

Research Paper

West Nile Virus Survey of Birds and Mosquitoes in the Dominican Republic

OLIVER KOMAR,^{1,5} MARK B. ROBBINS,¹ GAVINO GUZMÁN CONTRERAS,²
BRETT W. BENZ,¹ KACI KLENK,^{3,6} BRADLEY J. BLITVICH,⁴ NICOLE L. MARLENEE,⁴
KRISTEN L. BURKHALTER,³ SUSAN BECKETT,³ GUILLERMO GONZÁLVEZ,²
CARLOS J. PEÑA,² A. TOWNSEND PETERSON,¹ and NICHOLAS KOMAR³

ABSTRACT

We report West Nile virus (WNV) activity from a new area on Hispaniola, in the vicinity of Monte Cristi National Park in northwest Dominican Republic. Specific anti-WNV antibodies were detected in 12 of 58 (21%) resident birds sampled in March 2003, representing six species in the orders Cuculiformes (cuckoos), Strigiformes (owls), and Passeriformes (song birds). This seroprevalence is the highest reported from any site in the Caribbean Basin. Virus was not detected in any mosquitoes or tissues from bird specimens. Testing of 20 sick or dead birds was negative for WNV. Undetermined flavivirus antibodies were detected in four resident birds at Monte Cristi, as well as in five resident birds at Sierra de Baoruco National Park in southwest Dominican Republic. These data suggest that an unidentified flavivirus, as well as WNV, is active in the Dominican Republic. **Key Words:** West Nile virus—*Flavivirus*—Caribbean Basin—West Indies—Dominican Republic—Haiti—Birds—Epidemiology. *Vector-Borne Zoonotic Dis.* 5, 120–126.

INTRODUCTION

WEST NILE VIRUS (WNV; family *Flaviviridae*, genus *Flavivirus*) has been present in the Caribbean Basin region since at least 2001 (Dupuis et al. 2003, Komar et al. 2003b, Quirin et al. 2004). In our previous Dominican Republic survey, we detected WNV-specific antibodies in birds sampled at Los Haitises National Park in the northeast, but not in birds from Sierra de Baoruco National Park in the southwest, in November 2002 (Komar et al. 2003b). Here, we report on expanded surveil-

lance for flavivirus activity in the Dominican Republic in 2003, including surveys of wild bird and mosquito populations along the western border of the country, with some additional sampling from Santo Domingo.

MATERIALS AND METHODS

Study area

The field team surveyed birds and mosquitoes at two principal sites (Fig. 1): Parque Nacional Monte Cristi (20–30 March 2003, 19°43'N,

¹University of Kansas Natural History Museum and Biodiversity Research Center, Lawrence, Kansas.

²Centro Nacional de Control de Enfermedades Tropicales, Secretaría de Estado de Salud Pública y Asistencia Social, Santo Domingo, República Dominicana.

³Centers for Disease Control and Prevention, Division of Vector-Borne Infectious Diseases, Fort Collins, Colorado.

⁴Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado.

⁵Present address: SalvaNATURA, Conservation Science Program, San Salvador, El Salvador.

⁶Present address: USDA National Wildlife Research Center, Wildlife Disease Program, Fort Collins, Colorado.

