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#Blastocystis24

FLASH TALKS ABSTRACT BOOK

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***Blastocystis* carriage and molecular diversity in Zambian asymptomatic schoolchildren**

Mable Mutengo^{1,2}, Alejandro Dashti³, Michaela Kaduková^{3,4}, Namwiinga Mulunda^{1,2}, Stanley Chinyanta², James Mwansa⁵, Sergio Sánchez³, Pamela Carolina Köster^{3,6,7}, Javier Sotillo³, David Carmena^{3,8}

¹University Teaching Hospitals, Lusaka, Zambia. ²Levy Mwanawasa Medical University, Lusaka, Zambia. ³Health Institute Carlos III, Majadahonda, Spain. ⁴University of Veterinary Medicine and Pharmacy in Košice, Košice, Slovakia. ⁵Lusaka Apex Medical University, Lusaka, Zambia. ⁶Alfonso X El Sabio University (UAX), Villanueva de la Cañada, Spain. ⁷Women for Africa Foundation, Madrid, Spain. ⁸CIBER Infectious Diseases (CIBERINFEC), Madrid, Spain

Abstract

Little information is currently available on the presence and molecular diversity of *Blastocystis* sp. in human populations from sub-Saharan African countries. In Zambia, *Blastocystis* carriage and molecular diversity has been only investigated in individuals with clinical manifestations. In this cross-sectional survey, individual stool samples from 256 apparently healthy schoolchildren (median age: 9.0 years; range: 5–18; male/female ratio: 1.1) attending two public schools in the localities of Chongwe ($n = 111$) and Kafue ($n = 145$) were investigated during April–August 2023. Detection of *Blastocystis* was conducted by PCR using pan-*Blastocystis*, barcode primers BhRDr/RD5, and confirmation and subtype (ST) identification by Sanger sequence analyses.

Overall, 45.3% (116/256; 95% CI: 39.3–51.6) of the participating children were *Blastocystis*-positive. Sequence analyses revealed the presence of only three different STs including ST1 (33.6%, 39/116), ST2 (43.1%, 50/116) and ST3 (18.1%, 21/116). Five samples (4.3%, 5/116) corresponded to mixed infections by unknown STs (identified as overlapping chromatograms). A single sample (0.9%, 1/116) was untypable due to low sequence quality. Additionally, 51 samples (19.9%, 51/256) yielded unreadable chromatograms and were conservatively considered negative. Children living in Kafue were more likely to carry *Blastocystis* [$\chi^2(1, n = 256) = 11.61, p = 0.00066$]. Gender, age group, and contact with domestic animals were not significantly associated with an increased likelihood of harbouring *Blastocystis*.

Blastocystis carriage was a common finding in the surveyed Zambian, apparently healthy, schoolchildren population. Absence of animal-adapted subtypes (*e.g.*, ST5–ST8) seems to indicate that *Blastocystis* transmission is primarily anthroponotic in this epidemiological scenario.

***Blastocystis hominis* diagnosis in a low prevalence setting: evaluation of the impact of formalin-free fixative and the number of specimens/episode using a commercial single-vial in a referral laboratory**

Lidia Goterris, Elena Sulleiro

Microgiology Department, Vall d'Hebron Universitary Hospital, Barcelona, Spain

Abstract

Background: Commercial single-vials including fixative have simplified the workflow in the laboratory stool concentration to perform microscopic examination. Recently, new fixatives formalin-free have been appeared. In this study we evaluate the performance of a formalin-free fixative and the number of specimens per episode for *Blastocystis hominis* (BH) diagnosis using commercial single-vials.

Methods: A retrospective longitudinal study was performed at the Microbiology Department of Vall d'Hebron Universitary Hospital (Barcelona) from January 2018 to December 2019. From January-2018 to March-2019 stool samples were collected in formalin-based fixative vials (Midi Parasep® Solvent free) and three specimen/episode were preferred. From April-2019 to December-2019 an alcohol-based formalin free fixative (Midi Parasep® Alcorfix™) was used and one specimen/episode was preferred. Lugol staining was performed for microscopic examination.

Results: 56068 stool specimens concerning 27912 episodes for protozoa investigation were included. BH was detected in 18.8% of episodes (5260/27912).

Irrespective of the number of specimens per episode: comparing the formalin-based periods (January-March), no significant differences on BH detection were observed (21.8% versus 23.7%, $p=0.063$) whereas comparing formalin-based versus formalin-free fixative periods (May-December), a significant decrease was observed (22.4% versus 13.0%, $p=0.000$).

Significant higher BH detection rates were observed in 3-specimen/episode versus 1-specimen/episode (24.3% versus 18.9% with formalin-based; 15.6% versus 11.9% with Alcorfix™). Cumulative percentage of BH detection in formalin-based 3-specimen episodes based on adding 1st, 2nd and 3rd specimens were 22.8%, 23.9%, 24.4% ($p=0.007$).

Conclusions: BH detection was impaired by using Alcorfix™ fixative and by the reduction of the number of specimens per episode.

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Blastocystis hominis frequency: Effects on clinical manifestations and infection outcomes in humans

OLTIANA PETRI¹, Erjona Abazaj², Ridvana Mediu³, Albana Daka¹

¹Cambridge Clinical Laboratories, Tirana, Albania. ²Institute of Public Health, Tirana, Albania.

³Albanian University, Tirana, Albania

Abstract

Background

Lack of consistency in *Blastocystis hominis* protocols in *developing* countries, although *clinical manifestations of Blastocystis spp. are frequently ambiguous*, has resulted in missing and misinterpreted data. Our aim is to assess *B. hominis* prevalence and clinical symptoms in *suspected patients*.

Methods

This is a year-long descriptive study at Cambridge Clinical Laboratories. Stool samples from 842 gastrointestinal suspected patients were microscopically examined using zinc sulphate flotation and iodine-stained preparations.

Results

Out of 842 patients suspected of gastrointestinal parasite, the average resulted 32.5 ± 11.2 with min 3 years and max 72 years old. The prevalence rate for gastrointestinal parasites resulted 37.76% (318/842), where *Giardia lamblia* and *Blastocystis hominis* were the most prevalent parasites (19.6% and 6.17% respectively). Related the gender positivity, male 62.58% (199/318) and female 37.42% (119/318) without significant ($\chi^2=0.83$; $p=0.20$).

