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STUDY PROTOCOL

Relationship between *Blastocystis* infection and clinical outcomes: A scoping review protocol

[version 1; peer review: 4 approved]

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Abstract





*Blastocystis*, a common protist in the human gastrointestinal tract, exhibits substantial genetic diversity and has been linked to varying clinical outcomes. However, its role in human health remains debated, with studies suggesting both commensal and pathogenic interactions. This scoping review aims to systematically map the existing evidence on the association between *Blastocystis* presence and human clinical outcomes. Herein, we present our proposed protocol, where, using systematic search methods, studies will be identified from multiple databases, focusing on diagnostic procedures, clinical outcomes, and treatment options. Findings will provide a comprehensive evidence map, highlighting knowledge gaps and guiding future research. The resulting data is intended to inform clinical and public health perspectives on *Blastocystis* and its potential implications for human health.




Plain language summary

*Blastocystis*, a common protist in the human gastrointestinal tract, exhibits substantial genetic diversity and has been linked to varying clinical outcomes. However, its role in human health remains debated, with studies suggesting both commensal and pathogenic interactions. This scoping review aims to systematically map the existing evidence on the association between *Blastocystis* presence and human clinical

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Approval Status    

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version 1				
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Any reports and responses or comments on the article can be found at the end of the article.

outcomes. Herein, we present our proposed protocol, where, using systematic search methods, studies will be identified from multiple databases, focusing on diagnostic procedures, clinical outcomes, and treatment options. Findings will provide a comprehensive evidence map, highlighting knowledge gaps and guiding future research. The resulting data is intended to inform clinical and public health perspectives on Blastocystis and its potential implications for human health

### Keywords

Gut microbiota, Diagnostic methods, Subtype diversity, Intestinal health



This article is included in the [COST Actions gateway](#).

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**Author roles:** **Tunali V:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Nagavci B:** Investigation, Methodology, Project Administration, Software, Supervision, Validation, Writing – Review & Editing; **Carmena D:** Formal Analysis, Validation, Writing – Review & Editing; **Robertson LJ:** Formal Analysis, Visualization, Writing – Review & Editing; **Doğruman AI F:** Data Curation, Formal Analysis, Funding Acquisition, Supervision, Visualization, Writing – Review & Editing; **Gentekaki E:** Formal Analysis, Funding Acquisition, Resources, Supervision, Writing – Review & Editing; **Tsaousis AD:** Formal Analysis, Funding Acquisition, Resources, Supervision, Visualization, Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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## Introduction

*Blastocystis*, a protist commonly found in the human gastrointestinal tract, is one of the most prevalent eukaryotic microorganisms globally<sup>1</sup>. It exhibits significant genetic diversity, with at least 44 subtypes (STs) identified to date based on sequence analyses of the small subunit ribosomal RNA (SSU rRNA) gene<sup>2,3</sup>. Among these, ST1-ST10, ST12, ST14, ST16, ST23, ST35, and ST41 have been reported from humans, with ST1 to ST4 being the most frequently identified<sup>4,5</sup>. The interaction of *Blastocystis* with the gut microbiota and the host immune system has been linked to a range of clinical outcomes, highlighting its potential relevance to human health<sup>6</sup>.

*Blastocystis* is distributed worldwide, with varying occurrence rates across different regions. It has been reported globally, with variations likely influenced by differences in sanitation and hygiene practices<sup>7</sup>. *Blastocystis* is found to be present in both healthy individuals and those experiencing gastrointestinal symptoms. Some studies have reported associations between symptoms such as diarrhea, abdominal pain, bloating, and the presence of *Blastocystis*<sup>8</sup>. However, these associations are inconsistent across studies, and the public health significance of *Blastocystis* remains uncertain, primarily due to conflicting evidence regarding its role in health and disease.

The clinical significance of *Blastocystis* has been a topic of ongoing debate. Historically considered a parasitic organism, some studies have questioned whether certain subtypes or host interactions might contribute to health outcomes in specific contexts<sup>9</sup>. The genetic diversity of *Blastocystis* adds complexity, with some subtypes being more frequently detected in symptomatic individuals, while others are commonly found in asymptomatic populations. The interplay between *Blastocystis*, the host immune response, and the gut microbiota is believed to play a critical role in determining its impact on human health<sup>10</sup>.

The existing literature presents diverse perspectives on *Blastocystis*. Some studies suggest that *Blastocystis* presence may be associated with beneficial changes in the gut microbiota, such as increased bacterial diversity and anti-inflammatory responses<sup>8</sup>. Conversely, some studies report that *Blastocystis* may be associated with reduced beneficial bacteria and an altered gut microbiome composition<sup>11</sup>. Given the conflicting evidence regarding the pathogenicity of *Blastocystis*, a comprehensive scoping review is warranted to systematically map the existing evidence. This review will synthesize original research studies on human subjects, focusing on the association between *Blastocystis* presence and various clinical outcomes.

