Research Article

Genetic Diversity and Distribution of Blastocystis Subtype 3 in Human Populations, with Special Reference to a Rural Population in Central Mexico

Liliana Rojas-Velázquez,1,2 Patricia Morán,1 Angélica Serrano-Vázquez,1 Leonardo D. Fernández,1 Horacio Pérez-Juárez,1,2 Augusto C. Poot-Hernández,4 Tobias Portillo,5 Enrique González,1 Eric Hernández,1 Oswaldo Partida-Rodríguez,1 Miriam E. Nieves-Ramírez,1 Ulises Magaña,1,2 Javier Torres,6 Luis E. Eguiarte,7 Daniel Piñero,7 and Cecilia Ximénez1

1Unidad de Investigación en Medicina Experimental, Facultad de Medicina, Universidad Nacional Autónoma de México (UNAM), Dr. Balmis 148, Doctores, Cuauhtémoc, 06726 Ciudad de México, México
2Unidad de Posgrado, Universidad Nacional Autónoma de México (UNAM), Circuito de Posgrado S/N, Coyocacán, Coyoacán, Cd. Universitaria, 04510 Ciudad de México, México
3Centro de Investigación en Recursos Naturales y Sustentabilidad (CIRENYS), Universidad Bernardo O’Higgins, Avenida Viel 1497, Santiago, Chile
4Departamento de Ingeniería de Sistemas Computacionales y Automatización, Sección de Ingeniería de Sistemas Computacionales, Instituto de Investigaciones en Matemáticas Aplicadas y en Sistemas, Universidad Nacional Autónoma de México (UNAM), Circuito Escolar 3000, Cd. Universitaria, Coyoacán, 04510 Ciudad de México, México
5Unidad de Bioinformática, Bioestadística y Biología Computacional, Red de Apoyo a la Investigación, Coordinación de la Investigación Científica, UNAM, Instituto Nacional de Ciencias Médicas y Nutrición, Vasco de Quiroga 15, Tlalpan, 14080 Ciudad de México, México
6Unidad de Investigación Médica en Enfermedades Infecciosas y Parasitarias, Hospital de Pediatría, Centro Médico Siglo XXI Instituto Mexicano del Seguro Social (IMSS), Avenida Cuauhtémoc 330, Doctores, Cuauhtémoc, 06720 Ciudad de México, México
7Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México (UNAM), Circuito Exterior S/N, Junto al Jardín Botánico, Coyoacán, 04510 Ciudad de México, México

Correspondence should be addressed to Liliana Rojas-Velázquez; lhily@yahoo.com and Cecilia Ximénez; cximenez@unam.mx

Received 29 December 2017; Accepted 11 February 2018; Published 18 March 2018

Academic Editor: Daniele Corsaro

Copyright © 2018 Liliana Rojas-Velázquez et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Blastocystis subtype 3 (ST3) is a parasitic protist found in the digestive tract of symptomatic and asymptomatic humans around the world. While this parasite exhibits a high prevalence in the human population, its true geographic distribution and global genetic diversity are still unknown. This gap in knowledge limits the understanding of the spread mechanisms, epidemiology, and impact that this parasite has on human populations. Herein, we provided new data on the geographical distribution and genetic diversity of Blastocystis ST3 from a rural human population in Mexico. To do so, we collected and targeted the SSU-rDNA region in fecal samples from this population and further compared its genetic diversity and structure with that previously observed in populations of Blastocystis ST3 from other regions of the planet. Our analyses revealed that diversity of Blastocystis ST3 showed a high haplotype diversity and genetic structure to the world level; however, they were low in the Morelos population. The haplotype network revealed a common widespread haplotype from which the others were generated recently. Finally, our results suggested a recent expansion of the diversity of Blastocystis ST3 worldwide.
1. Introduction

Blastocystis (Heterokonta, Stramenopiles) is a genus comprising parasitic protozoans, which inhabit the digestive tract of several metazoans, such as fishes, amphibians, birds, reptiles, rodents, and humans [1–3]. Blastocystis is globally distributed, showing a high rate of infection from underdeveloped to developed countries [4, 5].