Out of 52 *B. hominis* positive patients, 55.77% (29/52) were males, 44.23% (23/52) were female without significant association ($\chi^2=0.71$; $p=0.26$). Patients in age 25-35 years resulted with high prevalence compared to other age groups (41.4%) ($\chi^2=89.2$; $p=0.01$). A significant association was found between the positivity for *B. hominis* and clinical manifestation vis asymptomatic patients for p value < 0.05 . Abdominal pain (31.9%) was the most frequent clinical manifestation, followed by acute diarrhea (23.5%), nausea (11.2%), anorexia (8.4%) and irritable bowel syndrome (5.7%).

Conclusion

Even though *B. hominis* is an underappreciated parasite in Albania, the clinical symptoms and infection outcomes in suspected patients are extremely concerning. To reduce the significant risk of transmission, up-to-date knowledge of laboratory must be applied.

***Blastocystis* infection in Polish Soldiers Stationed in the Republic of Kosovo and Lebanon**

Barbara Pietrzak-Makyla¹, Krzysztof Korzeniewski², Karolina Baranowicz¹, [Anna Lass](#)¹

¹Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdansk, Powstania Styczniowego 9b, 81-519 Gdynia, Poland, Gdańsk, Poland. ²Department of Epidemiology and Tropical Medicine, Military Institute of Medicine—National Research Institute, Szaserów 128, 04-141 Warsaw, Poland, Warsaw, Poland

Abstract

Blastocystis is the most common protozoan found in the intestines of humans and animals. Since soldiers participating in military operations are particularly vulnerable to infection, this study was undertaken to identify and subtype *Blastocystis* sp. in Polish troops participating in peacekeeping missions in the Republic of Kosovo and Lebanon.

Faecal samples were collected from 108 and 192 soldiers stationed respectively in Lebanon and Kosovo in a period of November 2020 – February 2021. Each soldier was tested twice on arrival and after four months of stay. Material was fixed in 70 % ethanol. After DNA extraction, the barcoding region of the small subunit ribosomal RNA (SSU-rRNA) gene was amplified and sequenced using Sanger method.

The DNA of *Blastocystis* was detected in six (3.13%) and three (1.10%) samples in the first batch and in thirty (15.16%) and nine (8.33%) samples in the second batch collected in Kosovo and Lebanon, respectively. Sequencing revealed infections with ST 2, 3, 4, and 7 in Kosovo and ST2 and 3 in Lebanon, with predominance of ST3. The results indicate that the visit to a new environment and prolonged stay in the area of military operations resulted in a significant increase in *Blastocystis* infections in soldiers. ST diversity was greater among soldiers stationed in Kosovo, which may be due to a different organization of bases, including contact with the civilian population and local food.

Financial support: project of the Military Institute of Medicine in Warsaw (No 573) and Polish Ministry of Science and Higher Education (MUG ST 02-0104/772).

***Blastocystis* is very rare in faeces of children with Crohn's disease: quantity, subtyping and association with the faecal bacteriome**

Klára Hubáčková¹, Jakub Hurych¹, Lucie Hlináková¹, Karolína Litošová¹, Tereza Lerchová², Hana Malcová³, Dita Cebecauerová³, Ondřej Hradský², Jiří Bronský², Ondřej Cinek^{1,2}, Jolana Havlová^{2,4}

¹Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University, University Hospital Motol, Prague, Czech Republic. ²Department of Paediatrics, 2nd Faculty of Medicine, Charles University, University Hospital Motol, Prague, Czech Republic. ³Department of Pediatric and Adult Rheumatology, University Hospital Motol, Prague, Czech Republic. ⁴Institute Immunology and Microbiology, First faculty of Medicine, Charles University, Prague, Czech Republic

Abstract

Background. As an indicator of gut health, *Blastocystis* is being studied among multiple diagnoses in adults; however, studies in children are generally lacking. Therefore, we aimed to assess its occurrence and subtypes in a longitudinal set of faecal samples from children with Crohn's disease (CD) and compare them to similar collections from children having other chronic immunopathological diseases without gut pathology (type 1 diabetes [T1D], and juvenile idiopathic arthritis [JIA]), and healthy controls.

Methods. Specific real-time PCR detected and quantified *Blastocystis*, whereas massively parallel amplicon sequencing showed its subtype. The relationship between *Blastocystis* and the bacterial community was assessed by 16S rDNA profiling.

Results. In total, 626 samples were collected from 156 subjects. Subject-wise positivity of *Blastocystis* in CD (3/40, 7.5%) was significantly lower than in either T1D (21/57, 37%), JIA (2/10, 20%), or in healthy controls (14/49, 29%). There was no notable difference in the subtype repertoires among diagnoses. *Blastocystis*-positive samples had higher alpha diversity and slightly yet significantly ($p < 0.001$) different overall community composition across all diagnoses, which was reflected by the differential abundance of several taxa.

Conclusions. *Blastocystis* is very rare among paediatric patients with CD, in contrast to two other chronic immunopathologic childhood diagnoses. Further longitudinal observation will show whether *Blastocystis* might return in patients with well-controlled CD upon ameliorating their bacteriome composition.

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***Blastocystis* occurrence and subtype diversity in wild ungulates in Spain**

Alejandro Dashti¹, Pamela C. Köster¹, Begoña Bailo¹, Ana Sánchez-de-las-Matas¹, Miguel Ángel Habela², Antonio Rivero-Juárez³, Joaquín Vicente⁴, Emmanuel Carrasco⁵, María C. Arnal⁶, Daniel Fernández de Luco⁶, Patrocinio Morrondo⁷, José A. Armenteros⁸, Ana Balseiro^{9,10}, Guillermo A. Cardona¹¹, Carlos Martínez-Carrasco¹², José A. Ortiz¹³, Antonio José Carpio^{14,3}, Rafael Calero-Bernal¹⁵, David González-Barrio¹, David Carmena^{1,16}

¹Health Institute Carlos III, Majadahonda, Spain. ²Extremadura University, Cáceres, Spain.

