Prevalence of Neonatal Cryptosporidiosis in Andean Alpacas (Vicugna pacos) in Peru

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Abstract: A national survey was conducted to evaluate the prevalence of Cryptosporidium sp. in alpacas 1 to 15 days-old of age (crias) in 105 Andean herds. Stool samples of 5,163 randomly selected crias from 7 representative geographical regions of Peru were screened using acid fast staining microscopy. Ninety percent of ~2.9 million Peruvian alpacas are raised in these areas. Data on geographical characteristics and husbandry practices by region were also collected. Prevalence was determined by study areas and by age (five categories set at three-day intervals). Cryptosporidium sp. was detected in crias from all 7 regions. The overall prevalence was 13%, ranging from 7 to 23% per region. Prevalence was 2% in the youngest and showed an increasing trend by age, reaching 20% in crias 12-15 days old. These oldest crias were more likely to have cryptosporidiosis (OR=2.2, p<0.01). Infections with Cryptosporidium were also significantly associated with low availability of grazing grounds and overcrowding at birth (p<0.01). This study demonstrates that Cryptosporidium is a frequent pathogen in Andean alpaca crias, and suggest that cryptosporidiosis may play a significant role in the alpaca neonatal diarrhea complex that causes significant morbidity and mortality among crias.

Keywords: Cryptosporidium, Cryptosporidiosis, Alpacas, Neonatal diarrhea.

INTRODUCTION

Alpacas (Vicugna pacos) are important for the economy of Peruvian peasants who live at ≥3,500 m above sea level (MASL) [1]. More than 70% of the world’s alpaca population is in the Andean “Altiplano”, located at ~3,500 to 4,100 MASL. The Altiplano is characterized by cold weather, limited rainfall, oxygen deficient environment (thin air) and large plateaus, with interspread mountains and valleys. Grazing areas are predominantly composed of native pastures rich in fiber and deficient in protein and key nutrients. Nonetheless, alpaca husbandry is a vital economic activity because it is the only potentially profitable farming alternative in these challenging conditions [2].

Cryptosporidium sp. is a ubiquitous and significant enteropathogen of both immunocompetent and immunocompromised vertebrate hosts [3, 4], and Cryptosporidium parvum has been identified as an important agent of the ruminant neonatal diarrhea syndrome [2, 5-10]. Cryptosporidium spp. are highly prevalent among several ruminant species [11], causing severe enteric disease, and economic losses [12, 13]. Cryptosporidium spp. have been described in young alpacas from 3 to 15 days of age [14, 15], and therapies or vaccines are not available to prevent or control this parasite in animals [16].

Neonatal alpacas (crias) are susceptible to enteritis and the neonatal diarrhea syndrome (NDS). This syndrome is characterized by self-limiting watering diarrhea lasting for up to 15 days, refractory to antibiotic treatments and, that is neither bacterial nor viral in origin [17, 18]. NDS is the most common and costly enteric disease affecting newborn llamas and alpacas [1, 19-21].

Cryptosporidium was first described as a fortuitous and innocuous parasite in alpaca crias, and its relationship with neonatal enteritis or diarrhea was not addressed [22]. Recently, however, C. parvum has been reported in diarrheic stools of young alpacas raised in small farms in the U.S.A. and England [23-26]. An experimental infection study using Cryptosporidium oocysts isolated from diarrheic stools from alpacas crias reproduced symptoms and course of the newborn alpaca atypical diarrhea reported by Moro in 1965 [18], this suggests that Cryptosporidium may be one of the etiologies of the alpaca NDS [27]. However, the prevalence of this parasite in alpaca crias raised under native husbandry practices in the Altiplano remained undetermined. According to the last national survey in Peru about 2,900,900 alpacas grazed in the Altiplano, at between 3,800 and 5,000 MASL [28]. Four regions of Peru have about 90% of the national population of alpacas: Puno (~1.7 million), Cuzco (~340,000) Huancavelica (~330,00), and Arequipa (~230,000 alpacas).

