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Research paper

Diversity of rickettsiae in a rural community in northern California

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ABSTRACT

Far northern California forests are highly biodiverse in wildlife reservoirs and arthropod vectors that may propagate rickettsial pathogens in nature. The proximity of small rural communities to these forests puts people and domestic animals at risk of vector-borne infection due to spillover from wildlife. The current study was conducted to document exposure to rickettsial pathogens in people and domestic animals in a rural community, and identify which rickettsiae are present in sylvatic and peri-domestic environments near this community. Blood samples from people, domestic animals (dogs, cats, and horses) and wild carnivores were tested for *Rickettsia* spp. antibodies and DNA (people and domestic animals only) by serology and real time (RT)-PCR, respectively. Ectoparasites were collected from dogs, wild carnivores and from vegetation by flagging, and tested for *Rickettsia* spp. DNA by RT-PCR. DNA sequencing of the rickettsial 17 kDa protein gene or the *ompA* gene was used for species identification. Despite a seroprevalence of 3% in people, 42% in dogs, 79% in cats, 33% in gray foxes, and 83% in bobcats, RT-PCR on blood was consistently negative, likely because the sensitivity of this test is low, as *Rickettsia* spp. do not often circulate in high numbers in the blood. *Rickettsia* spp. DNA was found in four flea species collected from bobcats and *Ctenocephalides felis* collected from domestic dogs. All amplicons sequenced from fleas were *R. felis*. *Ixodes pacificus* collected by flagging were commonly infected with a *Rickettsia* sp. endosymbiont. *Rickettsia rhipicephali* DNA was found in *Dermacentor variabilis* from dogs, black bears, a gray fox, and a *D. occidentalis* collected by flagging. *Dermacentor variabilis* from dogs and black bears also contained *R. montanensis* DNA. Multiple *Rickettsia* spp. (including species with zoonotic and pathogenic potential) were found among human biting arthropod vectors of both wild and domestic carnivores and on flags. Knowledge of the diversity of *Rickettsia* spp. that are present within arthropod vectors to which people and domestic animals are exposed is an essential first step is making an accurate diagnosis and in better understanding the epidemiology of these potential pathogens. Within-host and vector interaction among these species may play a role in spillover into human and domestic animals.

1. Introduction

Rickettsiae are obligately intracellular, pleomorphic bacteria that can cause disease in humans and other animals (Parola et al., 2005). These organisms can be transmitted by arthropod vectors (e.g. fleas, ticks, mites, and lice) to their mammal hosts. Various classifications have been proposed, but recently rickettsiae have been organized into four groups: the pathogenic spotted fever group (SFG) and typhus group, and the non-pathogenic *Rickettsia bellii* group and *Rickettsia canadensis* group (Merhej and Raoult, 2011). Multiple species of SFG rickettsiae are found in California, including *R. rickettsii* (the causative agent of Rocky Mountain spotted fever), *R. philippi, R. rhipicephali, R. felis* and *R. typhi* (Parola et al., 2005). Rickettsial infections can be fatal (even in otherwise healthy individuals), despite the availability of effective treatment, due to

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diagnostic challenges and delayed treatment (Bakken et al., 2006; Palmer and Azad, 2012).

Despite surveillance and research programs in many states targeting human cases and to a lesser extent testing of wildlife and vectors, numerous aspects of sylvatic cycles of these diseases remain poorly understood. These cycles comprise a high diversity of wildlife species and various pathogen strains, thus allowing opportunities for co-infection, and host-generalist and specialist vectors. The forests of northern California are highly biodiverse, providing habitat for numerous wildlife and arthropod reservoirs that maintain sylvatic cycles of rickettsial pathogens. In addition, many residents of rural northern California suffer from health disparities including lower socioeconomic status, being under-served minorities, and having limited access to health care. These same residents may also have high exposure to pathogen vectors through both occupation (forestry, agriculture, etc.) and recreation (hunting, fishing, hiking, camping). Early symptoms of vector-borne diseases are often mild or nonspecific, and this can lead to delayed, or misdiagnosis, even in at-risk communities.

We performed a cross-sectional epidemiologic study to determine exposure to rickettsial pathogens in people, domestic animals and wild carnivores in a rural community. In addition, we tested arthropod vectors (including human-biting ticks and fleas) collected from domestic dogs and wildlife to determine which rickettsiae are present in the sylvatic and peri-domestic environments near this community in Humboldt County, California, and could pose a risk to humans and domestic animals. By determining the presence of and exposure to multiple rickettsiae, we can better understand their impact on human and animal health in this rural northern California community and help pave the way for more effective surveillance, prevention, control and diagnostic measures.

