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Blastocystis Colonization and Gut Microbiota Composition in a Rural Colombian Community

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Abstract

Background: *Blastocystis*, a common intestinal protozoan, has been associated with specific gut microbiota signatures. However, the extent and specific shifts in microbiota composition and structure, particularly in rural populations, remain unclear.

Methods: Microbiota structure and composition were evaluated in *Blastocystis* carriers and non-carriers from a rural Colombian community (n=99 human samples). The experimental procedures involved microscopy and molecular techniques (e.g., qPCR) for protozoa detection. *Blastocystis* subtyping and gut microbiota was characterized by Illumina sequencing of 16S and 18S rRNA genes, to profile prokaryotic and eukaryotic microbiota. The resulting sequences were analyzed using a comprehensive bioinformatic pipeline.

Results: A high prevalence of *Blastocystis* (90%) was observed, with both monoinfections and co-infections with other parasites. Subtyping revealed a diverse array of *Blastocystis* subtypes and coexistence patterns, with ST2 being the most prevalent ST. At the bacterial genus level, *Blastocystis* carriers were enriched in *Prevotella*, *Ruminococcus*, *Blautia*, *Clostridium*, and *Oscillibacter*, whereas non-carriers were enriched in *Escherichia*. Eukaryotic enrichments included *Entamoeba* and *Hanseniaspora* in carriers, and *Ancylostoma*, *Malassezia*, *Candida*, and *Saccharomyces* in non-carriers.

Conclusions: This study supports a relationship between *Blastocystis* colonization and specific gut microbiota signatures in a rural population. This study is one of the few studies to characterize prokaryotic and eukaryotic microbiota members concurrently. Intriguingly, gut microbiota in *Blastocystis*-carriers Colombians from rural populations mirrored Western Europeans, despite vastly different environments and diets. This suggests that *Blastocystis* may strongly influence gut microbiota structure. Further studies with broader populations and longitudinal designs are required to confirm this potential modulatory role.

Uncovering *Blastocystis* subtype diversity in Portuguese ungulates: first insights into subtype specificity and cross-transmission patterns

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Abstract

Wild ungulate populations are expanding their geographic range across Europe, fostering interactions with domestic species and prompting the spread of infectious diseases, particularly those with zoonotic significance. Despite the increasing understanding of the faecal-oral transmitted protist *Blastocystis*, there is still a considerable gap concerning its epidemiology in free-ranging wild ungulate and possible transmission patterns with domestic hosts, especially with those sympatric domestic hosts raised in extensive grazing systems. Thus, we collected a total of 413 faecal samples from three widespread wild ungulates (wild boar, red deer, and roe deer) and domestic ungulates (cattle, goats, horses, and sheep) in Portugal to investigate *Blastocystis* occurrence and subtype (ST) diversity using PCR and next-generation amplicon sequencing. *Blastocystis* was present in 38.3% (158/413) of the total analysed samples (goats, 81.0%; sheep, 60.9%; red deer, 40.4%; wild boar, 34.3%; roe deer, 32.5%; cattle 32.2%), while none of the horse samples were *Blastocystis*-positive. Nineteen STs were identified, including ST1-ST3, ST5-ST7, ST10, ST13, ST14, ST21, ST23-ST26, ST30, ST31 and ST42-ST44. Mixed ST infections were the norm in domestic ungulates *Blastocystis*-positive samples (97.3%), while less common in the wild ungulates (39.0%). Our results demonstrate that Portuguese wild and domestic ungulates harbour a great diversity of *Blastocystis* STs, including STs with zoonotic potential, highlighting their role in the maintenance of the sylvatic-domestic cycle of this parasite and the associated public health risks. Furthermore, our data provide novel molecular evidence indicating that certain *Blastocystis* STs might exhibit distinctive host preferences.

