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Prevalence and subtype distribution of *Blastocystis* sp. infecting children from a rural community in Panama

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ABSTRACT

Blastocystis sp. is a commonly reported intestinal parasite with a worldwide distribution. Phylogenetic analyses describe at least 17 subtypes for this parasite, and nine of them have been found in humans. However, the prevalence and some epidemiological characteristics of this parasitic infection in rural communities are not well known. The objective of this cross-sectional study was to evaluate the prevalence, subtypes, and epidemiological factors related to *Blastocystis* sp. Infection in children from of a small rural community in the central area of Panama. For this, 66 fecal samples from children (1 to 12 years old), were initially analyzed for the presence of parasites by a formalin-ethyl acetate/concentration method. Molecular detection and identification of *Blastocystis* sp. subtypes were carried out by amplification and sequencing of a partial fragment of the small-subunit ribosomal RNA gene. Using data from a questionnaire, analyses of epidemiological conditions potentially associated with *Blastocystis* sp. transmission were also conducted. Microscopic diagnostics showed that 33.3% (22/66) of the analyzed samples presented entero-parasites. Among them, *Blastocystis* sp. was the most prevalent, with 21.2% (14/66), followed by the *E. histolytica/dispar/moshkovskii* complex 4.5% (3/66), *Giardia lamblia* 1.5% (1/66) and *Strongyloides stercoralis* 1.5% (1/66). PCR-based analyses detected a prevalence of *Blastocystis* sp. infection of 74.2% (49/66) in apparently healthy children. Phylogenetic analysis revealed two different subtypes of this parasite: ST1 with 42.2% (28/66) infected, and ST3 with 31.8% (21/66) infected. In addition, recent diarrhea was significantly associated with *Blastocystis* sp. infection. None of the other risk factors evaluated was statistically associated with infection. These results highlight the need to further investigate clinical, epidemiological, and genetic characteristics of *Blastocystis* sp. infections in this community.

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1. Introduction

Diseases caused by intestinal parasites are one of the main public health problems around the world (Chifunda and Kelly, 2019). These infections are frequent in children from developing countries, who often live in rural and indigenous communities (Echagüe et al., 2015; Sánchez et al., 2017). Usually, transmission of these enteropathogens is linked to environmental contamination with human/animal feces, directly via hand-mouth or indirectly through ingestion of contaminated food or water (Villamizar et al., 2019; Efstratiou et al., 2017; Khodabakhsh Arbat et al., 2018).

Blastocystis sp. is considered the most prevalent intestinal human protozoan in the world (Del Coco et al., 2017). There are many unknown characteristics of organism (Stensvold and Clark, 2016; Kurt et al., 2016), and whether it is pathogenic or not is controversial (Subirats and Borrás, 2018; Roberts et al., 2014). Consequently, there are debates over the need to treat infected people (Coyle et al., 2012; Andersen and Stensvold, 2016; Subirats and Borrás, 2018). The prevalence of this infection varies from one country to another, according to diagnostic methodology used and hygienic-sanitary conditions, exceeding 5% in industrialized countries and reaching 30–100% in poor countries (Del Coco et al., 2017). Although there are factors that favor its transmission, such as lack of hygiene, consumption of contaminated food and zoonotic contact, other specific conditions of particular epidemiological scenarios must be considered (Moura et al., 2018; Angelici et al., 2018; Rojas-Velázquez et al., 2018; Barbosa et al., 2018; Javanmard et al., 2018).

Blastocystis sp., like other potentially pathogenic protozoa, presents a high genetic diversity, and a significant number of subtypes (ST) have been described (Skotarczak, 2018; Barbosa et al., 2018; Valença-Barbosa et al., 2019), all of them with similar morphological characteristics. Ten of the described subtypes (ST1-ST9, ST12) have been reported in humans, while the subtypes ST10, ST11, ST13-ST17 have been found only in non-human hosts (Stensvold et al., 2007; Stensvold and Clark, 2016; Ramírez et al., 2016; Del Coco et al., 2017). The geographical distribution of subtypes and the distribution of patients with potential symptoms associated with this infection (Ramírez et al., 2017) are not homogeneous in North and South America (Jiménez et al., 2019). In this sense, it is important to characterize each epidemiological scenario before developing surveillance, prevention and control measures. In this same line, the genetic diversity of *Blastocystis* sp. has only been partially studied in Latin America; studies in

