

PATHOLOGY AND VIRUS DETECTION IN TISSUES OF NESTLING HOUSE SPARROWS NATURALLY INFECTED WITH BUGGY CREEK VIRUS (*TOGAVIRIDAE*)

Valerie A. O'Brien,^{1,3} Carol U. Meteyer,² Hon S. Ip,² Renee R. Long,² and Charles R. Brown¹

¹ Department of Biological Sciences, University of Tulsa, 800 South Tucker Drive, Tulsa, Oklahoma 74104, USA

² US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA

³ Corresponding author (email: valerie-obrien@utulsa.edu)

ABSTRACT: Alphaviruses (*Togaviridae*) infect wild birds, but clinical illness and death attributable to virus in naturally infected birds is rarely reported, particularly for small passerine species or nestlings. *Buggy Creek virus* is a unique alphavirus in the *Western equine encephalomyelitis virus* (WEEV) complex that is vectored by the cimicid swallow bug (*Oeciacus vicarius*), an ectoparasite of the colonially nesting Cliff Swallow (*Petrochelidon pyrrhonota*) and the introduced House Sparrow (*Passer domesticus*). While sampling birds for Buggy Creek virus (BCRV) during the summers of 2007 and 2008, we discovered large numbers of clinically ill or dead House Sparrow nestlings. Ill nestlings exhibited ataxia, torticollis, paresis, and lethargy. Histologic examination revealed that encephalitis was the most common finding, followed by myositis, myocarditis, and hepatic changes, but pathology was highly variable. We isolated BCRV from brain tissue in most of the ill or dead nestlings, and from blood, liver, kidney, spleen, lung, feather pulp, and skin in some birds. To our knowledge, this is the first report of clinical illness, gross pathology, and histopathology for a WEEV-complex alphavirus in a field-collected passerine species.

Key words: Alphavirus, *Buggy Creek virus*, clinical pathology, House Sparrow, *Passer domesticus*, viral encephalitis, virus ecology.

INTRODUCTION

Wild birds are often naturally infected with alphaviruses (*Togaviridae*), but the discovery of free-ranging birds with clinical illness attributable to these viruses is rare (Scott, 1988). Although alphavirus infection can result in clinical illness and death in naturally or experimentally infected birds, most of those showing pathogenic responses to infection are nonpasserine species. For example, *Eastern equine encephalitis virus* (EEEV) is known to cause morbidity and mortality in native wading birds (Spalding et al., 1994; Gottdenker et al., 2003) and in captive-raised Ring-necked Pheasants (*Phasianus colchicus*; Williams et al., 2000). *Eastern equine encephalitis virus* and *Highlands J virus* (HJV), an alphavirus in the *Western equine encephalomyelitis virus* (WEEV) complex, cause clinical illness and death in pen-raised Chukars (*Alectoris chukar*; Ranck et al., 1965) and domestic turkeys (Ficken et al., 1993). Experimental infection studies with wild passerine birds and alphaviruses generally have shown low

mortality and transient to no morbidity in adults of most species (Komar et al., 1999; Reisen et al., 2003; Huyvaert et al., 2008), although response to experimental infection can vary by species. For example, European Starlings (*Sturnus vulgaris*) inoculated with EEEV died more frequently than other species tested (Komar et al., 1999), and White-crowned Sparrows (*Zonotrichia leucophrys*), Golden-crowned Sparrows (*Zonotrichia atricapilla*), Tricolored Blackbirds (*Agelaius tricolor*), and Red-winged Blackbirds (*Agelaius phoeniceus*) died when inoculated with passaged strains of WEEV, whereas other passerines, including House Sparrows (*Passer domesticus*), were unaffected by experimental virus infection (Hardy and Reeves, 1990).

Although adults are considered the primary amplifying hosts of bird-associated alphaviruses (Reeves, 1990; Griffin, 2001), nestling birds may also be important in seasonal transmission cycles (Scott, 1988; Day, 2001; Unnasch et al., 2006). However, nestlings are difficult to locate for virus sampling, and sick or dead

nestlings with sequelae attributable to viruses are not typically discovered in field studies (McLean, 2006). Infection studies involving altricial nestling birds are rare, and challenges in husbandry of nestlings make following the clinical progression of disease and attributing mortality to virus infection problematic (Holden et al., 1973a; Scott et al., 1990; Mahmood et al., 2004).

