# HEMATOLOGY, PLASMA BIOCHEMISTRY, AND ANTIBODIES TO SELECT VIRUSES IN WILD-CAUGHT EASTERN MASSASAUGA RATTLESNAKES (SISTRURUS CATENATUS CATENATUS) FROM ILLINOIS

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# HEMATOLOGY, PLASMA BIOCHEMISTRY, AND ANTIBODIES TO SELECT VIRUSES IN WILD-CAUGHT EASTERN MASSASAUGA RATTLESNAKES (*SISTRURUS CATENATUS CATENATUS*) FROM ILLINOIS

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ABSTRACT: During the 2004 field season, blood was collected from Eastern massasauga rattlesnakes (*Sistrurus catenatus catenatus*) in the Carlyle Lake (Carlyle, Illinois, USA) and Allerton Park (Monticello, Illinois, USA) populations to derive baseline complete blood count and plasma biochemistry data and to assess the prevalence of antibodies to West Nile virus (WNV) and ophidian paramyxovirus (OPMV). Massasaugas were located for sampling through visual encounter surveys. Body weight, snout–vent length, total protein, globulins, sodium, and potassium were normally distributed among the survey population. Aspartate aminotransferase, creatine kinase, albumin, calcium, uric acid, white blood cell count, heterophils, lymphocytes, monocytes, eosinophils, and basophils were non-normally distributed within these animals. Female snakes had significantly shorter tail lengths; lower blood glucose, packed cell volumes, and absolute azurophil counts; and higher plasma calcium and phosphorus concentrations than did males. None of the snakes tested ($n=21$) were seropositive for WNV, whereas all ($n=20$) were seropositive for OPMV.

*Key words:* Biochemistry, hematology, massasauga, ophidian paramyxovirus, rattlesnake, serology, *Sistrurus catenatus catenatus*, West Nile virus.

## INTRODUCTION

Habitat fragmentation because of human-induced changes in the landscape is a major cause of population declines in wildlife species, including many reptiles, throughout the USA (Ricklefs, 1997, Pough et al., 1998). Environmental conditions that fail to meet physiologic requirements lead to stress, which may increase circulating levels of glucocorticoids, causing immune suppression (Oppliger et al., 1998). Impairment of immune function may increase mortality because of infectious diseases. Subclinical infections may also interfere with reproduction, undermining sustainability of reptile populations.

The eastern massasauga rattlesnake (EMR), *Sistrurus catenatus catenatus*, is a North American species adversely affected by landscape changes in recent years. Eastern massasauga rattlesnakes have a unique range for a North American reptile, distributed from Illinois and Missouri through the Great Lakes region to Ontario and New York (Conant and Collins, 1998; Johnson and Leopold, 1998). The species' distribution was previously more continuous throughout this range but, because of habitat loss and fragmentation, it now occurs only in isolated populations. Thus, the EMR is on the endangered, threatened, or special concern list in every state or province in which it occurs, and it is a candidate for the Federal Endangered Species List (Johnson and Leopold, 1998).

Despite a historic range in the northern two-thirds of Illinois, the EMR has been identified in only five counties since 1980 (Phillips et al., 1999). The largest population, estimated to be roughly 300 snakes, exists near the southernmost distribution of the subspecies, at Carlyle Lake, Clinton County, and is the only population believed to have a high likelihood of long-term viability. A smaller population of EMRs is present at Allerton Park, Piatt County. The Allerton Park area is undergoing changes related to ecological rehabilitation of adjacent agricultural areas and nearby residential development. Assessment of the impacts of these activities on EMR populations is in the early stages.

Hematologic and biochemistry data are needed to characterize the health status of EMR populations over time and to relate health to habitat quality. There is also a need to examine exposure of the endangered EMR to viral pathogens. Two viruses of potential concern in rattlesnakes are West Nile virus (WNV) and ophidian paramyxovirus (OPMV). Illinois has experienced numerous human fatalities from WNV as well as major death losses in wild birds (Centers for Disease Control, 2006). To our knowledge, no reptile or amphibian in Illinois has been reported as infected, but WNV has been isolated from American alligators (*Alligator mississipiensis*) and *Rana ridibunda* showing clinical signs (Kostyukov, 1986; Miller et al, 2003) and caused epizootics in alligators in 2001 and 2002 (Jacobson et al., 2005). Thus, WNV is a potential threat to EMRs in Illinois.