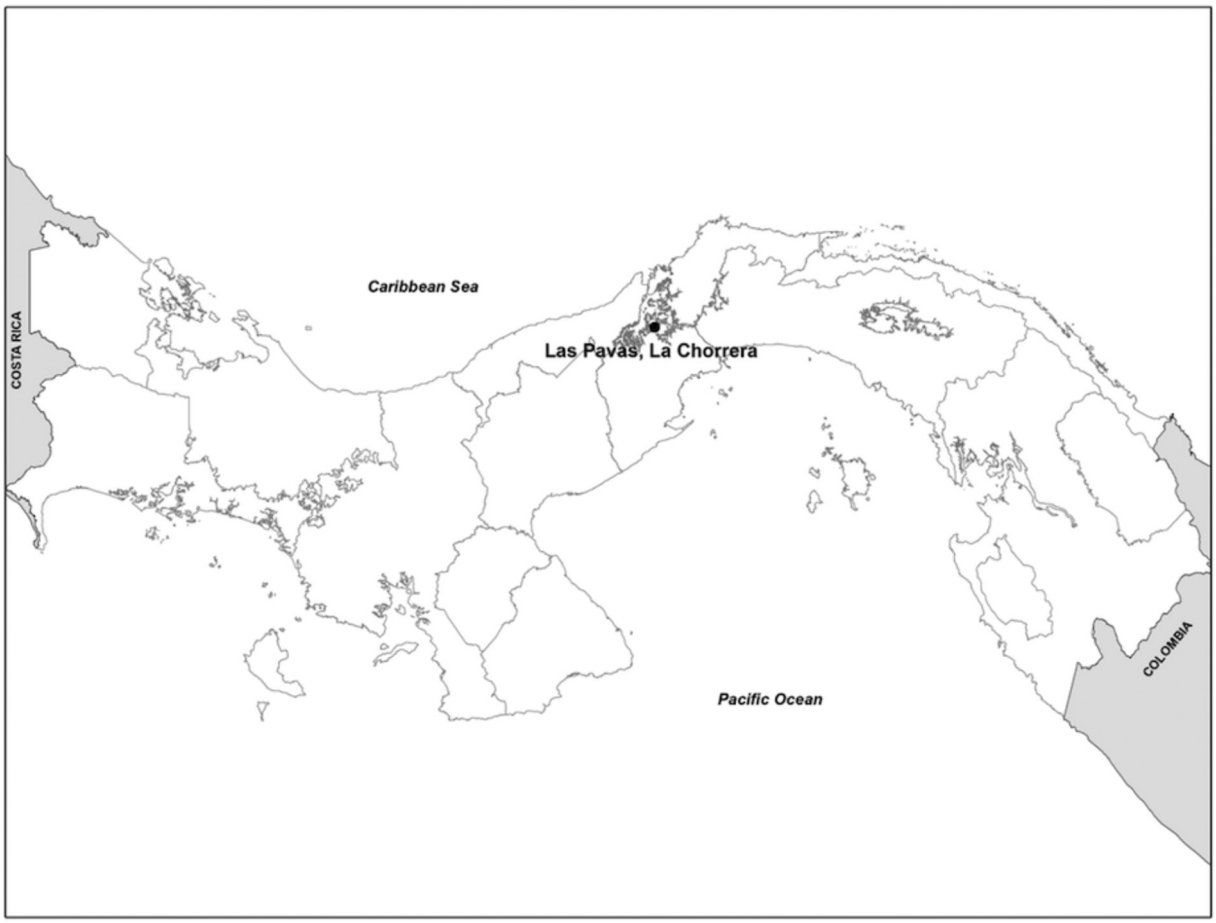

Fig. 1. Geographic location of the community of Las Pavas, Province of Panama Oeste, where children's stool samples were collected.

Colombia, Brazil, Argentina and Mexico have shown that humans are infected primarily with ST1-ST3 (Ramírez et al., 2016; Rojas-Velázquez et al., 2018). There are few investigations on the prevalence *Blastocystis* sp. in Panama (Sandoval et al., 2015), and, to our knowledge, there is no prior genetic typing study of this parasite in Panama. The objective of this cross-sectional study was to evaluate the prevalence, subtypes, and epidemiological factors related to infection with *Blastocystis* sp. in children from of a small rural community in the central area of the isthmus of Panamá.

2. Methods

2.1. Study area

A descriptive and cross-sectional study was conducted during the months of July and August 2017 in a rural community called Las Pavas (9°6′15″N, 79°53′9″W), located in the district of La Chorrera, province of Panamá Oeste, 80 km from the city of Panama (Fig. 1). It is a settlement of approximately 99 homes, 50–150 m above sea level, located on the west shore of the Panama Canal. The community is surrounded by pastures and some remnant forest patches. The climate of the region is considered humid tropical lowland, with an annual average temperature of 26 °C and an average annual precipitation of 2500 mm. Most homes are supplied with non-chlorinated water through a local aqueduct, however the use water from other sources (wells, streams, rain etc.) is common. A dairy and horse breeding farm is located practically in the middle of the town, close to the underground water source for the aqueduct and to the primary school. Much of the population is engaged in agricultural activities, raising domestic animals, and working on private nearby farms.

2.2. Study population, sample collection and questionnaire analysis

All students (94) from the only primary school in the community were invited to participate in the study. After presenting a basic talk and graphic material about intestinal parasitic diseases, each student was given a plastic container with a lid. The safe way to perform feces collection was explained to them, and these instructions were also sent in graphic and written forms to the parents. Recently collected fecal samples were brought to the school two days after the fecal collection instructions. Every house in the community where children live was also visited to carry out a questionnaire with the help of their parents. Information concerning age, sex, clinical symptoms, defecation habits, hygiene customs, water sources, presence of animals and dwelling characteristics were collected. Informed consent, approved by the Research Bioethics Committee of the Gorgas Memorial Institute (1028/CBI/ICGES/07), was obtained from all parents or legal guardians of minors. The study was designed as a cross-sectional study, and the results were analyzed using descriptive statistics to determine the frequency of *Blastocystis* sp. infection. Fisher's exact test or Chi-square were conducted to evaluate possible associations between *Blastocystis* sp. presence and each of the 13 variables collected during the survey (Fig. 2). A level of $p < 0.05$ was considered to be a statistically significant association. All statistical analyses were performed using the open source software OpenEpi (http://www.openepi.com/Menu/OE_Menu.htm).

2.3. Frequency and genetic characterization of Blastocystis sp.

At the time of collection, fresh samples were divided each into two portions. The first portion was frozen at −20 °C without any additives in a small plastic container. The second portion was stored with 5% formalin solution in preparation for fecal parasite concentration processing. Within a week of collection, the 66 preserved samples were processed by means of a formalin ethyl acetate sedimentation procedure (Garcia, 2007). DNA was extracted with QIAmp DNA Stool Minikit (Qiagen Inc., Germantown, MD) from 200 mg of each frozen stool sample, according to the manufacturer's instructions.

