

Serosurvey of vector-borne diseases in the Mexican wolf (*Canis lupus baileyi*) in captivity

Estudio serológico de enfermedades transmitidas por vectores en el lobo mexicano (*Canis lupus baileyi*) en cautiverio

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RESUMEN

Las enfermedades transmitidas por vectores afectan el estado de salud de los caninos en todo el mundo. Se examinaron muestras de suero de seis lobos mexicanos cautivos (*Canis lupus baileyi*) en la Reserva de la Biosfera La Michilí, Durango, México, para detectar anticuerpos contra *Anaplasma phagocytophilum*, *Ehrlichia canis* y *Borrelia burgdorferi*, así como el antígeno contra *Dirofilaria* spp. Las muestras de sangre se colectaron de la vena cefálica y se centrifugaron para obtener los sueros, que posteriormente fueron analizados utilizando pruebas de ELISA IgG disponibles en el mercado. Uno de los seis individuos resultó positivo para anticuerpos contra *A. phagocytophilum*. Este lobo positivo (macho, 13 años) nació en la Reserva, donde vivió toda su vida, pero nunca mostró signos de enfermedad. La conservación del lobo mexicano es una prioridad internacional y ante la evidencia de anticuerpos contra *A. phagocytophilum* registrados en un individuo de esta especie en la Reserva de la Biosfera de La Michilí se recomiendan estudios posteriores acerca de detección molecular de esta bacteria, el papel de los posibles vectores y la inmunidad de este lobo al patógeno.

Palabras clave: lobo, México, ELISA, *Anaplasma*.

SUMMARY

Vector-borne diseases affect the health status of canines worldwide. We examined serum samples from six captive Mexican wolves (*Canis lupus baileyi*) at the Reserva de la Biosfera La Michilí, Durango, México for antibodies against *Anaplasma phagocytophilum*, *Ehrlichia canis*, *Borrelia burgdorferi*, and the antigen against *Dirofilaria* spp. Blood samples were collected from the cephalic vein and centrifuged to obtain sera, to be assayed using commercially available IgG ELISA tests. One of the six individuals tested positive for antibodies to *A. phagocytophilum*. The positive-tested wolf (male, 13 year-old) was born at the Reserve and had lived there all his life, but had never showed signs of illness. Conservation of the Mexican wolf is an international priority and due to the evidence of antibodies against *A. phagocytophilum* found in this animal species at the Reserva de la Biosfera de la Michilí, further studies on molecular detection of this bacteria, the role of potential vectors, and wolf immunity to this pathogen are recommended.

Key words: wolf, México, ELISA, *Anaplasma*.

INTRODUCTION

The Mexican wolf is the smallest subspecies of gray wolf, originally found in semi-arid regions within temperate pine-oak forests of southwestern North America. In 1976 the Binational Mexican Wolf Recovery Program was initiated with the goal to produce genetically healthy captive groups of wolves which could then be reintroduced as wild populations within their historic range. The U.S.A and Mexico decided to remove the last wild wolves from the states of Durango and Chihuahua to begin an intensive captive breeding program (Servín-Martínez 2007). One of the objectives of the Reserva de la Biosfera La Michilí in Durango, Mexico, is to conserve animal diversity in representative natural ecosystems (Halffter

1984), and has participated in the Mexican wolf (*Canis lupus baileyi*) recovery program by keeping individuals in captivity within a pine-oak forest habitat. Today, this wolf is considered extinct in the wild in Mexico (SEMARNAT 2010). As with other species of canids, Mexican wolves are susceptible to vector-borne diseases, which are increasingly prevalent worldwide as tick and mosquitoes distributions expand through climate change and wildlife migration. In order to evaluate the occurrence of such diseases in the captive wolves at Michilí we evaluated their antibody reactions to the pathogens *Anaplasma phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi*, and the antigen against *Dirofilaria* spp.