This parasite is often transmitted via the oral-fecal route to people who work directly with animals, such as those involved in intensive animal farming or industrial livestock production [6]. In humans, the signs and symptoms associated with Blastocystis infection range from diarrhea to flatulence, bloating, and abdominal discomfort [7, 8], with the "irritable bowel syndrome" (IBS) being the most frequent clinical manifestation [8–11].

Molecular evidence based on the small subunit ribosomal RNA (SSU-rDNA) gene suggests that, at least, 17 genetic subtypes can be recognized within Blastocystis [12]. Nine of these subtypes are found in humans, with subtype 3 (ST3 hereafter) being the most common in epidemiological studies worldwide [12–18]. ST3 has been regarded to trigger IBS in humans [8], and recent research also suggests an association between this subtype and colorectal cancer [19]. Other studies, however, suggest a lack of association between the ST3 and some type of symptomatology in humans [7, 20]. While Blastocystis ST3 has medical importance and high prevalence in humans, the real magnitude of its genetic diversity and geographical distribution remains so far unknown [5, 17]. Apparently, the ST3 exhibits a broader geographical distribution and higher genetic diversity than other genetic subtypes of Blastocystis [16, 21], but this hypothesis still needs to be tested using genetic data and clinical cases from both well-studied and undersampled geographical areas. In this context, there are very few geographical and genetic data on Blastocystis ST3 from Mexico.

Herein, we aimed to provide new data on the geographical distribution and genetic diversity of Blastocystis ST3 from a rural and asymptomatic human population in Mexico. To do so, we collected and targeted the SSU-rDNA region in fecal samples from this population and further compared its genetic diversity and structure with those previously observed in populations of Blastocystis ST3 from other regions of the planet.

2. Materials and Methods

2.1. Ethical Considerations. The protocol used in this study was conducted under the ethical principles and approval of both the Mexican Commission on Ethics and Research of the Health Ministry of the State of Morelos (Comisiones de Ética y de Investigación del Ministerio de Salud del Estado de Morelos) and the Commission on Ethics in Research of the Facultad de Medicina of the Universidad Nacional Autónoma de México (UNAM) (Comité de Ética de Investigación de la Facultad de Medicina de la Universidad Nacional Autónoma de México). The guidelines of the committees are based on the Mexican Official Norm (Norma Oficial Mexicana NOM-012-SSA3-2007), which regulates the ethical principles of every research on humans and on laboratory animals, as well as on the Declaration of Helsinki, which set ethical principles regarding human experimentation developed by the World Health Organization (WHO).

Based on the abovementioned guidelines, our study only used samples from volunteers, who were respectively informed about the objectives of this research, the potential risks (if any), and the sampling procedures. We obtained an informed consent letter from all the participants.

2.2. Sampling and Analysis. Between May and November 2015, fecal samples were collected from 182 volunteers (86 males and 96 females) from Puente de Ixtla in the community of Xoxocotla, State of Morelos (Mexico), ranging in age from 2 to 51 years old. The asymptomatic status was defined according to the ROME III criteria. Three fecal samples were collected from each volunteer on three consecutive days. The samples were maintained at 4°C and transported to the laboratory in Mexico City on the same day of collection. A subsample of each fecal sample was smeared, stained with 4% Lugol’s iodine solution, and examined under a light microscope at 10x and 40x magnifications [22].

2.3. Amplification and Sequencing of SSU-rDNA. DNA was extracted from fresh fecal samples using QIAamp DNA stool kit (QIAGEN, Hilden, Germany) and following the manufacturer’s instructions. PCR protocol targeting the SSU-rDNA was conducted according to Scicluna et al. [23]. In brief, we used a total mixture of 20 µl: 20 µM of primers RD5 (5’-ATC TGG TTT ATC CTG CCAG T-3’) and BhRDr (5’-GAG CTT TTT AAC TGC AAC AAC G-3’) [23], as well as 0.025 U of polymerase (AmpliTaq Platinum Polymerase, Invitrogen). To verify the presence of a single band and the size of the amplified products (approximately 600 bp), the PCR products were separated by electrophoresis in agarose gel (1.5%) in the presence of ethidium bromide, visualized by ultraviolet transillumination, and photographed. The amplification product of a 600 bp fragment of the Blastocystis SSU-rDNA was purified and sequenced using a dideoxynucleotide-terminal method. Sequencing was carried out in a capillary sequencer (ABI-Avant 100, University of Washington). The sequences obtained were edited and/or analyzed with BioEdit, MEGA 5.0 software [24, 25], and ad hoc scripts from Python. These sequences were compared to sequences available in GenBank, employing BLAST to establish their identity. The final sequences were deposited in GenBank under accession numbers MF539962–MF540015.

