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Cross-sectional study on intestinal parasite infections in different ecological zones of the Department of La Paz, Bolivia

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ABSTRACT

We performed a cross-sectional parasitological survey to assess the prevalence of protozoa and helminth infection among 275 school-age children (SAC) living in rural and peri-urban areas located in different ecological zones of the Department of La Paz, Bolivia. Public health activities for the control of STH, based on the biannual administration of mebendazole to preschool and school children are implemented at national level since 1986.

We found an overall prevalence of 82.2% for intestinal parasites, including protozoa (80%) and helminths (23.3%). *Blastocystis* and *Entamoeba coli* were the most prevalent protozoa (found in 44% and 20.7% of the SAC enrolled); as for helminths, *Ascaris lumbricoides* and *Hymenolepis nana* were diagnosed in 14.5% and 3.3% of the children, respectively, followed by *Trichuris trichiura* 1.4%, *Enterobius vermicularis* 1.4%, *Strongyloides stercoralis* 0.7% and hookworms 0.7%.

Molecular characterization of *Blastocystis* positive samples evidenced three different subtypes (ST1, ST2, ST3) highlighting the risk of transmission also from animal reservoir. We found a significant difference in the distribution of intestinal parasitic infection (IPIs) by ecological zone (44/74. 59% in Andean highlands, 94/170, 88% in tropical lowlands and 88/94, 94% in the Yungas, $p < 0.001$). Access to potable water (OR 0.1 95%CI 0.02–0.5, $p = 0.004$) and the habit of boiling drinking water (OR 0.3, 95% CI 0.2–0.7, $p = 0.004$) showed an independent association with a lower risk of all IPIs and STHs, respectively. The very low prevalence of STH infections of moderate heavy intensity demonstrate that periodical deworming has been successful in reducing the morbidity due to these parasites, however the high prevalence of protozoa demonstrate that sanitation is still problematic and there is a relevant contamination of the environment with human faeces.

Significant efforts are still needed to reduce IPIs transmission and to improve health and sanitation in this area.

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1. Introduction

Intestinal parasitic infections (IPIs), including helminth and protozoa infections, are some of the most prevalent infections globally [1]. In particular, soil transmitted helminthiases (STHs) are highly endemic in low- and middle-income countries, affecting more than 1.5 billion people worldwide, or 24% of the world's population [2]. Although STHs are rarely fatal, however infections of moderate and heavy intensity are associated with significant morbidity in preschool and school-age children (SAC), women of childbearing age, and immunocompromised subjects, causing growth impairment and poor educational performance, micronutrient deficiencies and iron-deficiency anaemia [3]. Control programs for STHs, based on large-scale mass preventive chemotherapy (PC) administration in endemic areas, are recommended by the World Health Organization (WHO), and are associated with an important reduction in STH prevalence, infection intensity and, ultimately, morbidity [4]. In Bolivia, a mass deworming program, based on a 6-monthly single-dose mebendazole delivery system aimed at school-aged children (SAC), has been implemented by the Bolivian Ministry of Health since 1986. Studies conducted 30 years apart (1987–2017) in the Chaco area, located in the south-eastern Bolivia, showed an impressive reduction in STH prevalence [5–8]. At the same time protozoan infections, as well as other faecal-oral infections, are still highly endemic in this area, suggesting that PC played a major role in STH control, but hygienic and sanitary conditions are still inadequate [9].

As per WHO recommendations, a nationwide review of the current deworming program should be launched to adapt the drug administration in areas where the prevalence of STH has significantly decreased [10]. So far, epidemiological data in other Departments and different ecological zones of the Plurinational State of Bolivia are limited.

In this study we report the results of a cross-sectional study conducted in 2019 on the prevalence of STHs and other IPIs among SAC from different ecological zones in the Department of La Paz in Bolivia.

2. Materials and methods

2.1. Study area

Between May and September 2019, 275 SAC were enrolled in a cross-sectional study from six communities located in three ecological zones in the Department of La Paz, Bolivia: (i) Andean highlands, lower temperatures and humidity; (ii) tropical lowlands, warmer temperature and year-round humidity; and (iii) the Yungas, intermediate temperatures and precipitation (Fig. 1). Of the selected communities, five were in rural areas and one in a peri-urban setting (Table 1).