³University of Córdoba, Córdoba, Spain. ⁴Institute for Game and Wildlife Research, IREC, Ciudad Real, Spain. ⁵Autonomous University of Barcelona, Barcelona, Spain. ⁶University of Zaragoza, Zaragoza, Spain. ⁷University of Santiago de Compostela, Lugo, Spain. ⁸Council of Development, Territory Planning and the Environment of the Principado de Asturias, Oviedo, Spain. ⁹University of León, León, Spain. ¹⁰Mountain Livestock Institute, León, Spain. ¹¹Regional Government of Álava, Vitoria-Gasteiz, Spain. ¹²University of Murcia, Murcia, Spain. ¹³Medianilla S.L., Benalup-Casas Viejas, Spain. ¹⁴Institute for Research on Hunting Resources, Ciudad Real, Spain. ¹⁵Complutense University of Madrid, Madrid, Spain. ¹⁶Center for Biomedical Research Network in Infectious Diseases (CIBERINFEC), Madrid, Spain

Abstract

Blastocystis is a microeukaryote capable of colonising the gastrointestinal tract of humans and animals worldwide. Little information is currently available on the occurrence and molecular diversity of *Blastocystis* in wildlife and the role of free-living host species as sources of environmental contamination and, consequently, potential human infections. The increase of wild ungulate populations across Europe has raised concerns about their role in the spread of infectious diseases, particularly those with zoonotic significance. Faecal samples ($n = 1,059$) from wild ungulate species were collected across Spain during the period 1999–2021). Using conventional PCR and next-generation amplicon sequencing, *Blastocystis* was found in 9.3% (99/1,059; 95% CI: 7.7–11.3) of the samples analysed, including 10.0% (36/360; 95% CI: 7.31–13.5) of wild boars and 9.0% (63/699; 95% CI: 7.1–11.4%) of wild ruminants, respectively. Sixteen *Blastocystis* STs (ST2, ST5, ST10a/b, ST13, ST14, ST15, ST21, ST23, ST24a/b/c, ST25, ST26, ST30, ST31, ST42b, ST43, and ST44) were identified among the surveyed wild ungulate populations, with a higher variability of STs found in wild ruminants than in wild boars. ST5 was found in all *Blastocystis*-positive wild boars, supporting the host preference of this subtype. Mixed infections were found in 17.2% (17/99) of all *Blastocystis*-positive samples. These results improve considerably our current understanding of the *Blastocystis* epidemiology and ST diversity in wild ungulates from Spain, providing molecular-based evidence of i) cross-species transmission and ii) *Blastocystis* ST preference for certain host species.

***Blastocystis*: Its Role in Gastrointestinal Health and Post-Cholecystectomy Colonisation**

Daisy Shaw¹, Charlotte Burford², Abigail McNeill³, Ankur Shah², Anastasios D. Tsaousis¹

¹University of Kent, Canterbury, United Kingdom. ²East Kent Hospitals University NHS Foundation Trust, Canterbury, United Kingdom. ³Kent and Medway Medical School, Canterbury, United Kingdom

Abstract

Blastocystis is the most prevalent eukaryotic microbe in the human gut, colonising between one and two billion people worldwide. Its role in gastrointestinal health remains controversial; it is associated with both symptomatic enteric conditions and a healthy gut microbiome. Previous research has predominantly focused on its presence in individuals with conditions like irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). This study marks the first to investigate the changes in *Blastocystis* colonisation and overall microbiome composition in patients diagnosed with cholelithiasis (gallstones).

We collected at least 75 stool samples from participants before, immediately after, and six months following cholecystectomy. DNA extractions were carried out for 16S-amplicon metagenomic sequencing to monitor microbiome shifts post-surgery. Concurrently, we screened for *Blastocystis* presence, subtyping the positive cases. Metabolomic profiles were assessed using 1D-proton NMR.

Preliminary findings indicate an intriguing pattern: several patients that were *Blastocystis* negative prior to surgery showed colonisation postoperatively. We hypothesise that once we have fully analysed the data, including 16S-amplicon sequencing and metabolomics, a correlation will emerge linking *Blastocystis* colonisation with the restoration of a eubiotic gut microbiome. These results may support the notion that *Blastocystis* is a marker of gastrointestinal health, rather than a contributor to disease.

Clinical scoring of symptoms of chemically induced colitis in experimental rats during colonization of the gut protist *Blastocystis*

Oldřiška Kadlecová¹, Zuzana Lhotská¹, Vincent Billy², Milan Jirků¹, Laura Wegener Parfrey², Mark van der Giezen³, Kateřina Jirků¹

¹Biology Centre CAS, České Budějovice, Czech Republic. ²University of British Columbia, Vancouver, Canada. ³University of Stavanger, Stavanger, Norway

Abstract

Blastocystis, a common intestinal protist in humans and animals, has unclear impacts on health and the microbiome. While sometimes linked to gastrointestinal issues, it often occurs in healthy individuals. This study investigates the effects of *Blastocystis* ST3 colonization on the immune system and gut bacteriome, both alone and with DNBS-induced colitis. Female Wistar rats were inoculated with *Blastocystis* ST3 and colitis was induced at short-term (three weeks) and long-term (three months) intervals. We conducted comprehensive clinical scoring of the health status of rats, assessing weight loss, stool consistency, hematochezia, and post-dissection colon inflammation.

Results showed that short-term colonization did not affect intestinal inflammation, while long-term exposure significantly improved recovery from colitis. This was evidenced by reduced inflammatory markers (TNF α , IL-1 β) and enhanced clinical scores, indicating better overall health and reduced symptoms two days post-induction. Despite initial health declines after colitis induction, long-term colonized rats demonstrated rapid and marked recovery, with significant weight gain, reduced haematochezia, less intestinal inflammation, and increased gut length by the next day.

These findings suggest that long-term *Blastocystis* ST3 colonization may offer protective effects against intestinal inflammation, promoting faster recovery and better health outcomes. These results could potentially apply to humans, who also experience long-term colonization with *Blastocystis*. For further details on the gut bacterial microbiome, see Billy et al. (2021).

Acknowledgment: This project was financially supported by a grant from the Czech Science Foundation (no. 22-04837S) and by the grant from the Research Council of Norway (no. 324516).

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Comparative genotyping of *Blastocystis* infecting cattle and human in the south of Tunisia

Hafidh AKKARI

ENMV, Manouba, Tunisia

Abstract

Background

Blastocystis sp. is currently the most common eukaryotic parasite found in humans and many animals' intestinal tract. Despite its potential public health impact, epidemiological data regarding genotypes of *Blastocystis* infecting cattle and humans in the south of Tunisia.

Methods

A total of 200 human stool samples and 175 cattle stool samples were microscopically examined for *Blastocystis* infection. DNA was extracted from thirty-eight microscopically positive samples (33 humans and 48 cattle). PCR was performed on positive samples targeting the *Blastocystis*-specific SSU rDNA gene. PCR products of eight humans and eleven cattle samples were sequenced and compared with available reference sequences in GenBank by BLAST queries. Genetic diversity was measured for *Blastocystis* subtypes in human and cattle, based on haplotype and nucleotide diversities.

Results

The PCR detected *Blastocystis* in ten humans and twenty-four cattle samples. *Blastocystis* subtypes 1, 2, and 6 were found in humans whereas subtypes 5 and 10 were found in cattle. Subtype (ST) 2 was the most predominant subtypes in humans whereas, in cattle specimens, the ST5 was the most dominant subtype. Based on the *Blastocystis* sequences of SSU rDNA, 78 sites were polymorphic and 74 sites were parsimony informative, resulting in the identification of 15 haplotypes, 12 haplotypes in the cattle and 9 in humans. No haplotype was shared between cattle and human parasites.