Spatial and genetic diversity of *Blastocystis* sp. in Italy: a network analysis

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Abstract

The ubiquitous protist *Blastocystis* sp. can colonize the gastrointestinal tracts of humans and non-human hosts. Due to the apparent variable host specificity in different *Blastocystis* subtypes (STs), assessments of incidence and subtype diversity in specific geographic areas are significant to better understand *Blastocystis* zoonotic potential. We reported the pattern of genetic relationship analysis among different haplotypes of *Blastocystis* STs to investigate spatial and genetic diversity of this protist in Italy.
Isolates from 55 *Blastocystis*-positive samples (Azienda Ospedaliera Universitaria Policlinico Tor Vergata, detected by Allplex^{TM} Gastrointestinal Parasite Panel Assay) were genotyped by Sanger sequencing analysis (ssu rDNA gene), checked in PubMLST for allele attribution, aligned, and analyzed through phylogenetic analysis. To these, all clinical sequences available from Italy were added, and a total of 107 isolates was used to perform the Minimum-Spanning network calculation through a haplotype analysis on polymorphic sites.
The 55 new isolates were assigned as follows: 9 to ST1 (16.4%), 7 to ST2 (12.7%), 11 to ST3 (20%), 26 to ST4 (47.3%) 1 to ST6 (1.8%) and 1 to ST7 (1.8%). From the alleles analysis, 8 different variants were detected : allele 4 (ST1), alleles 9 and 12 (ST2), alleles 34 and 36 (ST3), allele 42 (ST4), allele 123 (ST6) and allele 137 (ST7). Forty-six haplotypes (hp) were identified across the 107 isolates, with hp 41 (29.9%) and hp 34 (15.9%) as the most representative. No spatial segregation has been observed.
The present work represents a further local contribution to the knowledge of *Blastocystis* sp. genetic diversity.

Occurrence and molecular characterization of *Blastocystis* spp. in humans and dogs in Slovenia

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Abstract

The occurrence of *Blastocystis* in humans and animals varies greatly from country to country, with little information on its presence and genetic diversity in Slovenia.

To determine the occurrence of *Blastocystis* spp. in patients with diarrhoea, we analysed faecal samples from 249 patients who had been tested for gastrointestinal pathogens in January and February 2023. To determine possible differences in the occurrence of *Blastocystis* spp. in sick and healthy populations of children, 379 DNA extracts from faecal specimens of the same number of children aged 0 to 6 years collected between October 2011 and October 2012 were analysed. Of the 397 children, 292 had diarrhoea and 87 represented a healthy control group without diarrhoea. The occurrence of *Blastocystis* spp. in dogs was determined by analysing 79 faecal samples from dogs with diarrhoea. The faecal samples were tested for the presence of *Blastocystis* spp. using *Taq*-man-based real-time PCR targeting the SSU rRNA gene, and the positive samples were sequenced using barcoding primers to determine the subtypes. Of the 249 patients with diarrhoea, 20 (8.0 %) tested positive. Subtypes were successfully determined in 11 of them (ST2-ST4). Of 397 children, nine (3.1 %) children with diarrhoea and six (6.9 %) children in the control group were positive; the difference is not statistically significant (p=0.11). We identified ST2-ST4 in three children with diarrhoea and ST2, ST3 and ST7 in five healthy children. *Blastocystis* spp. was detected in seven (8.9 %) samples from dogs, but the subtypes could not be determined.

Keywords: *Blastocystis*, humans, dogs, occurrence, subtypes

Prevalence and roles of *Blastocystis* sp. and *Dientamoeba fragilis* in a clinical trial involving Faecal Microbiota Transplantation in Irritable Bowel Syndrome

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Abstract

Background: *Blastocystis* sp. and *Dientamoeba fragilis* are intestinal protists often associated with diverse and thus "healthy" gut bacteriome. Therefore, we investigated their prevalence and association with gut bacteriome in patients with Irritable Bowel Syndrome (IBS), a functional disorder with inflammatory signs affecting 10% of the Europid population.

Methodology: Adult patients participated in a randomised, double-blind, cross-over, interventional trial of Faecal Microbiota Transplantation (FMT) for treating IBS. The FMT intervention was mixed microbiota from eight healthy donors; the placebo was the same mixture but autoclaved. Allocation groups varied in the order of FMT received. Specific real-time PCR detected and quantified the protists, and 16S rDNA profiling served for bacteriome analysis.

Results: Stool samples from IBS patients (n=58; median age 35) were analysed. The subject-wise prevalence was 10.3% for *Blastocystis* sp. (6/58) and 13.8% (8/58) for *D. fragilis*. The protists quantity was affected neither by active microbiota nor placebo. Positivity of the protists was marginally influenced by active microbiota (P=0.071) and associated with increased bacteriome diversity on genus level (P<0.044 across multiple indices) and with several bacterial genera (P<0.03) regardless of the intervention status.

Conclusion: The prevalence of *Blastocystis* sp. and *D. fragilis* in IBS patients is low, and their colonisation is unaffected by FMT. As expected, their presence was associated with high bacteriome diversity but was surprisingly inversely associated with some beneficial taxa.

