Serosurveillance for Japanese Encephalitis and West Nile Viruses in Resident Birds in Hawai‘i

Nicole M. Nemeth,1,4 Angela M. Bosco-Lauth,1 Rebecca H. Sciulli,2 Remedios B. Gose,2 Mark T. Nagata,2 and Richard A. Bowen3

1 Department of Microbiology, Immunology and Pathology, Colorado State University, 3801 W. Rampart Road, Fort Collins, Colorado 80523, USA; 2 Bioterrorism Response Laboratory, Hawai‘i Department of Health, State Laboratories Division, 2725 Waimano Home Road, Pearl City, Hawai‘i 96782, USA; 3 Department of Biomedical Sciences, Colorado State University, 3801 W. Rampart Road, Fort Collins, Colorado 80523, USA; 4 Corresponding author (email: nnemeth@colostate.edu)

ABSTRACT: Japanese encephalitis virus (JEV) and West Nile virus (WNV) are emerging zoonotic arboviruses that have recently undergone intercontinental expansion. Both JEV and WNV are naturally transmitted between mosquito vectors and vertebrate reservoir hosts, including birds. A potential route of JEV introduction from Asia to western North America is via the Hawaiian archipelago, while the spread of WNV from mainland North America to Hawai‘i is also considered an impending threat. We surveyed resident, non-native bird sera for antibodies to JEV and WNV on two Hawaiian Islands from 2004–2005. Three of 1,835 birds (0.16%) had evidence of antiflavivirus antibodies, demonstrating neutralizing activity to JEV and St. Louis encephalitis virus (SLEV). These detections could represent a limited transmission focus of either, or both, JEV and SLEV, or cross-reactive antibodies due to primary infection with an alternate flavivirus. Frequent air traffic from both Asia and North America to Hawai‘i, along with the presence of competent vectors and amplifying vertebrate hosts in Hawai‘i, increases the likelihood of introduction and maintenance of novel flaviviruses. Therefore, it is important to monitor for the presence of these viruses.

Key words: Bird, Hawai‘i, Japanese encephalitis virus, seroprevalence, St. Louis encephalitis virus, West Nile virus.

Hawai‘i and other remote islands are particularly vulnerable to the introduction of novel wildlife pathogens because of immunologically naïve vertebrate host populations. Peridomestic mosquitoes were introduced to Hawai‘i with the arrival of European human settlers and, as a result, devastating mosquito-borne pathogens (e.g., Plasmodium relictum [avian malaria] and Avipoxvirus [avian pox]) decimated native avian populations (LaPointe, 2007). Japanese encephalitis virus and West Nile virus (JEV and WNV, respectively; family Flaviviridae, genus Flavivirus) are globally emerging mosquito-borne viruses that cause fatal encephalitis in humans and other vertebrates and could pose a significant threat to the state of Hawai‘i (Kilpatrick et al., 2004; Mackenzie et al., 2004). Similar to WNV, JEV is transmitted primarily by mosquitoes, with birds as amplifying hosts. It is thought that WNV may have arrived in the United States via an infected mosquito or viremic vertebrate host (Gubler, 2007); similarly, these mechanisms have likely contributed to the geographic spread of JEV (Mackenzie et al., 2004).

Because of the risk of introduction of novel arboviruses to Hawai‘i, we screened free-ranging, resident birds for serologic evidence of past infection with several arboviruses within the Japanese encephalitis serocomplex including JEV, WNV, and St. Louis encephalitis virus (SLEV). Our objective was to determine the serum antibody prevalence of WNV and JEV among birds captured primarily at sites that receive frequent air traffic. We hypothesized that a local transmission focus could initiate if one of these viruses arrived via a viremic human or other vertebrate, or via an infected mosquito associated with air travel. Serologic evidence of flavivirus infection among resident birds may be suggestive of local transmission and would help establish baseline antibody prevalence for future research and surveillance efforts.

