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Original Article

Molecular survey of *Rickettsial* organisms in ectoparasites from a dog shelter in Northern Mexico

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ABSTRACT

The objective of this study was to screen and identify rickettsial organisms in ectoparasites collected from dogs in a shelter in Gomez Palacio, Durango, Mexico. One hundred dogs were inspected for ectoparasites. All the dogs were parasitized with *Rhipicephalus sanguineus* ticks, three with *Heterodoxus spiniger* lice and one with *Ctenocephalides felis* fleas. DNA was extracted from the ectoparasites found on each dog, and PCR with the primers for the Anaplasmataceae 16S rRNA and citrate synthase *gltA* genes were performed. Eight DNA samples obtained from ticks, three from lice and one from fleas were positive to 16S rRNA. Only one sample from *C. felis* and one from *H. spiniger* were positive to gltA. Sequence analysis of amplified products from *C. felis* showed identity to *Rickettsia felis, Wolbachia pipientis,* and *Wolbachia* spp., while a sequence from *H. spiniger* showed identity to *Wolbachia* spp. Herein we report the molecular detection of *R. felis, W. pipientis,* and *Wolbachia* spp. in *C. felis* and *H. spiniger* in northern Mexico. These results contribute to the knowledge of the microorganisms present in ectoparasites from dogs in Mexico.

1. Introduction

The order Rickettsiales comprises a group of obligate intracellular alphaproteobacteria that are common parasites of eukaryotes. Three families are included in this order: Anaplasmataceae, Holosporaceae, and Rickettsiaceae. Many alphaproteobacteria are zoonotic pathogens and cause severe human and animal diseases such as anaplasmosis, ehrlichiosis, and rickettsiosis (Guo et al., 2016). This order also includes the genus *Wolbachia*, which was identified for the first time in fleas in 2000 (Jeyaprakash and Hoy, 2000), and infects several arthropods and filarial nematodes (Hilgenboecker et al., 2008). *Wolbachia* spp. are mainly obligatory mutualists as they are essential for the survival of their hosts (Guo et al., 2016).

The genus *Rickettsia* includes a variety of bacteria causing disease in humans and animals. They are known as vector-borne rickettsioses and are mainly transmitted by fleas, mites, and ticks (Portillo et al., 2015). This genus includes among others the species *R. rickettsi, R. akari, R. typhi, R. prowazekii, R. australis, R. japonica, R. astrakhan,* and *R. felis* (Peniche-Lara et al., 2015a). These gram-negative bacteria require a vertebrate and invertebrate host to survive and reproduce (Yazid-Abdad et al., 2011). Clinical signs associated with infections from this group of microorganisms range from non-specific signs (such as fever, joint pain, headache, and vomiting) to more severe complications that sometimes even lead to death. These non-specific signs make diagnosis difficult and can lead to confusion with other diseases such as classic or haemorraghic dengue (Peniche-Lara et al., 2015a, 2015b).

Rickettsial pathogens can be successfully detected through a variety of animal and human clinical samples (biopsies, blood, and serum), as well as in many blood-sucking arthropods by ELISA, IFA, and PCR (Richards, 2012). As reviewed by Brown and Macaluso (2016), rickettsiosis has been documented in Africa, Asia, Australia, Europe, and North and South America. Specific countries in the Americas with reports of these pathogens include Argentina (Nava et al., 2008), Brazil (Horta et al., 2005), Chile (Labruna et al., 2007), Colombia (Ramírez-

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Hernández et al., 2013), Costa Rica, Guatemala (Troyo et al., 2012), Panama (Bermúdez et al., 2011), Peru (Flores-Mendoza et al., 2013), and Uruguay (Venzal et al., 2006).

In Mexico, few studies on rickettsial pathogens have been performed. Zavala-Velázquez et al. (2002) reported the cat-flea spotted fever agent *R. felis* by PCR/RFLP in *Ctenocephalides felis* obtained from dogs in the southeastern state of Yucatan (Zavala-Castro, 2013). Recently, Panti-May et al. (2014) detected *R. felis* DNA in the spleen of rodents and opossums by PCR, Peniche-Lara et al. (2015b) reported the agent in *C. felis* collected from dogs, and *R. felis* was reported by Pérez-Osorio et al. (2008) from fleas found inside houses. In contrast, in the northern part of the country, where the cases of rickettsiosis are increasing every year (Navarrete-Espinosa et al., 2015), no studies have been performed. Therefore, the objective of this study was to screen and identify rickettsial organisms in ectoparasites collected from dogs in a shelter in Gomez Palacio, Durango, Mexico.

2. Materials and methods

2.1. Study site

The study was performed in the city of Gomez Palacio, Durango, in the metropolitan area of La Laguna, located in the north central part of Mexico, latitude 25°34′ N and longitude − 103°30′O, at an altitude of 1150 m above the sea level, with a dry, semi-arid climate, summer rains ranging from 100 to 400 mm and mean temperature of 18–22 °C (INEGI, 2016) (Fig. 1).

