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COMPARISON OF IMMUNE RESPONSES OF BROWN-HEADED COWBIRD AND RELATED BLACKBIRDS TO WEST NILE AND OTHER MOSQUITO-BORNE ENCEPHALITIS VIRUSES

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ABSTRACT: The rapid geographic spread of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) across the United States has stimulated interest in comparative host infection studies to delineate competent avian hosts critical for viral amplification. We compared the host competence of four taxonomically related blackbird species (Icteridae) after experimental infection with WNV and with two endemic, mosquito-borne encephalitis viruses, western equine encephalomyelitis virus (family *Togaviridae*, genus *Alphavirus*, WEEV), and St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV). We predicted differences in disease resistance among the blackbird species based on differences in life history, because they differ in geographic range and life history traits that include mating and breeding systems. Differences were observed among the response of these hosts to all three viruses. Red-winged Blackbirds were more susceptible to SLEV than Brewer's Blackbirds, whereas Brewer's Blackbirds were more susceptible to WEEV than Red-winged Blackbirds. In response to WNV infection, cowbirds showed the lowest mean viremias, cleared their infections faster, and showed lower antibody levels than concurrently infected species. Brown-headed Cowbirds also exhibited significantly lower viremia responses after infection with SLEV and WEEV as well as coinfection with WEEV and WNV than concurrently infected icterids. We concluded that cowbirds may be more resistant to infection to both native and introduced viruses because they experience heightened exposure to a variety of pathogens of parenting birds during the course of their parasitic life style.
Key words: Blackbirds, Brown-headed Cowbird, Icteridae, immune system, parasite-mediated selection, West Nile virus, western equine encephalomyelitis virus.

INTRODUCTION

The rapid geographic spread of West Nile virus (family *Flaviviridae*, genus *Flavivirus*, WNV) across the United States has stimulated interest in comparative host infection studies of many avian species to delineate competent hosts critical for viral amplification (Komar et al., 2003; Reisen et al., 2005). Taxonomic differences in avian susceptibility have been noted, and they include heightened susceptibility and mortality of American Crows and other Corvidae (Komar et al., 2003; Brault et al., 2004; Reisen et al., 2005) or the refractory tendency of some galliforms such as adult quail and chickens that produce low-level viremias but respond well immunologically (Reisen et al., 1994, 2006; Langevin et al., 2001). Investigators can capitalize on differences in species' life history traits to strategically select study species that facilitate examination of particular aspects of pathogen virulence or host immunity (Brault et al., 2004).

We investigated species' differences in susceptibility and immunity by comparing the viremia and antibody responses of four blackbird species (Icteridae; Lanyon, 1994) from California, USA, after experimental infection with two endemic and one introduced arbovirus. These species differ in geographic range, breeding behavior, and mating system (Ehrlich et al., 1988); factors that are relevant to species-specific histories of exposure to pathogens and evolution of immune systems (Schmid-Hempel, 2003). Brown-headed Cowbirds (*Molothrus ater*) and Red-winged Blackbirds (*Agelaius phoeniceus*) have exceptionally large geographic ranges that cover most of North America and northern Central America (Sibley, 2000; Sauer et al., 2005); Brewer's Blackbirds (*Euphagus cyanocephalus*), although widespread and abundant, are not found in the eastern United States; and Tricolored Blackbirds (*Agelaius tricolor*) have a very limited range and are found only in the Central Valley of California. All four species have polygamous mating systems, although Brewer's Blackbirds occasionally are monogamous (Orians, 1980; Ehrlich et al., 1988). The breeding system of the Brown-headed Cowbird is different from that of the other blackbirds because it is an obligate brood parasite, that lays its eggs in the nests of >200 other songbird species (Hahn and Hatfield, 1995; Ortega, 1998; Rothstein and Robinson, 1998; Davies, 2000), whereas the other blackbird species are nonparasitic (Orians, 1980; Ehrlich et al., 1988).

