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ORIGINAL ARTICLE

Viremia profiles and host competence index for West Nile virus (*Flavivirus*, Flaviviridae) in three autochthonous birds species from Argentina

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Abstract West Nile virus (WNV) is an emerging *Flavivirus* dispersing throughout the American continent. It has emerged in the United States as an important medical and veterinary pathogen. It was introduced into Argentina late in 2004 with reported activity in human, wild birds and equines. Field evidence supports the hypothesis of an enzootic transmission cycle between free ranging birds and mosquitoes. The aim of this research was to analyze the role of autochthonous birds as maintenance hosts. Bay-Winged Cowbirds, Picui Ground Doves and Shiny Cowbirds were subcutaneously inoculated with an Argentinean isolate of WNV. Bay-Winged and Shiny Cowbirds developed relatively low mean peak viremias ($10^{2.7}$ and $10^{3.5}$ PFU/mL serum, respectively). Picui Ground Doves had the highest peak viremia titers of the longest duration [$10^{4.8}$ log PFU/mL serum (range $10^{2.9–6.2}$; 4–5 days duration)]. No mortality was observed during the study. The reservoir competence index for each species suggests that an infected Picui Ground Dove leads to ten times more infectious mosquitoes than one infected Shiny Cowbird, and that Bay-Winged Cowbird do not represent a source of infectious virus for mosquito vectors. This is the first study carried out in the region regarding the avian host of WNV in Argentina. However, additional studies, including seroprevalence and reservoir competence of resident birds as well as vector competence, are needed to shed light on the ecology of this pathogen in Argentina.

Keywords West Nile virus · Avian host · *Agelaioides badius* · *Columbina picui* · *Molothrus bonariensis*

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Introduction

West Nile virus (WNV; genus *Flavivirus*; family Flaviviridae) was detected in the western hemisphere in 1999 (Nash et al. 2001). By 2004, serological studies detected WNV-specific antibodies in birds and horses from Canada to northern South America (Komar and Clark 2006; Morales-Betoulle et al. 2006; Bosch et al. 2007). The first report of WNV activity in the southern cone of South America emerged in April 2006, when three horses died in Argentina (Morales et al. 2006). However, established transmission foci in Argentina were detected by Diaz et al. (2008a), as evidenced by resident birds that tested WNV positive. Based on serological data, these authors also pointed out that WNV had been introduced to Argentina by 2004 (Diaz et al. 2008a).

WNV is maintained primarily by a bird–mosquito–bird transmission cycle. Urban and rural viral activity has been detected throughout the United States (Komar et al. 2003). The mosquito species that serve as primary vectors of WNV vary by geographic distribution; for example, *Culex pipiens* and *Cx. restuans* are primary vectors in northeast and north-central US (Kilpatrick et al. 2005), *Cx. quinquefasciatus* in the southern US, and *Cx. tarsalis* in the western US (Reisen et al. 2004). Passeriformes birds are the main amplifying competent host, including Blue Jay (*Cyanocitta cristata*), American Crow (*Corvus brachyrhynchos*), Black-billed Magpie (*Pica hudsonia*), Common Grackle (*Quiscalus quiscula*), House Finch (*Carpodacus mexicanus*) and House Sparrow (*Passer domesticus*) (Kilpatrick et al. 2007).

The ecology of WNV in Argentina is completely unknown due to its recent introduction and absence of research regarding available competent vectors and hosts. This work was carried out with the aim of analyzing the potential role of three indigenous bird species as amplifying hosts: Bay-Winged Cowbird (*Agelaioides badius*), Picui Ground Dove (*Columbina picui*) and Shiny Cowbird (*Molothrus bonariensis*), all collected in a central region of Argentina.