For each sample, 5 μl of extracted DNA was subjected to PCR assay using the *Blastocystis* sp. specific primers BL18SPPF1 (5′-AGTAGTCATACGCTCGTCTCAAA-3′) and BL18SR2PP (5′-TCTTCGTT ACCCGTTACTGC-3′) (Poirier et al., 2011). These primers target a DNA fragment of 320 to 342 bp of the *Blastocystis* sp. SSU rDNA gene, depending on the ST. The amplified products were purified and directly sequenced on both strands by Sanger sequencing using BigDye™ Terminator v3.1 Cycle Sequencing Kit following manufacturer's instructions. The capillary electrophoresis of the sequencing fragments was performed in the ICGES-Panama sequencing facilities using an ABI 3500xL sequencer.

All electrophorograms were trimmed and edited using the Sequencher 4.1.4 software (Gene Codes Corp., Ann Arbor, Michigan). The FASTA results, along with the reference sequences obtained in the GenBank were then aligned using the program MAFFT under the bioinformatic package UGENE v1 31.1 (Okonechnikov et al., 2012) that was set up with a gap opening penalty of 2.03, an offset configuration of 2.00 and a maximum number of iterative refinements of 20. The acquired sequences were used for a gene homology search, with the SSU rDNA sequences accessible in the public databases from BLAST (NCBI, Bethesda, MD, USA).

2.4. Ethical considerations

The study was approved by the Research Bioethics Committee of the Gorgas Memorial Institute (1028/CBI/ICGES/07). All the information generated was of strict confidentiality and it was guaranteed that it was used only to comply with the objectives of the study.

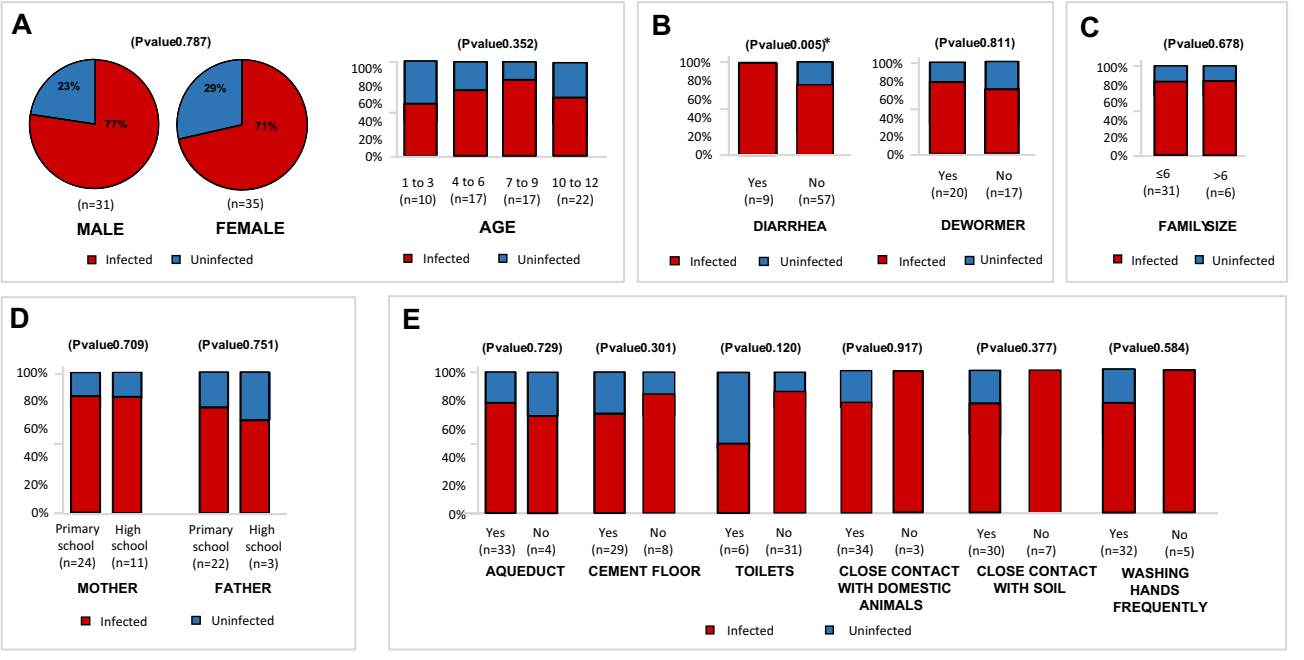

Fig. 2. Descriptive and statistical analysis of possible rick factors for *Blastocystis* sp. infection among children from the community of Las Pavas, Province of Panama Oeste. Gender/age (A) and recent diarrhea data (B) refer to the 66 evaluated children. The rest of the information were obtained from the survey conducted in 37 houses: having received an anti-intestinal parasites treatment during the last 2 months (B), number of people living in the house (C), parent's formal education (D), water source (E), floor construction materials (E), sanitary facilities (E), contact with domestic animals (E), contact with soil (E) and frequency of hand washing (E). *p values* were determined by the Fisher exact test or by chi-square test (age groups). Statically significant association are marked with an asterisk (*), $p < 0.05$.

3. Results

3.1. Frequency and genetic characterization of Blastocystis sp.

A total of 66 fecal samples were collected. Microscopic analysis revealed that 33.3% (22/66) of the analyzed samples had enteroparasites. Among the parasites found by microscopy, *Blastocystis* sp. was the most prevalent with 21.2% (14/66), followed by the *E. histolytica/dispar/moshkovskii* complex 4.5% (3/66), *Giardia lamblia* 1.5% (1/66) and *Strongyloides stercoralis* 1.5% (1/66). Only five samples had more than one species of intestinal parasite (Table 1). Further PCR-based analyses detected a prevalence of *Blastocystis* sp. infection of 74.2% (49/66) in apparently healthy children (Table 1). All 49 *Blastocystis* sp. positive samples confirmed by PCR were successfully subtyped by the SSU rDNA gene. BLAST search and phylogenetic analysis identified two subtypes, ST1 with 42.2% (28/66) and ST3 with 31.8% (21/66). No mixed infections were found with these two subtypes. The 49 *Blastocystis* SSU rDNA sequences were deposited in Gene Bank-NCBI 330 under accession numbers: MN503605-MN503653.

