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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.

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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 human 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 is 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.
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.
or woody species such as trees. The vegetation category assigned to each sample site was based on direct visual observation and supported by the Normalized Difference Vegetation Index developed by the National Agriculture Imagery Program (California Department of Fish and Wildlife, 2010). Lastly, the mode of human access to each sampling site was recorded as trail, if hiking was necessary to reach the site, or dirt/paved road if the site was accessible by vehicle. We used this variable as an index of human presence in the marmot habitats in order to evaluate its association with Cryptosporidium oocysts in the feces. In sampling locations accessible by a dirt/paved road, people are more likely to pass through the location in vehicles, while the presence of a hiking trail suggests more chances of people walking across the location and potentially defecating.

2.3. Detecting Cryptosporidium oocysts

The detection of Cryptosporidium oocysts from fecal samples was based on published methods (Kuczynska and Shelton, 1999; Pereira et al., 1999) with modifications. Briefly, feces were weighed, suspended in PBS, and strained into 50 ml centrifuge tubes through 4 layers of cotton gauze. Tubes were centrifuged at 1500 g for 15 min, supernatant was discarded, and pellet was resuspended in 5 ml of deionized water. The fecal solution was mixed with 30 ml of sucrose solution (specific gravity ~1.2) and centrifuged at 1000 g for 25 min. Oocysts were collected from the top of sucrose solution by overlying 5 ml of deionized water, gently stirring, and pipetting 5 ml from the top. Oocysts were washed by mixing with deionized water and centrifuging at 1500 g for 15 min. The supernatant was discarded by aspiration, leaving a 1:1 ratio of pellet to solution volume. This final solution was weighed and 10 μl was overlaid on commercially prepared slides (Waterborne Inc., New Orleans, LA) and weighed too. Smears were air dried, stained using a direct immuno-fluorescence antibody kit (Meridian Bioscience, Inc., Cincinnati, OH), and examined using an Olympus BX60 microscope at ×400 magnification. Slides containing one or more oocytes were recorded as positive while those without detectable oocytes were recorded as negative. The crude number of oocytes per gram feces (O), unadjusted by assay percent recovery, was calculated according to the formula: \( O = \frac{[N \times (W_{\text{in}}/W_{\text{sm}})]}{F} \), where \( N \) is the number of oocytes in the smear, \( W_{\text{in}} \) and \( W_{\text{sm}} \) are the suspension and smear weight respectively, and \( F \) is the fecal weight. Assuming daily consistent oocyst shedding, the Environmental Loading Rate (ELR) was calculated using the mean number of oocysts per gram of feces found in this study and a crude estimate for total daily fecal production estimated as ~3% of mean body mass or 0.02 kg feces wet weight per day for a typical adult marmot (Atwill et al., 2003).

2.4. Statistical analysis

In order to evaluate the effect of environmental parameters on the likelihood of detecting Cryptosporidium oocysts in marmot feces, a hierarchical Bayesian logistic regression model was constructed using JAGS 3.4.0 (http://mcmc-jags.sourceforge.net, [9–15–10], [Plummer, M.], in R 2.15.1 (http://www.r-project.org, [10–10–11], [Development Core Team]), with R2jags 0.03–08 (http://cran.r-project.org/web/packages/R2jags/index.html, [11–17–2012], [Yu-Su and Masanao]) as the interface. The outcome \( y_{ij} \) was the presence of Cryptosporidium oocysts (0 = no oocysts / 1 = oocysts present) in the \( n \) feces collected at the \( j \) location: \( y_{ij} \sim \text{Bernoulli} (p_{ij}) \), where the probability of Cryptosporidium oocysts being present in the ith yellow-bellied marmot feces sampled at location \( j \), \( p_{ij} \) was related to a suite of q fixed predictors at the feces level: elevation and vegetation.

\[
\logit (p_{ij}) = \alpha_{0j} + \beta X_{1j} + \cdots + \beta_{q} X_{qj},
\]

where \( \alpha_{0j} \) are random intercepts shared by feces belonging to location \( j \), defined as:

\[
a_{j} \sim \text{Normal} (\beta_{0} + \gamma_{1} r_{j}, \sigma^{2})
\]

where \( \gamma_{1} r_{j} \) is the group level predictor mode of human access used to reach each sampling location. We selected a hierarchical model because yellow-bellied marmot fecal samples were grouped by sampling location. This grouping was based on the assumption that the probability of Cryptosporidium infection in marmots from the same location may not be independent from each other and therefore may exhibit a level of positive correlation in and above what is accounted for in a fixed effects regression model. Moreover, a Bayesian approach is preferred over the frequentist because the former can avoid problems of identification when the multilevel model is complicated.

