Fecal shedding of Cryptosporidium oocysts in healthy alpaca crias and their dams

Alexandra J. Burton, BVSc, DACVIM; Daryl V. Nydam, DVM, PhD; Katharyn J. Mitchell, BVSc, DACVIM; Dwight D. Bowman, DVM, PhD

Objective—To determine the apparent prevalence of shedding of Cryptosporidium spp in healthy alpaca crias and their dams on 14 farms in New York and 1 farm in Pennsylvania.

Design—Cross-sectional study.

Animals—110 alpaca crias and their 110 dams.

Procedures—Fecal samples were obtained from 220 alpacas at 14 alpaca farms in New York and 1 farm in Pennsylvania. For each animal, age, sex, and health condition were recorded. A fecal score (1 = normally formed; 2 = soft or loose; 3 = diarrhetic) was recorded for each cria. Cryptosporidium oocysts were identified in fecal samples by a direct immunofluorescence assay.

Results—Apparent prevalence of fecal shedding of Cryptosporidium oocysts was 8% (95% confidence interval, 4% to 15%) in dams and was 7% (95% confidence interval, 3% to 13%) in crias. There was no significant difference in age between dams with positive fecal test results for Cryptosporidium oocysts (median age, 4 years; range, 3 to 8 years) and dams with negative results (median age, 4 years; range, 2.5 to 19 years). No significant difference was found in age between crias with positive fecal test results (median age, 20 days; range, 7 to 53 days) and those with negative results (median, 36 days; range, 2 to 111 days). No significant difference in fecal scores was found between crias with positive versus negative fecal test results.

Conclusions and Clinical Relevance—A higher than previously reported apparent prevalence of fecal shedding of Cryptosporidium oocysts in healthy alpacas was found. A zoonotic risk should be considered, especially for Cryptosporidium parvum. (J Am Vet Med Assoc 2012;241:496–498)

Cryptosporidium organisms are apicomplexan (protozoan) parasites that infect mammals, birds, reptiles, amphibians, and fish, causing enterocolitis and diarrhea. Several clinical reports have identified Cryptosporidium spp as etiologic agents of diarrhea in alpacas. However, few data exist on the prevalence of Cryptosporidium spp in apparently healthy domesticated South American camelids (llamas and alpacas). Examination of fecal samples from 354 llamas at 33 locations in California, by means of immunofluorescent microscopy, revealed no shedding of Cryptosporidium oocysts. In a similar study, Cryptosporidium oocysts were not detected in fecal samples from 61 alpacas (age, 10 weeks to 10 years) on 2 farms in Maryland. During an outbreak of cryptosporidiosis in some crias from 2 alpaca farms in the United Kingdom, examination of fecal samples via a fluorescent antibody test did reveal Cryptosporidium oocysts in 4 crias with normally formed feces.

From the Departments of Population Medicine and Diagnostic Sciences (Burton, Nydam), Clinical Sciences (Mitchell), and Microbiology and Immunology (Bowman), College of Veterinary Medicine, Cornell University, Ithaca, NY 14853. Dr. Burton’s present address is Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602.

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Abbreviation

CI Confidence interval

The purpose of the study reported here was to determine the apparent prevalence of shedding of Cryptosporidium spp in 110 healthy alpaca crias and their dams (total, 220 animals) on 14 farms in New York and 1 farm in Pennsylvania. Fecal samples from all 220 healthy alpacas (110 dams and their crias) were systematically collected and evaluated for Cryptosporidium oocysts. None of the tested animals had clinical signs of diarrhea that would have otherwise prompted fecal examination.