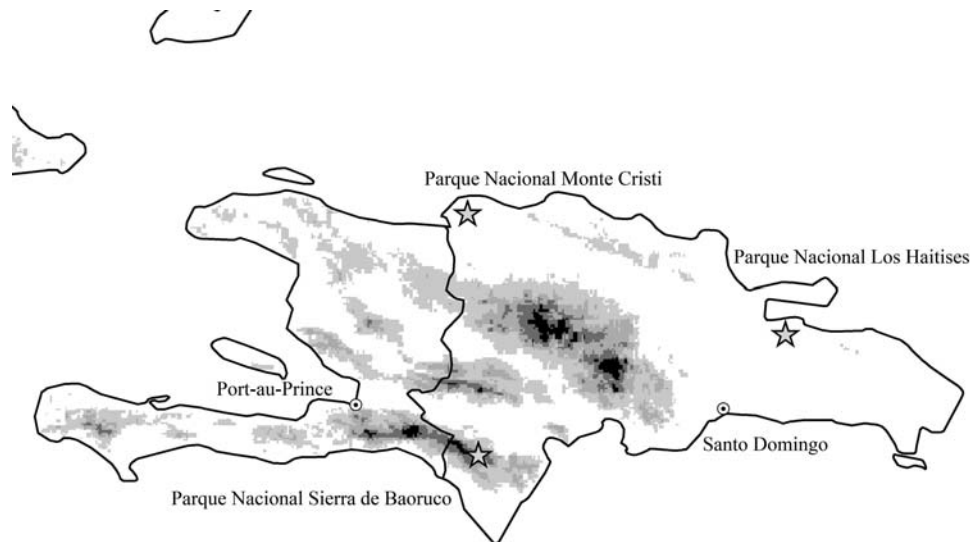


FIG. 1. Island of Hispaniola. West Nile virus transmission occurred at Parque Nacional Monte Cristi prior to March 2003. Also shown is Parque Nacional Los Haitises, where West Nile virus transmission occurred prior to November 2002. Shades of gray are 500-m intervals (e.g., 0–500, 501–1000).

71°40'W), and Parque Nacional Sierra de Baoruco (2–12 April 2003; 18°12'N, 71°32'W). We also tested birds from the city of Santo Domingo (18°29'N, 69°54'W). The habitat at Monte Cristi was dry thorn scrub adjacent to tidal estuaries and mangroves at sea level. At Sierra de Baoruco, the field team worked for 8 days at high elevations (1600–1750 m), in open-understory pine forest mixed with patches of dense humid broadleaf forest and for 3 days at lower elevations (350–450 m), in dry thorn scrub near Puerto Escondido.

Avian serum and tissue collections

Healthy wild birds were collected by standard methods (Gaunt and Oring 1997), in a manner qualitatively similar to methods used during the previous virus survey in the Dominican Republic (Komar et al. 2003b, University of Kansas unpublished data). Tissues (eye, spleen, kidney, and brain) were removed from 70 resident birds (16 species) and 20 migratory birds (four species) at Monte Cristi, from 90 resident birds (33 species) and 11 migratory birds (four species) at Sierra de Baoruco, and from three resident birds (three species) and one migratory bird at Santo Domingo. Blood samples were collected from a subsample of these birds, consisting of 58 resident birds (16 species) at Monte Cristi, 55 resident birds (27 species) at

Sierra de Baoruco, and two resident birds (two species) at Santo Domingo. Blood was generally not collected from migratory birds or from very small resident birds, such as hummingbirds. Blood and tissue specimens were frozen immediately in liquid nitrogen for transportation, and then stored at -70°C . Voucher specimens (including tissue samples) were prepared for all birds and were deposited at the University of Kansas Natural History Museum (KUNHM). Birds were aged by plumage, skull ossification, and presence or absence of a bursa of Fabricius. The migratory or resident status of each bird was determined based on standard references (American Ornithologists' Union 1998, Raffaele et al. 1998). For species with both migratory and resident populations, we treated individuals as migratory.

Mosquito collections

Mosquitoes were sampled following the method of Belkin et al. (1970), and were identified in the field or in the laboratory of the Centro de Control de Enfermedades Tropicales de la República Dominicana. Adult mosquitoes were grouped into pools of approximately 50 for testing. At Monte Cristi, 647 adult *Psorophora jamaicensis* were pooled into 13 groups. At Puerto Escondido (near Sierra de Baoruco), 124 adult *Culex quinquefasciatus* were

pooled into two groups. Larval mosquitoes were collected and identified but not tested for viral antigen.