2.2. Study design and population

A cross-sectional parasitological survey was performed in six primary schools, randomly selected from three ecological zones in the Department of La Paz, Bolivia. The study design, sampling methods and analysis techniques were defined according to WHO recommendations, aimed at obtaining data representative of each ecological zone.

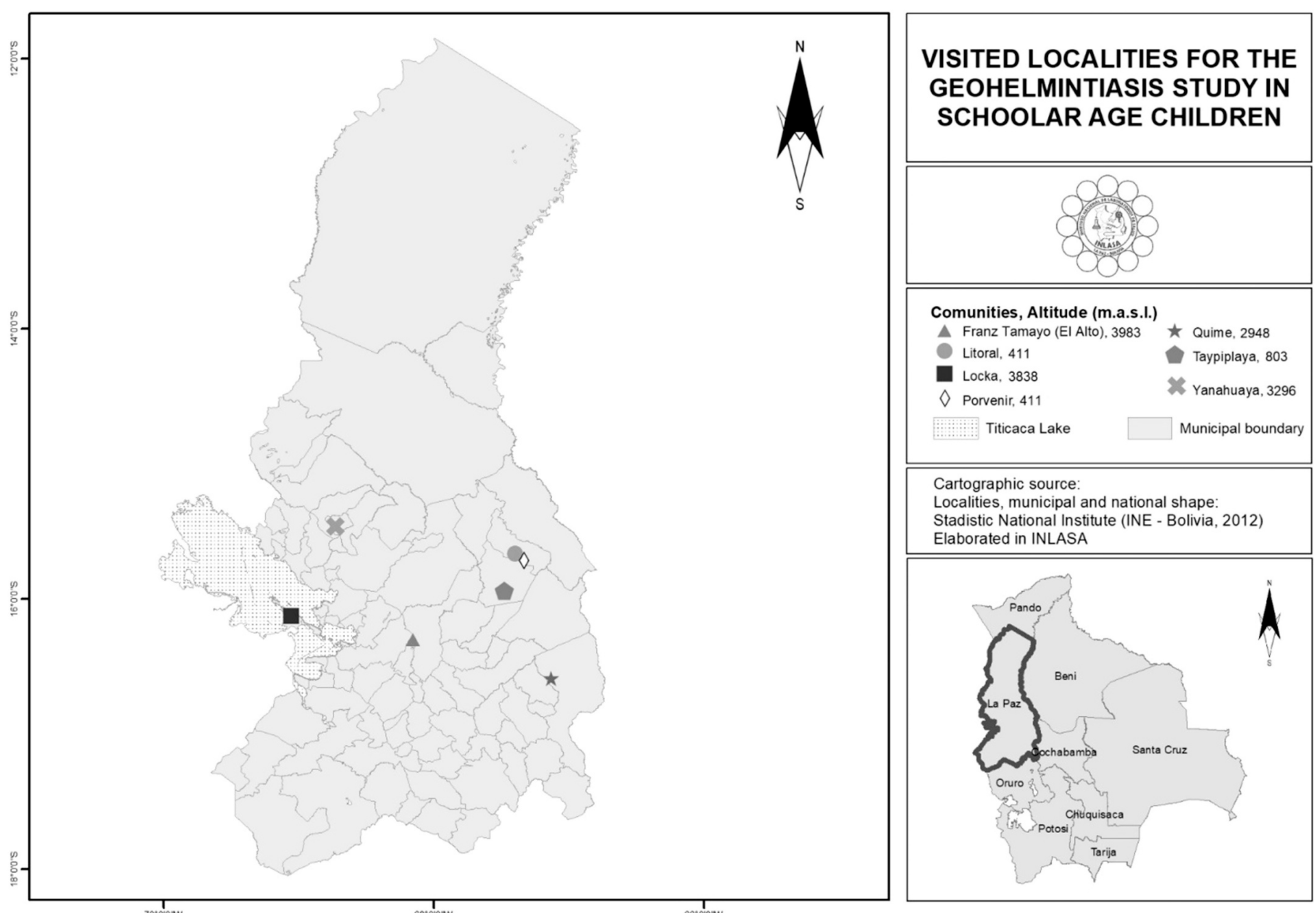

Fig. 1. The study area of the Department of La Paz with surveyed provinces and communities. Provinces of Murillo and Capocabana (communities: Franz Tamayo and Locka), Caranavi (communities: Villa Porvenir-Litoral Taipiplaya), Muñecas and Inquisivi (communities: Yanahuaya and Quime).