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Frequency of *Blastocystis* in vulnerable Mexican Population and its association with their health status

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Abstract

Blastocystis is considered the main protist that parasitizes the human intestine and a wide range of animals. Although *Blastocystis* has been implicated as a cause of several gastrointestinal tract symptoms, its role as a pathogen remains unclear. Factors that favor the transmission of *Blastocystis* have been established, such as lack of hygiene, consumption of contaminated food and zoonotic contact. But there are also other conditions that must be considered in the transmission of *Blastocystis*, such as the vulnerability of certain groups to becoming infected by *Blastocystis* and its probable impact on their health status. The objective of this work is to determine the frequency of *Blastocystis* infection in vulnerable groups of the Mexican population, schoolchildren from a subrural community in the state of Morelos and patients diagnosed with HIV/AIDS from a public hospital in Mexico City, and to analyze their association with their health status, 294 schoolchildren between 4-15 years old and 163 patients with HIV/AIDS were studied, in whom the presence of *Blastocystis* was determined by PCR (SSU-rDNA-barcoding). To establish the health status of the participants, the following variables were considered: body mass index (BMI) and hemoglobin level of the schoolchildren and the stage of the disease according to the classification of Castro K et al. for patients diagnosed with HIV/AIDS. Concluding, for our studied groups, being infected by *Blastocystis* had no impact on their health status, versus having a pathogenic character; it may be acting as a health indicator. Grants: IN217821, IV200420 and IN219624 from PAPIIT-DGAPA-UNAM.

Relationship Between Blastocystis Infection and Clinical Outcomes: A Comprehensive Systematic Review

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Abstract

Background:

Blastocystis is a common eukaryotic inhabitant of the human gastrointestinal tract, with variable prevalence rates globally. Despite the recent studies that suggest its commensal role, Blastocystis has been related to gastrointestinal symptoms, raising questions about its pathogenic potential. However, there is ongoing debate regarding the clinical significance of Blastocystis. Given the conflicting evidence and lack of consensus on its pathogenicity, there is a critical need for a systematic review to evaluate existing evidence and clarify its relationship with human health outcomes. This protocol aims to lay the foundations of a systematic review that comprehensively assesses the available literature, addressing knowledge gaps and providing insights for clinical practice and public health interventions.

Methods (Protocol):

This systematic review will follow PRISMA guidelines. Research question: "What is the relationship between Blastocystis infection and clinical outcomes in humans, and what evidence exists regarding its pathogenicity?" Include original research in humans, regardless of symptoms, confirming Blastocystis infection. Exclusion criteria: reviews, animal studies, non-original data, and non-English articles. Conduct a comprehensive search of PubMed, Embase, Scopus, Web of Science, and Cochrane Library using relevant terms. Selection: Two reviewers screen titles/abstracts, followed by a full-text review of potentially eligible studies. Any discrepancies will be resolved through discussion and consensus. Data Extraction: Standardized form for study characteristics, participant demographics, diagnostic methods, symptoms, and outcomes. Quality Assessment: Newcastle-Ottawa Scale for observational studies, Cochrane Risk of Bias tool for trials. Synthesis: Narrative synthesis; meta-analysis if possible. Reporting: Adhere to PRISMA. Consultation: Seek stakeholder feedback.

Molecular, structural and functional characterization of the superoxide dismutases of the enteric protozoa *Blastocystis* sp.

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Abstract

As part of the interaction between *Blastocystis* and its intestinal environment, the parasite defense against oxidative stress represents a key step in its survival and colonization. The study of this machinery was initiated by the characterization of the enzymes representing the first defense barrier of the parasite, the superoxide dismutases (SODs). Three SOD genes have been identified in the available *Blastocystis* genomes. SOD1 and SOD2 are dimeric iron-containing SODs (FeSODs) protecting the cytosol and mitochondria, respectively, while SOD3 would be a cytosolic dimeric copper/zinc-containg SOD (Cu/ZnSOD). However, certain residues conserved for all Cu/ZnSODs involved in the binding of cofactor metals are absent for this SOD3. A phylogenetic analysis demonstrated that these SODs would represent excellent molecular markers for studying the evolution of subtypes (STs) within the *Blastocystis* genus. Three-dimensional models of these SODs were built using artificial intelligence AlphaFold2. SOD1 and SOD2 share all characteristics of dimeric FeSODs and would be active whereas the mutations observed for SOD3 would not allow copper fixation as evidenced by ICP-MS. After production of the 3 SODs of *Blastocystis* ST4 in a bacterial expression system, enzymatic activity tests were carried out with the affinity chromatography-purified SODs, showing strong activity of SOD1 and SOD2 and no activity for SOD3. In parallel, overexpression of the *SOD1* and *SOD2* genes was observed in parasite cultures under oxidative stress. Our data suggest that SOD1 and SOD2 play a key role in protecting *Blastocystis* against oxidative stress while the potential role of SOD3 remains unknown.