3.2. Questionnaire survey

Descriptive and statistical analysis of possible risk factors for *Blastocystis* sp. infection are shown in Fig. 2. Of the 66 stool samples analyzed, 46.9% (31/66) were male and 53.0% (35/66) were female. Infection with *Blastocystis* sp. was similar in both genders (24 boys and 25 girls). The registered age range was 1–12 years, including 14 children who still did not attend school. None of the assigned age ranges were significantly associated with *Blastocystis* sp. infection. Significant associations were found between *Blastocystis* sp. infection and children who had experienced diarrhea events 1–4 weeks before the coprological evaluation ($p < 0.05$) (Fig. 2B). None of the other variables evaluated showed a significant relationship with in *Blastocystis* sp. infection. These variables were: water source (rural aqueduct, non-chlorinated), floor construction materials, sanitary facilities, contact with domestic animals, contact with soil, frequency of hand washing, having received an anti-intestinal parasites treatment during the last 2 months, family size, and parent's formal education (Fig. 2).

4. Discussion

Human infection with *Blastocystis* sp. occurs both in urban and rural areas worldwide, often affecting children (Nithyamathi et al., 2016). Despite being very frequent and with potential pathogenicity, this organism is mainly characterized by nonspecific gastrointestinal symptoms (Wawrzyniak et al., 2013). In rural environments, there are proposed factors that can favor the transmission of *Blastocystis* sp., such as lack of hygiene, consumption of contaminated food, and zoonotic contact (Rojas-Velázquez et al., 2018; Suntaravitun and Dokmaikaw, 2018; Barbosa et al., 2018). Several studies have suggested that this infection may be a consequence of food intake or water contaminated with feces of domestic animals (Angelici et al., 2018; Leelayoova et al., 2004; Lee et al., 2012). The high prevalence found for this infection in children from a rural community in Panama should be called to the attention of medical services due to the risk of illness and possible linkages with poor sanitary conditions or particular ecological conditions. In addition, as *Blastocystis* sp. is an organism with a proven genetic diversity (Skotarczak, 2018), it is also important to identify which genetic variants infect these children and the related epidemiological factors.

In this study, microscopic analysis showed that 33.3% (22/66) of the children were infected with some intestinal parasite. *Blastocystis* sp. accounted for 63.6% (14/22) of these positive samples. However, after SSU rDNA-PCR analysis, 35 additional samples were found positive for the presence of *Blastocystis* sp. This demonstrates the higher sensitivity of this molecular test and calls the attention to the frequency of the possibility of false negative results when using techniques such as direct stool examination and concentration with formaldehyde ethyl acetate for the diagnosis of this parasite. This issue has been corroborated by previous reports, indicating that studies on *Blastocystis* sp. prevalence that rely on microscopy of fecal samples are probably underestimating the true frequency of this infection (Menounos et al., 2008; Süli et al., 2018). In this sense, the reported *Blastocystis* sp. prevalence of 38.9% obtained in a previous study on intestinal parasitism in Panama (Sandoval et al., 2015), although by itself high, could be below the actual frequency considering that only one flotation concentration technique was used.

Table 1
Frequency of intestinal parasites infections among children from the community of Las Pavas, Province of Panama Oeste, Panama.

	No. of positive samples (%)
Blastocystis sp.	14 (21.2)[a]
Blastocystis sp.	49 (74.2)[b]
Blastocystis sp. -*E. histolytica/dispar/moshkovskii* complex	2 (3.0)[a]
Blastocystis sp. - *Giardia lamblia*	1 (1.5)[a]
Blastocystis sp. - *Strongyloides stercoralis*	1 (1.5)[a]
E. histolytica/dispar/moshkovskii complex	3 (4.5)[a]
Giardia lamblia	1 (1.5)[a]

[a] Microscopy analysis.
[b] PCR analysis.

The results of the questionnaire carried out in the 37 homes from which the children evaluated came noted that having diarrhea recently was significantly related to *Blastocystis* sp. infection in this study (Fig. 2). No other risk factors evaluated were statistically associated with *Blastocystis* sp. infection. Previous studies indicate that age is a risk factor associated with *Blastocystis* infection (Mohammad et al., 2017; El Safadi et al., 2014; Pipatsatitpong et al., 2015). However, it should be considered that the statistical power of our analysis may be affected by the low number of evaluated samples and the high prevalence of *Blastocystis* sp. infections. Regarding age as a risk factor, it is also will be important to assess the prevalence of infection among adults who live with children, especially considering that the presence of an infected family member may contribute to the transmission of *Blastocystis* sp. among other family members (Mohammad et al., 2017).

Our results suggest a high prevalence of *Blastocystis* sp. infection together with a low frequency of other intestinal parasites is a finding that should be considered uncommon for this type of rural population (Torres et al., 1992; Devera et al., 2003; Devera et al., 2006). In this regard, it is important to consider the recent administration of antiparasitic drugs, but without action against *Blastocystis* sp. Because two months before starting the present study, students from the primary school of Las Pavas received a single dose of albendazole. This treatment is usually done once a year without previous diagnoses, as part of a joint program between the Ministries of Education and Health of Panama. It is known that this drug is efficient against the most frequent intestinal helminthiases and giardiasis (Solaymani-Mohammadi et al., 2010), but is not a recognized drug for the treatment of blastocystosis (Horton, 2000). However, the effects of this antiparasitic drug, regarding *Blastocystis* sp. infection, are difficult to conclude based on the evaluated data. Thus, it is advisable that these deworming programs be carried out after performing the corresponding coproparasitological studies of all children (Taylor-Robinson et al., 2015). With these results, a better selection of the drugs to be used can be achieved; that in the case of infection with *Blastocystis* sp., metronidazole could be a better choice (Roberts et al., 2014).

