5th International *Blastocystis* Conference

COST Action CA21105

2nd September – 4th September 2026

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Abbey Ruffell, University of Kent, Division of Natural Sciences, United Kingdom

Blastocystis is a globally distributed intestinal protist with a broad host range, yet its presence in invertebrates remains poorly characterised. Here, I report the first detection and successful culturing of Blastocystis from Hermetia illucens (Black Soldier Fly; BSF), an insect of growing agricultural and bioconversion importance. Samples were collected from multiple commercial and research sources, representing all major life stages: eggs, larvae, prepupae, pupae, and adults. Microscopy and sequencing screening revealed 100% prevalence across all life stages and sources, indicating that Blastocystis is consistently associated with BSF populations and may be vertically or environmentally maintained within rearing systems. Following detection, I trialled multiple culturing approaches to establish viable Blastocystis isolates from BSF. Initial attempts using standard Jones' medium and modified anaerobic conditions were unsuccessful, prompting optimisation of media composition and incubation parameters. Ultimately, viable cultures were obtained, demonstrating that Blastocystis from BSF can be maintained ex vivo despite initial growth challenges. Nested PCR barcoding failed to produce subtype resolvable amplicons, due to host contamination. To overcome this, cultured isolates were submitted for full length 18S rRNA gene sequencing, enabling accurate identification and providing the first high resolution genetic data for Blastocystis associated with H. illucens. These findings expand the known ecological range of Blastocystis and raise important questions about its persistence, transmission, and potential functional role within insect-based waste conversion systems. This work contributes novel host range data and establishes a foundation for future investigations into the biology, diversity, and ecological significance of Blastocystis in invertebrate systems.

A longitudinal pilot study of Blastocystis in healthy participants

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Blastocystis is a highly prevalent gut eukaryote whose temporal dynamics and interactions with host lifestyle and the gut ecosystem remain poorly understood. Here, we present the first longitudinal pilot study to investigate the relationship between Blastocystis abundance, diet, the gut microbiome, and the metabolome in healthy individuals.

Fifteen asymptomatic participants were sampled daily over a two-week period. Stool samples were analysed using shotgun metagenomic sequencing and ^1H-NMR-based metabolomics, alongside dietary metadata. Blastocystis was detected using a sensitive probe-based molecular assay, enabling reliable detection across all samples. Notably, Blastocystis was identified in every participant, with marked intra-individual fluctuations in abundance observed across the sampling period.

Despite the limited cohort size, exploratory analyses revealed that individuals with a history of appendectomy exhibited lower overall Blastocystis abundance and greater temporal instability compared to appendix-intact participants. This observation suggests a potential role for the appendix in supporting stable Blastocystis colonisation, consistent with hypotheses proposing the appendix as a microbial reservoir.

Together, these findings highlight the dynamic nature of Blastocystis colonisation in healthy hosts and demonstrate the value of longitudinal, multi-omics approaches for capturing short-term ecological variation. This pilot study provides a foundation for larger, hypothesis-driven investigations into the factors shaping Blastocystis persistence and its interactions with diet, the gut microbiome, and host physiology.

First survey on the prevalence, subtype distribution and circulation of the enteric protozoan Blastocystis sp. in French broiler chicken farms

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Blastocystis sp. is the most frequently detected protozoan in the human population. Despite its well-established zoonotic potential, the occurrence of this parasite in broiler chickens remains poorly documented. Here, we report the first survey conducted in France and the largest performed in Europe, aiming to investigate the prevalence, subtype (ST) /genotype distribution, and circulation of Blastocystis sp. in French broiler chicken farms. Cecal samples from 967 broiler chickens originating from 66 farms representing three production systems (Label Rouge, Standard, and Certified) were collected. Blastocystis sp. was detected by real-time PCR in 49.7% of samples and in 83.3% of farms. Statistical analyses identified production system correlated with age of chickens and outdoor access, seasonality, and treatments as major factors influencing parasite prevalence. Among mono-infections identified by Sanger sequencing, ST7 was largely predominant, followed by ST6, supporting the avian origin of these two STs, which co-circulate within the same farms. Genotyping of the isolates revealed high genetic diversity within ST7. The predominance of specific ST7 and ST6 genotypes, likely adapted to broiler chickens, indicates extensive chicken-to-chicken transmission. In contrast, less frequent genotypes may reflect incidental contamination from transient avian species. Mixed infections were detected in nearly 20% of colonized animals. Next-generation sequencing of a subset of these samples revealed co-infections with ST6 and ST7 or, alternatively, with highly divergent ST7 genotypes. While avian STs are generally regarded as non-pathogenic in chickens, this is not the case in humans. Accordingly, the active circulation of Blastocystis sp. demonstrated in French broiler chickens highlights the substantial reservoir for zoonotic transmission represented by these animals. Such transmission may occur through direct contact between poultry industry workers and chickens, via consumption of water contaminated with bird droppings harboring Blastocystis sp., or potentially through consumer handling of contaminated carcasses—a yet unexplored route that warrants further investigation.

First epidemiological and molecular report of Blastocystis sp. in equines from the northern region of Algeria

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Blastocystis is a widespread parasite that can inhabit the intestines of humans and various animals, including equines. In this study, we characterised the frequency, risk factors, and subtypes of Blastocystis in faecal samples from equids (horses, donkeys, ponies, mules, and zebras) across four Algerian provinces. Of the 197 faecal samples included in this study, 37.56% (74/197) tested positive for Blastocystis using microscopic examination and PCR amplification of the SSU rRNA gene. Univariate statistical analysis showed that species, breeding system, and weather conditions had a significant effect (P<0.05). Horses (47/104, 45.19%) were the most infected equine species by Blastocystis, compared to donkeys (24/89, 26.96%). Signal cases were detected in zebras (1/1, 100%), ponies (1/2, 50%), and mules (100%), though these were based on limited sample sizes. Blastocystis infections were higher in semi-arid (48.71% (19/39)) and humid (30.63% (34/111)) areas, compared to arid (25.00% (7/28)) and sub-humid (15.78% (3/19)) regions. Animals raised in intensive systems (55.26% (21/38)) were more susceptible to infection than those in semi-intensive (36.66% (11/30)) and extensive systems (32.55% (42/129)). None of the factors remained significant in multivariate analysis. Positive qPCR products were amplified by nested PCR and sequenced to determine subtypes. Sequencing revealed seven Blastocystis subtypes: ST1 (n=3/20, 15%), ST2 (n=3/20, 15%), ST3 (n=3/20, 15%), ST4 (n=9/20, 45%), ST25 (n=1/20, 5%), and ST33 (n=3/20, 15%). Additionally, two sequences were newly observed and proposed as ST48-like. Horses exhibited six subtypes (ST1-4, ST33, and ST48-like), donkeys showed mixed infections with ST2 and ST4 and single infections with ST1, ST4, ST25, ST33, and ST48-like. Furthermore, three short sequences, each isolated from a horse, pony, and mule, clustered within the ST10 clade.

Survey of Blastocystis sp. and microsporidia in farmed avian species in Tuscany, Central Italy

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Blastocystis and microsporidia are common parasites in humans and animals and can be transmitted through contaminated water or food. Clinical disease caused by these organisms has mainly been reported in children, elderly, and immunocompromised individuals. Understanding their occurrence, distribution, and genotypes in birds is important from a One Health perspective, as birds may act as reservoirs of zoonotic genotypes and may themselves be affected by these parasites. This study evaluated the occurrence of Blastocystis sp. and microsporidia in farmed birds in Tuscany, Italy. Bird farms differing in flock size (30–2500 birds), housing conditions (indoor, outdoor, free-ranging), bird age (young/adult), and species (chickens, ducks, geese, quails) were investigated. Twenty pooled faecal samples, each consisting of 5–12 droppings, were collected and analysed using the Allplex™ Gastrointestinal Parasite Panel Assays (Seegene), multiplex real-time PCR assays targeting the small subunit ribosomal RNA gene for the simultaneous detection of gastrointestinal parasites, including Blastocystis sp. and Enterocytozoon spp./Encephalitozoon spp. Selected positive samples were sequenced for genotyping. All examined farms, samples, and bird species tested positive for Blastocystis. The zoonotic subtype ST7 was identified, confirming previous reports of the high prevalence of this parasite and the predominance of ST7, together with ST6, in birds. Blastocystis is frequently detected in healthy farmed birds and is considered part of the avian microbiome. Enterocytozoon spp. was detected only in young chickens. Enterocytozoon bieneusi, the species most frequently reported in chickens, has been associated with acute watery diarrhoea, whereas Encephalitozoon species are rarely identified in birds. Overall, this study provides new data on the occurrence of Blastocystis and microsporidia in farmed birds in Italy and highlights their potential role as reservoirs of zoonotic genotypes. Further studies are needed to assess their prevalence, zoonotic potential, and impact on animal health and production.