Resident birds were mist-netted year-round from June 2004–December 2005 at various sites on the islands of Oahu...
(Barber’s Point, Dillingham Airfield, Hickam Airfield, Honolulu Airport, Kalaeloa, and Kewalo; approximately 21° N, 157°–158° W) and Maui (Kahului Airport; 20°54’ N, 156°26’ W). Blood was collected by venipuncture of the jugular vein (approximately 0.1–0.2 ml) and either left undiluted or diluted 1:10 in phosphate buffered saline with 0.75% bovine serum albumin. Blood samples were centrifuged at 10,000 × G for 3 min, and sera were aliquotted into separate vials, heat inactivated at 56°C for 30 min, and stored at −20 or −80°C.

Sera were screened at the Hawai’i Department of Health Laboratory (HDHL), in Pearl City, Hawai’i, for anti-WNV antibodies by a blocking enzyme-linked immunosorbent assay (ELISA) targeting an immunodominant WNV epitope on the NS1 protein. Prior to establishing the ELISA as part of the WNV testing algorithm at the HDHL in February 2005, optimal titers for both antigen and antibody were determined by checkerboard titration. Sera were tested in triplicate at a 1:20 dilution for initial screening, with suspected positives (inhibition ≥50%) also tested at further dilutions (e.g., 1:40 and 1:80). Beginning in February 2005, a blocking ELISA protocol was established according to Hall et al. (1995), using inactivated Kunjin lysate antigen and WNV-specific monoclonal antibodies, with modifications for detection of anti-WNV antibodies as described in Jozan et al. (2003). All ELISA positives were considered presumptive until confirmatory testing by plaque reduction neutralization test (PRNT).

Samples were shipped overnight on ice to Colorado State University, Fort Collins, Colorado, where they were screened for anti-JEV antibodies by PRNT (Beaty et al., 1995). For PRNT, sera were tested at a 1:10 dilution in BA-1 medium (Hank’s M-199 salts, 1% bovine serum albumin, 350 mg/l sodium bicarbonate, 100 units/ml penicillin, 100 mg/l streptomycin, 1 mg/l Fungizone in 0.05 M Tris, pH 7.6) against a challenge dose of approximately 100 plaque-forming units of JEV strain 826309 (isolated from human brain in India). Serum samples that neutralized ≤70% of JEV were considered negative for anti-JEV antibodies. Samples with >70% neutralization of JEV were retested through a second JEV PRNT for confirmation of the initial result, and were also screened by PRNT at a 1:10 dilution for antibodies to WNV (using strain NY99-4132, isolated from crow brain in New York, USA) and SLEV (using strain TBH-28, isolated from human brain in Florida, USA) with the same criteria of >70% neutralization for a positive result. This 70% neutralization cut-off was selected to reasonably distinguish antiflavivirus antibody reactivity from clearly seronegative (i.e., <50% neutralization) samples.

A total of 1,835 birds of 11 species were sampled, most of which were nonnative residents of Hawai’i (Table 1). Serum samples from all birds included in the present study were negative for anti-WNV antibodies as determined by blocking ELISA, along with an additional >10,000 avian serum samples collected in Hawai’i from January 2006–October 2009. Sera from three birds (0.16%) exhibited flavivirus neutralizing activity with between 71–94% neutralization of JEV or SLEV. Sera from these three birds were clearly negative for antibodies to WNV by ELISA (<0% inhibition) and by PRNT (<50% neutralization). Sera with JEV- or SLEV-neutralizing activity were collected between June and September 2005 from a Spotted Dove (Streptopelia chinensis) and a Java Sparrow (Padda oryzivora) at the Honolulu International Airport and a Spotted Dove at Dillingham Airfield. In two of these birds (Spotted Dove and Java Sparrow), apparent cross-reactive antiflavivirus antibodies made it difficult to differentiate antibodies specific to JEV versus SLEV (Table 2).

Japanese encephalitis virus has been deemed the most important cause of
epidemic encephalitis worldwide and is the leading recognized cause of childhood encephalitis in Asia (Tsai, 1997; Solomon, 2003). Likewise, WNV is an important cause of arboviral disease in the United States and, since its arrival to New York in 1999, has led to neuro-invasive disease in >11,000 humans and the death of likely millions of birds (Reimann et al., 2008). Because transmission of these viruses involves primarily wild birds and mosquitoes, as well as pigs for JEV, they are difficult to control and eradicate once established. Therefore, ongoing monitoring for these viruses is important, especially in Hawai’i, where the climate favors year-round arbovirus transmission and, presumably, competent (and naïve) vectors and hosts are in abundance.