At least ten (ST1-ST9 and ST12) of the 17 described subtypes of *Blastocystis* sp. have been reported in humans, the rest have only been demonstrated so far in animal feces (Stensvold and Clark, 2016; Wang et al., 2018). Despite the large number of studies, the association of these subtypes with a certain clinical characteristic remains unclear (Dogruman-Al et al., 2008; Forsell et al., 2012; Taylor-Orozco et al., 2016). In the present study, it was possible to determine the subtype in 49 *Blastocystis* sp. positive samples. Twenty-eight samples were assigned to ST1 and 21 to ST3, being the first report on the genetic diversity of *Blastocystis* sp. in Panama. Unfortunately, with the methodology used by us, mixed subtype infections may not be evident in sequence chromatograms and can thus be undetected (Stensvold and Clark, 2016). In this regard, we must also emphasize that the presence of mixed subtype infections is an important characteristic, in order to explore the diversity and distribution of this parasite in the human gut (Scanlan et al., 2015). In addition, given the small number of samples evaluated, it is very likely that other subtypes could also be circulating in this or other geographic regions of the country. It has been reported that ST1 and ST3 infects a wide variety of domestic animal species including cattle, pigs and dogs and that therefore may be related to zoonotic transmission (Abe, 2004; Noël et al., 2005; Stensvold et al., 2009). As observed in our study, ST1 was the most common subtype found in some rural communities in Colombia (Ramírez et al., 2017; Sánchez et al., 2017). Although ST3 has been considered the most frequent subtype that infects humans worldwide (Meloni et al., 2011; Das et al., 2016), ST1 showed a higher prevalence in the child population studied. Some studies suggest that ST3 is more associated with disease than ST1 (Casero et al., 2015; Skotarczak, 2018), however both subtypes are frequent in people without symptoms (Yan et al., 2006; Jantermtor et al., 2013; Pandey et al., 2015; Ramírez et al., 2017). In this sense, none of the children evaluated in this study reported gastrointestinal symptoms on the day of the stool collection. On the other hand, the zoonotic potential of *Blastocystis* sp. has been described in several reports (Parkar et al., 2010; Cian et al., 2017). Both ST1 and ST3 have been found in cattle feces (Abe, 2004; Alfellani et al., 2013; Cian et al., 2017). This should be considered when establishing epidemiological measures to prevent blastocystosis in this community, since there is a private farm with many animals, including cows, in areas near the main facilities of the aqueduct that supplies water to the community. The presence of *Blastocystis* sp. in water for human consumption has been confirmed in several studies (Leelayoova et al., 2004; Angelici et al., 2018; Abdulsalam et al., 2012). Unfortunately, the water distributed through the aqueduct of Las Pavas does not receive any type of treatment. Thus, the evaluation of the presence or not of *Blastocystis* sp. and other enteropathogens in water for human consumption and in farm animal feces from this community is an important pending task.

Finally, it is important to remind the health personnel of this region that a high frequency of apparently *Blastocystis* sp. asymptomatic cases should not be an excuse for not providing the corresponding clinical care to these patients and organizing basic preventive measures for this infection.

5. Conclusions

The results indicate a high prevalence of infection with *Blastocystis* sp. in the studied child population, even with a low frequency of other intestinal parasites. Also, they confirm the added value of using molecular diagnostic methods for this parasitic infection. Additionally, it provides the first genetic characterization of *Blastocystis* sp. subtypes circulating in a rural area of Panama. Infections in this child population with subtypes ST1 and ST3 were demonstrated, with a slight predominance of ST1. Finally, the study highlights the need to investigate the clinical pictures of *Blastocystis* sp. infections, to establish educational interventions and to evaluate the presence of *Blastocystis* sp. in water sources, as well as the role of domestic and farm animals in the transmission of this parasite in this community.

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Contributors

Conception and design of the study: AS, JEC; data collection: MP, VP, VV; statistical analysis, data and sequence analysis and interpretation: MP, VV, FS, JEC; funding acquisition: AS, JEC; writing – original draft: MP, AS; writing – review & editing: AS, JEC. All authors approved the final version for publication.

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Ethics approval

The study was approved by the Research Bioethics Committee of the Gorgas Memorial Institute (1028/CBI/ICGES/07).

Declaration of competing interests

The authors declare that there is no conflict of interest during the execution of this work. The authors have not received salaries or benefits from distributors or manufacturers of the materials or reagents used in this study.

References

Abdulsalam, A.M., Ithoi, I., Al-Mekhlafi, H.M., Ahmed, A., Surin, J., Mak, J.W., 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. Parasitology 139 (8), 1014–1020. https://doi.org/10.1017/S0031182012000340.

Abe, N., 2004. Molecular and phylogenetic analysis of *Blastocystis* isolates from various hosts. Vet. Parasitol. 120 (3), 235–242. https://doi.org/10.1016/j.vetpar.2004.01.003.

Alfellani, M.A., Taner-Mulla, D., Jacob, A.S., Imeede, C.A., Yoshikawa, H., Stensvold, C.R., Clark, C.G., 2013. Genetic diversity of *Blastocystis* in livestock and zoo animals. Protist 164 (4), 497–509. https://doi.org/10.1016/j.protis.2013.05.003.

Andersen, L.O., Stensvold, C.R., 2016. *Blastocystis* in health and disease: are we moving from a clinical to a public health perspective? J. Clin. Microbiol. 54 (3), 524–528. https://doi.org/10.1128/JCM.02520-15.

Angelici, M.C., Nardis, C., Scarpelli, R., Ade, P., 2018. *Blastocystis hominis* transmission by non-potable water: a case report in Italy. New Microbiol. 41 (2), 173–177. https://doi.org/10.4269/ajtmh.2004.70.658.

