

## The prevalence of shedding of *Cryptosporidium* and *Giardia* spp. based on a single fecal sample collection from each of 91 horses used for backcountry recreation

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**Abstract.** *Cryptosporidium parvum* and *Giardia duodenalis* are now recognized as primary enteric pathogens in animals and humans. Regulatory agencies, such as the Environmental Protection Agency are under increasing pressure to reduce the concentration of these protozoa in surface waters. Given the popularity of recreational riding of horses on public land in California backcountry, concerns have been raised by various regulatory agencies as to whether horses used for backcountry recreation are a significant source of *C. parvum* and *G. duodenalis* for the environment. The prevalence of fecal shedding of *Cryptosporidium* and *Giardia* in horses with a history of being ridden in California backcountry during 1993 and 1994 was estimated. Using both direct fluorescent antibody and levitation centrifugation tests, none of 91 single-collection fecal samples from throughout California had *Cryptosporidium* oocysts or *Giardia* cysts. Horses ranged from 4 to 24 years of age. Because none of the 91 samples, collected 1 time from each horse, were positive and assuming that the sensitivity and specificity of the test methods employed were 100%, the highest probable prevalence of shedding for either protozoal pathogen was <3.2% for the cohort of horses studied.

*Cryptosporidium parvum* and *Giardia duodenalis* are now recognized as primary enteric pathogens in animals and humans.<sup>5,7,13,18,35,37-39</sup> The severity of enteric disease produced by either of these parasites can range from a mild, self-limiting to a fulminating diarrhea. Severe life-threatening disease is more commonly seen in young and aged or immunologically suppressed hosts. Each of these parasites can be transmitted by the direct fecal-oral route or through ingestion of contaminated food or water.<sup>3,10,21,25,30</sup>

With the recent large-scale waterborne outbreaks of cryptosporidiosis in metropolitan areas of the United States and the rising numbers of people that are immunodeficient, public attention is increasingly focused on reducing the concentration of these protozoa in drinking water.<sup>12,17,20,26,32,33</sup> Both of these parasites produce resistant stages that are passed out through the feces of infected hosts and are not easily destroyed with disinfectants or during normal water treatment processing.<sup>1,2,19,22</sup> Domestic animals, primarily dairy calves, have been incriminated as a source of contamination

of public water supplies.<sup>24,27,29</sup> Concern over these protozoa in drinking water has lead California agencies to limit access to public lands for recreational horseback riding. Such actions are being made in the absence of scientific data linking the presence of these pathogens in surface water to patterns of equestrian use in a watershed region. The first step in assessing the potential risk of surface water contamination by horses shedding these pathogens is to estimate the prevalence of shedding among equine populations with access to watersheds.

There are only a few reports of natural infection with these parasites in horses and fewer reports of surveys in targeted horse populations that have access to watersheds. *Cryptosporidium* oocysts have been recovered from both immunodeficient and immunocompetent foals.<sup>9,14,28,34</sup> Results of surveys for *Cryptosporidium* in the feces of neonatal foals have been conflicting. Two surveys did not detect infections in a total of 66 foals.<sup>30,37</sup> In 2 other surveys, *Cryptosporidium* was found in 27% (21/77) of normal foals and 29% (83/285) of diarrheic foals and from 69% (22/32) of fecal samples collected from foals raised under helminth-free conditions.<sup>8,10</sup> In the 2-year survey, 15% (8/55) of pasture-reared foals were also found to be infected with *Cryptosporidium* the first year, but the subsequent year's foals were negative, and differences were attributed to variations in the weather conditions observed between the 2 years of the survey.<sup>10</sup> The most recent survey found *Cryptosporidium* shed by 15-31% of foals, 0-4.8% of weanlings, and 0% of yearlings and mares.<sup>39</sup>

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In the same survey, *Giardia* was shed by 17–35% of foals, 4.8–16.7% of weanlings, 0–9.1% of yearlings, 1.9–27.8% of mares. Natural infections of horses with *Giardia* have been documented in 2 foals and a 4-year-old Thoroughbred.<sup>6,21</sup> Although these prevalences suggest that under appropriate conditions horses could contaminate surface water with these protozoa, the actual prevalence of shedding by horses used in backcountry watersheds or headwater regions is unknown. The following survey was undertaken to determine the prevalence of shedding of *Cryptosporidium* and *Giardia* in horses used for recreational riding on public lands in California backcountry.