2.4. Global Genetic Diversity and Haplotype Network for Blastocystis ST3. We investigated the global genetic diversity (i.e., Latin America, Europe, and Asia) within Blastocystis ST3 using the novel SSU-rDNA sequences reported in the present study and those previously reported within the literature. We provided an exhaustive list of the latter sequences (n = 169) and sources in the Supplementary Material (available here). We investigated the following descriptive statistics of genetic diversity and geographical distribution:
were coinfected with other parasites (Figure 1). 99 (67%) out of 148 positive samples, and 7% of the samples out of 148 positive samples. It was also the unique parasite in to cystis had the greatest frequency, occurring in 109 (74%) of 148 samples collected in Morelos, Mexico. Blastocystis was the only parasitic infection found in 67% of individuals and in 7% in coinfection with other parasites. Bsp: Blastocystis; OP: parasites other than Blastocystis; Bsp + OP: coinfection of Blastocystis and other parasites; Negative: no parasite found. Among OP: Chm, Chilomastix mesnili; Ec, Entamoeba coli; En, Endolimax nana; Hn, Hymenolepis nana; Gl, Giardia lamblia; Ib, Iodamoeba bütschlii.

3. Results
3.1. Frequency of Blastocystis ST3 in Morelos, Mexico. A microscopic analysis revealed that 148 (81.32%) of the 182 fecal samples collected in Morelos (Mexico) exhibited at least some type of intestinal parasite. These 148 samples (positive samples hereafter) harbored different parasites, including representatives of Blastocystis, Chilomastix mesnili, Entamoeba coli, Hymenolepis nana, Iodamoeba bütschlii, Endolimax nana, the Entamoeba histolytica/Entamoeba dispar complex, and Giardia lamblia. Among the abovementioned parasites, Blastocystis had the greatest frequency, occurring in 109 (74%) of 148 positive samples. It was also the unique parasite in 99 (67%) out of 148 positive samples, and 7% of the samples (10/148) were coinfected with other parasites (Figure 1).

Further PCR and sequencing procedures successfully confirmed the presence of three different Blastocystis subtypes in 72 of the 148 positive samples collected in Morelos. These three Blastocystis subtypes (ST) were recorded according to the following frequencies: Blastocystis ST1, 9.7% (n = 7 samples); ST2, 15.3% (n = 11 samples); and ST3, 75% (n = 54 samples) (Figure 2).

3.2. Genetic Diversity of ST3 and Haplotype Network. Genetic diversity indices revealed a total of 44 segregating sites (S) and 20 haplotypes (h), as well as a total haplotype diversity (Hd) of 0.563 and nucleotide diversity (π) of 0.019. Tajima’s D test provided values ranging between −1.303 and −2.363 (Table 1). A pairwise Fst analysis revealed that there is very low genetic differentiation between all geographical populations of Blastocystis ST3 (Table 2).

The number of haplotypes ranged from 3 to 15 between human populations, the number of segregating sites ranged between 1 and 35, haplotype diversity ranged between 0.142 and 0.740, and nucleotide diversity ranged between 0.001 and 0.045 (Table 1). The ST3 genetic diversity of Latin American populations (except Morelos’s population) and Eurasia exhibited the highest values of genetic diversity indices in contrast to Morelos’s population, where low haplotype diversity (three haplotypes) was detected (Table 1).

The haplotype network showed the haplotype distribution of the ST3 (Figure 3). In general, the worldwide haplotype network evidenced large levels of diversity, with a total of 20 haplotypes, and haplotype 1 was the dominant. The network showed a star topology radial distribution (Figure 3). Also, haplotype 1 was the most frequently found in Morelos’s population and this haplotype is commonly distributed in American populations.

4. Discussion
In the present study, we analyzed the frequency and distribution of Blastocystis subtypes in an asymptomatic rural population. The results revealed a great frequency of Blastocystis
Blastocystis observed between different geographical populations of the parasite Blastocystis [32]. Around the world, studies in South America have described similar frequency of Blastocystis varies from 23% to 61% in Mexico [20, 28, 29]. Recent from 74%, above the national average, as the frequency of this permutation test with 50,000 replicates. ns: not significant.

