CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, May 2005, p. 665–667 Vol. 12, No. 5
1071-412X/05/$08.00+0 doi:10.1128/CDLI.12.5.665–667.2005

NOTES

Persistence of Antibodies to West Nile Virus in Naturally Infected Rock Pigeons (*Columba livia*)

Samantha E. J. Gibbs,[1,2]* Douglas M. Hoffman,[3] Lillian M. Stark,[4] Nicole L. Marlenee,[5] Bradley J. Blitvich,[5] Barry J. Beaty,[5] and David E. Stallknecht[1,2]

Southeastern Cooperative Wildlife Disease Study, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602[1]; Department of Infectious Diseases, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602[2]; U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Wildlife Services, School of Forest Resources, Athens, Georgia 30602[3]; Florida Department of Health, Bureau of Laboratories—Tampa, 3602 Spectrum Blvd., Tampa, Florida 33612[4]; and Arthropod-Borne and Infectious Diseases Laboratory, Department of Microbiology, Immunology, and Pathology, Colorado State University, Fort Collins, Colorado 80523[5]

Received 10 January 2005/Accepted 1 March 2005

Wild caught rock pigeons (*Columba livia*) with antibodies to West Nile virus were monitored for 15 months to determine antibody persistence and compare results of three serologic techniques. Antibodies persisted for the entire study as detected by epitope-blocking enzyme-linked immunosorbent assay and plaque reduction neutralization test. Maternal antibodies in squabs derived from seropositive birds persisted for an average of 27 days.

West Nile virus (WNV) (*Flaviviridae* family, *Flavivirus* genus) is maintained in a bird-mosquito transmission cycle, and wild bird surveillance has proven effective in tracking the spread of this virus in North America. Since extensive avian mortality has been associated with WNV infection in North America, much of this surveillance has concentrated on dead-bird testing (16). As demonstrated with other arboviruses, such as St. Louis encephalitis virus (SLEV), eastern equine encephalitis virus, and western equine encephalitis virus, serologic testing of birds represents another tool for further investigating WNV epidemiology (7, 8, 14).

The duration of the antibody response, test performance, and persistence of maternal antibodies can complicate interpretation of serologic results. Information on the persistence of antibodies to WNV in avian species is currently limited. Experimentally, persistence of neutralizing antibodies to the North American strain of WNV in rock pigeons (*Columba livia*) was demonstrated over a 9-week period postinoculation and in chickens over a 28-day period postinoculation (9, 11). Pigeons inoculated with an African strain of WNV maintained antibodies for 16 months (12). Recaptured naturally infected wild birds in South Africa with initial WNV antibody titers of >40 lost demonstrable antibody by hemagglutination inhibition (HAI) in as few as 3 weeks (13).

The objectives of this study were the following: (i) to determine the long-term persistence of antibodies to WNV in naturally infected rock pigeons, (ii) to compare the long-term utility of commonly used WNV serologic techniques (plaque reduction neutralization test [PRNT], HAI, and epitope-blocking enzyme-linked immunosorbent assay [ELISA]), and (iii) to determine the persistence of maternal antibodies to WNV in squabs derived from these naturally infected birds.

Thirty rock pigeons, 20 seropositive for WNV and 10 negative controls, were captured in April 2003 in Atlanta, Georgia. All birds were banded and housed in a mosquito-free facility for 60 weeks. Venipuncture was performed on each bird upon entry and at 3-week intervals by wing vein. Serum samples were stored at −70°C.

Using WNV (Georgia isolate DES-107-01) and SLEV (strain TBH-28), PRNTs were performed following standard protocols (1, 10). Titers were expressed as the reciprocal of serum dilutions reducing the number of plaques >90% ($PRNT_{90}$). Samples with $PRNT_{90}$ titers to WNV which were fourfold greater than titers to SLEV were considered seropositive for WNV. HAI assays were performed at the Florida Department of Health using a published protocol (5). The antigen used for HAI, SLEV (TBH-28), was prepared by following the sucrose-acetone procedure (4). Epitope-blocking ELISAs were performed using the WNV-specific monoclonal antibody (MAb) 3.1112G (Chemicon International, Inc., Temecula, CA) and the flavivirus-specific MAb 6B6C-1 (provided by the Centers for Disease Control and Prevention, Fort Collins, CO) as previously described (2). MAb 3.1112G detects an NS1 protein epitope; MAb 6B6C-1 detects an envelope protein epitope.

All serum samples collected over the 60-week period were tested by PRNT using WNV to determine persistence and antibody titers. Serum samples collected on day 0 were also tested by PRNT using SLEV. To compare antibody persistence as measured by PRNT, HAI, and epitope-blocking ELISA, a subset of samples collected from five positive birds on day 0 to week 45 were tested with all three serologic tests. To compare

* Corresponding author. Mailing address: Wildlife Health Building, College of Veterinary Medicine, University of Georgia, Athens, GA 30602-4393. Phone: (706) 542-1741. Fax: (706) 542-5865. E-mail: sgibbs@vet.uga.edu.