Conclusion

Human-derived *Blastocystis* subtypes were different from cattle subtypes in southern Tunisia. Nevertheless, subtype 5 in cattle can be a risk factor for human infection.

Cross-Species detection of select intestinal Pathogenic Parasites Under One Health Approach in Algeria.

Yaseen Majid Salman Al-Adilee^{1,2}, Reghaissia Nassiba³, Laatamna Abdelkarim⁴, Eleni Gentekaki⁵, Anastasios Tsaousis¹

¹University of Kent, Kent, Canterbury, United Kingdom. ²Ninevah University, Ninevah, Iraq.

³University of souk ahras, souk ahras, Algeria. ⁴University of Djelfa, Djelfa, Algeria. ⁵University of Nicosia, Nicosia, Cyprus

Abstract

Cross-Species detection of select intestinal Protists in Algeria

Blastocystis, *Giardia*, *Cryptosporidium*, and *Entamoeba histolytica* are common intestinal eukaryotes of humans and other animals. *Blastocystis* is a parasite of controversial pathogenicity, while the other three are causative agents of diarrhoea, particularly in areas with poor sanitation and hygiene.

This study took place in rural community in Djelfa Province in the center south of Algeria. In total, 208 fecal samples were collected, of which 25 were from humans, and 183 from their livestock (cattle=45, sheep=96, and goat=42). All samples were screened using qPCR. In humans, *Blastocystis* was detected in 31% of the samples, *Giardia* in 16%, while *Entamoeba histolytica* was not detected. In animals, the corresponding rates were 67%, 61%, and 38% for *Blastocystis*, *Cryptosporidium*, and *Giardia*, respectively. Subsequently, for subtyping, nested PCR was used on the positive samples to amplify fragments of the *18S rRNA* and *gp60* of *Cryptosporidium*, the *18S rRNA* of *Blastocystis*, the beta-giardin (*bg*) and triosephosphate isomerase (*tpi*) of *Giardia*. These findings reveal the widespread distribution of these parasites in humans and their livestock. Co-infections of *Blastocystis* and other eukaryotic parasites were also noted.

This study highlights the importance of considering human and animal hosts when studying disease transmission and control strategies between humans and animals.

Detection and molecular characterization of *Blastocystis* species in school children living in Kosovo

Karolina Baranowicz¹, Anna Lass¹, Barbara Pietrzak-Makyla¹, Krzysztof Korzeniewski²

¹Department of Tropical Parasitology, Institute of Maritime and Tropical Medicine in Gdynia, Medical University of Gdańsk, Gdańsk, Poland. ²Department of Epidemiology and Tropical Medicine, Military Institute of Medicine — National Research Institute, Warsaw, Poland

Abstract

Background

Blastocystis is a unicellular anaerobic protozoan commonly found in the digestive tract of both humans and animals. Although it has been linked to different gastrointestinal disorders, the role of *Blastocystis* as a commensal or parasitic factor is not clearly defined. The aim of this study was to estimate occurrence of *Blastocystis* infection among children living in Kosovo.

Material and Methods

In total, 478 faecal samples fixed in 70% ethyl alcohol were collected from school children aged 6-15 in the community of Kačanik, southern part of Kosovo in 2018. The samples were investigated using molecular methods including the amplification of barcoding region of the small subunit ribosomal RNA (SSU-rRNA) gene and Sanger sequencing.

Results

DNA of *Blastocystis* was detected in 126 (26.4%) samples tested. Sequencing of the PCR products revealed infections with ST 3, ST1 and ST2 in 45.7 %, 31.3 % and 22.9 samples tested, respectively.

Conclusions

The determination of the *Blastocystis* ST3 subtype as the most frequently isolated ST in the studied population, is consistent with the literature data. The wide spectrum of animal species that are carriers of the ST3 subtype may suggest zoonotic infections. Molecular studies confirmed high level of contamination of investigated school children with *Blastocystis* that corresponds most probably with poor sanitary conditions.

This study was supported and co-funded by the project of the Military Institute of Medicine in Warsaw (No 573) and by the Ministry of Science and Higher Education in Poland (MUG ST 02-0104/772).

Disentangling the epidemiology of *Blastocystis* in Nicaragua: preliminary data from a cross-sectional study

Jacklyn Comas-Murillo^{1,2}, Mónica Santín³, Jenny G. Maloney³, Pamela C. Köster^{4,5,6}, Sergio Sánchez⁴, Carla Muñoz-Antoli¹, [David Carmena](#)^{4,7}

¹University of Valencia, Valencia, Spain. ²University of Chocó Diego Luis Córdoba, Chocó, Colombia. ³United States Department of Agriculture, Beltsville, USA. ⁴Health Institute Carlos III, Majadahonda, Spain. ⁵Alfonso X El Sabio University, Villanueva de la Cañada, Spain. ⁶Women for Africa Foundation, Madrid, Spain. ⁷CIBER Infectious Diseases (CIBERINFEC), Madrid, Spain

Abstract

Background: The epidemiology of *Blastocystis* in Nicaragua is largely unknown. Molecular-based data are necessary to identify subtypes, which is key to understanding the transmission dynamics of the protist.

Methods: In this cross-sectional survey, individual stool samples from 88 apparently healthy children (median age: 6 years; range: 1–13; male/female ratio: 1.0) residing on a rural Nicaraguan island were collected during January 2023. Initial detection of *Blastocystis* was conducted by PCR. Subtype assignment (including identification of mixed infections) was carried out by next-generation sequencing using Illumina technology.

Results: *Blastocystis* carriage was confirmed in 27.3% (24/88; 95% CI: 18.3–37.8) of the participating children. Sequence analyses identified four known subtypes (ST1, ST2, ST3, and ST7) and a potentially novel subtype. Mono-subtype infections (70.8%; 17/24) were more common than mixed subtype infections (29.2%; 7/24) among *Blastocystis*-positive samples. Mono-infections included ST1 (45.8%, 11/24), ST2 (4.2%, 1/24), and ST3 (20.8%, 5/24). The potentially novel ST was identified in combination with ST2 (12.5%, 3/24) or with ST1+ST2 (4.2%, 1/24). Other coinfections included ST1+ST3 (8.3%, 2/24) and ST3+ST7 (4.2%, 1/24). The dominance of ST1/ST3 coincides with previous data from the Americas region. The finding of avian-adapted ST7 is indicative of a potential avian-human zoonotic transmission event, although lack of prevalence and genotyping data from domestic animals and wildlife precluded us to confirm this hypothesis.