Towards harmonised sampling and molecular detection of Blastocystis spp.: a pilot workflow addressing temporal shedding variability

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Blastocystis spp. is one of the most frequently detected intestinal eukaryotic microorganisms in humans, yet its epidemiology, biological significance, and clinical interpretation remain influenced by methodological limitations. One underexplored source of bias is the temporal variability of parasite shedding, which may contribute to false-negative results when only a single stool sample is examined. In parallel, the marked molecular heterogeneity of Blastocystis spp. may contribute to variable detectability and complicate comparisons between diagnostic approaches. This abstract presents the rationale and planned workflow of a pilot study designed to assess how short-term shedding variability and molecular heterogeneity may influence the detection of Blastocystis spp. in human stool samples. Consistent with Polish PTDL/PTP recommendations for parasitological stool examination, which advise examining three samples collected at 2–3-day intervals due to intermittent parasite shedding, the workflow is based on repeated sampling from adult participants, with three stool samples collected at 48–72-hour intervals. Each sample is planned to be examined using complementary diagnostic approaches: direct microscopy, xenic in vitro culture in Jones' medium, and molecular detection targeting the SSU rRNA gene. Nested PCR will be applied systematically to all samples to reduce false-negative results and improve detection of low-intensity or intermittently shed colonisation. Positive amplicons will undergo Sanger sequencing for subtype assignment and exploratory assessment of molecular heterogeneity.

The planned analytical framework includes assessment of method agreement, incremental diagnostic yield across consecutive samples, and temporal variability of detection. By integrating repeated sampling, culture-based detection, and molecular characterisation, this workflow aims to address a key methodological gap in Blastocystis research and support harmonisation of sampling and detection strategies in future multicentre studies aligned with COST Action CA21105 priorities.

Blastocystis spp. and Dientamoeba fragilis as the most common protist co-colonisation in a single Czech tertiary-care cohort

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Blastocystis spp. (SAR: Stramenopiles) and Dientamoeba fragilis (Metamonada: Parabasalia) are among the most frequently detected intestinal protists in routine parasitological diagnostics, yet their clinical significance remains unclear. Whereas Blastocystis spp. are increasingly interpreted as common intestinal colonisers, studies evaluating D. fragilis report inconsistent associations with gastrointestinal symptoms. We analysed prevalence patterns and co-detection of both protists in patients referred for parasitological examination at the Regional Hospital Liberec, Czech Republic. Stool samples from 166 paediatric and adult patients referred for routine parasitological examination were analysed using multiplex real-time PCR together with demographic and clinical metadata. Blastocystis spp. were detected in 15.1% of individuals (25/166), whereas D. fragilis was detected in 25.3% (42/166). Co-detection occurred in 10.2% of patients (17/166). Blastocystis spp. positivity showed no significant association with age group or sex, whereas D. fragilis positivity was more frequent in male and paediatric patients, particularly among children aged 0–10 years. Abdominal pain was associated with Blastocystis spp. positivity after adjustment for age group and sex (OR 3.67, 95% CI 1.25–10.79; $p = 0.018$), but the association weakened after additional adjustment for D. fragilis positivity (OR 2.62, 95% CI 0.82–8.38; $p = 0.104$). Co-detection with D. fragilis represented the strongest association observed (adjusted OR 10.49, 95% CI 3.88–28.31; $p < 0.001$). These findings support a close ecological overlap between both protists within the gut eukaryotic microbiota and suggest that the observed clinical associations may reflect complex co-colonisation patterns and broader microbiome interactions.

Blastocystis Characterization in Rodent Populations in Türkiye: The Ovacık/Tunceli Experience Through a One Health Approach

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Blastocystis is the most prevalent intestinal eukaryote in humans, yet its transmission dynamics, zoonotic potential, and subtype diversity remain incompletely understood, particularly in closed ecosystems characterized by close human-animal-environment contact. This study investigates the environmental spread and genetic diversity of Blastocystis in Ovacık district, Tunceli, Türkiye, within a One Health framework. The district represents a geographically isolated ecosystem with minimal industrial pollution, active livestock transhumance, and documented wildlife activity, making it a suitable natural model for studying cross-species transmission. Fecal samples are collected from humans, rodents, and domestic animals, alongside environmental samples including soil, sediment, river water, and shared water sources. Blastocystis is detected using real-time PCR, followed by subtype and allele determination through conventional barcoding PCR and sequencing. Metagenomic sequencing is applied for population-level genetic diversity analysis. Geographic Information Systems (GIS) are used to generate risk maps, and compartmental epidemiological simulation models are developed to evaluate transmission scenarios under varying environmental and intervention conditions. This is the first study in Türkiye to systematically examine Blastocystis in rodent populations within a closed ecosystem. Rodents are hypothesized to serve as significant reservoirs for subtypes also detected in humans, with genetic analysis expected to reveal shared alleles supporting zoonotic transmission. The project is funded by TÜBİTAK under the 2515 (project no 325S024) COST Support Programme and is directly aligned with the CA21105 OneHealthBlastocystis Action, contributing specifically to WG1 (Mapping Blastocystis epidemiology and diagnostics) and WG2 (Blastocystis collection and database). Results will provide novel molecular epidemiological data from Türkiye in rodent populations, contribute to the COST Action's international database, and elucidate the transmission dynamics of Blastocystis in rural, high-contact settings within a One Health perspective.

Genetic engineering of Blastocystis ST7-B

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Blastocystis is metabolically unconventional, ecologically ubiquitous, and clinically contentious — yet until recently, it has remained refractory to molecular-level experimental manipulation due to lack of genetic access. Building on the first demonstration of transient transfection in Blastocystis ST7-B, we developed a combinatorial genetic toolkit that enables stable episomal transgenesis and recovery of colony-derived transgenic line in this organism. Using a proteomics-guided candidate screen, we benchmarked 23 endogenous promoter–terminator combinations from 14 loci with a NanoLuc luciferase reporter, resolving them into robust, moderate, weak, and near-background expression tiers, expanding the regulatory toolkit well beyond the single legumain-derived cassette previously available. We replaced time-constant electroporation with optimized square-wave conditions, which eliminated arcing and improved post-transfection recovery. Drug sensitivity profiling identified puromycin and trimethoprim as the most reliable selectable systems. Bicistronic P2A constructs enabled selection-linked co-expression of anaerobic-compatible reporters and combined with a three-stage workflow — stepwise liquid-phase enrichment, solid-phase colony isolation, and liquid re-expansion — supported recovery of colony-derived transgenic lines. These lines remained drug-resistant and reporter-positive after more than 15 passages and one year of cryopreservation. Together, these tools make Blastocystis ST7-B markedly more amenable to molecular investigation, with the toolkit potentially transferable to other Blastocystis subtypes.

Tracing Blastocystis from Epidemiology to Experimental Function

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Blastocystis represents an exceptionally suitable model for studying intestinal protists because it concentrates many of the key questions shaping the field today. It is common in humans and animals, genetically diverse, frequently present in gut-healthy hosts, associated with different microbiome states, and still debated as a potential pathogen, commensal organism, or marker of gut eubiosis. This ambiguity makes it an ideal organism for exploring what the presence of an intestinal protist actually means in different host and ecological contexts.

Our work follows this question across several interconnected levels. Epidemiological data from gut-healthy human populations show that Blastocystis is not a marginal finding, but a common and subtype-diverse colonizer whose occurrence is shaped by exposure, lifestyle, and host-related factors. Microbiome analyses extend this picture further: the presence of Blastocystis is not merely an isolated diagnostic finding, but is associated with specific bacterial community patterns, and may therefore reflect, or possibly contribute to, a particular state of the gut ecosystem.