TABLE 1. Persistence of antibodies to WNV in naturally infected rock pigeons (*Columba livia*)[a]

Bird ID[b]	Assay	Result for wk[c]:																			
		0	3	6	9	12	15	18	21	24	27	30	33	36	39	42	45	48	51	54	60
13	PRNT	80	160	80	160	160	160	160	80	160	160	160	160	160	160	160	160	160	160	160	80
	ELISA	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+				
	HAI	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10				
31	PRNT	40	80	40	80	40	80	80	80	80	40	40	80	80	80	80	80	40	80	80	40
	ELISA	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+				
	HAI	80	80	80	80	40	40	40	40	80	40	40	40	40	20	40	20				
42	PRNT	640	640	640	1280	1280	1280	1280	640	640	640	320	640	640	320	640	640	640	640	640	640
	ELISA	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+				
	HAI	20	20	20	10	20	20	10	10	10	10	10	10	10	10	10	10				
192	PRNT	80	160	160	160	160	80	80	160	160	160	160	160	160	160	160	160	160	160	160	160
	ELISA	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+				
	HAI	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10				
200	PRNT	80	160	160	160	160	160	160	160	160	80	80	80	80	160	160	160	80	160	80	160
	ELISA	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+				
	HAI	<10	10	10	10	10	10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10				

[a] Serum samples from five birds were tested.
[b] Bird identification no.
[c] Titers of antibody for $PRNT_{90}$ and HAI are given. ELISA results are for monoclonal antibodies (6B6C-1/3.1112G).

performance of serologic assays, a second subset consisting of samples collected from all birds on weeks 3, 24, and 45 were tested by PRNT, HAI, and epitope-blocking ELISA. Concordance of results was determined using the Kappa statistic (15).

Persistence of maternal antibodies to WNV was determined in five squabs that hatched during the study. Blood samples were taken from all squabs 8 days after hatching and every several days thereafter until 6 weeks of age. PRNT was used to test all samples.

The 20 birds that had antibodies to WNV at the time of capture remained antibody positive during the 60-week study period; the 10 control birds that had no detectable antibody to WNV remained antibody negative (data not shown). From the first subset of samples, antibodies to flaviviruses were detected in two of the five PRNT-positive birds by HAI and in all five birds by ELISA at all time points tested (Table 1).

The initial WNV $PRNT_{90}$ titers ranged from 40 to 640 for the 20 birds that had antibodies to WNV. $PRNT_{90}$ titers for 16 of these birds did not vary by more than a twofold dilution throughout the 60-week testing period. The titers for the remaining four pigeons varied only fourfold (two dilutions). The 60-week $PRNT_{90}$ titers for 18 birds were within a twofold dilution of the day-zero titer. The two HAI-positive samples in the first subset remained positive, with a steadily decreasing trend in HAI titer.

Comparative results for serologic assays are shown in Table 2. HAI results were inconsistent with PRNT results (kappa = 0.14) (a kappa value of 0.8 to 1.0 indicates almost perfect agreement between tests). While good agreement was observed between ELISA (when positive with both MAbs) and PRNT results (kappa = 0.91), agreement improved slightly (kappa = 0.95) when results were considered positive by either MAb.

Neutralizing maternal antibodies to WNV in the squabs lasted for an average of 27 days (Table 3).

The pigeons used in this study were naturally infected field-collected birds. The dates of WNV infection are therefore unknown, and an absolute estimate of antibody persistence could not be determined. This study has shown, however, that the minimum duration of persistence of antibody to WNV in rock pigeons is 15 months, there is little long-term variation in antibody titers, and there is no serological evidence of viral recrudescence. Based on these findings, the population immu-

TABLE 2. Comparison of serologic assays in determining flavivirus antibody titer or status of naturally infected rock pigeons (*Columba livia*)

