

## *Hymenolepis nana*—An Emerging Intestinal Parasite Associated with Anemia in School Children from the Bolivian Chaco

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**Abstract.** Tropical anemia can have multiple causes, whether socioeconomic, dietary, or infectious. In the Bolivian Chaco, soil-transmitted helminthiasis (STH), malaria, and Chagas disease are potential infectious causes of anemia among school-aged children (SAC). Following years of preventive chemotherapy with mebendazole, the prevalence of STH among SAC living in that area is now negligible, whereas protozoan infections are still highly prevalent (81%); *Hymenolepis nana* is the most frequent intestinal helminth (~13%). We present results of hemoglobin (Hb) assessment and the association between parasitic infections and Hb levels of that SAC population. Overall, 511 SAC (girls:boys ratio 1:1, mean age 9.4 years [95% confidence interval {CI}: 9.3–9.5]) had Hb levels measured by using a point of care testing (HemoCue® Hb 301 System; HemoCue, Angelhome, Sweden). The prevalence of anemia was 23% (117/511), with mean and median Hb level = 12.2 g/dL (95% CI: 12.1–12.3; range 9.2–15.4 g/dL). By multivariate analysis, *H. nana* infection was associated with an increased risk of anemia (odds ratio 2.9, 95% CI: 1.5–5.7,  $P = 0.002$ ). Two samples (0.5%) were positive for *Trypanosoma cruzi* and none for *Plasmodium* spp. by polymerase chain reaction of the 439 children tested. Anemia is still a concern among SAC living in the Bolivian Chaco. Our findings call for a greater attention to fecal–oral emerging pathogens, such as *H. nana*, and highlight the importance of water, sanitation, and hygiene improvements for disadvantaged population such as those living in the Bolivian Chaco.

### INTRODUCTION

Tropical anemia can have multiple causes—iron deficiency being the most common, especially among women of reproductive age and children, whether caused by inadequate iron dietary intake (the main cause) or contributed to by infectious diseases such as malaria and soil-transmitted helminthiasis (STH).<sup>1</sup> Hookworm infection in particular is known to be associated with chronic gastrointestinal blood loss and micronutrients depletion, and thus anemia, chiefly in pregnancy.<sup>2</sup> Regular deworming through preventive chemotherapy (PC) of at-risk groups (preschool and schoolchildren and women of reproductive age) is expected to reduce worm burden and STH-induced morbidity in the infected individuals and overall prevalence.<sup>3</sup> In the Bolivian Chaco, a recent cross-sectional survey found that the prevalence of hookworm infection in school-age children has dropped from 50% in the 1990s to 0.7% in 2016, and no cases of *Ascaris lumbricoides* and *Trichuris trichiura*—likely the result of 30 years of PC,<sup>4</sup> and this all the while protozoan infections continue to be prevalent (81% of children had at least one) and the minute tapeworm *Hymenolepis nana* having become the most frequent helminthic infection (~13%).

*Hymenolepis nana* has cosmopolitan distribution, but prevalence is highest among children living in low-income countries with poor Water, Sanitation, and Hygiene standards.<sup>5</sup> The adult tapeworm parasitizes the mucosal surface of the small intestine. Eggs of *H. nana* are immediately infective when excreted with the stools, but they cannot survive more than 2 weeks in the environment. Transmission does not

require an intermediate host and infection can be passed directly from one infected person to another by hand-to-mouth transmission. Ingested eggs release motile embryos, the oncospheres, which invade the small intestinal mucosa, encysting within villi. In 3–4 days, they develop into the larval cysticercoids, and then evaginate, destroying the villi they invaded; attached to the mucosal surface, cysticercoids develop into the adult tapeworm in about 1 month. Cycles of autoinfection, both internal and external, can also occur, sustaining chronic infection. The impact on the health of infected children is still unclear, but the invasive nature of *H. nana* infection is likely to have been overlooked, especially in children with heavy and/or chronic infection.<sup>5</sup> During the survey carried out in 2016, we also measured hemoglobin (Hb) concentrations and explored the potential association between parasitic infections and anemia.