Given the continuous nature of the variable elevation, we evaluated graphically the assumption of linearity with respect to the log odds of a fecal sample testing positive for Cryptosporidium oocysts by categorizing elevation into 4 strata based on quartiles of the likelihood of detecting Cryptosporidium oocysts across 4 strata based on quartiles to get an equal number of observations per stratum elevation: 6,985–9,265 (reference level), 8,265–9,068, 9,068–10,057, and >10,057 ft. Across these four strata, there was a non-linear association between elevation and the log odds of Cryptosporidium oocysts shedding, therefore a quadratic term for elevation was considered. Moreover, to determine if effect modification was present between vegetation and elevation, the full dataset was stratified by vegetation status and the relationship reevaluated between elevation and the log odds of Cryptosporidium shedding within each strata.

### Table 1

The locations where marmot scat samples were collected in Sierra Nevada Mountains.

| Area                        | Sampling location                 | Elevation range | Latitude    | Longitude   |
|-----------------------------|----------------------------------|-----------------|-------------|-------------|
| Sierra National Forest      | Courtright Reservoir             | 8131-8365       | 37° 10' 8.94'' N | 118° 33' 57.06'' W |
| Sequoia National Park       | Mineral King                     | 7567-8089       | 36° 27' 2.25'' N | 118° 35' 41.1216'' W |
|                            | Eagle Lake                       | 9319-10144      | 36° 24' 52.6824'' N | 118° 36' 22.7664'' W |
|                            | White Chief Creek                | 8980-9561       | 36° 25' 25.1364'' N | 118° 34' 30.2412'' W |
|                            | Elk Kaweah River                 | 8316-9380       | 36° 24' 53.1072'' N | 118° 35' 11.0976'' W |
|                            | Coyote Creek                     | 8801-9286       | 36° 39' 23.5296'' N | 118° 43' 24.7224'' W |
| Inyo Forest                 | Cottonwood Lakes                | 11003-11073     | 36° 29' 30.7428'' N | 118° 12' 24.6168'' W |
|                            | Gilbert Lake                     | 10478-10538     | 36° 46' 11.4492'' N | 118° 21' 20.6928'' W |
|                            | Chocolate Lakes                  | 10112           | 37° 0' 1.728'' N   | 118° 58' 21.9036'' W |
|                            | Little Lakes Valley              | 10092-10728     | 37° 24' 20.2356'' N | 118° 45' 31.5612'' W |
| Yosemite National Park      | Tenaya Creek                     | 8421-8696       | 37° 48' 45.6228'' N | 119° 29' 6.7632'' W |
|                            | Bridalveil Creek                 | 6985-7164       | 37° 39' 41.76'' N   | 119° 37' 7.464'' W |
|                            | Pothole Dome                     | 8704-9688       | 37° 52' 56.5428'' N | 119° 22' 31.9224'' W |
|                            | Lembert Dome                     | 8610-8941       | 37° 52' 49.0088'' N | 119° 21' 2.7756'' W |

\(^{a}\) Only one sample.
For model construction, the variable elevation was centered at its mean: 9,068 ft, and the quadratic elevation term was constructed from these centered values. Non-informative priors or hyperpriors were assigned for $\beta_0$, $\beta_1$ and $\tau$ and $\sigma^2$: Normal (0, 25) for the first three parameters and LogNormal (0, 1) for the last one. Posterior distributions for all parameters were sampled from each of three chains for 60,000 iterations following a 10,000 iteration burn-in, and thinning set to 5, for a total of 30,000 samples. Each of three chains for 60,000 iterations following a 10,000 iteration burn-in, and thinning set to 5, for a total of 30,000 samples. Each chain was assigned random start values from a Normal (0, 1) distribution for $\beta_0$ and $\beta_1$ and a Uniform (0,1) for $\sigma$. Convergence was assessed by the Gelman–Rubin statistic (Gelman and Rubin, 1992). A backward stepping procedure was used to select terms for the final model, starting with a full model containing the random in-

### 3. Results

#### 3.1. Fecal shedding of Cryptosporidium oocysts

A total of 224 fecal samples were collected. Thirty three (~15%) fecal samples had detectable levels of *Cryptosporidium* oocysts (95% CI: 10.0–19.4%). Among these 33 positive fecal samples, 22 had one oocyst, 6 had 2 oocysts, 2 had 4 oocysts and one of each had 8, 26 and 35 oocysts per smear. This distribution of oocyst shedding intensity resulted in a mean of 535 oocysts per gram of feces wet weight (95% CI: 204–865), ranging from 35 to 1,646 and a median value of 129 oocysts per gram of feces. The mean ELR was estimated as 10,693 *Cryptosporidium* oocysts per adult marmot per day.