## Objectives

The primary objective of this scoping review is to systematically map the existing evidence on *Blastocystis* presence and clinical outcomes in humans. This review will help clarify the current state of knowledge, identify research gaps, and

provide a foundation for future studies that can further explore this relationship. The following research questions will be assessed:

1. What evidence exists on the relationship between *Blastocystis* occurrence and human clinical outcomes?
2. What evidence exists regarding the diagnostic methods used for *Blastocystis* detection, and their performance and accuracy?
3. What evidence is available on the treatment and management options for *Blastocystis*?

## Methods

This scoping review will be conducted and reported in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) extension for scoping reviews (PRISMA-ScR)<sup>12</sup>.

### Literature searches

Systematic searches for relevant published studies will be conducted in PubMed, EMBASE, Web of Science, and the Cochrane Library. An experienced methodologist will develop and validate search strategies to ensure maximum sensitivity. Searches will be performed without time restrictions, and the date of the search, search strategy, and number of search results will be documented for each database.

### Selection of studies

The selection process will involve two stages: screening and eligibility assessment. Titles and abstracts will be screened by two independent reviewers to identify potentially relevant studies, using Rayyan. Full-text articles of studies deemed relevant will be retrieved and assessed for eligibility based on predefined criteria, by two reviewers. Disagreements will be resolved through discussion or by consulting a third reviewer. Screening results and reasons for exclusion will be documented in PRISMA flowcharts.

### Eligibility criteria

To be included in this scoping review, studies must involve human participants with confirmed (or, for the diagnostic question, suspected) *Blastocystis* infection. To be a confirmed case, the infection must be diagnosed using reliable methods such as direct microscopy, culture, conventional and qPCR, or next-generation sequencing (NGS). Studies that primarily focus on other parasitic infections, where *Blastocystis* is not the main focus, will be excluded.

Studies that include both symptomatic and asymptomatic individuals with *Blastocystis* infection will be eligible, as this should assist in providing a comprehensive understanding of the organism's potential pathogenicity and its association with various clinical outcomes (Table 1).

**Table 1. Inclusion and exclusion criteria for each research question.**

	Research Question	Population	Exposure	Comparator	Outcomes	Study Design	Exclusion criteria
1	What evidence exists on the relationship between <i>Blastocystis</i> infection and clinical outcomes, and pathogenicity in humans?	Humans of all ages	<i>Blastocystis</i> infection	No <i>Blastocystis</i> infection	All reported clinical outcomes (e.g., gastrointestinal/extraintestinal symptoms, pathogenicity etc.)	Cross-sectional, cohort studies, case-control studies, prevalence studies, systematic reviews.	<b>Study Type:</b> Editorials, letters, commentaries, conference abstracts, case series, case reports, clinical guidelines, narrative reviews (though reference lists will be screened), and non-peer-reviewed works (e.g., theses, preprints). <b>Subject:</b> Animal studies or research conducted solely in non-human settings (e.g., laboratory/animal models). <b>Confirmation:</b> Studies where <i>Blastocystis</i> infection is neither confirmed nor suspected. Results: Studies reporting combined treatment outcomes without <i>Blastocystis</i> -specific results <b>Focus:</b> Studies where <i>Blastocystis</i> is not the main focus, including those primarily addressing other parasitic infections or treatments not targeting <i>Blastocystis</i> specifically. <b>Language:</b> Except English, Greek, Spanish, German, French, Norwegian, Danish, Russian, and Turkish.
2	What evidence exists regarding the diagnostic methods used for <i>Blastocystis</i> diagnosis, and their performance?	Humans of all ages suspected of <i>Blastocystis</i> infection	Index Test	Reference Test	Outcomes	Study Design	
			Diagnostic methods (e.g., microscopy, culture, molecular diagnostics)	Any reference standard or another diagnostic method	All reported test accuracy outcomes.	Diagnostic accuracy studies, clinical trials, systematic reviews.	
3	What evidence is available on the treatment options for <i>Blastocystis</i> infection, and how effective are these interventions in managing the infection and associated symptoms?	Humans of all ages with confirmed <i>Blastocystis</i> infection	Intervention	Comparator	Outcomes	Study Design	
			Any treatment for <i>Blastocystis</i>	No treatment, placebo, or alternative strategies	All reported outcomes (e.g. eradication of <i>Blastocystis</i> , symptom resolution etc.)	Clinical trials, cross-sectional, cohort studies, case-control studies, systematic reviews.	

Studies published in all languages spoken by the panel members will be considered (English, Greek, Spanish, German, French, Norwegian, Danish, Russian, and Turkish), in order to maximize data capture.