A preliminary insight in the gut microbiome profile in *Blastocystis*-carrier patients with diarrhoea in Italy

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Abstract

Background and Aim: Gut microbiome (GM) is a diverse and complex ecosystem involved in beneficial physiological functions as well as disease pathogenesis. *Blastocystis* sp. is a common protozoan parasite and is increasingly recognized as an important GM component. Despite recent studies suggesting *Blastocystis* sp. decreases the beneficial bacteria abundance, the understanding of their relationship with other GM communities is still so far. This work aims trying to better understand the role of *Blastocystis* sp. and the interactions with the GM community in a cohort of patients with diarrheal diseases in Italy.

Methods: Over a period 2022-2024, consecutive, non-replicated diarrhoeic faecal samples were subjected to Allplex™ GI Parasite Platform. Furthermore, 16S rRNA gene amplification and sequencing by Oxford Nanopore Technologies platform (EPI2ME software) was performed on a sub-sample of ten *Blastocystis*-carrier patients, homogenous by age and gender, to profile and compare their GM.

Results: Out of 1181 faecal samples, 75 (6.3%) were found positive to *Blastocystis* sp., (73.2% single infection and 26.8% enteric protozoa co-infection). A higher abundant bacterial diversity was recorded for *Streptococcus*, *Bacteroides* and *Oscillibacter* (at genus level) and *Escherichia fergusonii*, *Faecalibacterium prausnitzii* and *Succinivibrio dextrinosolvens* (at species level) were found within our sub-samples and with a significant association with eosinophilia level (p=0.02359).

Conclusions: Our results are not in accordance with previous evidence according to which, *Blastocystis* sp. colonisation would be linked with an eubiotic gut characterized by potentially beneficial species such as *Prevotella* and *Ruminococcus*, Further investigations with larger samples size are needed to better clarify the eubiotic or dysbiotic state of *Blastocystis* sp.

No association between *Blastocystis* carriage and colorectal cancer in a cohort of Colombian patients

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Abstract

Background: *Blastocystis* is a major colonizer of the human gastrointestinal tract whose role as an agent of gastroenteritis remains controversial. However, some studies have suggested its contribution to the severity of multiple conditions, such as AIDs, irritable bowel syndrome, and cancer, in particular colorectal cancer (CRC). CRC is the third most common cancer and the second leading cause of cancer-related deaths worldwide. This study investigates whether *Blastocystis* and other intestinal microorganisms could increase the likelihood of developing CRC.

Methods: Stool samples were collected from patients undergoing CRC screening with a normal colonoscopy (non-CRC group) and patients with cancer (CRC group) at five hospitals in Medellín, Colombia. Informed consents and epidemiologic surveys were collected from all the participants. The presence of intestinal parasitic and bacterial agents was investigated by conventional (microscopy, Jones' medium culture for *Blastocystis*) and molecular (PCR and next-generation sequencing) techniques.

Results: A total of 308 (154 non-CRC, 154 CRC) patients paired for sex and age were included in the study. *Blastocystis* was the most prevalent agent found (111, 36.0%), followed by enteroaggregative *Escherichia coli* (48, 15.6%), diffusely adherent *E. coli* (39, 12.7%), and *Giardia duodenalis* (23, 7.5%). *Blastocystis* was detected at similar proportions in both non-CRC and CRC groups. However, CRC patients were more likely to harbour *Streptococcus gallolyticus* subsp. *pasteurianus* (9.7%, 15/154 vs. 2.6%, 4/154, p = 0.009) and *Morganella morganii* (6.5%, 10/154 vs. 1.3%, 2/154, p = 0.018) than non-CRC patients.

Conclusion: According to our results, *Blastocystis* infection does not predispose to the development of CCR.

From land to sea: The long journey of *Blastocystis* sp.

