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Estimating Environmental Loading Rates of the Waterborne Pathogenic Protozoa, ***Cryptosporidium Parvum***, in Certain Domestic and Wildlife Species in California

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Introduction

Cryptosporidium parvum (*C. parvum*) is a protozoal parasite that can cause gastrointestinal illness in a wide variety of mammals, including humans, livestock, companion animals, and wildlife. New species of *Cryptosporidium* are constantly being discovered, such as *C. canis* and *C. felis*, but their significance relative to the large role that *C. parvum* plays in livestock and human cryptosporidiosis is still unclear. In the majority of livestock species, clinical disease and shedding of *C. parvum* typically occurs in youngstock under a few months of age, but fecal shedding of oocysts can also occur in healthy older animals which can then serve as a source of infection for these younger animals. In humans, clinical disease and shedding can appear at all ages, but is typically more common among children. The predominant clinical sign is profuse, watery diarrhea lasting from a few days to several weeks in normal (immunocompetent) individuals, but can be prolonged and life threatening among immunocompromised hosts such as AIDS patients. Modes of transmission range from direct fecal-oral transmission, as might occur between infected and susceptible calves during lay behavior, or ingestion of food or water inadvertently contaminated with oocysts from the feces of an infected host.

Waterborne transmission of the pathogenic protozoa, *Cryptosporidium parvum*, has emerged as an important public health concern. Because the infectious stage of *C. parvum* (oocysts) is resistant to conventional water treatment processes, public health agencies and water districts are actively seeking methods of reducing surface water contamination with this parasite. Protection of source water such as rivers and lakes has the potential to reduce the risk of transmission to humans and animals through drinking water, as well as through human recreational contact with untreated water. Given that the parasite readily infects a large number of mammalian hosts (Fayer et al. 1997), there are a number of possible contributing sources of oocysts present for any given watershed. Unfortunately, the primary quantitative sources of waterborne *C. parvum* oocysts are not well defined, and our methods of prioritizing point and non-point vertebrate sources of this zoonotic parasite are lacking.

Our objective is to develop a standardized methodology for comparing environmental loading rates for different populations of vertebrate hosts for *C. parvum*. Such a comparison would help form the basis of a rational decision making process for evaluating land use practices and vertebrate populations with respect to their relative environmental loading rates for important waterborne microbial pathogens. Both domestic and wild animal populations are infected by and can shed in their feces the infectious stage of this parasite. Attempting to characterize or assess the risk of point

and non-point source protozoal contamination requires numerous parameters to be estimated, the most important being a valid and precise estimate of the oocyst loading rate per animal unit (Atwill et al. 2001; Hoar et al. 2000). The oocyst loading rate, which can be defined as the total number of oocysts excreted by a defined cohort of animals for a specific period of time, can be calculated directly by measuring the kinetics of total oocyst shedding, that is, duration and intensity per Kg feces, multiplied by fecal production. This direct measurement method is very difficult for free-ranging wildlife and some species of livestock. An alternative approximation for determining the oocyst loading rate for cohorts of mammals is to measure the prevalence of infection and the intensity of shedding using cross-sectional surveys of the mammalian population, and then relying on experimental or laboratory estimates of fecal production (Hoar et al. 2000). We applied these concepts to a variety of domestic and wild animal species to generate a set of comparative loading rates for the waterborne pathogen, *C. parvum*.

Methods

For livestock, fecal samples were obtained either per rectum during herd visits or from freshly voided samples on pasture or rangeland. For wildlife species, the animal was dispatched according to the American Veterinary Medicinal Association's guidelines for harvesting wildlife, and fecal samples then obtained post-mortem. Fecal samples were shipped or delivered on ice to the Veterinary Medical Teaching and Research Center, Tulare, CA, where they were refrigerated at 4 C until examined for presence of *C. parvum* by means of a direct immunofluorescent assay as described elsewhere (Atwill et al. 1999). This assay generates an estimate of number of oocysts per fecal smear. In order to rescale this parameter to oocysts per gram of feces, we estimate the mean mass of a fecal smear (usually 17.0 to 18.0 mg) from 20 to 30 slides and the percent recovery of the immunofluorescent assay through spiking known negative fecal samples with known oocysts concentrations, as described in Atwill et al. 1998 and Pereira et al. 1999. Estimates for total fecal production wet weight per animal unit were either estimated from experimental feeding trials (California ground squirrels, coyotes), the literature (beef and dairy cattle), or were very crude estimates of using 2 to 4% of mean body mass (striped skunks, yellow-bellied marmots). Estimates of daily fecal production for the different species is the parameter with the greatest error at this time and in need of future improvement. The final equation for oocysts per gram of feces was: [(mean oocyst concentration per fecal smear)/(mean smear weight multiplied by percent recovery)]. The final equation for oocyst loading rate per animal unit was: [(mean oocyst concentration per Kg feces multiplied times total daily fecal production (Kg))].

