Cross-sectional study of faecal shedding of *Giardia duodenalis* and *Cryptosporidium parvum* among packstock in the Sierra Nevada Range

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**Keywords:** horse; cross-sectional; *Cryptosporidium parvum*; *Giardia*

**Summary**

Faecal specimens from 305 horses and mules used as packstock at one of 17 commercial or governmental (National Park Service, US Forest Service) operations were examined for *Giardia duodenalis* and *Cryptosporidium parvum* using immunofluorescent microscopy. Fourteen packstock (4.6%) were shedding *G. duodenalis* cysts, with herd-level prevalences ranging 0–22%. Number of packstock in the corral, size of corral and density of packstock in the corral were associated with the odds of shedding *G. duodenalis* cysts. None of the horses had detectable *C. parvum* oocysts. Assuming a sensitivity of at least 43% and a specificity of 100% for our assay, the estimated maximum true prevalence of shedding of *C. parvum* for packstock would be ≤ 2.3% of the population. These data suggest that faecal dispersal of *C. parvum* on back country watersheds is unlikely with packstock.

**Introduction**

*Giardia duodenalis* and *Cryptosporidium parvum* can be transmitted between hosts by direct faecal-oral route or through ingestion of contaminated food or water (Archer and Young 1988; Craun 1990; Smith and Rose 1990). Given recent waterborne outbreaks of cryptosporidiosis in metropolitan areas of the United States and Europe, water districts and public health agencies are increasingly focused on reducing the concentration of these protozoa in drinking water (D’Antonio et al. 1985; Hayes et al. 1989; Smith and Rose 1990; MacKenzie et al. 1994; Atherton et al. 1995). *C. parvum* shed by infected foals may be infectious for man, based on anecdotal data in which veterinary students acquired cryptosporidiosis following exposure to infected foals (Cohen and Snowden 1997). In contrast, the zoonotic potential of *G. duodenalis*, shed by livestock, remains somewhat inconclusive (Erlandsen 1994), in part due to the ongoing confusion regarding the taxonomy of this genus (Lymbery and Thayere 1994). Equestrian or packstock activity in watershed areas has come under greater scrutiny as regulatory agencies seek ways to reduce the concentration of *G. duodenalis* and *C. parvum* in source water supplies. Overnight pack trips to the mountainous back country with either horses or mules (packstock) is under particular scrutiny since surface water originating from these regions is often used as a drinking water source.

The first step in assessing the potential risk of surface water contamination by packstock is to estimate the prevalence of faecal shedding among horses and mules being actively used in the back country, where few surveys on the prevalence of faecal shedding with these protozoa have been conducted. Johnson et al. (1997) determined that none of 91 horses used for back country recreation in California, USA, were positive for either *G. duodenalis* and *C. parvum*. A recent survey on trail horses utilising public trails in Colorado, USA, found that 0.7% and 0.3% had detectable concentrations of *G. duodenalis* and *C. parvum*, respectively (Forde et al. 1997). Among the general equine population, 2 surveys detected *C. parvum* in 27% (21/77) of normal foals, 29% (83/285) of diarrhoeic foals and from 69% (22/32) of faecal samples collected from foals raised under helminth-free conditions (Coleman et al. 1989; Browning et al. 1991). In the 2 year survey by Coleman et al. (1989), 15% (8/55) of pasture-reared foals were found to be infected with *C. parvum* the first year, but the subsequent year foals were negative. A more recent survey found *C. parvum* being shed by 15–31% of foals, 0–5% of weanlings and 0% of yearlings and mares (Xiao and Herd 1994). In the same survey, *G. duodenalis* was shed by 17–35% foals, 5–17% weanlings, 0–10% yearlings, and 2–28% of mares. Cole et al. (1998) determined that 7% (5/70) of foals on breeding farms and 0.3% (1/366) of geldings, intact males and mares were infected with *C. parvum* as determined by immunofluorescent microscopy. Acid fast staining and flow cytometry were also used in this study, both of which detected a higher prevalence of infection suggesting that these diagnostic procedures were either more sensitive or less specific compared to immunofluorescent microscopy.