2. Methods

2.1. Study area

Humboldt County is a densely forested, mountainous, rural county located in northern California along the coastline. Elevations range from sea level along the Pacific coast to 1170 m in the nearby mountain ranges. Vegetation in forested areas primarily consists of redwood (*Sequoia sempervirens*), Ponderosa pine (*Pinus ponderosa*), Douglas fir (*Pseudotsuga menziesii*), tanoak (*Notholithocarpus densiflorus*), madrone (*Arbutus menziesii*), Oregon white oak (*Quercus garryana*), California black oak (*Quercus kelloggii*), evergreen huckleberry (*Vaccinium ovatum*), tobacco brush (*Ceanothus velutinus*), salal (*Gaultheria shallon*), and poison oak (*Toxicodendron diversilobum*). The non-forested areas include residential areas, natural prairies, large rock outcrops, and costal beaches. Forestry is a major industry in Humboldt County: Humboldt is the largest timber-producing county in California, responsible for almost 20% of all timber production in the state (Laaksonen-Craig et al., 2003).

Humboldt County includes eight Native American reservations (United States Census Bureau, 2010) and several areas are designated medically underserved including the Arcata, Ferndale, Garberville, Redway, Mckinleyville, and North Coastal service areas (Healthcare Workforce Development Division, 2010). 19.5% of the population of Humboldt County was living below the poverty level in 2000 compared to 14.2 statewide and 12.4 nationally (VanArsdale and Barry, 2008). The median household income for the county was 42,153 in 2010 compare to 61, 489 for California (United States Census Bureau, 2010).

2.2. Participant recruitment and sampling

Participants and their domestic animals were recruited throughout Humboldt County, California by word-of-mouth and by posting flyers in visible, high-traffic areas. During previous fieldwork and interactions with residents of Humboldt County, several groups of individuals had expressed a concern for their exposure to ticks and potential tick-borne diseases and an effort was made to include them in the study. These groups included forestry workers, wildlife biologists, college students interested in wildlife research, and Native Americans living in close proximity to forested areas. Recruitment events were scheduled to coincide with other community activities where participants were also recruited using a script. After receiving informed consent from participants, basic demographic information and whole blood samples were collected by a licensed nurse. In conjunction, veterinarians and veterinary students collected blood samples and ectoparasites from participant's dogs, cats, and horses as part of no-cost rural area veterinary clinics offering routine veterinary care and vaccinations. Blood samples and ectoparasites were collected from wild carnivores and from vegetation by flagging as part of various other studies. Collected ectoparasites were stored in 70% ethanol at room temperature. Blood samples were collected into sterile tubes containing EDTA and stored at 4 °C until plasma was separated and stored at −20 °C until testing. All work was performed under appropriate guidance and permits from the University of California, Davis Institutional Review Board, Institutional Animal Care and Use Committee and the California Department of Fish and Wildlife scientific collection permits.

2.3. Blood DNA extraction and real time-PCR

DNA was extracted from 100 μl of whole blood using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following the blood, spin-column protocol. A sensitive and specific real time (RT)-PCR assay for the detection of *Rickettsia* genus DNA (including both SFG and typhus group) was performed as described (Stenos et al., 2005) (Table 1). Samples were considered positive if the threshold cycle (CT) <40 with a characteristic amplification curve. Three negative water controls and a sequence-confirmed *R. prowazekii* positive control were included in each run.

2.4. Serology

Indirect immunofluorescence assays (IFA) were performed for antibodies to *R. rickettsii* on all human and domestic animals and on wild carnivores when a blood samples was available. Due to cross-reactivity, positive results are not considered species-specific (Bakken et al., 2006). Plasma was diluted in phosphate-buffered saline (PBS) at 1:64 and applied to commercial slides (VMRD, Pullman, WA). Slides were incubated at 37 °C with moisture for 30 min and washed three times with PBS. They were then incubated for 30 min with fluorescein-labeled, immunoglobulin G heavy and light chain conjugate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) diluted in PBS at 1:100 for humans and domestic animals. Wherever possible, we used species-specific secondary antibodies. For wildlife for which species-specific antibodies were not available, anti-dog conjugate was used for gray fox and raccoons, and for bobcats we used anti-cat conjugate following the same procedure. Slides were washed three additional times and counter-stained with eriochrome black. Positive and negative control serum was included in each batch. Samples were considered positive if they had strong fluorescence detected compatible with the morphology of the antigen on the slide.

2.5. Ectoparasite identification, DNA extraction, and real-time PCR

Ticks were identified to species and sex using California taxonomic keys (Furman and Loomis, 1984). DNA was extracted from

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