Serologic assays

Avian serum samples were diluted 1:10 and screened for WNV-neutralizing antibodies by plaque-reduction neutralization test (PRNT) according to standard methods (Beaty et al. 1995). The specific protocol and virus stocks used were reported in our earlier study (Komar et al. 2003b). We also tested for neutralizing antibodies to Saint Louis encephalitis virus (SLEV), because this avian virus has been detected in the Caribbean Basin (Belle et al. 1980), and is known to cross-react to anti-WNV neutralizing antibodies (Komar et al. 2001b). Specimens that neutralized a challenge dose of approximately 100 plaque-forming units (pfu) of virus by at least 90% were further titrated in duplicate. Sera were also tested by epitope-blocking enzyme-linked immunosorbent assay (blocking ELISA) as previously described (Blitvich et al. 2003). Briefly, blocking ELISAs were performed using the WNV-specific monoclonal antibody (MAb) 3.1112G and the flavivirus group-reactive MAb 6B6C-1. All sera were tested in the assay that utilized MAb 3.1112G (unless there was insufficient serum), and any serum that was positive was tested at least once more for confirmation. The ELISA that utilized MAb 6B6C-1 was only used to test sera that screened positive for neutralizing antibodies to WNV or SLEV. Again, ELISA-positive sera were tested at least once more for confirmation.

Serologic case definition

Due to the lack of information on flaviviruses currently in the Dominican Republic, we considered it important to test the serum samples for antibodies to WNV using two serologic techniques. Results were categorized as follows:

WN. Sera that neutralized WNV plaque formation by at least 90% (PRNT₉₀) at a dilution of $\geq 1:10$, that exhibited a PRNT₉₀ titer at least fourfold greater than its corresponding SLEV PRNT₉₀ titer, and that blocked the reaction of

MAb 3.1112G by $\geq 30\%$ were classified as positive for antibodies to WNV.

SLE. Sera that exhibited SLEV PRNT₉₀ titers of $\geq 1:10$ and at least fourfold greater than its corresponding WNV PRNT₉₀ titer, and that blocked the binding of MAb 6B6C-1 (but not MAb 3.1112G) by $\geq 30\%$ were classified as positive for antibodies to SLEV.

FLAV. Sera that reacted to flaviviruses but could not be categorized as either WN or SLE were considered positive for antibodies to an undetermined flavivirus. These included sera that had positive PRNT₉₀ titers for both WNV and SLEV, sera that were reactive to either WNV or SLEV by PRNT₉₀ but negative by blocking ELISA, and sera that were reactive to either WNV or all flaviviruses by blocking ELISA but negative by PRNT₉₀.

Negative. Sera that were negative for reactivity to flaviviruses by PRNT₉₀ and by blocking ELISA were considered negative.

Virus detection

Tissue homogenates from apparently healthy wild birds were tested for viral infections by Vero plaque assay, as described earlier (Komar et al. 2003b). Tissue homogenates obtained from flavivirus-seropositive birds were assayed for flavivirus RNA by TaqMan reverse-transcription polymerase chain reaction (RT-PCR) with flavivirus-specific and WNV-specific primers (Lanciotti et al. 2000).

Dead birds collected through virus surveillance activities by health authorities during March, 2003, and mosquito pools were tested by VecTest (Ryan et al. 2003). For birds, 20 individuals were sampled first from the oral and then from the cloacal cavity by a dry dacron polyester tipped swab. The swab was agitated in a cryotube containing 1 ml of "grinding" solution provided with the commercial VecTest package (Medical Analysis Systems, Inc., Camarillo, CA), and then the swab was discarded. Swabs from additional bird carcasses were agitated into the same cryotube, until only 250 μ L of solution remained, the rest having been absorbed by the swab tips. This occurred after

about five samples had been added to the tube. A VecTest test strip was then inserted into the tube and left for 15 min. Then the strip was aired dry on a paper towel, and read once dry. If any of the pooled bird samples were positive, birds within the positive pool would have been tested individually by VecTest. For mosquitoes, the commercially recommended protocol was followed. Pools of approximately 50 mosquitoes were ground in solution prior to inserting the VecTest test strip into the solution.

RESULTS

We found 21 of 115 (18%) resident birds sampled in the Dominican Republic in March 2003 with some evidence of past flavivirus infection (Table 1). Twelve (10%) of these birds were confirmed positive for antibodies to WNV by two serologic techniques: PRNT and blocking ELISA. Nine birds were considered positive for flavivirus-reactive antibodies, but could not be classified as infected with either WNV or SLEV with certainty. Some of these were strongly reactive for antibodies to flaviviruses, as determined by ELISA, and were negative for WNV antibodies by ELISA or had low neutralizing antibody titers to WNV (PRNT₉₀ titers of 20–80).