We compared the relative susceptibility of these four blackbird species to the NY99 strain of WNV that is highly virulent for many passeriform birds (Komar et al., 2003). Previously, we had experimentally infected three of these species (all except Tricolored Blackbirds) with two North American mosquito-borne encephalitis viruses, western equine encephalomyelitis virus (family *Togaviridae*, genus *Alphavirus*, WEEV) and St. Louis encephalitis virus (family *Flaviviridae*, genus *Flavivirus*, SLEV) (Reisen et al., 2003). Both SLEV and WEEV occur intermittently in the western United States (Reeves et al., 1990), are transmitted in rural environments by the same vector mosquito, *Culex tarsalis*, and are known to naturally infect Brown-headed Cowbirds (Milby and Reeves, 1990; Reisen et al., 2000b). The previous experiments indicated that adult Brown-headed Cowbirds survived infection, developed a minimal viremic response, and were considered to be incompetent hosts. In this study, we compare the viremia, mortality, and antibody responses of these four related blackbird species after infection with these three arboviruses.

MATERIALS AND METHODS

Birds

Brewer's Blackbirds, Red-winged Blackbirds, and Brown-headed Cowbirds were collected from mixed winter foraging flocks at several dairies near Bakersfield in Kern County, California, during 2003–05 (Fig. 1). Additional Brown-headed Cowbirds were obtained from a removal program in the Prado Basin, Orange County, California, during 2004, and from grain-baited traps in the Coachella Valley, Riverside County, California, during 2004. Tricolored Blackbirds were collected by mist netting after the completion of nesting at a colony along the Kern River, Kern County, California, during 2005. Brewer's Blackbirds, Red-winged Blackbirds, and Tricolored Blackbirds are long-established natives of California, whereas the Brown-headed Cowbird arrived in California in 1900, as part of its range expansion associated with anthropogenic change related to agriculture (Laymon, 1987).

All birds were brought to the Arbovirus Field Station in Bakersfield, prebled to establish that they did not have previous infection with the viruses to be tested, banded, and then released into a mosquito-proof outdoor aviary to observe general health and adaptation to captivity. Birds were fed a mixture of mixed wild birdseed and adjusted well to captivity. The collection and infection of birds was done under Protocol 11184 approved by the Animal Use and Care Administrative Advisory Committee of the University of California, Davis, California Resident Scientific Collection Permit 801049-02 from the State of California Department of Fish and Game, and Federal Fish and Wildlife Permit MB082812-0 from the Department of the Interior. Use of arboviruses for avian infection was approved under Biological Use Authorization 0554 by Environmental Health and Safety of the University of California, Davis, and USDA Permit 47901.

Viruses

We used the NY99 strain of WNV isolated from a flamingo that died in the Bronx Zoo (strain 35211 AAF 9/23/99) and that was passaged twice in Vero cells. For the endemic encephalitides, we used sympatric strains of viruses isolated from *Cx. tarsalis* collected from the same localities as the birds. The Kern217 strain of SLEV was isolated in Bakersfield during the outbreak of 1989 (Reisen et al., 1992), whereas the COA608 strain was isolated from the Coachella Valley

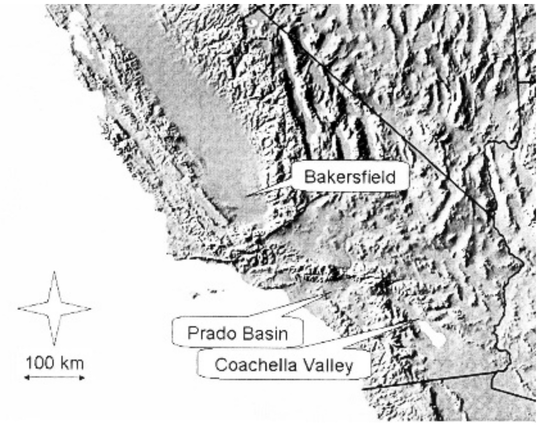

FIGURE 1. Map of southern California showing locations of bird and virus collections.

during 1992. Both SLEV strains were similar genetically (Reisen et al., 2002). The Kern5547 and BFS1703 strains of WEEV were isolated from Bakersfield during 1983 and 1953, respectively, whereas the COA592 strain was isolated in the Coachella Valley during 1992. These virus strains were at Vero passage 2–3, and they have been used extensively in previous host competence studies (Reisen et al., 2005).

In one experiment, Brown-headed Cowbirds and Brewer's Blackbirds were coinfected with approximately 3 $\log_{10}$ plaque-forming units (PFU)/ml of WNV(NY99 strain) and 0.5 $\log_{10}$ PFU/ml of WEEV (BFS1703 strain). Coinfection permitted the analysis of the host species response to more complex infection and assessed the relative fitness of the competing viruses. Although simultaneous infection occurs infrequently, we recently captured Mourning Doves (*Zenaida macroura*) in Kern County with antibodies against WNV that were viremic for WEEV.