We conducted a cross-sectional survey on horses and mules used as packstock at commercial or governmental (National Park Service, US Forest Service) operations in the Sierra Nevada Range, California, USA, to determine the point prevalence of shedding of *G. duodenalis* and *C. parvum*. In addition, information on each animal and on current management practices were also collected for risk factor analysis.
TABLE 1: Host factors evaluated for an association with shedding of Giardia duodenalis and Cryptosporidium by horses and mules used as packstock at commercial and governmental operations in California

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. sampled</th>
<th>G. duodenalis</th>
<th>C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Horse</td>
<td>223</td>
<td>9 (4.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Mule</td>
<td>66</td>
<td>4 (6.1%)</td>
<td>0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Gender</th>
<th>No. positive for G. duodenalis and C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>172</td>
</tr>
<tr>
<td>Female</td>
<td>118</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>No. sampled</th>
<th>G. duodenalis</th>
<th>C. parvum</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0–7.9</td>
<td>51</td>
<td>3 (5.9%)</td>
<td>0</td>
</tr>
<tr>
<td>8.0–13.9</td>
<td>90</td>
<td>4 (4.4%)</td>
<td>0</td>
</tr>
<tr>
<td>14.0–19.9</td>
<td>91</td>
<td>5 (4.4%)</td>
<td>0</td>
</tr>
<tr>
<td>20.0–25.9</td>
<td>27</td>
<td>0 (0.0%)</td>
<td>0</td>
</tr>
<tr>
<td>&gt;26.0</td>
<td>31</td>
<td>2 (6.5%)</td>
<td>0</td>
</tr>
</tbody>
</table>

Materials and methods

Study population

Commercial and governmental packstock operations which were in the southern Sierra Nevada mountains, California, USA, were enrolled voluntarily into the study. On the scheduled day of sampling, horses and mules were tied up in the corral for 2–4 h, during which time 50 g faeces were collected off the ground from each horse that had defaecated.

Detection of C. parvum and G. duodenalis

Approximately 5 g of faeces was placed into a paper cup and mixed with 30 ml deionised water. The sample was strained through 2 layers of cotton gauze into a 50 ml centrifuge tube. Tubes were centrifuged at 1000 g for 10 min and the supernatant was aspirated. The resulting pellet was resuspended in an equal volume of deionised water. A 10 µl transfer loop was used to transfer a drop of faecal material to a treated slide well. The slide was air dried overnight and stained according to the manufacturer’s instructions (Merifluor Cryptosporidium/Giardia). The entire smear was examined with epifluorescent microscopy at x400 magnification for C. parvum oocysts and G. duodenalis cysts. Samples containing one or more 4–6 µm diameter oocysts (C. parvum) or one or more 10 x 15 µm cysts (G. duodenalis) were recorded as positive. If no oocysts or cysts were seen, the sample was recorded as negative.

Statistical analyses

Fixed effects logistic regression (Mehta and Patel 1996) was used to test and quantify the association between host factors (age, gender, species), stock use (e.g. total trips during the previous 8 weeks, exposure to cattle), management practices (e.g. manure disposal, stock density in the corral), and the probability of shedding C. parvum oocysts or G. duodenalis cysts. Forward stepping algorithm was used, with P-value ≤ 0.05 for inclusion of the term in the model using the likelihood ratio test (LRT). Goodness-of-fit for the final model was calculated using both the deviance and the Hosmer-Lemeshow test, with a Chi-square test performed on the appropriate degrees of freedom to determine P values (Mehta and Patel 1996).

