

INFECTIOUS DISEASE SEROLOGIC SURVEY IN FREE-RANGING VENEZUELAN ANACONDAS (*EUNECTES MURINUS*)

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Abstract: Reptiles can harbor pathogenic microorganisms asymptotically and serve as potential reservoirs of infection for humans, domestic animals, and other reptiles. Infectious diseases are also problematic for free-ranging reptile populations and are an important consideration in reptile reintroduction and translocation projects. There have been limited serologic studies of free-ranging reptiles for evidence of exposure to potential pathogens. In the present study, serum or plasma samples from five male and five female free-ranging Venezuelan anacondas (*Eunectes murinus*) were screened for antibodies to eastern, western, and Venezuelan equine encephalitis viruses, vesicular stomatitis virus, ophidian paramyxovirus, 19 *Leptospira interrogans* serovars, and *Cryptosporidium serpentes*. Antibodies to these agents were not detected, or antibody titers were low and possibly nonspecific. These results for the limited number of anacondas surveyed suggest that they do not serve as significant reservoirs for these infectious agents at this location.

Key words: Anaconda, *Eunectes murinus*, cryptosporidia, health assessment, serology, virology.

INTRODUCTION

Reptiles may harbor viral, bacterial, and protozoal pathogens asymptotically and serve as reservoirs of infection for humans, domestic animals, and other reptiles. Potential pathogens include such arboviruses of economic importance in agricultural species as vesicular stomatitis (VS) virus and Venezuelan, eastern, and western equine encephalitis (VEE, EEE, and WEE, respectively) viruses.^{1,19,30,31} *Leptospira interrogans* is a bacterium that can be harbored by reptiles and can affect agricultural species, humans, and reptiles.^{12,16,21,32,34} Other pathogens, such as ophidian paramyxovirus^{6,19} and *Cryptosporidium serpentes*,^{5,13,33} are specific to snakes and have caused considerable morbidity and mortality in captive specimens.

Infectious diseases are also factors in the health of free-ranging populations^{7–9,28} and must be considered when attempting reptilian reintroduction or translocation.¹⁸ Reports of serologic studies documenting exposure of free-ranging reptile populations to potential pathogens are limited.^{3,12,15,20,28,34}

As part of a long-term study of the biology and conservation of anacondas (*Eunectes murinus*) in

the Venezuelan llanos,^{2,24–26} health assessments were performed in conjunction with implantation of radiotelemetry equipment in free-ranging anacondas in March 1992.^{2,22,25} Results of hematologic, biochemical, vitamin, mineral, toxicologic, and parasite studies of these snakes have been reported previously.² Serum and heparinized plasma samples obtained during the project were utilized for viral, bacterial, and protozoal serologic assays to investigate the prevalence of exposure to potential pathogens in the study population.

MATERIALS AND METHODS

Five male and five female anacondas were collected during the 1992 dry season at the site of an ongoing study of anaconda ecology and conservation (Hato El Cedral in the seasonally flooded Venezuelan llanos).^{2,24–26} The snakes were manually restrained, physical examinations were performed, and blood samples were obtained from the ventral coccygeal vein.² Serum and heparinized plasma samples were separated by centrifugation, transported to the USA, and frozen at -70°C until analyzed.² Samples were then assessed for exposure to various infectious agents.

Antibody titers to VEE, EEE, and WEE viruses were determined for all the anacondas by hemagglutination inhibition (HI) testing.^{14,29} The HI titer was the reciprocal of the highest dilution of sample that inhibited 8 hemagglutinating units of virus. A positive result was defined as a titer of ≥ 20 . Sera from seven anacondas (four female and three male) were screened for titers to VS virus serovars Indiana and New Jersey by serum neutralization (SN) testing.¹¹ The SN titer was the reciprocal of the highest dilution of sample that inhibited cytopathic effect. A positive result was defined as a titer of

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Table 1. Positive antibody titers to *Leptospira interrogans* serovars and Venezuelan equine encephalitis (VEE) virus in free-ranging Venezuelan anacondas (*Eumectes murinus*). All anacondas tested had negative antibody titers to 14 other *L. interrogans* serovars, eastern equine encephalitis virus, western equine encephalitis virus, vesicular stomatitis virus, ophidian paramyxovirus, and *Cryptosporidium serpentes* (data not shown).

Anaconda no.	<i>L. interrogans</i> serovars ^a						VEE virus ^b
	icterohaemorrhagiae/copenhageni	autumnalis	bratislava	icterohaemorrhagiae/icterohaemorrhagiae	kennewicki		
49		100	200				
73	100	200	200	100			
75	100						
76						20	
78		100	100	100	100		
79	100		100				

^a Antibody titers determined by microscopic agglutination testing.
^b Antibody titers determined by hemagglutination inhibition testing.

≥8. Testing for VEE, EEE, WEE, and VS virus antibodies was performed at the Texas Veterinary Medical Diagnostic Laboratory (College Station, Texas 77841, USA).