Barbosa, C.V., Barreto, M.M., de Jesus Andrade, R., Sodré, F., d'Avila-Levy, C.M., Peralta, J.M., Igreja, R.P., de Macedo, H.W., Santos, H.L.C., 2018. Intestinal parasite infections in a rural community of Rio de Janeiro (Brazil): prevalence and genetic diversity of *Blastocystis* subtypes. PloS One 13 (3), e0193860. https://doi.org/10.1371/journal.pone.0193860.

Casero, R.D., Mongi, F., Sánchez, A., Ramírez, J.D., 2015. *Blastocystis* and urticaria: examination of subtypes and morphotypes in an unusual clinical manifestation. Acta Trop. 148, 156–161. https://doi.org/10.1016/j.actatropica.2015.05.004.

Chifunda, K., Kelly, P., 2019. Parasitic infections of the gut in children. Paediatr Int Child Health 39 (1), 65–72. https://doi.org/10.1080/20469047.2018.1479055.

Cian, A., El Safadi, D., Osman, M., Moriniere, R., Gantois, N., Benamrouz-Vanneste, S., Delgado-Viscogliosi, P., Guyoy, K., Luen Li, L., Monchy, S., Noël, C., Poirier, P., Nourrisson, C., Wawrzyniak, I., Delbac, F., Bosc, S., Chabé, M., Petit, T., Certad, G., Viscogliosi, E., 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French zoos and evaluation of potential zoonotic risk. PLoS One 12 (1). https://doi.org/10.1371/journal.pone.0169659.

Coyle, C.M., Varughese, J., Weiss, L.M., Tanowitz, H.B., 2012. *Blastocystis*: to treat or not to treat. Clin Infect Dis 54 (1), 105–110. https://doi.org/10.1093/cid/cir810.

Das, R., Khalil, S., Mirdha, B.R., Makharia, G.K., Dattagupta, S., Chaudhry, R., 2016. Molecular characterization and subtyping of *Blastocystis* species in irritable bowel syndrome patients from north India. PLoS One 11 (1). https://doi.org/10.1371/journal.pone.0147055.

Del Coco, V.F., Molina, N.B., Basualdo, J.A., Córdoba, M.A., 2017. *Blastocystis* spp.: advances, controversies and future challenges. Rev. Argent. Microbiol. 49 (1), 110–118. https://doi.org/10.1016/j.ram.2016.08.004.

Devera, R., Cermeño, J.R., Blanco, Y., Bello Morales, M.C., Guerra, X., De Sousa, M., Maitan, E., 2003. Prevalencia de *Blastocistosis* y otras parasitosis intestinales en una comunidad rural del Estado Anzoátegui. Venezuela. Parasitol Latinoam 58, 95–100. https://doi.org/10.4067/s0717-77122003000300001.

Devera, R., Angulo, V., Amaro, E., Finali, M., Franceschi, G., Blanco, Y., Tedesco, R.M., Requena, I., Velásquez, V., 2006. Parásitos intestinales en habitantes de una comunidad rural del Estado Bolívar. Venezuela. Rev Biomed 17 (4), 259–268.

Dogruman-Al, F., Dagci, H., Yoshikawa, H., Kurt, Ö., Demirel, M., 2008. A possible link between subtype 2 and asymptomatic infections of *Blastocystis hominis*. Parasitol. Res. 103 (3), 685–689. https://doi.org/10.1007/s00436-008-1031-3.

Echagüe, G., Sosa, L., Díaz, V., Ruiz, I., Rivas, L., Granado, D., Funes, P., Zenteno, J., Pistilli, N., Ramírez, M., 2015. Enteric parasitic disease in children under 5 years of age, indigenous and non-indigenous, from rural communities in Paraguay. Rev Chilena Infectol. 32 (6), 649–657. https://doi.org/10.4067/S0716-10182015000700006.

Efstratiou, A., Ongerth, J.E., Karanis, P., 2017. Waterborne transmission of protozoan parasites: review of worldwide outbreaks-an update 2011–2016. Water Res 1 (114), 14–22. https://doi.org/10.1016/j.watres.2017.01.036.

El Safadi, D., Gaayeb, L., Meloni, D., Cian, A., Poirier, P., Wawrzyniak, I., Dabboussi, F., Delhaes, L., Seck, M., Hamze, M., Riveau, G., Viscogliosi, E., 2014. Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infect Dis 25 (14), 164. https://doi.org/10.1186/1471-2334-14-164.

Forsell, J., Granlund, M., Stensvold, C.R., Clark, C.G., Evengård, B., 2012. Subtype analysis of *Blastocystis* isolates in Swedish patients. Eur J Clin Microbiol Infect Dis. (7), 1689–1696 https://doi.org/10.1007/s10096-011-1416-6.

Garcia, L.S., 2007. Diagnostic medical parasitology, 5TH Edition. Shock 27 (6), 707–708. https://doi.org/10.1097/01.shk.0000270192.88120.53.

Horton, J., 2000. Albendazole: a review of anthelmintic efficacy and safety in humans. Parasitology 121 (S1), S113–S132. https://doi.org/10.1017/S0031182000007290.