In a broader One Health context, Blastocystis emerges as part of interconnected host networks, in which shared environments, contact between humans and animals, and hidden subtype diversity influence its circulation across hosts. Experimental models then enable a shift from questions of occurrence and association toward questions of function. They show that colonization can be asymptomatic and long-lasting, while its impact on the microbiome and host response depends on time, context, and the state of the gut environment.

Together, this line of work shifts the interpretation of Blastocystis away from the simple category of pathogen versus commensal and toward a more precise question: under which host, microbial, environmental, and temporal conditions does its presence become harmful, neutral, or potentially beneficial?

The Art of Seeing: Advanced Fluorescence Imaging for Blastocystis ST7-B and ST4-WR1

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Here, we report advanced fluorescence microscopy workflow to visualize the cell surface, lipid, and cytoskeletal dynamics of two contrasting Blastocystis subtypes, ST7-B and ST4-WR1, across multiple time points. We tested and optimized several fluorescent probes, including wheat germ agglutinin conjugates for surface carbohydrate labelling, BODIPY-based dyes for lipid visualization, and Hoechst 33342 and SYTOX Deep Red for DNA staining. In parallel, we used immunofluorescence assays to examine actin and tubulin organization, allowing subtype-specific cytoskeletal features to be compared over time. To move beyond conventional diffraction-limited imaging, we also adapted expansion microscopy for Blastocystis ST7-B. By embedding fixed cells in a swellable gel matrix, this approach enabled intracellular and surface-associated structures to be resolved in greater detail. Together, this workflow transforms Blastocystis from a morphologically familiar but cell-biologically obscure protist into a tractable system for high-resolution imaging. It provides a practical platform for comparing subtype-specific architecture, cytoskeletal organization, and surface features in one of the most prevalent microbial eukaryotes of the gut.

Expanding the genomic landscape of Blastocystis hominis species in the human gut microbiome

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Blastocystis is one of the most prevalent single-celled eukaryotes found in the human gastrointestinal tract, and its presence and role in the human microbiome is still debated.

Currently, only eight human-associated subtypes (ST1 - ST9) of Blastocystis are described by complete genomes, but they still exhibit large genomic diversity with inter-subtype average nucleotide identity (ANI) values below 80%. Nonetheless, over 40 different subtypes have been described by their 18S ribosomal RNA gene across many animal hosts. Studying Blastocystis in purely axenic isolation is still challenging, while the supplementation of a diverse group of bacterial species improves cultivation but limits genomic reconstruction due to the significant residual bacterial presence. Here we sequenced, assembled and phylogenetically characterized the genetic diversity of human-associated Blastocystis subtypes. We assembled from 65 xenic cultures as well as from more than 60 metagenomic datasets, retrieving more than 400 high-quality Blastocystis genomes MAGs. Phylogenetic analysis of these MAGs revealed a high level of diversity within the human-associated subtypes. Multiple Sequence Alignment (MSA) of shared genomic regions confirmed the ANI comparisons, showing significant phylogenetic distances between Blastocystis subtypes, with exceptions for ST6 and ST9 and a putative new subtype closely related to ST7 (ANI >90%).

Our results showed that recovering high-quality MAGs of Blastocystis from metagenomic samples is possible and allows for a broader definition and phylogenetic contextualization of the complex genetic landscapes. Additionally, these results demonstrate the viability of exploiting metagenomic data for the reconstruction of high-resolution eukaryotic genomes, which extends beyond conventional bacterial profiling. Considering current associations of Blastocystis with diet, BMI, and clinical outcomes, the retrieval of ST-specific MAGs from large-scale metagenomic datasets will enable refining the role of this protist in human health.

Implementation of a novel droplet digital PCR for Blastocystis detection in environmental samples

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Background: Blastocystis is a ubiquitous protist colonizing humans and a wide range of animal hosts, with an unclear clinical role. Its detection is complicated by its morphological variability and genetic diversity, including multiple molecular subtypes. While frequently reported in wildlife, its presence in environmental matrices such as water remains poorly understood. From a One Health perspective, environmental reservoirs are crucial for understanding transmission dynamics. In this study, a novel droplet digital PCR (ddPCR) was developed to detect Blastocystis in environmental and clinical samples.

Methods: The ddPCR assay was developed using a primer pair adapted from Stensvold et al. Analytical performance was assessed through optimization of primer concentrations and annealing temperature, as well as the assay efficiency and limit of detection. Thirteen water samples were collected in June 2025 from a natural wastewater treatment system (Fuerteventura) and a coastal lagoon ecosystem (La Charca de Maspalomas, Gran Canaria) as part of the NATALIE project, which seeks to analyze and replicate nature-based solutions (NBS) to combat climate change in vulnerable regions.

Results: The optimized analysis employed primer concentrations of 250nM, with an annealing temperature of 58ºC. Two out of thirteen samples analyzed were positive for Blastocystis, both corresponding to 2 distinct parts of the wastewater treatment system in Fuerteventura. No positive results were detected in the remaining samples.

Conclusions: The ddPCR method proved to be a rapid, sensitive, and efficient diagnostic approach for Blastocystis detection in environmental samples. The presence of this pathogen in a wastewater treatment system underscores the potential role of anthropogenic water systems as environmental reservoirs and pathways for dissemination. The persistence of protist DNA throughout the different treatment stages further suggests a potential tolerance to depuration processes, highlighting the importance of incorporating environmental molecular surveillance to improve understanding of its ecology and implications for public health.

Drug discovery against Blastocystis: applying anti-protozoal screening with AI and Plant bioguided fractionation

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Blastocystis sp. is one of the most prevalent intestinal protists globally, colonizing humans and diverse animal hosts. Although its pathogenic role remains debated, specific subtypes have been linked to intestinal barrier disruption, microbial dysbiosis, and gastrointestinal disorders. To advance mechanistic and therapeutic research, the current project aims to establish Blastocystis sp. as a standardized experimental model within the Antiparasitic Chemotherapy Laboratory of the University Institute of Tropical Diseases and Public Health of the Canary Islands.

The present study proposes an interdisciplinary approach that merges technology with ethnopharmacological exploration. The project will implement a dual-phase strategy: (1) development of an AI-enhanced high-content imaging platform to automate life-stage identification and viability quantification post-treatment, and (2) systematic screening of endemic plant extracts from the Canary Islands and Tunisia. Bioactive fractions will be isolated via bio-guided fractionation and structurally characterized to identify novel antiparasitic leads.

Intestinal eukaryotes associate with taxon-specific, age-independent bacteriome gut microbiome signatures across the developmental stages of production pigs

Yohannes Seyoum Demissie, University of Stavanger

Intestinal eukaryotes co-exist with gut bacterial communities throughout the mammalian lifespan, yet whether they shape microbiome development or instead depend on it remains unresolved. We characterised the intestinal eukaryotes and gut microbiota of 111 production pigs across four developmental stages using combined 16S and 18S rRNA metabarcoding and taxon-specific PCR, applying a joint deconfounding framework to identify genus-level bacterial associations independent of host age and co-occurring eukaryotes. Eukaryotic carriage was common: Blastocystis, Entamoeba, Balantidioides, parabasalids, Iodamoeba, Enterocytozoon bieneusi, and Cryptosporidium spp. all varied by host developmental stage. We identified 94 deconfounded genus-eukaryote associations across eight eukaryotic variables; Cryptosporidium did not retain deconfounded genus-level associations, but was the only eukaryote associated with lower bacterial richness after age adjustment and was linked to reduced microbiome maturity in sows. Eukaryotic carriage was widespread and strongly structured by developmental stage. Extracellular microeukaryotes were consistently associated with enrichment of butyrate-producing and fibre-fermenting bacterial genera, whereas E. bieneusi showed the opposite pattern, selectively depleting taxa that increased with host age. In contrast, , including Balantidoides, parabasalids, Entamoeba, Iodamoeba, and Blastocystis ST3, were consistently associated with enrichment of butyrate-producing and fibre-fermenting genera, with high directional concordance across taxa. In contrast, E. bieneusi showed an inverse pattern, selectively depleting nine of the ten genera that increased most strongly with host age, a signal that persisted after age residualisation of the co-abundance network. Causal mediation analysis indicated that gut microbiota maturity mediated 71.5% of the total age effect on eukaryotic richness, while the reciprocal pathway accounted for 2.6%. These findings suggest that microbiome developmental state may influence eukaryotic colonization, while eukaryotic colonization and life stages shape distinct gut bacteria associations.