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Abstract

Blastocystis sp. is frequently identified in humans and several animal hosts and exhibits a large genetic diversity. In the perspective of the One Health approach, data on the distribution of *Blastocystis* sp. and its circulating subtypes from the terrestrial environment are available, while those from the marine environment remain still unknown. In order to better understand the epidemiology of this microorganism across different host species and its potential zoonotic transmission, a large-scale survey was conducted from land to sea by screening faecal samples from humans and four species of free-ranging marine mammals (sperm, fin, long-finned pilot and Cuvier's beaked whales). The samples were collected from Northern to Southern Italy and subjected to real-time Allplex™ GI Parasites Platform and SSU-rDNA gene amplification and sequencing over the period 2022-2024. Out of 1181 human faecal samples, 75 (6.3%) were found positive to *Blastocystis* sp., with ST1 (allele 4), ST2 (alleles 9, 13), ST3 (alleles 34, 36) and ST4 (allele 92) detected, with varying distribution across human samples. Out of 43 marine-mammal faecal samples, 10 (23.2%) were found positive to *Blastocystis* sp. with ST3 the most detected subtype among the different species of marine mammals. Furthermore, all of the ST3 sequences were phylogenetically placed within a unique clade. The present survey provides new insights regarding *Blastocystis* prevalence and its circulating subtypes from the marine environment with a potential zoonotic transmission to human. In addition, these results present the first new hosts record and extend the known host range of this zoonotic protozoan parasite.

From Parasite to Protector? *Blastocystis* and Gut Health: Insights from the DanFunD Study

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Abstract

Blastocystis is one of the world's most successful human parasites and yet the presence of *Blastocystis* may provide a protective effect against the development of intestinal disease, including inflammatory bowel disease (IBD). This project investigates the relationship between *Blastocystis,* gut health, and eubiosis through the analysis of 2,460 participant fecal samples from the Danish epidemiological study of Functional Disorders (DanFunD). DanFunD samples are accompanied by comprehensive epidemiological data, including biochemical analysis of blood and urine, health examination data, diet, and other environmental factors. Fecal samples will be analyzed via 16S/18S small subunit rRNA amplicon sequencing of the V3, V4, and V5 hyper-variable regions, allowing for subtype-level resolution of *Blastocystis* species. Raw sequencing data will be processed using the BION metagenomic pipeline and mapped to the RDP and SILVA reference databases for taxonomic assignment. Results will be paired with the DanFunD's robust epidemiological data creating a comprehensive analysis of the impact of *Blastocystis* on bacterial microbiota, other gut eukaryotes, alpha and beta diversity, and gut health. While this project is ongoing, preliminary results are available for analysis. Expected results include detailed descriptions of: 1) *Blastocystis* prevalence in the Danish population; and 2) the characteristics of *Blastocystis* carriers, including their microbiome profile, clinical parameters, and overall health. Given the statistical power afforded by the sample size and the extensive epidemiological data, this project will help to reshape our understanding of the role *Blastocystis* plays within the human gut and opportunities for treating e.g., IBD.

Genetic diversity of Blastocystis and associated microbiome among patients with immune-mediated inflammatory diseases, Saudi Arabia

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Abstract

Introduction

There is a worldwide unexplained increase in both gut and systemic immune-mediated inflammatory diseases (IMID). This study's goal is to detect the genetic diversity of *Blastocystis* in patients with IMID. Also, to assess the association between *Blastocystis* colonization and patient data, including gut microbiome.

Methodology

Fecal specimens and related data were collected from >2,500 patients attending a university hospital in the Eastern Province of Saudi Arabia. Stool specimens were microscopically examined for pathogens and cultured for *Blastocystis*. *Blastocystis* and protozoal DNAs were amplified using PCR assays, followed by genotyping. Obtained DNA products are in the process of being amplicon sequenced for gut bacteria, targeting the 16S rRNA gene, and gut eukaryotes (fungi), targeting the ITS sequence.

Results

Blastocystis species were the most prevailing pathogen of studied populations, and much more prevalent in healthy individuals than patients with IMIDs. Other parasitic infections were sporadic among the study population. Both IMIDs and *Blastocystis* distribution showed patterns among different age groups and sex. Data from microbiome analysis and its relation to the occurrence of *Blastocystis* will be presented.

Discussion

To our knowledge, this is the first extensive study in Saudi Arabia identifying *Blastocystis* species in IMIDs. There is a high prevalence of IMIDs and *Blastocystis* infection rate among study populations. *Blastocystis* was more predominant in healthy individuals than in patients with GIT disorders. Pathogenicity may be related to *Blastocystis* subtypes. Further genetic studies to assess *Blastocystis* distribution and role in health and disease will provide a better understanding of *Blastocystis* transmission dynamics.

Application of the anti-amoebic methodology in drug discovery against Blastocystis

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Abstract

One of our main research lines is the drug discovery against various protozoal organisms including pathogenic Free Living Amoebae (FLA) genus. FLA are ubiquitous protozoa parasite widely distributed in the environment, including some that can cause opportunistic infections in humans and other animals. Among the known FLA genus, *Acanthamoeba* genus was the most isolated amoeba from environmental habitats and clinical cases.