Table 1
Environmental and demographic features of the 6 communities surveyed.

Ecological zone	Community (municipality, province)	Setting	Elevation (m.a.s.l.)	Total population (*n*)	Recruited into the study (*n*)
Andean highlands	Zona Franz Tamayo (El Alto, Murillo)	Peri-urban	3983	922,598	37
	Locka (Copacabana, Manco Kapac)	Rural	3838	3000	37
Tropical lowlands	Villa Provenir-Litoral (Alto Beni, Caranavi)	Rural	411	1502	51
	Taipiplaya (Caranavi)	Rural	803	2926	56
Yungas	Yanahuaya (Aucapata, Muñecas)	Rural	3296	5495	44
	Quime (Inquisivi)	Rural	2948	5142	50

The sample size recommended by WHO for cluster sampling was used ($n = 50$), assuming a design effect equal to 2. This sample size enables estimates to be obtained with an absolute precision of 5% and a confidence level of 95%, which is considered sufficient for monitoring purposes [10–12]. In brief, approximately 50 children were planned to be recruited in each sentinel site, among those attending the third-year class, usually aged 8–9 years; children from higher classes were enrolled when the number of third-year pupils was < 50.

2.3. Field procedures

Recruitment and collection of stool samples were carried out in the schools in collaboration with local authorities. For each participant, a structured questionnaire was completed in the presence of a parent or legal guardian, including data on SAC demographics, personal hygiene behaviours and adherence to PC programs. In all cases, a single stool sample was obtained per participant. Children received a stool container labelled with an identification number with instructions on how to collect the specimen.

The study was approved by a local ethics committee (INLASA), and written informed consent was obtained from a parent or a legal guardian of each child enrolled.

2.4. Laboratory procedures

2.4.1. Examination of faecal samples

Stool samples were analysed for intestinal parasites using direct microscopic examination in a drop of iodine solution and the Kato-Katz method [13]. The Kato-Katz thick smear stool examination was based on duplicate slides prepared from a fresh stool specimen, which was sieved and calibrated using of a nylon wire mesh and 41.7 mg plastic templates [13]. Particulate matter was dissolved by overlaying the stool with a cellophane coverslip previously soaked in 50% glycerin and 3% malachite green stain. The parasite load was determined in terms of eggs per gram (epg) of faeces. The epg value was obtained by multiplying the number of eggs counted on the slide by a fixed multiplication factor provided with the kit (WHO Ginevra Switzerland). The infection intensity of individual parasites was interpreted as light, moderate or heavy infection, based on the following World Health Organization guidelines [10]: *A. lumbricoides* light (1–4999 epg), moderate (5000–49,999 epg), heavy (≥ 50,000 epg); *T. trichiura* light (1–999 epg), moderate (1000–9999 epg), heavy (≥ 10,000 epg); hookworm light (1–1999 epg), moderate (2000–3999 epg), heavy (≥ 4000 epg). Samples from Locka, Yanahuaya, Villa Porvenir-Litoral and Taipiplaya (201 out of the total number of 275 stool samples) were further examined with the modified Ritchie concentration technique (MRCT) [14].

2.4.2. Molecular analysis

Genomic DNA was extracted from a sub-population of *Blastocystis* positive samples to the microscopic observation, selected on the basis of abundance, using a faecal DNA kit (Bioline, UK) according to the manufacturer's protocol. A fragment of about 600 bp from the SSU rDNA gene was amplified using the primers RD5 (5'-ATCTGGTTGATCCTGCCAGT-3′) and BhRDr (5'-GAGCTTTTTAACTGCAACAACG-3′), following the PCR-conditions previously described [15]. Amplicons were sequenced using the forward amplification primer (Bio-Fab Research, Rome, Italy). The resulting chromatograms were analysed and edited using the software Chromas version 2.33 (Technelysium Pty Ltd., Australia). The sequences obtained were compared to those of *Blastocystis* STs previously deposited in GenBank using the BLAST application (www.ncbi.nlm.nih.gov/BLAST). The STs were identified by determining the exact match or closest identity (99%), according to the classification given by Stensvold [16] (https://pubmlst.org/blastocystis/).