All confirmed WNV-positive birds were collected at Monte Cristi, where the community-wide seroprevalence rate for resident birds was 12 of 58 (21%; 95% confidence interval [CI], 12–33%). The seropositive birds were of the orders Cuculiformes ($n = 2$), Strigiformes ($n = 1$), and Passeriformes ($n = 9$). Undetermined flavivirus-positive resident birds (Table 1) were found at a similar frequency at Monte Cristi (four of 58 resident birds) and at Sierra de Baoruco (five of 55 resident birds).

Virus antigen was not detected in any oral and cloacal swabs of dead birds collected through public health virus surveillance efforts in Santo Domingo. No virus antigen was detected from mosquito pools collected at Monte Cristi or Sierra de Baoruco. Virus could not be isolated from any tissues of the healthy 163 resident and 32 migratory birds assayed. The migratory birds tested for virus antigen represented the following families: Threskiornithidae ($n = 1$), Ardeidae ($n = 3$), Tyrannidae ($n =$

3), Hirundinidae ($n = 6$), Turdidae ($n = 2$), and Parulidae ($n = 17$). In addition, we did not detect flavivirus RNA in any of the tissue homogenates from flavivirus-seropositive birds by TaqMan RT-PCR amplification.

At Monte Cristi, the highest WNV seroprevalence rate for an individual bird species (black-crowned palm-tanager) was 80% ($n = 5$). That rate was significantly higher than the rate for all other species combined (15%, $n = 53$, $p = 0.0028$, Barnard's Unconditional Test of Superiority using difference of two binomial proportions).

All mosquito pools were negative for viral antigen. Only *Psorophora jamaicensis* ($n = 647$) was tested at Monte Cristi, although very low numbers of seven other species (*Anopheles albimanus*, *Culex duplicator*, *Cx. quinquefasciatus*, *Cx. secutor*, *Ochlerotatus hemisurus*, *Oc. sollicitans*, and *Oc. taeniorhynchus*) were present. Only larval forms of two species (*Cx. secutor* and *Wyeomyia* sp.) were found in the highlands of Sierra de Baoruco, but *Cx. quinquefasciatus* adults ($n = 124$) were tested at nearby Puerto Escondido.

DISCUSSION

Although only 12 resident birds at Monte Cristi were seropositive for WNV infection, the seroprevalence rate (21%) at that site is the highest reported so far in wild bird communities of the Caribbean region. Seroprevalence was 15% (CI 5–32%) at Parque Nacional Los Haitises in northeastern Dominican Republic in late 2002 (Komar et al. 2003b), and 4.9% (CI 2.6–7.2%) in Jamaica in early 2002 (Dupuis et al. 2003). Seroprevalence detected in resident birds at Staten Island, New York, after a WNV outbreak in 2000 was 23% (CI 18–29%) (Komar et al. 2001a). Given the similar seroprevalences between Staten Island in 2000 our Monte Cristi in 2003, we believe that epizootic transmission may have occurred recently at Monte Cristi. We used more conservative criteria for determining a positive result than the New York study, which used only PRNT results and not blocking ELISA as well.

Overall, the PRNT and ELISA data were in concordance, although several disparities were