Materials and Methods

Study design and procedures—The study protocol was approved by the Institutional Animal Care and Use Committee at Cornell University. A cross-sectional study was performed to assess the prevalence of Cryptosporidium spp in alpaca crias and their dams from farms in New York and Pennsylvania. Alpaca breeding farms with > 10 crias born/y (30 farms) were selected from the database of the Empire Alpaca Association, a New York affiliate of the Alpaca Owners and Breeders Association, and contacted via e-mail or telephone. Owners of 15 of the 30 farms agreed to participate in the study. On each
farm, fecal samples were collected from all crias < 16 weeks of age and from their respective dams. Between March 24 and August 24, 2009, fecal samples were obtained from alpacas on 14 farms in New York and 1 farm in northern Pennsylvania. Prior to fecal sample collection, physical examinations were performed on each alpaca by an author (AJB or KJM). Historical health status of the herd was assessed by an owner questionnaire. Questions included those pertaining to any biosecurity measures taken for new and or sick animals, routine testing for infectious disease, neonatal cria management, parasite control and vaccination programs in place, morbidity and mortality rate during the previous 12 months, and the suspected causes. Owners were also asked specifically whether Salmonella or Cryptosporidium infections were diagnosed in herd members during the past 5 years and whether they had heard of cryptosporidiosis and knew what it was. Fecal samples were obtained via digital rectal technique, placed into empty plastic containers, refrigerated for transport, and stored at 4°C prior to examination. A simple fecal score at the time of fecal sample collection was recorded for the crias, with 1 being normally formed feces, 2 being soft or loose feces, and 3 being overt diarrhea.

All fecal samples were screened for the presence of *Cryptosporidium* oocysts via a commercially available direct immunofluorescence assay kit. A rapid screening method developed within the authors’ laboratory was used. Briefly, a thin fecal smear was made on a glass microscope slide by use of a sterile cotton swab. Seven microliters of detection reagent (ie, fluorescein isothiocyanate–labeled anti- *Cryptosporidium* and anti-Giardia monoclonal antibodies) was immediately placed on top of the fresh fecal smear and covered with a 22-mm glass coverslip. After 1 minute, the glass microscope slide was examined at 200X magnification under a fluorescence microscope. This modified method to detect *Cryptosporidium* oocysts was assessed previously in the authors’ laboratory by use of spiked calf fecal samples containing known quantities of oocysts. The modified method was comparable in sensitivity with the conventional procedure detailed by the manufacturers of the direct immunofluorescence assay kit, with a sensitivity of 90% and a specificity of 100%, although with both methods, threshold of detection was > 1,000 oocysts/g of feces in naive oocyst-seeded feces.

**Statistical analysis**—Data were analyzed by use of statistical software. Continuous data were nonnormally distributed as assessed by the Shapiro–Wilk test. A Wilcoxon rank sum test was used to compare sets of continuous data, and the Fisher exact test was used for comparison of dichotomous variables. Values of *P* ≤ 0.05 were considered significant. For the apparent prevalence of fecal shedding of *Cryptosporidium* oocysts, the exact binomial 95% CI was calculated.

**Results**

Fecal samples were obtained from dams (110 adults) and their unweaned crias (110) on 15 farms, with a median of 8 fecal samples collected/farm (range, 7 to 9 fecal samples/farm). Total number of adult camelids (including males and nonbreeding animals) on all farms at the time of sampling ranged from 21 to 276, with a median of 69, and number of crias ranged from 7 to 30, with a median of 10. The median age of adults was 5 years (range, 2.5 to 19 years) and of the crias was 36 days (range, 2 to 111 days). There was no significant difference (*P* = 0.4) in the number of male (n = 52) versus female (58) crias that underwent fecal testing. There was also no significant (*P* = 0.3) difference in the proportion of male (2/52) versus female (6/58) crias that had positive fecal test results for *Cryptosporidium* oocysts.