Although WNV infections have been sparsely identified in reptiles, OPMV has been well documented in highly susceptible viper species, including species of *Crotalus*, *Bitis*, *Vipera*, *Bothrops*, *Agkistrodon, Elaphe, Sistrurus, Porthridium, Lachesis, Naja, Spilotes*, and *Dendroaspis*. (Ahne et al., 1987; Cranfield and Graczyk, 1996). Individuals with OPMV infection have signs of generalized neurologic and respiratory dysfunction (Jacobson, 1980; Jacobson et al., 1981). Epizootics have occurred in various zoological institutions with high mortality in vipers (Jacobson et al., 1981; Potgieter et al., 1987; Jacobson et al., 1992). One outbreak in a colony of 438 individuals resulted in 8% mortality in various genera, all within the family Viperidae (Jacobson et al., 1981). Ophidian paramyxovirus has been confirmed immunohistochemically in a captive *Sistrurus* sp. that died during a zoological outbreak (Homer et al., 1995). Although the epidemiology of OPMV is not well understood in wild populations, the potential effects of an outbreak of OPMV in a susceptible wild population of endangered reptiles could be devastating. Thus, surveillance for OPMV in free-ranging EMRs is needed.

## MATERIALS AND METHODS

Eastern massasauga rattlesnakes were collected from South Shore State Park (SSSP) (38.620°N, 89.304°W), from Eldon Hazlet State Park (EHSP) (38.658°N, 89.331°W), from land surrounding EHSP (EHSP field 3) managed by the US Army Corps of Engineers near Carlyle, Illinois, USA, and from Allerton Park (39.993°N, 88.641°W) near Monticello, Illinois, USA. Some study sites were subjected to a controlled burn in fall 2003 or early spring 2004. Controlled burns in the Carlyle area were in accordance with the ecological restoration plan developed by the Illinois Department of Natural Resources and the US Army Corps of Engineers.

Individual snakes were captured by the Carlyle Lake and Allerton Park EMR research team of the Illinois Natural History Survey at the time of emergence from hibernation from late March through mid-May 2004. Individuals randomly encountered during searches in the study sites were captured, placed in pillowcases that were tied securely and placed in lockable plastic containers with air ventilation holes, and transported to the field station for examination and blood collection. Snakes were identified using photographs of the dorsum of the head and subcutaneous implantation of passive integrated transponder (PIT) tags on the dorsum of the body. Individual physical measurements were recorded, including total length, snout–vent length (SVL), and tail length.

Snakes were held for blood collection with the head and cranial half of the body in a transparent open-ended PVC tube. In this position, the ventral tail vein was accessible. A 23-gauge or 25-gauge sterile hypodermic needle and a 1- or 3-ml syringe were used to obtain blood samples up to a maximum of 0.8% of the body weight. Blood was immediately transferred to a lithium heparin-coated 400-μl tube. Snakes were each placed into a clean container and returned to the site of collection. Each snake was sampled only once.

Standard methods were used to determine packed-cell volume (PCV). Total solids in plasma were measured with a refractometer as a correlate of plasma protein. An eosinophil Unopette system (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA) was used to estimate the white blood cell count. Blood smears were stained with Wright's stain and 100-cell differential counts were evaluated under a light microscope using oil immersion. For comparison and quality assurance, a second stained blood smear was similarly examined at Louisiana State University.

Whole heparinized blood was centrifuged and no less than 0.5 ml of plasma was placed in 2-ml cryovials that were held in a conventional freezer at −18.5 C until transported on wet ice to the Louisiana State University School of Veterinary Medicine for biochemistry analyses. Testing was performed using the avian–reptilian rotor on the VetScan analyzer (Abaxis, Inc. Whipple City, California, USA). Plasma specimens were analyzed for calcium, phosphorus, sodium, potassium, aspartate aminotransferase, creatine kinase, uric acid, glucose, total protein, and albumin. Globulin was calculated by subtracting albumin from total protein.

