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West Nile Virus Seroprevalence Study of Bird Handlers in Virginia, 2004

Introduction

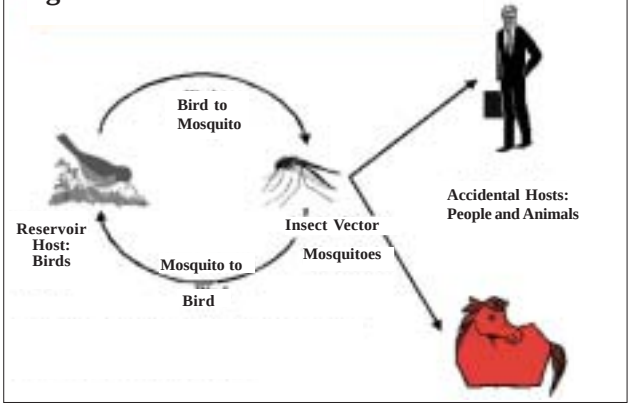
While the number of human West Nile Virus (WNV) cases in Virginia has been relatively low (Table 1), mosquito pool and dead bird surveillance indicate that WNV is established in the Commonwealth. Transmission between bird-biting mosquitoes and wild birds is thought to be the primary mechanism for WNV maintenance and amplification in nature. Infection in humans occurs primarily through "bridge vectors": mosquitoes (such as *Culex pipiens*) that feed on both humans and birds (Figure 1). Alternative mechanisms of WNV transmission have been previously documented, such as human-to-human transmission of WNV through blood transfusions and organ transplants,[2,3] and direct transmission to laboratory workers who acquired infection through percutaneous inoculation.[4] In addition, non-mosquito transmission of WNV from birds to humans has been previously implicated in occupational settings, but this has not been thoroughly explored.[5] There is also evidence of direct transmission of WNV among birds.[6] Several bird species are known to shed large amounts of the virus in their oral secretions and feces,[7] creating the potential for bird-to-human transmission, especially among persons who regularly handle such birds.

Although the extent of the risk of WNV transmission from birds to humans is unknown, certain individuals (e.g., wildlife rehabilitators, local health department staff

Table 1. WNV Human Cases, US vs. VA, 2002-2004

Year	US	VA
2002	4,146	29
2003	9,737	26
2004	2,539	5

Figure 1. WNV TRANSMISSION CYCLE[1]



who handle dead wild birds as part of surveillance for WNV) may be at increased risk of contracting WNV infection as a result of their occupational or recreational activities. [Wildlife rehabilitators are individuals who volunteer their time to care for injured or orphaned wildlife. They are licensed by the Virginia Department of Game & Inland Fisheries to rehabilitate various types of animals such as small mammals, reptiles and birds.] This article presents the results of a 2004 study designed to examine a potential risk factor for acquiring WNV: bird handling. Additionally, the study attempted to identify risk and protective factors for acquiring WNV among the bird handling groups.

Table 2. Characteristics of Bird and Non-bird Handler Participants, Pre- and Post-WNV Season, 2004

	Pre-Season		Post-Season	
Characteristic	**Bird handlers n = 77 (%)**	**Non-bird handlers n = 65 (%)**	**Bird handlers n = 62 (%)**	**Non-bird handlers n = 50 (%)**
Median age (range)	44 (20 - 66)	47 (19 - 67)	46 (22 - 66)	47 (22 - 63)
Gender				
Female	62 (80.5)	49 (75.4)	50 (80.6)	38 (76.0)
Male	15 (19.5)	16 (24.6)	12 (19.4)	12 (24.0)
Race				
White/Caucasian	75 (97.4)	62 (95.4)	60 (96.8)	48 (96.0)
Black/African American	2 (2.6)	2 (3.1)	2 (3.2)	1 (2.0)
Refused	0 (0.0)	1 (1.5)	0 (0.0)	1 (2.0)

Methods

Study Design and Recruitment

A prospective cohort study design was employed. The exposed person cohort (bird handlers) was recruited in the spring of 2004 with help from wildlife rehabilitator regional leaders and district health department staff. Exposed persons included wildlife rehabilitators, veterinarians, mosquito control and environmental health personnel. Participants were recruited from the five different health regions of Virginia (West, Southwest, North, East, and Central). Regional meetings were arranged to facilitate recruitment of study participants and included lectures on WNV. The unexposed person cohort (non-bird handlers) consisted of friends and neighbors of the wildlife rehabilitators and non-bird handler employees from local health departments, matched on age, sex, and location of residence. Only adults over the age of 18 years and non-pregnant women were recruited for participation in the study.

Questionnaires

Pre- and post-WNV season standardized questionnaires were administered to each member of each cohort. [A WNV season is defined as the period of potential WNV transmission. In Virginia this is typically between June and late September.] Interviewers were trained to administer questionnaires to study participants. Collected data included information on demographics and activities as they related to WNV exposure. Bird handlers were asked details about their bird handling activities.