Nepal, Switzerland, Iraq, Italy, and France). Probability obtained by a that of Morelos. Eurasia: Blastocystis populations of Europe and Asia (i.e., Nepal, Switzerland, Iraq, Italy, and France).

Table 2: Estimates of $F_{ST}$ based on the SSU-rDNA variation observed between different geographical populations of the parasite Blastocystis ST3.

| Population | Morelos | Latin America | Eurasia |
|------------|---------|---------------|---------|
| Morelos    | 0.04165*ns | 0.09164*ns | 0.05975*ns |
| Latin America | ------ | ------ | ------ |
| Eurasia | ------ | ------ | ------ |

N: number of sequences; S: number of segregating sites; $h$: number of haplotypes; Hd: haplotype diversity; $\pi$: nucleotide diversity; ns: not significant. **$P < 0.01$.

Latin America: Blastocystis populations of North and South America (i.e., Mexico, Colombia, Brazil, Ecuador, Bolivia, Peru, and Argentina), except that of Morelos. Eurasia: Blastocystis populations of Europe and Asia (i.e., Nepal, Switzerland, Iraq, Italy, and France). Probability obtained by a permutation test with 50,000 replicates. ns: not significant.

from 74%, above the national average, as the frequency of this parasite varies from 23% to 61% in Mexico [20, 28, 29]. Recent studies in South America have described similar frequency of Blastocystis in the human population (21% to 67%) [30–32]. Around the world, Blastocystis exhibits a frequency range of 0.5% to 62% [4]. The higher prevalence of Blastocystis has been linked to hygiene factors including the consumption of food/water contaminated with Blastocystis and exposure to domestic and peri-domestic animals infected too with this parasite [4, 33].

Many years ago, Blastocystis was considered as saprophytic yeast of the digestive tract, innocuous for the host [34]. Nowadays, we can observe that this parasite is widely distributed in the human population in the world and it is similarly distributed in symptomatic and asymptomatic individuals. For instance, Morelos's population (Mexico) showed a high infection frequency of Blastocystis although the participants were asymptomatic, suggesting tolerance to this parasite, as reported elsewhere [20, 35, 36].

Blastocystis has a high worldwide genetic diversity, represented by 17 subtypes (ST1–ST17) [5]. It is possible that there are other subtypes capable of infecting humans and other vertebrates [4, 5]. Regarding the distribution of subtypes in the present study, ST1 (9.7%), ST2 (15.3%), and ST3 (75%) were identified among 72 human isolates successfully genotyped. Globally, Blastocystis ST3 is the most prevalent subtype in humans found in different geographic areas [5, 37]. In our study, the frequency of ST3 was 75%, high compared to other populations of Mexico as the state of Michoacan, where the frequency of this subtype was 21%, and in Mexico City it was 42% [38, 39].

In Latin America, the frequency of ST3 is high [4, 37], with the following frequencies reported for Blastocystis ST3 in this political region: 14% in Colombia, 36% in Brazil, 84% in Ecuador, 30% in Bolivia, 92% in Peru, and 63% in Argentina [40]. While ST3 is amply distributed worldwide, it is more prevalent in Latin America [37], which opens the possibility that this subtype was generated in this geographic area and spread to the rest of the continents.

To improve our knowledge on the magnitude of the genetic diversity of Blastocystis ST3 on the planet, we analyzed sequences of Mexico City, South America, France, Switzerland, Italy, Nepal, and Iraq and the sequences obtained in the present study (169 total sequences, 54 from Morelos and 115 from the NCBI database). The genetic parameters calculated for these sequences suggested a recent population increase or directional/purifying selection. These results are supported by the haplotype network, which showed a star topology with the haplotypes distributed in a radial way supporting inferences of a recent geographical expansion in Blastocystis.
distribution of parasites. It is known that these factors can influence the epidemiology and the causal factors that contribute to its dispersion dynamics and distribution.