Plasma, prepared as above, was transported on wet ice to Louisiana State University School of Veterinary Medicine for WNV testing. Samples were tested using a plaque reduction neutralizing titer assay (PRNT) (Beaty, 1989). Plasma was heat-inactivated at 56 C for 30 min, diluted 1:5 in BA-1, and then serially diluted to 1:160. An equal volume of WNV stock was added to each plasma dilution to provide 100 plaque-forming units (PFU) of WNV and dilutions were incubated for 1 hr. Six-well plates containing Vero cell monolayers were inoculated with one plasma–virus dilution per well. A back-titration was made by adding 100 μl of BA-1, 1 PFU/100 μl, 10 PFU/100 μl (to two different wells), 100 PFU/100 μl, and 50 μl of 200 PFU/100 μl to a six-well plate. Plates were incubated for 90 min, then media containing 2X-M199, distilled water, and 1% agarose was added. After 48 hr incubation, 2X-M199, 0.33% neutral red solution, and 1% agarose were added and the plates were incubated for another 24 hr. After final incubation, plaques were counted and compared to the average count of the two 10 PFU/100 μl wells from the back-titration. Any well with up to the number of plaques from the average of 10 PFU/100 μl wells demonstrated at least 90% viral neutralization. Samples that did not show at least 90% neutralization were considered negative, whereas samples showing at least 90% neutralization were considered positive for WNV antibodies.

Plasma was transported on wet ice to the University of Tennessee for OPMV testing as described in Burleson (1992). A hemagglutination inhibition assay was used to determine seropositivity with a titer >80 considered to reflect definitive exposure. Plasma was heat-inactivated at 56 C, and treated with 0.01 M potassium periodate and 0.6% glycerol, diluting the samples to 1:10. Guinea pig red blood cells (Lampire Biological Labs, Pipersville, Pennsylvania, USA) were washed three times in sterile phosphate-buffered saline (PBS) (Invitrogen, Carlsbad, California, USA) and a 1% dilution was made in PBS. For hemagglutination-inhibition, two strains of OPMV, San Lucan rattlesnake and green tree python, were titrated and diluted to eight hemagglutinating (HA) units each. Each plasma sample was tested, in duplicate, with both OPMV strains separately. Two-fold serial dilutions in PBS were prepared. Titrated virus (8 HA units) was added and incubated for 1 hr at room temperature. Finally, 1% guinea pig red blood cell solution was added and incubated for 1 hr at room temperature. The titer was reported as the last dilution that formed a pellet.

The distribution of each physical measurement, hematologic, and plasma biochemistry variable was evaluated separately for the sample population, each sex, and the different trapping locations. Statistical analyses were performed using SPSS 8.0 (SPSS Inc., Chicago, Illinois, USA). The mean, standard deviation, median, 25% and 75% quartiles, and range were determined. Distribution of data was evaluated using a Shapiro-Wilk test. For normally distributed measures, a 95% confidence interval was calculated. In cases where the prevalence estimate was 0, the 95% confidence intervals were calculated with the technique described by Van Belle and Millard (1998). Levene's test for equality of variances was used to determine if the data were homogeneous. Comparisons were made between sexes and trapping locations. A one-way analysis of variance (ANOVA) was used to assess between group differences for normally distributed data. Specific between-group differences were evaluated using a Tukey's test. For data that were not normally distributed,

TABLE 1. Normally distributed body measurements and biochemistries for eastern massasauga rattlesnakes captured in Illinois.

| Test (units) | Mean±SD | 95% CI | Min/Max |
| --- | --- | --- | --- |
| Body weight (g) | 211.3±77.4 | 175.1–247.5 | 117.0–380.0 |
| Snout–vent length (cm) | 56.8±6.7 | 53.7–60.0 | 43.2–68.1 |
| Total protein (g/dl) | 4.6±0.9 | 4.2–5.0 | 3.1–6.7 |
| Globulins (g/dl) | 3.4±0.8 | 3.0–3.8 | 1.9–4.9 |
| Potassium (mmol/l) | 4.6±0.7 | 4.3–5.0 | 3.7–6.7 |
| Sodium (mmol/l) | 148.5±8.1 | 144.8–152.2 | 132.0–166.0 |

a Kruskal–Wallis one-way ANOVA, and Dunn's test were used to assess differences between and within groups, respectively. After completion of the crude analysis, a univariate general linear model was used to identify interactions between sex and trapping location. Values of $P<0.05$ were considered significant.

