

## PASSIVE TRANSFER OF MATERNAL ANTIBODIES TO WEST NILE VIRUS IN FLAMINGO CHICKS (*PHOENICOPTERUS CHILENSIS* AND *PHOENICOPTERUS RUBER RUBER*)

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**Abstract:** Passive transfer of maternal antibodies against West Nile virus (WNV) was studied in a captive population of Chilean (*Phoenicopterus chilensis*) and Caribbean flamingos (*Phoenicopterus ruber ruber*). Transfer of WNV antibodies from hens to chicks was documented and measured by plaque-reduction neutralization test. Hen titers were significantly correlated to chick titers. Mean half-life of maternal WNV antibodies was 13.4 days in chicks for which half-life was measurable.

**Key words:** Flamingo, maternal antibodies, *Phoenicopterus chilensis*, *Phoenicopterus ruber ruber*, West Nile virus.

### BRIEF COMMUNICATION

Since 1999, West Nile virus (WNV), in the genus *Flavivirus*, family *Flaviviridae*, has become well established in North America and remains a significant disease concern for susceptible species including flamingos.<sup>2,10,13,15</sup> Juvenile birds are especially susceptible to new infections because of their immature and naïve immune systems. In August of 2002, four of seven parent-reared chicks from an outdoor mixed flock of Chilean (*Phoenicopterus chilensis*) and Caribbean flamingos (*Phoenicopterus ruber ruber*), aged 3–6 wk, died of WNV infection at Zoo New England, in Boston, Massachusetts. This event led to the decision to hand-rear all subsequent chicks indoors.

Vaccination studies conducted in adult flamingos have yielded mixed results.<sup>9,13</sup> However, vaccination protocols using a series of three 1-ml intramuscular injections in flamingos have shown high seroconversion rates compared to other species tested.<sup>9</sup> Optimal timing of vaccination protocols in young birds should provide protection during the vulnerable period of waning passive immunity, yet avoid interference of maternal antibodies. The purpose of this study was to document passive transfer of maternal antibodies to WNV in flamingos.

Eggs were pulled from flamingo nests during the months of June through September in 2004 and 2005, typically 27–28 days post-laying, and were artificially incubated (Humidaire® Model 20 Incubator/Hatcher, New Madison, Ohio 45346, USA)

thereafter. After hatching, chicks were hand-reared indoors in a mosquito-free environment.

Blood samples were collected from flamingo chicks of a mixed population of captive Caribbean and Chilean flamingos from both hatch seasons. Sampling of chicks occurred during the first and fourth weeks after hatching. In the first season, two Caribbean and two Chilean flamingo chicks were sampled. In the second season, six Caribbean flamingo chicks were sampled.

Blood samples were also collected from the respective hens, including two Chilean and five Caribbean flamingo females ranging in age from 4 to 36 yr. One Caribbean flamingo hen (hen C) produced one chick in both seasons (chicks 3 and 7). A second Caribbean hen (hen D) produced one chick (chick 4) in season 1 and two chicks (chicks 5 and 10) from separate clutches in season 2. To avoid disturbing breeding pairs, samples were collected at the end of each laying season, typically in September, ranging 24–106 days (mean = 64 ± 28.1 days) after their eggs were laid. Hens that produced chicks in both seasons were sampled at the end of each respective season. Medical records were reviewed for each hen to determine history of WNV vaccination and exposure status.

Approximately 0.5–1.0 ml of blood was drawn from the jugular vein and serum was separated by centrifugation after the sample clotted. Serum samples were stored at –20°C until the sampling for each season was completed (maximum of 21 days) and were shipped as a batch to the New York State Animal Health Diagnostic Laboratory at the Cornell University College of Veterinary Medicine for measurement of WNV antibody titers. Assays were performed using a plaque-reduction neutralization test on batches from each season.

The minimum dilution measured for positive titers was 1:20. During the initial assays, samples

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**Table 1.** West Nile virus antibody titers of Chilean (*Phoenicopterus chilensis*) and Caribbean (*Phoenicopterus ruber*) flamingo chicks and their immunized hens, measured by plaque-reduction neutralization.