Samples from all 10 anacondas were tested at the University of Florida College of Veterinary Medicine (Gainesville, Florida 32610, USA) for ophidian paramyxovirus antibody titers with an HI test developed and validated for snakes.²³ HI titers were the reciprocal of the highest dilution of sample that inhibited agglutination. Negative results were defined as titers of ≤20, titers of 40–80 were considered suspicious, and titers of >80 were considered positive.

Antibody titers to *L. interrogans* serovars canicola, hardjo, grippityphosa, icterohaemorrhagiae/copenhageni, pomona, australis, autumnalis, ballum, bataviae, bratislava, icterohaemorrhagiae/icterohaemorrhagiae, javanica, pyrogenes, sejroe, saxkoebing, szwajizak, tarassovi, kennewicki, and wolffi were determined for all anacondas by microscopic agglutination testing (MAT) performed at the Diagnostic Laboratory, New York State College of Veterinary Medicine (Ithaca, New York 14852, USA).^{4,10,27} Samples were considered positive if ≥50% of the live *Leptospira* antigen/cells agglutinated at the screening dilution of 1:100. Titers of samples reacting at this dilution were determined by assay of serial dilutions of sample.

Samples from five female and three male anacondas were analyzed at the Animal Diagnostic Laboratory (Baltimore, Maryland 21228, USA) for *C. serpentes* antibody titers by an indirect enzyme-linked immunosorbent assay (ELISA) developed and validated for snakes.¹³ Positive results were defined as an absorbance value greater than 3 SDs above the mean absorbance value of the negative control samples.

RESULTS

Positive results are listed in Table 1. All 10 anacondas had negative HI titers of 10 for EEE and WEE viruses. Nine had negative VEE virus titers of 10, and one had a titer of 20 (Table 1). All seven anacondas tested had negative titers for VS virus serovars Indiana and New Jersey. All anacondas had negative HI titers of ≤10 for ophidian paramyxovirus. Five anacondas were seropositive with titers of 100 or 200 for one to four *L. interrogans* serovars (Table 1). All 10 anacondas had negative ELISA titers for *C. serpentes*.

DISCUSSION

The anacondas studied concentrate in small bodies of water during the dry season but disperse broadly (over >3,000 m²) across the flooded savanna during the rainy season.^{24,25} The anacondas' ranges are within an active ranch housing cattle, horses, and capybara (*Hydrochoerus hydrochaeris*).²⁴ Reptiles may serve as reservoir hosts for such zoonotic arboviruses of importance in domestic agricultural species as VEE, WEE, EEE, and VS viruses.^{1,19,20,30,31} None of the anacondas tested had elevated titers to any of these viruses. The one anaconda with a VEE titer of 20 may represent a true VEE titer or may result from cross-reaction in the HI test with antibodies to a related virus. The serologic test was not validated for this species, so the titer could have resulted from a nonspecific response in the test assay.

Ophidian paramyxovirus can cause severe morbidity and mortality in infected snakes and has caused multiple devastating outbreaks in captive snake collections but has not yet been identified in a free-ranging snake population.^{6,19,23} There was no

serologic evidence of exposure to this virus in these anacondas.

Leptospira interrogans spirochetes can infect reptiles, humans, and agricultural and other domestic animals and are often associated with aquatic or moist environments.^{12,16,21,32,34} Reptiles are usually not clinically ill when infected by *Leptospira* spirochetes. A number of seropositive snakes of several species, including captive snakes in Brazil, have been reported (with titers of up to 6,400).¹⁷ The low-level titers detected in the anacondas in the present study may represent early infection, waning titers from previous infection, antibody responses to *Leptospira* antigens present in ingested prey items, or antibodies to *Leptospira* serovars that were not included in the serologic panel. Alternatively, rather than being diagnostic for active infection with a pathogenic *Leptospira* serovar, they may be nonspecific responses resulting from cross-reaction with the nonpathogenic saprophyte *Leptospira biflexa*, as has been reported in chelonians. However, this type of cross-reaction has not yet been documented in snake species.^{3,16,21,34}

Cryptosporidium serpentes infection of captive snakes has resulted in chronic morbidity and eventual mortality of affected specimens.^{5,13} *Cryptosporidium* spp. have also been documented in many free-ranging reptile species, although morbidity and mortality have not been recognized in these free-ranging individuals.³³ The apparent lack of *C. serpentes* exposure in this anaconda population suggests that the disease may not be present at this time but could have health consequences if it were to be introduced.

Infectious diseases are important factors for the health of both captive and free-ranging reptile populations. Many infectious disease outbreaks have been documented in captive reptile collections.^{5,6,18,19} Although the impact of infectious diseases in free-ranging populations is not as well documented, its importance is being increasingly recognized.^{7-9,18,28} The results of this limited survey for serologic evidence of exposure to some bacteria, viruses, and protozoa suggest that these anacondas do not serve as significant reservoirs for selected arboviruses, ophidian paramyxovirus, *C. serpentes*, or pathogenic *Leptospira* spirochetes at this location.

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