In the event that the observed prevalence was zero, the highest probable prevalence of shedding would be calculated from the binomial distribution by solving Psuch that,

\[ P \leq 1 - 0.05^{1/305} \]  

(2)

Such a calculation assumes sensitivity and specificity of the diagnostic test are 100%. In order to calculate the maximum true prevalence of shedding based upon the maximum apparent prevalence of shedding and the sensitivity and specificity of the diagnostic test, the maximum true prevalence can be calculated as (Schwabe et al. 1977),

\[ \text{Maximum true prevalence} = \frac{\text{Maximum apparent prevalence} + \text{Sp} - 1}{\text{Se} + \text{Sp} - 1} \]  

(3)

where the maximum apparent prevalence, P, is derived from equation 2, and specificity (Sp) and sensitivity (Se) are the diagnostic attributes of the immunofluorescent assay when applied to our study population of mature packstock.

Results

A single faecal sample was collected from 305 horses and mules...
used as packstock at one of 17 commercial or governmental (National Park Service, US Forest Service) operations. Host demographics were available for most packstock (Table 1): 73% were horses and 19% were mules; 56% were male and 37% were female. The median age was 14 years, range 2–33 years.

Fourteen packstock (4.6%) had detectable levels of *G. duodenalis* cysts. Herd-level prevalences were 0–22%, with 11 herds having all horses testing negative and 6 herds having one or more horses testing positive (Table 3). The odds of shedding of *G. duodenalis* cysts were not associated with breed, gender or age of horse (Table 1), and were also not associated with frequency of manure removal from the corral or total number of trips into the back country during the previous 2 months (data not shown).

Using logistic regression to test the association between various management factors and the likelihood of shedding *G. duodenalis* cysts, we developed 2 alternative statistical models for the association between corral management and *G. duodenalis* shedding among packstock populations. For the first, we found that density of packstock (No. of animals per 9 sq. m) housed in the corral was significantly associated with the odds of shedding *G. duodenalis* cysts (Table 2, Fig 1) (LRT = 13.5 on 1 df, P <0.001). The odds of shedding *G. duodenalis* cysts increased 2-fold for every additional pack animal per 9 sq. m (odds ratio = 1.98, 95% CI 1.4–2.8). The overall model did not significantly differ from the raw data (Table 3). Goodness-of-fit for the packstock density model: deviance = 16.1 on 16 df, P = 0.37; Hosmer-Lemeshow statistic = 9.6 on 6 df, P = 0.14.

A more complicated but better fitting logistic regression model for the association between corral management and the odds of shedding *G. duodenalis* cysts was a model based on the total number of packstock in the corral and the size of corral (Table 2, Fig 2) (LRT for total number of packstock = 13.7 on 1 df, P<0.001; LRT for corral size = 5.2 on 1 df, P = 0.02).

### TABLE 2: Factors associated with shedding of *G. duodenalis* by horses and mules used as packstock at commercial and governmental operations in California

<table>
<thead>
<tr>
<th>Presence of <em>G. duodenalis</em> cysts</th>
<th>No. of packstock in corral</th>
<th>Stocking density + no. of packstock</th>
<th>Corral size</th>
<th>Predicted prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–10</td>
<td>1/70 (1.4%)</td>
<td>13</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>11–20</td>
<td>1/46 (2.2%)</td>
<td>15</td>
<td>2.2%</td>
</tr>
<tr>
<td></td>
<td>21–30</td>
<td>1/42 (2.4%)</td>
<td>17</td>
<td>2.8%</td>
</tr>
<tr>
<td></td>
<td>31–40</td>
<td>0/56 (0.0%)</td>
<td>19</td>
<td>2.0%</td>
</tr>
<tr>
<td></td>
<td>41–60</td>
<td>10/100 (10%)</td>
<td>21</td>
<td>3.7%</td>
</tr>
<tr>
<td></td>
<td>&gt; 60</td>
<td>0/28 (0.0%)</td>
<td>23</td>
<td>2.0%</td>
</tr>
</tbody>
</table>

### TABLE 3: Observed herd-level prevalences of shedding of *G. duodenalis* by horses and mules used as packstock at commercial and governmental operations in California, and predicted prevalences from 2 logistic regression models