Jantermtor, S., Pinlaor, P., Sawadpanich, K., Pinlaor, S., Sangka, A., Wilailuckana, C., Wongsena, W., Yoshikawa, H., 2013. Subtype identification of *Blastocystis* spp. isolated from patients in a major hospital in northeastern Thailand. Parasitol. Res. 112 (4), 1781–1786. https://doi.org/10.1007/s00436-012-3218-x.
Javanmard, E., Niyyati, M., Ghasemi, E., Mirjalali, H., Aghdaei, H.A., Zali, M.R., 2018. Impacts of human development index and climate conditions on prevalence of *Blastocystis*: a systematic review and meta-analysis. Acta Trop. 185, 193–203. https://doi.org/10.1016/j.actatropica.2018.05.014.
Jiménez, P.A., Jaimes, J.E., Ramírez, J.D., 2019. A summary of *Blastocystis* subtypes in North and South America. Parasit Vectors. 29 (12), 376. https://doi.org/10.1186/s13071-019-3641-2.
Khodabakhsh Arbat, S., Hooshyar, H., Arbabi, M., Eslami, M., Abani, B., Poor, Movayed R., 2018. Prevalence of intestinal parasites among food handlers in Kashan, central Iran 2017-2018. J. Parasit. Dis. 42 (4), 577–581. https://doi.org/10.1007/s12639-018-1037-4.
Kurt, Ö., Doğruman, Al F., Tanyüksel, M., 2016. Eradication of *Blastocystis* in humans: really necessary for all? Parasitol Int. 65 (6Pt B), 797–801. https://doi.org/10.1016/j.parint.2016.01.010.
Lee, L.I., Chye, T.T., Karmacharya, B.M., Govind, S.K., 2012. *Blastocystis* sp.: waterborne zoonotic organism, a possibility? Parasit Vectors 28 (5), 130. https://doi.org/10.1186/1756-3305-5-130.
Leelayoova, S., Rangsin, R., Taamasri, P., Naaglor, T., Thathaisong, U., Mungthin, M., 2004. Evidence of waterborne transmission of *Blastocystis hominis*. Am J Trop Med Hyg 70 (6), 658–662. https://doi.org/10.4269/ajtmh.2004.70.658.
Meloni, D., Sanciu, G., Poirier, P., El Alaoui, H., Chabé, M., Delhaes, L., Dei-Cas, E., Delbac, F., Pier, L.F., Di Cave, D., Viscogliosi, E., 2011. Molecular subtyping of *Blastocystis* sp. isolates from symptomatic patients in Italy. Parasitol. Res. 109 (3), 613–619. https://doi.org/10.1007/s00436-011-2294-7.
Menounos, P.G., Spanakos, G., Tegos, N., Vassalos, C.M., Papadopoulou, C., Vakalis, N.C., 2008. Direct detection of *Blastocystis* sp. in human faecal samples and subtype assignment using single strand conformational polymorphism and sequencing. Mol Cell Probes 22 (1), 24–29. https://doi.org/10.1016/j.mcp.2007.06.007.
Mohammad, N.A., Al-Mekhlafi, H.M., Moktar, N., Anuar, T.S., 2017. Prevalence and risk factors of *Blastocystis* infection among underprivileged communities in rural Malaysia. Asian Pac J Trop Med 10 (5), 491–497. https://doi.org/10.1016/j.apjtm.2017.05.001.
Moura, R.G.F., Oliveira-Silva, M.B.D., Pedrosa, A.L., Nascentes, G.A.N., Cabrine-Santos, M., 2018. Occurrence of *Blastocystis* spp. in domestic animals in Triangulo Mineiro area of Brazil. Rev. Soc. Bras. Med. Trop. 51 (2), 240–243. https://doi.org/10.1590/0037-8682-0484-2016.
Nithyamathi, K., Chandramathi, S., Kumar, S., 2016. Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. PloS One 25 (11(2)), e0136709. https://doi.org/10.1371/journal.pone.0136709.
Noël, C., Dufernez, F., Gerbod, D., Edgcomb, V.P., Delgado-Viscogliosi, P., Lip-Chuen, Ho, Mulkit, S., Wintjens, R., Sogin, M.L., Capron, M., Pierce, R., Zenner, L., Viscogliosi, E., 2005. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol 43 (1), 348–355. https://doi.org/10.1128/JCM.43.1.348-355.2005.
Okonechnikov, K., Golosova, O., Fursov, M., 2012. The UGENE team. Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28, 1166–1167. https://doi.org/10.1093/bioinformatics/bts091.
Pandey, P.K., Verma, P., Marathe, N., Shetty, S., Bavdekar, A., Patole, M.S., Stensvold, C.R., Shouche, Y.S., 2015. Prevalence and subtype analysis of *Blastocystis* in healthy Indian individuals. Infection, Genet Evol 31, 296–299. https://doi.org/10.1016/j.meegid.2015.02.012.
Parkar, U., Traub, R.J., Kumar, S., Mungthin, M., Vitali, S., Leelayoova, S., Morris, K., Thompson, R.C.A., 2010. Direct characterization of *Blastocystis* from zoo animals and their animal-keepers. Vet. Parasitol. 169 (1–2), 8–17. https://doi.org/10.1017/S0031182006001582.
Pipatsatitpong, D., Leelayoova, S., Mungthin, M., Aunpad, R., Naaglor, T., Rangsin, R., 2015. Prevalence and risk factors for *Blastocystis* infection among children and caregivers in a child care center, Bangkok, Thailand. Am J Trop Med Hyg 93 (2), 310–315. https://doi.org/10.4269/ajtmh.14-0492.
Poirier, P., Wawrzyniak, I., Albert, A., El Alaoui, H., Delbac, F., Livrelli, V., 2011. Development and evaluation of a real-time PCR assay for detection and quantification of *Blastocystis* parasites in human stool samples: prospective study of patients with hematological malignancies. J Clin Microbiol 49 (3), 975–983. https://doi.org/10.1128/JCM.01392-10.
Ramírez, J.D., Sánchez, A., Hernández, C., Flórez, C., Bernal, M.C., Giraldo, J.C., Reyes, P., López, M.C., García, L., Cooper, P.J., Vicuña, Y., Mongi, F., Casero, R.D., 2016. Geographic distribution of human *Blastocystis* subtypes in South America. Infect. Genet. Evol. 41, 32–35. https://doi.org/10.1016/j.meegid.2016.03.017.