Experimental infection

Birds were inoculated subcutaneously in the cervical region with 100 µl of virus diluent containing 100 to 1,000 PFU of virus. This dose is comparable with the amount of virus expectorated by infectious *Cx. tarsalis*, and it was found to produce a similar viremia response as infection by mosquito bite (Reisen et al., 2000a). Infection was determined by the detection of a viremia or antibody response. Viremia was monitored by collection of blood daily for 5–8 days postinfection (dpi) by jugular puncture using a 28-gauge needle. The 100-µl blood sample was expelled immediately into 400 µl of virus diluent (phosphate-buffered saline containing 20% fetal calf serum and antibiotics), clarified by centrifugation, and then stored at −80 C until tested. Antibody levels were measured by collecting blood from birds weekly for 6 wk postinfection by jugular puncture (100 µl of blood expelled immediately into 900 µl of saline). Surviving birds were necropsied 6 wk postinfection and blood, lung, spleen, and kidney tissues tested for virus.

Diagnostics

Mortality was recorded daily. Viremia was measured by standard plaque assay using Vero cell culture (Kramer et al., 2002), with a minimal threshold of detection of 2 PFU/0.1 ml or 1.7 $\log_{10}$ PFU/ml. Antibody in surviving birds was measured weekly using an enzyme immunoassay (EIA) (Chiles and Reisen, 1998), and antibody level is reported as the ratio of the mean optical density of two positive wells over a negative control well (P/N ratio) for each bird. At 6 wk postinfection, neutralizing antibody titers were measured using a plaque reduction neutralization test (PRNT). For coinfected birds, we used a multiplex real-time polymerase chain reaction (RT-PCR) assay to measure the relative amounts of WEEV and WNV in each sample; combined viremias were measured by plaque assay. The RNA was extracted from necropsy tissues by using the RNEasy kit (QIAGEN, Valencia, California, USA). Real-time PCR was done with a TaqMan platform (Shi et al., 2001) by using previously published primers derived from sequences from envelope and nonstructural (NS-5) portions of the genome (Lanciotti et al., 2000). Quantity of RNA present was expressed as TaqMan cycle threshold (Ct) scores or the number of PCR thermal cycles required to exceed a positive threshold; a lower number of cycles implies more viral RNA and therefore virus in the original sample. Virus isolation was attempted on Vero cells after blind passage of samples in mosquito cells (*Aedes albopictus* C6/36) from birds positive for WNV RNA by using primers encoding for the NS-5 region.

RESULTS

Mortality

Mortality among all species was minimal in both single and coinfection experiments. Only Brewer's Blackbirds died after infection with WNV alone (one of eight birds died) and after coinfection with WNV-WEEV (two of 10 birds died). All negative control birds that were concurrently maintained and bled survived. None of the other species died, including five Tricolored Blackbirds, 62 Brown-headed Cowbirds, and 25 Red-winged Blackbirds; nor did any of the 15 Brewer's Blackbirds die after infection with WEEV or SLEV.

Viremia: single virus infection

Viremia profiles for species infected with WEEV, SLEV, and WNV are shown in Fig. 2. Infections in Brown-headed Cowbirds produced the lowest or among the lowest mean viremias.

Two Brown-headed Cowbird populations infected with sympatric strains of WEEV showed differing results; five birds from Kern County failed to show any viremia after infection with KERN5547, whereas five birds from Riverside County showed a low-level viremia peaking on 1 and 2 dpi (mean=2.8 $\log_{10}$ PFU/ml) after infection with COA592 (Fig. 2A). Interestingly, of birds tested from Coachella Valley, two birds had maximum viremias of 5.1 and 4.5 $\log_{10}$ PFU/ml, one bird peaked at 2.8 $\log_{10}$ PFU/mL, and two birds remained viremia negative; all birds produced measurable antibody, indicating infection. In comparison, populations of Brewer's Blackbirds from the same localities infected with the same WEEV strains did not exhibit a statistically different viremia response during 1–2 dpi when