<table>
<thead>
<tr>
<th>No. of packstock per 9 sq. m of corral</th>
<th>Stocking density + 200</th>
<th>Corral size</th>
<th>Predicted prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9a</td>
<td>0/16 (0.0%)</td>
<td>9</td>
<td>0.0%</td>
</tr>
<tr>
<td>0.1–0.5</td>
<td>3/168 (1.8%)</td>
<td>31</td>
<td>1.1%</td>
</tr>
<tr>
<td>0.6–1.0</td>
<td>0/27 (0.0%)</td>
<td>9</td>
<td>0.0%</td>
</tr>
<tr>
<td>1.1–1.5</td>
<td>0/38 (0.0%)</td>
<td>31</td>
<td>1.1%</td>
</tr>
<tr>
<td>1.6–2.0</td>
<td>6/52 (12%)</td>
<td>9</td>
<td>1.8%</td>
</tr>
<tr>
<td>&gt;2.0</td>
<td>4/18 (22%)</td>
<td>31</td>
<td>0.3%</td>
</tr>
</tbody>
</table>

*Packstock were kept in a pasture rather than in a corral.*

*ND = not determined since number of packstock in corral was not determined.*
negligible once the corral exceeded 600 sq. m (Fig 2) (odds ratio for total number of packstock = 1.1, 95% CI 1.03–1.17; odds ratio for size of corral in 9 sq. m increments = 1.03, 95% CI 1.01–1.05; odds ratio for interaction term = 0.998, 95% CI 0.997–0.999). The overall model did not significantly differ from the raw data and provided a better fit compared to the packstock density model (Table 3). Goodness-of-fit for the model: deviance = 6.2 on 16 df, $P = 0.98$; Hosmer-Lemeshow statistic = 2.3 on 6 df, $P = 0.89$.

None of the 305 horses had detectable concentrations of *C. parvum* oocysts (Table 1). The maximum apparent prevalence of shedding *C. parvum*, setting our confidence to be ≥ 5% (i.e. eliminating scenarios with less than a 5% probability of occurrence) was 0.98%. In order to calculate the maximum true prevalence, estimates for the sensitivity and specificity of the diagnostic assay in this population of horses are needed. Given that the observed number of positive tests was zero, there was no opportunity for false positives. Hence, specificity should approximate 100% in this cohort of animals. The specificity of the Merifluor assay was recently estimated at 100% using flow cytometry as the gold standard for a population of 95 horses (Cole et al. 1998). Three independent evaluations of the Merifluor assay on human faecal samples estimated a value of 100% for the specificity (Garcia et al. 1992; Kehl et al. 1995; Garcia and Shimizu 1997). The sensitivity of the Merifluor assay, defined in our study as the probability of detecting one or more oocysts per faecal smear for positive faecals (Jones and Atwill 1998), was recently estimated at 43% using flow cytometry as the gold standard for a population of 95 horses (Cole et al. 1998). In addition, 4 independent determinations of the sensitivity of the Merifluor assay for human faecal samples ranged 83–100% (Garcia et al. 1992; MacPherson and McQueen 1993; Kehl et al. 1995; Garcia and Shimizu 1997). For our calculations we use the more conservative of these point estimates, 43%, as an approximation of the sensitivity of this assay in mature packstock. Based on these assumptions, the estimated maximum true prevalence of shedding of *C. parvum* for high Sierra packstock would be ≤ 2.3% of the population.