Materials and methods

Bird collection and husbandry

Avian species selected for study was based on previous detection of evidence of WNV infection in nature (Diaz et al. 2008a; L.A.D. unpublished data), abundance in urban/periurban areas, and ease of maintenance in captivity. Bay-Winged Cowbird (*Agelaioides badius*, Family Icteridae, Orden Passeriformes), Picui Ground-Dove (*Columbina picui*, Family Columbidae, Orden Columbiformes) and Shiny Cowbird (*Molothrus* bonariensis, Family Icteridae, Orden Passeriformes) were collected in November 2008 at a rural site 10 km away from La Para city (Province of Córdoba, Argentina) using mist nets. This collection site was selected to represent an area of known endemic WNV activity (Diaz et al. 2008a). The respective authorization was obtained from Wildlife Division—Agencia Cordoba Ambiente, Córdoba, Argentina. Birds were maintained at the Virology Institute biosafety facilities under semi-natural conditions (photoperiod and temperature depended upon environmental conditions), and were fed mixed grains ad libitum. After collection, birds were bled and banded; 200 µL blood was removed from each bird, and blood was stored at room temperature for 30 min to coagulate, and then centrifuged for separation of serum from packed cells. The sera were analyzed by plaque reduction neutralization test (PRNT) for antibodies against WNV and St. Louis encephalitis virus (SLEV). Only seronegative birds were used for the experiment.

Viral strain

Birds were experimentally infected with WNV strain E/7229/06 isolated from a dead horse in the Province of Buenos Aires (Argentina) (Morales et al. 2006) and birds were housed at the Virology Institute at the Universidad Nacional de Córdoba. The viral suspension was prepared from infected suckling-mouse brain diluted 10% in minimum essential medium (MEM) with Earle's salts and L-glutamine, 10% fetal calf serum (FCS) and 1% gentamicin centrifuged at 11,400 *g* at 4°C for 30 min. The viral suspension was titered by Vero cell plaque assay and 100 µL aliquots were stored at −80°C. The viral titer was expressed as plaque-forming units per milliliter of serum (PFU/mL) (Diaz et al. 2008b).

Inoculation, sampling, and virus isolation

Birds were subcutaneously inoculated in the abdominal region with approximately 300 PFU WNV diluted in 0.1 mL MEM supplemented with 10% fetal bovine serum (FBS). Following inoculation, birds were observed every 12 h to detect any clinical signs of illness. Birds were bled (200 µL) daily for 7 days via jugular or brachial venipuncture. Whole blood was diluted in 0.9 mL of refrigerated phosphate buffer solution (PBS) with 10% FCS and 1% gentamicin to avoid bacterial contamination, centrifuged at 1,500 *g* for 15 min, and the supernatant stored at −80°C. Viremia titers were determined by plaque assay on Vero cells and expressed as PFU/mL with a detection threshold of 10^2 PFU/mL (Diaz et al. 2008b).

Serology

To verify seroconversion to WNV in inoculated birds, all survivors were bled at 14 days post-inoculation (dpi). Blood was allowed to coagulate at room temperature for 30 min, followed by centrifugation to separate the serum. Samples were stored at −20°C, and heat inactivated at 56°C for 30 min prior to testing. For PRNT, sera were diluted 1:10 in MEM, and endpoint antibody titers were determined using serial twofold dilutions (Diaz et al. 2008b).

Host competence index

In order to quantify and compare the role as host for WNV of each bird species tested in the assay, the host competence index (C_i) was calculated. Host competence index is a term that describes the infectiousness of an infected host and provides an estimate of the number of infectious mosquitoes generated for each individual of a given species per day (Komar et al. 2003).

One of the main parameters included in the C_i is "i" (mean daily infectiousness: the proportion of exposed vectors that become infectious per day). Since there was no data available at the moment for indigenous *Culex* mosquitoes in Argentina, we used derived data obtained for *Culex* mosquitoes in the US (Kilpatrick et al. 2005): $i = 0.1349 \times \text{Log10 (Viremia)} - 0.6235$.

