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

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Abstract: Waterborne transmission of the pathogenic protozoa *Cryptosporidium parvum* has emerged as an important public health concern. To develop focused strategies to minimize the risk of waterborne transmission of this parasite to humans or animals, a standardized methodology is needed for comparing environmental loading rates for different populations of vertebrate hosts for *C. parvum*. A reasonable approximation for an estimate of the environmental loading rate is to measure the prevalence of infection and the intensity of shedding using cross-sectional surveys of the mammalian population, and then multiplying by an estimate of fecal production. We applied this concept to a variety of livestock and wildlife species found throughout California. In general we found that regardless of age, striped skunks, coyotes, California ground squirrels, and yellow-bellied marmots were substantial sources of *C. parvum* oocysts. In contrast, only the young stock of beef and dairy cattle were substantial sources of oocysts; adult cattle appear to excrete only limited numbers oocysts relative to either calves or wildlife. Watershed management plans that endeavor to minimize contamination of drinking water with *C. parvum* need to focus on appropriate management of wildlife reservoirs of *C. parvum* in addition to the traditional concern of animal agriculture.

Key Words: *Cryptosporidium parvum*, waterborne, protozoa, zoonoses, cattle, livestock, skunks, ground squirrels, marmots, coyotes, disease, public health

Proc. 20th Vertebr. Pest Conf. (R. M. Timm and R. H. Schmidt, Eds.)
Published at Univ. of Calif., Davis. 2002. Pp. 241-243.

INTRODUCTION

Cryptosporidium 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 young stock 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 it 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 *C. 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 results in Table 1 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. Two parameters, mean oocyst concentration per Kg feces and total daily fecal production (Kg), 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*.

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

Table 1. Estimates of environmental loading rate of *Cryptosporidium*, by species and age class.

Species	Oocysts /kg feces	Kg feces /day	Oocysts excreted /day	Oocysts /kg feces	Kg feces /day	Oocysts excreted /day
	Cows			**Calves**		
San Joaquin dairy cattle - Holstein (*Bos taurus*)	67	60	4000	3,000,000,000	1	3,000,000,000
Calif. beef cattle - mixed breeds (*Bos taurus*)	150	40	6,000	150,000	4	600,000
	Adults			**Foals and weanlings**		
Calif. horses - various breeds (*Equus caballus*)	Similar to adult beef and dairy cattle			Not done adequately		
	Adults			**Juveniles**		
Striped skunks (*Mephitis mephitis*)	2,800,000	0.05	140,000	4,400,000	0.02	88,000
California ground squirrels (*Spermophilus beecheyi*)	6,500,000	0.012	78,000	10,300,000	0.004	41,200
Coyotes (*Canis latrans*)	205,000	0.2	41,000	505,000	0.07	35,000
Yellow-bellied marmots (*Marmota flaviventris*)	10,400,000	0.02	208,000	Not done		

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 (young calves) needs to be carefully managed. 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, 1999, 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.

ACKNOWLEDGEMENTS

We would like to express our sincere thanks to the dedicated staff of USDA Wildlife Services, Kern County Mosquito and Vector Control District, and numerous private and governmental trappers throughout the state of California for assisting in this project. In particular, we are indebted to Joe Bennet and Scott Little, USDA WS, for their tireless efforts on this project.

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Current Control Strategies to Combat Lyme Disease in the North-Central and Eastern U.S.

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Abstract: Lyme disease is an emerging infectious disease accounting for more than 90% of all reported vector-borne diseases in the United States. In the eastern U.S., the deer tick *Ixodes scapularis* carries the spirochete *Borrelia burgdorferi*, which causes the disease. The main reservoir for the spirochete in the wild is the white-footed mouse *Peromyscus leucopus,* which serves as the most common blood-meal host for the larval and nymphal life stages of the tick. Additionally, the enzootic cycle includes the white-tailed deer *Odocoileus virginianus.* As the human incidence of Lyme disease continues to increase, effective intervention methods are needed. Control methods for decreasing risk of contracting Lyme disease have been developed and center on targeting the tick or the wildlife hosts that harbor the tick vector. Personal protective measures have also been developed to protect individuals potentially exposed.