Conclusion: Data indicate that *Blastocystis* is a common finding in Nicaraguan children. More molecular-based studies targeting human, animal, and environmental samples are needed to disentangle the epidemiology of *Blastocystis* in Nicaragua.

Evaluation of the diagnostic performance of microscopy, xenic culturing, and next generation sequencing techniques for the detection of *Blastocystis*

Carolina Hernández-Castro^{1,2}, Miguel Ángel Toro-Londoño¹, Sonia del Pilar Agudelo-López¹, Mónica Santín-Durán³, Sergio Sánchez², David Carmena²

¹Universidad de Antioquia, Medellín, Colombia. ²Health Institute Carlos III, Majadahonda, Spain. ³United States Department of Agriculture., Beltsville, Maryland, USA

Abstract

Background: Microscopy and xenic culturing remain the first-line diagnostic methods for the detection of *Blastocystis* in many clinical laboratories globally. However, highly sensitive PCR-based methods with high throughput and rapid turnaround times are increasingly replacing conventional methods in a cost-effective manner. This study compares the diagnostic performance of microscopy, xenic culturing, and next-generation sequencing (NGS) for the detection of *Blastocystis* in human stool samples.

Methods: *Blastocystis* sp. was investigated in stool samples from patients ($n = 338$) undergoing colorectal cancer screening in Medellín (Colombia) by direct microscopy examination of concentrated material vs. microscopy of xenic culture in Jones medium vs. PCR plus NGS methods. Statistical analyses were conducted to assess the agreement between tests.

Results: *Blastocystis* was identified by direct microscopy examination and concentrated material in 33.1% (112/338; 95% CI: 28.0–38.3), by microscopy of xenic culture in Jones medium in 33.4% (113/338; 95% CI: 28.3–38.6) and by PCR-NGS in 34.6% (117/338; 95% CI: 29.4–40.0). Kappa indexes of 0.967 (direct microscopy vs. xenic culturing), 0.782 (direct microscopy vs. PCR-NGS), and 0.789 (xenic culturing vs. PCR-NGS) confirmed the concordance of the results obtained with the different techniques compared.

Conclusion: Contrary to what might be expected, direct microscopy examination (either of concentrated material or from xenic cultures) performed equally well than PCR-NGS in the detection of *Blastocystis* in stool samples. Such a striking result highlights the importance of having highly trained and experienced personnel in clinical microbiology laboratories where conventional microscopy cannot be replaced by molecular-based techniques.

Frequency and molecular diversity of *Blastocystis* in patients with and without colorectal cancer from Medellín, Colombia

Carolina Hernández-Castro^{1,2}, Miguel Angel Toro-Londoño¹, Sonia del Pilar Agudelo-López¹, Jorge Humberto Botero-Garcés¹, Alonso Martínez¹, Winston Rojas-Montoya¹, Maria Victoria Parra-Marín¹, Santiago Rojas-Restrepo^{3,4}, Luis José Palacios-Fuenmayor⁵, Juan Camilo Correa-Cote^{1,6}, Alejandro Múnera-Duque^{1,6,7}, Mónica Santín-Durán⁸, Sergio Sánchez², David Carmena²

¹Universidad de Antioquia, Medellín, Colombia. ²Health Institute Carlos III, Majadahonda, Spain. ³Hospital San Vicente Fundación, Medellín, Colombia. ⁴Clínica El Rosario, Medellín, Colombia. ⁵Clínica Las Américas AUNA, Medellín, Colombia. ⁶Clínica Medellín Quirónsalud, Medellín, Colombia. ⁷Hospital Alma Máter de Antioquia, Medellín, Colombia. ⁸United States Department of Agriculture, Beltsville, Maryland, USA

Abstract

Background: *Blastocystis* is a major colonizer of the human gastrointestinal tract with a global distribution. Prevalences range from 0.5–24% in developed nations to 50–100% in developing countries. Based on sequence analyses of the small subunit ribosomal RNA (*ssu-rRNA*) gene, at least 40 *Blastocystis* subtypes (STs) are currently considered.

Methods: Stool samples ($n = 338$) were collected from patients with a normal colonoscopy (non-CRC group, $n = 184$) and patients with cancer (CRC group, $n = 154$) in five hospitals in Medellín, Colombia. Initial detection of *Blastocystis* sp. was conducted by *ssu-rRNA* PCR and confirmation and ST identification (including mixed infections) by next-generation sequencing (Illumina).

Results: The frequency of *Blastocystis* sp. was 33.7% (62/184) in the non-CRC group and 35.7% (55/154) in the CRC group. A total of 11 different STs were identified. ST3 was the most common ST (59.0%) followed by ST1 (31.6%), ST2 (27.4%), ST4 (10.3%), ST6, ST7, and ST10a (1.7% each), and ST14, ST30, ST41, and ST44 (0.9% each). There were no statistically significant differences between non-CRC and CRC in relation to specific STs. Mixed infections involving different STs were common (24.8%), with 8 cases being infected by 2 different STs, 5 cases with 3 STs, one case with 4 STs and even one case infected by 6 different STs.

Conclusion: *Blastocystis* infection was highly frequent in both non-CRC and CRC groups and the substantial molecular diversity observed was independent of the clinical status.

Genetic diversity at the *ssu* rDNA gene does not play a role in the pathogenicity of *Blastocystis* infection in clinical patients

Laura Seijas-Pereda^{1,2}, Pamela C. Köster^{3,4,5}, Alejandro Dashti³, Begoña Bailo³, Isabel Guadano-Procesi^{6,3}, Carlos Rescalvo-Casas^{1,2}, Marcos Hernando-Gozalo^{1,2}, Juan Cuadros-González^{1,2}, David Carmena^{3,7}, Ramón Pérez-Tanoira^{1,2}

¹Príncipe de Asturias University Hospital, Alcalá de Henares, Spain. ²University of Alcalá, Alcalá de Henares, Spain. ³Spanish National Centre for Microbiology, Majadahonda, Spain. ⁴Alfonso X El Sabio University (UAX), Villanueva de la Cañada, Spain. ⁵Women for Africa Foundation, Madrid, Spain. ⁶University of “Tor Vergata”, Rome, Italy. ⁷CIBER Infectious Diseases (CIBERINFEC), Madrid, Spain