Serologic Testing

Blood samples were drawn before and after the 2004 WNV season from all study participants to determine WNV infection status. Blood samples were tested by the Virginia Department of General Services, Division of Consolidated Laboratory Services (DCLS) for the presence of reactive WNV IgM and IgG antibodies using an IgM Antibody Capture Enzyme-linked Immunosorbent Assay (MAC-ELISA) and an IgG ELISA.

FIGURE 2. Previously injured red-tailed hawk rehabilitated at the Wildlife Center of Virginia.[8]

Data Analysis

Data were entered and managed in Microsoft Access and Excel 2002 (Microsoft Corporation, Redmond, WA) and analyzed using STATA 7.0 (Stata Corporation, College Station, TX) and EpiInfo 2000 (Centers for Disease Control and Prevention, Atlanta, GA).

Results

Demographic Information

A total of 142 persons were recruited during the pre-season phase of the study; 77 (54.2%) were bird handlers. During the post-season phase, 112 of the original 142 study participants returned for follow-up; 62 (55.4%) were bird handlers. Study participant characteristics are summarized in Table 2. There was no significant difference in age, race, sex or locality between bird and non-bird handlers, pre- or post-season. Bird and non-bird handlers differed significantly with regards to licensed rehabilitator status; licensed rehabilitators made up 64.9% of the exposed cohort, compared to 6.2% of the unexposed cohort (Test of proportions, p-value < 0.00).

Serology

During pre-season testing, two persons from the bird handler cohort had positive WNV IgM and WNV IgG ELISAs, indicative of relatively recent infection (Table 3). [WNV IgM antibodies may persist for well over a year after WNV infection.[9]] In addition, one person from the bird handler cohort tested during the pre-season was positive for WNV IgG only, indicating past infection. The majority of the pre-season study participants (85.2%) were negative for WNV infection.

During post-season testing, there were no new WNV seroconversions in either cohort. The two persons that were WNV IgM and WNV IgG positive during pre-season testing were only positive for WNV IgG at post-season testing. The person positive only for WNV IgG during pre-season testing remained WNV IgG positive at post-season testing. Overall, seroprevalence of WNV among bird handlers was 4.2% (95% CI = 0.9% - 11.9%); there were no seropositive results for WNV among non-bird handlers (Table 3). [Uninterpretables were removed from the analysis.] There was no significant difference in seroprevalence rates between bird

handlers (4.2%) and non-bird handlers (0.0%) (Test of proportions, p = 0.94) and serologic evidence of previous WNV infection was not associated with being a bird handler (Fisher's exact, p = 0.184).

Table 3. Serology Results, Pre- and Post-Season, 2004

	Pre-Season		Post-Season	
Serostatus	**Bird Handler**	**Non-bird Handler**	**Bird Handler**	**Non-bird Handler**
WNV IgM^{+}/IgG^{+}	2	0	0	0
WNV IgM^{-}/IgG^{+}	1	0	3	0
WNV IgM^{-}/IgG^{-}	68	53	57	46
WNV IgM^{-}/IgG$^{Uninterp.}$	6	12	2	4
Total	77	65	62	50

ELISA results are interpreted as WNV-positive to WNV-negative control (P/N) ratios. P/N values <3.0: negative; P/N values = 3.0-4.0: equivocal or uninterpretable; P/N values > 4.0: positive.

Risk and Protective Factors

Bird handlers spent significantly more time outdoors than non-bird handlers, during both the pre- and post-season study phases (two-sample t-test, p < 0.00). Bird handlers and non-bird handlers did not differ significantly in either the pre- or post-season study phases with regard to mosquito repellent use and awareness of mosquito bites (Table 4). There was no significant association between WNV infection status and any risk or protective factors (e.g., mosquito repellent use, presence of known mosquito bites, travel outside state or country, etc.).

Bird Handler Practices

Nearly 18% of bird handlers rehabilitated at least 200 birds during the 2004 WNV season. Approximately 19.4% reported handling 10 birds or fewer during the same WNV season. Over 26% of birds handled were blue jays and crows. Other birds commonly handled included sparrows (16.9%), raptors (8.3%), and robins (6.8%).

Use of different types of personal protective equipment (gloves, masks, glasses and clothing) among bird handlers varied greatly for both pre- and post-season. The top three most commonly reported bird handling protective practices were washing hands after handling birds, covering wounds while handling birds, and use of gloves. However, there was no marked difference between pre- and post-season with regards to use of protective equipment (data not shown).

Discussion

The fact that this study did not find a statistically significant difference between the WNV seroprevalence rates for bird handlers and non-bird handlers suggests that bird handlers are not at increased risk of acquiring the virus.

