Potential pathogens in feces from unweaned llamas and alpacas with diarrhea

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Objective—To identify potential pathogens in feces from llama and alpaca crias with diarrhea. Design—Prospective observational study. Animals—45 unweaned crias with diarrhea. Procedure—Fecal samples were evaluated for Eimeria spp, Giardia spp, Cryptosporidium spp, enteric viruses, and Salmonella spp. A questionnaire yielded information concerning herd management and presence of other affected camels. Results—28 crias were ≤ 31 days old, 11 were 32 to 62 days old, and 6 were 63 to 210 days old. Potential pathogens were isolated from feces from 32 of the 45 crias. A total of 59 potential pathogens were obtained, including coronavirus (n = 19 crias; 42%), Giardia spp (8; 18%), Eimeria spp (6; 13%), Cryptosporidium spp (4; 9%), rotavirus (1; 2%), and nematode ova (1; 2%). Salmonella spp were not isolated. Most crias from which potential pathogens were isolated were identified during outbreaks of diarrhea involving other camels, although only coronavirus was isolated from crias identified during outbreaks involving adult camels. Coronavirus was detected throughout the year, whereas protozoa were most commonly isolated during the fall and winter. Conclusions and Clinical Relevance—Results suggest that a variety of potential pathogens may be isolated from young crias with diarrhea. Many crias shed coronavirus, which may also have been affecting older camels. Protozoa were isolated most often during wetter months, suggesting that crias born during these months may have greater exposure to protozoal pathogens. (J Am Vet Med Assoc 2003;223:1806–1808)

Diarrhea is 1 of the most common and most important causes of death and disease among neonatal and juvenile domestic hoofstock. In many cases, diarrhea is thought to result from an intestinal microbial infection, with transmission of the causative organism from older animals to younger ones and from younger animals to each other. Llamas and alpacas are domesticated native South American herbivores that are physiologically and anatomically similar in many ways to domestic ruminants. In North America, they are managed similarly to flocks of small ruminants, with the major exception being that live animals are typically the only market product. A growing number of camelids are being used to improve health care in these species. Among the subjects about which little information is available are causes of diarrhea in unweaned llamas and alpacas. Bacterial, viral, protozoal, and helminth pathogens are all associated with diarrhea in domestic hoofstock. The presence of some of these potential pathogens, particularly protozoa and helminths, has been studied epidemiologically in llamas and alpacas, but information on their pathogenicity, clinical importance, and shedding patterns is generally lacking or limited to brief case reports. The purposes of the study reported here were to identify potential microbial and helminth pathogens in feces from unweaned llama and alpaca crias with diarrhea and determine factors possibly associated with identification of such potential pathogens.

Materials and Methods Animals—Unweaned llama and alpaca crias up to 7 months old that had had diarrhea for < 3 days, for which adequately sized fecal samples had been submitted to the Oregon State University Veterinary Diagnostic Laboratory between July 1, 1999, and June 30, 2002, were eligible for inclusion in the study. Fecal samples were collected by veterinarians or other individuals on the farm, chilled, and transported within 24 hours to the laboratory or, for animals hospitalized at the Oregon State University Veterinary Teaching Hospital, were immediately transported to the laboratory. If multiple samples were obtained from a cria because of a relapse of diarrhea or long-term diarrhea, information only for the first sample submitted was used. To promote participation, the study was described in direct mailings to local veterinarians, announced at veterinary meetings and lay presentations, and advertised on electronic mailing lists devoted to camelids and in lay newsletters.