Abstract

The clinical significance of *Blastocystis* sp. is a question that still needs to be solved. This study assesses whether *Blastocystis* subtype diversity can affect the outcome of the infection and the occurrence of clinical manifestations in infected individuals. Stool samples from 219 *Blastocystis*-positive patients diagnosed by PCR (targeting the *ssu* rDNA gene) were collected from a tertiary public hospital in Madrid (Spain) and fully genotyped by Sanger sequencing analyses. Co-infections by other parasitic, viral, and bacterial enteropathogens were identified by molecular and culture methods. Epidemiological and clinical data from patients was collected. Sequence analyses revealed the presence of six *Blastocystis* subtypes including ST1 (21.5%), ST2 (17.8%), ST3 (29.7%), ST4 (22.8%), ST6 (5.5%), and ST7 (2.3%), with a single sample harbouring a ST1+ST3 co-infection (0.5%). Overall, 81.7% of the patients presented with some gastrointestinal symptom. Multivariate risk factor analyses using logistic regression models indicated that neither *Blastocystis* subtypes nor patient-associated variables including sex, country of origin, travelling history, and presence of nonspecific symptoms were positively associated with a higher likelihood of developing gastrointestinal symptoms (abdominal pain and diarrhoea). However, being of a young age (p -value: 0.003), experiencing skin pruritus (p -value < 0.001) and showing eosinophilia (p -value: 0.016) were found to increase the odds of presenting gastrointestinal symptoms in *Blastocystis* infection. We conclude that the occurrence of gastrointestinal manifestations is independent of the *Blastocystis* subtype involved in the infection, based on variability within the *ssu* rDNA gene.

High prevalence of *Blastocystis* in non-human primates from a zoo in Serbia

Danijela Petrovic¹, Vuk Vračar², Gordana Kozoderović¹, Ivan Galić², Marko Lazić³, Vesna Lalošević²

¹University of Novi Sad, Faculty of Education in Sombor, Sombor, Serbia. ²University of Novi Sad, Faculty of Agriculture, Novi Sad, Serbia. ³Zoo Palić, Palić, Serbia

Abstract

Background: *Blastocystis* is an anaerobic protozoa with controversial pathogenic significance, registered in gastrointestinal tract of both humans and animals. The infection can be self-limited but some studies have shown that in humans *Blastocystis* is related to inflammatory bowel disease, while in animals to diarrhea, abdominal pain, nausea and gastric distension.

Method: To assess the presence of *Blastocystis* sp. we performed xenic *in vitro* cultivation in modified Jones' medium supplemented with 10% horse serum and molecular analysis identifying a specific region of the small subunit ribosomal RNA (SSU rRNA). We collected 17 fecal samples of eight captive non-human primate's species (Barbery macaque, Ring-tailed lemur, Red-bellied lemur, Tufted capucin, Common patas monkey, Chimpanzee, De Brazza's monkey, Common marmoset) in Palić Zoo, located in the north of Serbia. DNA extraction was performed using commercial stool DNA Extraction Kit EURx, Poland. *Blastocystis* sp. was determined through PCR amplification of an approximately 600 bp region of the SSU rRNA gene using the forward RD5 and reverse BhrDr primers. **Results:** Among 17 samples, 6 (35,3%) were positive by xenic *in vitro* cultivation. Molecular analysis showed the high prevalence of *Blastocystis*, 88,2% (15/17) present in different species. **Conclusion:** The two diagnostic methods were in line in 8 samples (6 positive and 2 negative), while 9 samples were only PCR positive. The PCR method showed higher sensitivity when compared with cultivation and should be encouraged in *Blastocystis* diagnosis. The presence of the potential zoonotic *Blastocystis* subtypes could be possible, hence their transmission between non-human primates and humans.

Investigation of Blastocystis Positivity and Subtypes in Stool Samples from Afyonkarahisar Health Sciences University Faculty of Medicine Microbiology Laboratory

Yeliz Cetinkol¹, [Erdogan Malatyali](#)², Savas Aslan¹, Betul Fatmanur Yildirim¹

¹Afyon Karahisar Health Sciences University, Afyon, Turkey. ²Aydin Adnan Menderes University, Aydin, Turkey

Abstract

Background: Despite being a common intestinal protozoan in Turkey, studies on Blastocystis are limited to certain regions. The main objective of this study is to investigate Blastocystis positivity using PCR in samples from a University Faculty of Medicine Microbiology laboratory for routine parasitology examination, and to determine their genotypes.

Methods: The present study included 190 fecal samples and has been supported by Afyon Karahisar Health Sciences University, with ongoing research. Genomic DNA isolation was performed on the samples using a commercial kit, and the barcode region was amplified. The alignment and querying of partial 18S rRNA sequences were submitted to the pubmlst.org database. Demographic characteristics of patients were also analyzed.

Results: Among the patients, 48.1% were male, and the majority were under 18 years old. Additionally, it was observed that patients from the city center accounted for the highest proportion of all. Blastocystis positivity was found to be 4.2% with PCR, with three subtypes detected: ST3, ST2, and ST1. There was a predominance of samples from pediatric services.

Conclusions: The findings of this project are consistent with others in our country. The forthcoming findings will contribute to a clearer understanding of Blastocystis in this region. The low rate of Blastocystis in children age group was also needs more attention.

The study was supported by AFSU Scientific Research Commission with project id:23genel004.

Non-Symptomatic Children Harbor Blastocystis and Dientamoeba fragilis More: Outcomes of a Field Study from Nepal

Tuana Tabak¹, Aral Sürmeli¹, Yeşim Yasin², Özgür Kurt³

¹Acibadem University School of Medicine, Istanbul, Turkey. ²Acibadem University School of Medicine Dept. of Public Health, Istanbul, Turkey. ³Acibadem University School of Medicine Dept. of Medical Microbiology, Istanbul, Turkey

Abstract

Background: Blastocystis spp. and Dientamoeba fragilis are blamed for infections in humans, while application of PCR indicated high prevalence rates for Dientamoeba fragilis (43% in Denmark) and Blastocystis (100% in Senegalese children) in healthy individuals, which questions their roles in human health. Here, we present the related outcomes of our stool examinations of children during the field studies in rural Nepal between 2013 and 2015.

Material/methods: Stools of 203 children aged 2-15 years were collected between 2013-2015 in rural Nepal. Routine O&P examination and Kinyoun staining were done for all samples kept and transported in fixative solutions, while Dientamoeba fragilis was sought using Real-Time PCR, as described (Verweij et al., 2007). A detailed inquiry form was filled in by the voluntary staff having questions on both personal information and physical examination.