Bird ID[a]	Titer of antibody or antibody positivity for wk:								
	3			24			45		
	PRNT	ELISA[c]	HAI	PRNT	ELISA	HAI	PRNT	ELISA	HAI
13	160	+/+	—	160	+/+	—	160	+/+	—
15	160	+/+	—	160	+/+	—	160	+/+	—
24	320	+/+	—	160	+/+	—	160	+/+	—
31	80	+/+	80	80	+/+	80	80	+/+	20
34	160	+/+	—	160	+/+	—	80	+/+	—
42	640	+/+	20	640	+/+	10	640	+/+	10
43	320	+/+	—	640	+/+	—	320	+/+	—
179	640	+/+	10	640	+/+	10	640	+/+	—
180	40	+/+	—	80	+/+	—	40	+/+	—
181	320	+/−	—	320	−/−	—	160	+/−	—
185	320	+/+	80	320	+/+	40	320	+/+	20
186	320	+/+	—	160	+/+	—	320	+/−	—
187	320	+/+	—	640	+/+	10	640	+/+	—
189	40	+/+	—	40	+/+	—	10	+/−	—
190	160	+/+	—	160	+/+	—	320	+/+	—
192	160	+/+	—	160	+/+	—	160	+/+	—
194	320	+/+	—	320	+/+	—	320	+/+	—
196	160	+/+	—	80	+/+	—	80	+/+	—
199	160	+/+	—	160	+/+	—	160	+/+	—
200	160	+/+	10	160	+/+	—	160	+/+	—
1	—[b]	−/−	—	—	−/−	—	—	−/−	—
5	—	−/−	—	—	−/−	—	—	−/−	—
21	—	−/−	—	—	−/−	—	—	−/+[d]	—
26	—	−/−	—	—	−/−	—	—	−/−	—
37	—	−/−	—	—	−/−	—	—	−/−	—
39	—	−/−	—	—	−/−	—	—	−/−	—
41	—	−/−	—	—	−/−	—	—	−/−	—
178	—	−/−	—	—	−/−	—	—	−/−	—
188	—	−/−	—	—	−/−	—	—	−/−	—
197	—	−/−	—	—	−/−	—	—	−/−	—

[a] Bird identification no.
[b] —, $PRNT_{90}$ or HAI titer of antibody of <10.
[c] Results for monoclonal antibodies (6B6C-1/3.1112G).
[d] This sample classified as negative due to negative screen by 6B6C-1.

TABLE 3. Persistence of maternal antibodies to WNV in squabs of naturally infected rock pigeons (*Columba livia*)[a]

Bird ID[b]	Hatch date	Titer of antibody for bleed date[c]								Duration of antibody persistence (days)
		6/10	6/14	6/18	6/21	6/24	6/29	7/2	7/12	
56	6/2	10	10	10	10	<10	<10	<10	<10	19
57	6/2	20	20	20	20	10	<10	<10	<10	22
61	5/30	20	40	20	20	20	20	20	<10	33
62	5/30	40	20	20	20	10	10	<10	<10	30
58	6/2	40	40	40	40	20	10	10	<10	30

[a] Dates are expressed as month/day.
[b] Bird identification no.
[c] Ninety-percent PRNT titer.

nity to WNV can be expected to increase as WNV establishes itself in North America.

The consistency of antibody titers observed over time during this study contrasts with the findings for experimentally infected pigeons (9). The antibody responses of those birds reflect an acute postinfection immunologic response, while the present study most likely reflects older infections. As a result, a direct comparison cannot be made between the two studies.

The persistence of antibodies to WNV in an avian species for more than a year complicates interpretation of multiyear studies involving serologic surveillance of wild bird populations. Because the antibody titers in this study remained at high levels, it suggests that pigeons maintain neutralizing-antibody titers to WNV for several years. Seroprevalence of WNV in avian populations may therefore increase while transmission of the virus in an area remains stable over time. Because species variation in the persistence of antibodies to WNV may exist, antibody persistence in other avian species should be evaluated.

The results in this study proved to be highly test dependent, and serologic results should be interpreted with this in mind. The HAI test was not as effective as the PRNT or ELISA in this study. Neutralizing antibodies are generally considered to persist longer than HAI antibodies, however, so the results of this study may reflect differences in the timing of infection in individual birds (3). Those pigeons positive by HAI in this study potentially represent more-recent infections. Additionally, the HAI assay was performed using SLEV antigen as a flavivirus group reactive antigen, rather than a specific WNV antigen, which may have affected test sensitivity.

To our knowledge, this is the first report detailing the persistence of avian maternal antibodies to the North American strain of WNV. Columbiformes are unique in that in addition to the maternal antibodies transferred through the egg yolk, they receive both maternal and paternal antibodies through crop milk after hatching. Immunoglobulin A and immunoglobulin G antibodies are present in the crop milk and are absorbed by 1-day-old squabs; further transfer of antibodies past day 1 appears to be limited (6).

The role of nestlings in WNV amplification cycles may be reduced by maternal-antibody persistence. In the case of pigeons, the additional opportunity for transfer of passive immunity from not only the hen but also the cock increases the proportion of squabs with resistance to WNV infection. How maternal antibody persistence in pigeons compares to that in indigenous North American avian species is unknown. When determined, this information will help to elucidate variations in WNV disease resistance among avian populations.