## RESULTS

Twenty-one EMRs, 12 males and nine females, were collected for this study. Sufficient blood was obtained from most individuals for complete blood counts ($n=21$), plasma biochemistries ($n=21$), antibody titers to WNV ($n=21$), and antibody titers to OPMV ($n=20$). Physical measurements and hematologic data that were normally distributed, and not found to be significantly different between sexes, were pooled (Table 1). Hematologic data not normally distributed or not significantly different between sexes were also pooled (Table 2).

The avian–reptile rotor used with the VetScan analyzer is unable to measure plasma calcium concentrations >16 mg/dl. Four female EMRs in this study had calcium concentrations >16 mg/dl. To determine plasma calcium in these animals, samples were retested using the Olympus AU 600 (Olympus America, Inc. Melville, New York, USA) and values were found to be 29.8, 32.8, 19.8, and 25.5 mg/dl. Plasma samples from five additional snakes, with calcium levels <16 mg/dl, were also tested using the Olympus analyzer, and a Spearman rho correlation coefficient of 0.997 suggested that values obtained using the different machines were highly correlated. Accordingly, the four calcium values obtained with the Olympus machine were combined with the VetScan results for the final analysis of the calcium in the EMRs sampled.

Female snakes had shorter tail lengths ($F=19.9$, $P=0.002$), lower PCVs ($F=10.9$, $P=0.004$), lower absolute azurophil counts ($F=53.0$, $P=0.02$), lower blood glucose ($F=5.9$, $P=0.03$), and higher plasma calcium ($F=5.6$, $P=0.03$) and phosphorus concentrations ($F=5.5$, $P=0.03$) than males (Table 3). Aspartate aminotransferase was significantly different ($F=4.8$, $P=0.04$) between the SSSP (Mean 26.4, 95% confidence interval (CI): 12.6–40.2,

TABLE 2. Non-normally distributed hematologic parameters and biochemistries for eastern massasauga rattlesnakes captured in Illinois.

| Test (units) | Median | 25–75% quartiles | Min/max |
| --- | --- | --- | --- |
| White blood cell count (/µl) | 8116 | 4267–11853 | 1778–25,778 |
| Heterophils (/µl) | 1168 | 504–2203 | 53–3530 |
| Lymphocytes (/µl) | 2635 | 1634–4331 | 782–8917 |
| Monocytes (/µl) | 815 | 450–1887 | 231–5376 |
| Eosinophils (/µl) | 109 | 62–195 | 18–773 |
| Basophils (/µl) | 81 | 0–195 | 0–1176 |
| Creatine kinase (IU/l) | 166 | 67–898 | 46.0–5479 |
| Uric acid (mg/dl) | 4.2 | 3.0–6.5 | 1.8–23.3 |
| Albumin (g/dl) | 1.2 | 1.1–1.2 | 1.0–1.8 |

TABLE 3. Significant differences in body measurement, hematologic, and plasma biochemistry values between male and female eastern massasauga rattlesnakes from Illinois.

| Test (units) | Sex | Mean±SD | 95% CI | Min/Max |
|---|---|---|---|---|
| Tail length (cm) | Male | 6.8±0.9 | 6.0–7.6 | 5.8–7.8 |
| | Female | 4.6±0.5 | 3.7–5.5 | 4.0–5.2 |
| Packed cell volume (%) | Male | 27.4±4.9 | 24.3–30.6 | 17.0–34.0 |
| | Female | 17.6±6.8 | 11.9–23.2 | 7.0–25.0 |
| Azurophils (/µl) | Male | 3264[a] | 2169–4925[b] | 806–17013 |
| | Female | 919[a] | 561–2415[b] | 522–4065 |
| Glucose (mg/dl) | Male | 90.0±42.0 | 63.2–116.7 | 29.0–189.0 |
| | Female | 50.9±20.5 | 32.2–68.7 | 28.0–89.0 |
| Calcium (mg/dl) | Male | 11.9±1.0 | 11.3–12.5 | 10.5–14.3 |
| | Female | 18.2±9.2 | 11.1–25.3 | 7.2–32.8 |
| Phosphorus (mg/dl) | Male | 2.6±1.4 | 1.7–3.5 | 1.0–6.3 |
| | Female | 4.3±1.7 | 2.8–5.7 | 1.6–6.2 |

[a] Median, not normally distributed.