Results: O&P examinations indicated Blastocystis in 58 (28.6%) of 203 children. Adequate DNA was extracted from 153 children stools and D. fragilis DNA was identified in 110 (71.9%). Other identified intestinal parasites were Entamoeba histolytica/dispar (n=38), Giardia lamblia (n=34) Cryptosporidium spp (n=24), Cyclospora cayetanensis (n=14) and Hymenolepis nana (n=3). It was noted that D. fragilis and Blastocystis-positive children were predominantly non-symptomatic.

Conclusions: Children in developing countries are under the threat of parasitic diseases that may disturb their physical and mental development. Therefore, public health measures are required to improve infrastructure and life standards of citizens. Both D. fragilis and Blastocystis-positive children were mostly non-symptomatic during the study; this may contribute to the discussions on their roles in human health.

Occurrence of *Blastocystis* subtype ST1 in primates in ZOO Košice, Slovakia

Michaela Kaduková, Andrea Schreiberová, Gabriela Štrkolcová

University of veterinary medicine and pharmacy in Košice, Košice, Slovakia

Abstract

Blastocystis sp. poses a public health problem due to its global distribution among many animal hosts, including humans. This study aimed to investigate the subtype diversity of *Blastocystis* spp. in the Košice ZOO. Fecal samples were collected from 23 animals (5 brown bears, 2 pandas, 7 leopards, 2 lemurs, and 7 primates) showing no clinical symptoms. In addition to conventional parasitological examination using the flotation method, molecular characterization of *Blastocystis* sp. was conducted using PCR method. DNA amplification was carried out using the small subunit ribosomal RNA gene (18S rRNA gene) and forward and reverse primers (RD5/BhRDr).

Overall, *Blastocystis* sp. was detected in only 2 primates out of 23 samples. Sanger sequencing analysis revealed 100 % similarity with subtype ST1. Furthermore, co-infection with *Trichuris* spp. was confirmed in these two primates using the flotation method.

Primates serve as significant hosts for *Blastocystis* sp., indicating the importance of screening measures. Given the potential for direct contact between zoo personnel, veterinarians, and zoo visitors, there is an increased risk of *Blastocystis* sp. transmission. Therefore, regular screening of primates in captive settings is recommended to mitigate transmission risks.

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Occurrence of *Blastocystis* sp. in a Gut-Healthy Human Population and Their Animals in the Czech Republic and its impact on the gut microbiota.

Zuzana Pavlíčková¹, Barbora Pafčo², Ilík Vladislav², Lee O'Brien Andersen³, Milan Jirků¹, Kristýna Brožová¹, Oldřiška Kadlecová¹, Christen Rune Stensvold³, Kateřina Jirků¹

¹Institute of Parasitology, Biology Centre, the Czech Academy of Sciences, České Budějovice, Czech Republic. ²Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic. ³Department of Bacteria, Parasites and Fungi, Statens Serum Institut, Copenhagen, Denmark

Abstract

Blastocystis sp. is a widespread intestinal protist and its prevalence in humans varies between low- and high-income countries. Its role in the human gut ecosystem is still unclear as knowledge about its epidemiology and the factors influencing intestinal colonization is incomplete. To date, very few studies have addressed the question of whether there is a link between the incidence of *Blastocystis* in humans in high-income countries and contact with pets and farm animals. In this study, we also focused on zoonotic potential of *Blastocystis* and whether its presence and other factors have an impact on the composition of the gut microbiota.

This study provides data on the prevalence and subtype diversity of *Blastocystis* sp. in a gut-healthy human population and in their pet or farm animals. A total of 288 stool samples were obtained from asymptomatic individuals across the wide age-range and 136 samples from animals. *Blastocystis* was detected by PCR and its subtypes determined based on the obtained sequences and phylogenetic analyzes. In humans, the prevalence was 24% and eight subtypes were found; in animals, the prevalence was 10%, and only five subtypes were detected. A higher incidence of *Blastocystis* was observed in people with frequent contact with farm animals and in travelers outside Europe. We also analyzed the microbial diversity of selected samples. Our results show that living in a village and the presence or absence of *Blastocystis* has an impact on the composition of the gut microbiota.

Presence of *Blastocystis* in pre-washed vegetables from a supermarket in South East England

Daphne Mavrides¹, Sadiya Maxamhud², Aisha Jamo Suleiman², Eleni Gentekaki¹, Anastasios Tsaousis²

¹University of Nicosia Veterinary School, Nicosia, Cyprus. ²University of Kent, Canterbury, United Kingdom

Abstract

Blastocystis is a widespread protist which colonises the gut of various species including humans, wildlife, livestock, and companion animals. In humans it is the most prevalent eukaryotic microbe of the gastrointestinal tract and has been found in individuals with symptomatic and asymptomatic infections. *Blastocystis* has been shown to contaminate various leafy vegetables that are destined for human consumption worldwide, however studies assessing the presence of this organism in pre-washed and ready-to-eat vegetables (RTE) are limited. Our study aim was to assess the presence of *Blastocystis* contamination in RTE from different supermarkets in the UK. Between May 2023 and July 2023, a total of 36 RTE were purchased from four major supermarkets in Canterbury, UK. Following DNA extraction, 24 out of the 36 samples had sufficient DNA. These samples underwent nested PCR to amplify the *Blastocystis* small subunit (SSU) rRNA gene sequence and gel extraction of the amplified products was subsequently carried out and sent for Sanger sequencing. Twenty one percent (5/24) of the RTE samples were PCR-positive and sequence positive for *Blastocystis*. Two of the positive samples were identified to be subtype (ST) 1 and three were identified as ST2. None of the samples were positive for both subtypes. All positive samples originated from one supermarket. This study underscores the importance of RTE vegetables as a potential vehicle for *Blastocystis* transmission to humans, highlighting the need for targeted interventions to identify contamination sources and develop effective prevention strategies.

Prevalence of intestinal parasites with a focus on molecular identification of *Giardia duodenalis*, *Blastocystis* sp., and *Cryptosporidium* spp. in children hospitalised at the Children's Faculty Hospital in Košice

Gabriela Štrkolcová¹, Michaela Kaduková¹, Andrea Schreiberová¹, Miroslava Petrášová², Daniela Fiľakovská Bobáková²

¹University of veterinary medicine and pharmacy in Košice, Košice, Slovakia. ²PJ Safarik University, Košice, Slovakia

Abstract

Intestinal parasitic infections are prevalent worldwide and have been identified as one of the most significant causes of diseases in human populations, particularly in children. Several studies concerning the detection of intestinal protozoa and helminths in the human population have been conducted in Slovakia; however, no survey of the occurrence of *Blastocystis* sp. in humans has been carried out so far.

