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THE PREVALENCE OF *CRYPTOSPORIDIUM/GIARDIA* IN THE TRAIL HORSE POPULATION UTILIZING PUBLIC LANDS IN COLORADO

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SUMMARY

Cryptosporidium and *Giardia* spp. are small coccidian parasites that infect the intestinal tract of birds, reptiles and mammals including humans. Oocysts of *Cryptosporidium parvum* and cysts of *Giardia duodenalis* are shed in the feces of infected animals and directly infect subsequent hosts following ingestion. The broad host range, resistant nature, and small size of the parasites in the environment may potentially contribute to contamination of water supplies. The perception that recreational riding horses may increase the prevalence of *Cryptosporidium* and *Giardia* in large watersheds adjacent to common trails, indirectly infecting humans, has resulted in banning recreational riding horses from some trails. However, there is currently no scientific data linking the recreational trail horse to the incidence or increased risk of human cryptosporidiosis or giardiasis. This survey was conducted to determine the prevalence of *Cryptosporidium* and *Giardia* spp. in recreational horses utilizing trails located near large watershed areas in Colorado. At the trail heads, approximately 10 g of fresh feces were collected from recreational horses (n=300) that were utilizing the trails on the day they were sampled. Fecal samples were mixed with 10% formalin at a dilution rate of 1 part feces to 4 parts formalin and were transported to the Veterinary Diagnostic Laboratory at Colorado State University. Samples were evaluated using the Merifluor™ *Cryptosporidium/Giardia* Direct Immunofluorescent Detection Procedure. One horse (0.33%) was detected positive for *Cryptosporidium* and two horses (0.66%) were positive for *Giardia*. The low prevalence of *Cryptosporidia* and *Giardia* in the trail horse population surveyed indicates that the adult recreational trail horse population is not likely to be a significant source of *Cryptosporidium* and *Giardia* environmental contamination in watershed areas.

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INTRODUCTION

Cryptosporidium parvum and *Giardia duodenalis* are small coccidian, single celled, parasites that commonly infect the intestinal tract of birds, reptiles, and mammals including humans. Sporulated *Cryptosporidia* oocysts and *Giardia* cysts, the infective form of both of these parasites, are shed in the feces of infected carriers. These parasites are infective to a new host as soon as they are passed and ingested. Thus, infection of the new host occurs through the ingestion of food and/or water contaminated with the *Cryptosporidia* oocysts and/or *Giardia* cysts.

Common clinical symptoms among infected humans include watery diarrhea, abdominal cramps, nausea, dehydration, weight loss, and a low grade fever. In healthy individuals, these symptoms usually resolve within two weeks; however, in the very young or elderly, these symptoms may prove to be dangerous, if persistent, leading to severe dehydration. In immunocompromised individuals, the ingestion of these parasites may be life-threatening.[1,2]

The prevalence of cryptosporidiosis in horses varies with age and immune status. *Cryptosporidia* is much more prevalent within the first two months of the foal's life but has been identified in the feces of adult horses, older foals, weanlings, and yearlings.[3] Infected horses may appear normal, but

infection in foals can result in diarrhea and malabsorption of the absorptive surface in the small intestine. In some cases, infection progresses rapidly and can result in death within two weeks of onset of clinical symptoms.[3,4,5,6] *Giardia* has also been identified in the feces of adult horses, yearlings, weanlings, and foals.[7]

The broad host range, coupled with the resistant nature and small size of these parasites in the environment may potentially contribute to environmental contamination and waterborne transmission. Oocysts and cysts are present in much of the surface water and their concentration may be increased after heavy rainfall due to an increased runoff of wild and domestic animal wastes.[8] Both of these parasites produce resistant stages which are not easily destroyed by chlorination and can escape routine water filtering and treatment processes. In 1993, an outbreak of cryptosporidiosis affected people in the Milwaukee, Wisconsin area. An estimated 400,000 people were infected and there were 100 deaths attributed to infection with *Cryptosporidia* sp. Since 1993, five other outbreaks of cryptosporidiosis in humans in the United States have been identified.[9]

There is a need to protect the nation's water supply from *Cryptosporidia* and *Giardia* contamination. Animals that are potential carriers of these diseases should be kept safe distances from watersheds. At this time, there is currently no scientific evidence linking the adult recreational trail horse to contamination of water with *Cryptosporidia* or *Giardia*. However, there are reports of *Cryptosporidium* and *Giardia* infections occurring in foals during their first two months of life.[5,6] Because horses were perceived by water quality officials to pose a risk of contaminating water supplies with these protozoa, the trail riding of horses in certain recreational areas in California was banned. As most of these horses would be adults, these recreational horses may have been blamed falsely as posing a risk of shedding *Cryptosporidia* and *Giardia* into the water supply.[7]

The objective of this study was to conduct a population survey of adult horses utilizing Colorado's recreational trails to determine the prevalence of shedding *Cryptosporidium* oocysts and *Giardia* cysts in feces.

MATERIALS AND METHODS

Collection of fecal samples. Fresh, uncontaminated, ground deposit, fecal samples were collected from adult trail horses (n = 300), ranging in age from 3 to 30 years, at various trail heads near large watersheds in Colorado. Samples were collected between July and November, 1996 and the demographics associated with sample collection represented 23 different trail heads in the state of Colorado. The collection of fecal samples from recreational horses was done at random, assuming that the sampled population adequately represented a much larger, total population of recreational trail horses in the state of Colorado.

Of the 300 horses sampled, 37% were female horses and 63% were male horses. None of the horses were younger than 3 years, 30% of the horses were between the ages of 3 and 8; 42% of the horses were between the ages of 9 and 14; 20% of the horses were between the ages of 15 and 20 and 8% of the horses were over the age of 21. In addition, 46% were Quarter Horses, 43% were mixed or rare breeds, and 11% were Arabians. The frequency of trail use in over 80% of the horses sampled was greater than 4 days per week during the peak season.

