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

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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 Tibayrenc 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 and by horses and mules used as packstock at commercial and governmental operations in California

	No. sampled	No. positive for *G. duodenalis*	No. positive for *C. parvum*
Breed			
Horse	223	9 (4.0%)	0
Mule	66	4 (6.1%)	0
Gender			
Male	172	9 (5.2%)	0
Female	118	4 (3.4%)	0
Age (years)			
2.0–7.9	51	3 (5.9%)	0
8.0–13.9	90	4 (4.4%)	0
14.0–19.9	91	5 (4.4%)	0
20.0–25.9	27	0 (0.0%)	0
>26.0	31	2 (6.5%)	0

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)*[1]. 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 Pvalue ≤ 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).

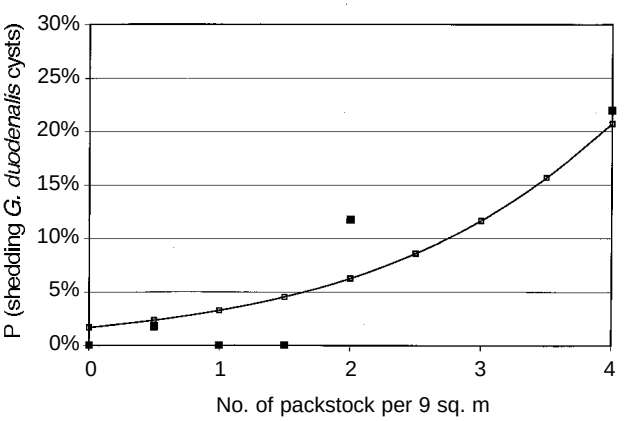

Fig 1: Association between total number of packstock per 9 sq. m in a corral and the probability of shedding Giardia duodenalis *cysts (* ■ *= raw data categorised into intervals of 0, 0.1–0.5, 0.6–1.0, 1.1–1.5, 1.6–2.0, 2.1–4.0 of packstock per 9 sq. m,* □ *= predicted prevalence of shedding from packstock density logistic regression model).*

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,

$$\binom{n}{x}(P)^x (1 - P)^{n-x} \geq \alpha \qquad (1)$$

where n is the number of total samples (in this case $n = 305$), x is the number of observed positive samples (in this case $x = 0$), P is the calculated maximum apparent prevalence of shedding for these protozoa for the sampled population of horses, and the probability of observing no positive samples among n samples given our point estimate of P (Johnson *et al.* 1997). Setting $n = 305$, $x = 0$, and $\alpha \geq 0.05$, the upper level for P is calculated by solving,

$$P \leq 1 - 0.05^{1/305} \qquad (2)$$

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),

$$\text{Maximum true prevalence} = \frac{\text{Maximum apparent prevalence} + Sp - 1}{Se + Sp - 1} \qquad (3)$$

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

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