**Disclosure**

Liliana Rojas-Velázquez is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México (UNAM), and received Fellowship 348424/239901 from CONACYT. This paper constitutes a partial fulfillment of the Graduate Program.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Acknowledgments**

The present work was supported by Grants IN218214 and IN-226511 from PAPIIT (DGAPA), Universidad Nacional Autónoma de México (UNAM); CONACyT 210-C01-140990 from the National Council for Science and Technology in Mexico (CONACyT); and FIS/IMSS/Prot/699 from the Mexican Institute of Social Security (IMSS). The authors would like to appreciate the collaboration of the Health Ministry of the State of Morelos, Mexico, for authorizing the establishments of the center of activity in the facilities and in the community of Xoxocotla. They also acknowledge the technical support provided by M.S. Martha Zaragoza, the Chemist Angeles Padilla, and Alejandro Flores. They also highly appreciate the informatics assistance of Angélica Serrano-Ahumada and Marco Gudiño. Leonardo D. Fernández is supported by CONICYT (FONDECYT Project no. 11170927) and by Universidad Bernardo O’Higgins (Project UBO/VRIP170201).

**Supplementary Materials**

List of the sequences reported in the present study and those previously reported within the literature. *(Supplementary Materials)*

**References**

[1] H. Yoshikawa, Z. Wu, J. Howe, T. Hashimoto, N. Geok-Choo, and K. S. W. Tan, “Ultrastructural and phylogenetic studies on Blastocystis isolates from cockroaches,” *Journal of Eukaryotic Microbiology*, vol. 54, no. 1, pp. 33–37, 2007.

[2] J. D. Silberman, M. L. Sogin, and D. D. Leipe, “Human parasite finds taxonomic home,” *Nature*, vol. 380, no. 6573, p. 398, 1996.

[3] A. Stechmann, K. Hamblin, V. Pérez-Brocail et al., “Organelles in blastocystis that blur the distinction between mitochondria and hydrogenosomes,” *Current Biology*, vol. 18, no. 8, pp. 580–585, 2008.

[4] C. G. Clark, M. van der Giezen, M. A. Alfellani, and C. R. Stensvold, *Recent Developments in Blastocystis Research*, Elsevier, Amsterdam, Netherlands, 2013.
[5] M. A. Alfellani, D. Taner-Mulla, A. S. Jacob et al., “Genetic diversity of blastocystis in livestock and zoo animals,” *Protist*, vol. 164, no. 4, pp. 497–509, 2013.

[6] K. S. W. Tan, “New insights on classification, identification, and clinical relevance of Blastocystis spp.” *Clinical Microbiology Reviews*, vol. 21, no. 4, pp. 639–665, 2008.

[7] C. V. Barbosa, R. D. J. Batista, R. P. Igreja, C. M. D. Levy, H. W. D. Macedo, and H. L. C. Santos, “Distribution of Blastocystis subtypes isolated from humans from an urban community in Rio de Janeiro, Brazil,” *Parasites and Vectors*, vol. 10, no. 1, p. 518, 2017.

[8] A. A. El-Badry, W. M. Abd ElWahab, D. A. Hamdy, and A. Aboud, “Blastocystis subtypes isolated from irritable bowel syndrome patients and co-infection with Helicobacter pylori,” *Parasitology Research*, pp. 1–11, 2017.

[9] A. Giacometti, O. Cirioni, A. Fiorentini, M. Fortuna, and G. Scalice, “Irritable bowel syndrome in patients with Blastocystis hominis infection,” *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 18, no. 6, pp. 436–439, 1999.

[10] J. Yakob, W. Jafari, M. A. Beg et al., “Irritable bowel syndrome: is it associated with genotypes of Blastocystis hominis,” *Parasitology Research*, vol. 106, no. 5, pp. 1033–1038, 2010.

[11] K. A. Jadallah, L. F. Nimri, and R. A. Ghanem, “Protozoan parasites in irritable bowel syndrome: a case-control study,” *World Journal of Gastrointestinal Pharmacology and Therapeutics*, vol. 8, no. 4, pp. 201–207, 2017.

[12] C. R. Stensvold, G. K. Suresh, K. S. W. Tan et al., “Terminology for Blastocystis subtypes - a consensus,” *Trends in Parasitology*, vol. 23, no. 3, pp. 93–96, 2007.

