HIGH CRYPTOSPORIDIUM PREVALENCES IN HEALTHY AYMARA CHILDREN FROM THE NORTHERN BOLIVIAN ALTIPLANO

JOSE-GUILLERMO ESTEBAN, CARLOS AGUIRRE, ANGELA FLORES, WILMA STRAUSS, RENE ANGLES, AND SANTIAGO MAS-COMA

Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Burjassot, Valencia, Spain; Unidad de Parasitología, Instituto Nacional de Laboratorios de Salud Nestor Morales Villazon (INLASA), Miraflorres, La Paz, Bolivia

Abstract. The prevalence of Cryptosporidium infection was determined in four Aymara communities in the Bolivian Altiplano, between the city of La Paz and Lake Titicaca, at an altitude of 3,800–4,200 meters. Single stool specimens were randomly collected from 377 5–19-year-old students, all apparently asymptomatic. The total prevalence (31.6%) is possibly the highest reported among healthy humans (a maximum of 9.8% and 2.0% in coprologic surveys in underdeveloped and developed countries, respectively) and one of the highest even in symptomatic subjects. No significant age and sex differences were observed. Such an infection prevalence is probably related to the poor sanitation conditions, contaminated water supplies, overcrowding, and close contact with domestic animals. Continuous exposure to the parasite could be associated with protection against parasite-related symptoms in the children examined.

Cryptosporidium (Apicomplexa: Eucoccida: Cryptosporidiidae), a common enteric protozoan, has since 1907 been known to parasitize a wide range of animals.1,3 The first human cases were reported in the United States in 1976 in an immunologically competent child3 and in an immunosuppressed adult.4 Since then there have been a substantial number of reported cases, and this sporozoan parasite is now recognized as a ubiquitous and significant enteropathogen of immunocompromised patients (including congenital immunodeficiency, those receiving immunosuppressive drugs, concurrent infections, and especially acquired immunodeficiency syndrome [AIDS]).5 It has also been seen in healthy individuals in water-borne outbreaks.5–8 One of the important aspects of cryptosporidiosis is its higher prevalence in young children with gastrointestinal symptoms, especially diarrhea, in both rural and urban environments throughout the world.9–11 However, several recent reports have identified appreciable numbers of asymptomatic infected subjects.2,12,13

This investigation is part of a multidisciplinary project to study the epidemiology of fascioliasis in the Bolivian Altiplano, the endemic region with the highest human prevalences14–16 and intensities.16 Studies on other parasitic diseases in this area were undertaken to ascertain their coexistence with fascioliasis in humans. The aim of the present paper is to report the prevalence data on cryptosporidiosis in the northern Bolivian Altiplano, in the northern region of the Department of La Paz. This region extends between the valley of the city of La Paz and Lake Titicaca, at an altitude of 3,800–4,200 meters. To date, cryptosporidiosis in Bolivia had only been reported in the communities of Camiri, Boyuibe, and Gutierrez in the Department of Santa Cruz in southeast Bolivia.17,18

SUBJECTS, MATERIALS, AND METHODS

Study population. The coprologic study was made in February 1993 and involved 377 Aymara students (205 boys and 172 girls) 5–19 years of age (mean ± SD = 10.2 ± 3.0 years) from the schools of the communities of Huacullani, Quiripujo, Caleria, and Corapata in the northern Bolivian Altiplano (Figure 1). Surveys were carried out so that the sample size in each school was representative of both the student enrollment in the school (at least 50%) and the number of children present on the day of the survey (at least 75%). These villages are rural and culturally traditional, and their economies are dependent upon agriculture and livestock, mainly cattle. There are no significant socioeconomic differences among these communities. Village sanitation is poor, and indiscriminate defecation is commonly practiced. No AIDS nor human immunodeficiency virus infection had been previously recorded among the Aymara inhabitants of the northern Altiplano, according to Bolivian health officials in La Paz (National Bolivian Reference Center for AIDS Studies, unpublished data). The coprologic surveys were made on randomly selected persons on a given day among all participating students. Despite the high spontaneous rate of participation of the students, the relatively low receptivity of Aymara adults (i.e., the parents of the students) discouraged a similar collection of samples the day after or a few days later. Thus, from the beginning of the study it was known that two problems could not be avoided: the reporting of false-negative results due to intermittent cyst shedding, by means of serially repeated stool examination, and the inability to obtain stool samples from students absent from school on the day of the study. It can be supposed that ill children did not attend school, so that in principle, only healthy students were analyzed on the day of the examination.