TABLE 1. FLAVIVIRUS-POSITIVE SEROLOGY RESULTS FOR BIRDS COLLECTED IN THE DOMINICAN REPUBLIC IN 2003^a

Species	KUNHM identification no.	Date sampled	Age	Locality	PRNT ₉₀ titer ^b			Percentage inhibition by ELISA ^c		Result
					WNV	SLEV	6B6C-1	3.1112G		
Hispaniolan lizard cuckoo (<i>Saurothera longirostris</i>)	8001	25 Mar.	Adult	Monte Cristi	80	<10	76	82	WN	
Hispaniolan lizard cuckoo	8049	30 Mar.	Adult	Monte Cristi	≥320	<10	56	98	WN	
Burrowing owl (<i>Athene cunicularia</i>)	8012	26 Mar.	Adult	Monte Cristi	≥320	<10	79	90	WN	
Northern mockingbird (<i>Mimus polyglottos</i>)	7970	23 Mar.	Adult	Monte Cristi	40	<10	82	45	WN	
Black-crowned palm-tanager (<i>Phaenicophilus palmarum</i>)	7984	24 Mar.	Adult	Monte Cristi	80	<10	71	91	WN	
Black-crowned palm-tanager	7994	25 Mar.	Undetermined	Monte Cristi	160	<10	95	81	WN	
Black-crowned palm-tanager	8033	28 Mar.	Adult	Monte Cristi	80	<10	94	76	WN	
Black-crowned palm-tanager	8038	28 Mar.	Adult	Monte Cristi	160	<10	86	93	WN	
Greater Antillean bullfinch (<i>Loxigilla violacea</i>)	7993	25 Mar.	Adult	Monte Cristi	160	<10	87	97	WN	
Greater Antillean bullfinch	8000	25 Mar.	Adult	Monte Cristi	≥320	<10	76	90	WN	
Village weaver (<i>Ploceus cucullatus</i>)	7973	23 Mar.	Adult	Monte Cristi	40	<10	43	58	WN	
Village weaver	7989	24 Mar.	Adult	Monte Cristi	160	<10	92	98	WN	
Hispaniolan lizard cuckoo	8050	30 Mar.	Adult	Monte Cristi	160	640	53	91	FLAV ^d	
Bay-breasted cuckoo (<i>Hyetornis rufigularis</i>)	8140	11 Apr.	Adult	Baoruco	10	<10	86	10	FLAV	
Hispaniolan woodpecker (<i>Melanerpes striatus</i>)	7979	24 Mar.	Adult	Monte Cristi	<10	<10	45	1	FLAV	
Greater Antillean elaenia (<i>Elaenia fallax</i>)	8133	09 Apr.	Adult	Baoruco	<10	<10	55	0	FLAV	
White-necked crow (<i>Corvus leucognathus</i>)	8148	11 Apr.	Adult	Baoruco	40	<10	79	13	FLAV	
Pine warbler (<i>Dendroica pinus</i>)	8118	08 Apr.	Adult	Baoruco	≥320	<10	NT	NT	FLAV	
Hispaniolan crossbill (<i>Loxia megalaga</i>)	8096	06 Apr.	Adult	Baoruco	<10	<10	47	24	FLAV	
Village weaver	7972	23 Mar.	Adult	Monte Cristi	20	<10	31	8	FLAV	
Village weaver	7974	23 Mar.	Adult	Monte Cristi	20	<10	40	11	FLAV	

^aELISA, enzyme-linked immunosorbent assay; FLAV, undetermined flavivirus; KUNHM, University of Kansas Natural History Museum, Division of Ornithology; NT, not tested; PRNT₉₀, reciprocal 90% plaque reduction neutralization titer; SLEV, Saint Louis encephalitis virus; WNV, West Nile virus.

^bValues represent reciprocal titers; threshold of detection was 1:10.
^cInhibition values for samples with less blocking than the mean readings of the negative control samples are presented as zeros. Positive readings and some negatives were duplicated; mean values from all repeated tests are presented.

^dAlthough traditional serology based upon PRNT would suggest that this specimen be identified as SLEV antibody-positive, the WNV antibody-positive result in the blocking ELISA test indicates that this specimen was possibly positive for both SLEV and WNV. However, secondary flavivirus infections are notorious for heterologous reactivity, so infection by WNV or other flaviviruses causing these reactions could not be ruled out. Hence, the determination as “FLAV.”

observed. For example, four birds had WNV PRNT₉₀ titers that were greater than the corresponding SLEV titer, but all were negative in the ELISA that utilized the WNV-specific MAb. However, it is important to note that all four birds had low WNV PRNT titers (between 10 and 40), and all were positive in the ELISA that used the group-reactive MAb. Furthermore, a Hispaniolan lizard cuckoo (KUNHM 8050) had PRNT₉₀ titers to SLEV and WNV of 640 and 160, respectively, consistent with an SLEV infection, but was positive in the WNV-specific ELISA. It is possible that this bird had been exposed to both viruses. Differences in the assay sensitivities and specificities may have contributed to the inability to classify all of the flavivirus-reactive sera as either WNV- or SLEV-reactive. Alternatively, one or more flavivirus(es), other than WNV and SLEV, may be active in the Dominican Republic.