Elevations of the sample collection sites ranged from 6,985 to 11,070 ft, with a median elevation of 9,068 ft. Total sample size of sites with and without vegetation was 158 and 66 respectively, with a median elevation of 8,657 and 9,374 ft, respectively. Bridaveil Creek, Chocolate Lakes, Courtright Reservoir, Lambert Dome, Pothole Dome, and Tenaya Creek were accessed from dirt/paved roads and the remaining locations were reached through hiking trails. The prevalences of *Cryptosporidium* oocysts in fecal samples collected at sites with and without vegetation were 11% and 23%, respectively. A summary of the number of samples collected by location and the positive proportion is presented in Table 2.

#### 3.2. Environmental parameters on fecal shedding of oocysts

Parameter estimates and odds ratios [OR] from the final regression model are presented in Table 3. In the final regression model, the estimates for both two-way interactions were evaluated: the centered elevation and vegetation status, and the square of centered elevation with vegetation status, resulting in credible OR. As a consequence of these credible OR, the variables elevation, quadratic elevation and vegetation status were retained in the selected final model. Simpler models without these interaction terms resulted in larger DICs and the non-credibility of the remaining parameters. The mode of human access to the marmot site was kept in the final model as a confounder because it adjusted elevation’s mean parameter estimate by approximately 14%.

The association between elevation and the odds for *Cryptosporidium* oocysts in marmot feces was strongly influenced by the presence or absence of vegetation at the sampling sites. When vegetation was present, the odds of detecting oocysts decreased as

### Table 2

| Location          | Total | Overall prev. [%] (95% CI) | Vegetation | No vegetation |
|-------------------|-------|---------------------------|------------|---------------|
|                   | Total | Pos. | Prev. [%] (95% CI) | Total | Pos. | Prev. [%] (95% CI) |
| Bridaveil Creek   | 11    | 18 (2–52) | 6 (2) | 33 (4–78) | 5 | 0 | 0 (0–52) |
| Elk Kaweah River  | 11    | 18 (2–52) | 10 (2) | 20 (3–56) | 1 | 0 | 0 (0–98) |
| Courtright Reservoir | 39   | 10 (3–24) | 29 (3) | 10 (2–27) | 10 | 1 | 10 (0–45) |
| Tenaya Creek      | 20    | 30 (12–54) | 1 (0) | 0 (0–98) | 19 | 6 | 32 (13–57) |
| Lambert Dome      | 12    | 25 (5–57) | 3 (1) | 33 (1–91) | 9 | 2 | 22 (3–60) |
| Pothole Dome      | 7     | 43 (10–82) | 0 (0) | - | 7 | 3 | 43 (10–82) |
| Mineral King      | 16    | 19 (4–46) | 16 (3) | 19 (4–46) | 0 | 0 | - |
| Clover Creek      | 7     | 43 (10–82) | 0 (0) | - | 7 | 3 | 43 (10–82) |
| White Chief Creek | 25    | 12 (3–31) | 24 (3) | 12 (3–32) | 1 | 0 | 0 (0–98) |
| Eagle Lake        | 23    | 0 (0–15) | 18 (0) | 0 (0–19) | 5 | 0 | 0 (0–52) |
| Chocolate Lakes   | 1     | 0 (0–98) | 1 (0) | 0 (0–98) | 0 | 0 | - |
| Gilbert Lake      | 3     | 0 (0–71) | 1 (0) | 0 (0–98) | 2 | 0 | 0 (0–84) |
| Little Lakes Valley | 6 | 0 (0–46) | 6 (0) | 0 (0–46) | 0 | 0 | - |
| Cottonwood Lakes  | 43    | 9 (3–22) | 43 (4) | 9 (3–22) | 0 | 0 | - |
| **TOTAL**         | 224   | 15 (10–20) | 158 (18 | 11 (7–17) | 66 | 15 | 23 (13–35) |

in the GenBank using the National Center for Biotechnology Information [NCBI] online blasting tool (http://blast.ncbi.nlm.nih.gov/).
Table 3
Estimated Bayesian hierarchical logistic regression coefficients for environmental factors associated with Cryptosporidium oocysts in feces of yellow-bellied marmots in the Sierra Nevada Mountains, California.