Institutional ethical review procedure. The surveys were carried out after informed consent was obtained from the local authorities of the community (among Aymaras, community authorities are responsible for transmitting parental consent after previous meetings), as well as from the Director and teachers of each school. The project was approved by the Secretaria Nacional de Salud del Ministerio de Desarrollo Humano (La Paz, Bolivia) and was performed in collaboration with the INLASA Institute in La Paz, the official disease reference center for Bolivia. Moreover, ethical aspects of the study were reviewed and approved by the European Commission as the project funding institution.

Stool collection and preparation. A clean, 30-ml plastic, wide-mouth, numbered container with a snap-on lid was given to every child. The students were then asked to try to fill the container with their own feces and to return it immediately. Stool specimens were collected from all participating schoolchildren and personal data (name, sex, age, etc.) were
noted on delivery of the container. Fecal specimens were transported to the Parasitology Laboratory of the Instituto Nacional de Laboratorios de Salud (La Paz, Bolivia) within 5 hr. In this laboratory, a Kato-Katz slide\textsuperscript{19, 20} was made and two aliquots were preserved in merthiolate-iodine-formalin fixative (1:3) and 10\% formalin solution (1:3)\textsuperscript{21} from each specimen. Consistency (formed, soft, loose, watery) of each stool sample was evaluated and noted. Later, parasitologic studies were carried out at the Departamento de Parasitología (Valencia, Spain). Samples fixed in 10\% formalin were processed by a formol-ether concentration technique\textsuperscript{22} and the sediment was stained using a modified Ziehl-Neelsen technique.\textsuperscript{23} Each sample was examined by two technicians and the by one of the authors (J-GE). Microscopic slides and materials from the human parasite collection of the Parasitology Department of the University of Valencia were used for quality control when needed.

Statistical analysis. Statistical analyses were done using SPSS 6.1 (SPSS Institute, Chicago, IL) for Windows. For the evaluation of categorical variables, we used the chi-square test or Fisher’s exact test when required by data scarcity. A \(P\) value less than 0.05 was considered significant.

RESULTS

In smears examined microscopically with an oil-immersion lens, oocysts (Figure 2) were classified as belonging to the genus \textit{Cryptosporidium} on the basis of their morphology (ovoid to elliptical and containing four sporozoites not enclosed with a sporocyst), staining characteristics, and small size. Their measurements were \(4.5 \pm 0.5 \mu m\) by \(4.0 \pm 0.6 \mu m\) (mean \(\pm SD\)), with a range of variation of \(3.8-5.5 \mu m\) (large diameter) and \(3.3-5.1 \mu m\) (small diameter). These measurements were consistent with those of \textit{C. parvum} Tyzzer, 1912.\textsuperscript{24} However, the species identification of our material as \textit{C. parvum} cannot be considered definitive because some investigators do not accept oocyst size as a sufficient criterion for species differentiation among the genus \textit{Cryptosporidium}.\textsuperscript{2}

Of the 377 samples collected, only two loose and one watery stool specimens were reported (0.8\%).

The total prevalence of cryptosporidiosis in the 377 students studied was 31.6\% (Huacullani = 32.1\%, Quiripujo = 31.0\%, Caleria = 31.4\%, and Corapata = 30.2\%) (Table 1). No statistically significant differences (\(\chi^2 = 0.98, P = 0.992\)) in the prevalence of infection were noted among these communities.