### MATERIALS AND METHODS

In September 2016, 519 school-aged children (SAC) from nine rural communities of the Bolivian Chaco, a semiarid and sparsely populated region, located in the southeast of the Plurinational State of Bolivia (lat. 17°58'–22°20'S, long. 64°30'–58°50'W) were enrolled in a cross-sectional parasitological survey, whose methodology and results have been previously described in detail.<sup>4</sup> Briefly, a minimum of 50 third to fifth graders, usually 8–11 years old, were enrolled in each school. Stool samples were analyzed for intestinal parasites by direct wet mount and Kato-Katz technique (one thick smear on a single stool sample from each individual), by double-blinded readers. At the same time, they were all invited to undergo a single measurement of Hb using a portable Hb analyzing system (HemoCue® Hb 301 System; HemoCue, Angelhome, Sweden). Of these nine communities, four were in

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the Cordillera Province (Santa Cruz department) and five in the Gran Chaco Province (Tarija department) with 203/242 and 215/269 participants providing a blood sample for Hb measurement, respectively.

In eight communities ( $N = 439$  SAC), it was also possible to collect dried blood spots (DBS), through finger prick on filter paper, to detect *Plasmodium* spp. and *Trypanosoma cruzi* by quantitative polymerase chain reaction (qPCR) (qPCR kits; Genesig, United Kingdom). Demographic data were recorded using a standardized questionnaire. Diagnosis and classification of anemia were established according to the World Health Organization guidelines.<sup>6</sup>

Data were analyzed using STATA 11.0 (StataCorp, College Station, TX). Frequencies, percentages, and means with 95% confidence intervals (CI) were calculated. Multivariate analysis was performed by logistic and linear regression, to establish the association of anemia (categorical) and Hb levels (continuous), respectively, with intestinal parasitic infections (excluding those with prevalence < 1%), allowing for the province where the communities were located. Results were considered significant when the  $P$  value was < 0.05.

The study, conducted with the support of the Bolivian Ministry of Health (Convenio Ministerio de Salud, Estado Plurinacional de Bolivia/Cátedra de Enfermedades Infecciosas, Universidad de Florencia, Italia) and the Guarani political organization (Asamblea del Pueblo Guarani), was approved by the local Ethic Committee. A written informed consent was obtained by a relative or a legal guardian of each participating child.

## RESULTS

Overall, 511 children had an Hb measurement (girls:boys ratio 1:1); the mean age was 9.4 years (95% CI: 9.3–9.5, range 7–16). The prevalence of anemia (Hb < 11.5 g/dL) was 23% (117/511), which was mild (11.5–11.0 g/dL) in 54 cases (46%) and moderate (11.0–8.0 g/dL) in 63 (54%); there was no case of severe anemia (< 8.0 g/dL). The mean and median Hb level was 12.2 g/dL (95% CI: 12.1–12.3; range 9.2–15.4 g/dL).

School-aged children living in communities of the Cordillera Province (department of Santa Cruz) had a higher prevalence rate of anemia than those living in the communities of the Gran Chaco Province (department of Tarija) (30% versus 17%, respectively;  $P < 0.001$ ) as well as lower median Hb values (11.9 versus 12.4 g/dL;  $P < 0.001$ , Mann–Whitney test). No between-sex statistical differences were found in the frequency of anemia and Hb levels.

Of the 511 participating children, 418 (82%) also provided a stool sample. The prevalence of anemia in this subset (21.3%, 89/418) was not statistically different from that of the overall group (22.9%, 117/511) ( $P = 0.56$ ).

The prevalence of infections and anemia is presented in Table 1: 82% of children had at least one parasite and 58% had two or more; the most frequent protozoan species was *Blastocystis* and the most frequent helminthic species was *H. nana*.