Key Words: zoonosis, public health, Lyme disease, *Borrelia burgdorferi*, white-footed mouse, *Peromyscus leucopus*, white-tailed deer, *Odocoileus virginianus*, deer tick, *Ixodes scapularis*

Proc. 20th Vertebr. Pest Conf. (R. M. Timm and R. H. Schmidt, Eds.)
Published at Univ. of Calif., Davis. 2002. Pp. 244-248.

INTRODUCTION

Considered an Emerging Infectious Disease by the CDC, human Lyme disease cases in the United States have increased about 25-fold since national surveillance began in 1982. The yearly average number of human cases reported is approximately 16,000 (CDC 2002a). The incidence of the disease is increasing; the number of cases in the year 2000 was greater than 17,000, the most of any year reported. Between 1991 and 2000, the reported incidence has almost doubled (CDC 2002b). The CDC reports that Lyme disease accounts for more than 95% of all reported vector-borne illnesses in the U.S., and more than 145,000 human cases have been reported to health authorities (CDC 2002c). The disease is primarily localized to states in the northeastern, mid-Atlantic, and upper north-central regions and to several areas in northwestern California (Dennis 1998). This paper discusses the Lyme disease cycle and strategies to control human infections in the north-central and eastern portions of the U.S.

In the eastern United States, the deer tick *Ixodes scapularis* is implicated in the transfer of the spirochete to humans. This tick is in a 2-year enzootic cycle with small mammals and deer, with the most common hosts being the white-footed mouse, *Peromyscus leucopus* and the white-tailed deer, *Odocoileus virginianus.*

ENZOOTIC CYCLE OF LYME DISEASE

As summarized in Sigal (1993), the 2-year life cycle of *I. scapularis* cycle begins when larvae hatch spirochete free in the summer (Figure 1). The larval ticks feed on small mammals in the summer and fall (July-September). The white-footed mouse is the most common host at this point. If the host is infected with *B. burgdorferi*, the larvae have the opportunity to acquire the infection. After feeding, the larvae remain dormant over winter, and the following spring they molt into nymphs and begin questing to feed. The spring and summer (May-July) are the primary months when nymphs seek a host for feeding, and it is the most common time for humans to become infected. If the nymphs are infected, then spirochete transfer to a host can occur. The most common host at this feeding is, again, the white-footed mouse. After feeding, the nymphs drop to the ground and molt into adults. In the fall of the same year, the adults seek an additional blood meal, and infection to human hosts can occur here as well. At this point, the adult ticks seek larger hosts, because they are questing at higher (~1 m) levels in the foliage. The most common host at this feeding is the white-tailed deer, and it is during this blood meal that the adult ticks breed. Following the adult blood meal in the fall, the adult ticks drop to the ground, remain dormant in the winter, and the females emerge in the spring to lay eggs. The female ticks die after laying eggs.

Humans acquire *B. burgdorferi* infection from infected ticks at the time the tick takes a blood meal (Piesman 1993). This nymphal stage of the tick is responsible for nearly 90% of Lyme disease cases each year (Fish 1993). As nymphs, the ticks are very small and difficult to detect. Studies have shown that it takes at least 24-48 hours for inoculation to the host to occur (Falco et al. 1996).

Although deer are not competent reservoirs of *B. burgdorferi*, they are the principal maintenance hosts for adult deer ticks, and the presence of deer appears to be a prerequisite for the establishment of *I. scapularis* in any area (Wilson et al. 1985). The rapid repopulation of white-tailed deer in the eastern United States in the recent decades has been linked to the spread of *I. scapularis* ticks and of Lyme disease in this region, and the future