2.5. Statistical analysis

Data were entered into Microsoft Excel 2010 software (Microsoft, Redmond, WA, USA); frequencies and percentages with 95% confidence intervals (CI) were calculated. Statistical analysis included univariate (chi-square or Fisher's exact tests, as appropriate) and multivariate analyses (logistic regression). *p* values <0.05 were considered significant. Cohen's K index was calculated to evaluate the agreement between the methods used.

3. Results

A total of 275 children (51.6% females; median age 8 years, IQR 7–9) were enrolled in the study from the six sentinel sites: Locka ($N = 37$), Franz Tamayo (N = 37), Yanahuaya ($N = 44$), Quime ($N = 50$), Villa Porvenir-Litoral ($N = 51$), Taipiplaya ($N = 56$). In three sentinel sites (Locka, Franz Tamayo and Yanahuaya), due to a low response rate (<80% of children returned their stool sample), the number of examined samples did not reach 50, although all available pupils aged <12 year-old were enrolled.

The overall IPI prevalence was 82.2% (226/275), including protozoa (80.0%, 220/275) and helminth infections (23.3% 64/275). Coinfections with protozoa and helminth were detected in 11.9% of SAC. No significant gender difference in IPI prevalence was reported.

The helminth prevalence detected in stool samples with the Kato-Katz technique were: *Ascaris lumbricoides* 14.5% (40/275), *Hymenolepis nana* 3.3% (9/275), *Strongyloides stercoralis* 0.7% (2/275), hookworms 1.1% (3/275), *Trichuris trichiura* 1.5% (4/275), *Enterobius vermicularis* 1.5% (4/275) (Table 2). The prevalence of any STH infection was 17.1%. Infections by *A. lumbricoides* were heavy (2/275, 0.7%) moderate (8/275, 2.9%) or light intensity (30/275, 10.9%) with a mean epg of 8755 (SD ±2765); infections by *T. trichiura* consisted of one moderate (0.4%) and three light (mean epg 372, SD ±260), and only two light hookworm infections were found (mean epg 2412, SD ±2316). The prevalence of STH infection of moderate/heavy intensity was of 4.0%.

Using the MRCT, the *Ascaris lumbricoides* prevalence was 11.9%, *Hymenolepis nana* 6.0%, *Strongyloides stercoralis* 3.5%, hookworms 1.5%, *Trichuris trichiura* 0.5%, *Enterobius vermicularis* 1.0% (Table 2). Overall, STHs were detected in 46/275 SAC (16.7%).

Blastocystis was the most prevalent protozoa, detected in 44.0% of the samples by direct microscopic examination, followed by *Entamoeba coli* (20.7%) *Endolimax nana* (19.6%) and *Giardia intestinalis* (17.8%) (Table 2).

A higher prevalence of IPI was detected with the MRCT, which identified *Blastocystis* in 64.2%, *Endolimax nana in* 42.3%, *Entamoeba coli*

Table 2
Intestinal helminths *N* (%) identified by Kato-Katz (KK) and the modified Ritchie concentration technique (MRCT) in stool samples from children living in the Department of La Paz, Bolivia.