Prevalence between boys and girls was not significantly different (30.7\% versus 32.6\%; \(\chi^2 = 0.144, P = 0.704\)). Similar results were obtained in each community studied (Huacullani: 31.7\% versus 32.7\%; \(\chi^2 = 0.025, P = 0.873\); Quiripujo: 36.0\% versus 23.5\%; \(\chi^2 = 0.736, P = 0.391\); Caleria: 31.0\% versus 31.8\%; \(\chi^2 = 0.004, P = 0.952\); and Corapata: 22.6\% versus 37.5\%; \(\chi^2 = 1.664, P = 0.197\)) (Table 2).

The distribution of prevalences according to age groups is shown in Table 3. Age groups were established according to the school criterion (5–8 years old: basic level; 9–12 years old: intermediate; 13–16 years old: medium; and 17–19 years old: students still in school but eligible for military
service). The highest prevalences according to age groups were detected in those individuals between five and eight years of age: 41.3% in Huacullani, 33.3% in Quiripujo, and 44.4% in Corapata, and 35.7% for the four surveyed schools combined. There was no significant difference between the prevalence rates among the age groups of each community.

**DISCUSSION**

To date, the only reports of *Cryptosporidium* from Bolivia consist of two coprologic studies in apparently healthy subjects from three communities (Camiri, Boyuibe, and Gutiérrez) in the Cordillera Province, Santa Cruz Department in southeast Bolivia, reporting a maximum prevalence of 2.1%. The present coprologic study has shown one of the highest prevalences (31.6%) of human cryptosporidiosis in the world. The most common clinical manifestation of cryptosporidiosis is diarrhea, which is characteristically profuse and loose to watery and often contains mucus but rarely blood or leukocytes. Excluding reports on AIDS patients and specific outbreak patients situations, with infection rates of 27–61%, prevalences in samples from diarrheic patients have ranged from 0.1% to 27.1% in industrialized countries, compared with 0.1–32% in less developed countries. Serologic surveys have obtained much higher rates ranging from 15% to 44%. The almost total absence of a relationship between chronic or severe diarrhea with *Cryptosporidium* oocyst shedding in the Aymara students is worth mentioning. Appreciable diarrhea symptoms at the time of sampling (only three [0.8%] of 377 samples had loose or watery consistencies) were not detected in the students. However, the interpretation of this result becomes difficult because of the absence of data of previous exposures and clinical histories. The possibility that some students had a recent history of diarrhea and that many were still shedding oocysts after resolving the diarrhea cannot be disregarded. The students examined could have developed diarrhea after the first exposure to (or infection with) *Cryptosporidium* months or years before and because of continual exposure, developed some level of immunity such that mild or asymptomatic infections were seen. Children are more susceptible to *Cryptosporidium* infection than adults, presumably because their immune systems are still developing, and the risk of infection is greater as a result of common unhygienic behavior. This and the unsanitary living conditions in this region suggest that children regularly suffer high-intensity exposure to the protozoan from a very early age. This continuous exposure to the parasite could be associated with protection against parasite-related symptoms in the students examined. Previous studies had indicated that the students in this region were not severely malnourished. Such a situation in which continuously exposed humans appear to be asymptomatic have already been reported in other surveys. Coprologic samples from asymptomatic individuals have also been examined in recent surveys and prevalence reports have ranged from 0% to 2% in developed countries, especially in settings such as day-care centers and areas with unreliable water supplies, compared with 0–9.8% in underdeveloped countries. The high prevalence of *Cryptosporidium* infection obtained in this coprologic study may be explained by taking into account the biology of this coccidian (lack of host specificity, one-host life cycle, oocysts are excreted fully sporulated and are resistant to common disinfectants and environmental factors, oral infection route, direct transmission) and the life style of the Aymara Indians. The Aymaras are adapted to the particular physiographic and extreme environmental characteristics of the high altitude of the Altiplano, and live under poor health conditions.