[b] 25–75% quartiles.

SD: 14.9, Range: 11–49) and EHSP (Mean 11.5, 95% CI: 7.8–14.5, SD: 3.2, Range: 6–15) populations.

None of the snakes was seropositive for WNV (0/21, 95% CI: 0–14). By contrast, all of the snakes (20/20) were seropositive for OPMV. For OPMV, low titers (20–80) were recorded in four snakes (19%), 11 snakes (52%) had low–moderate titers (160), and five snakes (24%) had moderate titers (320–640). Nine (45%) of the titers were higher using the San Lucan rattlesnake strain, and the remaining 11 (55%) titers were similar between the strains.

## DISCUSSION

Hematologic and biochemistry values and antibody titers to select viruses for 21 wild-caught EMRs were generated as baseline information and for comparison in future studies of this species. All animals were adults in good body condition and appeared well hydrated; however, a few individuals had minor cutaneous lesions that were not investigated. The range of the SVL recorded for this population was slightly shorter than the SVLs reported by Conant and Collins (1998).

Lymphocytes were more numerous than heterophils in most snakes in this study. This is consistent with values from the International Species Information System (ISIS) (2002) database for this species (heterophils, 1035/µl, $n=16$; lymphocytes, 6132/µl, $n=16$). Lymphocytes also tend to be the predominant peripheral white blood cell in other reptiles, including other snakes (Campbell, 1996; Lamirande et al., 1999; Salakij et al., 2002). Although every snake examined for antibodies to OPMV tested positive, and circulating lymphocytes are often increased in response to viral infections, we cannot assert that OPMV directly influenced lymphocyte counts. Lymphocyte numbers can be influenced not only by infectious disease, but also by sex, noninfectious disease, and nutritional status (Campbell, 1996).

The azurophil counts in the EMRs of this study were higher than the heterophil counts, as reported in many snake species (Campbell, 1996; Lamirande et al., 1999; Salakij et al., 2002). Values reported in the ISIS database (2002) for this species also reveal a higher count of azurophils (1646/µl, $n=12$) than heterophils Azurophil counts differed significantly between sexes in our study, with males having higher counts. Although increases in the azurophil count can be associated with granulomatous reactions to bacteria or parasites (Campbell, 1996), higher counts in male EMRs in this study were not accompanied by clinical disease. Male EMRs have larger home ranges and travel farther than females in search of food and mates, which would potentially increase the likelihood of exposure to infectious agents, possibly resulting in a higher baseline count.

There were also significant differences between sexes in tail length, PCV, calcium, phosphorus, and glucose. Shorter tail lengths found in the females were expected, because tails of male snakes are generally wider and longer to accommodate the hemipenes. Differences in the PCV between genders may have been associated with limited sample size and very low PCVs (7% and 8%) in two female snakes (25%). Although lymph dilution is often a concern when collecting blood samples from reptiles, results for other hematologic and biochemistry parameters from the two snakes with low PCVs were not consistent with lymph dilution. Accordingly, it appears that these two female snakes were anemic. Because the erythron was not evaluated in this study, it was not possible to determine if the anemia was regenerative or nonregenerative.

Increased calcium and phosphorus have been associated with folliculogenesis, as the associated increases in estrogen mobilize calcium from the bone (Campbell, 1996). Both calcium and phosphorus levels were significantly higher in females than in males. It is thought that this species reproduces biennially and therefore, the changes in the calcium and phosphorus noted in this population may have been because of reproductive calcium and phosphorus mobilization. Four of the female snakes had calcium levels >19.5 mg/dl, suggesting reproductive activity at the time of collection. Significant differences between sexes in glucose cannot be explained, but may have been because of energy partitioning for reproduction related to both folliculogenesis and replenishing body stores after parturition. Similarity in hematologic and plasma biochemistry results for the EMRs captured and captive EMRs represented in ISIS (2002) suggest that the two populations are in a similar plane of health.

