

Original Article

Outbreak of diarrhea among preweaning alpacas (*Vicugna pacos*) in the southern Peruvian highland

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Abstract

Introduction: Infections, particularly diarrheal infections, are a major cause of neonatal death in South American camelids. The aim of this study was to identify the pathogens that could have caused the recent diarrhea outbreak among the alpacas in Silli, Cusco, located in the southern Peruvian highland.

Methodology: Spleen, kidney, and intestine tissue along with fecal and intestinal lavage samples were obtained from 50 one- to five-week-old alpacas and analyzed for the presence of parasites, bacteria, and viruses.

Results: Laboratory testing of the 50 crias included in this study revealed that 80% were infected with *Eimeria* spp., 40% with coronavirus, 34% with *E. coli*, 32% with rotavirus, 22% with *Clostridium* spp., and 20% with *Cryptosporidium* spp. Of these 50 alpaca crias, 20 presented with a single infection (19 positive for *Eimeria* spp. and 1 positive for rotavirus). Co-infections with up to four pathogens occurred in 60% of the samples. The significance of such infections is not clear, but it is noteworthy that the animals suffering from necrotic and/or hemorrhagic enteritis presented with quadruple infections. It is likely that co-infections increase the severity of the disease.

Conclusions: These data show that multiple pathogens circulate among young alpaca crias and could be associated with diarrheal disease in these animals. The findings from this study warrant the provision of subsidies for future assessment of the potential economic impact of these infections on the productivity of the Peruvian alpaca industry.

Key words: alpacas; bacteria; diarrhea; Peru; protozoa; virus.

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Introduction

Alpacas (*Vicugna pacos*) are a domesticated species of camelids indigenous to the Andean region of South America. Peru has the largest alpaca herd in South America and the world (4–4.5 million alpacas) [1]. The largest proportion of these alpacas lives in the southern Peruvian highland, which represents one of the nation's most important regions of economic activity [2]. Commercialization of alpaca breeding is also growing outside South America, particularly in Australia, the United States, and the United Kingdom [3,4].

According to the National Congress of South American Camelids, the primary source of income for at least 1.5 million people living in the highlands of the Peruvian Andes is the breeding of South American domestic camelids [5]. Inhabitants of the Andean

highlands raise alpacas for food and fiber, and the feces of these animals are used as fuel for cooking food and as fertilizer. Peru produces an average of about 3.5 million kg of alpaca fiber annually, which represents 90% of total world production [6]. Cusco is the second-most important center for alpaca breeding in Peru. Almost 90% of the alpaca population in this region is generated and cared for by small producers, who have inadequate health management training due to their extreme poverty [1,2,7]. The breeding of alpacas in this environment is characterized by low productivity, reduced fertility rates, and high neonatal losses that are not always included in the analysis of industrial productivity and in health records [1].

During the first week of February 2010, the Tropical and High-Altitude Veterinary Research

Institute of the National University of San Marcos, Peru, was notified of a diarrhea outbreak that resulted in high rates of morbidity and mortality among neonatal alpacas in Silli, Cusco. Silli is home to approximately 790 alpacas. On average, 100 animals are born in Silli each year, and the mortality rates for neonatal and adult alpacas are about 18% and 7%, respectively. In Silli, 101 families depend on the flocks of farm alpacas for subsistence. Loss of crias due to infection imposes a major economic burden on this population.

Alpacas are regarded as very resilient animals. However, several diseases, the prevalence of which depends mainly on the climate and management conditions, can endanger neonatal crias and ultimately limit productivity [3,7-9]. A six-year study of neonatal crias from communal herds in Puno Department, the largest alpaca-producing area in Peru, determined that enteric disorders (especially enterotoxemia) were responsible for 44% of the mortalities [10]. Diarrhea is an important disease in alpacas, and it is the most common cause of morbidity during the preweaning period, affecting about 23% of crias [8,9,11]. The most significant infectious pathogens causing diarrhea in alpacas are *Coccidia* (affecting crias 21–60 days of age), *Cryptosporidium* spp. (affecting crias 7–45 days of age), *Giardia* spp. (affecting crias 7–120 days of age), *Salmonella* spp. (affecting crias at any age), *Escherichia coli* (affecting crias younger than 7 days of age), rotavirus (affecting crias at any age), and coronavirus (affecting crias 10–150 days of age) [8,9,11]. *Clostridium* spp. have also been detected in diarrheic alpacas younger than 6 months of age [1,9]. Identification of the infectious agent involved in the disease is important for proper treatment. Bacterial infections may require antibiotic administration [8,9], while antiparasitic drugs have been effective in treating giardiasis and coccidiosis in alpacas [4,9,12]. Treatment for viral diarrhea is mainly supportive. Nevertheless, it is important to rule out viral diarrhea and, if necessary, treat other pathogens that may be concurrently involved in the disease pathogenesis [8,9].