By multivariate analysis, of all intestinal parasitic infections, only *H. nana* was associated with an increased risk of anemia (odds ratio 2.9, 95% CI: 1.5–5.7,  $P = 0.002$ ) and inversely correlated with the Hb level ( $P = 0.016$ ). No correlation was found between *H. nana* egg counts and Hb levels (age-adjusted linear regression) or anemia (age-adjusted logistic regression).

Among 439 DBS analyzed by PCR, two (0.5%) were positive for *T. cruzi* and none for *Plasmodium* spp.

## DISCUSSION

Today, approximately one in four to one in five school-age children living in the Bolivian Chaco is anemic, half of them having moderate anemia (Hb levels 11 to 8 g/dL). The situation has not improved in the past quarter of a century: a 1990 study carried out in two rural communities of the same area showed rates of anemia of 22% and 16%, and mean Hb 11.8 and 12.4 g/dL, among pre-SAC (2–5 years old) and SAC (6–9 years old), respectively.<sup>7</sup> At that time, STH infections were highly prevalent (41.0–64.5%), with hookworm prevalence reaching 50%, 5.6% of which of heavy or moderate intensity (> 2,000 eggs

TABLE 1  
Risk factor for anemia: univariate and multivariate analysis

	Total N (%)	Anemia* N (%)†	Univariate analysis		Multivariate analysis	
			OR (95% CI)	P value	OR (95% CI)	P value
Age in years mean (95% CI)	9.4 (9.3–9.5)	–	–	–	<b>0.8 (0.6–0.9)</b>	<b>0.01‡</b>
Gender female	256 (50)	64 (25)	–	–	1.3 (0.8–2.1)	0.31
Parasitism (any one infection)	342 (82)	78 (23)	1.7 (0.9–3.8)	0.11	–	–
Polyparasitism (any two or more infections)	241 (58)	58 (24)	1.5 (0.9–2.5)	0.11	–	–
Province						
Cordillera (Dep. Santa Cruz)	242 (47)	72 (30)	<b>2.1 (1.4–3.2)</b>	<b>0.001</b>	<b>1.8 (1.1–3.0)</b>	<b>0.02</b>
Gran Chaco (Dep. Tarija)	269 (53)	45 (17)	1	–	1	–
<i>Hymenolepis nana</i>	49 (12)	20 (41)	<b>3.0 (1.5–5.8)</b>	<b>&lt; 0.001</b>	<b>2.9 (1.5–5.7)</b>	<b>0.002</b>
<i>Entamoeba histolytica</i> complex	30 (7)	5 (17)	0.7 (0.2–2.0)	0.52	1.0 (0.3–2.8)	0.97
<i>Giardia intestinalis</i>	99 (24)	21 (21)	1.0 (0.5–1.8)	0.98	0.9 (0.5–1.6)	0.74
<i>Blastocystis</i> spp.	209 (50)	54 (26)	<b>1.7 (1.0–2.9)</b>	<b>0.02</b>	1.6 (0.9–2.6)	0.08
<i>Entamoeba nana</i>	110 (26)	28 (25)	1.4 (0.8–2.4)	0.21	1.2 (0.7–2.2)	0.48
<i>Entamoeba coli</i>	111 (27)	27 (24)	1.3 (0.7–2.2)	0.36	1.2 (0.7–2.2)	0.45
<i>Entamoeba hartmanni</i>	118 (28)	27 (23)	1.1 (0.7–1.9)	0.62	1.0 (0.6–1.8)	0.96
<i>Iodamoeba bütschlii</i>	24 (6)	3 (13)	0.5 (0.1–1.8)	0.28	0.3 (0.1–1.2)	0.09

CI = confidence interval; N = number; OR = odds ratio. Significant associations are highlighted in bold. Overall, 117 cases of anemia (23%) were detected of 511 school-aged children enrolled in the study; among them, 418 provided a fecal sample. Parasites detected at less than 1% prevalence were arbitrarily excluded from the model (Hookworm: 0.7%; *Enterobius vermicularis*: 0.7%; *Chilomastix mesnili*: 0.5%; *Ascaris lumbricoides*, *Trichuris trichiura*, *Strongyloides stercoralis*: no cases).