How common is the transmission of Blastocystis via deep-frozen faecal microbiota transplantation? Observations from a randomised study with 50 patients, 100 faecal microbiota transplantations and 555 stool samples.

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Background: Traditionally, Blastocystis has been a reason for exclusion from stool donation for faecal microbiota transplantation (FMT), although evidence from clinical studies is very limited. We recently analysed Blastocystis data from an irritable bowel syndrome (IBS) trial, evaluating whether FMT using material positive for Blastocystis leads to transmission of the protist. Methods: The quantitative PCR data of Blastocystis originate from a three-arm, randomised, double-blind, placebo-controlled crossover clinical trial that tested the clinical effects of FMT in 50 patients with IBS. The live faecal material used for FMT remained consistent throughout the study, having been prepared in a single batch by mixing stools from eight donors and then frozen at -80°C. The placebo was the same material that had been autoclaved before freezing. The transferred material was highly positive for Blastocystis ST3 DNA, yielding a mean threshold cycle of 22.2. A total of 100 FMT interventions were performed, with a mean of 11 stool samples from each participant tested for Blastocystis.
Results: One case of potential transmission was noted among the 31 patients who were initially Blastocystis-negative and received live FMT. This patient developed high positivity for Blastocystis ST3 DNA nine weeks after FMT, and this positivity persisted without any relevant adverse effects. One additional patient had a single positive sample for an untypeable trace load (Ct=38.6) at week 24 post-FMT, transmission being considered unlikely as all other stool samples were negative. Pre-existing long-term colonisations with various subtypes (observed in five subjects) were unaffected by placebo or live FMT.
Conclusions: The frequency of Blastocystis transmission from a PCR-positive live FMT was very low and had no adverse clinical correlates. This may be related to the likely decreased viability of the protist, given that all faecal doses for FMT were frozen at -80°C for at least three months during quarantine. This observation has implications for the selection of FMT donors.

Circulation of Blastocystis sp. in pigs, dairy cattle and farm water sources from a high-density livestock area

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Blastocystis sp. is one of the most widespread intestinal protists in humans and animals and is increasingly investigated because of its zoonotic potential. High prevalences have been reported in livestock worldwide, particularly in pigs and cattle, which are considered reservoirs of potentially zoonotic subtypes. In Italy, intensive farming systems may facilitate the circulation and environmental dissemination of Blastocystis sp., although data on associated water sources remain limited. This study aimed to investigate the occurrence of Blastocystis sp. in pigs, cattle, and drinking water sources from farms located in a high-density livestock area in Northern Italy.

Six farms (three pig farms and three dairy cattle farms) located in Mantua Province were selected. Pig farms included different production systems (breeding, farrow-to-finish, and fattening). Seasonal sampling is ongoing from February to October 2026, with 20 fecal samples collected per farm at each sampling time. To date, 120 fecal samples from the first sampling campaign have been analyzed. In addition, 10-L water samples were collected from the drinking water sources supplying the animals. DNA was extracted from fecal and sedimented water samples and analyzed by SSU-rDNA gene PCR plus sequencing for Blastocystis sp. (Scicluna et al., 2006).

Blastocystis sp. was detected in all pig farms, with prevalences of 10% (2/20) in the breeding farm, 75% (15/20) in the farrow-to-finish farm, and 100% (20/20) in the fattening farm. In dairy cattle farms, 3.3% (2/60) of animals tested positive, with both positive samples originating from the same farm. Preliminary phylogenetic analyses showed that bovine and swine sequences clustered together in a distinct clade, closely related to ST14, a subtype previously reported in cattle and potentially associated with zoonotic transmission. Deeply subtyping analyses of fecal isolates and water samples are currently ongoing to investigate possible environmental circulation patterns and relationships between animal and water-associated isolates.

Observed Prevalence and Successful Culturing of Blastocystis from the rumen of cattle (Bos taurus)

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Blastocystis is a globally distributed intestinal protist and has long been presumed to reside in the rumen, potentially being correlated with methanogenic microbes. Reported in this study is the first detection and successful culturing of Blastocystis from cattle rumen fluid. Samples were collected from cannulated cattle. Microscopy and sequencing screening revealed 100% prevalence of Blastocystis in all 4 cattle sampled and from both strained/unstrained rumen fluid, indicating that Blastocystis may be consistently associated with the rumen and potentially involved in the function of the rumen as a commensal.

Following detection, viable Blastocystis cultures were established from rumen fluid using standard Jones' medium and modified anaerobic conditions. Conformation of Blastocystis was performed using light microscopy and 18s amplicon sequencing. Ultimately, viable cultures were obtained, demonstrating that cultures of Blastocystis obtained from cattle rumen fluid can be produced and maintained.

Due to the high economic importance of both cattle, and their methane emissions (predominantly produced via rumen anaerobes), the presence of Blastocystis and possibility of its role as commensal could further research into the functioning of eukaryotes in the rumen microbiome. These findings expand the known ecological range of Blastocystis and raise important questions about its persistence, transmission, and potential functional role within the rumen of cattle. This work contributes novel host range data and establishes a foundation for future investigations into the biology, diversity, and ecological significance of Blastocystis in ruminants.

Blastocystis in Children with and without Diarrhoea: Molecular Prevalence and the Association with Bacteriome Community Composition

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Background: Although Blastocystis is a common protist of the human intestine, its clinical significance in health and disease remains unclear, and its interactions with the intestinal microbiome continue to attract scientific interest. This study aimed to determine the molecular characterization of Blastocystis in children aged 2–12 years with diarrhoea and in healthy controls, and to evaluate the potential association of this protist with dysbiosis.

Methods: Stool samples from a total of 163 participants (n=97 children with diarrhoea; n=66 healthy controls) were analyzed for Blastocystis. Fecal calprotectin levels were measured to assess the association between Blastocystis and intestinal inflammation. Microbiome interactions were evaluated by determining the Bacillota/Bacteroidota ratio as dysbiotic index using qPCR (dysbiosis defined as index > 1.5), and by 16S rDNA profiling of the community composition.

Results: The prevalence of Blastocystis was not different between children with diarrhoea (10.3%) and healthy controls (12%), p=0.914. Subtype analysis identified ST2, ST3, and ST7, with ST2 being the predominant subtype in both groups. PCR-detected dysbiosis was strongly associated with diarrhoea, being detected in 25% of patients but only 7.6% healthy controls, OR = 4.1 [95%CI 1.4 -14.6], P=0.0060. No such association was found between dysbiosis and the positivity for Blastocystis, OR = 1.3 [95%CI 0.21-5.3], P=0.72.

The alpha (intra-sample) diversity of the bacteriome was higher in control children than in patients with diarrhoea (P<10-5 for all indices), and higher in samples having high PCR positivity for Blastocystis (observed species, Chao1 and Shannon index). Several taxa were differentially abundant in children with high Blastocystis positivity (namely, Bifidobacterium, Entgerobacteriaceae and Strepotococcaceae were depleted), and expectedly, diarrhoea was associated with significantly disturbed bacteriome composition and also with increased concentrations of fecal calprotectin (p<0.001).

Conclusion: The presence of Blastocystis was not associated with elevated calprotectin levels, suggesting that Blastocystis may colonize the gut microbiome in a compatible and non-inflammatory manner. Diarrhoea and Blastocystis were both associated with compositional changes in the bacterial community, which often occurred in contradictory directions.

Development of loop-mediated isothermal amplification (LAMP) assay combined with lateral flow dipstick for rapid and sensitive detection of Blastocystis spp.