Chick	Species	Hen	Hen titer	Sample 1		Sample 2		Half-life (days)
				Age (days)	Titer	Age (days)	Titer	
Season 1								
1.	Chilean	A	1:80	4	1:20	21	0	ND <sup>a</sup>
2.	Chilean	B	1:640	4	1:40	24	1:20	20
3.	Caribbean	C <sup>b</sup>	1:160	6	1:40	21	0	ND
4.	Caribbean	D <sup>c</sup>	1:640	7	1:320	21	1:160	14
Season 2								
5.	Caribbean	D <sup>c</sup>	1:640	3	1:1280	23	1:80	10.7
6.	Caribbean	E	1:160	3	1:80	21	1:20	12
7.	Caribbean	C <sup>b</sup>	1:160	2	1:160	20	1:20	10.3
8.	Caribbean	F	1:160	3	1:40	22	0	ND
9.	Caribbean	G	0	3	0	21	0	ND
10.	Caribbean	D <sup>c</sup>	1:640	3	>1:640	21	1:80	ND

<sup>a</sup> ND = Not determined.

<sup>b</sup> Hen C produced a chick in both season 1 and season 2.

<sup>c</sup> Hen D produced one chick in season 1 and two chicks in separate clutches in season 2.

with titers greater than 1:640 were not measured to their end point. In order to calculate the half-life of antibodies, samples with titers greater than 1:640 were retested with banked serum. For any sample that was retested, the entire series was retested (hen titer, chick first and second titers) so that calculations for that series could be based on the same assay. A Kolmogorov-Smirnov test was used to determine normality of data. Means and 95% confidence intervals (C.I.s) were calculated for hen and chick titers and a Spearman rank order correlation for nonparametric data was applied to determine correlations between hen and chick titers for both sample periods. WNV antibody half-life was calculated for chicks with measurable titers for both sample periods. Half-life was calculated by the following equation: half-life =  $(T_1/2) \div (T_1 - T_2)/D_\Delta$ , where  $T_1$  = initial chick titer,  $T_2$  = second chick titer, and  $D_\Delta$  = number of days between titers. All calculations were performed with SigmaStat 3.5 (Systat Software Inc., Point Richmond, California 94804, USA) and Microsoft Excel (Microsoft Excel, Microsoft Corporation, Redmond, Washington 98052, USA).

Review of adult female histories revealed that all hens in this study were WNV seronegative prior to 2002. During the spring of 2002, all adult birds in the flock were vaccinated with a series of three intramuscular injections of a commercial inactivated WNV vaccine (West Nile-Innovator®, Fort Dodge Animal Health, Fort Dodge, Iowa 50501, USA), given 3 wk apart, with 0.25 ml administered for the first two injections and 1 ml for the third injection.

Only one of the birds was WNV antibody positive (1:40, hen D) following vaccination, which is similar to findings in other studies using vaccination doses less than 1 ml.<sup>13</sup> During the two summers following vaccination, sporadic episodes of neurologic presentations involving ataxia, lethargy, and anorexia occurred among birds in the flock and birds were suspected of having diseases caused by WNV. In August of 2002, four of the five Caribbean flamingo hens (hens C, D, E, and F) were affected. All birds recovered after hospitalization and intensive supportive care. In January 2003, the entire flock received a booster vaccination, administered as 1 ml i.m. In September of 2003, both Chilean flamingo hens (hens A and B) were affected and one of the previously affected Caribbean flamingos (hen C) presented again with neurologic signs. Clinical signs of birds affected in 2003 were much milder than the previous year and all birds recovered without treatment. One of the Caribbean flamingos (hen G), hen to chick 9, was not clinically affected in either year. All birds were WNV antibody positive following these clinical episodes and the single unaffected hen remained WNV antibody negative. The Caribbean flamingo (hen C) that was seemingly affected twice could not be confirmed as reinfected, because WNV antibody titer was already markedly elevated prior to repeat presentation.

Results for WNV antibody titers from hens and chicks are shown in Table 1. The mean titer of the hens, expressed as the inverse of the titer dilution factor, was  $364.4 \pm 171.6$  (mean  $\pm$  95% C.I.).