### Materials and methods

**Study population.** The required sample size needed to estimate the prevalence of shedding of *Cryptosporidium* and *Giardia*, assuming the true prevalence was around 0.01 and setting  $\alpha = 0.05$ , error = 0.02, was 91 samples.<sup>11</sup> Because widespread geographic sampling was desired, California was divided into 16 regions, and within each region the members of the Backcountry Horsemen of California were solicited for voluntary participation. Five or 6 horses from each location were sampled (Fig. 1). For inclusion in the study, each horse must have been ridden for recreation on public land in California backcountry during 1993 and 1994. Fecal samples were submitted from August 25, 1994 through October 3, 1994.

**Examination for *Cryptosporidium* and *Giardia*.** Approximately 10 g of a single fecal sample collected from each horse was placed in 10% neutral formalin (1 part feces/4 parts formalin) and shipped to the Parasitology Laboratory, Veterinary Medical Teaching Hospital (VMTH), School of Veterinary Medicine, University of California, Davis.

The fecal samples were resuspended, strained and washed through a tea strainer into 50-ml conical centrifuge tubes and spun at 1,500 rpm for 6 min. The supernatant was poured off, and the pellet was resuspended in an equal volume of deionized water to the volume of sediment. Ten-microliter aliquots of the fecal suspensions were prepared for direct fluorescent antibody staining using a commercial system.<sup>4</sup> Because the monoclonal antibody for *C. parvum* used in this system cross-reacts with *C. muris* and *C. meleagridis*<sup>4</sup> and studies comparing methods for detection of *Cryptosporidium* and *Giardia* in equine samples were not available, samples were also subjected to a standardized centrifugation levitation procedure using a 70% solution of sodium dichromate with a specific gravity of 1.40, which routinely detects cyst and oocyst stages of both of these protozoa from a broad range of animal species in clinical samples at the VMTH.

**Statistical analysis.** Prevalence of infection was estimated as the number of positive samples out of all submitted samples. In the event that the observed prevalence was zero, the highest probable prevalence of shedding, given that none of the samples were positive, would be calculated from the binomial distribution by solving

$$\binom{n}{X} (P)^X (1 - P)^{n-X} > \alpha$$



Figure 1. The location of 16 premises in California from which single fecal samples collected from 5 or 6 adult horses per premise during the fall of 1994 were examined for the presence of *Cryptosporidium parvum* and *Giardia duodenalis*.

where  $n$  is the number of total samples (in this case  $n = 91$ ),  $X$  is the number of observed positive samples (in this case  $X = 0$ ),  $P$  is the assumed true prevalence of shedding these protozoa in the population of horses from which samples were drawn, and  $\alpha$  the probability for observing no positive samples among  $n$  total samples given  $P$ .<sup>20</sup> Setting  $n = 91$ ,  $X = 0$ , and  $\alpha > 0.05$ , the upper level for  $P$  is determined by solving

$$P < 1 - 0.05^{1/91}.$$

For  $n = 91$  and  $X = 0$ , the relationship between the assumed true prevalence of shedding and  $\alpha$  was modeled for  $0.0 \leq P \leq 0.10$ .

### Results

A single fecal sample collected from each of 91 horses was submitted; 41% were from female horses and 59% were from male horses. None of the horses were younger than 4 years; the mean age was 11 years (SD = 4.5 years; range = 4–24 years) (Table 1).