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Blastocystis is a common protist in the intestines of humans and animals. Although the diagnosis of this protist is most often based on microscopic examination, this method may lead to false negative results. An effective, accurate and appropriate diagnostic approach is of great importance in both epidemiological and clinical studies of Blastocystis. The aim of this study was to evaluate the sensitivity and specificity of the LAMP-LFD test, which was developed by designing primers and probes specific to the LAMP method, which is an easier, faster, more reliable and sensitive method for Blastocystis, which is commonly found in humans and animals, and combining it with the lateral flow dipstick (LFD). With the LAMP-LFD test developed in this study, bands were observed in agarose gel electrophoresis and LFD within 30 minutes at 65 °C. The minimum detection limit of the LAMP-LFD assay was determined as 100 fg/µl of genomic DNA and 1x101 plasmid copies/reacts. These results showed that it was 10 times more sensitive compared to cPCR. Moreover, this method showed good specificity by not giving any cross-reaction. The developed LAMP-LFD test was tested with 48 human stool samples and compared with the cPCR method. These results confirm that the newly developed LAMP-LFD test is faster, more sensitive, more reliable and more easily applicable and can be used in the diagnosis of Blastocystis.

Re-reading the Blastocystis SSU rRNA database

Raul Tito, KUL-VIB

Background. Blastocystis subtyping is built on a chain of trust that ends at GenBank: every new isolate is identified by its closest deposited neighbour, and that neighbour's subtype label was assigned by an earlier submitter. With more than forty subtypes (STs) now recognised, recent reassignments within the ST10 group, and several contested STs still circulating in the database, this chain has never been audited at scale.

Methods. We developed an open-source pipeline that retrieves every Blastocystis SSU rRNA record from NCBI in a single pass and applies a transparent curation logic to all of them at once. Each record is filtered for sequence length and metadata quality, then assigned to a subtype in two stages: first by parsing its own GenBank annotations for explicit ST tags, and second, for records without a tag, by sequence-based placement against a self-bootstrapped reference set. Records that fit neither route are clustered among themselves into provisional novel groups. Every assignment carries the provenance needed to reproduce or contest it.

Results. The broad query returned 17,717 records; quality and length filtering, followed by deduplication, produced a final reference set of 1,045 near-full-length sequences covering 43 known STs and 17 provisional novel clusters. Subtype-host associations recapitulate expected biology across humans, pigs, and ruminants. The largest novel cluster groups six tortoise species (Testudo, Geochelone, Stigmochelys, Astrochelys, Agrionemys) from Japan and the Czech Republic into a single coherent lineage, extending the chelonian Blastocystis clade recently described by Lind et al. (2025) from three Testudo strains to six tortoise genera across two continents.

Conclusion. A single transparent curation pass converts the public database from an opaque resource into an auditable, traceable reference set, and surfaces a candidate chelonian Blastocystis lineage worth formal description.

Evaluation of 16S-18S Amplicon NGS for Detection and Genetic Diversity Analysis of Blastocystis in Hospitalized Patients

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The development of new methodologies to detect Blastocystis and study genomic diversity is critical to identifying its role in health and disease. Amplicon Next-Generation Sequencing (NGS) provides a promising diagnostic method that enables simultaneous detection of multiple Blastocystis subtypes (STs). Here, 93 faecal samples from hospitalized patients with gastrointestinal symptoms (mostly diarrhoea) were subjected to 16S-18S-NGS platform and the positivity results compared with those (Ct values) obtained from Allplex™ GI-Parasite Assay RT-PCR, considering as rapid and highly sensitive gold standard for detecting specific known targets, to which the samples were previously subjected. By NGS, 30.1% (28/93) and 69.9% (65/93) were detected positive and negative to Blastocystis, respectively with a higher prevalence of ST3 (32.1%) followed by ST4 (28.6%), ST1 (21.4%), ST6-ST9 (7.1%) and ST2 (3.5%). Mixed subtypes (ST2/ST4) and (ST1/ST2/ST4) were also recorded for one sample each (3.5%). Among the 28 Blastocystis positive, 26 were also positive by RT-PCR (Ct value range, 20–33). Worth nothing, two samples registered as negative by RT-PCR (Ct values > 35) were detected by NGS (ST3 both) showing a better performance in deep detection and subtype profiling compared with RT-PCR. These preliminary results support the use of 16S-18S NGS as a useful diagnostic tool to improving the detection of undetectable samples and single/mixed infections revealing profound Blastocystis genetic diversity in clinical isolates. The characterization of the microbial composition in relation to patients with different subtypes and with different geographical origin will be useful for a better understanding of the combined effect on gastrointestinal ecology in health and disease.

Longitudinal observation of Blastocystis sp. in children and young persons: a very rare occurrence in chronic diseases with gut dysbiosis

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Background: Blastocystis sp. is currently viewed as an indicator of a diverse gut microbiome rather than a pathogen. However, longitudinal data on its presence and stability remain limited. This study aimed to assess its presence and stability in a longitudinal series of faecal samples from paediatric patients with inflammatory bowel disease (IBD) and cystic fibrosis (CF), contrasting these with gut-healthy children with type 1 diabetes (T1D), juvenile idiopathic arthritis (JIA), and healthy controls.

Methods: Stool samples were collected at tertiary centres in Czechia and Finland. Internally controlled real-time PCR was used to detect and quantify Blastocystis sp. Massively parallel amplicon sequencing was used to determine Blastocystis subtypes (partial sequence of 18S rDNA) and bacteriome profiles (16S rDNA, V3-V4 amplicon).

Results: A total of 372 subjects (mean age 13.9, 47% female) and their 1884 stool samples were analysed: 135 IBD subjects (597 samples) and 22 JIA subjects (96 samples) from anti-TNF therapy studies, 116 T1D subjects from interventional dietary studies (626 samples), and 45 CF subjects from a study on CFTR modulator therapy (237 samples). Subject-wise positivity rate was significantly lower in IBD (5.9%) and CF (none positive) than in healthy controls (26.7%), T1D (16.8%), or JIA (9.1%), corrected $P< 0.01$. ST2 was the most common subtype, ST4 showed a suggestive enrichment among IBD; no clear subtype-specific pattern was observed across diagnoses.

Conclusions: Blastocystis sp. is frequently detected in gut-healthy individuals (T1D, JIA, healthy), including asymptomatic long-term co-colonisation in some cases. In contrast, it is rare in IBD and entirely absent in CF, likely reflecting their severely dysbiotic, inhospitable, low-diversity microbiomes. Notably, this pattern persisted despite clinical improvement with anti-TNF therapy in IBD or the initiation of CFTR-modulating therapy in CF. Subtype distribution was largely heterogeneous.

Comparison of Blastocystis Subtype Analysis from Culture and Direct Stool Samples

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Introduction: Blastocystis is a protist that widely colonizes the gastrointestinal tract of humans and animals, exhibiting exceptionally high genetic diversity. Accurate subtype (ST) analysis is essential for understanding the epidemiology, transmission routes, and potential pathogenicity of this organism. The aim of this study was to compare ST analysis results obtained from culture-derived and direct stool samples of Blastocystis-positive specimens.

Methods: Jones medium culture materials supplemented with 10% horse serum were prepared from 30 stool samples that had previously undergone direct ST analysis. DNA extracted from these cultures was subjected to barcoding PCR using RD5 and BhRDr primers to amplify the Blastocystis small subunit (SSU) rRNA gene region. Amplicons containing the target band were submitted for Sanger sequencing for further analysis.

Results: PCR analysis of culture-derived DNA demonstrated the presence of the expected target band at approximately 600 bp in all 30 samples. Subtype analysis is currently ongoing, and the results will be discussed accordingly.

Conclusion: This study is significant in that it aims to determine whether Jones medium culture materials represent a suitable substrate for the molecular characterization of Blastocystis. Successful amplification of the target region across all culture samples may demonstrate that culture-derived DNA serves as a reliable template for ST analysis. Upon completion of the sequencing process, subtype data obtained from culture will be compared with those previously derived directly from stool samples of the same patients. This comparison will provide preliminary insight into the potential selective effects of culture enrichment on Blastocystis subtyping outcomes.