**TABLE 1**

<table>
<thead>
<tr>
<th>Community</th>
<th>Age range (mean ± SD) (years)</th>
<th>No. examined</th>
<th>No. (%) positive</th>
<th>(95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huacullani</td>
<td>5–19 (10.8 ± 3.2)</td>
<td>221</td>
<td>71 (32.1)</td>
<td>(25.9–38.3)</td>
</tr>
<tr>
<td>Quiripujo</td>
<td>6–12 (7.8 ± 2.0)</td>
<td>42</td>
<td>13 (31.0)</td>
<td>(17.0–45.0)</td>
</tr>
<tr>
<td>Caleria</td>
<td>6–14 (9.1 ± 2.3)</td>
<td>51</td>
<td>16 (31.4)</td>
<td>(18.7–44.1)</td>
</tr>
<tr>
<td>Corapata</td>
<td>6–15 (10.9 ± 2.4)</td>
<td>63</td>
<td>19 (30.2)</td>
<td>(18.9–41.5)</td>
</tr>
<tr>
<td>Total</td>
<td>5–19 (10.2 ± 3.0)</td>
<td>377</td>
<td>119 (31.6)</td>
<td>(26.9–36.3)</td>
</tr>
</tbody>
</table>

* CI = confidence interval.
Today, the mode of transmission of Cryptosporidium is assumed to be the fecal-oral route. Several epidemiologic studies have suggested transmission by direct human-to-human and/or animal-to-human contacts, or by indirect contamination of the environment, food, or water supplies. Living conditions in this Bolivian Altiplano region are unsanitary, with most villages having no protected water supply; there are also no proper waste or sewage disposal facilities and indiscriminate defecation is commonly practiced. Conditions are overcrowded with poor household sanitation and deficient personal hygiene. Domestic animals, especially cattle (the most valuable property of these families), are kept adjacent to or inside the houses, and direct contact between children and animals is common.

Further studies are needed to elucidate risk factors and sources of Cryptosporidium infections in children. Due to the well-known low host specificity of Cryptosporidium species, the possible involvement of different host species and the potential role of domestic animals as reservoirs in the occurrence of this human disease in the Bolivian Altiplano need clarification. Our findings demonstrate that cryptosporidiosis is endemic, at least in the communities surveyed and probably all over this region, based on the transmission characteristics of this coccidiosis. Further studies in other communities and in different seasons other than the summer are needed to characterize its geographic extent and possible prevalence variations over the year.

Acknowledgments: We thank Dr. J. A. Oviedo (Valencia, Spain) and M. Magarino (La Paz, Bolivia) for technical assistance in the schools surveyed. We also acknowledge the facilities and collaboration received from the following Bolivian organizations, institutions, and centers, as well as from their respective representatives or directors: Instituto Nacional de Laboratorios de Salud (INLASA) of La Paz, Dirección Nacional de Relaciones Internacionales and the Dirección Nacional de Epidemiología of the Ministerio de Previsión Social y Salud Publica (now known as the Secretaría Nacional de Salud of the new Ministerio de Desarrollo Humano) in La Paz; Comité Regional de Zoonosis and Centro Piloto La Paz of the Unidad Sanitaria La Paz; Dirección Nacional de Producción Pecuaria and the Instituto Nacional de Biología Animal of Chasquiampa-Calacoto, both of the Ministerio de Asuntos Campesinos y Agropecuarios (MACA) in La Paz; Granja de Mejoramiento Ganadero de Kallutaca related to the Programa de Fomento Lechero of the Corporacion Regional de Desarrollo de La Paz (CORDEPAZ); Instituto de Ecología of the Facultad de Ciencias Pías y Naturales of the Universidad Mayor de San Andrés (UMSA) in Calacoto-La Paz; Centro de Datos para la Conservacion (de America Latina y el Caribe) in Calacoto-La Paz; Office of the Pan American Health Organization in La Paz; and Danish NGO Danhurchaid-Bolivia in La Paz. This research was performed in partial fulfillment of a doctoral degree by Carlos Aguirre at the Department of Parasitology of the University of Valencia, Spain.