Here we describe the first major epidemiologic survey of Cryptosporidium in alpaca crias <15 days-old in the Peru-
vian Andes, the natural habitat for almost 80% of the world population of alpacas.

**MATERIALS AND METHODOLOGY**

**Study Sites**

The survey was conducted in 7 regions that have more than 90% of the alpaca population in Peru. Data on management practices, spacing in calving areas and the availability of grazing grounds were collected from each region. Good management practices were defined as use of iodine disinfection of the umbilical cord at birth, colostrum intake shortly after birth, availability and use of weather shelters, having a pasture rotation plan, and use of animal identification methods. Overcrowding at birth was defined as less than 2 meters of distance between alpaca mothers in the calving areas. The availability of grazing grounds was considered high if there were four or fewer alpacas/hectare.

In 1995 we conducted a preliminary study in 6 herds of alpacas in the region of Cuzco. Data from that survey was used to optimize procedures of this study and calculate the sample size for the national survey. Stool samples from 241 alpaca crias (<15 days-old) were collected; 24 crias had Cryptosporidium in their stools, with a resulting prevalence of 10% (Table 2). This preliminary study also showed variation among herds (0-30%), finding that was also used to design the national survey and evaluate potential predictors of infection.

**Table 2. Prevalence of Cryptosporidium in Alpaca Crias: Preliminary Study in Cuzco 1995**

<table>
<thead>
<tr>
<th>Herd</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1/69 (1)</td>
</tr>
<tr>
<td>P2</td>
<td>2/63 (3)</td>
</tr>
<tr>
<td>P3</td>
<td>18/61 (30)</td>
</tr>
<tr>
<td>P4</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>P5</td>
<td>2/24 (8)</td>
</tr>
<tr>
<td>P6</td>
<td>1/7 (14)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>24/241 (10)</td>
</tr>
</tbody>
</table>

**Study Animals**

Alpaca crias up to 15 days of age were randomly selected from 105 herds in 7 regions of Peru (Fig. 1), and they were not selected by breeds or sex. Age was the only selection parameter. This age was based on findings from the preliminary study, and reports from neonatal lambs [29-31], calves [32], and case reports from alpacas [33-37].

![Fig. (1). Physical map of Peru and location of the regions surveyed.  1 – Pasco, 2 – Junin, 3 – Huancavelica, 4 – Ayacucho, 5 – Arequipa, 6 – Cuzco, 7 – Puno.](image-url)
Table 3. Prevalence of Cryptosporidium in Fecal Samples of 5,163 Alpaca Crias from 105 Herds in 7 Regions of the Peruvian Andes

<table>
<thead>
<tr>
<th>Region (Number of Herds Surveyed)</th>
<th>Prevalence (%)</th>
<th>Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ayacucho (13)</td>
<td>46/672 (7)</td>
<td>4.9-8.8</td>
</tr>
<tr>
<td>Cuzco (8)</td>
<td>62/686 (9)</td>
<td>6.9-11.2</td>
</tr>
<tr>
<td>Arequipa (37)</td>
<td>86/940 (9)</td>
<td>7.3-11.0</td>
</tr>
<tr>
<td>Pasco (16)</td>
<td>55/526 (10)</td>
<td>7.8-13.1</td>
</tr>
<tr>
<td>Junín (7)</td>
<td>125/1,030 (12)</td>
<td>10.1-14.1</td>
</tr>
<tr>
<td>Huancavelica (18)</td>
<td>140/639 (22)</td>
<td>18.7-25.1</td>
</tr>
<tr>
<td>Puno (6)</td>
<td>152/670 (23)</td>
<td>19.5-25.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td>666/5,163 (13)</td>
<td>12.0-13.8</td>
</tr>
</tbody>
</table>

Sample and Data Collection

Stool samples were collected directly from the rectum of each alpaca and placed into labeled new polyethylene bags. Data collected included date, age, sex and location of each animal. The geographical characteristics and husbandry practices were also recorded for each site.