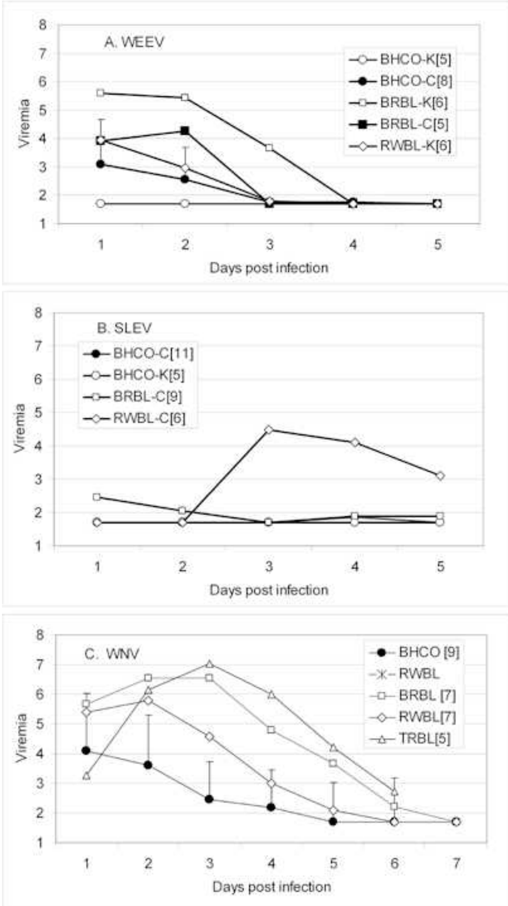

FIGURE 2. Mean viremias in $\log_{10}$ plaque-forming units (PFU)/ml for bird species infected with (A) western equine encephalomyelitis virus (WEEV), (B) St. Louis encephalitis virus (SLEV), and (C) West Nile virus (WNV). SE shown for Brown-headed Cowbird (BHCO) only. BRBL = Brewer's Blackbird; RWBL = Red-winged Blackbird; TRBL = Tricolored Blackbird. Localities were Kern County (K), Orange County (O), or Coachella Valley (C). *n* values in brackets.

tested by a repeated measure analysis of variance (ANOVA) ($P=0.09$). The among-species effect in an ANOVA of viremia titers comparing all bird species on 1–2 dpi was significant ($F=11.1$; df=2,48; $P<0.001$), with Brown-headed Cowbirds having the significantly (least significant difference [LSD], $P<0.05$) lowest mean viremia titers (2.3 $\log_{10}$ PFU/ml), followed by Red-winged Blackbirds (3.5 $\log_{10}$ PFU/

ml) and Brewer's Blackbirds (4.9 $\log_{10}$ PFU/ml), respectively.

Brown-headed Cowbirds showed no detectable viremia after inoculation with sympatric strains of SLEV (Fig. 2B). Brewer's Blackbirds were similarly negative, but Red-winged Blackbirds in the same experiment produced a viremia that peaked at >4 $\log_{10}$ PFU/ml on 3 and 4 dpi. Data were not analyzed statistically, because most birds failed to produce a detectable viremia.

All species inoculated with WNV produced a measurable viremia (Fig. 2C), and consistent with the results with WEEV, Brown-headed Cowbirds had the lowest mean viremia response. There was significant among-species variation in an ANOVA of viremia during 1–4 dpi (F=13.0; df=3,96; P<0.001), with Brown-headed Cowbirds having significantly (LSD, P<0.05) the lowest mean viremia (mean = 3.1 $\log_{10}$ PFU/ml), followed by Red-winged Blackbirds (4.7 $\log_{10}$ PFU/ml), Tricolored Blackbirds (5.6 $\log_{10}$ PFU/ml), and Brewer's Blackbirds (5.9 $\log_{10}$ PFU/ml), respectively. As shown in Fig. 2C, viremia changed significantly over time (F=3.5; df=3,96; P=0.02), and this pattern differed significantly among species, as indicated by the significant interaction effect in the ANOVA (F=2.7; df=9,96; P=0.008). Brown-headed Cowbirds not only had the lowest mean viremias but also cleared their infections most rapidly. There also was notable variability among individual Brown-headed Cowbird response to WNV: three of nine Brown-headed Cowbirds failed to produce a detectable viremia, whereas two birds developed viremias as high as 4.8 $\log_{10}$ PFU/ml on 2 dpi.