As a member of a research group with a long track record in the study of free-living amoebae including drug discovery. Our main objective is to apply our knowledge in anti-amoebic field towards *Blastocystis* spp. Although, the life cycle of this unicellular parasite is more complex and still not well defined, the existence of an amoeboid form would allow us to study the *in vitro* activity using the same methodology as FLA. Still, we need to understand much better the life cycle of this protozoa parasite to be able to optimize the in vitro activity.

Investigating parasitic diseases in Iraq under One Health Perspective

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Abstract

This study investigates the prevalence of *Cryptosporidium*, *Blastocystis*, *Giardia*, and *Entamoeba histolytica* in humans, animals, and the environment in a rural area in Iraq.

In total, 140 samples were collected from a village near the Iraqi-Irani border of which 50 were from humans, 50 from their animals (35 sheep and 15 goats), 20 from soil, and another 20 from water. Microscopic examination of human stool revealed infection rates of 12% in the case of *Cryptosporidium*, 16% for *Blastocystis,* and 10% for *Giardia*. In animals, the rates were *Cryptosporidium* 26%, *Blastocystis* 78%, and *Giardia* 8%. Soil samples showed *Cryptosporidium* 5%, *Blastocystis* 45%, while 15% of water samples were positive for *Cryptosporidium* and 5% for *Blastocystis*. Polymerase chain reaction (PCR) was used on all samples to amplify the *18S rRNA* gene and 60-kDa glycoprotein gene (*gp60*) of *Cryptosporidium*, the *18S rRNA* gene of *Blastocystis* and the beta-giardin (*bg*) and triosephosphate isomerase (*tpi*) of *Giardia.* Using qPCR of the bg gene, 30% of human samples were positive for *Giardia*, 14% of animals, and 2% of soil. *Entamoeba histolytica* was not detected in any of the samples. Sanger sequencing has so far revealed that 2% of sheep were infected with *Cryptosporidium ubiquitum*, while *Cryptosporidium parvum* was found in 16% of humans and 2% of animals all of which were sheep. *Blastocystis* subtype (ST)1 was found in humans, whereas ST4 and ST10 were detected in sheep. For *Giardia intestinalis*, only one type was found in sheep. The presence of *C. parvum* in humans and animals living in the same community suggests zoonosis.

Meta-Analysis of Blastocystis Subtype Distribution and Prevalence Across Hosts and Geographic regions: Advancing Knowledge Through Interdisciplinary Collaboration and Data Visualisation Abi Girl Sanda, Eleni Gentekaki and Anastasios D. Tsaousis

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Abstract

Blastocystis is a controversial parasite found in the intestinal tract in both human and non-human hosts. The diversity of *Blastocystis* has allowed for scientists to classify numerous subtypes (STs) over the past few years. Interdisciplinary studies have significantly advanced our understanding of the parasite, elucidating some of its behavioural patterns and genetic characteristics. However, despite the studies constantly referencing each other, over the last decade there has not been a single study focusing on all the STs identified by data already submitted, providing a comprehensive understanding of their prevalence and distribution across countries and hosts. This research is a meta-analysis of 18S rRNA sequences submitted to GenBank, which will provide a study where scientists interested in Blastocystis can access previous data categorised based on the STs identified, the country/continent the data was collected from, the host the parasite as found in and access numbers in case further details are needed from the original database (such as full sequences). The second aim is to provide visual representations of trends and patterns detected using data analysis software such as RStudio and Python, allowing the easy recognition of subtype prevalence patterns in each country already studied. As of yet, the analysis of the data and graphs generated have allowed the recognition of patterns that suggest host specificity in ST prevalence. It is hoped that the introduction of statistical analysis (Bayesian statistics) will not only further explain the relationship between ST prevalence, host and country, but also allow the prediction of future infection patterns.

Molecular Characterization of Blastocystis from Human, Animal And Environmental Samples from Turkiye

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Abstract

Blastocystis is a protist of controversial pathogenicity commonly found in the gastrointestinal tract of a variety of vertebrate hosts, including humans and other mammals, as well as birds, reptiles, fish and insects. Despite intense research efforts, our understanding of its biology and transmission dynamics remains incomplete. Previous studies in Colombia and Thailand indicated circulation of the organism between vertebrate hosts and the environment. In this study, we undertake a One Health approach to investigate the transmission dynamics of Blastocystis between human-animal and environmental ecosystems in a rural area in Turkiye. The study area comprised of a settlement near the Seyhan Dam Lake of Kırıklı village, Adana Province. A total of 429 stool samples were collected, of which 124 were from humans, 151 from sheep, 89 from cattle and 65 from goats. Forty dam water samples were also collected. All samples were screened for *Blastocystis* using real-time PCR (RT-PCR). The prevalence of *Blastocystis* was found to be 89,5% (111/124) in humans and 100% in cattle (89/89), sheep (151/151), goats (64/64) and also dam water materials (40/40). Subtype analysis is ongoing and will be discussed. This research highlights the significance of a One Health viewpoint in uncovering the transmission dynamics of *Blastocystis.*