Despite the importance of these animals to the Peruvian economy, studies examining the frequency of enteric infections (especially viral infections) among these herds are scarce. A better understanding of the epidemiology of enteric infections is essential for the development of preventive measures. Therefore, the aim of this study was to identify the pathogens that could have caused the recent diarrhea outbreak among the alpacas in Silli, Cusco, located in the southern Peruvian highland.

Methodology

Spleen, kidney, and intestine tissue along with fecal and intestinal lavage samples were obtained from 50 preweaning (one- to five-week-old) alpacas suffering from dehydrating diarrhea during an outbreak that occurred between January and February 2010. The alpacas were from an Andean alpaca herd in Silli, Cusco, Peru, approximately 4,000 meters above sea level. The animals were subjected to postmortem examination at the Laboratory of Histology, Embryology and Veterinary Pathology, Universidad Nacional Mayor de San Marcos, Peru.

Bacteriological and parasitological analyses were performed in the Laboratory of Microbiology and Parasitology, Faculty of Veterinary Medicine, Universidad Nacional Mayor de San Marcos, Peru. Virological analyses were carried out in the Institute of Microbiology Prof. Paulo de Goes, Federal University of Rio de Janeiro, Brazil. Samples of spleen, kidney, and intestine obtained during the necropsy procedure were streaked onto MacConkey agar plates (Oxoid Limited, Hampshire, UK) and thioglycollate broth (Oxoid Limited) for *E. coli* and *Clostridium* spp. isolation, respectively. Fecal samples were collected from the rectum, and the McMaster flotation procedure and light microscopy were used to identify *Eimeria* spp. oocysts [13]. Smears of intestinal tissue stained with the modified Ziehl-Neelsen technique were examined for *Cryptosporidium* spp. [14]. Intestinal lavage samples were obtained during necropsy by thoroughly washing the intestines with warm water and were then used for viral detection. The same samples were taken from all of the animals tested. The lavage samples were diluted in 10% v/v sterile phosphate-buffered saline and clarified by low speed centrifugation (2,500×g) for five minutes. Total RNA was extracted from 300 µL supernatant using a kit according to the manufacturer's instructions (Applied Biosystems/Ambion, Austin, USA). Viral RNA detection was performed by conventional reverse transcription polymerase chain reaction (RT-PCR). All primers were synthesized by IDT (Integrated DNA Technologies, Coralville, USA). Reactions were performed in a Veriti 96-well thermocycler (Applied Biosystems, Foster City, USA). Rotavirus detection was done using a set of conserved primers that amplify a conserved region (379 base pairs [bp]) of the VP6-encoding gene present in rotavirus strains belonging to species A (RVA) [15]. Coronavirus was detected by using a set of conserved primers that amplify a 251 bp fragment of the polymerase gene present in all coronavirus strains [16]. The RT-PCR products were separated by 1.2% (w/v) agarose gel

electrophoresis, stained with ethidium bromide, and visualized under UV light [17]. A 100 bp DNA ladder (Fermentas/Thermo Fischer Scientific, Waltham, Massachusetts, USA) was used to determine molecular size.