Molecular Diversity of Blastocystis in Farmed Red Deer (Cervus elaphus) in Southern Spain

Ana Manuel Bastos Figueiredo, University of Aveiro, Department of Biology, Portugal

Wild red deer farming is a common practice for meat production in Spain, which is the world's second-largest exporter of venison. However, the zoonotic infectious diseases present in these populations are not well understood, potentially increasing public health risks. Blastocystis is a widespread zoonotic enteric protist characterised by high genetic diversity, a broad host range, and uncertain pathogenicity. To address these concerns, we investigated the occurrence, genetic diversity, and colonisation patterns of Blastocystis in a semi-extensively managed red deer population in southern Spain. A total of 319 faecal samples from 247 individuals were collected between 2012 and 2014 and analysed using next-generation amplicon sequencing. Blastocystis was detected in 14.7% of the samples, and thirteen previously described subtypes (ST5, ST10, ST14, ST21, ST23-ST26, ST30, ST32, ST42-ST44), along with one novel subtype, were identified. This novel subtype was validated through phylogenetic and pairwise distance analyses of the full-length SSU rRNA gene sequences generated by Oxford Nanopore sequencing. All Blastocystis-positive samples exhibited 4 to 16 subtype combinations, with up to 44 unique variants, indicating substantial intra-subtype variability. A longitudinal study of five deer revealed both persistent and intermittent colonisation patterns among various subtypes. Potentially zoonotic subtypes ST10a and ST14, as well as ST21, ST25, ST43, and ST44, were present in 90–100% of the longitudinally analysed samples. These findings demonstrate that farmed wild red deer harbour a high diversity of Blastocystis subtypes and exhibit evidence of long-term colonisation and complex transmission dynamics. This highlights the need to thoroughly understand Blastocystis's ecological role in the cervid gut microbiome.

Long-term effects of Blastocystis colonization on the rat caecal microbiome

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Blastocystis is a widespread gut endobiont in humans and other animals, yet many aspects of its biology remain elusive – including its possible influence on the host. These may involve direct interactions with the host tissue or immune system, but also indirect effects through alteration of the gut microbiome. In our three-month experimental colonization assay, we estimated differences in the bacterial microbiome of Blastocystis-positive (n = 14) and negative (n = 16) rats. This unique long-term exposure with sampling across multiple time points enables us to address the effect of Blastocystis during the early acute phase as well as after stable colonization is established. We combined Illumina profiling of rat caecum metagenome and meta transcriptome with Oxford Nanopore PromethION DNA sequencing of the same material. The former provided standard-length reads for mapping and quantification, while the latter produced long reads to generate a tailored de novo reference assembly and reconstruct several near-complete bacterial MAGs (metagenome-assembled genomes). This strategy resulted in over 90% of meta transcriptomic reads mapping to the reference, representing a substantial improvement over standard taxonomic assignment tools. We focus our analysis on identifying features that best discriminate between control and experimental groups and, notably, on uncovering not only taxonomic but also functional shifts through differential gene expression.

Emerging Roles of Protistan 'Dark Matter' in Microbiota-Host Interactions

Kevin SW TAN, National University of Singapore

Blastocystis is one of the most prevalent eukaryotes in the human gut, yet its contribution to host physiology remains unresolved. Rather than fitting a simple commensal-versus-pathogen model, Blastocystis appears to act in a subtype-dependent manner, with distinct lineages exerting divergent effects on microbial ecology, metabolite networks and mucosal immunity.

Using integrated in vitro, in vivo, microbiome and metabolomic approaches, we define contrasting host-associated signatures of Blastocystis ST4 and ST7. ST4 is associated with increased bacterial diversity, enhanced short-chain fatty acid production and protection against experimental colitis. By contrast, ST7 drives dysbiosis, depletes beneficial taxa including lactobacilli and bifidobacteria, exacerbates intestinal pathology and promotes Th1/Th17-skewed mucosal inflammation.

Mechanistically, we identify a protist-derived immunometabolic axis centred on tryptophan metabolism. Blastocystis ST7 produces indole-3-acetaldehyde, a metabolite that suppresses inducible regulatory T-cell differentiation while promoting pro-inflammatory CD4+ T-cell responses, providing a direct link between protist metabolism and host immune dysregulation. We further propose that ST7-mediated disruption of microbiota capable of metabolising this intermediate amplifies inflammatory outcomes by reshaping the downstream indole landscape.

Together, these findings position Blastocystis as an active and underappreciated component of the gut ecosystem whose effects depend on subtype-specific interactions with the microbiome and host immune system. More broadly, this work expands prevailing microbiome frameworks by placing eukaryotic microbes at the centre of host-microbiota crosstalk in health and disease.

Mapping Blastocystis in Spain: Molecular insights from a multicentre clinical study (2017–2019)

David Carmena, Spanish National Centre for Microbiology, Health Institute Carlos III, Spain

Background: Blastocystis is a highly prevalent enteric protist with debated clinical significance and substantial genetic diversity. In Spain, molecular epidemiological data from clinical settings remain limited and fragmented.

Methods: A prospective multicentre study was conducted between June 2018 and August 2019 across four tertiary hospitals in Spain. Stool samples testing positive for Blastocystis by routine diagnostic methods (conventional microscopy, PCR) were included. Sociodemographic and clinical data were retrieved from medical records. Molecular characterization was performed by PCR amplification of the SSU rRNA gene followed by Sanger sequencing for subtype identification.

Results: A total of 384 Blastocystis-positive samples were analysed, of which 307 (79.9%) were successfully subtyped. Molecular analysis identified seven distinct subtypes including ST1 (30.6%), ST3 (23.8%), ST2 (20.8%), ST4 (16.6%), ST6 (6.5%), ST7 (1.3%), and ST8 (0.3%). Human-associated subtypes ST1–ST4 accounted for 91.9% (282/307) of all typable isolates. Notable geographic variability in subtype distribution was observed across participating centres. Less frequent subtypes (ST6–ST8) were detected mainly in one hospital. Sociodemographic and clinical data were available for 331 patients, who were predominantly adults aged 25–64 years (48.9%), with a male/female ratio of 1.1. While 56.5% were Spanish-born, a substantial proportion originated from Latin America (%) and Africa (%). Diarrhoea (31.4%) and abdominal pain (26.0%) were the most frequently reported clinical manifestations, although 23.9% of patients were asymptomatic. Parasitic co-infections were common (24.2%), mainly involving intestinal protozoa including Giardia duodenalis (5.7%) and Dientamoeba fragilis (5.1%).

Conclusion: Blastocystis in clinical settings in Spain shows high subtype diversity dominated by ST1–ST4, with marked inter-centre variability. The frequent occurrence of asymptomatic cases and co-infections highlights the difficulty of attributing pathogenicity. These findings underscore the need for standardized, large-scale studies integrating molecular, clinical, and microbiome data to clarify the epidemiological and clinical role of Blastocystis.

Gut feelings: How diet influences Blastocystis colonization and diversity

David Carmena, Spanish National Centre for Microbiology, Health Institute Carlos III, Spain

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Unravelling the Role of Blastocystis in Colorectal Cancer Development

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Blastocystis is one of the most prevalent intestinal protists worldwide and is frequently identified in the Colombian population. Although its role as gastrointestinal pathogen remains controversial, persistent colonization of the large intestine has led to speculation regarding its potential involvement in colorectal cancer (CRC) development through inflammation or dysbiosis-related mechanisms. Several case-control studies have reported an association between Blastocystis faecal carriage and CRC; however, most of them present important methodological limitations, including insufficient sample sizes, lack of appropriate matching between cases and controls, heterogeneous study populations, or reliance on microscopy for detection purposes without molecular confirmation.

To critically address this controversy, we designed a robust case-control study in a Colombian population, aiming to maximize the sample size, up to 308 participants (154 cases and 154 controls), matched by sex and age in a 1:1 ratio. To ensure population homogeneity, both individual and population level ancestral genetic composition were evaluated. Blastocystis detection was performed using microscopy and molecular methods, including PCR and NGS.

No statistically significant differences were observed in Blastocystis frequency between cases and controls, either by microscopy (36.4% vs. 32.5%; p=0.472) or molecular methods (35.7% vs. 36.4%; p=0.906). Likewise, no significant differences were identified in the distribution of any of the 11 Blastocystis subtypes between cases and controls. Although substantial intra-subtype variability was observed in both absolute and relative terms, the distribution of unique variants of a particular subtype was remarkably homogeneous between cases and controls, and therefore no intra-subtype variants associated with CRC could be identified.