Ramírez, J.D., Flórez, C., Olivera, M., Bernal, M.C., Giraldo, J.C., 2017. *Blastocystis* subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. PLoS ONE 21 (12(2)), e072586. https://doi.org/10.1371/journal.pone.0172586.
Roberts, T., Stark, D., Harkness, J., Ellis, J., 2014. Update on the pathogenic potential and treatment options for *Blastocystis* sp. gut pathogens. BioMed 6 (1), 17. https://doi.org/10.1186/1757-4749-6-17.
Rojas-Velázquez, L., Morán, P., Serrano-Vázquez, A., Fernández, L.D., Pérez-Juárez, H., Poot-Hernández, A.C., Portillo, T., González, E., Hernández, E., Partida-Rodríguez, O., Nieves-Ramírez, M.E., Magaña, U., Torres, J., Eguiarte, L.E., Piñero, D., Ximénez, C., 2018. Genetic diversity and distribution of *Blastocystis* subtype 3 in human populations, with special reference to a rural population in central Mexico. Biomed Res Int 18 (2018), 3916263. https://doi.org/10.1155/2018/3916263.
Sánchez, A., Munoz, M., Gómez, N., Tabares, J., Segura, L., Salazar, Á., Restrepo, C., Ruíz, M., Reyes, P., Qian, Y., Xiao, L., López, M.C., Ramírez, J.D., 2017. Molecular epidemiology of *Giardia*, *Blastocystis* and *Cryptosporidium* among indigenous children from the Colombian Amazon Basin. Front Microbiol 21 (8), 248. https://doi.org/10.3389/fmicb.2017.00248.
Sandoval, N. R., Ríos, N., Mena, A., Fernández, R., Perea, M., Manzano-Román, R., Siles-Lucas, M., 2015. A survey of intestinal parasites including associated risk factors in humans in Panama. Acta Tropica, 147, 54–63. doi:https://doi.org/10.1016/j.actatropica.2015.03.024
Scanlan, P.D., Stensvold, C.R., Cotter, P.D., 2015. Development and application of a *Blastocystis* subtype-specific PCR assay reveals that mixed-subtype infections are common in a healthy human population. Appl Environ Microbiol. 15 (81(12)), 4071–4076. https://doi.org/10.1128/AEM.00520-15.
Skotarczak, B., 2018. Genetic diversity and pathogenicity of *Blastocystis*. Ann Agric Environ Med. 25 (25(3)), 411–416. https://doi.org/10.26444/aaem/81315.
Solaymani-Mohammadi, S., Genkinger, J.M., Loffredo, C.A., Singer, S.M., 2010. A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with *Giardia duodenalis*. PLoS Negl. Trop. Dis. 4 (5), e682. https://doi.org/10.1371/journal.pntd.0000682.
Stensvold, C.R., Clark, C.G., 2016. Molecular identification and subtype analysis of *Blastocystis*. Curr Protoc Microbiol 18 (43), 20A.2.1–20A.2.10. https://doi.org/10.1002/cpmc.17.
Stensvold, C.R., Suresh, G.K., Tan, K.S., Thompson, R.A., Traub, R.J., Viscogliosi, E., Clark, C.G., 2007. Terminology for *Blastocystis* subtypes–a consensus. Trends Parasitol 23 (3), 93–96. https://doi.org/10.1016/j.pt.2007.01.004.
Stensvold, C.R., Alfellani, M.A., Nørskov-Lauritsen, S., Prip, K., Victory, E.L., Maddox, Nielsen H.V., Clark, C.G., 2009. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int. J. Parasitol. 39 (4), 473–479. https://doi.org/10.1016/j.ijpara.2008.07.006.
Subirats, M., Borrás, R., 2018. *Blastocystis* sp., an emerging parasite with controversial pathogenicity. Should all human cases be treated? Rev. Clin. Esp. 218 (3), 133–134. https://doi.org/10.1016/j.rce.2018.03.003.
Süli, T., Kozoderović, G., Potkonjak, A., Simin, S., Simin, V., Lalošević, V., 2018. Comparison of conventional and molecular diagnostic techniques for detection of *Blastocystis* sp. in pig faeces. Iran J Parasitol 13 (4), 594–601. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6348225/.
Suntaravitun, P., Dokmaikaw, A., 2018. Prevalence of intestinal parasites and associated risk factors for infection among rural communities of Chachoengsao Province, Thailand. Korean J Parasitol 56 (1), 33. https://doi.org/10.3347/kjp.2018.56.1.33.
Taylor-Orozco, V., López-Fajardo, A., Muñoz-Marroquín, I., Hurtado-Benítez, M., Ríos-Ramírez, K., 2016. *Blastocystis* sp: evidencias de su rol patógeno. Biosalud 15 (2), 69–86. https://doi.org/10.17151/biosa.2016.15.2.8.
Taylor-Robinson, D.C., Maayan, N., Soares-Weiser, K., Donegan, S., Garner, P., 2015. Deworming drugs for soil-transmitted intestinal worms in children: effects on nutritional indicators, haemoglobin, and school performance. Cochrane Database Syst Rev 23 (7), CD000371. https://doi.org/10.1002/14651858.CD000371.pub6.
Torres, P., Miranda, J.C., Flores, L., Riquelme, J., Franjola, R., Perez, J., Auad, S., Hermosilla, C., Riquelme, S., 1992. Blastocystosis and other intestinal protozoan infections in human riverside communities from-Valdivia River Basin. Chile. Rev Inst Med Trop 34 (6), 557–564. https://doi.org/10.1590/S0036-46651992000600010.
Valença-Barbosa, C., do Bomfim, T.C.B., Teixeira, B.R., Gentile, R., Neto, S.F.D.C., Magalhães, B.S.N., Balthazar, D.A., da Silva, F.A., Biot, R., d'Avila, Levy C.M., Santos, H.L.C., 2019. Molecular epidemiology of *Blastocystis* isolated from animals in the state of Rio de Janeiro, Brazil. PLoS One 25 (14(1)), e0210740. https://doi.org/10.1371/journal.pone.0210740.