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Elevation and vegetation determine *Cryptosporidium* oocyst shedding by yellow-bellied marmots (*Marmota flaviventris*) in the Sierra Nevada Mountains

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ABSTRACT

Wildlife are increasingly recognized as important biological reservoirs of zoonotic species of *Cryptosporidium* that might contaminate water and cause human exposure to this protozoal parasite. The habitat range of the yellow-bellied marmot (*Marmota flaviventris*) overlaps extensively with the watershed boundaries of municipal water supplies for California communities along the foothills of the Sierra Nevada. We conducted a cross-sectional epidemiological study to estimate the fecal shedding of *Cryptosporidium* oocysts by yellow-bellied marmots and to quantify the environmental loading rate and determine risk factors for *Cryptosporidium* fecal shedding in this montane wildlife species. The observed proportion of *Cryptosporidium* positive fecal samples was 14.7% (33/224, positive number relative to total number samples) and the environmental loading rate was estimated to be 10,693 oocysts animal^{-1} day^{-1}. Fecal shedding was associated with the elevation and vegetation status of their habitat. Based on a portion of the 18s rRNA gene sequence of 2 isolates, the *Cryptosporidium* found in *Marmota flaviventris* were 99.88%–100% match to multiple isolates of *C. parvum* in the GenBank.

1. Introduction

The protozoa of the genus *Cryptosporidium* are obligate, intracellular parasites affecting a large number of vertebrate species including humans. *Cryptosporidium* oocysts are resistant to chlorine disinfection and can cause waterborne enteric disease. Even though humans and livestock are considered major biological reservoirs of *Cryptosporidium* oocysts (Mac Kenzie et al., 1994; Xiao and Ryan, 2004; Atwill et al., 2006a; Feltus et al., 2006; Nichols et al., 2006; Brook et al., 2009; Santin, 2013), wildlife are increasingly recognized as significant sources of water contamination with this parasite (Jiang et al., 2005; Feng et al., 2007; Ruecker et al., 2007, 2012; Chalmers et al., 2010). Wild rodents have been identified as hosts for several *Cryptosporidium* species, including species causing disease in humans (Morgan et al., 1999; Hajdusek et al., 2004; Feng et al., 2007; Kimura et al., 2007; Meireles et al., 2007; Raskova et al., 2013). Specifically in the *Marmota* genus, reported *Cryptosporidium* species include *C. andersoni* and *C. ubiquitum* (Ryan et al., 2003; Feng et al., 2007), suspected *C. parvum* (Atwill et al., 2003), as well as undetermined species (Ziegler et al., 2007).

The Sierra Nevada is a mountain range extending along the border between California and Nevada with the Sacramento and San Joaquin Valleys to the west and the Basin and Range region to the east (Fig. 1). The Sierra Nevada Range annual snowpack and the surface water generated from snowmelt constitute major sources of municipal and recreational water for California. Above 6,000 feet (~1830 meters), this mountain range encompasses the habitats of several species of mammals including the yellow-bellied marmot (*Marmota flaviventris*) (Sumner and Dixon, 1953). Given the extensive spatial overlap between the range of this rodent and the watershed boundaries for municipal water supplies for California communities along the foothills of the Sierra Nevada, we conducted a cross-sectional epidemiological study to estimate the prevalence of *Cryptosporidium* in yellow-bellied marmots and quantify the Environmental Loading Rate [ELR] (Atwill et al., 2003, 2004) of oocysts from these rodents. Through the construction of a hierarchical Bayesian logistic regression, we tested if habitat characteristics are associated with *Cryptosporidium* oocyst shedding in these rodents.