Hawai’i has been recognized as a potential location for JEV emergence that could facilitate spread from Asia to western North America (Quisenberry and Wallace, 1959). The possibility of introduction of SLEV, WNV, JEV, or other zoonotic viruses to Hawai’i via viremic captive birds or infected mosquitoes is an important consideration in quarantine and other control measures. Accordingly, WNV surveillance has been ongoing in Hawai’i since November 2004, and embargoes and quarantines have been implemented to mitigate the threat of WNV introduction (Marra et al., 2004). Quantitative analysis suggested that the most likely source for the introduction of WNV to Hawai’i is a human-transported infected mosquito, or an infectious bird or other vertebrate host (Kilpatrick et al., 2004). Similarly, JEV introduction to the West coast of the United States was deemed most likely to occur via an infected

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Status</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Java Sparrow</td>
<td>Padda oryzivora</td>
<td>Resident</td>
<td>807</td>
</tr>
<tr>
<td>Spotted Dove</td>
<td>Streptopelia chinensis</td>
<td>Resident</td>
<td>517</td>
</tr>
<tr>
<td>House Finch</td>
<td>Carpodacus mexicanus</td>
<td>Resident</td>
<td>255</td>
</tr>
<tr>
<td>House Sparrow</td>
<td>Passer domesticus</td>
<td>Resident</td>
<td>86</td>
</tr>
<tr>
<td>Red-crested Cardinal</td>
<td>Paroaria coronata</td>
<td>Resident</td>
<td>56</td>
</tr>
<tr>
<td>Zebra Dove</td>
<td>Geopelia striata</td>
<td>Resident</td>
<td>53</td>
</tr>
<tr>
<td>Northern Cardinal</td>
<td>Cardinalis cardinalis</td>
<td>Resident</td>
<td>32</td>
</tr>
<tr>
<td>Common Myna</td>
<td>Acridotheres tristis</td>
<td>Resident</td>
<td>21</td>
</tr>
<tr>
<td>Saffron Finch</td>
<td>Sicalis flaveola</td>
<td>Resident</td>
<td>6</td>
</tr>
<tr>
<td>Francolin</td>
<td>Francolinus sp.</td>
<td>Resident</td>
<td>1</td>
</tr>
<tr>
<td>Pacific Golden Plover</td>
<td>Pluvialis fulica</td>
<td>Migrant</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1,835</td>
</tr>
</tbody>
</table>

### Table 1. Avian species and numbers tested for antibodies to West Nile and Japanese encephalitis viruses in Hawai’i, 2004–2005.

### Table 2. Birds with neutralizing antibodies to Japanese encephalitis and St. Louis encephalitis viruses (JEV and SLEV, respectively) in Hawai’i, 2004–2005.

<table>
<thead>
<tr>
<th>Species (age)</th>
<th>Date blood collected</th>
<th>JEV</th>
<th>SLEV</th>
<th>WNV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spotted Dove (immature)</td>
<td>1 June 2005</td>
<td>74%</td>
<td>&lt;50%</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>Spotted Dove (adult)</td>
<td>30 August 2005</td>
<td>77%</td>
<td>80%</td>
<td>&lt;50%</td>
</tr>
<tr>
<td>Java Sparrow (adult)</td>
<td>20 September 2005</td>
<td>94%</td>
<td>71%</td>
<td>&lt;50%</td>
</tr>
</tbody>
</table>

*a Sera with >70% neutralization of the respective virus were considered antibody positive.

*b All three birds were negative for antibodies to West Nile virus (WNV) by ELISA and PRNT.
mosquito arriving by air or marine transport, via a viremic bird by importation or migration, or via a viremic human traveler; the latter two could serve as a source of infection for blood-feeding mosquitoes (Nett et al., 2008).