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Blastocystis in cattle: a providential game changer to limit global warming

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Abstract

Blastocystis, the most prevalent protozoan in human stools, is increasingly recognized as a commensal member of our gut microbiome, being associated with hallmarks of a healthy gut, like an increased gut bacterial diversity in asymptomatic individuals. In this study, we focused on the impact of *Blastocystis* in dairy cattle. Thanks to a collaboration with a company specializing in livestock genomic selection, we had access to a large cohort of 1,581 fecal samples from non-pregnant, non-diarrheic Prim'Holstein dairy cows bred in 20 commercial herds from the North of France. The cows were sampled between 2017 and 2018 but their zootechnical performances were surveyed until the end of their dairy production career. Fecal samples were tested for the presence of *Blastocystis* and their associated fecal bacterial microbiota. We found a prevalence of *Blastocystis* of 54.8 % in the dairy cows. As in humans, we demonstrated that bacterial alpha-diversity was higher in the fecal microbiota of *Blastocystis* colonized cows than in cows without it. Also, by studying heifers specifically, we found that the presence of *Blastocystis* at the heifer stage was associated with a 22% higher productive longevity without compromising the daily milk yield and a 17.5 % lower carbon contribution per kg of milk produced, than in *Blastocystis*-free dairy cows. Thus, we were able to conclude that *Blastocystis* was an early indicator for productive longevity in dairy cows and a game changer to limit cattle CH4 emissions. This result became an invention, which we protected with a European patent, currently under PCT.

Worldwide presence of *Blastocystis* in the human gut microbiome is linked to healthier diets and more favorable cardiometabolic outcomes.

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Abstract

The human gut microbiome is a diverse community of microorganisms that inhabit the human gastrointestinal tract and influence host physiology. Shotgun metagenomic sequencing enables high-resolution profiling of all the members of this community. While the bacterial fraction has been extensively investigated in relation to human health, organisms from other domains are currently underexplored. *Blastocystis* is a micro-eukaryote that has shown contrasting associations with both gastrointestinal symptoms and favorable cardiometabolic profiles. To investigate *Blastocystis*' role in host health, we performed a computational analysis of over 60,000 metagenomic samples. We observed a widespread presence of *Blastocystis* among healthy individuals, with prevalence variability linked to host age, lifestyle, and geographical provenance. We found that individuals consuming healthier diets rich in plant-based and unprocessed foods were more likely to be *Blastocystis*-positive. Furthermore, *Blastocystis* presence was associated with more favorable short-term cardiometabolic markers, such as lower GlycA and higher HDL levels. Meta-analyses revealed a robust association between *Blastocystis* carriage and lower BMI values, as well as reduced risk for disorders linked to altered gut ecology. Moreover, in a diet intervention study of 1,124 individuals, we observed that improvements in dietary quality were linked to weight reductions and increases in *Blastocystis* prevalence and abundance. Finally, machine learning models accurately predicted *Blastocystis* presence and revealed a common signature of previously undescribed microbiome species across datasets. Overall, our results suggest a potentially beneficial role for gut *Blastocystis*.

Further genomic and functional analyses are needed to characterize different *Blastocystis* subtypes and to elucidate acquisition and transmission routes.

Blastocystis and the gut microbiome: metagenomic and metatranscriptomic study of Blastocystis-colonized rat gut

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Abstract

Blastocystis hominis, an anaerobic microbial eukaryote of the lineage Stramenopila, lives as an intestinal endobiont in various animals, including humans. While traditionally considered a pathogen associated with gastrointestinal symptoms such as nausea, diarrhea, and vomiting, recent evidence challenges this view due to its high prevalence among healthy, asymptomatic individuals. Despite the growing number of studies, the question of Blastocystis' influence on the human gut microbiome and human health remains unresolved. Building upon our previous study that demonstrated the protective effect of Blastocystis against induced colitis in rats, we employed a similar experimental laboratory model to investigate its relationship with the prokaryotic gut microflora. Utilizing metagenomic and metatranscriptomic data from rat ceca infected and uninfected with Blastocystis, we aim to elucidate the impact of Blastocystis presence on the composition and transcriptional profile of the gut microbiome.