| Parameter | Level | Coefficient (90% CrI) | Odds ratio (90% CrI) |
|-----------|-------|-----------------------|---------------------|
| Intercept |       | -2.110 (−2.942, −1.291) | 0.121 (0.053, 0.275) |
| Vegetation | Absence | 1.111 (−0.363, 2.454) | 3.038 (0.695, 11.638) |
| Elevation | Presence | 0 | 1 |
| Elevation² | Presence | −0.524 (−1.050, −0.018) | 0.592 (0.350, 0.982) |
| Elevation × Vegetation | Absence | −3.013 (−6.555, −0.046) | 0.049 (0.001, 0.955) |
| Elevation² × Vegetation | Absence | −5.208 (−9.357, −1.828) | 0.005 (0.0001, 0.161) |
| Access Road | Presence | −0.346 (−1.549, 0.867) | 1.413 (0.213, 2.380) |
| Access Trail | Presence | 0 | 1 |

σ² among locations: 0.473 (0.659, 0.750)

3.3. PCR and sequencing
Amplification and sequencing of a fragment of the 18S rRNA gene were successful for two of the microscopic positive samples. The two samples were collected from Bridalveil Creek in Yosemite National Park (isolate 1) and Mineral King in Sequoia National Park (isolate 2). Sequence analysis of the two Cryptosporidium isolates demonstrates that they were 99.76% (828/830 bases) similar to each other. Given that the Cryptosporidium oocysts were isolated from Marmota flaviventris in 2012, the two isolates were named as Mflav12a(1) and Mflav12a(2), respectively. The GenBank accession numbers of the two isolates are KF626380 for Mflav 12a (1) (isolate 1) and KF626381 for Mflav 12a (2) (isolate 2), respectively. Using the NCBI's default algorithm parameters to target maximum 100 sequences in GenBank, the Mflav12a(1) isolate was 100% similar to a C. parvum isolate (KM225275) and 99.88% similar to C. parvum isolates of the most closely related 10 entries in GenBank (Table 4). Similarly, the Mflav12a(2) isolate was 100% identical to two C. parvum isolates (JN247404 and KC476546) and 99.88% similar to C. parvum isolates of the other 8 entries in GenBank (Table 4) (based on BLAST analysis completed on December 29, 2014).

4. Discussion
To standardize the sampling procedure, we searched for scat samples at each location during a similar period of time; however, the number of samples by location varied due to the difference in abundance of marmots at each location. We sampled naturally voided fecal deposits and collected only one scat sample per site so as to site elevation increased (Table 3). For example, at sites where access was via a hiking trail, the odds of detecting oocysts in marmot feces was about 5 times more likely at sites at 7,000 ft elevation compared to feces collected at sites of 9,000 ft elevation. In contrast, for fecal samples collected at rocky location with minimal vegetation, it was rare to find fecal samples with Cryptosporidium oocysts from sites at the extreme lower or higher elevations. Instead, positive samples clustered in the middle to upper 8,000 ft range (Fig. 2). The variance among random intercepts was estimated at 0.473; however, the CrI for this estimate covered zero, suggesting that significantly different random intercepts for locations were not present.
minimize repeated sampling of the same animal. The home range of yellow-bellied marmots can vary in size and shape (Armitage, 2009) and they can live in colonies or as satellites (Armitage and Downhower, 1974), thus it is not possible to guarantee that each fecal sample belongs to different individuals. Nevertheless, point prevalence estimated in this study (~15%) is similar to the prevalence reported from studies on related mammalian species (Feng et al., 2007; Ziegler et al., 2007; Feng, 2010). The ELR as reported previously for this host species was 208,000 oocysts animal⁻¹ day⁻¹ (Atwill et al., 2003), which represents a 20-fold higher value compared with the value calculated for this study. However, values reported in the present work are the crude number of oocysts per gram of feces which is unadjusted by percent recovery. In our laboratory, the percent recovery can typically range from 9 to over 30% depending on the vertebrate species being tested (Atwill et al., 2003, 2006a), which results in a 3.3- to 11.1-fold increase in the estimated ELR as reported previously for this host species (Atwill et al., 2003). The estimated ELR from this study is less than the values reported previously for horses (Equus caballus), striped skunks (Mephitis mephitis), coyotes (Canis latrans), and California ground squirrels (Spermophilus beecheyi), but higher than the daily loads reported for adult dairy cattle (Bos taurus) (Atwill et al., 2003, 2004).

