Acta Tropica 167 (2017) 183–190

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Multiple-host pathogens in domestic hunting dogs in Nicaragua's Bosawás Biosphere Reserve

Christine V. Fiorello[a,*], Mary H. Straub[b], Laura M. Schwartz[c], James Liu[c,1], Amanda Campbell[c], Alexa K. Kownacki[b,2], Janet E. Foley[b]

[a] One Health Institute, USA
[b] Department of Medicine and Epidemiology, USA
[c] Veterinary Medical Teaching Hospital, One Shields Avenue, School of Veterinary Medicine, University of California, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form
18 December 2016
Accepted 18 December 2016
Available online 21 December 2016

Keywords:
Canine distemper virus
Disease spillover
Dog health
Hunting dogs
Jaguars
Nicaragua

ABSTRACT

Nicaragua's Bosawás Biosphere Reserve is a vast forested area inhabited largely by indigenous Mayangna and Miskitu people. Most Bosawás residents rely on subsistence hunting and swidden agriculture, and hunting dogs are important for finding and securing wild game. We investigated the health of hunting dogs in three communities differing in location, size, and economy. Dogs in all communities were nutritionally compromised and experienced a heavy burden of disease. Seroprevalence of canine distemper, canine parvovirus, *Rickettsia rickettsii*, and *Leptospira* spp. exceeded 50% of dogs. At least one dog was actively shedding leptospires in urine, and many dogs were anemic and/or dehydrated. These dogs interact with wildlife in the forest and humans and domestic livestock in the communities, and may therefore serve as sources of zoonotic and wildlife diseases. Bosawás represents one of the largest intact tracts of habitat for jaguars (*Panthera onca*) in Central America, and given that these communities are located within the forest, jaguars may be at risk from disease spillover from hunting dogs. Dog owners reported that four of 49 dogs had been attacked and killed by jaguars in the past year, and that retaliatory killing of jaguars was sometimes practiced. Disease spillover from dogs to wildlife could occur both in the course of dogs' hunting activities as well as during jaguar attacks. A better understanding of dog depredation by jaguars, pathogen exposure in jaguars, and a management strategy for the hunting dog population, are urgently needed to mitigate these dual threats to jaguars, improve the lives of hunting dogs, and safeguard the health of their owners.

1. Introduction

In the Bosawás Biosphere Reserve of Nicaragua, most residents are members of the Mayangna or Miskitu indigenous communities. Human and veterinary health infrastructures are severely limited, and the economy is largely based on subsistence hunting and agriculture (Stocks et al., 2007). Domestic dogs are integral to hunting activities and valued by their owners (Koster, 2008). Dogs in these communities are owned, but receive virtually no veterinary care and have short lifespans (Koster, 2007). Most households have at least one dog, and the typical dog spends about two hours per day outside of the community, accompanying its owner while assisting in locating and capturing prey species or for other reasons (e.g., collecting firewood) that allow for opportunistic prey encounters (Koster, 2007; Koster and Noss, 2014).

Although the dogs of Bosawás, like those of many indigenous communities, appear to be in poor health (Fiorello et al., 2006; Koster and Noss, 2014), there is no information in the literature on the disease burden of the canine population. Therefore, we conducted a pilot study to investigate the health and pathogen exposure of domestic dogs in the Reserve. We interviewed dog owners, subjected dogs to physical examinations, and collected biological samples from the dogs. We focused on zoonotic pathogens and those pathogens likely to infect both domestic and wild species, including canine distemper virus (CDV), canine parvovirus (CPV), *Rickettsia rickettsii*, *Ehrlichia* spp., *Anaplasma* spp., *Borrelia burgdorferi*, and *Leptospira* spp.

* Corresponding author at: One Health Institute, School of Veterinary Medicine, 1089 Veterinary Medicine Drive, Davis, CA 95616, USA.
E-mail address: cvfiorello@ucdavis.edu (C.V. Fiorello).
[1] Present address: Turtle Conservancy, 49 Bleeker St, Ste 601, New York, NY 10012, USA.
[2] Present address: Marine Mammal and Turtle Division, Southwest Fisheries Science Center, National Marine Fisheries Service, National Oceanic and Atmospheric Administration, La Jolla, CA 92037, USA.

http://dx.doi.org/10.1016/j.actatropica.2016.12.020
0001-706X/

2. Materials and methods

2.1. Study site

The Bosawás Reserve is located in wet lowland tropical forest in the North Caribbean Coast Autonomous Region (RACCN) of Nicaragua. Human population density is relatively low and communities consist of mestizo, Mayangna, and Miskitu settlements, although the indigenous communities predominate. We worked in one Miskitu and two Mayangna communities during June of 2013. Arang Dak (N14.515833, W84.999444) is a remote Mayangna settlement of about 35 households situated on the Rio Lakus (Fig. 1). Raiti (N14.594639, W85.027722) is a Miskitu town of approximately 300 households. Amak (N14.065417, W85.142233) is a busy Mayangna community of about 110 households located at the confluence of the Rios Amak and Bocay. Average household size is roughly five to seven people. Access to these communities is limited to riverways.

2.2. Recruitment of dogs

Upon arrival in a community, our team met with the community coordinator (highest local authority) to explain our intent and request permission to proceed with the study. After permission was granted, the community leaders disseminated the information throughout the town by word of mouth. Participation was voluntary and consent was given verbally. Many residents are functionally illiterate, making printed materials for written consent an inefficient use of resources. Dog owners were paid the equivalent of US$1 for the first dog and US$0.5 for each subsequent dog that they brought to participate in the project. Only dogs six months of age and older were enrolled to avoid interference with serologic tests by maternal antibodies.