Data collection—The herd veterinarian or owner was asked to complete a questionnaire for each fecal sample submitted. The questionnaire solicited information on age of the animal, duration of illness, clinical signs, whether the animal had been transported recently, time of year, diet, whether the animal had been treated with anthelmintics, and environment (ie, whether the animal was exposed to other camels of various age groups, other camels with diarrhea, or other domestic species). Fecal analysis—Fecal samples were divided into aliquots, and the following assays were performed: routine procedures were used. The McMaster flotation procedure was used to identify nematode ova and Eimeria spp cysts. Direct fecal smears stained with fluorescent antibody were examined for Giardia duodenalis, and smears stained with TB Auramine M were examined for Cryptosporidium parvum. Samples were streaked on xylene-lysine-tergitol 4 and MacConkey agar plates, as well as incubated overnight in selenite enrichment broth and then streaked on xylene-lysine-tergitol 4 plates, and plates were examined for Salmonella spp. For detection of viruses, fecal samples were prepared in a 10% suspension and clarified by centrifugation. The supernatant...
was centrifuged to concentrate viruses, and the pellet was suspended in 4 to 5 drops of distilled water. The preparation was stained with potassium phosphotungstate and viewed with an electron microscope. Coronaviruses and rotaviruses were identified on the basis of characteristic morphology.

**Results**

Fecal samples were collected from 45 unweaned llama and alpaca crias up to 7 months old that had had diarrhea for < 3 days. Completed questionnaires were obtained for all 45 crias. All crias lived in northwestern Oregon. Crias came from 25 different herds, with herd size ranging from < 10 to > 100 camelids.

Potential pathogens were isolated from feces from 32 of the 45 crias. A total of 39 potential pathogens were obtained, including coronavirus (n = 19 crias; 42%), *Giardia* spp (8; 18%), *Eimeria* spp (6; 13%), *Cryptosporidium* spp (4; 9%), rotavirus (1; 2%), and nematode ova (1; 2%). *Salmonella* spp were not isolated.

Coronavirus was identified in fecal samples from 16 of the 23 (69%) herds, with 3 herds each having 2 crias from which coronavirus was isolated. *Giardia* spp were identified in samples from 5 (20%) herds, with 1 herd having 4 crias from which *Giardia* spp were isolated. The remaining potential pathogens were each identified in single samples from individual herds. The 2 herds from which the most samples were obtained (6 and 5) each had at least 3 different potential pathogens identified. One of these herds had only 16 camelids; the other had > 100.

Twenty-eight of the crias were ≤ 31 days old, 11 were 32 to 62 days old, and 6 were 63 to 210 days old (Fig 1). Coronavirus was identified in crias between 10 and 150 days old; *Giardia* spp were identified in crias between 10 and 120 days old, with 7 of the 8 being > 30 days old; *Eimeria* spp were identified in crias between 21 and 60 days old; and *Cryptosporidium* spp were identified in crias between 10 and 45 days old, with 3 of the 4 being < 14 days old. The cria in which nematode ova were identified was 60 days old, and the cria in which rotavirus was identified was 210 days old.

Fifteen samples from crias > 31 days old that were obtained during October or November yielded 11 potential pathogens, including coronavirus (n = 5) and protozoa (6; Fig 2). Nine samples from crias ≤ 31 days old that were obtained during June through August yielded coronavirus (n = 6) and protozoa (3).

Seven samples from crias > 31 days old that were obtained during October through December yielded 8 potential pathogens, including coronavirus (n = 3) and *Giardia* spp (3; Fig 3). Seven samples from crias > 31 days old that were obtained during February through March yielded 8 potential pathogens.

None of the herds reported that camelids were exposed to other species of hoofstock, although many herds were exposed to cats or dogs and 1 was exposed to poultry. All but 1 herd had camelids that had been to a show or had been to or arrived from another herd for reproductive or other purposes within the past 2 months. The herd with no reported movement yielded no potential pathogens. Herds from which coronavirus was isolated reported simultaneous or recent diarrhea in other crias (13/15 herds; 1 questionnaire was incomplete) and older camelids, including adults (10/15 reporting herds). Many of these affected camelids or herdmates not included in the study had been moved within 2 weeks of the onset of signs in the herd. Simultaneous or recent diarrhea was reported in other crias from herds from which *Giardia* spp (3/5 herds), *Eimeria* spp (4/6 herds), and *Cryptosporidium* spp (2/4 herds) were isolated, but reports of diarrhea in older camelids in these herds were rare, unless crias with coronavirus also came from that herd.