Buggy Creek virus, also known as *Fort Morgan virus* (FMV; Calisher et al., 1988; Pfeffer et al., 2006; Padhi et al., 2008), is a unique alphavirus in the WEEV antigenic complex. Although BCRV shares structural similarities with other alphaviruses in the WEEV complex, it is primarily transmitted by the cimicid swallow bug (Hemiptera: Cimicidae, *Oeciacus vicarius*), rather than by mosquitoes, and is not considered a pathogen of humans or domesticated animals (Griffin, 2001). Buggy Creek virus (BCRV) is known to be amplified by only two vertebrate hosts, the Cliff Swallow (*Petrochelidon pyrrhonota*) and the introduced House Sparrow that usurps the mud nests that Cliff Swallows build (Hayes et al., 1977; Hopla et al., 1993). Adult and nestling House Sparrows are exposed to and fed upon by the hematophagous swallow bugs (O'Brien, pers. obs.), which live in and behind Cliff Swallow nests (Brown and Brown, 1996). Investigators have isolated BCRV/FMV from the brains of House Sparrow nestlings found sick or dead below nests (Hayes et al., 1977; Scott et al., 1984), but none have characterized the pathology and tissue tropism associated with BCRV infection in nestlings. In a study of FMV at three colony sites in Colorado, USA, Scott et al. (1984) reported that dead or clinically ill, 10-day-old House Sparrow nestlings were more likely than older sparrows to have virus in the brain (based on virus isolation from needle-aspirated brain samples), but they attributed the main cause of House Sparrow nestling mortality to nests falling from the substrate.

While sampling Cliff Swallows and House Sparrows for BCRV infection at 17 swallow colonies in southwestern Nebraska, USA, in the summers of 2007 and 2008, we found large numbers of dead and moribund House Sparrow nestlings either inside nests or on the ground near nests. Clinical signs of disease in ill nestlings included lethargy, ruffled feathers, gasping, generalized weakness, emaciation, ataxia, torticollis, unilateral weakness or paresis of legs, and ascites (Fig. 1). In this study, we collected sick and dead nestlings, examined the carcasses for gross and microscopic abnormalities, and attempted virus isolation from various tissues to determine whether BCRV or another arbovirus infection may have contributed to House Sparrow nestling illness or mortality.

MATERIALS AND METHODS

Study area and animal collection

Our study area was centered at the Cedar Point Biological Station (41°13'N, 101°39'W), in Keith County, Nebraska, USA, and included portions of Lincoln, Garden, Deuel, and Morrill counties. The study area is described in detail by Brown and Brown (1996). During the summers of 2007 and 2008, we monitored 17 Cliff Swallow colonies on bridges or in culverts under highways or railroads for House Sparrow and Cliff Swallow nesting activity. Occupied nests were numbered and visited every 4–6 days throughout the birds' summer nesting season. Nestling Cliff Swallows and House Sparrows aged 4–17 days were systematically blood-sampled by jugular venipuncture, in which 0.1 ml of blood was placed in 0.4 ml of diluent (Moore et al., 2007) for virus assay.

From 26 May 2007 to 21 June 2007, we collected nine clinically ill or dead House Sparrow nestlings aged 6–12 days at five colony sites. Birds were aged by nest records, if the nest of origin could be identified, or based on our experience with nestlings of known age if found outside the nest. Ill nestlings ($n=4$) were euthanized and carcasses were stored on wet ice and then frozen at -70°C until shipment to the US Geological Survey (USGS) National Wildlife Health Center (NWHC) for necropsy and virus isolation. From 8 June 2008 to 10 August 2008, six dead



FIGURE 1. Paresis, generalized weakness, and torticollis in a House Sparrow nestling, age 6 days (right), alongside nest mate, collected 25 July 2008 in Keith County, Nebraska, USA. Buggy Creek virus was isolated from multiple tissues in the clinically ill nestling. Note engorged swallow bug on leg of nestling on right.

and three ill nestlings aged 5–15 days were collected at four colony sites. Ill nestlings were blood-sampled and euthanized. For comparison, two outwardly healthy House Sparrow nestlings aged 7 and 12 days were removed from nests in a Cliff Swallow colony that had been fumigated to control parasites as part of a long-term study (Brown and Brown, 1996). The “control” nestlings were processed the same as ill nestlings. In 2008, carcasses were stored on wet ice in the field and then at 4 C until shipment to the NWHC within 24 hr of collection.

Gross and microscopic pathology

Necropsies on nestling House Sparrows were performed at the NWHC the day of their arrival. Body condition was evaluated and scored as good, fair, poor, or emaciated based on extent of body fat stores. External and internal lesions were recorded, and a subset of brain, heart, trachea, lung, kidney, liver, pancreas, esophagus, proventriculus, intestine, spleen, thymus, bursa, bone marrow, adrenal gland, skeletal muscle, and skin were collected for histopathology. All tissues were not sampled from every nestling. Tissues were placed in 10% neutral-buffered formalin, trimmed and embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin. In addition, heart sections were stained with phosphotungstic acid–hematoxylin using a standard method to better visualize cardiac

muscle cross-striations and fibrin. Lung and liver were cultured for aerobic bacteria using 5% sheep blood and eosin-methylene-blue agars. Inoculated plates were incubated at 36 C for 48 hr and bacterial isolates were identified using standard methods. Brain cholinesterase activity was evaluated in a subset of nestlings using methods previously reported (Hill and Fleming, 1982).