Viremia: coinfection

Brown-headed Cowbirds experienced significantly lower viremias than concurrently infected Brewer's Blackbirds. The combined viremias in coinfected Brown-headed Cowbirds and Brewer's Blackbirds exceeded viremias produced against either virus independently (Figs. 3A, 4). In a multifactorial ANOVA with species (Brewer's Blackbird, Brown-headed Cowbird), virus (WEEV, WNV, mixed) and dpi (1 and 2) as main effects, species differences accounted for 33% of the total variation, with mean viremia for Brown-headed Cowbirds (3.5 $\log_{10}$ PFU/ml) significantly less (F=57.5; df=1,97; P< 0.001) than for Brewer's Blackbirds (5.7 $\log_{10}$ PFU/ml). Virus titers also varied significantly among virus treatments (F=14.0; df=2,97; P<0.001), with coinfection titers (5.3 $\log_{10}$ PFU/ml) significantly (LSD, P<0.05) greater than in individual WNV (4.8 $\log_{10}$ PFU/ml) or WEEV (3.6 $\log_{10}$ PFU/ml) infections. These relationships were consistent over species during 1 and 2 dpi, because the interaction terms were not significant (P>0.05).

Virus from birds infected concurrently with WEEV and WNV produced plaques in Vero cultures that were not distinguishable morphologically or in their time of appearance, so the viremia levels of each virus had to be measured using a multiplex RT-PCR. Examination of RT-PCR results indicated that most of the plaques observed during days 1 – 3 were attributable to WEEV (Fig. 3B). Therefore, although in the single infection studies, both species had viremia levels for WNV greater than for WEEV (Fig. 4), in this coinfection study, both species showed higher levels of WEEV than WNV during 1 – 2 dpi. These data implied that WEEV initially out competed WNV, even though the inoculating dose was markedly lower for WEEV than for WNV.

Antibody

Antibody response at 6 wk postinfection varied markedly among and within bird species and viruses (Fig. 5). There were no marked differences in the response of Brown-headed Cowbirds compared with other species, except for WNV where the mean P/N ratio was lower than for Brewer's and Red-winged Blackbirds,