This study was primarily supported by the Georgia Department of Human Resources through the Centers for Disease Control and Prevention's "Epidemiology and Laboratory Capacity for Infectious Diseases" grant program, contract no. 427-93-25328, and by CDC contract CCu820510 to Colorado State University. Additional support was provided by the Federal Aid to Wildlife Restoration Act (50 Stat. 917) and through sponsorship from fish and wildlife agencies in Alabama, Arkansas, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Mississippi, Missouri, North Carolina, Oklahoma, Puerto Rico, South Carolina, Tennessee, Virginia, and West Virginia.

We thank Ben Wilcox for assistance with animal care and handling, Robert Lanciotti for supplying SLEV, and Maribel Casteneda, Rita Judge, and Eddie Tensley for their HAI expertise.

REFERENCES

1. **Allison, A. B., D. G. Mead, S. E. J. Gibbs, D. M. Hoffman, and D. E. Stallknecht.** 2004. West Nile virus viremia in wild rock pigeons. Emerg. Infect. Dis. **10:**2252–2255.
2. **Blitvich, B. J., N. L. Marlenee, R. A. Hall, C. H. Calisher, R. A. Bowen, J. T. Roehrig, N. Komar, S. A. Langevin, and B. J. Beaty.** 2003. Epitope-blocking enzyme-linked immunosorbent assays for the detection of serum antibodies to West Nile virus in multiple avian species. J. Clin. Microbiol. **41:**1041–1047.
3. **Casals, J.** 1973 Arbovirus infections, p. 99–117. *In* J. R. Paul and C. White (ed.), Serological epidemiology. Academic Press, New York, N.Y.
4. **Clarke, D. H., and J. Casals.** 1958. Techniques for hemagglutination and hemagglutination-inhibition with arthropod-borne viruses. Am. J. Trop. Med. Hyg. **7:**561–573.
5. **Day, J. F., and L. M. Stark.** 1996. Transmission patterns of St. Louis encephalitis and eastern equine encephalitis viruses in Florida: 1978–1993. J. Med. Entomol. **33:**132–139.
6. **Engberg, R. M., B. Kaspers, I. Shranner, J. Kösters, and U. Lösch.** 1992. Quantification of the immunoglobulin classes IgG and IgA in the young and adult pigeon (*Columba livia*). Avian Pathol. **21:**409–420.
7. **Gruwell, J. A., C. L. Fogarty, S. G. Bennett, G. L. Challet, K. S. Vanderpool, M. Jozan, and J. P. Webb, Jr.** 2000. Role of peridomestic birds in the transmission of St. Louis encephalitis virus in southern California. J. Wildl. Dis. **36:**13–34.
8. **Howard, J. J., J. Oliver, and M. A. Grayson.** 2004. Antibody response of wild birds to natural infection with alphaviruses. J. Med. Entomol. **41:**1090–1103.
9. **Komar, N., S. Langevin, S. Hinten, N. Nemeth, E. Edwards, D. Hettler, B. Davis, R. Bowen, and M. Bunning.** 2003. Experimental infection of North American birds with the New York 1999 strain of West Nile virus. Emerg. Infect. Dis. **9:**311–322.
10. **Komar, N., N. A. Panella, J. E. Burns, S. W. Dusza, T. M. Mascarenhas, and T. O. Talbot.** 2001. Serologic evidence for West Nile virus infection in birds in the New York City vicinity during an outbreak in 1999. Emerg. Infect. Dis. **7:**621–625.
11. **Langevin, S. A., M. Bunning, B. Davis, and N. Komar.** 2001. Experimental infection of chickens as candidate sentinels for West Nile virus. Emerg. Infect. Dis. **7:**726–729.
12. **McIntosh, B. M., W. Madsen, and D. B. Dickinson.** 1969. Ecological studies on Sindbis and West Nile viruses in South Africa. VI. The antibody response of wild birds. S. Afr. J. Med. Sci. **34:**83–91.
13. **McIntosh, B. M., G. M. McGillivray, D. B. Dickinson, and J. J. Taljaard.** 1968. S. Afr. J. Med. Sci. **33:**105–112.
14. **Reisen, W. K., J. O. Lundstrom, T. W. Scott, B. F. Eldridge, R. E. Chiles, R. Cusak, V. M. Martinez, H. D. Lothrop, D. Gutierrez, S. E. Wright, K. Boyce, and B. R. Hill.** 2000. Patterns of avian seroprevalence to west equine encephalomyelitis and Saint Louis encephalitis viruses in California, USA. J. Med. Entomol. **37:**507–527.
15. **Smith, R. D.** 1995. Veterinary clinical epidemiology: a problem-oriented approach, 2nd ed., p. 149–150. CRC Press, Boca Raton, Fla.
16. **Steele, K. E., M. J. Linn, R. J. Schoepp, N. Komar, T. W. Geisbert, and R. M. Manduca.** 2000. Pathology of fatal West Nile virus infections in native and exotic birds during the 1999 outbreak in New York City, New York. Vet. Pathol. **37:**208–224.