Virus isolation

Virus isolation was attempted on brain tissue for all birds and on combinations of tissues (brain, liver, kidney/spleen, lung, feather pulp, and skin) for 15 birds. Tissues were chosen for virus isolation based on our objective of identifying foci of BCRV infection in nestlings in general, not to specifically match histopathology with virus isolation in individual birds. Briefly, tissues were homogenized in a Stomacher 400 Circulator (Seward Ltd., Norfolk, UK) in 10 volumes of viral transport media. The suspensions were centrifuged at 800 \times G for 30 min at 4 C, and 1 ml of the supernatant was inoculated onto Vero cell (CRL-1587, American Type Cell Culture [ATCC], Manassas, Virginia, USA) monolayers in 12-cm² flasks. The flasks were incubated at 37 C and 2% CO₂ and examined daily for cytopathic effect (CPE). Virus isolation was also attempted on blood samples as part of our wider study of BCRV in House Sparrows.

TABLE 1. Pathology, virus isolation (VI), and Buggy Creek virus (BCRV)-specific reverse-transcription polymerase chain reaction (RT-PCR) in virus-positive House Sparrow nestlings. Not all tissues were examined for pathology nor all birds subjected to virus isolation.

Tissue	Pathology ^a	VI ^{b,c}	RT-PCR
Blood		6/11	6/11
Brain	Nonsuppurative encephalitis, 7/8	14/15	14/14
Heart	Myocarditis, 4/8	nd ^d	nd
Spinal cord	Meningoencephalitis, 1/3	nd	nd
Liver	Multifocal necrosis, 1/7	6/7	nd
Kidney	Multifocal nephritis, mild nephrosis, 2/7	7/11 ^e	1/1 ^e
Spleen	Splenomegaly, 1/9	— ^e	— ^e
Lung	Interstitial changes, 5/12	5/8	nd
Trachea	Submucosal hemorrhage, 1/7	nd	nd
Bone marrow	Decrease in cellularity, 1/3	nd	nd
Skin	Mild inflammation, 1/6	4/8	nd
Feather pulp	Mild inflammation, 1/6	3/6	nd
Skeletal muscle	Myositis, 6/7	nd	nd

^a No. affected/No. examined.

^b No. positive/No. tested.

^c Positive = evidence of cytopathic effect; *West Nile virus* ruled out.

^d nd = not done.

^e Kidney and spleen tissue combined for VI and RT-PCR.

Polymerase chain reaction

We performed BCRV-specific reverse-transcription polymerase chain reaction (RT-PCR) on blood and on virus-positive harvests or tissue samples (one per bird) to identify virus as BCRV. Sera and isolates or tissue samples were thawed, and 25 μ l was added to 100 μ l of a guanidine thiocyanate-based lysis buffer. After the addition of 100 μ l of 100% ethanol, RNA was extracted using the QIAmp Viral RNA Mini Kit (Qiagen, Valencia, California, USA) following the manufacturer's protocol. A positive BCRV control (BCRV isolates from swallow bugs that were passaged once in Vero cells) was included in each extraction, and negative controls were placed between every five samples. Reverse-transcription PCR was performed using the Qiagen OneStep RT-PCR Kit 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. Primer sequences and thermocycler conditions are given in Moore et al. (2007). Product (10 μ l) was electrophoresed on a 4% Nusieve/agarose gel using at least one BCRV-positive control on each gel and a 100-bp ladder. This protocol has been used for detecting BCRV in swallow bug pools and in sera of nestling House Sparrows (Moore et al., 2007; V. O'Brien and Brown, unpubl. data). *West Nile virus* (WNV) RT-PCR was performed according to Docherty et al. (2004) and avian influenza RT-PCR according to Ip et al. (2008).

RESULTS

Gross and microscopic pathology

One of the 15 nestlings positive for BCRV was emaciated, eight were in poor body condition, three were in fair condition, and three were in good body condition. Overall, 80% (12 of 15) of BCRV-positive birds were in suboptimal body condition; however, one virus-negative nestling was also in poor condition. Gross findings in BCRV-positive birds included splenomegaly, ascites (one bird), and mild nephrosis (Table 1). No gross findings were consistent across a majority of the carcasses. The single, emaciated, BCRV-positive nestling had rare, pin-point submucosal hemorrhages in the mouth and on the skin of the toe and hemorrhage in the intestine. The bone marrow was severely depleted, which may explain the small hemorrhagic foci seen in the mouth and skin. Starvation can lead to a marked decrease in bone marrow cellularity; this bird was the oldest collected (15 days) and would be considered near fledging (Lowther and Cink, 1992). The virus-negative bird had myocardial inflammation, ascites, and hepatomegaly. One ill