The prevalence of shedding of *Cryptosporidium* and *Giardia* was zero (Table 1). The highest probable prevalence of shedding for either protozoan pathogen, given that 91 samples were negative, was  $< 3.2\%$  when  $\alpha$

**Table 1.** Prevalence of *Cryptosporidium parvum* and *Giardia duodenalis* in single-collection fecal samples from 91 horses that had been used during 1993 and 1994 for recreational riding on public land in California backcountry.

	<i>C. parvum</i>	<i>G. duodenalis</i>
Gender		
Female	0% (0/37)	0% (0/37)
Male	0% (0/54)	0% (0/54)
Age (yr)*		
4.0–7.9	0% (0/22)	0% (0/22)
8.0–12.9	0% (0/29)	0% (0/29)
13.0–15.9	0% (0/23)	0% (0/23)
16.0–19.9	0% (0/13)	0% (0/13)
20.0–23.9	0% (0/3)	0% (0/3)
>24.0	0% (0/1)	0% (0/1)

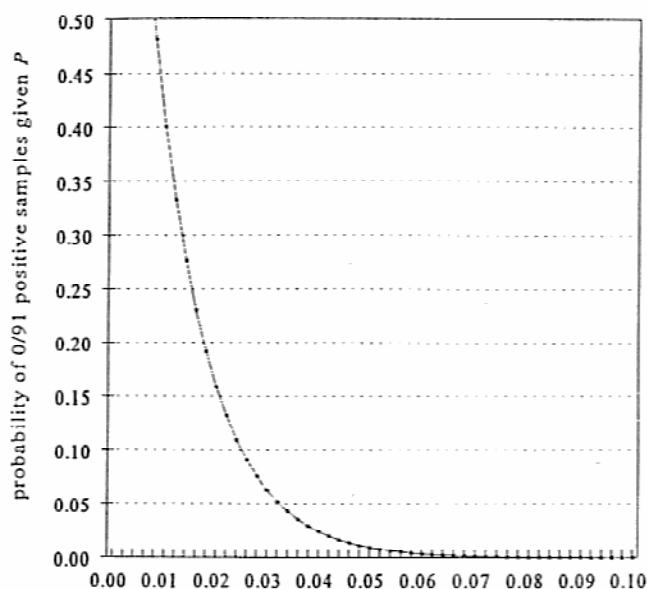
\* All horses were >4 yr of age ( $\bar{x}$  = 11 yr).

was restricted to be greater than 0.05 ( $1 - 0.05^{1/91} = 0.032$ ) (Fig. 2).

### Discussion

Horses used for recreational riding in the backcountry of California during both 1993 and 1994 were not shedding *Cryptosporidium* oocysts or *Giardia* cysts at the time of sampling (August 28–October 3, 1994). Because almost all backcountry riding occurs from May through October, the longest duration between riding in 1994 and fecal sampling would be 4 months. Although the prevalence of shedding at the time these horses were actually being ridden in the backcountry was not known, the observation that none of these horses were shedding at the farm suggests that the likelihood of shedding from an existing infection would be very low during backcountry rides and thus that backcountry recreation with horses does not pose a significant risk of contamination of regional surface water supplies with either of these protozoans.

Relatively high rates of giardiasis among foals (17–35%) and lactating mares (1.9–27.8%) has been documented using the fluorescent antibody method employed in the present study.<sup>39</sup> Lower rates were observed in weanlings (0–9.1%). These categories of horses are not typically used for backcountry recreation. The horses used in this study were 4–24 years old, and lactating mares were not used for backcountry recreation. Typical backcountry trips in California last 4–7 days.<sup>25</sup> The incubation period for *G. duodenalis* is 1–2 weeks.<sup>31</sup> In 1 study, infected foals started excreting cysts between 2 and 22 weeks of age, with the longest duration of excretion lasting 16 weeks.<sup>39</sup> Based on these incubation periods for experimental and natural *Giardia* infections, a horse acquiring a novel infection during a backcountry trip would most likely start shedding cysts 1–2 weeks later, near or after the conclusion of the trip.