Helminths	Franz Tamayo ($n = 37$)		Locka ($n = 37$)		Porvenir y Litoral ($n = 51$)		Tapiplaya ($n = 56$)		Yanahuaya ($n = 44$)		Quime ($n = 50$)		Total ($n = 275$)	
	KK	MRCT	KK	MRCT	KK	MRCT	KK	MRCT	KK	MRCT	KK	MRCT	KK	MRCT
Ascaris lumbricoides	0 (0)	N/D	7 (18.9)	N/D	2 (3.9)	1 (2.0)	12 (21.4)	7 (12.5)	18 (40.9)	15 (34.1)	1 (2.0)	1 (2.0)	40 (14.5)	24 (11.9)
Hookworms	0 (0)	N/D	0 (0)	N/D	0 (0)	1 (2)	1 (1.8)	1 (1.8)	0 (0)	0 (0)	1 (2.0)	1 (2.0)	2 (0.7)	3 (1.5)
Trichuris trichiura	0 (0)	N/D	0 (0)	N/D	1 (2.0)	0 (0)	2 (3.6)	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	4 (1.5)	1 (0.5)
Strongyloides stercoralis	0 (0)	N/D	0 (0)	N/D	3 (5.8)	6 (11.8)	0 (0)	0 (0)	1 (2.3)	1 (2.3)	0 (0)	0 (0)	2 (0.7)	7 (3.5)
Enterobius vermicularis	0 (0)	N/D	0 (0)	N/D	1 (2.0)	0 (0)	3 (5.4)	1 (1.8)	0 (0)	0 (0)	0 (0)	1 (2.0)	4 (1.5)	2 (1.0)
Hymenolepis nana	0 (0)	N/D	0 (0)	N/D	7 (13.7)	7 (13.7)	0 (0)	1 (1.8)	1 (2.3)	2 (4.5)	1 (2.0)	2 (4.0)	9 (3.3)	12 (6.0)

in 32.3%, and *Giardia intestinalis* in 27.4% of the 201 children examined with this technique (Table 3).Overall, the MRCT showed a good agreement with both microscopic examination (kappa coefficient = 0.93) and Kato-Katz technique (k = 0.92).

A total of 37 Blastocystis-positive samples (29%) were successfully DNA extracted and subtyped by sequences analysis of the SSU-rDNA. BLAST searches identified three different STs, with distinctive percentage values. The most common subtype was ST1 identified in $N = 20$ (54.05%) of the subjects, followed by ST2 ($N = 14$, 37.83%) and ST3 ($N = 3$, 8.10%). No significant correlation was found between ST distribution, patient gender, age or community (data not shown).

At the time of sampling, less than half of the SAC (126/275, 45.8%) reported having received PC within the last 3 months. PC coverage widely varied among communities, ranging from <10% in Yanahuaya, Locka, and Villa Porvenir-Litoral up to >90% in Tapiplaya and Quime. However, lower PC coverage did not necessarily correspond to higher STH prevalence by community, and vice versa. Likewise, the overall prevalence of STHs was similar between children treated (17/126, 13.4%) and not treated (29/149, 19.4%) with mebendazole within the last 3 months ($p = 0.186$).

Altogether, IPIs were significantly less prevalent in the Andean highlands (44/74, 59%), than in other zones (94/170, 55.3% in tropical lowlands and 88/94, 94% in the Yungas) ($p < 0.001$). As for STH distribution, no statistically significant difference emerged among the three areas, although a trend to lower prevalence was observed in the Andean highlands (9%, vs. 17% and 22% in the tropical area and the Yungas, respectively), ($p = 0.085$).

The association of IPIs and STHs with at-risk behaviour was investigated by univariate and multivariate analyses (Table 4). Access to potable water (OR 0.1 95%CI 0.02–0.5, $p = 0.004$) and the habit of boiling drinking water (OR 0.3, 95% CI 0.2–0.7, $p = 0.004$) were independently associated with a lower risk of all IPIs and STHs, respectively.

4. Discussion

Our study evidenced a high prevalence of IPIs (82.2%) among SAC across three different ecological zones of the Department of La Paz, Bolivia. In Andean areas (above 3800 m), a lower, although not negligible, burden of IPIs (59%) and STHs (9%) was found in comparison with areas located at lower altitudes. The climate conditions of the highlands, characterized by colder temperatures throughout the year (a mean of about 10 °C), intense solar radiation and lower partial oxygen pressure, are likely to be less suitable for the lifecycle and transmission of parasites. Coprological surveys carried out during the 6-year period between 1992 and 1997 in 24 localities of a region in the Northern Bolivian Altiplano documented a STH prevalence of 18% among 2521 SAC aged 5–19 at a very high altitude (3800–4200 m). Interestingly, almost all infections were due to *A. lumbricoides* and *T. trichiura*, while hookworm infections were sporadic [17]. Two decades later, in 2013, helminth, protozoal and mixed infection rates found in the highlands around La Paz were 1.8%, 39.1% and 2.9%, respectively, among 274 samples examined by the formalin-ether medical sedimentation method [18].