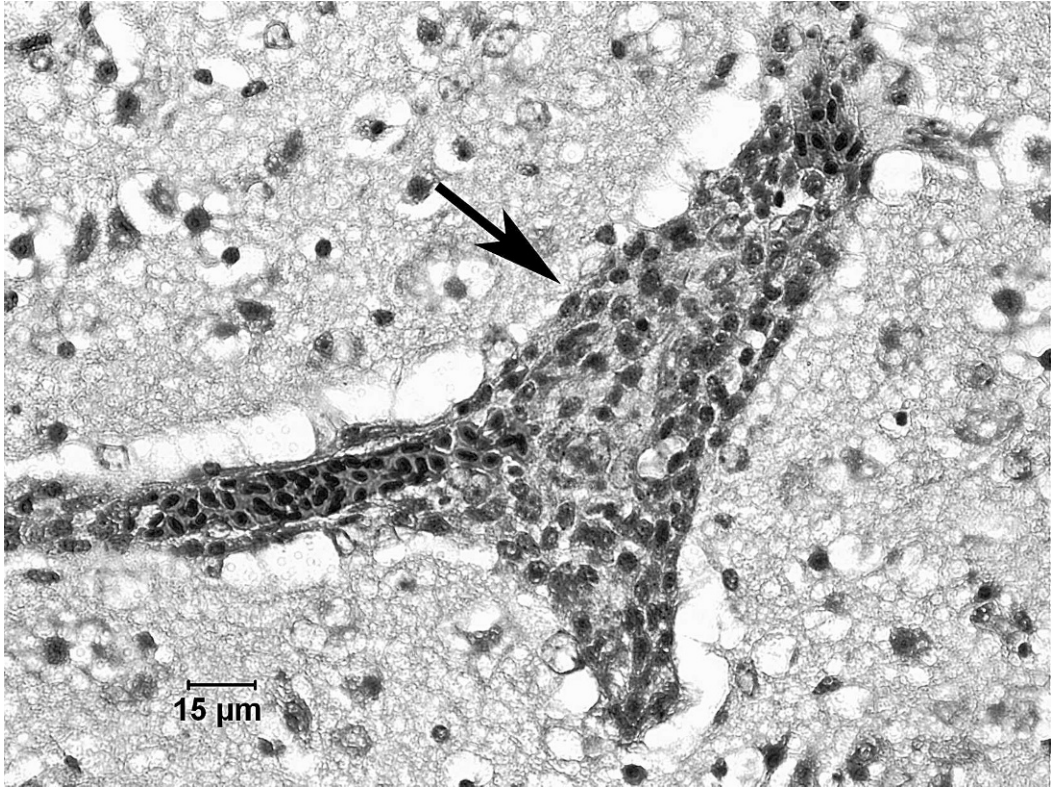


FIGURE 2. Photomicrograph of brain from 7-day-old House Sparrow nestling found dead and confirmed to be infected with Buggy Creek virus. Encephalitis with dense cluster of lymphocytes and plasma cells surround a vessel in the cerebrum (arrow). Clear vacuoles that border the vessel suggest perivascular edema. Hematoxylin and eosin stain.

nestling had an engorged swallow bug attached to its left leg (Fig. 1); no other birds had visible ectoparasites when collected. No endoparasites were detected in any of the necropsied nestlings. The two controls appeared healthy, were in good body condition, and had no external or internal pathology.

Numerous histologic lesions were found in BCRV-infected nestlings. Detection of lesions was difficult for the birds collected in 2007 because of freeze artifact and autolysis. Quantification of nestlings with lesions discussed in this section includes only those that had adequate fixation of tissues. Although organs affected varied among individuals in severity and occurrence, the most common finding was encephalitis, followed by myositis and myocarditis (Table 1). Inflammation in

the brain and spinal cord was usually mild, but the lymphoplasmacytic perivascular cuffing could be multifocally moderate with occasional heterophils (Fig. 2). Perivascular cuffing could be seen in all regions of the brain, but gliosis was most obvious in the central gray layer of the optic lobe. Myocardial pathology was mild to moderate and included fragmentation, increased waviness, and loss of cross-striations in myofibers. Lymphoplasmacytic inflammation was seen multifocally in the endocardium, and cuffing was seen in medium-sized vessels in the myocardium (Fig. 3). Unless there was necrosis or inflammation in the heart, the phosphotungstic acid–hematoxylin staining of heart tissues did not show significant differences in myocardial cross-striations or fibrin deposition between BCRV-positive nest-