Results

On physical examination, the alpacas were prostrate; the body condition scoring (BCS) was determined to evaluate the animals' fat stores and long-term energy balance; the sick alpacas were given a BCS of 3/10, which means that they had no visible or palpable fat or muscle between skin and bones and extreme loss of muscle [18]. The alpacas exhibited cachexia, dehydration of varying severity, and fever (Figure 1A). The alpaca fibers and ocular, labial, vulvar, and penile mucus membranes were dull and pale (Figure 1B), and capillary refill times were more than four seconds. In the most severe cases, the animals exhibited hypothermia (6 animals). Most animals (27/50) presented with profuse, foul-smelling, watery, mostly greenish feces, whereas the others (23/50) presented with grayish or yellowish feces. Of the 50 affected animals, 6 died and 44 were euthanized to prevent the spread of infection to other alpacas.

The 6 dead animals were examined by gross necropsy (macroscopic examination of the animal), which revealed marked muscle wasting and loss of body fat. All of the mesenteric lymph nodes were enlarged and dark red. The intestinal contents contained gas and were watery, yellowish, and mixed with mucus (Figure 1C). Their livers had areas of infarction in the caudate lobe. The lungs had signs consistent with pneumonia. Necropsy of the 44 euthanized animals revealed intestinal congestion, inflammation, and watery feces, findings consistent with all 6 of the dead alpacas. Samples of spleen, kidney, intestine, intestinal lavage, and feces were submitted for laboratory investigation.

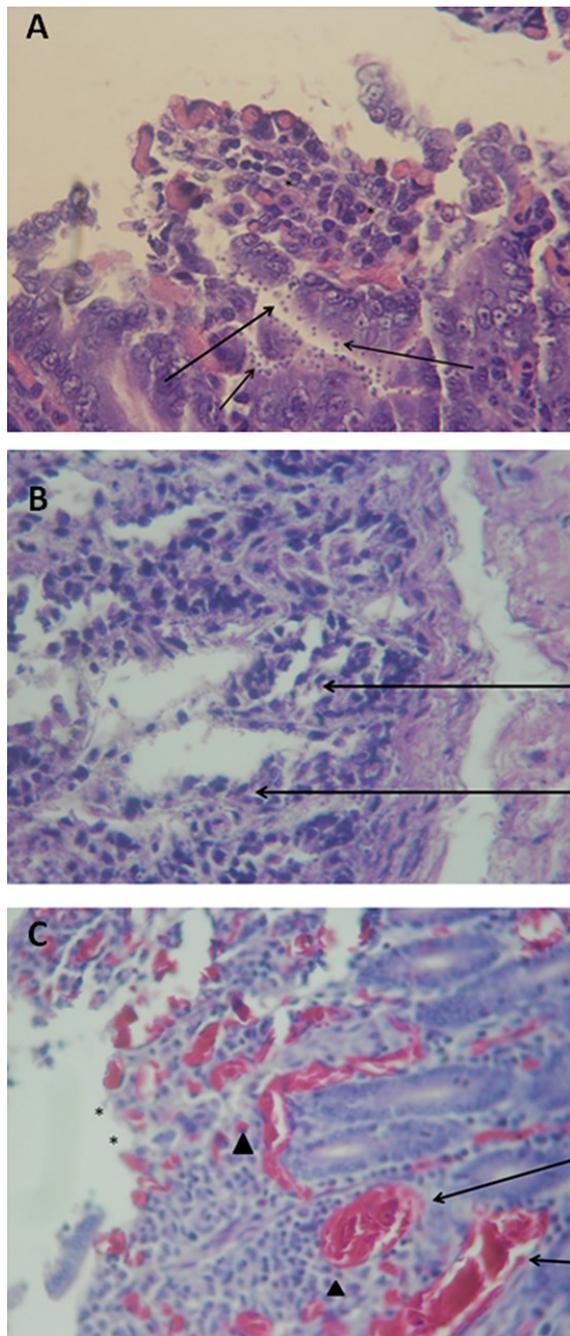
Histopathological examination of intestinal specimens revealed that most of the animals (44/50) demonstrated catarrhal enteritis (Figure 2A), while only 4 animals presented with necrotic enteritis (Figure 2B), and 2 presented with hemorrhagic necrotic enteritis (Figure 2C). Those animals with necrotic and/or hemorrhagic enteritis presented with multiple infections consisting of rotavirus + coronavirus + *Eimeria* spp. + *Clostridium* spp. (4 crias) or coronavirus + *Eimeria* spp. + *E. coli* + *Clostridium* spp. (2 crias). Laboratory testing of the 50 crias included in this study revealed that 80% were infected with *Eimeria* spp., 40% with coronavirus, 34% with *E. coli*, 32% with