These findings, supported by a rigorous and thorough study design, allow us to conclude definitively that there is no association between faecal carriage of Blastocystis, whether at the species, subtype, or intra-subtype variant level, and the development of CRC.

Occurrence and molecular characterization of Blastocystis sp. isolates in dairy cattle from northern Italy: insights from a gastrointestinal parasite survey

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Blastocystis sp. is an intestinal protist commonly detected in humans and animals, with increasing attention toward its zoonotic potential and epidemiological role in livestock systems.

The present study investigated its occurrence and subtype characterization in a dairy cattle farm located in northern Italy, evaluating associations with other gastrointestinal parasites and assessing the possible zoonotic implications of the detected subtypes.

A total of 109 Holstein-Friesian cattle belonging to different production categories were sampled. Fecal samples were analyzed by the FLOTAC® technique for copromicroscopic detection of parasites and by molecular assays. For Blastocystis sp., PCR amplification and sequencing of the SSU-rDNA gene were performed (Scicluna et al., 2006). In addition, Cryptosporidium spp. and Enterocytozoon bieneusi were investigated using previously described nested PCR protocols (Xiao et al., 1999; Buckholt et al., 2002).

Blastocystis sp. was detected in 21/109 animals (19.3%). Preliminary phylogenetic analysis showed that the obtained sequences clustered with reference sequences of the subtypes ST2 and ST3, which are among the most frequently reported subtypes in humans. None of the Blastocystis-positive animals tested positive for Cryptosporidium spp. or Enterocytozoon bieneusi. In contrast, five Blastocystis-positive cattle were co-infected with Eimeria spp., the most frequently detected parasite overall (50/109; 45.9%). Molecular analysis identified two Cryptosporidium-positive samples (C. parvum and C. bovis) and three Enterocytozoon bieneusi-positive samples.

These preliminary findings provide new evidence of the circulation of Blastocystis sp. isolates in Italian dairy cattle and support the role of cattle as reservoirs of subtypes with potential zoonotic relevance. Further studies involving larger sample sizes and additional farms are needed to better clarify transmission dynamics and zoonotic risk.

The Blastocystis Biobank: a curated, multi-host biological resource of cultures, nucleic acids, metabolites, and stool-derived aliquots with a controlled-access virtual catalogue for global research

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Background: Blastocystis is among the most prevalent gut-associated microbial eukaryotes in humans and animals worldwide, yet reproducible cross-laboratory research remains hampered by the absence of widely accessible, well-annotated reference materials. Subtype (ST) diversity spanning at least 45 recognised STs (ST1–ST17, ST21, ST23–ST49), methodological heterogeneity in detection, culture, and nucleic-acid extraction, and the dispersal of valuable biological materials across individual laboratories represent ongoing obstacles to comparative analyses, assay benchmarking, and inter-laboratory reproducibility.

Methods: We established a physical collection and a linked virtual catalogue at the University of Kent. The collection holds cryopreserved xenic and axenic Blastocystis cultures spanning multiple STs and host categories, DNA extracts and, for a subset of accessions, matched RNA extracts, prepared either from culture or directly from faecal material, and stool-derived aliquots from both human and animal donors, at present predominantly of animal origin. Each deposit is assigned a persistent accession identifier linking all associated physical assets, subtype assignments with an explicit confidence level and mixed-colonisation flags, contamination screening outcomes, and quality-control metrics. The virtual catalogue provides a harmonised, versioned metadata framework for discovery and for controlled access under a material transfer agreement, with tiered visibility that accommodates ethical, permit-related and depositor constraints.

Data Available: Physical materials are available on request through the University of Kent Biobank Coordinator, subject to a material transfer agreement. The virtual catalogue and its metadata schema are versioned and citable. Contact details and access procedures are provided in this manuscript.

Reuse Potential: The resource supplies standardised, well-annotated reference materials for Blastocystis research. It is intended to support cross-laboratory comparison, assay benchmarking and inter-laboratory ring trials spanning a range of subtypes, hosts and methods.

Bacterial and fungal biomarkers in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD): trans-kingdom interactions, Blastocystis carriage, and enterotype–succinotype stratification

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The gut microbiome, encompassing bacteria, fungi, and protists, is integral to gastrointestinal (GI) health and disease. While bacterial dysbiosis is well documented in irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD), fungal contributions and bacterial-fungal interactions remain underexplored, particularly in non-Western populations. We profiled bacterial (16S rRNA) and fungal (ITS) communities from stool samples of Saudi participants with IBS, ulcerative colitis (UC), Crohn's disease (CD), and controls. Analyses incorporated bacterial enterotypes, succinotypes, and Blastocystis carriage to explore ecological and metabolic stratification. Differences in community composition were largely explained by GI disorder, host factors, enterotype, and Blastocystis carriage, accounting for 1–9% of bacterial and fungal variation. IBS patients exhibited increased bacterial richness and evenness, whereas UC and CD displayed stronger fungal dysbiosis. Enterotyping identified two Bacteroides-dominant clusters: Bact1 (with Faecalibacterium and Alistipes) and Bact2 (enriched in Escherichia-Shigella and Streptococcus), with Bact2 linked to reduced diversity and elevated calprotectin. Succinotyping distinguished Dialister-dominant (D-type) and Phascolarctobacterium-dominant (P-type) communities; UC was enriched in fragile, bridge-dependent D-type networks, in contrast to resilient, short-chain fatty acid (SCFA)- and H2S-producing P-type networks. Blastocystis was detected in 48% of participants (mainly subtypes 1 (ST1) and ST3). It was associated with enrichment of butyrate-producing bacteria (Roseburia, Anaerobutyricum, Butyribacter) and distinct fungal genera, with subtype-specific signatures (ST1 enriched in Alistipes, ST3 in Escherichia-Shigella). Disease-associated biomarkers included UC-linked depletion of butyrate producers and enrichment of Cutibacterium, Comamonas, and Dialister; depletion of Clostridia UCG-014 in CD; and enrichment of fermentative fungi (Candida, Meyerozyma) and bacteria (Coprococcus, Catenibacterium) in IBS. Cross-domain networks were resilient and modular in controls and IBS but fragile in UC and intermediate in CD. These findings highlight trans-kingdom interactions and Blastocystis carriage as ecological features of the gut microbiome in IBS and IBD, with enterotype-succinotype stratification offering complementary biomarkers for disease classification in a non-Western cohort.

Knowledge and awareness of Blastocystis spp. among graduate nursing students at a Croatian university: a cross-sectional survey analysis

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Background: Although Blastocystis spp. is one of the most frequently detected intestinal protozoa worldwide, its clinical significance remains controversial, while awareness among healthcare professionals is often limited (which is especially pertinent for nursing professionals). To our knowledge, this is the first study that aimed to assess the knowledge and awareness of Blastocystis spp. among graduate nursing students.

Materials and Methods: A cross-sectional anonymous survey was conducted among students enrolled in the Master of Nursing study programme at University North in Croatia. A total of 113 students participated, including 62 first-year and 51 second-year graduate students. All participants had previously completed undergraduate microbiology course as part of their prior education. The questionnaire assessed knowledge regarding transmission, epidemiology, pathogenicity, diagnosis and treatment of Blastocystis spp. Descriptive statistics and chi-square testing were performed, with $p<0.05$ (two-tailed) considered statistically significant.

Results: Overall awareness of Blastocystis spp. was low. Less than half of students correctly identified Blastocystis spp. as an intestinal protozoan (40.7%); moreover, many respondents were uncertain regarding its pathogenic potential, asymptomatic carriage and indications for treatment. Knowledge of diagnostic methods was particularly limited, with only a minority recognizing stool microscopy (28.3%) as the standard diagnostic approach. Second-year students achieved slightly higher knowledge scores than first-year students, although the difference was not statistically significant ($\chi^2=1.842$, $p=0.175$). Similarly, no significant differences in overall awareness were observed between male and female students ($\chi^2=0.964$, $p=0.326$). A large majority of students stated that additional education on Blastocystis spp. and intestinal parasitology is needed within nursing curricula (92.9%).