Table 3
Intestinal protozoa *N* (%) identified by direct microscopic examination (M) and the modified Ritchie concentration technique (MRCT) in stool samples from children living in the Department of La Paz, Bolivia.

Helminths	Franz Tamayo ($n = 37$)		Locka ($n = 37$)		Porvenir y Litoral ($n = 51$)		Tapiplaya ($n = 56$)		Yanahuaya ($n = 44$)		Quime ($n = 50$)		Total ($n = 275$)	
	M	MRCT	M	MRCT	M	MRCT	M	MRCT	M	MRCT	M	MRCT	M	MRCT
Blastocystis sp.	11 (29.7)	N/D	15 (40.5)	N/D	20 (39.2)	32 (62.7)	22 (39.3)	33 (58.9)	29 (65.9)	32 (72.7)	24 (48.0)	32 (64.0)	121 (44.0)	129 (64.2)
Entamoeba coli	12 (32.4)	N/D	6 (16.2)	N/D	12 (23.5)	13 (25.5)	7 (12.5)	10 (17.9)	9 (20.4)	20 (45.5)	11 (22.0)	22 (44.0)	57 (20.7)	65 (32.3)
Endolimax nana	6 (16.2)	N/D	6 () 16.2	N/D	10 (19.6)	20 (39.2)	10 (17.9)	17 (30.4)	10 (22.7)	25 (56.8)	12 (24.0)	23 (46.0)	54 (19.6)	85 (42.3)
Giardia intestinalis	4 (10.8)	N/D	2 (5.4)	N/D	17 (33.3)	20 (39.2)	12 (21.4)	12 (21.4)	6 (13.6)	10 (22.7)	8 (16.0)	13 (26.0)	49 (17.8)	55 (27.4)
Chilomastix masnili	0 (0)	N/D	3 (8.1)	N/D	1 (2.0)	2 (3.9)	0 (0)	1 (1.8)	1 (2.3)	2 (4.5)	5 (10.0)	6 (12.0)	10 (3.6)	11 (5.5)
E. histolytica/ dispar	5 (13.5)	N/D	0 (0)	N/D	4 (7.8)	4 (7.8)	0 (0)	1 (1.8)	0 (0)	0 (0)	0 (0)	0 (0)	9 (3.3)	5 (2.5)
Iodamoeba buetschlii	2 (5.4)	N/D	0 (0)	N/D	0 (0)	3 (5.9)	1 (1.8)	1 (1.8)	0 (0)	2 (4.5)	0 (0)	0 (0)	3 (1.1)	6 (3.0)
Entamoeba hartmanni	3 (8.1)	N/D	0 (0)	N/D	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (1.1)	0 (0)

Table 4
Risk factor for intestinal parasite and soil-transmitted helminth infections, by univariate and multivariate analysis.

Variable	Intestinal parasite infections			Soil transmitted helminthiasis		
	Total (%)	Univariate *p*-value	Multivariate[a] OR (95% CI), *p*-value	Total	Univariate *p*-value	Multivariate[a] OR (95% CI), *p*-value
Gender						
Female	118/142 (83%)			27/142 (19%)		
Male	108/133 (81%)	0.681	1.2 (0.5–2.5), 0.673	19/133 (14%)	0.294	0.7 (0.4–1.4), 0.345
PC in the last 3 months						
No	124/149 (83%)			29/149 (19%)		
Yes	102/126 (81%)	0.624		17/126 (13%)	0.186	
Ecological zone						
Andean highlands	44/74 (59%)			7/74 (9%)		
Tropical lowlands	94/107 (88%)			18/107 (17%)		
Yungas	88/94 (94%)	**< 0.001**		21/94 (22%)	0.085	
At-risk behaviours						
Boil drinking water						
No	98/113 (87%)			34/113 (30%)		
Yes	105/125 (84%)	0.553	1.0 (0.4–2.8), 0.941	12/125 (10%)	**<0.001**	**0.3 (0.2–0.7), 0.004**
Access to potable water						
No	75/77 (97%)			21/77 (27%)		
Yes	128/161 (80%)	**<0.001**	**0.1 (0.02–0.5), 0.004**	25/161 (16%)	**0.032**	0.8 (0.4–1.8), 0.619
Barefoot walking						
No	115/134 (86%)			33/134 (25%)		
Yes	88/104 (84%)	0.795	1.5 (0.5–2.4), 0.719	13/104 (13%)	**0.019**	0.5 (0.2–1.0), 0.066
Access to basic sanitation						
No	50/53 (94%)			15/53 (28%)		
Yes	153/185 (83%)	**0.035**	0.4 (0.1–1.6), $p = 0.201$	31/185 (17%)	0.061	0.8 (0.3–1.7), 0.519
Hand washing habit						
No	64/76 (84%)			20/76 (26%)		
Yes	139/162 (86%)	0.746	1.2 (0.4–3.4), 0.725	26/162 (16%)	0.061	1.1 (0.5–2.3), 0.894