Figure 1. Gross appearance of diarrheic alpacas. **A:** Prostrate animal showing muscle wasting, cachexia, and dull fiber; **B:** Pale mucus membranes and corneal opacity; **C:** Representative gross necropsy of a diarrheic alpaca. The intestinal contents were watery, yellowish, and mixed with mucus.



rotavirus, 22% with *Clostridium* spp., and 20% with *Cryptosporidium* spp. Co-infections with up to four pathogens occurred in 60% of the animals and included the following pathogens: *Eimeria* spp., *Cryptosporidium* spp., *Clostridium* spp., coronavirus, rotavirus, and *E. coli* (Figure 3). Of the 20 alpacas with

Figure 2. Histopathology of intestinal tissue from diarrheic alpacas. Modified Ziehl-Neelsen. Magnification × 400. **A:** Jejunum of a 15-day-old alpaca with catarrhal enteritis. Villous epithelium shows developmental stages of *Cryptosporidium* spp. (arrows). Epithelial cells have sloughed into the intestinal lumen, leaving the lamina propria of villi with congested blood vessels exposed. Mononuclear cells (macrophages/monocytes and lymphocytes) are present in the subepithelium (*). **B:** Jejunum of a 15-day-old alpaca showing early stage necrotic enteritis. Karyorrhexis and karyolysis of crypt cells and villous epithelium are present (arrows). **C:** Ileum of a 7-day-old alpaca with hemorrhagic necrotic enteritis. Loss of the villous epithelium has left the lamina propria (*) exposed. Dilated blood vessels (→), inflammatory cells, and extravasations of red blood cells (◄) are also present.



single infections, 19 tested positive for *Eimeria* spp., and 1 tested positive for rotavirus (Table 1).

Discussion

The purpose of this study was to investigate a diarrhea outbreak among Peruvian alpacas that occurred in Silli, Cusco, using histopathological and microbiological methods. We found that the majority of the 50 crias (80%) affected by the diarrhea outbreak tested positive for *Eimeria* spp., and nearly half (47.5%) of these animals had no dual infection. Coccidiosis in camelids is caused by several different species belonging to the genus *Eimeria* [1,8,9,11,19]. It is the main diarrheal pathogen reported in Peruvian camelids and is regarded as the most common cause of death in alpacas younger than 4 months of age [20-22]. Similar to our findings, previous necropsies of diarrheic crias infected with *Eimeria* have revealed intestines distended by gas, reddened areas affecting primarily the jejunum and ileum, and enlarged mesenteric lymph nodes [20-22]. Congestion has been found in other internal organs, including the kidneys, thymus, liver, and lungs. In another study, histopathological findings in the alpaca intestine ranged from moderate catarrhal enteritis to severe necrotic enteritis and locally extensive hemorrhagic and necrotic enteritis [21].

Cryptosporidium has been detected in diarrheic alpacas [11,23-25] and has been recognized as diarrhea-causing pathogen in preweaning crias younger than 3 weeks of age. Subclinical infection with *Cryptosporidium* has also been reported in alpacas [26]. In Peru, one study detected *Cryptosporidium* in 4.4% of fecal samples (n = 274) collected from 12 herds in Puno

Figure 3. Photomicrograph of a section of jejunum from a 21-day-old alpaca infected with *Eimeria* spp. Modified Ziehl-Neelsen. Magnification × 1000. Note the numerous stages of parasite development.