Six of 15 alpaca farms had dams or their cria with positive fecal test results for *Cryptosporidium* oocysts. Overall, 9 dams and 8 crias had positive fecal test results for *Cryptosporidium* oocysts and 101 adults and 102 crias had negative results. The apparent prevalence of fecal shedding of *Cryptosporidium* oocysts in dams was 8% (95% CI, 4% to 15%) and in their crias was 7% (95% CI, 3% to 13%). There was no significant (*P* = 0.8) difference in age between dams with positive fecal test results for *Cryptosporidium* oocysts (median age, 4 years; range, 3 to 8 years) and dams with negative results (median age, 4 years; range, 2.5 to 19 years). Similarly, no significant (*P* = 0.2) difference was found in age between crias with positive fecal test results for *Cryptosporidium* oocysts (median age, 20 days; range, 7 to 53 days) and those with negative results (median, 36 days; range, 2 to 111 days). When crias with fecal scores of 2 or 3 (ie, feces with soft to diarrhetic consistency) were grouped, there was no significant (*P* = 0.5) difference in fecal scores (score of 1 vs score of 2 or 3) between crias with positive fecal test results for *Cryptosporidium* oocysts and those with negative results. Similarly, when crias with fecal scores of 1 or 2 (ie, feces with normal to soft consistency) were grouped, there was no significant (*P* = 0.7) difference in fecal scores (score of 1 or 2 vs scores of 3) between crias with positive fecal test results for *Cryptosporidium* oocysts and those with negative results. Although both adult alpacas and crias shed *Cryptosporidium* oocysts in feces, no related dam-cria pairs were found to be simultaneously shedding oocysts. Only owners of 2 farms reported having identified *Cryptosporidia* oocysts previously in fecal samples from alpacas. Both farms were in conjunction with an outbreak of diarrhea in crias. *Cryptosporidium* oocysts were identified in cria fecal samples with normal consistency from 1 of these 2 farms only. Although none of the other farms, to the best of farm staff knowledge, had previous cases of *Cryptosporidium* infections, the staff at 6 of these 13 farms had not heard of cryptosporidiosis (or crypto) before.

**Discussion**

In the present study, we found a higher apparent prevalence of fecal shedding of *Cryptosporidium* oocysts in apparently healthy alpacas than has been identified previously. Information on subclinical fecal shedding of *Cryptosporidium* oocysts is useful for 2 reasons. Firstly, subclinical fecal shedding of *Cryptosporidium* oocysts could present a greater zoonotic and biosecurity risk, compared with oocyst shedding by an alpaca with signs of outward illness, simply due to caretaker and veterinarian awareness. Secondly, it may be that some *Cryptosporidium* oocysts in apparently healthy alpacas than has been identified previously.
Ruminants/Camelids

Cryptosporidium infections in camelids are not pathogenic but are cited as the cause of diarrhea due to their identification, superimposed with another etiologic agent. Although no association was identified between fecal score in crias and the presence of Cryptosporidium oocysts, we cannot infer any relationship to clinical disease from the data of the present study because the prevalence was low and only 1 sample was collected at a single time point. It is possible that alpacas infected with Cryptosporidium spp could have had unnoticed diarrhea in the past or might develop diarrhea or reduced weight gain or weight loss in the future. Finally, although only 2 of 15 owners reported having Cryptosporidium spp identified on their farm previously, the staff at 6 of these farms had never heard of cryptosporidiosis before. This factor, combined with the low likelihood of detecting oocysts in a standard fecal floatation test, could mean that some cases went undiagnosed. To assess these possibilities, a longitudinal study, with examination for all other pathogens that might be responsible for diarrhea and weight loss, would have to be performed.

To date, the sparse molecular research on Cryptosporidium spp from South American camelid feces has identified the zoonotic species Cryptosporidium parvum, formerly the cervine genotype, which has also been identified in alpaca feces. The oocysts seen in our samples were the correct size and morphology for C. parvum, so this species, or the cervine genotype, are candidates. This study indicates that apparently healthy alpacas with normally formed feces can be shedding Cryptosporidium oocysts and thus could represent a zoonotic risk, especially if the species were C. parvum, the most commonly identified zoonotic species in human cryptosporidiosis, although C. ubiquitum has been detected in people also.

References