Conclusions: In this monocentric cross-sectional study, graduate nursing students demonstrated insufficient awareness and important knowledge gaps regarding Blastocystis spp., despite previous microbiology education. Greater emphasis on intestinal parasitology and clinically relevant microbiology topics within nursing curricula may improve future healthcare professionals' diagnostic understanding and clinical decision-making.

Changing epidemiology of Blastocystis spp. In the Republic of Croatia: comparison of nationwide stool sample analyses from 2022 and 2025

Mario Sviben, Croatian National Institute of Public Health

Background: The epidemiology and clinical significance of Blastocystis spp. remain controversial, particularly regarding its association with gastrointestinal symptoms. This study evaluated the prevalence of Blastocystis spp. in Croatia using nationwide data from 2025 and compared the findings with a previously analyzed 2022 cohort.

Materials and Methods: A total of 9,749 stool samples collected across Croatia during 2025 were analyzed using routine microscopic methods. The study population included 4,095 male and 5,654 female participants. Results were compared with a 2022 cohort comprising 2,560 stool samples. Statistical analyses were performed using chi-square testing in R software, with $p<0.05$ (two-tailed) considered statistically significant.

Results: In 2025, Blastocystis spp. was detected in 438/9,749 stool samples, corresponding to an overall prevalence of 4.49%. This represented a significant decrease compared to the 7.27% prevalence observed in 2022 (186/2,560; $\chi^2=31.82$, $p<0.001$). Positivity rates did not differ significantly between male and female participants in 2025 (4.30% vs. 4.63%, respectively; $p=0.459$), consistent with findings from 2022 (7.93% vs. 6.67%; $p=0.218$). Age-related trends remained evident in both cohorts. In 2022, positivity increased significantly with age, from 4.24% in children aged 0–18 years to 14.26% in individuals aged ≥65 years ($\chi^2=50.292$, $p<0.001$). In 2025, adults aged 19–64 years accounted for 50.0% of all positive samples, followed by individuals aged ≥65 years (24.9%) and children aged 0–18 years (25.1%). Asymptomatic individuals showed significantly higher positivity rates than symptomatic patients in both study periods. In 2025, positivity was observed in 6.20% of asymptomatic individuals compared to 3.10% of symptomatic patients. Similar findings were observed in 2022 (10.54% vs. 5.04%; $\chi^2=22.1315$, $p<0.001$).

Conclusions: The prevalence of Blastocystis spp. in Croatia decreased significantly between 2022 and 2025, while asymptomatic carriage remained common, supporting the uncertain pathogenic role of this organism.

What does not work in Blastocystis research: a community-sourced inventory of pitfalls and failed experiments

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Blastocystis sp. is the most prevalent intestinal eukaryote of humans, yet its biology has resisted many of the cellular, molecular, and microbiological tools that protistologists apply routinely to related model systems. Researchers new to the field frequently underestimate how often established protocols fail when applied to Blastocystis, and negative results rarely reach publication. The consequence is that the same dead-end experiments are repeated across laboratories, and reviewers routinely request controls that the community has shown, in practice, to be unworkable. This Perspective consolidates the collective experience of twelve laboratories working on Blastocystis to produce a critical, evidence-based inventory of what does not work. We describe recurrent failures in culturing and axenization; in bacterial-load reduction by filtration and density gradients; in flow-cytometric sorting; in sample preservation and DNA extraction; in nested and barcoding PCR-based detection and subtyping; in housekeeping-gene selection for expression studies; in fixation for downstream cell biology; and in the interpretive limits of amplicon-based next-generation sequencing (NGS) surveys performed without culture or microscopy support. Recent progress in genetic tools, notably the transient transfection protocol for ST7, is acknowledged, but a routine knock-down or CRISPR toolkit remains absent. We close with recommendations for authors, reviewers, and funders, and identify community priorities for the next five years, including the construction of a curated, full-length 18S rRNA reference alignment. The aim is to spare newcomers from predictable dead ends and to provide a citable

reference for authors defending their manuscripts against reviewer requests that the field has learned are not realistic.

Blastocystis occurrence and subtype diversity in a cohort of individuals with HIV, HCV, and HIV/HCV coinfection in Latvia

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Background and Aim. People living with human immunodeficiency virus (HIV) exhibit well-documented alterations in gut microbiota composition, with similar changes also reported in patients with chronic hepatitis C virus (HCV) infection. However, data on Blastocystis, a protist with debated opportunistic potential, remain limited. This study aimed to assess the occurrence and to characterise the genetic diversity of Blastocystis in a cohort of individuals with HIV and/or HCV infection in Latvia.

Materials and methods. The study enrolled individuals with HIV (n = 17), HCV (n = 10), and HIV/HCV coinfection (n = 11) hospitalised at Riga East Clinical University Hospital (Latvia); DNA was isolated from faecal samples. Blastocystis was detected by polymerase chain reaction targeting a 300 bp fragment of the small subunit ribosomal RNA (SSU rRNA) gene, and positive samples were genotyped by Sanger sequencing. Sequence analysis and subtype assignment were performed using MEGA6, FinchTV, and BLAST.

Results. Overall, Blastocystis was detected in 2 of 17 individuals with HIV, in 4/10 with HCV, and in 0/11 with HIV/HCV coinfection. All sequences exhibited ≥98% identity to Blastocystis SSU rRNA reference sequences in GenBank. Sequence analysis identified three subtypes: ST3 (4/6, 66.7%), ST2 (1/6, 16.7%), and ST1 (1/6, 16.7%); no mixed cases were detected.

Conclusions. The obtained results are consistent with earlier reports from European populations regarding circulating Blastocystis subtypes, with ST3 as the predominant subtype in this specific cohort. Given the absence of data on gastrointestinal signs and symptoms, further studies are warranted to clarify the clinical relevance of these findings.

BLASTOCYSTIS SP. IN URBAN WILD BOARS INHABITING NORTHERN POLAND

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Blastocystis is a protozoan frequently present in the gastrointestinal tract of both humans and animals. It shows unclear pathogenicity and exists in over 30 genetic subtypes. The aim of the study was to determine the frequency of Blastocystis occurrence in a specific urban population of wild boars living in the region of Northern Poland and to investigate its molecular structure.

Fecal samples were collected from 118 wild boars during necropsy. These animals died naturally or were hunted as part of a planned cull. The material was analyzed using nested PCR and Sanger sequencing to specifically detect Blastocystis DNA and determine its genotypes.

Presence of Blastocystis was confirmed in 37 (31.35%) samples analysed. ST3 subtype infection predominated. It accounted for 91.89 % of the positive results followed by the ST5 (8.11 %). There was no statistically significant difference in the number of ST3 subtype infections in relation to animals sex (p=0.508) or age (p=0.956).

The results of this study confirm that the wild boars studied harbor Blastocystis subtypes frequently found in humans, as well as those primarily found in farm pigs. This is due to the genetic proximity of wild boars and farm pigs on the one hand, and the fact that areas inhabited by humans also become natural habitats for wild boars on the other hand. These results seem to support the thesis of anthroponotic transmission, meaning that the ST3 subtype is acquired by animals through contact with humans.

A multi-omics analysis of Blastocystis ST3 in xenic culture

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Despite being the most commonly found subtype in humans, Blastocystis ST3 is one of the many subtypes that has not been successfully axenised in vitro. A previous preliminary study observed that the bacterial composition and the metabolite profile for xenic ST3 was distinct from other xenic subtypes maintained in culture. Here, a further investigation was carried out to observe changes in prokaryotic composition and metabolic activity, with the addition of gene expression via transcriptomics, using 16S-amplicon sequencing, ^{1}H-NMR metabolomics and mRNA sequencing, to observe microenvironmental changes across six days in static culture. Alpha and beta diversity showed that there was no significant change in microbial composition between timepoints, and the metabolite profile also remained fairly stable, although there is a clear depletion of sugars from the media at the later timepoints. Analysis of the Blastocystis transcriptome showed upregulation of gene expression related to mitochondrial activity towards the end of the culturing period. These together are suggestive of a shift from energy acquisition via nutrient scavenging in fresh media to internal mitochondrial processes as nutrients become depleted. These methodologies can be extended to other STs of Blastocystis to understand its behaviour in culture and better inform further in vitro investigations.