**Figure 2.** The relationship between the assumed true prevalence of fecal shedding of *Cryptosporidium parvum* and *Giardia duodenalis* in adult horses and the likelihood of observing no shedding in 91 single-collection fecal samples.

Results of surveys for *Cryptosporidium* in horses have been conflicting. A recent study found 15–31% of foals, 0–4.8% of weanlings, and 0% of yearlings and mares shedding *Cryptosporidium* oocysts at the time of sampling.<sup>39</sup> In 2 studies, no oocysts were found in 66 diarrheic foals.<sup>30,37</sup> Foals experimentally infected with a bovine isolate of *Cryptosporidium* did contract sub-clinical disease.<sup>37</sup> Other surveys found oocysts in 21/77 (27%) normal foals, 83/285 (29%) diarrheic foals, and 0% (0/58) to 15% (8/55) of pastured foals.<sup>8,10</sup> In ponies raised in helminth-free conditions, oocysts were detected between 9 and 28 days after birth and shedding of oocysts lasted for 2–18 days.<sup>10</sup> Although these prevalences do warrant attention, foals are typically not brought on backcountry rides. An adult horse acquiring an infection from contaminated surface waters during a backcountry trip would most likely not start shedding oocysts during the typical backcountry ride of 4–7 days in California.

Although none of the samples had *Cryptosporidium* oocysts or *Giardia* cysts, the true prevalence for the population of interest (horses used for recreational riding in the backcountry) may not be zero. Given the study results, the highest probable prevalence of protozoal shedding for this population of horses would be <3.2%. Population prevalences >3.2% make the observation of no positive fecal samples among 91 samples very improbable. For instance, if the underlying population prevalence was 1%, a 40% chance ( $[1 - 0.01]^{91}$ ) of seeing no positive samples (0/91) existed. In comparison, if the underlying population prevalence was 8%, a 0.05% ( $1/20 \times 1\%$ ) chance of seeing no

positive samples existed. There was no reason to believe that such a rare sample from the underlying population was obtained. It is more likely that the prevalence was very low.

In the studies of the immunofluorescent antibody test, the sensitivity for *C. parvum* was determined to be 94% and the specificity was 100%.<sup>4</sup> Fecal samples tested were a combination of 281 human specimen in 10% formalin and 30 positive controls (20 human and 10 calf). The sensitivity and specificity for *G. duodenalis* were both 100% when 60 positive and 105 negative human fecal samples in 10% formalin were tested.<sup>15</sup> In a recent study evaluating the commercial enzyme immunoassay and immunofluorescent antibody test kits for detection of *Cryptosporidium* oocysts of species other than *C. parvum*, the sensitivity of both tests to *C. parvum* oocysts was 100%.<sup>16</sup> The sensitivity and specificity of the immunofluorescent tests to non-*C. parvum* isolates obtained from birds, snakes, lizards, camels, and a hyrax were 64% and 76%, respectively. There was less cross-reactivity with the enzyme immunoassay.

To date, there is only circumstantial evidence to support the claim that horses in general are a source of *C. parvum* or *G. duodenalis* infections for humans, either through direct contact or through contamination of a watershed. The most convincing evidence that horses might be a source of *C. parvum* infection for humans was the experimental infections of foals with a bovine isolate of *Cryptosporidium*.<sup>36,37</sup> The results of the present survey for the prevalence of *Giardia* and *Cryptosporidium* in adult immunocompetent horses are consistent with previous findings. Previous work and the present study indicate that backcountry use of horses for recreational riding is unlikely to pose a significant risk of environmental contamination from *Cryptosporidium* oocysts or *Giardia* cysts of equine origin nor is it likely to create a significant threat to human health from either of these protozoans.

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