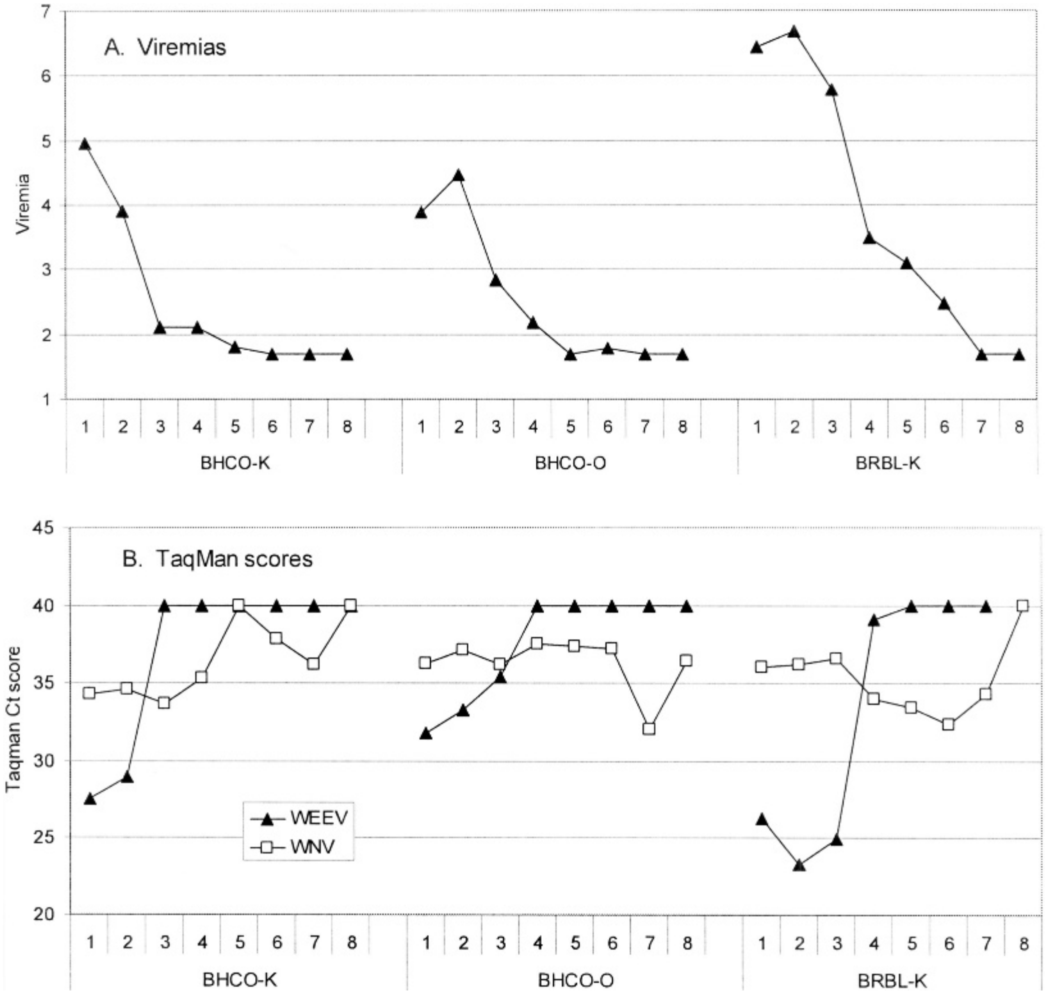

FIGURE 3. Bird species coinfected with western equine encephalomyelitis virus (WEEV) and West Nile virus (WNV). (A) Mean viremias in $\log_{10}$ plaque-forming units/ml for WEEV and WNV based on plaque assays on Vero cell culture. (B) Mean TaqMan cycle threshold (Ct) scores for each virus by using a multiplex assay. BHCO = Brown-headed Cowbird; BRBL = Brewer's Blackbird. Localities were Kern (K) and Orange Counties (O).

agreeing with the viremia data presented above. In the mixed infection experiment, the WNV antibody PRNT titers for Brown-headed Cowbirds were significantly lower than for Brewer's Blackbirds (Table 1). Interestingly, Brown-headed Cowbirds, which showed the lowest viremias, also exhibited the lowest PRNT responses, indicating that the Brown-headed Cowbird resistance to infection was not associated with an immediate and elevated antibody response.

Necropsy

Birds surviving 6 wk were euthanized, and blood, lung, spleen, and kidney tissues were tested for viral RNA by using RT-PCR. None of the birds were positive for WEEV or SLEV, and all blood specimens were negative, indicating that all birds were not viremic at necropsy. In contrast, some birds retained WNV RNA for 6 wk postinfection (Table 2), although there was no significant difference in the prevalence of persistent infection among

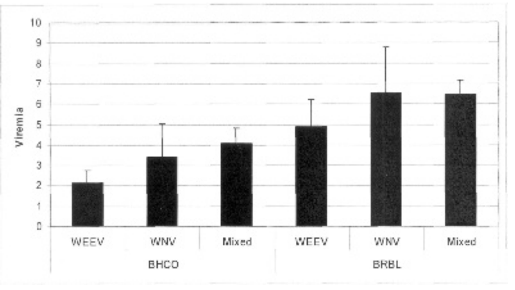

FIGURE 4. Mean viremias (+95% confidence limit) in $\log_{10}$ plaque-forming units (PFU)/mL on day 2 postinfection for bird species infected with western equine encephalomyelitis virus (WEEV), West Nile virus (WNV), or both. BHCO = Brown-headed Cowbird; BRBL = Brewer's Blackbird.

TABLE 1. Inverse of the geometric mean plaque reduction neutralization test (PRNT) titers for birds concurrently infected with West Nile virus (WNV) and western equine encephalomyelitis virus (WEEV). Brown-headed Cowbirds were from Kern and Orange counties, and Brewer's Blackbirds were from Kern County.

Virus	Brown-headed Cowbirds (Kern County) $n=4$	Brown-headed Cowbirds (Orange County) $n=10$	Brewer's Blackbirds (Kern County) $n=10$
WNV	3 (0–49)[a]	6 (2–24)	136 (82–226)
WEEV	20 (na[b])	20 (na)	12 (3–42)

[a] Geometric mean PRNT titers (95% confidence limit).
[b] na = not applicable (all titers were the same).

species (χ^2 =4.3, df=3, P=0.2). Infectious virus (3.4 $\log_{10}$ PFU/ml;) was isolated on Vero cells from the spleen of one of three Brewer's Blackbirds positive by RT-PCR after blind passage in C6/36 cells. These data indicated that the positive RT-PCR results may have indicated the persistence of infectious WNV.