The samples were directly transported to the local laboratory, and processed for microscopy examination. Briefly, direct smears covering the surface of a glass slide were made using about 50 μl of stools, fixed in methanol, and stained using the Ziehl-Neelsen modified acid fast stain [38]. The whole smear on each slide was microscopically analyzed at 400x magnification.

Bio-Statistical Analyses

The sample size for the preliminary study was determined at 214 using the formula \( N = \left(\frac{Z^2pq}{E^2}\right) \), where \( p=0.167 \) was from previously reported prevalence (Rojas 1988), \( Z= 1.96 \) for the 95% confidence interval, and \( E=0.05 \) was the significance level. The prevalence value from the preliminary study (10%) was used to calculate the sample size for the national survey: ≥130 crias per region. Due to the high variation in prevalence among herds, the sample size was arbitrarily increased to ~600 crias per region.

Data were organized into 5 age categories set at 3 day-increments, from day 1 to 15, and analyzed using the EpiInfo software (http://www.cdc.gov/epiinfo/, Centers for Disease Control and Prevention, Atlanta, GA). The total prevalence rates were determined using frequency tables, determining 95% confidence limits as previously described [39, 40]. Risk factors such as husbandry practices, density at calving and availability of grazing areas were evaluated for its association with cryptosporidiosis using Chi-square analysis, with \( p<0.05 \) as the significance level.

RESULTS

A total of 5,163 stool samples were analyzed for the presence of Cryptosporidium spp. and 666 crias had Cryptosporidium in their stools, with an overall prevalence of 13% (Table 3). The prevalence rates varied among herds and were proportional to the alpaca population in each region, ranging from 7 to 23% (Table 3). Chi-Square analyses showed significant associations between the detection of Cryptosporidium and low availability of grazing grounds (Chi Square, OR=1.37, \( p<0.01 \)). The highest prevalence rates (23 and 22%) were observed in Puno and Huancavelica, respectively, and both regions had low availability of grazing grounds (Tables 1 and 3). In contrast, the lowest prevalence was observed in Ayacucho (7%) a region with high availability of grazing grounds (Tables 1 and 3). The availability of grazing grounds was significantly associated with lower rates of cryptosporidiosis (Chi-square analysis, OR=0.37, confidence interval 0.33-0.44, \( p<0.001 \)).

The prevalence of Cryptosporidium also varied by age groups, showing an incremental trend by age with significant higher prevalence (\( p<0.001 \)) in older crias (Table 4). Newborns (1-3 days old) had 2% prevalence, and this rate increased with age, reaching up to 20% in crias 13-15 days of age. The older crias were 2.2 times more likely to have Cryptosporidium (Chi-square analysis, OR=2.27, confidence in-

Table 4. Prevalence of Cryptosporidium spp. in Crias by Age Categories

<table>
<thead>
<tr>
<th>Age (days)</th>
<th>Prevalence (%)</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 – 3</td>
<td>17/704 (2)</td>
<td>1.84-2.99</td>
</tr>
<tr>
<td>4 – 6</td>
<td>95/1,087 (9)</td>
<td>7.88-9.6</td>
</tr>
<tr>
<td>7 – 9</td>
<td>110/944 (12)</td>
<td>10.61-12.7</td>
</tr>
<tr>
<td>10 – 12</td>
<td>163/1,053 (15)</td>
<td>14.36-16.59</td>
</tr>
<tr>
<td>13 – 15</td>
<td>281/1,375 (20)</td>
<td>19.3521.520</td>
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terval 1.91-2.78, p<0.001) than alpacas 12 days old or younger.

**DISCUSSION**

This study evaluated the presence of *Cryptosporidium* in 5,163 alpaca crias showing a total prevalence of 13% (Table 3). *Cryptosporidium* was prevalent in all regions studied, which were representative of the natural habitat of alpacas in the Andes. Results from the 1995 survey in Cuzco (baseline data), and this national survey, showed that the prevalence rates were relatively similar in both years (10 and 13% respectively, Tables 2 and 3) suggesting that *Cryptosporidium* is indeed endemic among alpaca crias.