Statistically significant associations are highlighted in bold,
[a] Only 238 children included in multivariate analysis (not available data from community of Franz Tamayo).

One thousand meters lower, the overall prevalence of IPIs was significantly higher. However, while the overall IPI distribution seems to be mainly influenced by the ecological zone, STH prevalence was primarily associated with the environmental setting (rural vs. peri-urban) and socio-sanitary condition. In the Andean highlands, no cases of STH infection were detected in a peri-urban district of El Alto, while 19% *A. lumbricoides* prevalence was found in the rural community of Locka, characterized by low socio-economic conditions and a lack of basic sanitation and hygiene measures, similar to most rural areas of low- and middle-income countries [19]. In the Yungas, a 40% STHs prevalence was found in Yanahuaya community, ten folds higher than that of Quime community (4%). Both communities are in rural areas, but basic sanitation and living condition are better in Quime. Moreover, Quime and Yanahuaya reported a PC coverage within the 3 months before the survey at the opposite extremes (100% and 0% of the surveyed SAC, respectively). Limited access to safe water supplies is a known driver of infection, and it was significantly linked in our study to the detection of both IPIs and STHs. *Blastocystis* was the most prevalent protozoon in all ecological zones. Molecular analysis identified *Blastocystis* ST1, ST2, ST3 which are the most common subtypes in humans. The high prevalence of ST1, observed here confirmed the trend reported in South America, where ST1 was found to be more abundant than ST3, which showed the highest overall prevalence in humans (44.1%) and underlined the animal-to-human and human-to-animal spill-over of *Blastocystis* in these communities [20,21]. This finding highlights once again the importance of a One Health approach for the control of intestinal parasitic infections.

The study also provides data on the performance of different parasitological methods, such as direct microscopic examination, MRCT and Kato-Katz, for the detection of protozoa and helminths in faecal samples. Although the MRCT was performed only in a subset of communities due to insufficient samples, it showed a good concordance rate with the other two methods. As expected, the MRCT allowed to detect a greater number of protozoa infections, in comparison with direct microscopic examination, while the two methods were comparable for the diagnosis of helminths.

5. Conclusions

In conclusion, updated epidemiological information is crucial to inform policy for STH control. For instance, in the Bolivian Chaco, where previous surveys have documented a consistently low STH prevalence throughout the region (<2%), a scaled-down program of PC delivery, accompanied by annual cross-sectional parasitological surveillance surveys were started in 2016, as per WHO recommendations [8]. In the Department of La Paz, the current overall STH prevalence is higher

(17.1%) and heterogeneous. Firstly, PC coverage should be significantly improved in all the at-risk populations, whereas currently more than half of the SAC included in our study had not received PC. Secondly, the frequency and strategy of PC delivery should be tailored to local conditions, as shown by the patchy distribution of STHs, irrespective of the ecological zone. Where the STH prevalence is lower than 20% the frequency of preventive chemotherapy with mebendazole can be reduced to once a year [10]. Thirdly, the prevalence of STH infections of moderate and heavy intensity show that the morbidity due to these parasites has not been eliminated yet (WHO target is to reduce these levels of STH infections to under 2%) [22]. Finally, significant efforts are still needed to reduce transmission and to improve health and sanitation in this area. Interventions should integrate expertise and collaboration across different sectors, targeting humans, animals and environment health, according to a One Health approach. Moreover, the efforts should be inspired by the WASH principles, endorsed by the WHO, and focused on access to safe drinking water, improvements in sanitation and hygiene promotion through health education.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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