[13] F. Dogruman-Al, S. Kustimur, H. Yoshikawa et al., “Blastocystis subtypes in irritable bowel syndrome and inflammatory bowel disease in Ankara, Turkey,” *Memoria do Instituto Oswaldo Cruz*, vol. 104, no. 5, pp. 724–727, 2009.

[14] A. Moosavi, A. Haghhi, E. N. Mojarrad et al., “Genetic variability of Blastocystis sp. isolated from symptomatic and asymptomatic individuals in Iran,” *Parasitology Research*, vol. 111, no. 6, pp. 2311–2315, 2012.

[15] H. Yoshikawa, Z. Wu, I. Kimata et al., “Polymerase chain reaction-based genotype classification among human Blastocystis hominis populations isolated from different countries,” *Parasitology Research*, vol. 92, no. 1, pp. 22–29, 2004.

[16] C. R. Stensvold, M. Alfellani, and C. G. Clark, “Levels of genetic diversity vary dramatically between Blastocystis subtypes,” *Infection, Genetics and Evolution*, vol. 12, no. 2, pp. 263–273, 2012.

[17] M. A. Alfellani, C. R. Stensvold, A. Vidal-Lapiedra, E. S. U. Onuoha, A. F. Fagbenro-Beyioku, and C. G. Clark, “Variable geographic distribution of Blastocystis subtypes and its potential implications,” *Acta Tropica*, vol. 126, no. 1, pp. 11–18, 2013.

[18] C. G. Clark, “Extensive genetic diversity in Blastocystis hominis,” *Molecular and Biochemical Parasitology*, vol. 87, no. 1, pp. 79–83, 1997.

[19] S. Padukone, J. Mandal, and S. Parija, “Severe Blastocystis subtype 3 infection in a patient with colorectal cancer,” *Tropical Parasitology*, vol. 7, no. 2, pp. 122–124, 2017.

[20] L. Rojas, P. Morán, A. Valadez et al., “Entamoeba histolytica and Entamoeba dispar infection in Mexican school children: Genotyping and phylogenetic relationship,” *BMC Infectious Diseases*, vol. 16, no. 1, article no. 485, 2016.

[21] D. Meloni, P. Poirier, C. Mantini et al., “Mixed human intra- and inter-subtype infections with the parasite Blastocystis sp.,” *Parasitology International*, vol. 61, no. 4, pp. 719–722, 2012.

[22] L. R. Ash and T. C. Orihel, “Parasites: a guide to laboratory procedures and identification,” *American Society of Clinical Pathologists Press*, vol. 4, no. 2, p. 1988, 1987.

[23] S. M. Scicluna, B. Tawari, and C. G. Clark, “DNA barcoding of Blastocystis,” *Protist*, vol. 157, no. 1, pp. 77–85, 2006.

[24] T. A. Hall, “BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT,” *Nucleic Acids Research*, vol. 41, pp. 95–98, 1999.

[25] K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, and S. Kumar, “MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods,” *Molecular Biology and Evolution*, vol. 28, no. 10, pp. 2731–2739, 2011.

[26] P. Librado and J. Rozas, “DnaSP v5: a software for comprehensive analysis of DNA polymorphism data,” *Bioinformatics*, vol. 25, no. 11, pp. 1451–1452, 2009.

[27] M. Clement, D. Posada, and K. A. Crandall, “TCS: a computer program to estimate genealogies,” *Molecular Ecology*, vol. 9, no. 10, pp. 1657–1669, 2000.

[28] M. E. Ramirez-Miranda, D. E. Jimenez-González, and M. E. Rodriguez-Campa, “Síndrome de intestino irritable: frecuencia y relación filogenética de Blastocystis sp. de pacientes mexicanos,” *Revista de Gastroenterología de México*, vol. 76, no. 4, pp. 309–315, 2011.

[29] E. Rodriguez, B. Mateos, and J. C. Gonzalez, “Transición parasitaria a Blastocystis hominis en niños de la zona centro del estado de Guerrero, México,” *Parasitologia Latinoamericana*, 2008.

[30] R. D. Casero, F. Mongi, A. Sánchez, and J. D. Ramírez, “Blastocystis y urticaria: Examination of subtypes and morphotypes in an unusual clinical manifestation,” *Acta Tropica*, vol. 148, pp. 156–161, 2015.