Financial support: This work was supported by funding from the STD Program of the Commission of the European Communities (DG XII: Science, Research and Development) (Contract no. TSS-CT94-0294), Brussels, EU, and the Program of Scientific Cooperation with Latin America, Instituto de Cooperacion Iberoamericana, Agencia Espanola de Cooperacion Internacional (ICI-AECI), Carlos Aguirre thanks the MUTIS Scholarship Program of the Instituto de Cooperacion Iberoamericana of the Agencia Espanola de Cooperacion Internacional (ICI-AECI), Madrid for a fellowship.

Authors' addresses: Jose-Guillermo Esteban, Carlos Aguirre, Angela Flores, and Santiago Mas-Coma, Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andres Estelles s/n, 46100 Burjassot, Valencia, Spain. Wilma Strauss and Rene Angles, Unidad de Parasitología, Instituto Nacional de Laboratorios de Salud Nestor Morales Villazon (INLASA), Pasaje Rafael Subieta No. 1889, Miraflores, La Paz, Bolivia.

Reprint requests: Jose-Guillermo Esteban, Departamento de Parasitología, Facultad de Farmacia, Universidad de Valencia, Av. Vicente Andres Estelles s/n, 46100 Burjassot, Valencia, Spain.

REFERENCES


---

**Table 2**

Prevalence of cryptosporidiosis by sex

<table>
<thead>
<tr>
<th></th>
<th>Huacullani</th>
<th>Quiripujo</th>
<th>Caleria</th>
<th>Corapata</th>
<th>Total prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/ no. tested</td>
<td>%</td>
<td>No. positive/ no. tested</td>
<td>%</td>
<td>No. positive/ no. tested</td>
</tr>
<tr>
<td>Males</td>
<td>38/120</td>
<td>31.7</td>
<td>9/25</td>
<td>36.0</td>
<td>9/29</td>
</tr>
<tr>
<td>Females</td>
<td>33/101</td>
<td>32.7</td>
<td>4/17</td>
<td>23.5</td>
<td>7/22</td>
</tr>
<tr>
<td>Total</td>
<td>71/221</td>
<td>32.1</td>
<td>13/42</td>
<td>31.0</td>
<td>16/51</td>
</tr>
</tbody>
</table>

* CI = confidence interval.

---

**Table 3**

Prevalence of cryptosporidiosis by age groups

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>Huacullani</th>
<th>Quiripujo</th>
<th>Caleria</th>
<th>Corapata</th>
<th>Total prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive/ no. tested</td>
<td>%</td>
<td>No. positive/ no. tested</td>
<td>%</td>
<td>No. positive/ no. tested</td>
</tr>
<tr>
<td>5–8</td>
<td>26/66</td>
<td>41.3</td>
<td>10/30</td>
<td>33.3</td>
<td>5/24</td>
</tr>
<tr>
<td>9–12</td>
<td>28/85</td>
<td>32.9</td>
<td>3/12</td>
<td>25.0</td>
<td>9/23</td>
</tr>
<tr>
<td>13–16</td>
<td>14/55</td>
<td>25.5</td>
<td>NC –</td>
<td>NC –</td>
<td>2/4</td>
</tr>
<tr>
<td>17–19</td>
<td>1/10</td>
<td>10.0</td>
<td>NC –</td>
<td>NC –</td>
<td>NC –</td>
</tr>
<tr>
<td>Unknown</td>
<td>2/8</td>
<td>25.0</td>
<td>NC –</td>
<td>NC –</td>
<td>1/3</td>
</tr>
<tr>
<td>Total</td>
<td>71/221</td>
<td>32.1</td>
<td>13/42</td>
<td>31.0</td>
<td>16/51</td>
</tr>
</tbody>
</table>

* CI = confidence interval; NC = not collected.

Established according to school criterion (5–8 years old: basic level; 9–12 years old: intermediate; 13–16 years old: medium; 17–19 years old: students still in school but eligible for military service).