**Discussion**

The major finding in the present study was how frequently coronaviruses could be isolated from fecal samples from young llamas and alpacas with diarrhea. To our knowledge, coronaviruses have not previously been isolated from the feces of llamas and alpacas, and a similar study10 in South America did not find any evidence of coronaviruses. That same study found serologic evidence of rotavirus infection in 93% of young
guanacos, whereas rotavirus was isolated from only a single cria in the present study. Contagious spread of potential pathogens from herd to herd could not be documented in the present study because most herds in this study had commuter populations.

Many of the potential pathogens identified in the present study were isolated during outbreaks of diarrhea in the affected herds. This supports the suggestion that these organisms had a pathogenic role, although healthy control populations were not examined for comparison. Greater concern on the part of the owners dealing with outbreaks of diarrhea may also have biased our results toward infectious or parasitic agents, but this also allowed us to concentrate on agents that might be controlled through prophylactic or therapeutic means. Of the agents that we identified, coronavirus was the only one commonly seen in crias from herds with outbreaks of diarrhea involving adult camels. Given that enteric coronavirus disease is uncommon in adults of other large ruminant species 1 and has not been described previously in New World camels, we surmise that these outbreaks were the result of naive camels being exposed to a novel pathogen. Further studies to support this hypothesis are needed, however. In addition, the source of the coronaviruses identified in the present study was not determined, but none of the herds had direct contact with other species of livestock.

The high number of crias from which *Giardia* spp were isolated in the present study was another interesting finding. Prior to this report, the most commonly described intestinal pathogens in llamas and alpacas were *Eimeria* spp and nematodes. 2 3 *Eimeria* spp are widely known parasites of the intestine and are thought to cause disease in camels under similar conditions as in domestic ruminants, with disease being more common among groups of animals housed in dirty, overcrowded buildings or pasture and among populations that have a large proportion of young, immunologically naive animals. Nematode infections occur under similar conditions, with greater emphasis on contaminated pastures. With both types of parasite, high intestinal loads may be necessary to cause diarrhea and smaller loads may be clinically unimportant. Although we found rare evidence of both nematode and *Eimeria* infestations, prevalence of infection could not be estimated because both are capable of inducing disease before ova or cysts are present in the feces. However, it is also possible that the higher awareness of these parasites has led to effective countermeasures in herds or that they are not as common causes of diarrhea in crias as previously believed.

*Giardia* spp and *Cryptosporidium* spp have both been identified previously in feces from New World camels. *Cryptosporidium* spp are zoonotic pathogens associated with diarrhea in young crias 4 5 but are generally rare and typically not isolated from healthy camels. 6 *Giardia* spp are potentially zoonotic pathogens that previously have been recovered from up to 25% of young llamas 7 and, in 1 instance, 8 from an unthrifty llama with soft feces. Whether any of the crias in the previous study had diarrhea was not reported. Our work suggests that giardiasis could be common in certain herds but is not widespread. More work is necessary to determine the clinical importance of giardiasis in young camels.

*Salmonella* spp were not found in this study or in a previous study 9 of healthy camelids of all ages. On the basis of results of these 2 studies, we suggest that enteric salmonellosis is rare in llamas and alpacas.

In the present study, we received the highest number of fecal samples from crias ≤ 31 days old during the fall, whereas peak sample submission times for crias > 31 days old were the winter and spring. These peaks coincided with isolation of protozoa, as coronaviruses were isolated throughout the year. Other researchers investigating other species have found increases in fecal parasite ova and *Eimeria* oocyst shedding 10 and increases in contamination of water sources by *Cryptosporidium* spp and *Giardia* spp during periods of high rainfall. 11 Although relative risk could not be determined in the present study, the weather conditions in the Pacific Northwest could have contributed to why most protozoal isolates were identified during the wet season. Fall birthing has been promoted as a strategy to avoid gestational hypovitaminosis D. However, in mild winter environments such as the Pacific Northwest, this strategy may increase the number of vulnerable crias during the wet fall and winter periods when protozoa may be more common and, hence, may increase problems with diarrhea.


References