MATERIAL AND METHODS

The Reserva de la Biosfera La Michilí is located in the southeast of Durango, Mexico, municipalities of

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Suchil and El Mezquital (23°, 24'02" to 23° 32'02" N, and 104° 08' 45" to 104° 19' 52" W). In September 2013 Mexican wolves at the Reserve were captured using nets, using care to ensure the safety of the animals as well as the veterinarians and biologists handling wolves (protocols for large canines suggested by the Association of Zoos and Aquariums, AZA Canid Tag 2012). Each animal was given a physical examination, and any signs of diseases were recorded. For each animal blood samples were collected from the cephalic vein using 3 ml Vacutainer® tubes. Samples were transported at 4 °C to the field laboratory. Blood was centrifuged at 1.500 rpm for 10 minutes to obtain serum. We assayed serum samples for antibodies against *A. phagocytophilum*, *Ehrlichia canis*, and *Borrelia burgdorferi*, and the antigen against *Dirofilaria* spp. using SNAP® 4Dx® ELISA IgG tests commercially available from IDEXX®. This test uses purified antigens that provide greater sensitivity and specificity when employing whole cell tests (IFA and Western blot); this is because peptide-based technology only evaluates the presence of highly specific antibodies against agents, which eliminates false positives. Using whole cells to detect all antibodies produced against these microorganisms can give false positives (IDEXX®).

RESULTS AND DISCUSSION

Six adult Mexican wolves were captured (3 females and 3 males). Based on physical inspections, all wolves were considered healthy; however one Mexican wolf, a 13-yr-old male, was found to be seropositive to antibodies for *A. phagocytophilum*. The other five Mexican wolves were seronegative to *A. phagocytophilum*, and all six were negative to *E. canis* and *B. burgdorferi* antibodies, as well as to the *Dirofilaria* antigen. The present study is the first to report antibodies against *A. phagocytophilum* in a Mexican wolf in Mexico.

The *A. phagocytophilum* bacteria causes granulocytic anaplasmosis, a disease that can affect humans as well as domestic and wild animals (De la Fuente *et al* 2005, Woldehiwet 2010). The bacterium infects and survives within neutrophils by disabling key neutrophil functions, including neutrophil motility, phagocytosis, the oxidative burst mechanism, and neutrophil-endothelial cell interactions, as well as interfering with neutrophil apoptosis; clinical signs in dogs, and presumably wolves, include fever, lethargy, lack of appetite, lameness, coughing, polydipsia, intermittent vomiting, and hemorrhages (Carrade *et al* 2009).

There is a report of acute granulocytic anaplasmosis in a captive timber wolf kept in an outdoor enclosure in Austria showing anorexia, depression, and fever. According to Leschnik *et al* (2012) granulocytic anaplasmosis is known to be a common infectious disease in dogs (although not all animals develop clinical signs), but in wildlife, symptomatic anaplasmosis might be a rare disease.

In Mexico, *A. phagocytophilum* antibodies in dogs have been reported. Recently, Salinas-Meléndez *et al* (2014) and Silva *et al* (2014) reported 3.0% and 7.4% of dogs from Monterrey and Oaxaca, Mexico, had anti-*A. phagocytophilum* antibodies. Recently, Leschnik *et al* (2012) reported for the first time acute granulocytic anaplasmosis in a captive timber wolf (*Canis lupus occidentalis*) in Austria. Clinical symptoms and temporary changes in blood parameters in this wolf were similar to granulocytic anaplasmosis seen in dogs. This was the first report on granulocytic anaplasmosis in a wolf, indicating that *A. phagocytophilum* might cause clinical disease in this species.

Anaplasma phagocytophilum is transmitted by hard ticks of the *Ixodes persulcatus*-complex. *I. scapularis* is present in the north, central and south regions of Mexico, including the states of Durango and Coahuila (Guzmán-Cornejo and Robbins 2010). It has been reported on Bovidae, Canidae, Cervidae and Leporidae (Guzmán-Cornejo *et al* 2007, Romero-Castañón *et al* 2008, Gordillo-Pérez *et al* 2009). For Michilía there are *I. scapularis* records on coyotes (*Canis latrans*), grey foxes (*Urocyon cinereoargenteus*), white-tailed deer (*Odocoileus virginianus*), and eastern cottontails (*Sylvilagus floridanus*) (Sosa-Fernández 1998), as well as on domestic dogs. Due to *I. scapularis*' range of host preferences and the presence of other susceptible species in Durango, the potential is there to transmit *A. phagocytophilum* to the Mexican wolf in the Reserva de la Biosfera La Michilía. According to the wolves' keeper at Michilía, the wolf that was positive to antibodies for *A. phagocytophilum* was born at the Reserve and lived there all his life, but never showed signs of illness. It is possible that he was previously exposed to tick bite and then developed immune defense mechanisms. Because conservation of the Mexican wolf is an international priority and the evidence of antibodies against *A. phagocytophilum* found in this animal species at the Reserva de la Biosfera de la Michilía, further studies on molecular detection of this bacteria, the role of potential vectors (i.e. *I. scapularis*), and wolf immunity to this pathogen are recommended.

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