While local transmission could initiate following JEV or WNV introduction to Hawai‘i, maintenance or amplification of transmission could subsequently fail due to lack of immediate and sufficient numbers of competent vectors or hosts. Alternately, flaviviruses that are yet to be detected or identified could occasionally infect birds. For example, “insect-only” flaviviruses have recently been identified in Africa, Asia, and the Americas (reviewed recently in Cook et al., 2009) and could be immunogenic in some birds. Documented viruses such as Murray Valley encephalitis virus, also a member of the JEV serocomplex that circulates between mosquito vectors and avian hosts in Australia, Papua New Guinea, and Irian Java, may also be capable of unexpected spread (Coelen and Mackenzie, 1988). The lack of 100% virus neutralization by sera from flavivirus-positive birds in the present study may suggest a primary infecting virus other than WNV, SLEV, or JEV. We detected a minimal percentage (<0.2%) of birds with flavivirus-neutralizing antibodies in Hawai‘i from 2004–2005. Low prevalence of antibodies to arboviruses among birds in Hawai‘i, as well as difficulty distinguishing the infective virus, is consistent with previously reported data. In 1959, surveillance for JEV, SLEV, and Eastern and Western equine encephalitis viruses on Oahu revealed five birds (0.66%; n = 754), all residents, with evidence of nonspecific virus neutralization (Wallace et al., 1964). In contrast, WNV antibody prevalence among some avian species has been 10–25% in the southern mainland United States, several years or more after the introduction of WNV (e.g., Louisiana in 2002 [Komar et al., 2005], Georgia in 2004 [Gibbs et al., 2006]).

Native Hawaiian birds are potentially vulnerable to disease associated with infection with novel arboviruses (Warner, 1968; Van Riper et al., 1986; LaPointe et al., 2009). However, introduced birds thrive on the Islands, and many of these are likely competent reservoir hosts of WNV and SLEV, such as the House Sparrow (Passer domesticus) and House Finch (Carpodacus mexicanus), as well as the native Hawai‘i ‘Amakihi (Hemignathus virens; McLean et al., 1983; Reisen et al., 2001; Komar et al., 2003; LaPointe et al., 2009). Potential JEV reservoirs are also present in Hawai‘i, such as the resident Black-crowned Night Heron (Nycticorax nycticorax hoactli) and introduced Cattle Egret (Bubulcus ibis), as well as feral swine (Sus scrofa) (Buescher et al., 1959; Mayer and Brisbin, 1991; Mackenzie et al., 2004; Marra et al., 2004; LaPointe, 2007). In addition, potentially competent mosquito vectors are found in Hawai‘i including Aedes albopictus, Ochlerotatus japonicus, and Culex quinquefasciatus (Reeves and Hammon, 1946; Quisenberry and Wallace, 1959; Joyce, 1961; Larish and Savage, 2005; LaPointe, 2007; LaPointe et al., 2009). Further, Cx. annulirostris and Cx. tritaeniorhynchus have been intercepted on numerous occasions from aircraft arriving in Honolulu (Joyce, 1961). Finally, unlike some birds in the mainland United States in which cross-protective anti-WNV antibodies may inhibit JEV amplification in blood (Nemeth et al., 2009), Hawaiian birds are likely immunologically naive and, therefore, more susceptible to adverse effects of flavivirus infection. The effects of JEV infection in birds remain unknown.

Recent flavivirus dispersal includes WNV spread from the Middle East to the United States and JEV from Southeast Asia to Australia. While the mechanism of spread remains unknown, these invasions signify the perpetual threat of pathogen emergence. In addition to global travel, human movement into previously uninhabited areas and associated changes in...
land and water use may facilitate pathogen emergence and establishment (Mackenzie et al., 2004; Marra et al., 2004). While the geographic spread of highly pathogenic Asian H5N1 avian influenza virus is closely monitored on a global scale, JEV and other arboviruses merit worldwide attention as significant zoonotic pathogens. The possibility of rapid and wide-ranging pathogen spread is greater than ever, and surveillance for novel invasions, especially involving wildlife, may provide an early warning and lead to mitigation of epidemic threats.

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