DISCUSSION

Examining disease in wildlife species with contrasting life history strategies can provide insight into both virulence of the pathogen and immunity of the host. Our results showed contrasting responses among species in terms of susceptibility and immune responses. Brewer's Blackbirds were more susceptible to WEEV than Red-winged Blackbirds, whereas Red-winged Blackbirds were more susceptible to SLEV than were the Brewer's Blackbirds. The Brown-headed Cowbirds were the least susceptible of all the blackbird species, exhibiting significantly lower viremia responses after infection with all three viruses individually as well as after coinfection with both WEEV and WNV than did three other icterid species. These data indicated that Brown-headed Cowbirds were innately more resistant than the other blackbirds tested to infection from both native and introduced viruses.

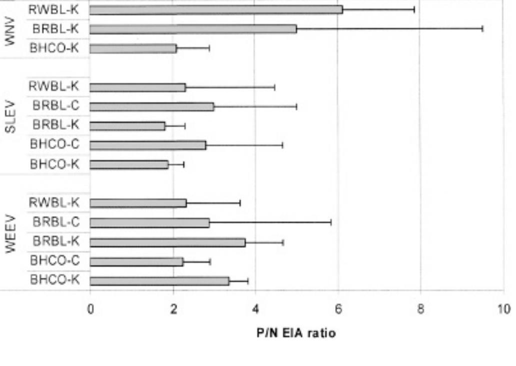

FIGURE 5. Mean antibody responses measured at week 6 postinfection for Red-winged Blackbird (RWBL), Brewer's Blackbird (RWBL), and Brown-head Cowbird (BHCO) from Kern County (K) or Coachella Valley (C). Similar mean antibody responses were noted despite between-species differences in viremia responses. Antibody measured by enzyme immunoassay (EIA) as the mean positive over negative well ratio for each bird.

In terms of immune responses to WNV, WEEV, and SLEV, the principal between-species differences were between the Brown-headed Cowbird and the nonparasitic species. The Brown-headed Cowbirds showed the lowest mean viremias, cleared their infections faster, and showed lower antibody levels than the other species. The three nonparasitic blackbirds showed a correlation between levels of viremia and antibodies, whereas there was no correlation between levels of viremia and antibodies in Brown-headed Cowbirds (Table 3).

TABLE 2. Number of each species with spleen, lung, kidney, or a combination of tissues positive for West Nile virus RNA at necropsy 6 wk postinfection.

Species	Virus	No. tested	No. WNV RNA positive[a]
Brown-headed Cowbird	WNV	9	2
	WNV-WEEV	10	1
Brewer's Blackbird	WNV	8	4
	WNV-WEEV	10	2
Red-winged Blackbird	WNV	5	3
Tricolored Blackbird	WNV	5	2

[a] One or more tissues positive for screening primer set from the envelope gene and confirmed by a second more specific primer set from the nonstructural region of the virus genome.

To put our results into context, we reviewed several comparative studies in which many avian species were experimentally infected, and all studies support our current results showing that cowbirds are exceptionally disease resistant. Reisen et al. (2003) measured the number days that viremia levels were high enough to infect mosquitoes: Brown-headed Cowbirds ranked 16th of 20 species tested for WEEV and were among eight of 22 species that did not produce a viremia in response to inoculation with SLEV. Similarly, Brown-headed Cowbird viremia for WNV was comparable with the six lowest of 25 species infected by mosquito bite (Komar et al., 2003) and with two of the lowest of seven species infected by needle inoculation (Reisen et al., 2005). Notably, all the less susceptible species were in the orders Psittaciformes (parakeets), Galliformes (quail, chickens, and pheasants), and Columbiformes (Rock Doves), orders taxonomically distant from Passeriformes (songbirds), the order in which Brown-headed Cowbirds are placed. Brown-headed Cowbirds consistently were the least susceptible of the Passeriformes tested.

In evaluating the between-species dif-

TABLE 3. Summary of the response of four icterid species to infection with western equine encephalitis virus (WEEV), eastern equine encephalitis virus (EEV), St. Louis encephalitis virus (SLEV), and West Nile virus (WNV).

	Virus								
	WEEV[d]			SLEV[d]			WNV		
Species	Peak viremia during 1–2 dpi[a]	Time to clearance (days)	Antibody response[b]	Peak viremia during 2–4 dpi[a]	Time to clearance (days)	Antibody response[b]	Peak viremia during 1–4 dpi[a]	Time to clearance (days)	Antibody response[b]
Brown-headed Cowbird	2.3	3	2.8	<1.7	0	2.4	3.1	5	2.1
Brewer's Blackbird	4.9	4	3.3	2.5	3	2.4	4.7	7	5
Red-winged Blackbird	3.5	3	2.3	4.5	>5	2.3	5.9	6	6.1
Tricolored Blackbird	nd[c]			nd[c]			5.6	>6	4.8

[a] Log_{10} plaque-forming units/ml; dpi = days postinfection.