**Discussion**

We found 4.6% (14/305) of packstock to be shedding *G. duodenalis* cysts in their faeces, with herd-level prevalences of 0–22%. This range of prevalences for shedding *G. duodenalis* cysts among horses and mules used as packstock at commercial and governmental operations was reasonably predicted by a logistic regression model based on the total number of stock housed in the corral and the overall size of the corral for the 17 facilities enrolled in the study. As the number of stock was increased for corrals in the 100–600 sq. m size, we found that the probability or prevalence of *G. duodenalis* shedding likewise increased. Given the faecal-oral route of transmission of this parasite, it is feasible that higher concentrations of horses or mules created environmental conditions conducive to animal-to-animal transmission of cysts. Once the size of the corral exceeded 600 sq. m, increasing the number of stock in the corral was no longer associated with an increase in *G. duodenalis* shedding, but instead tended toward a shedding prevalence of zero. Therefore, the association between stocking density and the prevalence of *G. duodenalis* shedding was dependent on the underlying size of the corral irrespective of the total number of stock in the corral, or in epidemiological terms, effect modification existed between these 2 variables (Kleinbaum et al. 1982). We can see this tendency in the raw data displayed in Table 2 whereby the prevalence of shedding was 10–12% for packstock housed in smaller corrals, but dropped to 0–2.5% for packstock housed in larger corrals, irrespective of the number of stock in the corral. This presence of effect modification explains why the logistic regression model based on stocking density alone was able to explain some of the variation in herd-level prevalence of *G. duodenalis* shedding, but provided poor predictions for several facilities (e.g. herds 11 and 15).

The observed 4.6% prevalence of *G. duodenalis* shedding for the horses and mules at commercial facilities in the Sierra Nevada Range was significantly higher than the 0% (0/91) prevalence of *G. duodenalis* shedding among horses used for back country recreation in California, USA (Johnson et al. 1997) (2-sided $P = 0.047$, based on Fisher’s exact test) and significantly higher than the 0.7% (2/300) prevalence of *G. duodenalis* shedding among recreational trail horses sampled in Colorado (Forde et al. 1997) (2-sided $P$-value = 0.004, based on Fisher’s exact test). We can only speculate as to why there existed higher prevalences of infection among these commercial and governmental packstock compared to recreational horses. The diagnostic methods and, in particular, the method of cleaning the faecal sample and serial dilution, were similar between our 1997 (Johnson et al. 1997) and the current study. In contrast, Forde et al. (1997) diluted their faecal samples 1 part faeces to 4 parts 10% formalin, effectively diluting the sample twice as much compared to our study. A 2-fold dilution of the faecal suspension relative to our procedure of approximately 1:1 dilution may have reduced the sensitivity of their assay. Other explanations would be that the sample populations are sufficiently different in their underlying risk factors, such as stocking density or corral size, to result in differences in *G. duodenalis* infection levels.

In contrast to these low observed prevalences of *G. duodenalis* shedding for mature horses used for private or commercial back country recreation, Xiao and Herd (1994) found among broodmare facilities that *G. duodenalis* was shed by 17–35% foals, 5–17% weanlings, 0–10% yearlings, and 2–28% of mares. There was a tendency for the prevalence of *G. duodenalis* cyst shedding to be higher among the younger horses on these broodmare facilities, but a Canadian study determined that 0% (0/10) of foals and 25% (6/25) of horses older than age 6 months were shedding *G. duodenalis* cysts (Olson et al. 1997). We did not detect an association between age and the prevalence of *G. duodenalis* shedding in our study, but our range of ages was limited to horses age 2 years or older. The higher overall prevalence of shedding among horses on broodmare facilities compared to our cohort of horses and mules could be due to a wide range of factors, including differences in age, stocking density, corral or pen size, or total number of animals per group.