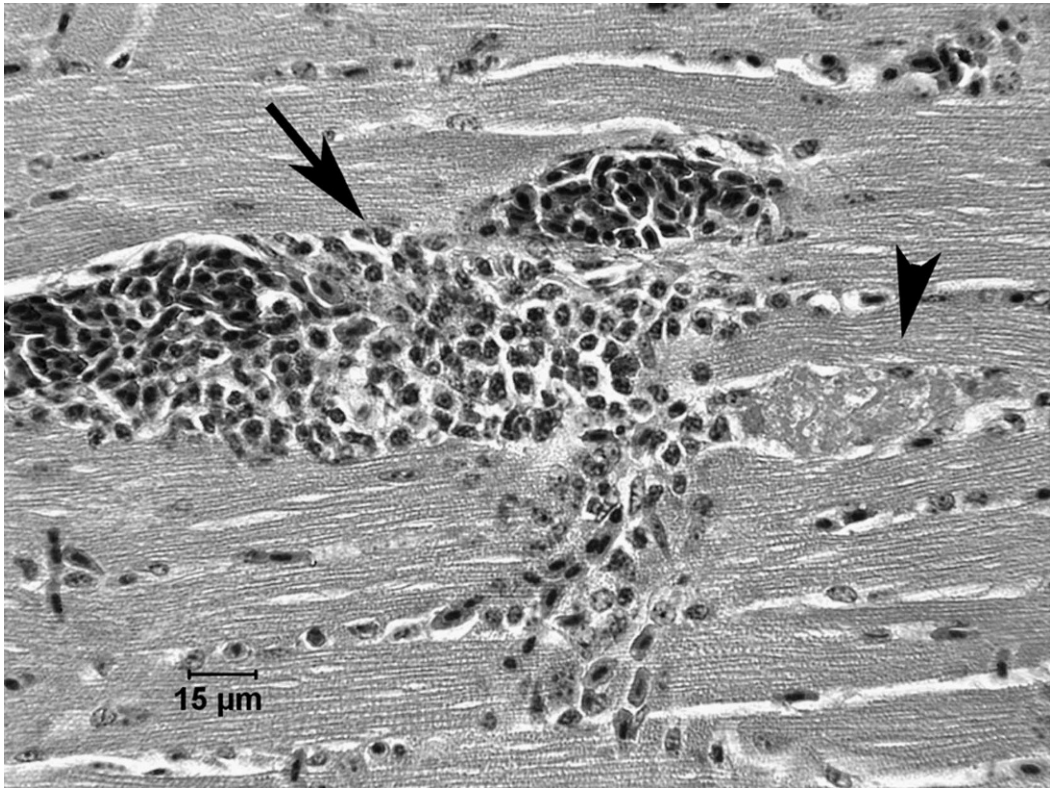


FIGURE 3. Photomicrograph of heart from House Sparrow in Figure 2. Lymphocytes and plasma cells form a perivascular cuff around a vessel deep within the heart (arrow). Necrosis of a muscle fiber is characterized by hyper-eosinophilia, fragmentation of the myofiber, and loss of cross-striations (arrowhead). Hematoxylin and eosin stain.

lings and age-matched controls. Skeletal muscle necrosis was often accompanied by subacute inflammation with lymphocytes, plasma cells, and heterophils. There were rare, small areas of liver necrosis in two BCRV-positive birds, and one of these had a multifocal, moderate, nonsuppurative interstitial pneumonia. Heterophilic inflammation in the feather pulp was seen in the skin of one of five nestlings examined. No notable histopathology lesions were seen in any of the 13 organs examined in each of the control, age-matched, uninfected House Sparrows.

One BCRV-positive bird was also positive by cloacal swab for avian influenza in matrix RT-PCR tests. Additional tests for H5 and H7 avian influenza virus subtypes were negative, which is consistent with nonpathogenic avian influenza virus in

birds. Bacteria unrelated to postmortem overgrowth were not isolated from liver, lung, or intestine of any bird. Cholinesterase activity was not appreciably depressed in two birds tested, making exposure to organophosphate pesticides unlikely.

Virus isolation and identification

An RNA virus was isolated from tissue in 17 of 18 birds that were ill or dead at the time of collection, and RT-PCR confirmed BCRV in brain tissue from 14 of these birds and in kidney/spleen tissue from one bird. Seven birds were BCRV-positive in sera by virus isolation or RT-PCR. Of birds positive for BCRV where multiple tissues were tested ($n=13$), 85% had virus in more than one tissue, and 71% of tissues (46 of 65) tested from these

TABLE 2. Relationship between observed pathology and virus isolation in tissues in field-collected House Sparrows, Nebraska, USA.

Tissue (<i>n</i>) ^a	Pathology only	Virus isolation only	Both (%)	Neither (%)
Brain (8)	0	1	7 (87.5)	0
Liver (2)	0	2	0	0
Kidney/spleen (6)	0	3	2 (33.3)	1 (16.7)
Lung (6)	2	2	2 (33.3)	0

^a No. birds tested.

birds were virus-positive. Buggy Creek virus was most commonly isolated from brain tissue, followed by blood and liver (Table 1). Virus isolation from organs may be related to concurrent viremia; of the 11 birds from which blood was collected within 5 days before natural death or euthanization, seven were positive for BCRV in blood and at least one other tissue, three were virus-negative in blood and virus-positive in at least one other tissue, and one was virus-negative in both blood and other tissues. There was a difference between the proportion of BCRV-positive organ tissues in birds with ($n=7$; mean \pm SE, 0.833 ± 0.096) and without ($n=4$; 0.417 ± 0.144) BCRV in blood (Wilcoxon two-sample test; $Z=-1.94$, $P=0.052$). Virus was isolated from brain tissue in all of the birds found dead ($n=6$), and virus was isolated from multiple tissues (brain, liver, kidney/spleen, feather pulp, and skin) in one bird found dead.

In birds with sufficient nonautolysed tissue available for pathologic examination and with the same tissue types submitted for virus isolation, BCRV isolation was not concordant with pathology, except in the brain (Table 2). *West Nile virus* was confirmed by RT-PCR on brain isolates from two 14-day-old nestlings likely from the same nest; these birds were negative for BCRV on all tests. No coinfections with WNV and BCRV were detected in any birds.