Results

Inoculated birds included two Shiny Cowbirds, three Bay Winged Cowbirds and seven Picui Ground Doves. Both inoculated Shiny Cowbirds developed peak viremia ranging from $10^{2.9}$ to $10^{4.9}$ PFU/mL serum (Fig. 1); viremia was detectable from 1 to 2 dpi. Two out of three inoculated Bay-Winged Cowbirds developed viremia ($10^{2.7}$ PFU/mL) limited to 2 dpi (Fig. 1). Picui Ground Doves developed viremia of the highest titers and longest duration. All seven inoculated individuals developed peak viremia between $10^{3.0}$ and $10^{6.2}$ PFU/mL serum (mean peak viremia $10^{4.8}$ PFU/mL) from 1 to 5 dpi inclusive (Fig. 1). The mean duration of peak viremia was 4.3 days (from 3 to 5 days). No mortality occurred during the study. All birds had neutralizing antibodies to WNV by 14 dpi (titers = 1/40), confirming that they had been infected with WNV.

The Picui Ground Dove had the highest C_i (Table 1), while Bay-Winged Cowbirds had a C_i of zero.

Discussion

In general, viremia titers results were consistent with previous studies in the US for related species (e.g., Common Ground Dove—*Columbina passerine*, Brown-headed Cowbird—*Molothrus ater*, Redwinged Blackbird—*Agelaius phoeniceus*, and Tricolored Blackbird—*Agelaius tricolor*) (viremia titers ranged $10^{2.0–5.0}$ PFU/mL serum) (Reisen et al. 2005; Reisen and Hahn 2007). However, differences in the duration of detectable viremia were observed between Argentinean and US cowbirds. For example, Bay-Winged and Shiny Cowbirds developed detectable viremia titers on 1 and 2 dpi, respectively, while Brown-headed Cowbirds developed viremia of 5 days duration (Reisen and Hahn 2007). Our results suggest that these species are not susceptible to WNV-associated morbidity and mortality, as none showed any clinical signs of illness during the study. Therefore, the WNV strains used appears to be avirulent in the species tested.

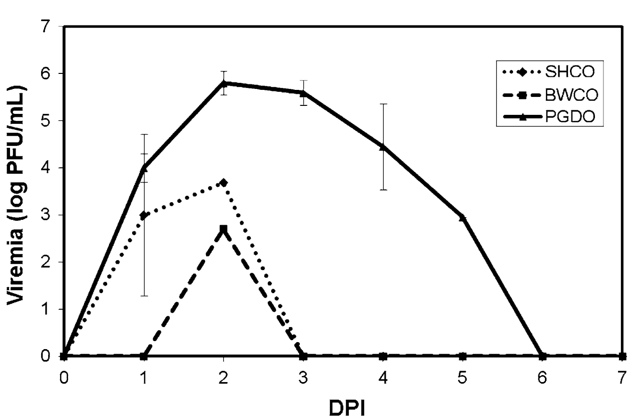

Fig. 1 West Nile virus (WNV) viremia detected in three resident bird species as a function of time. *BWCO* Bay-Winged Cowbird, *PGDO* Picui Ground Dove, *SHCO Shiny Cowbird*, *DPI* days post-inoculation

West Nile virus viremia profiles among birds in the present study were similar to those obtained for SLEV in the same species (Diaz 2009). In contrast, WNV and SLEV viremia profiles differed from each other in related species in California in the US (i.e., Common Ground Dove, Brown-headed Cowbirds, Redwinged Blackbirds, Tricolored Blackbirds) (Reisen et al. 2005).

Host competence is a term that describes the infectiousness of an infected host and provides an estimate of the number of infectious mosquitoes generated for each individual of a given species per day (Komar et al. 2003). Hence, hosts that develop long viremic periods and high-titered viremias are highly competent. These calculations predict that a Picui Ground Dove would promote infection of approximately ten times more infectious mosquitoes than an infected Shiny Cowbird, and that Bay-Winged Cowbirds do not represent a viable source for virus transmission.

Unfortunately, vector competence of indigenous Argentinean mosquitoes and the threshold WNV dose needed for infection in birds is unknown, so the C_i provided in the present study may be mis-estimated (Kilpatrick et al. 2005).

The viral strain used to inoculate birds in the present study was isolated along with an additional strain from a horse in 2006 during small epizootics in the province of Buenos Aires (Morales et al. 2006). When these WNV strains were molecularly characterized, phylogenetic analyses showed a high relatedness to the NY99 strain (Fabbri et al. 2008), which is the virulent WNV genotype associated with avian, equine and human mortality events in the US. However, no large scale avian or equine mortality has been observed after the isolation of WNV in Argentina.