Given the 4.6% prevalence of *G. duodenalis* shedding for commercial or governmental packstock, small amounts of *G. duodenalis* dispersal may occur while the packstock are in the back country. It is important to mention, though, that the zoonotic potential of equine *G. duodenalis* remains controversial. Two reviews of the scientific literature both concluded that evidence for the zoonotic potential of *G. duodenalis* is incomplete (Erlandsen 1994; Thompson and Boreham 1994). On the other hand, *G. duodenalis* obtained from a Gambian giant pouched rat (*Critetomys gambianus*) was infectious for a human volunteer (Majewska 1994). Two recent
molecular epidemiological studies using enzyme electrophoresis and PCR-RFLP indicated that isolates from human subjects and several domestic and wild animal hosts were genetically similar (Meloni et al. 1995; Ey et al. 1996), but genetic similarity is not evidence that cross-transmission is occurring. While this evidence is interesting, its relevance is unclear with respect to the risk that equine G. duodenalis poses to man in the back country under standard sanitary and culinary conditions (e.g., filtering or boiling water, adequate personal hygiene, cooking rehydrated food). For corrals smaller than 600 sq. m, keeping stocking densities as low as possible may serve as a proactive good management practice for reducing packstock infection of G. duodenalis and thereby reducing faecal dispersal of G. duodenalis cysts in the back country, but additional studies are needed to confirm this suggestion.

Of the 305 packstock from the 17 facilities that we tested, none were found to be shedding detectable concentrations of C. parvum oocysts. This low apparent prevalence of faecal shedding of C. parvum among horses used in the back country is consistent with previous studies in which either none (0/91) (Johnson et al. 1997) or only 0.3% (1/300) (Forde et al. 1997) of trail horses were found to be shedding detectable concentrations of C. parvum. In addition, several surveys among broodmare facilities and among the general equine population detected very low prevalences of C. parvum oocyst shedding among mature horses. Two recent surveys found C. parvum being shed by 0% of yearlings (0/46) and mares (0/71) (Xiao and Herd 1994) and 0.3% (1/366) of geldings, intact males and mares (Cole et al. 1998), as determined by immunofluorescent microscopy. In contrast, Olson et al. (1997) found 21% (5/24) of horses older than 6 months were shedding C. parvum oocysts. There were no foals present in the packstock facilities enrolled in our study which helped minimise the possibility of C. parvum contamination from packstock manure. Faecal shedding of C. parvum among foal populations appears to be much higher compared to mature horses or mules. Cole et al. (1998), found 7% (5/70) of foals on breeding farms to be infected with C. parvum. In addition, C. parvum was present in 27% (21/77) and 29% (83/285) of normal and diarrhoeic foals, respectively, and from 69% (22/32) of faecal samples collected from foals raised under helminth-free conditions (Coleman et al. 1989; Browning et al. 1991). A more recent survey found C. parvum being shed by 15-31% of foals and 0 to 5% of weanings (Xiao and Herd 1994).

Although the apparent prevalence of faecal shedding of C. parvum was 0% in our study, we believe that it is more useful under these low prevalent conditions to calculate the maximum true prevalence of faecal shedding rather than to limit inferences to the apparent prevalence (Johnson et al. 1997; Jones and Atwill 1998). We estimated that the maximum true prevalence of faecal shedding of C. parvum would be 2.3% for this population of horses and mules. This estimate eliminates shedding prevalences that have a less than 5% chance of occurring given our data and our assumption that the sensitivity and specificity of immunofluorescent microscopy was 43 and 100%, respectively. If the sensitivity of detecting oocysts in a single faecal smear was less than 43% for this population of packstock, then the estimated maximum true prevalence of faecal shedding of C. parvum would be higher. A lower sensitivity would result if the median concentration of oocysts shed by adult packstock is very low. Our data cannot rule out this possibility. Nevertheless, based on our estimate of 2.3% for the maximum true prevalence of faecal shedding among this population of horses and very low estimates of apparent shedding from 2 earlier studies (0.0 to 0.3%), it is unlikely that California packstock are capable of contaminating back country watersheds with significant levels of C. parvum.

Acknowledgement
This project was supported in part by the Center for Equine Health with funds by the Oak Tree Racing Association, the State of California satellite wagering fund, and contributions from private donors.

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1Meridian Diagnostics, Cincinnati, Ohio, USA.

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Faecal shedding of Giardia duodenalis and Cryptosporidium

Received for publication: 9.11.98
Accepted: 1.9.99