2.2. Ethics statement and sample collection

The sampling procedures were carried out according to the guidelines of the Federal Law for Animal Health (LFSA, 2007). Samples were collected during three visits to the shelter from June to October 2011, and each animal was sampled only one time. One hundred dogs of different breeds and age, ranging from 1 month to 10 years, from the dog shelter at Gomez Palacio, state of Durango, Mexico, were inspected for the presence of ectoparasites, as described by Jafari-Shoorijeh et al. (2008). When ectoparasites (fleas, lice, or ticks) were found, a representative sample of five specimens of each parasite was collected, deposited in identified tubes, and transported live to the laboratory for identification using a stereoscopic microscope according to the keys for fleas (CDC, 1984), lice (Price and Graham, 1997) and ticks (Estrada-Peña et al., 2004). Once identified, the specimens of each arthropod group were deposited in 1.5 ml plastic sterile tubes and stored at − 30 °C until DNA extraction.

2.2. DNA extraction and PCR analysis.

DNA was extracted from each pool of arthropod tissues. The Tri-Reagent method was used following the specifications of the manufacturer (Invitrogen, Carlsban, CA, USA). The DNA was precipitated with ethanol, resuspended in nuclease free water (Ambion, Foster City, CA, USA), and stored at − 30 °C. PCR was performed on each sample to amplify the 16S rRNA, using oligonucleotides 16SANA-F (5′-CAG AGT

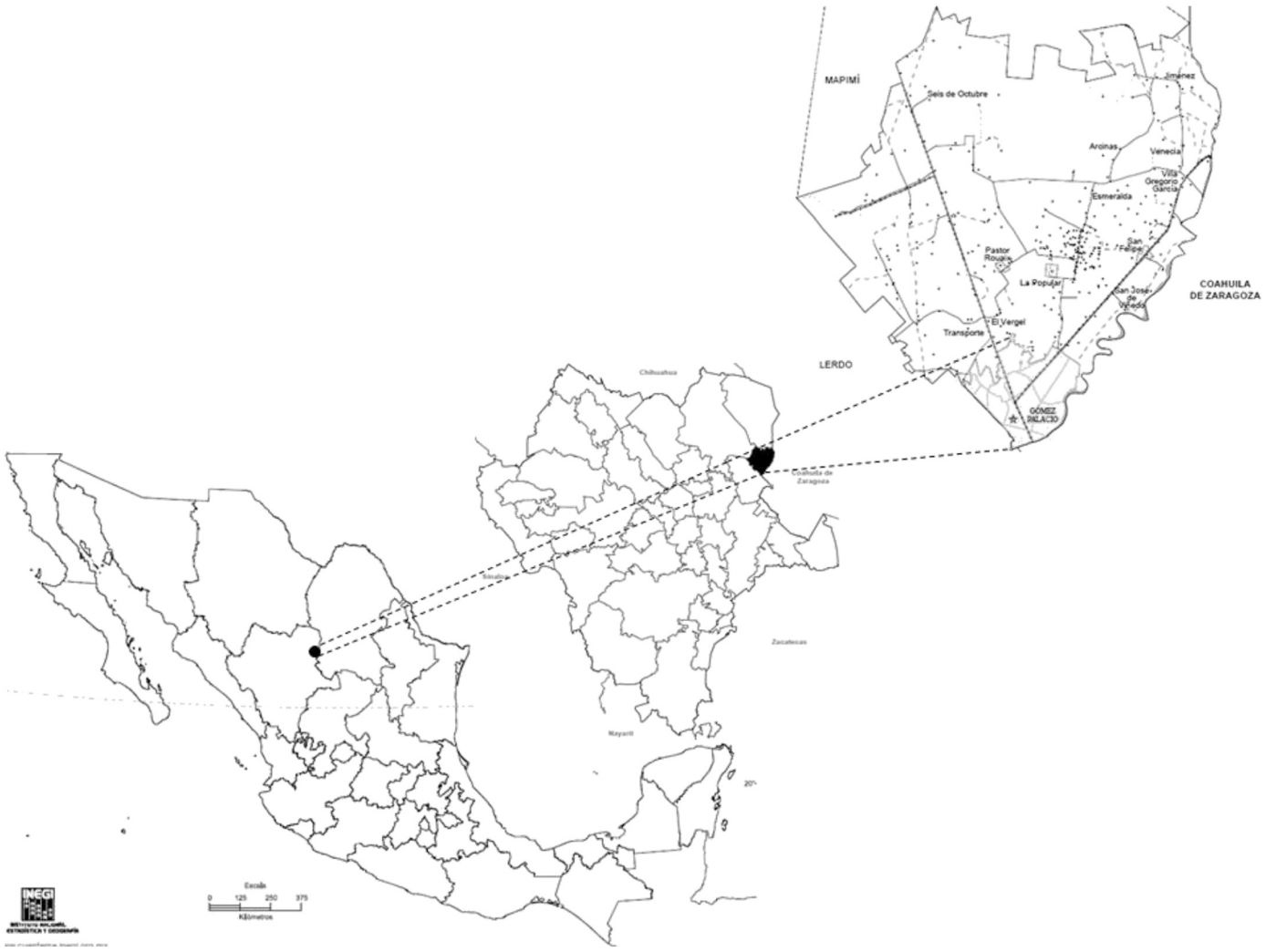

Fig. 1. Location of the study area.