We presume that the 12 WNV-seropositive resident birds were infected locally because the species are not migratory (Raffaele et al. 1998). All seropositive birds were adults, thus active virus transmission could have taken place >1 year prior to sampling. We found no evidence of active virus in bird tissues of flavivirus-seropositive resident birds, and we are unaware of any evidence that virus transmission occurred during the present study. We did not test serum samples from most migrants, because presence of antibodies would not be informative, given the history of these birds traveling through areas of WNV transmission in or near North American breeding grounds.

Although active WNV infections were not detected, we cannot conclude that virus transmission was *not* active during our study. Samples were small, such that active infections may have been missed by chance. There is no evidence yet that *Ps. jamaicensis*, the only mosquito tested at Monte Cristi, is involved in the local transmission cycle for WNV. The dead birds tested may have been collected outside of transmission foci. In fact, the probability of recovering WNV-infected dead birds for testing may be much lower in tropical ecosystems than in temperate areas: not only are dead birds likely to be scavenged more quickly, but avian mortality from WNV is likely to be lower for a variety of reasons (Peterson et al. 2004). Even

if WNV caused significant avian mortality in the Dominican Republic, reporting and testing networks would be less sensitive than in developed countries.

Lack of evident transmission activity may also be due to the timing of our study in March and April. WNV transmission may be most active at other times of year depending upon a variety of influencing factors, such as local climatic conditions, and abundance of local vectors and amplifying hosts (Marra et al. 2004), all of which remain unknown in the Dominican Republic. WNV may behave similarly to SLEV in the Caribbean Basin. In Jamaica, SLEV was isolated in June and July from mosquitoes and wild birds (Belle et al. 1980). Larval density of *Culex nigripalpus*, the suspected vector of SLEV and potentially WNV, reportedly peaks during the months of November to February in Cuba (Alonso Navarro et al. 1990). The influx of wintering birds from North America could also provide susceptible hosts for amplifying the virus during the fall and winter months (September–March). All of these relationships need to be further studied in the Dominican Republic.

Only one bird species, black-crowned palm-tanager (family Thraupidae, order Passeriformes), had a sufficiently large sample size and seroprevalence at Monte Cristi to allow detection of a significant difference when compared to the background seroprevalence rate for anti-WNV antibodies. Given that other members of Passeriformes have high reservoir competence for WNV in North America (Komar et al. 2003a), this palm-tanager may also be a competent amplifying host. It was fairly common in both matorral and mangrove habitat at Monte Cristi. The importance of the palm-tanager in particular, but also the other WNV-seropositive species, in the amplification cycle of WNV in the Dominican Republic should be investigated.

Finally, we note that nine of 21 (43%) flavivirus-seropositive birds could not be assigned with certainty as either WNV- or SLEV-antibody positive. In contrast, five of 147 (3%) flavivirus-seropositive bird sera in New York City remained unassigned to either WN or SLE (Komar et al. 2001b). Recent studies in the Caribbean also found evidence of flavivirus in-

fections that could not be attributed to those viruses or Ilhéus virus (Dupuis et al. 2003, Komar et al. 2003b, Ulloa et al. 2003). The unattributed flavivirus seroprevalence rate (7% at Monte Cristi and 9% at Sierra de Baoruco) is comparable to the rate of 6% reported at Los Haitises (Komar et al. 2003b). Dupuis et al. (2003) reported low frequencies of undetermined flavivirus-positive sera from Puerto Rico and Jamaica. These results suggest that an unknown flavivirus that cross-reacts with antibodies for WNV is enzootic in bird populations throughout Hispaniola and perhaps the Caribbean Basin.

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Address reprint requests to:

Oliver Komar

SalvaNATURA

33 Ave. Sur #640

Colonia Flor Blanca, San Salvador, El Salvador

E-mail: okomar@salvanatura.org