[b] Mean immunoglobulin G enzyme immunoassay ratio of the mean optical density of two positive wells over a negative control well (P/N ratio) on week 6 postinfection.

[c] nd = not done.

[d] Reanalyzed from Reisen et al. (2003).

ferences observed in our current study, we considered the potential effects of geographic range, breeding system, and mating system, because these factors affect the historic exposure patterns that lead to the unique portfolio of pathogen defense components that each species evolves (Schmid-Hempel, 2003). Read (2001) examined the role of mating systems and showed that there is a decreased level of blood parasites in passerine species having polygamous versus monogamous mating systems, but the species we studied were predominantly polygamous (Orians, 1980; Ehrlich et al., 1988; Searcy and Yasukawa, 1995). Nor do the differences in disease resistance shown in our results correspond to differences in the species' geographic ranges, because Brown-headed Cowbirds, Red-winged Blackbirds, and Brewer's Blackbirds all have similar, unusually large ranges (Sibley, 2000; Sauer et al., 2005), but they showed significant differences in susceptibility and immune responses to the arboviruses tested. Tricolored Blackbirds had a distinctly smaller range compared with the other blackbirds (Sibley, 2000; Sauer et al., 2005), but they did not differ in infection results from Red-winged Blackbirds and Brewer's Blackbirds. The principal differences in infection among the blackbird species tested corresponded to the difference in parasitic versus nonparasitic breeding systems, the principal factor differentiating the Brown-headed Cowbird from its nonparasitic relatives.

A growing literature in evolutionary ecology discusses the power of parasite-mediated selection on the evolution of immune responses and disease resistance (Moller, 1997; Sheldon and Verhulst, 1996; Schmid-Hempel, 2003; Schmid-Hempel and Ebert, 2003), and the Brown-headed Cowbird offers an opportunity to determine whether a life history strategy that incurs increased exposure to parasites leads to the evolution of enhanced disease resistance. Due to the Brown-headed Cowbird's breeding system, it regularly comes into intimate physical contact with the songbird species it parasitizes, a phenomenon that nonparasitic birds rarely experience. Female cowbirds settle into other birds' nests while laying their parasitic eggs, and the resulting nestlings are brooded by their host parents' kin for a few days and then fed by mouth for several weeks until fledging (Johnsgard, 1997; Ortega, 1998; Davies, 2000). As a result of their parasitic lifestyle, cowbirds experience higher infection levels with host birds' pathogens. For example, Hahn et al. (2000) and Hahn and Price (2001) showed that cowbirds experience infestation with a significantly greater diversity of louse species than do other songbirds. Recently fledged cowbirds were infested with a diversity of louse species that corresponded to the diversity of louse species found on the songbird parent species in the local community; adult cowbirds also had higher diversity of louse ectoparasites than nonparasitic blackbirds and other songbirds (Hahn et al., 2000; Price et al., 2004). A broad review of ectoparasite infestations concluded that ectoparasites have a direct negative effect on the health and fitness of their hosts (Lehmann, 1993); so, the increased exposure of Brown-headed Cowbird to infectious organisms from their parenting species could affect the evolution of a robust immune system and innate disease resistance. Further investigation of the immune system of the Brown-headed Cowbird may be a fruitful area of study for the genetic basis of immune defenses in songbirds.

The public health priority to identify avian species serving as reservoirs for WNV was the original impetus for our investigating the response of the Brown-headed Cowbird to infection. Based on our hypothesis that the Brown-headed Cowbird is unusually disease resistant, we were concerned that if the Brown-headed Cowbird tolerated a high viremia, then it could be a significant factor in the transmission and local spread of WNV, given its broad ecological niche. This species is very abundant (Sauer et al., 2005), found in an extremely wide range of ecological habitats (Hahn and O'Connor, 2001), and commutes long distances among varied landscapes from breeding habitats and feeding grounds in agricultural and suburban habitats (Thompson, 1994; Hahn et al., 1999; Curson et al., 2000). However, our results showed that the Brown-headed Cowbird generally maintains a low viremia, which means this species most likely will not be able to pass its infection on to vector mosquitoes and therefore does not pose a concern for public health in the spread of WNV.

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