\* The cutoff for the diagnosis of anemia was 11.5 g/dL in the age group 7–11 and 12 g/dL in 12–14.

† Row percentage.

‡ Risk of an anemia decreases with age (OR 0.8 for each 1-year increase).

per gram), and *T. trichiura* prevalence ranged from 14.5% to 19.3% (all of light intensity).<sup>8</sup> Today, STH prevalence is < 1%, yet this dramatic decrease does not seem to have modified the risk of anemia in school-age children in the past three decades (16% in 1990 versus 23% 2016, Pearson's  $\chi^2$  test  $P = 0.20$ ), possibly because STH infections were of low intensity in 1990.<sup>4</sup> Yet, still today more than 80% of children have at least one intestinal parasitic infection and nearly 60% have two or more, essentially protozoa; about every other child is infected with *Blastocystis* spp., and one in four is infected with an *Entamoeba* species. Protozoan infections do not seem to be associated with anemia.

We found that today the most prevalent helminth species is *H. nana* (a situation shared with other reports from India, Africa, and Latin America) and that *H. nana* infection is associated with anemia and decreased Hb.<sup>9–11</sup> Despite it emerging as a leading infection in children in tropical areas, our knowledge of the impact of *H. nana* infection on their health is limited. Symptoms that have been attributed to *H. nana* infection in children are generic, such as diarrhea, abdominal pain, irritability, headaches, fever, fatigue and stunting<sup>9,10,12</sup>; only few studies reported an association with anemia.<sup>13,14</sup> Infection is hand-to-mouth, and autoinfection is common, whereby continuous injury of the intestinal villi is sustained over a long time if untreated, causing enteritis and impaired intestinal permeability, with micronutrient intestinal leakage and low levels of vitamin B12 and folate in blood.<sup>15</sup> This could explain the lack of correlation between egg counts in the feces and degree of anemia.

Presently, there are no control programs specifically addressing *H. nana* or other tapeworms, although they can be treated, such as schistosomiasis, with a single dose of praziquantel.<sup>12,16</sup> The persistently high prevalence of anemia in the region is of concern. Clearly socioeconomic conditions in this area have not improved significantly over the past three decades. As for infections, considering that schistosomiasis is absent in this area, we looked for *Plasmodium* and *T. cruzi*. By PCR, all 439 samples tested were negative for malaria, possibly because they were collected during the dry season, whereas two of 439 were positive for *T. cruzi* (0.5%). This confirms that active transmission of *T. cruzi* continues within this area, where house infestation rates by the vector *Triatoma infestans* remain high, despite decades long efforts, and hints to the possibility of chronic iron-deficiency anemia following repeat Triatominae bites.<sup>17,18</sup> Recently, *Babesia microti* has also been reported in rural population of the Bolivian Chaco.<sup>19</sup>

Poverty and low socioeconomic conditions, which translate into inadequate dietary intake of micronutrients, remain the leading causes of anemia. The imbalance in the prevalence of anemia between SAC living in the Cordillera Province and those from the Gran Chaco Province is probably related to the different socioeconomic levels of the two provinces (poverty rate 60% versus 38%, respectively).<sup>20</sup> However, an excess risk of anemia in children infected with *H. nana* emerged, independently from other factors, at a time when hookworms and other soil-transmitted helminths have virtually disappeared in these communities. Because the prevalence of *H. nana* infection is relatively low (12%), ultimately it is associated with anemia in only 5% of children in the community.

However, our findings call for a greater attention to *H. nana*, and more broadly to fecal–oral protozoa and helminths as emerging pathogens, especially in areas where STH infections

are successfully controlled and other parasitic infections classically related to anemia have declined. Water, Sanitation, and Hygiene interventions are critical to achieve sustainable improvements in health conditions of these population, including anemia.<sup>21</sup>

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