The mean values for aspartate aminotransferase (AST) in the groups at SSSP, EHSP, EHSP field 3, and Allerton Park, and their ranges, are consistent with other snake populations. Although the difference between groups sampled was statistically significant, the values for EMRs at these locations were not in an elevated range; thus, the difference was not considered clinically important.

Antibodies to WNV were not detected by PRNT. It has been reported that WNV replicates poorly in reptiles and amphibians (Klenk and Komar, 2003). However, it also has been reported that reptiles are potential reservoirs for mosquito-borne diseases, including western equine encephalitis (Thomas et al., 1980). The habitats of these snakes include standing water and extreme humidity during the summer that sustain mosquito populations, and WNV has been reported in mosquito, equine, and human populations throughout Illinois. However, this population of EMRs apparently has not been exposed to WNV nor is it serving as a reservoir for transmission at this time.

Antibody titers to OPMV indicate that individuals in this population have been exposed to this virus. No die-offs related to disease processes have been recognized in this population; however, histopathology, ultrastructural studies, and virus isolation have not been pursued in snakes found dead in the environment. Whether OPMV infection might increase the probability of death from trauma may warrant further study. In addition, it is possible that the seropositive snakes were exposed to a different species of paramyxovirus that is similar enough to cause a positive reaction in the OPMV assay. False positives may also occur in any hemagglutination inhibition test because of nonantibody hemagglutination inhibitors, such as certain carbohydrates and lipoproteins (Janaway et al., 1999). To the authors' knowledge, there have been no published reports of OPMV affecting free-ranging snakes. However, if this population has been exposed to OPMV, the titers in apparently healthy individuals indicate that EMRs may be able to mount an adequate immune response.

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## LITERATURE CITED

AHNE, W., W. J. NEUBERT, AND I. THOMSEN. 1987. Reptilian viruses: Isolation of myxovirus-like particles from the snake *Elaphe oxycephala*. Journal of Veterinary Medicine B 34: 607–612.

BEATY, B. J., C. H. CALISHER, AND R. S. SHOPE. 1989. Arboviruses. *In* Diagnostic procedures for viral, rickettsial and chlamydial infections, 6th ed., N. J. Schmidt and R. W. Emmons (eds.). American Public Health Association, Washington, DC, pp. 797–856.

BURLESON, F. G., T. M. CHAMBERS, AND D. L. WEIDBRAUK. 1992. Virology: A Laboratory Manual. Academic Press, San Diego, California, 616 pp.

CAMPBELL, T. 1996. Clinical Pathology. *In* Reptile medicine and surgery, D. Mader (ed.). W.B. Saunders Publishing, Philadelphia, Pennsylvania, pp. 263–264.

CENTERS FOR DISEASE CONTROL AND PREVENTION. Statistics, surveillance, and control, www.cdc.gov/ncidod/dvbid/westnile/surv&control06Maps.htm. Accessed February 2006.

CONANT, R., AND J. T. COLLINS. 1998. Reptiles and amphibians; Eastern and Central North America. Houghton Mifflin Publishing, Boston, Massachusets and New York, New York, 616 pp.

CRANFIELD, M. R., AND T. K. GRACZYK. 1996. Ophidian paramyxoviruses. *In* Reptile medicine and surgery, D. Mader (ed.). W.B. Saunders Co. Publishing, Philadelphia, Pennsylvania, pp. 392–394.

HOMER, B. L., J. P. SUNDBERG, J. M. GASKIN, AND J. SCHUMACHER. 1995. Immunoperoxidase detection of ophidian paramyxovirus in snake lung using polyclonal antibody. Journal of Veterinary Diagnostic Investigation 7: 72–77.

INTERNATIONAL SPECIES INFORMATION SYSTEM. 2002. *Sistrurus catenatus*, Massasauga physiologic data reference values. Apple Valley, Minnesota.

JACOBSON, E. R. 1980. Paramyxo-like virus infection in a rock rattlesnake. Journal of the American Veterinary Medical Association 177: 796–799.

———, J. M. GASKIN, D. PAGE, W. O. IVERSEN, AND J. W. JOHNSON. 1981. Illness associated with paramyxo-like virus infection in a zoologic collection of snakes. Journal of the American Veterinary Medical Association 179: 1227–1230.