However, there were limitations to the study that may affect these conclusions. In particular, the sample size of participants may have been too small to capture any new seroconversions among the population at risk and/or to capture significant differences in seroprevalence rates between the two study cohorts. WNV activity during the 2004 season was low. Although the reasons are unknown, part of the cause may have been that the most likely vectors of WNV (e.g., *Cx. pipiens/restuans*) breed in standing water in storm sewers and culverts. In the 2004 summer season, Virginia experienced above average rainfall,[11] and this may have washed out breeding habitats, leading to a decrease in the mosquito population available to transmit WNV. Additionally, it is known that higher than normal ambient temperatures increase the rate of WNV replication in mosquitoes. However, during the 2004 summer season, below average temperatures were recorded, and this could have contributed to lower than normal viral replication rates among mosquitoes.[11,12] Other factors, such as the level and persistence of viremia among birds, the extent of viral shedding among birds, and precautions taken by bird handlers to prevent bird to human transmission of the virus, may need to be further evaluated by future studies.

Although this study did not find bird handlers to be at a greater risk of acquiring WNV than non-bird handlers, it would be valuable to repeat the study with a larger number of study participants in an area where there is more WNV activity. Despite our findings rehabilitators should always take precautions to protect themselves while handling birds to decrease their risk of acquiring WNV and other zoonotic diseases.

Table 4. Risk and Protective Factors

	Pre-Season 2004			Post-Season 2004		
Mean Total Time in Minutes Spent Outdoors* (Range)						
	Bird Handler	**Non-bird Handler**	**p-value**	**Bird Handler**	**Non-bird Handler**	**p-value**
	331.5 (40 - 1020)	213.4 (40 - 660)	p < 0.00	169.0 (30 - 810)	197.4 (30 - 690)	p < 0.00
Mosquito Repellant Use for 30 minutes or more? n (%)**						
Yes	18 (23.4)	13 (20.0)	p = 0.687	19 (30.7)	14 (28.0)	p = 0.836
No	59 (76.6)	52 (80.0)		43 (69.4)	36 (72.0)	
Any known mosquito bites? n (%)**						
Yes	49 (63.6)	40 (61.5)	p = 0.757	51 (82.3)	12 (24.0)	p = 0.192
No	24 (31.2)	23 (35.4)		11 (17.7)	36 (72.0)	
DK/DR	4 (5.2)	2 (3.1)		0 (0.0)	2 (4.0)	

** Two-sample t-test*
***Fisher's Exact test*

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(continued on page 4)

Cases of Selected Notifiable Diseases Reported in Virginia*

Disease	Total Cases Reported, August 2005						Total Cases Reported Statewide, January - August		
		Regions							
	State	NW	N	SW	C	E	This Year	Last Year	5 Yr Avg
AIDS	27	6	5	1	7	8	368	468	492
Campylobacteriosis	63	17	13	16	10	7	372	419	423
E. coli O157:H7	8	1	2	2	0	3	25	23	33
Giardiasis	64	6	22	14	15	7	355	309	243
Gonorrhea	832	54	67	92	267	352	5,590	5,857	6,490
Hepatitis, Viral									
A	4	0	1	0	1	2	56	70	78
B, acute	13	2	2	4	2	3	112	155	122
C, acute	1	0	0	1	0	0	10	12	6
HIV Infection	59	6	20	3	15	15	493	571	567
Lead in Children†	52	15	10	8	12	7	332	496	478
Legionellosis	6	2	1	2	0	1	31	30	29
Lyme Disease	53	18	23	5	3	4	138	94	81
Measles	0	0	0	0	0	0	0	0	<1
Meningococcal Infection	2	1	0	0	0	1	22	12	25
Mumps	0	0	0	0	0	0	0	5	5
Pertussis	17	4	4	3	1	5	255	107	72
Rabies in Animals	39	9	7	14	5	4	323	347	361
Rocky Mountain Spotted Fever	22	3	0	5	5	9	47	17	14
Rubella	0	0	0	0	0	0	0	0	0
Salmonellosis	186	24	57	29	32	44	714	747	709
Shigellosis	24	1	9	1	11	2	85	101	299
Syphilis, Early§	13	2	3	0	4	4	174	134	148
Tuberculosis	26	1	17	3	0	5	197	155	176

Localities Reporting Animal Rabies This Month: Albemarle 1 bat; Arlington 1 bat; Augusta 1 bat; Bath 1 raccoon; Bedford 1 skunk; Botetourt 1 skunk; Campbell 1 skunk; Carroll 1 fox, 1 raccoon, 1 skunk; Chesterfield 2 raccoons; Culpeper 1 skunk; Fairfax 2 bats, 1 dog, 1 groundhog; Fauquier 1 raccoon; Grayson 1 skunk; Hanover 1 fox, 1 raccoon; Henry 1 raccoon; Isle of Wight 1 fox; Lancaster 1 fox; Loudoun 1 fox; Lunenburg 1 groundhog; Patrick 1 fox, 1 raccoon; Prince William 1 bat; Richmond 1 raccoon; Roanoke 1 skunk; Rockingham 1 cat, 1 horse, 1 raccoon; Russell 1 raccoon; Shenandoah 1 skunk; Smyth 1 skunk; York 1 raccoon.

Toxic Substance-related Illnesses: Adult Lead Exposure 16; Asbestosis 1; Pneumoconiosis 7.

*Data for 2005 are provisional. †Elevated blood lead levels ≥10µg/dL. §Includes primary, secondary, and early latent.

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