Metagenomics, metabolomics and transcriptomics as a tool to better understand the role of *Blastocystis* in the gastrointestinal environment

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Abstract

The fundamental role of *Blastocystis* in the gastrointestinal environment remains elusive: it is unclear whether this microbial eukaryote engineers the composition of the surrounding ecosystem, or whether it is merely responsive to changes in this environment. Metagenomics, metabolomics and transcriptomics are three key 'omics' techniques we will use to uncover the *in vitro* biology of *Blastocystis*. Xenic cultures of *Blastocystis* ST1-ST9 were maintained over six days to investigate any changes to the microbiome, metabolome and transcriptome. DNA extractions were performed and sent for 16S-amplicon metagenomic sequencing to decipher the prokaryotic composition present in *in vitro* cultures. Metabolite extractions were carried out, and relative metabolite abundances were detected using 1D proton NMR, to help map the metabolic processes taking place between *Blastocystis* and the surrounding microorganisms in static culture. Preliminary microbiome analysis shows that the prokaryotic composition differed significantly from the rest of the subtypes for ST3 at feature-level and ST6 at species level. Similarly, differences were identified in the metabolome data. This study's integration of metagenomics, metabolomics, and transcriptomics provides a multi-dimensional view of *Blastocystis'* interactions within the microbial ecosystem, illustrating subtype-specific relationships and their potential impacts on microbial community structure and function. The distinct microbial and metabolic signatures associated with different *Blastocystis* subtypes suggest that this protist may play a role in shaping the gastrointestinal environment, rather than merely reacting to it. These insights pave the way for future research to explore the causal mechanisms behind these interactions and their implications for host health and disease.

Artificial intelligence and Diagnosis of Blastocystis in the hospital setting: Road back to Microscopy

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Abstract

Diagnosis of Blastocystis species in clinical fecal specimens continues to be challenging. Clinical implications, challenges, and limitations of old and new fecal diagnostic methods will be discussed based on our two decades of clinical experience both in hospital settings and in clinical research. We will present our newly developed molecular nano-based technique that can be used as a point-of-care test.

Currently, many diagnostic approaches are used for the diagnosis of *Blastocystis* species in fecal clinical samples, including conventional microscopy and immunodiagnostics as well as molecular diagnostics, all of which contain challenges and limitations.

In the last few years, Artificial Intelligence (AI)-based automated digital imaging, syndromic panels, and next-generation genome sequencing techniques coupled with bioinformatics data analysis have undergone huge advances. They have revolutionized diagnosis, are one of the most promising applications, and will continue to be the next frontier in diagnostic parasitology. These new techniques with automation open new horizons in developing affordable, versatile, and user-friendly interface applications. This makes sophisticated steps of diagnostics easy to perform without expertise and decreases the need for pre-analytical sample preparation. This facilitates the implementation of these novel diagnostic techniques in different healthcare facilities, including limited resource settings, with a positive impact on patient care. Notwithstanding, the challenges and limitations of these techniques need to be addressed.

Despite the benefits of these new technologies, expert parasitologists will always be needed throughout incorporating and implementing these new techniques.

An overview of Blastocystis epidemiology from One Healthperspective

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Abstract

Blastocystis, a common intestinal protist found in humans andanimals, presents significant challenges in epidemiology dueto its diverse host range and unclear pathogenicity. From a "One Health" perspective, which integrates human, animal, and environmental health, understanding Blastocystis is crucial for understanding transmission dynamics and host-protist interactions, thereby clarifying the clinical and publichealth significance.

Blastocystis is globally distributed, with prevalence ratesvarying by region, often higher in developing areas. Currently, at least 42 genetic subtypes (STs) exist and ST1 toST4 are predominantly found in humans, while others infect a range of animals and rarely humans.

To improve of the knowledge for *Blastocystis* epidemiologyis needed a One Health approach, involving surveillanceacross human, animal, and environmental components. New strategies for focusing on the molecular epidemiology of Blastocystis are essential, especially for detecting *Blastocystis*subtypes and intrasubtype variations, providing an understanding of the characteristics of Blastocystis.

Developing and disseminating strategies to determine themolecular epidemiology of *Blastocystis* within a One Healthperspective will enable the identification of the distribution of subtypes and intrasubtype variations across differentgeographic regions, thereby clarifying the transmissiondynamics and zoonotic potential, monitoring the reservoirs of this protist.

In providing the necessary data for *Blastocystis* epidemiology, it is very important for researchers to use standardizedmethods for an effective analysis of the results, preventingvariability due to methodology.

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