Mean titer of chicks was  $247.5 \pm 297.2$  at the first sample, and  $42.2 \pm 35.5$  at the second sample. Data from chick 9 and its hen (hen G) were not included in calculation of means, because no titer was detectable in these birds. Sufficient quantities of banked serum were not available to retest the initial titer sample for chick 10. Therefore, the initial titer result for chick 10 remains greater than 1:640 and this result was not included in calculation of the mean, because no end titer was available. The titers in chicks were significantly correlated to those in their respective hens for both the first (correlation coefficient = 0.77,  $P = .01$ ) and second samples (0.84,  $P < .01$ ). Three of the chicks (chicks 1, 3, and 8) that were seropositive at the initial sampling period had an undetectable titer at the second sampling (mean =  $21.5 \pm 0.7$  days). Average half-life of antibodies in chicks for which it could be calculated ( $n = 5$ ) was 13.4 days.

The results show that maternal antibodies to WNV are transferred to flamingo chicks from seropositive hens. It is notable that chicks 5 and 10, which shared the same hen (hen D) and were hatched 36 days apart during the same laying season, also shared very similar titer results. It would appear that, at least in this case, passive transfer of immunoglobulin is not diminished during a multiple clutch season. Levels of passive transfer from year to year, however, were not as consistent in this study, as seen with the chick titers from hens C and D. Maternal WNV antibodies transferred to chicks in this study were likely in response to natural infection of the hens, as only one hen was seropositive following vaccination. Vaccination of hens may still be a suitable means of providing passive immunity to progeny if different, more immunogenic vaccination protocols were employed instead of that used with this group.

Maternal antibodies persisted for several weeks in most chicks. Correlation coefficient between hen and chick titers was strongest at the second titer, as chicks from hens with the highest titers had titers that persisted longer than chicks from hens of lower titers. Functional loss of titer may be earlier than the measured loss in this study, because collecting two samples per chick only allows for limited analysis. Maternal antibodies decline at rates unique to each species and type of immunoglobulin and may decline in linear or logarithmic patterns.<sup>1,7,11,14</sup> An additional sample taken during the second or third week after hatch may have helped to more accurately calculate rate and pattern of decay. The only other report found in the literature on waning maternal WNV antibody in an avian species was performed with pigeon squabs,<sup>5</sup> which reported the du-

ration of antibody persistence to be an average of 27 days.

Flamingos, like pigeons, feed their young via esophageal secretions, or crop milk. Columbiform crop milk is known to contain parental antibodies that are absorbed by 1-day-old squabs and likely continue to provide local mucosal immunity thereafter.<sup>5</sup> It is not known whether immunoglobulins are present in flamingo crop milk, or if similar immunoglobulin transfer occurs in flamingo chicks. Flamingo crop secretions contain approximately 8% protein, some of which could possibly be immunoglobulin.<sup>3</sup> It may be that parent-reared flamingo chicks would have longer durations of antibody persistence or might continue to have at least some local mucosal immunity after serum antibody titers are depleted, but while the chick is still being fed by the parents. This condition would potentially expand duration of parental immunologic protection of chicks through weaning age, which is approximately 75 days in wild flamingos, or up to 4 mo–1 yr in captive flamingos.<sup>3</sup> Evaluation of antibody titers in sire serum and esophageal secretions of both hen and sires that participate in feeding of young, may contribute additional information regarding immunoglobulin transfer.

In the flamingo chicks of this present study, three (chicks 1, 3, and 8) of nine birds have undetectable titers by the fourth week after hatch and only three birds (chicks 4, 5, and 10) had titers greater than 1:20. The minimal WNV antibody titer at which birds are protected from WNV disease is not known. Maternal antibodies have provided protection to poultry chicks from disease caused by pathogens such as *Eimeria*, *Salmonella*, and infectious bursal disease virus, and levels of maternal antibody have correlated to level of protection.<sup>1,8,14</sup> Correlation of titers between hens and chicks found in this and other studies indicate that serologic assessment of the female breeding population in a collection will help to determine the general level of protection in offspring.<sup>6</sup>

To determine timing of the initial chick vaccination, consideration also needs to be given to the level of maternal antibody that will interfere with active immunization. Studies in poultry have shown that vaccination of chicks during the first week after hatch, when passive immunity is at its highest level, results in reduced vaccine effectiveness and hastens the depletion of maternal antibodies.<sup>4,8,12</sup> Further studies of WNV immunity in flamingo chicks should be directed toward administration of vaccine and monitoring titer reaction to determine the most suitable WNV vaccination protocol for flamingo chicks and breeding flocks. Establishment of opti-

mal timing for WNV vaccination may allow chicks to be parent-reared in an outdoor setting in areas where WNV is endemic.

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