Ecological differences among avian hosts for other flaviviruses (e.g., SLEV) in the US and Argentina were previously pointed out by Sabattini et al. (1998) and Diaz et al. (2008b). Therefore, is not surprising that WNV should have a different eco-epidemiological profile in Argentina versus the US.

The eco-epidemiology of WNV in South America, and particularly in Argentina, is completely unknown. Previous data indicate that the virus maintains itself in enzootic fashion by resident birds in temperate and subtropical regions in Argentina (Diaz et al. 2008a), but the role of birds

Table 1 West Nile virus (WNV) host competence index (C_i) values for three autochthonous bird species in Córdoba, Argentina

Species	Susceptibility (s)[a]	Infectiousness (i)	Duration (d)[b]	C_i[c]
Bay-Winged Cowbird (*Agelaioides badius*)	1	0	1	0
Shiny Cowbird (*Molothrus bonariensis*)	1	0.04	1.5	0.06
Picui Ground Dove (*Columbina picui*)	1	0.13	4.3	0.56

[a] Ratio of infected and/or viremic individuals/inoculated individuals

[b] Viremia mean duration in days

[c] $C_i = s \times i \times d$. $i =$ infectiousness for a given bird species

as WNV amplifying hosts in Argentina remain unknown. This study is the first to be carried out regarding the avian host of WNV in Argentina. At the moment, evidence indicates that the Picui Ground Dove acts as an amplifying host for WNV in Argentina. However, additional studies, including more resident bird species (*Furnarius rufus*, *Pseudoseisura lophotes*, *Turdus amaurochalinus*), as well as vector competence are needed to shed light on the ecology of this *flavivirus* in Argentina.

Zusammenfassung

Virämie Profile und Wirtskompetenz Index für West-Nil Virus (WNV) (Flavivirus, Flaviviridae) in drei einheimischen argentinischen Vogelarten

Der West-Nil Virus (WNV) ist ein Flavivirus, der im Begriff ist, sich über den gesamten amerikanischen Kontinent ausbreitet. In den USA hat er sich zu einem bedeutenden medizinischen und tiermedizinischen Krankheitserreger entwickelt. Ende 2004 kam der WNV nach Argentinien, wo er schon in Menschen, wilden Vögeln und Pferden nachgewiesen wurde. In Feldversuchen wurde festgestellt, dass die Viren in einem enzootischen Zyklus zwischen wildlebenden Vögeln und Stechmücken übertragen werden. Das Ziel dieser Studie war, die Rolle der einheimischen Vögel als Zwischenwirte zu klären. Braunkuhstärlinge, Picuitäubchen und Glanzkuhstärlinge wurden subkutan mit einem argentinischen Isolat von WNV inokuliert. Braunkuhstärlinge und Glanzkuhstärlinge entwickelten schwache Virämie (102.7 bzw. 103.5 PFU/mL Serum). Picuitäubchen hatten die höchsten Titer-Werte (104.8 log PFU/mL Serum (Spannweite 102.9-6.2; Dauer: 4–5 Tage)). In keinem der Fälle verlief die Krankheit tödlich. Der Reservoir Kompetenz Index (das Produkt aus Ansteckungsfähigkeit und Dauer der Virämie) für die einzelnen Arten zeigt, dass ein infiziertes Picuitäubchen zehnmal mehr ansteckende Stechmücken hervorbringen würde, als ein kranker Glanzkuhstärling und dass Braunkuhstärlinge keine Überträger von WNV sind. Dies ist die erste Studie aus dieser Region, die Vögel als Überträger von WNV in Argentinien untersuchte. Weitere Studien zur Seroprävalenz und Reservoir Kompetenz der ortsansässigen Vögel und Studien zur Vektorkompetenz sind notwendig, um die Ökologie dieses Erregers in Argentinien besser zu verstehen.

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Conflict of interest statement The authors declare no conflict of interest related to this article.

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