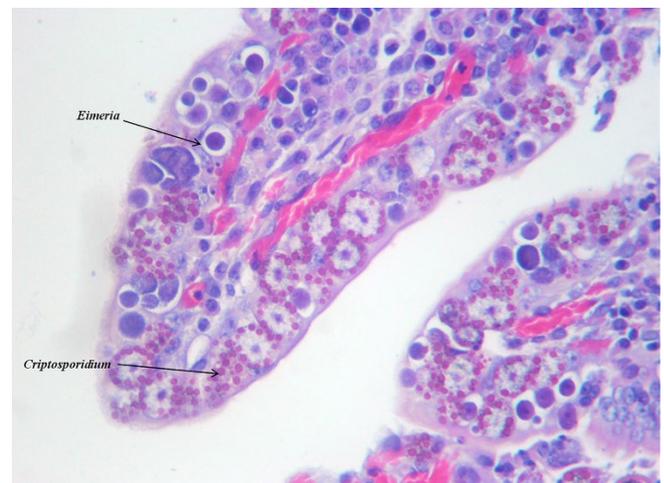


Table 1. Parasites, bacteria, and viruses detected in alpaca crias.

Pathogen	Number of samples
<i>Eimeria</i> spp.	19
RVA	1
<i>Eimeria</i> spp. + CoV	1
RVA + <i>E. coli</i>	5
CoV + <i>Cryptosporidium</i> spp.	1
<i>Eimeria</i> spp. + CoV + <i>Clostridium</i> spp.	4
CoV + <i>Cryptosporidium</i> spp. + <i>E. coli</i>	3
<i>Eimeria</i> spp. + CoV + <i>E. coli</i>	2
<i>Eimeria</i> spp. + CoV + <i>Cryptosporidium</i> spp.	2
<i>Eimeria</i> spp. + RVA + <i>E. coli</i>	1
<i>Eimeria</i> spp. + RVA + CoV + <i>Clostridium</i> spp.	4
<i>Eimeria</i> spp. + RVA + <i>Cryptosporidium</i> spp. + <i>E. coli</i>	3
<i>Eimeria</i> spp. + CoV + <i>E. coli</i> + <i>Clostridium</i> spp.	2
<i>Eimeria</i> spp. + RVA + CoV + <i>Cryptosporidium</i> spp.	1
<i>Eimeria</i> spp. + RVA + <i>E. coli</i> + <i>Clostridium</i> spp.	1

RVA: rotavirus species A; CoV: coronavirus

and Cusco [27]. However, in that study it was not possible to establish an association between *Cryptosporidium* and diarrhea, because it was also detected among non-diarrheic animals. The age of the animals in that study was greater than 5 weeks. In the present study, we detected *Cryptosporidium* in 20% of diarrheic crias 1–5 weeks of age, but co-infection with other pathogens known to cause enteric disease in camelids was present in all cases, making it difficult to determine the role of *Cryptosporidium* in the illness.

Previous studies have shown that *Clostridium perfringens* and *E. coli* can cause enterotoxemia in Peruvian alpacas [1,28]. We detected one or the other of these bacterial species in 25 of 50 crias. In 3 cases, both bacterial species were present; however, all of those 25 animals also presented with co-infections by other pathogens.

Both coronavirus and rotavirus have been identified as diarrheal pathogens in neonatal alpacas [1,8,9,11,19,29]. In a study conducted in Oregon, USA [11], coronavirus was the most common diarrhea-causing pathogen in llama and alpaca crias younger than 31 days of age. Another study detected rotavirus and coronavirus in 16% and 23%, respectively, of 14 alpacas from the Centro Experimental de La Raya, Universidad Nacional de San Antonio Abad del Cusco, Peru [29]. In that study, virus detection was performed using immunochromatographic tests. By contrast, we used a more sensitive molecular amplification method (RT-PCR) that detected rotavirus and coronavirus in a larger percentage of alpacas (32% and 40%, respectively). One alpaca was positive for only rotavirus, whereas the others presented with multiple pathogens such as *Eimeria* spp., *E. coli*., *Clostridium*

spp., and coronavirus (Table 1). Coronavirus was only detected in co-infected animals.

Conclusions

Data presented in this report demonstrate that multiple pathogens co-circulate among young alpaca crias in Peru. Alone or in combination, it is likely that these pathogens could be associated with diarrhea. We observed a high rate of mixed infections (60%). We found a high number of triple and quadruple infections as well. The significance of such infections is not clear, but it is noteworthy that the animals suffering from necrotic and/or hemorrhagic enteritis presented with quadruple infections. It is likely that co-infections increase the severity of the disease. Future work will incorporate assessments of the potential economic impact of these infections on the productivity of the Peruvian alpaca industry.

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