DISCUSSION

Infection with BCRV appears to be strongly associated with clinical illness and

death in House Sparrow nestlings. Fifteen of the 18 nestlings (83.3%) found ill or dead were positive for BCRV by virus isolation and RT-PCR from tissues. Studies of WEEV and BCRV have shown little isolation of active virus in adult birds, with most field-collected isolates obtained from brain tissue or blood of nestlings (Holden et al., 1973b; Scott et al., 1984; McLean et al., 1989; Milby and Reeves, 1990; O'Brien and Brown, unpubl. data). Huyvaert et al. (2008) experimentally inoculated 16 adult House Sparrows with BCRV. No birds showed clinical signs of infection, and the birds were not necropsied. At 15 days postinoculation, tissue samples were obtained from brain, spleen, liver, lungs, and skin of 15 birds and tested by RT-PCR for BCRV. Viral RNA was detected in brain tissue of four birds and in skin of four birds (Huyvaert et al., 2008). In a comprehensive study of arboviruses in California, Milby and Reeves (1990) sampled 1,308 birds and obtained 15 isolations of WEEV. Of those, 14 were from nestlings, and virus was isolated from brain, lung, heart, liver, and blood. Holden et al. (1973b) also recovered WEEV from brain samples of dead nestlings. Our findings of BCRV in the brains of ill and dead House Sparrow nestlings support the earlier studies of Hayes et al. (1977) and Scott et al. (1984), where only brain tissue was tested for virus.

Clinically ill, BCRV-infected House Sparrow nestlings exhibited the lethargy, weakness, unilateral paresis, torticollis, and ataxia associated with neurologic disease. Domestic turkeys also showed

leg paralysis, lethargy, and ataxia when infected with WEEV, HJV, or EEEV (Ficken et al., 1993; Guy et al., 1993). To our knowledge, no studies exist that report symptoms of neurologic disease in alphavirus-infected, small passerines or altricial nestling birds. Most birds infected with alphaviruses do not show widespread gross lesions (Ficken et al., 1993), although Chukars had heart and spleen enlargement (Ranck et al., 1965), and a Great Egret (*Casmerodius albus*) exhibited liver necrosis and heart abnormalities when found naturally infected with EEEV (Gottdenker et al., 2003). Nestlings with BCRV had few gross lesions on necropsy; the single BCRV-negative nestling had the most remarkable gross pathology, with hepatomegaly, ascites, and heart inflammation, which suggests that gross lesions are not reliable diagnostic criteria in suspected BCRV infection. A cause for the pathology in this BCRV-negative nestling from 2007 was not identified.

Although all of the BCRV-positive nestlings had histologic lesions in more than one organ, microscopic pathology was inconsistent in these birds, except in the brain, where all but one bird with brain tissue available for examination showed encephalitic changes. The brain tissue sample from the single virus-positive nestling with no evidence of encephalitis was autolyzed and may have been inadequate to reliably detect encephalitic changes. Lack of agreement of virus isolation from tissue with pathology among birds sampled for both (Table 2) could reflect differences in duration and stage of infection, age at onset of infection, individual condition upon virus infection, or peracute mortality before the appearance of pathology. Larger sample sizes or experimental infection studies are necessary to identify a typical course of BCRV infection in House Sparrow nestlings, but the encephalitis and muscle inflammation found in these birds is an indication that House Sparrow nestlings experience a systemic, likely severe infection.

The mild, nonsuppurative encephalitis found in nestlings with BCRV is similar to the brain pathology found in birds with other encephalitic avian viruses, such as EEEV in Chukars and Ring-necked Pheasants (Ranck et al., 1965; Williams et al., 2000), neurotropic velogenic *Newcastle disease virus* in Double-crested Cormorants (*Phalacrocorax auritus*; Meteyer et al., 1997), and *West Nile virus* in owls (Fitzgerald et al., 2003). Because of the concordance between brain lesions and virus isolation in nestlings infected with BCRV, brain tissue should be targeted when BCRV is suspected as a cause of death in House Sparrows collected near Cliff Swallow colonies. However, our isolation of WNV from the brains of two House Sparrow nestlings demonstrates the need to rule out WNV when examining ill or dead birds found in or near swallow colonies. An immunohistochemistry (IHC) test has not yet been developed for BCRV; having IHC tests available would greatly improve the differential diagnosis and would allow histologic abnormalities in organs to be definitively linked to BCRV antigen.

ACKNOWLEDGMENTS

This work was funded by NIH grant AI057569 and NSF grant DEB-0514824. The Cedar Point Biological Station of the University of Nebraska-Lincoln provided logistical support. Kenton Miller, William Reisen, and two anonymous reviewers provided helpful comments on the manuscript. Use of trade or product names does not imply endorsement of the US Government.