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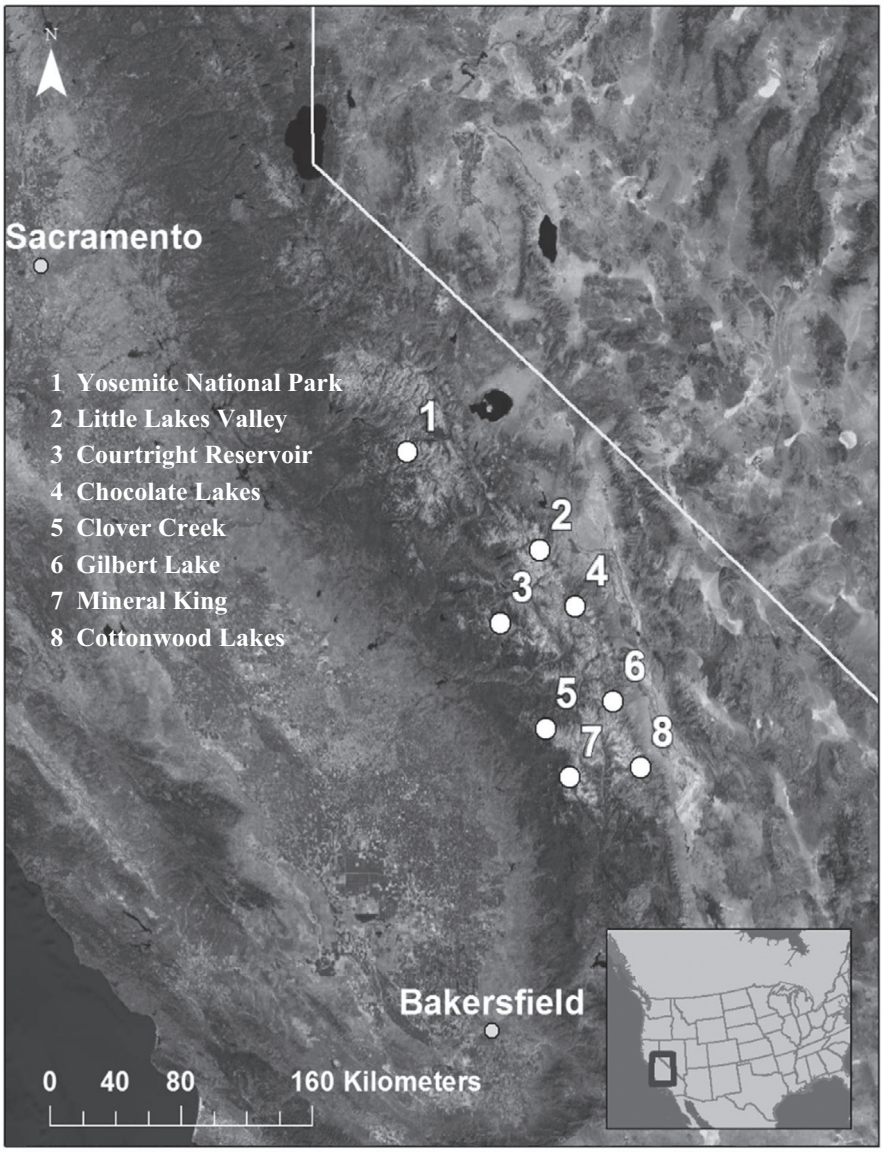

Fig. 1. Locations in the Sierra Nevada Mountains where scat samples from yellow-bellied marmot were collected. The map contains 14 locations, but some locations clustered together due to the shorter distances between them and the scale of the map. The cluster in Yosemite National Park includes Tenaya Creek, Bridaveil Creek, Pothole Dome, and Lembert Dome. The cluster at Mineral King includes Mineral King, Eagle Lake, White Chief Creek, and Elk Kaweah River.

2. Materials and methods

2.1. Sample collection

Naturally voided fecal samples were collected opportunistically at 14 different locations between June and July 2012 from sites where yellow-bellied marmots defecate. These locations were in the Sequoia National Park, Inyo National Forest, Sierra National Forest, and Yosemite National Park, extending approximately 200 kilometers from north to south, at the eastern and western sides of the Sierra Nevada, California (Table 1, Fig. 1). These locations were selected as suitable habitat for yellow-bellied marmots or were locations where marmots were previously reported. We visited each location once, collecting fresh scat samples found within 4–5 hours. The recognition of feces as from yellow-bellied marmot was based on sighting individuals at every location and by using descriptions and characterizations of marmot scat (Murie and Elbroch, 2005). Only adult sized scat samples were collected in order to avoid collecting feces from other smaller rodent species. To obtain only fresh fecal samples, samples were selected from scat that were moist and with flies present. In order to minimize the chance of sampling feces from the same animal, only one scat was selected from each site within a location, and no additional samples were collected within 20 m diameter surrounding the scat sampled. Approximately 1.6 g of fecal sample was collected from each sampling site.

Fecal samples were placed into 50 ml polypropylene sterile centrifuge tubes containing 5 ml of antibiotic storage solution (0.1 ml 10% Tween 20, 0.006 g penicillin G, 0.01 g streptomycin sulfate, 1.0 ml amphotericin B solution in 100 ml reagent-grade water). Tubes were kept on ice at 4 °C until transported to the laboratory at University of California at Davis. For each sampling site, coordinates and elevation (ft) above sea level were obtained using a GPS device GPSMAP® 76Cx (Garmin, Olathe, KS, USA) with an accuracy of ≤ 5 m. Location and date of collection were recorded.

2.2. Environmental parameters

Occurrence of vegetation at the sampling sites was recorded as two categories: no vegetation due to predominately rocky substrate; and vegetation, predominately meadow of grasses and sedges