Results

The following results are a tally of the estimates of the mean daily *C. parvum* oocyst excretion rate (or environmental loading rate) per animal per species. The phylogenetics of this genus of protozoa are in a state of flux for the time, so exact species designation of *Cryptosporidium* from these various hosts may be revised in the future. In parentheses following the loading rate are the two parameters, mean oocyst concentration per Kg feces, total daily fecal production (Kg), that generated the estimate of the daily loading rate of *C. parvum*-like oocysts. These estimates should be considered crude estimates at this time, but they do allow a rough species-to-species comparison of how different vertebrate animals load a watershed with *C. parvum*.

1. **San Joaquin Dairy Cattle (Holstein, *Bos taurus*)**
   Cows: 4,000 oocysts per day (67 oocysts/Kg; 60 Kg feces)
   Calves: 3,000,000,000 oocysts per day (3,000,000,000 oocysts/Kg; 1 Kg feces)

2. **California Beef Cattle (mixed breeds, *Bos taurus*)**
   Cows: 6,000 oocysts per day (150 oocysts/Kg; 40 Kg feces)
   Calves: 600,000 oocysts per day (150,000 oocysts/Kg; 4 Kg feces)

3. **California Horses (various breeds, *Equus caballus*)**
   Adults: similar to adult beef and dairy cattle
   Foals and weanlings: not done adequately

4. **Striped skunk (*Mephitis mephitis*)**
   Adults: 140,000 oocysts per day (2,800,000 oocysts/Kg; 0.05 Kg feces)
   Juveniles: 88,000 oocysts per day (4,400,000 oocysts/Kg; 0.02 Kg feces)

5. **California ground squirrels (*Spermophilus beecheyi*)**
   Adults: 78,000 oocysts per day (6,500,000 oocysts/Kg; 0.012 Kg feces)
   Juveniles: 41,200 oocysts per day (10,300,000 oocysts/Kg; 0.004 Kg feces)

6. **Coyotes (*Canis latrans*)**
   Adults: 41,000 oocysts per day (205,000 oocysts/Kg; 0.2 Kg feces)
   Juveniles: 35,000 oocysts per day (505,000 oocysts/Kg; 0.07 Kg feces)

7. **Yellow-bellied marmot (*Marmota flaviventris*)**
   Adult: 208,000 oocysts per day (10,400,000 oocysts/Kg; 0.02 Kg feces).
   Juvenile: not done

Discussion

Several inferences can be generated from this list of estimates of environmental loading of *C. parvum*. First, there exists a very wide difference between the excretion rate of oocysts by young-stock compared to adult animals for cattle populations. For example, for dairy cattle in the San Joaquin Valley, dairy calves can produce as much as 750,000 times more oocysts compared to dairy cows, despite that fact that dairy cows defecate 30 to 60 times more feces per day compared to calves. The ramifications of this difference in shedding across different age groups is that the vast majority of *C. parvum* oocysts produced by a dairy herd occurs in a very limited age group, that being calves from 1 to 30 days of age. This facilitates the management of *C. parvum* contamination on dairies because the manure from only a small subset of the population needs to be carefully managed, that being young calves. For beef cattle, given their seasonal calving patterns, the majority of protozoal contamination is limited to the time when young calves are present in the herd, allowing for very strategic grazing practices to be implemented. In contrast, both younger and older members of the wildlife populations examined in this study appear to shed appreciable amounts of oocysts, with adults in some populations shedding more oocysts compared to the young. This suggests that not only is the entire wildlife population at risk of contaminating watersheds with *C. parvum* if population densities are excessive, but that we do not have a seasonal reprieve of protozoal contamination as we do with some livestock populations such as

beef cattle, horses, and mules (Atwill et al. 1998; Atwill et al. 1999; Atwill et al. 2000; Hoar et al. 2000). Given the fact that juveniles and adult wildlife shed oocysts, we can assume that pastures and rangeland are seeded with *C. parvum* prior to beef calving, thereby potentially serving as a source of infection for susceptible beef calves. Finally, it is worthy to note that both young and older striped skunks, coyotes, California ground squirrels, and yellow-bellied marmots produce more oocysts per individual animal than either beef cows or dairy cows. Much regulatory attention is being placed on the role that livestock play in contaminating watersheds with *C. parvum*. Assuming that collective our goal is to protect water quality and to minimize waterborne transmission of this parasite, it would be prudent to equally focus on the role that wildlife play in loading watersheds with this pathogenic protozoa if we are going to successfully protect the public's health from this pathogen.

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