LITERATURE CITED

- BROWN, C. R., AND M. B. BROWN. 1996. Coloniality in the Cliff Swallow: the effect of group size on social behavior. University of Chicago Press, Chicago, Illinois, 566 pp.
- CALISHER, C. H., N. KARABATSOS, J. S. LAZUICK, T. P. MONATH, AND K. L. WOLFF. 1988. Reevaluation of the western equine encephalitis antigenic complex of alphaviruses (family *Togaviridae*) as determined by neutralization tests. *American Journal of Tropical Medicine and Hygiene* 38: 447–452.

- DAY, J. F. 2001. Predicting St. Louis encephalitis virus epidemics: Lessons from recent, and not so recent, outbreaks. *Annual Review of Entomology* 46: 111–138.
- DOCHERTY, D. E., R. R. LONG, K. M. GRIFFIN, AND E. K. SAITO. 2004. Corvidae feather pulp and West Nile virus detection. *Emerging Infectious Diseases* 10: 907–909.
- FICKEN, M. D., D. P. WAGES, J. S. GUY, J. A. QUINN, AND W. H. EMORY. 1993. High mortality of domestic turkeys associated with Highlands J virus and eastern equine encephalitis virus infections. *Avian Diseases* 37: 585–590.
- FITZGERALD, S. D., J. S. PATTERSON, M. KIPEL, H. A. SIMMONS, S. D. GRIMES, C. F. SARVER, R. M. FULTON, B. A. STEFICEK, T. M. COOLEY, J. P. MASSEY, AND J. G. SIKARSKIE. 2003. Clinical and pathologic features of West Nile virus infection in native North American owls (family Strigidae). *Avian Diseases* 47: 602–610.
- GOTTENKER, N. L., E. W. HOWERTH, AND D. G. MEAD. 2003. Natural infection of a great egret (*Casmerodius albus*) with eastern equine encephalitis virus. *Journal of Wildlife Diseases* 39: 702–706.
- GRIFFIN, D. E. 2001. Alphaviruses. In *Fields virology*, D. M. Knipe and P. M. Howley (eds.). Lippincott, Williams & Wilkins, Philadelphia, Pennsylvania, pp. 917–962.
- GUY, J. S., M. D. FICKEN, H. J. BARNES, D. P. WAGES, AND L. G. SMITH. 1993. Experimental infection of young turkeys with eastern equine encephalitis virus and Highlands J virus. *Avian Diseases* 37: 389–395.
- HARDY, J. L., AND W. C. REEVES. 1990. Experimental studies on infection in vertebrate hosts. In *Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987*, W. C. Reeves (ed.). California Mosquito and Vector Control Association, Inc., Sacramento, California, pp. 66–127.
- HAYES, R. O., D. B. FRANCY, J. S. LAZUICK, G. C. SMITH, AND E. P. J. GIBBS. 1977. Role of the cliff swallow bug (*Oeciacus vicarius*) in the natural cycle of a western equine encephalitis-related alphavirus. *Journal of Medical Entomology* 14: 257–262.
- HILL, E. F., AND W. J. FLEMING. 1982. Anticholinesterase poisoning of birds: Field monitoring and diagnosis of acute poisoning. *Environmental Toxicology and Chemistry* 1: 27–38.
- HOLDEN, P., D. B. FRANCY, C. J. MITCHELL, R. O. HAYES, J. S. LAZUICK, AND T. B. HUGHES. 1973a. House sparrows, *Passer domesticus* (L.), as hosts of arboviruses in Hale County, Texas, II: Laboratory studies with western equine encephalitis virus. *American Journal of Tropical Medicine and Hygiene* 22: 254–262.
- , R. O. HAYES, C. J. MITCHELL, D. B. FRANCY, J. S. LAZUICK, AND T. B. HUGHES. 1973b. House sparrows, *Passer domesticus* (L.), as hosts of arboviruses in Hale County, Texas, I: Field studies, 1965–1969. *American Journal of Tropical Medicine and Hygiene* 22: 244–253.
- HOPLA, C. E., D. B. FRANCY, C. H. CALISHER, AND J. S. LAZUICK. 1993. Relationship of cliff swallows, ectoparasites, and an alphavirus in west-central Oklahoma. *Journal of Medical Entomology* 30: 267–272.
- HUYVAERT, K. P., A. T. MOORE, N. A. PANELLA, E. A. EDWARDS, M. B. BROWN, N. KOMAR, AND C. R. BROWN. 2008. Experimental inoculation of house sparrows (*Passer domesticus*) with Buggy Creek virus. *Journal of Wildlife Diseases* 44: 331–340.
- IP, H., P. FLINT, J. C. FRANSON, R. DUSEK, D. DERKSEN, R. GILL, C. ELY, J. PEARCE, R. LANCTOT, S. MATSUOKA, D. IRONS, J. FISCHER, R. OATES, M. PETERSEN, T. FONDELL, D. ROCQUE, J. PEDERSEN, AND T. ROTHE. 2008. Prevalence of Influenza A viruses in wild migratory birds in Alaska: Patterns of variation in detection at a crossroads of intercontinental flyways. *Virology Journal* 5: 71.
- KOMAR, N., D. J. DOHM, M. J. TURELL, AND A. SPIELMAN. 1999. Eastern equine encephalitis virus in birds: Relative competence of European starlings (*Sturnus vulgaris*). *American Journal of Tropical Medicine and Hygiene* 60: 387–391.
- LOWTHER, P. E., AND C. L. CINK. 1992. House sparrow. In *Birds of North America* No. 12, A. Poole, P. Stettenheim and F. Gill (eds.). Academy of Natural Sciences, Philadelphia, Pennsylvania, and American Ornithologists' Union, Washington, D.C., 20 pp.
- MAHMOOD, F., R. E. CHILES, Y. FANG, C. M. BARKER, AND W. K. REISEN. 2004. Role of nestling mourning doves and house finches as amplifying hosts of St. Louis encephalitis virus. *Journal of Medical Entomology* 41: 965–972.
- MCLEAN, R. G. 2006. West Nile virus in North American birds. *Ornithological Monographs* 60: 44–64.
- , R. B. SHRINER, L. J. KIRK, AND D. J. MUTH. 1989. Western equine encephalitis in avian populations in North Dakota, 1975. *Journal of Wildlife Diseases* 25: 481–489.
- METEYER, C. U., D. E. DOCHERTY, L. C. GLASER, J. C. FRANSON, D. A. SENNE, AND R. DUNCAN. 1997. Diagnostic findings in the 1992 epornitic of neurotropic velogenic Newcastle disease in double-crested cormorants from the upper midwestern United States. *Avian Diseases* 41: 171–180.
- MILBY, M. M., AND W. C. REEVES. 1990. Natural infection in vertebrate hosts other than man. In *Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987*, W. C. Reeves (ed.). California Mosquito and Vector Control Association, Inc., Sacramento, California, pp. 26–65.