Ten grams of feces from each horse was mixed with 10% formalin at a dilution rate of 1 part feces to 4 parts formalin and transported in individually labeled, screw top, plastic containers to the Veterinary Diagnostic Laboratory at Colorado State University, Fort Collins for analysis. The individual labels included the assigned identification number, age, sex, and breed of the respective horse the sample was taken from. These samples were kept at room temperature until analyzed. Another 10 g of feces sample was stored as reserves in individually labeled plastic bags and transported to the walk-in freezer for future studies at the Department of Animal Sciences, Colorado State University, Fort Collins. In addition to sample collection, a separate questionnaire was completed for each of the 300 horses. Through the use of these questionnaires, information gathered included: age, sex, and breed of the horse, stabling, frequency of trail use, and occurrence of diarrhea.

Detection of Cryptosporidium and Giardia in feces. Upon arrival of the feces in formalin at the Veterinary Diagnostic Laboratory, samples were evaluated for *Cryptosporidium* and *Giardia* using the Merifluor™ Direct Immunofluorescent Detection Procedure.[a] Each evaluation was performed using the same protocol by the same laboratory professional, thus reducing variation of test results.

For each of the 300 alliquots of feces in formalin, two separate transfer loops were used to transfer one drop of fecal suspension and one drop of Positive Control to two different, treated slide wells, respectively. Each slide was allowed to air dry at room temperature. One drop each of Detection Reagent and Counterstain was then placed into each well, mixed, and spread across the slide with an applicator stick. The treated slide was then incubated in a humidified chamber at room temperature for 30 min. After incubation, the slide was gently rinsed with 1x Wash Buffer until excess Detection Reagent, Counterstain, and unbound antibodies were removed. Subsequently, excess buffer was removed by tapping the slide on a clean paper towel, paying particular attention to avoid cross contamination of the sample and the Positive Control. Mounting medium was then added to each well along with a coverslip and examined underneath 100-200x

[a]The sensitivity and specificity of the Merifluor™ *Cryptosporidium* test is 100% and 99%, respectively Both the sensitivity and specificity of the Merifluor™ *Giardia* test is 100%. Meridian Diagnostics, Inc., 3471 River Hills Drive, Cincinnati, Ohio 45244.

magnification for an apple green color and characteristic morphology of *Cryptosporidium* oocysts and *Giardia* cysts and confirmed at 450x magnification using a fluorescent microscope.[10]

In the case of positive *Cryptosporidium* results, the EM Scan/Elisa test was used to detect viruses and the Modified Stoll Technique[b] was used to identify the level of parasitism in the horse. The Modified Stoll Technique is the method used to perform fecal egg counts. It is a quantitative technique that uses a known quantity of feces (4 g) in a known amount of water (56 ml). A 1.5 ml aliquot of this solution is mixed with a salt solution, $MgSO_4$, covered with a coverslip and centrifuged for 5 min at 2000 rpm. The cover slip is then removed, placed on a slide and scanned for parasite eggs which are counted and differentiated.

No further analysis through the use of population statistics was performed on the sampled population due to lack of population statistics for recreational horses in the state of Colorado.

RESULTS AND DISCUSSION

Results from tests performed on the 300 fecal samples revealed that one horse (0.33%) had one *Cryptosporidium* oocyst and two other horses (0.66%) had *Giardia* cysts. The horses that were detected positive with *Giardia* cysts were both male Quarter Horses, aged 11 and 14. These prevalence results are consistent with the *Cryptosporidium/Giardia* Study conducted in California.[7]

Detailed information on the positive Cryptosporidium *horse.* Only a single oocyst was detected in the fecal sample from this horse, suggesting that potentially the oocyst was ingested and then expelled without undergoing development within the microvillous border of the intestine of this horse. Three months after the initial laboratory detection of the *Cryptosporidia* oocyst, another fresh fecal sample was obtained from this same horse and retested for *Cryptosporidium* and *Giardia* using the same procedure. Results from the second Merifluor™ Direct Immunofluorescent Detection Procedure indicated that the horse was negative for both *Cryptosporidium* and *Giardia.* An aerobic fecal culture on the second sample indicated normal flora growth and the EM Scan/Elisa test was negative; in addition, no *Salmonella* was detected. The fecal exam results quantitatively indicated that there were 130 strongyle eggs per gram, suggesting that the horse was parasitized to some degree.

This female Quarter Horse was 24 years old, immunocompromised, had bad teeth and poor digestion. In addition, the horse was ridden on trails on a daily basis as a part of a commercial riding establishment. At the time the first sample was collected, the horse had no abnormal, clinical signs associated with *Cryptosporidium*, with the exception of weight loss, per the owner. However, based on the case history of this horse, weight loss could conceivably be due to the 130 strongyle eggs per gram found in the fecal exam coupled with immunocompromisation, poor digestion, and bad teeth. The horse was taken out of the working string due to the weight loss and placed on pasture where it regained weight. The occurrence of the *Cryptosporidia* oocyst found in the first fecal sample could be attributed to the horse being allowed to drink water from the stream during long trail rides and the nearby high beaver population and running stream where the horse was housed.

Overall Conclusion. Based on the low prevalence of *Cryptosporidia* in the trail horse population surveyed, it can be concluded that the adult recreational trail horse population in Colorado is not likely to be a significant source of *Cryptosporidium* environmental contamination in watershed areas.

[b]Technique used at the Veterinary Diagnostic Laboratory at Colorado State University, Fort Collins.

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