The endemnicity of *Cryptosporidium* varied significantly among regions. The highest prevalence rates were found in Huancavelica and Puno, both regions with low availability of grazing grounds, a risk factor significantly associated with cryptosporidiosis in the present study. In contrast the region of Ayacucho presented the lowest prevalence and higher availability of grazing grounds. Junin also had a relatively low prevalence of cryptosporidiosis (12%) in the 7 herds evaluated but it also had higher availability of grazing grounds and good management practices, which were reported to decrease the incidence of cryptosporidiosis in other ruminants [41-43].

In Puno, alpacas are usually kept in large herds that are managed by community-owned enterprises, resulting in a higher density of alpacas and less efficient or individual control of crias [44]. Huancavelica was another region with very high infection rates (22%). In contrast to Puno, most alpacas belonged to small size companies, thus were raised at lower animal densities. However, the accessibility to pastures was as limited as in Puno. Thus, the limited availability of grazing areas in both regions seems to be a likely predictor of *Cryptosporidium* in alpaca crias. The harsh weather and ecological features of the Altiplano preclude the widespread establishment of improved pastures, thus, grazing areas are more a geographical feature than a farming practice. Therefore, the availability of grazing grounds could be indirect indicators of animal crowding, lower nutrient consumption, concentration of parasites in hot spots, or combinations thereof. Thus, further studies would be needed to elucidate the role of availability of grazing grounds, overcrowding at birth and other potential risk factors.

The prevalence rates in alpaca crias ranged from 7 to 23%, and may be lower than previously reported in other ruminant species. In calves for example, the prevalence of *C. parvum* in France among 1628 selected calves was 17.9%, although the rates of diarrhea were only 5.3%. Another study conducted in 1996 reported *Cryptosporidium* in 55.6% of 189 livestock farms on samples from calves ages 4 to 21 days. Among the 440 calves examined, of which 398 (90.5%) presented diarrhea, cryptosporidia were found in 191 animals, i.e. a prevalence of 43.4% (C.I. 95%=[38. 8-48.0%]) [7]. These data, however are from farms in industrialized nations where dairy or beef cattle are raised using highly efficient practices that include high density production for dairy animals and defined pastures for beef cattle.

The alpacas in our survey, however, were usually raised in herds that roamed independently or semi-independently from each other for 10-11 months each year. However, once a year all herds converge on defined sites for a few weeks for several key activities: delivery of newborns, mating of breeders, shearing of selected adults, and deworming of the herds. Thus, despite the differences in husbandry practices and wide geographical setting, *Cryptosporidium* was still detected in ~13% of the alpaca crias.

Several husbandry practices have been reported to influence the prevalence of cryptosporidiosis, such as herd size, sanitation practices and geographic location [8, 44]. For example, in Ayacucho, the department with the lowest prevalence, farmers emphasize the consumption of colostrum by crias, while they have also implemented a rotation scheme of sleeping sites. These practices may have influenced the relatively low rates (7%) of *Cryptosporidium* in this region. Other husbandry practices for newborns, such as adequate rotation of calving areas has been reported to prevent cryptosporidiosis [44-47], however this is not frequent among alpaca farmers and may be considered in future studies.

How do alpacas acquire *Cryptosporidium*, or its role in the alpaca NDS remains unknown. As with other ruminants, the sources of infection could be other infected animals of the same species or others, such as cattle, rodents or even wildlife. However, the recent finding that *C. parvum* is primarily found among very young cattle suggests that transmission of *Cryptosporidium* could occur primarily between alpaca crias. Nevertheless, the species or genotypes of *Cryptosporidium* infecting alpacas, and the role of other alpacas, including mothers, either as reservoirs or asymptomatic carriers would need to be elucidated in future studies.

**CONCLUSION**

The present study shows that alpaca crias can have *Cryptosporidium* spp. shortly after birth. The prevalence of this parasite was associated with low availability of pastures and can also be influenced by management practices in the calving area. Because cryptosporidiosis occurs in young crias, this parasite may also play a significant role in the alpaca neonatal diarrhea syndrome. A case control study should be necessary to clearly elucidate the role of *Cryptosporidium* in this syndrome.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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