- MOORE, A. T., E. A. EDWARDS, M. B. BROWN, N. KOMAR, AND C. R. BROWN. 2007. Ecological correlates of Buggy Creek virus infection in *Oeciacus vicarius*, southwestern Nebraska, 2004. *Journal of Medical Entomology* 44: 42–49.
- PADHI, A., A. T. MOORE, M. B. BROWN, J. E. FOSTER, M. PFEFFER, K. P. GAINES, V. A. O'BRIEN, S. A. STRICKLER, A. E. JOHNSON, AND C. R. BROWN. 2008. Phylogeographical structure and evolutionary history of two Buggy Creek virus lineages in the western Great Plains of North America. *Journal of General Virology* 89: 2122–2131.
- PFEFFER, M., J. E. FOSTER, E. A. EDWARDS, M. B. BROWN, N. KOMAR, AND C. R. BROWN. 2006. Phylogenetic analysis of Buggy Creek virus: Evidence for multiple clades in the western Great Plains, United States of America. *Applied and Environmental Microbiology* 72: 6886–6893.
- RANCK, F. M., JR., J. H. GAINER, J. E. HANLEY, AND S. L. NELSON. 1965. Natural outbreak of eastern and western encephalitis in pen-raised chukars in Florida. *Avian Diseases* 9: 8–20.
- REEVES W. C. (ed.). 1990. *Epidemiology and control of mosquito-borne arboviruses in California, 1943–1987*. California Mosquito and Vector Control Association, Inc., Sacramento, California, 508 pp.
- REISEN, W. K., R. E. CHILES, V. M. MARTINEZ, Y. FANG, AND E. N. GREEN. 2003. Experimental infection of California birds with western equine encephalomyelitis and St. Louis encephalitis viruses. *Journal of Medical Entomology* 40: 968–982.
- SCOTT, T. W. 1988. Vertebrate host ecology. In *The arboviruses: Epidemiology and ecology*, Vol. 5, T. P. Monath (ed.). CRC Press, Inc., Boca Raton, Florida, pp. 257–280.
- , G. S. BOWEN, AND T. P. MONATH. 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. *American Journal of Tropical Medicine and Hygiene* 33: 981–991.
- , L. H. LORENZ, AND J. D. EDMAN. 1990. Effects of house sparrow age and arbovirus infection on attraction of mosquitoes. *Journal of Medical Entomology* 27: 856–863.
- SPALDING, M. G., R. G. MCLEAN, J. H. BURGESS, AND L. J. KIRK. 1994. Arboviruses in water birds (Ciconiiformes, Pelecaniformes) from Florida. *Journal of Wildlife Diseases* 30: 216–221.
- UNNASCH, R. S., T. SPRENGER, C. R. KATHOLI, E. W. CUPP, G. E. HILL, AND T. R. UNNASCH. 2006. A dynamic transmission model of eastern equine encephalitis virus. *Ecological Modelling* 192: 425–440.
- WILLIAMS, S. M., R. M. FULTON, S. P. JON, AND W. M. REED. 2000. Diagnosis of eastern equine encephalitis by immunohistochemistry in two flocks of Michigan ring-neck pheasants. *Avian Diseases* 44: 1012–1016.

Received for publication 12 June 2009.