The present research focused mainly on the detection of *Blastocystis* sp., as well as other protozoan species *Giardia duodenalis* and *Cryptosporidium* spp. in children hospitalised at the Children's Faculty Hospital in Košice. A total of 76 faecal samples were collected from symptomatic and asymptomatic children. Detection of *Blastocystis* sp. was performed using PCR with the SSU rRNA gene and RD5/BhRDr primers with negative results so far. The samples were examined for the presence of *Giardia* cysts using the flotation method, while the molecular detection of *G. duodenalis* was carried out with the use of the *bg*, *tpi*, *gdh* genes. Out of 14 samples, 7 were identified as assemblage A and 5 as assemblage B; mixed assemblages (A,F) and (B, F) were also identified. The ELISA test was used for proof of the coproantigen in the detection of *Cryptosporidium* spp. from faecal specimens, with negative results (VEGA No. 1/0709/23 and APVV-19-0493).

Our preliminary results indicate a higher prevalence of *G. duodenalis* in the studied population of children compared to other protozoan species. As the study is still ongoing, possible occurrence of *Cryptosporidium* spp. and *Blastocystis* sp. cannot be ruled out.

023

Primer spacers (staggering) improve the performance of *Blastocystis* subtyping using the massively parallel amplicon sequencing of the informative 18S rDNA region

Ondrej Cinek, Klara Hubackova, [Karolina Litosova](#), Jolana Havlova

Department of Medical Microbiology, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic

Abstract

The subtypes of *Blastocystis* can be efficiently determined using massively parallel amplicon sequencing as specified by Maloney et al [Infect Genet Evol. 2019;73:119-25], i.e. with primers originally developed by Santin et al [Parasitol Res. 2011;109:205-12] extended with Illumina Nextera tails. However, the amplicons exhibit high similarity at numerous locations. This homogeneity presents a challenge to the acquisition of signal on the Illumina MiSeq platform that fails to provide quality sequencing signal unless a very large concentration of the *PhiX* heterogeneity library is added.

A systematic, algorithm-driven approach was therefore employed to design heterogeneity spacers for primers. These were tested *in silico* and *in vitro* in marker gene profiling applications, including *Blastocystis* subtyping. We aimed to design a primer set that would produce no more than 60% dominant base in any sequencing cycle.

Additional bases (spacers) were inserted into the first-round amplification primer construct between the binding site of the Illumina sequencing primer and the target-specific portion. The aforementioned primers were then used similarly to their non-staggered counterparts. Of note, the polymerase chain reaction (PCR) was conducted as a single multiplexed reaction per sample. A simple script was used to remove the heterogeneity spacers from the reads, and to verify that the primer combinations were equally represented. A total of sixteen staggered primer variants were designed for bi-directional amplicon profiling of the 18S rDNA region informative for *Blastocystis* subtypes. The efficacy of our design can be evidenced by an increase in the heterogeneity of the sequencing signal, and the increase in the effective sequencing capacity.

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The link between parasitic infection *Blastocystis hominis* and *Helicobacter pylori*: A descriptive study

Erjona Abazaj¹, Oltiana Petri², Ridvana Mediu^{3,4}

¹Institute of Public Health,, Tirana, Albania. ²Cambridge Laboratory, Tirana, Albania. ³Albania University, Tirana, Albania. ⁴LOGOS University College, Tirana, Albania

Abstract

Background: Some researchers have reported the attendant link between parasitic infections including *Blastocystis hominis* (*B. hominis*) and *Helicobacter pylori* (*H. pylori*) infection. The study aimed to evaluate the frequency of intestinal parasites (*B.hominis* and *H. pylori*) and associated clinical symptoms in human faecal samples.

Methods: This cross-sectional study occurred during the periods January 2023 to March 2024. Approximately 258 adult faecal samples with gastrointestinal concerns, for more than 6 months were examined in the parasitology laboratory for parasites (with Formalin-ether sedimentation by microscopy) and *H. pylori* (Stool Antigen Test).

Results: Patients belong to both sexes, ranging in age from 25 to 75 years. Parasitic infection was found in 33.7% of patients, with *Giardia lamblia* and *B. hominis* being the most often discovered species in 19.8% (51/258) and 5.8% (15/258), respectively. Among *H. pylori* positives, 23.1% (31/134) had a co-infection, and *B.hominis-H. pylori* was the most common coinfection followed by *G. lamblia-H. pylori*. Based on the clinical complaints of patients, the positive rate for *H. pylori* was 51.9% (134/258). Clinical complaints were associated with the disease (p-value < 0.05), where irritable bowel syndrome (25.2%) and gastric reflux resulted as the most common complaints followed by abdominal discomfort (19.37%), diarrhea (8.5%), anorexia (3.1%) and nausea (2.7%).

Conclusion: Our data showed a link between *H. pylori* and *B. hominis*. Given that the evidence is still uncertain as to how *H. pylori* promote intestinal parasitosis or inversely, caution should be exercised when we screen the faeces for parasitic diseases. More research is needed, specifically to determine the link with intestinal microbial ecosystems.

Zoonotic intestinal protists: A cross-sectional survey of non-human primates and caregivers in Zoos

Kristýna Brožová^{1,2}, Anna Šejnohová^{1,2}, Monika Koutenská^{1,2}, Zuzana Lhotská¹, Milan Jirků¹, Oldřiška Kadlecová¹, Ondřej Cinek³, Klára J Petřelková^{1,4}, Kateřina Jirků¹

¹Institute of Parasitology, Biology Centre, the Czech Academy of Sciences, České Budějovice, Czech Republic. ²Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic. ³Department of Medical Microbiology, Charles University and Motol University Hospital, Praha, Czech Republic. ⁴Institute of Vertebrate Biology, Czech Academy of Sciences, Brno, Czech Republic

Abstract

We investigated the prevalence, genetic diversity and potential zoonotic transmission of two intestinal protists - *Blastocystis* sp. and *Giardia intestinalis* - in 37 species of non-human primates (NHPs) and their caregivers in six zoos in the Czech Republic. We analyzed 179 fecal samples (159 from NHPs, 20 from humans) by qPCR. The overall prevalence of *Blastocystis* sp. detected using qPCR in caregivers was 35% (7/20) and in the NHP hosts 57% (91/159). The prevalence of *Giardia intestinalis* was 30% (6/20) in caregivers and 47% in the NHP hosts (74/159). Using next generation sequencing we identified a range of *Blastocystis* subtypes (ST1-ST5, ST7, ST8) of which some were shared between NHPs and their caregivers, suggesting possible zoonotic transmission. In the case of *Giardia*, we found assemblages A and B. Our study emphasizes the critical role of molecular diagnostics and calls for further research on the epidemiology and transmission of these protists to better understand their public health impact.



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