2.3. Physical examinations and sample collection

Dogs were muzzled and manually restrained for the physical examination, which was performed by a veterinary student under the supervision of a veterinarian. Due to the presence of the muzzle, only a limited oral exam was possible. Hydration was rated on a scale of 1–3 (1 = adequate, 2 = mild-moderate (≤5%) dehydration and 3 = severe (>5%) dehydration). Body condition score (BCS) was assigned on a nine point scale (Mawby et al., 2004). Blood was collected from the jugular or cephalic vein and placed into EDTA and serum separator tubes. Urine was collected by cystocentesis. Ectoparasites were manually removed and placed into 70% ethanol for preservation prior to identification.

2.4. Sample processing

Blood in EDTA was placed into microhematocrit tubes for packed cell volume (PCV) and total protein (TP) determination, and used for patient-side testing for *Ehrlichia* (*E. canis* and *E. ewingii*) antibodies, *Anaplasma* (*A. platys* and *A. phagocytophilum*) antibodies, *Dirofilaria immitis* antigen, and *Borrelia burgdorferi* antibodies (SNAP 4 DDX test kits, IDEXX Laboratories, Westbrook, ME, USA). Remaining EDTA blood was aliquoted into cryotubes with 1.3 mL of a nucleic acid stabilizer (RNALater, Qiagen, Germantown, MD, USA). After centrifugation of serum separator tubes, serum was placed into cryotubes. Immediately after collection, a few drops of urine were placed directly into *Leptospira* Ellinghausen-McCullough-Johnson-Harris (EMJH) semi-solid culture media with 0.02% 5-fluorouracil and 0.01% cyclohexamide. All materials were stored at ambient temperature for up to two weeks, until arrival in the United States, when serum and whole blood were stored at minus 80 °C.

Whole blood from all dogs with detectable anti-*Ehrlichia* antibodies was tested for the presence of *E. canis* DNA using a Taqman PCR protocol (Doyle et al., 2005). First, DNA was extracted from blood using a commercially available kit (DNeasy Blood and Tissue Kit, Qiagen, Germantown, MD, USA). Then, 1 μL of DNA was added to a 12 μL reaction consisting of 6 μL Maxima Probe/ROX qPCR Master Mix (Thermo Scientific, Waltham, MA, USA), 0.6 μL Primer Probe Mix (yielding a final concentration of 0.4 μM for each primer and 0.2 μM for the probe), and 4.4 μL water. Two primers were used, following Doyle et al. (2005): *E. canis* DsbFor 5′TTGCAAAATGATGTCTGAAGATATGAAACA and *E. canis* DsbRev 5′GCTGCACCACCGATAAATGTATCCCCTA. The probe was *E. canis* Dsb 5′AGCTAGTGCTGCTGCTTGGGCAACTTTGAGTGAA. Amplification conditions were as follows: 50 °C for two minutes; 95 °C for 10 min; and 50 cycles of 95 °C for 15 s, followed by 60 °C for one minute.

Antibodies against CDV, CPV, and *Rickettsia rickettsii* (causative agent of Rocky Mountain spotted fever, RMSF) were detected using indirect fluorescent antibody (IFA) tests. Specificity and sensitivity of IFA are considered comparable to that of serum neutralization, the gold standard (Greene and Appel, 2006). Serum was diluted to 1:25 in phosphate-buffered saline (PBS) and applied to the wells of commercially available substrate slides containing cells infected with the agent of interest (VMRD, Pullman, WA, USA). Slides were incubated at 37 °C for 25 min, washed three times in PBS and blotted dry before adding goat anti-dog IgG heavy and light chain conjugated to FITC (Kierkegaard-Perry, Gaithersburg, MD, USA) at 1:100 dilution, and incubating them at 37 °C for 25 min. They were then washed three additional times in PBS, counterstained with Eriochrome black, cover-slipped with 10% glycerol, and examined under ultraviolet epifluorescent microscopy. All slides were compared to both positive and negative controls, and were evaluated by at least two people. Weak positive results were repeated for confirmation.

Urine leptospire culture tubes were incubated at 30 °C and evaluated by dark-field microscopy for leptospiral growth every two weeks for six months. Cultures were also evaluated by real-time PCR every month for the presence of leptospiral DNA (Stoddard et al., 2009) by first boiling 100 μL of culture media for 10 min and then extracting DNA using a kit following manufacturer's instructions for blood extraction (Qiagen DNeasy Blood and Tissue Kit, Qiagen, Germantown, MD, USA). Positive culture samples were analyzed genetically using a multilocus sequence typing (MLST) scheme to further characterize the organism (Boonsilp et al., 2013). The microscopic agglutination test (MAT) (WHO Lepto Guide, 2006) was used to evaluate dogs for antibodies against seven serovars of *Leptospira*; *L. interrogans* serovars Pomona (serogroup Pomona), Hardjo type Prajitno (serogroup Sejroe), Canicola (serogroup Canicola), Copenhageni (serogroup Icterohaemorrhagiae), and Bratislava (serogroup Australis); *L. kirschneri* serovar Grippotyphosa (serogroup Grippotyphosa); and *L. santarosai* serovar Georgia (serogroup Mini). Samples with a reciprocal titer of 100 or greater to any serovar were considered positive.

2.5. Survey

We administered a semi-structured survey to dog owners whose dogs were enrolled in the study (Appendix A). The surveys were executed orally using an interpreter, who spoke Spanish, Mayangna, and Miskitu.

2.6. Statistics

Statistical analyses were performed using SPSS Version 22.0 for Macintosh (IBM Corp. 2013) and R (R Core Team, 2013). Summary statistics were calculated for demographic and health parameter