The role of elevation as a risk factor for Cryptosporidium oocysts fecal shedding could be explained by the effects of variables that vary with elevation, such as temperature, desiccation and ultraviolet (UV) radiation. In general, as elevation increases: a) temperature decreases as a result of the air expansion under lower pressure; b) relative humidity decreases as a consequence of lower air temperature and pressure; and c) UV radiation increases due to decreasing density of molecules and particles in the atmosphere. Temperature is a key factor for the survival of intact oocysts on the ground (Fayer, 1994; Jenkins et al., 1999; Olson et al., 1999; Nasser et al., 2007) and in feces (Jenkins et al., 1999; Olson et al., 1999; Kato et al., 2002; Li et al., 2005, 2010). Exposure to ambient temperatures in excess of 30 °C to 40 °C has been shown to inactivate or reduce oocyst infectivity (Anderson, 1985; Fayer, 1994; Li et al., 2005, 2010). Moreover, oocysts have been shown to be susceptible to freeze/thaw cycles (Jenkins et al., 1999) and freezing (Kato et al., 2002). At high elevations, freeze/thaw cycles might occur, but it seems oocysts are more likely affected by heat than cold (Olson et al., 1999; Nasser et al., 2007). Desiccation has been shown to strongly reduce oocyst viability (Anderson, 1986; Robertson et al., 1992; Deng and Cliver, 1999; Nasser et al., 2007). UV radiation inactivates or reduces infectivity of *C. parvum* oocysts (Lorenzo-Lorenzo et al., 1993; Campbell et al., 1995; Craik et al., 2001; Drescher et al., 2001; Linden et al., 2001; Shin et al., 2001; Morita et al., 2002; Rochelle et al., 2004; Connelly et al., 2007; King et al., 2008, 2010; Lee et al., 2008). In this cross-sectional study, we did not include variables of temperature, desiccation, or UV radiation, but these should be considered in future studies of fecal shedding of oocysts by yellow-bellied marmots. In addition, it would be necessary to consider other factors potentially influencing these variables, for example, slope direction.

Buffer strips of vegetation have been shown to retain *Cryptosporidium* oocysts (Davies et al., 2004; Tate et al., 2004; Trask et al., 2004; Atwill et al., 2006b; McLaughlin et al., 2013); therefore, sites with vegetation could potentially have higher oocyst loads to which marmots could become exposed and infected. Vegetation could also influence oocyst inactivation rates by protecting them from higher temperatures, desiccation and UV radiation. We speculate that the negative association between the odds for oocyst shedding and elevation for marmots located in vegetated habitat (Fig. 2) may be the consequence of vegetation reducing oocyst exposure to UV radiation at lower elevation. The effect of vegetation protection is probably related with changes of plant species as elevation increases which lead to higher inactivation rates of oocysts in the environment. With respect to sites with no vegetation, oocysts in fecal material can be removed by the runoff from precipitation, thus reducing oocysts loads (Davies et al., 2004; Tate et al., 2004; Trask et al., 2004; McLaughlin et al., 2013; Davidson et al., 2014). Therefore in these areas, infection of susceptible individuals would likely occur in zones with higher density of marmots and closer contact between animals. According to our results, clusters of positive fecal samples existed at locations harboring a higher density of individuals.

This work describes what we believe to be the first detection of *Cryptosporidium* from yellow-bellied marmots in the unique high elevation locations in Sierra Nevada Mountains. PCR amplification of DNA of *Cryptosporidium* oocysts from fecal samples is frequently hampered due to the many inhibitors present in fecal samples, as experienced by our laboratory and reported by others (Kostrzynska et al., 1999; Xiao et al., 2003; Adamska et al., 2012; Elmore et al., 2013). Although only two isolates were successfully sequenced, our results demonstrate that some of these oocysts recovered are likely to be *C. parvum*. Because *C. parvum* is a species that infects humans and a wide range of vertebrate animals (Fayer, 2010), there is the potential of transmission between mammal species in the Sierra Nevada Mountains, such as marmots, horses, wildlife, and humans. In addition, *Cryptosporidium* shed by yellow-bellied marmots could potentially contaminate water sources used for drinking or recreation in the mountains. However, a valid risk assessment of waterborne outbreaks needs to take into consideration the density of the local yellow-bellied marmot population, the low loads of oocysts shed by these animals, the environmental factors that influence the survival and viability of the oocysts, and hydrological transport of oocysts in the environment.

In summary, yellow-bellied marmots in the Sierra Nevada Mountains are hosts of *Cryptosporidium* spp. with estimated prevalence of 10–20%. Some of the marmots in these populations are likely the hosts of *C. parvum*. The estimated ELR for these animals is lower than values reported for other mammals in California. Fecal shedding of *Cryptosporidium* oocysts by yellow-bellied marmots is associated with elevation and strongly influenced by the presence or absence of vegetation.

### Conflict of interest

The authors declared that there is no conflict of interest.

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