———, P. E. GINN, S. WELLS, B. S. BOWLES, AND J. SCHUMACHER. 1992. Epizootic of ophidian paramyxovirus in a zoological collection: pathological, microbiological, and serological findings. Journal of Zoo and Wildlife Medicine 23: 318–327.

———, ———, J. M. TROUTMAN, L. FARINA, L. STARK, K. KLENK, K. L. BURKHALTER, AND N. KOMAR. 2005. West Nile infection in farmed American alligators (*Alligator mississippiensis*) in Florida. Journal of Wildlife Diseases 41: 96–106.

JANEWAY, C. A., P. TRAVERS, M. WALPORT, AND J. D. CAPRA. 1999. The induction, measurement, and manipulation of the immune respise *In* Immunobiology: The immune system in health and disease. Elsevier Science Ltd./Garland Publishing, New York, New York, pp. 34–48.

JOHNSON, G., AND D. J. LEOPOLD. 1998. Habitat management for the eastern massasauga in a central New York peatland. Journal of Wildlife Management 62: 84–97.

KLENK, K., AND N. KOMAR. 2003. Poor replication of West Nile virus (New York 1999 Strain) in three reptilian and one amphibian species. American Journal of Tropical Medicine Hygiene 69: 260–262.

KOSTYUKOV, M. A., A. N. ALEKSEEV, V. P. BULYCHEV, AND Z. E. GORDEEVA. 1986. Experimental infection of *Culex pipiens* mosquitoes with West Nile virus by feeding on infected *Rana ridibunda* frogs and its subsequent transmission. Meditsinskaia Parazitologiia I Parazitarnye Bolezni (Moskva) 6: 76–78. [In Russian.]

LAMIRANDE, E. W., A. D. BRATTHAUER, D. C. FISCHER, AND D. K. NICHOLS. 1999. Reference hematologic and plasma chemistry values of brown tree snakes (*Boiga irregularis*). Journal of Zoo and Wildlife Medicine 30: 516–520.

MILLER, D. L., M. J. MAUEL, C. BALDWIN, G. BURTLE, D. INGRAM, M. E. HINES, 2nd, AND K. S. FRAZIER. 2003. West Nile virus in farmed alligators. Emerging Infectious Diseases 9(7): 794–799.

OPPLIGER, A., J. CLOBERT, J. LECOMTE, P. LORENZON, K. BOUDJEMADI, AND H. JOHN-ALDER. 1998. Environmental stress increases the prevalence and intensity of blood parasite infection in the common lizard *Lacerta vivipara*. Ecology Letters 1: 129–138.

PHILLIPS, C. A., R. A. BRANDON, AND E. O. MOLL. 1999. Field guide to amphibians and reptiles of Illinois. Illinois Natural History Survey, Champaign, Illinois, 288 pp.

POTGIETER, L. N., R. E. SIGLER, AND R. G. RUSSELL. 1987. Pneumonia in Ottoman vipers (*Vipera xanthena xanthena*) associated with a parainfluenza 2-like virus. Journal of Wildlife Diseases 23: 355–360.

POUGH, F. H., R. M. ANDREWS, J. E. CADLE, M. L. CRUMP, A. H. SAVITSKY, AND K. D. WELLS. 1998. Herpetology. Prentice Hall, Upper Saddle River New Jersey, 575 pp.

RICKLEFS, R. E. 1997. The economy of nature, 4th ed. W.H. Freeman and Company, New York, New York, 678 pp.

SALAKIJ, C., J. SALAKIJ, S. APIBAL, N.-A. NARKING, L. CHANHOME, AND N. ROCHANAPAT. 2002. Hematology, morphology, cytochemical staining, and ultrastructural characteristics of blood cells in king cobras (*Ophiophagus hannah*). Veterinary Clinical Pathology 31: 116–126.

THOMAS, L. A., E. R. PATZER, J. C. CORY, AND J. E. COE. 1980. Antibody development in garter snakes (*Thamnophis* spp.) experimentally infected with western equine encephalitis. American Journal of Tropical Medicine Hygiene 29: 112–117.

VAN BELLE, G., AND S. P. MILLARD. 1998. STRUTS: Statistical rules of thumb. www.nrcse.washington.edu/research/struts/chaptoz.pdf. Seattle, Washington, 14 pp.

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