampling dates ensured that birds at both sites were newly arrived. Both colony sites sampled had been fumigated multiple times per summer during the previous 10 seasons to remove swallow bugs, suggesting that few bugs were present in April and the likelihood of any bird being infected by a bug after arrival and before sampling was low. The insecticide used is highly effective against swallow bugs (Brown and Brown 2004). Fumigation procedures are described by Brown and Brown (1996).

Birds caught were bled by jugular or brachial venipuncture, in which 0.1 ml of blood was collected and placed in 0.4 ml of BA-1 diluent (Moore et al. 2007). Samples were centrifuged and 25 μl of supernatant was added to 100 μl of a guanidine thiocyanate-based lysis buffer. RNA was extracted after the addition of 100 μl of 100% ethanol using the QIAmp Viral RNA Mini Kit (Qiagen), following the manufacturer's protocol. A positive BCRV control was included in each extraction.

Reverse-transcription PCR (RT-PCR) was performed using the OneStep RT-PCR Kit (Qiagen) following the manufacturer's protocol. We used BCRV-specific primers that yielded a 208-bp fragment from the E2 region of the viral genome. Primer sequences and thermocycler conditions are described in Moore et al. (2007). Product (6.5 μl) was electrophoresed on a 4% Nusieve/agarose gel to identify any positive pools, using at least one BCRV positive control on each gel and a 100-bp ladder. This protocol was used for detecting BCRV in both swallow bug pools and sera of nestling House Sparrows, which are commonly infected (about 25% of bug pools and >20% of nestling sparrows; Moore et al. 2007; V. A. O'Brien and C. R. Brown, unpubl. data). Our RT-PCR methods have also detected BCRV in sera of Cliff Swallows during the summer nesting season, including samples confirmed by both RT-PCR and plaque assay on Vero cells (V. A. O'Brien and C. R. Brown, unpubl.data).

RESULTS

We captured 100 Cliff Swallows during the sampling periods in the 2 years. None of the 100 birds had evidence of circulating BCRV

RNA in blood, as judged from RT-PCR. All but 14 birds had been banded in the study area in a previous breeding season. Eighty-one were at least 2 years of age and their history of breeding-colony use was known for at least one previous year. Five had been banded the previous year as recently fledged juveniles. Fifty-nine of the 86 birds with past histories were known to have been resident at only fumigated colonies in the past (the same sites sampled in this study), 13 had used only non-fumigated sites in the past, and 9 had used both fumigated and non-fumigated sites in previous seasons. The 5 juveniles had been captured at fumigated colonies a few days after fledging.

DISCUSSION

Birds that had used parasite-free sites in past seasons were unlikely to have been exposed to BCRV in a previous summer and therefore not likely to show latent, chronic infections (as seen for some arboviruses; Reisen et al. 2003). The 47 birds sampled in 2007 were tested for BCRV-specific antibodies using a plaque reduction neutralization test (Huyavert et al. 2008); none of these birds was seropositive (G. R. Young and N. Komar, pers. comm.). Thus, the individuals sampled in this study were well suited to studying whether virus could be introduced by migrants that were infected prior to arrival in breeding areas.

Hayes et al. (1977) sampled 52 adult Cliff Swallows for the Fort Morgan strain of BCRV on 30 May 1974 in northeastern Colorado, ~215 km from our study area. That study used plaque assay and found no evidence of BCRV in swallows. Hayes et al. (1977) concluded that no evidence existed for spring re-introduction of virus by returning birds, although their samples were taken sufficiently late in the season that birds had begun egg-laying at time of sampling. Using a more sensitive assay (RT-PCR), we also found no evidence of circulating BCRV (i.e., viral RNA) in blood of adult Cliff Swallows, and our birds had arrived at most only a few days before sampling.

Most birds we sampled had probably not been exposed to BCRV in breeding areas, by virtue of their use of fumigated colony sites in past years (and, for some, their lack of antibodies to BCRV). Thus, they were prime candidates for transporting virus from wintering areas or from stopover sites en route. Surveys for BCRV have not been conducted in South America, and whether it occurs in wintering areas is unknown. BCRV is found at Cliff Swallow colony sites south of our study area, for example in west central Oklahoma, about 750 km from the Nebraska study area (Hopla et al. 1993; C. R. Brown, V. A. O'Brien, and A. T. Moore, unpubl. data). Migrating Cliff Swallows conceivably could be infected there and move the virus north to Nebraska.

Our results are consistent with the absence of direct evidence that migrating birds, re-introduce arboviruses to temperate localities. It is more likely these viruses persist annually by over-wintering in insect vectors or alternative resident hosts. Over-wintering of BCRV in swallow bugs is known to occur (Hayes et al. 1977, Rush et al. 1980, Strickler 2006). House Sparrows may be more suitable hosts for BCRV than Cliff Swallows, at least in summer (V. A. O'Brien and C. R. Brown, unpubl. data), and may provide another mechanism for annual persistence of virus. This is especially true if BCRV is maintained via latent, chronic infections in vertebrate tissue over long periods of time (Huyvaert et al. 2008). The role of migratory birds in re-introducing arboviruses to temperate latitudes in spring is unclear, and we urge all studies finding even negative evidence for re-introduction be reported.

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