[31] A. F. Malheiro, C. R. Stensvold, C. G. Clark, G. B. Braga, and J. J. Shaw, “Short report: Molecular characterization of Blastocystis obtained from members of the indigenous tapirapé ethnic group from the Brazilian Amazon Region, Brazil,” *The American Journal of Tropical Medicine and Hygiene*, vol. 85, no. 6, pp. 1050–1053, 2011.

[32] J. D. Ramírez, L. V. Sánchez, D. C. Bautista, A. F. Corredor, A. C. Flórez, and C. R. Stensvold, “Blastocystis subtypes detected in humans and animals from Colombia,” *Infection, Genetics and Evolution*, vol. 22, pp. 223–228, 2014.

[33] T. C. Tán, K. G. Suresh, and H. V. Smith, “Phenotypic and genotypic characterisation of Blastocystis hominis isolates implicates subtype 3 as a subtype with pathogenic potential,” *Parasitology Research*, vol. 104, no. 1, pp. 85–93, 2008.

[34] D. J. Stenzel and P. F. L. Boreham, “Blastocystis hominis revisited,” *Clinical Microbiology Reviews*, vol. 9, no. 4, pp. 563–584, 1996.

[35] R. AbuOdeh, S. Ezzedine, A. Samie, C. R. Stensvold, and A. ElBakri, “Prevalence and subtype distribution of Blastocystis in healthy individuals in Sharjah, United Arab Emirates,” *Infection, Genetics and Evolution*, vol. 37, pp. 158–162, 2016.

[36] É. B. David, S. Guimarães, A. P. Oliveira et al., “Molecular characterization of intestinal protozoa in two poor communities in the State of São Paulo, Brazil,” *Parasites & Vectors*, vol. 8, no. 1, article no. 103, 2015.

[37] C. R. Stensvold and C. G. Clark, “Current status of Blastocystis: a personal view,” *Parasitology International*, vol. 65, no. 6, pp. 763–771, 2016.
[38] G.-B. Vargas-Sanchez, M. Romero-Valdovinos, C. Ramirez-Guerrero et al., “Blastocystis isolates from patients with irritable bowel syndrome and from asymptomatic carriers exhibit similar parasitological loads, but significantly different generation times and genetic variability across multiple subtypes,” PLoS ONE, vol. 10, no. 4, pp. 1–13, 2015.

[39] G. Villalobos, G. E. R. Orozco-Mosqueda, M. Lopez-Perez et al., “Suitability of internal transcribed spacers (ITS) as markers for the population genetic structure of Blastocystis spp,” Parasites & Vectors, vol. 7, p. 461, 2014.

[40] J. D. Ramirez, A. Sanchez, C. Hernandez et al., “Geographic distribution of human Blastocystis subtypes in South America,” Infection, Genetics and Evolution, vol. 41, pp. 32–35, 2016.

[41] D. A. Joy, “Early origin and recent expansion of Plasmodium falciparum,” Science, vol. 300, no. 5617, pp. 318–321, 2003.

[42] J. C. Yomb, S. Jonckheere, G. Colin et al., “Imported malaria in a tertiary hospital in Belgium: epidemiological and clinical analysis,” Acta clinica Belgica, vol. 68, no. 2, pp. 101–106, 2013.

[43] A. P. Oliveira-Arbez, E. B. David, and S. Guimaraes, “Blastocystis genetic diversity among children of low-income daycare center in Southeastern Brazil,” Infection, Genetics and Evolution, vol. 57, pp. 59–63, 2018.

[44] S. H. F. Hagmann, P. V. Han, W. M. Stauffer et al., “Travel-associated disease among US residents visiting US GeoSentinel clinics after return from international travel,” Journal of Family Practice, vol. 31, no. 6, pp. 678–687, 2014.

[45] S. Bühler, R. Rüegg, R. Steffen, C. Hatz, and V. K. Jaeger, “A profile of travelers - An analysis from a large Swiss travel clinic,” Journal of Travel Medicine, vol. 21, no. 5, pp. 324–331, 2014.

[46] H. S. Cheong, K.-T. Kwon, J.-Y. Rhee et al., “Imported malaria in Korea: a 13-year experience in a single center,” The Korean Journal of Parasitology, vol. 47, no. 3, pp. 299–302, 2009.