Eligible study types include randomized and non-randomized trials, cohort studies, case-control, systematic reviews, and cross-sectional studies (with or without a control group). Editorials, letters, commentaries, conference abstracts, case series, case reports, clinical guidelines, narrative reviews, and non-peer-reviewed work (e.g. theses), will be excluded. Narrative review articles will not be included, but their reference lists will be explored for further data sources (Table 1).

No date limit will be used for the inclusion criteria, in order to provide a full evidence map on this topic.

### Data extraction

Data from included studies will be charted using a standardized data extraction form. The form will be piloted to ensure consistency and reliability. Data extraction will be performed independently by two reviewers, with cross-checking to resolve discrepancies. Additional data or clarifications will be sought from study authors when required.

### Data items

The data items to be extracted will include:

- Study Characteristics: Author, year of publication, study type, design, setting, and geographical location.
- Population Details: Number of participants, age, gender
- Details of *Blastocystis* Infection: Detection methods used, subtype identification, presence or absence of symptoms.
- Clinical Outcomes: All reported symptoms (including gastrointestinal, dermatologic etc. symptoms),

associations with *Blastocystis* subtypes (if reported), any given treatment and treatment outcome, and any other clinically relevant outcome

- Subgroups: studies will be presented in subgroups based on study design and patient population.

### Results

The results section will describe the evidence of the relationship between *Blastocystis* infection and clinical outcomes, variations in outcomes based on diagnostic methods, and the pathogenicity of specific *Blastocystis* subtypes. Identified and included studies will be presented in both tabular and graphical forms, offering a complete evidence map on this topic. Research gaps will also be identified, and recommendations for future studies will be provided.

### Critical appraisal of individual sources of evidence

As critical appraisal is not a mandatory step in scoping reviews, it is not planned to be conducted.

### Discussion and conclusions

The findings of this scoping review will provide a comprehensive synthesis of the current evidence on *Blastocystis*'s potential associations with clinical outcomes. Despite its high prevalence and global distribution, *Blastocystis* remains poorly understood, with conflicting perspectives regarding its clinical relevance<sup>13</sup>.

In conclusion, this scoping review aims to serve as a foundational resource for researchers and clinicians, guiding the design of targeted studies and providing a robust evidence base for *Blastocystis* research.

### Ethics and consent

Ethics and consent were not required.

### Data availability statement

No data are associated with this article

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# Open Peer Review

Current Peer Review Status:    

## Version 1

Reviewer Report 03 March 2025

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**Renzo S Salazar-Sánchez** 

Heidelberg University Hospital Institute of Medical Biometrics (Ringgold ID: 222689), Heidelberg, Baden-Württemberg, Germany

This study protocol, entitled "Relationship between *Blastocystis* Infection and Clinical Outcomes: A Scoping Review Protocol," is a well-structured and meticulously designed proposal. It outlines a standardized approach to conducting a systematic review across multiple databases, with a particular focus on *Blastocystis*, a globally distributed protozoan whose role in human health remains uncertain. The primary objective of this review is to establish a comprehensive understanding of the existing evidence concerning its clinical implications in humans. This proposal aims to address three critical research questions supported by scientific evidence related to:

1. The association between *Blastocystis* occurrence and human clinical outcomes.
2. The diagnostic methodologies employed for *Blastocystis* detection, including their performance and accuracy.
3. The availability and efficacy of treatment and management strategies for *Blastocystis* infections.

Given the controversial nature and complex biological characteristics of *Blastocystis*, it is imperative to synthesize and critically appraise the available literature. This will facilitate a comprehensive and evidence-based understanding of its clinical significance.

### Methodological Considerations

The proposed protocol delineates a logical and well-defined workflow, incorporating key methodological criteria to ensure the quality and reliability of the findings. However, I would like to highlight two potential limitations that were not explicitly addressed in the methodology and could introduce bias into the conclusions:

1. Literature Search Strategy: The authors indicate that systematic searches will be conducted using PubMed, EMBASE, Web of Science, and the Cochrane Library. To enhance the breadth and comprehensiveness of the review, I suggest including Scopus and SciELO to provide broader coverage, particularly for Latin American and regional studies.
2. Eligibility Criteria: The inclusion criteria define eligible studies as those involving human participants with confirmed *Blastocystis* infection, irrespective of symptomatology (symptomatic or

asymptomatic). However, the protocol does not clearly outline how studies involving multiple *Blastocystis* subtypes, cases of mixed infections, or coinfections with other commensal protozoa will be managed. This is particularly relevant given the prevalence of coinfections reported in Latin American studies. Such polyparasitic infections can contribute to symptomatology, making it essential to address this aspect explicitly to avoid misinterpretations in the findings.

Table 1 provides a clear and coherent summary of the study design and key points.

#### Conclusion

In conclusion, this study protocol represents a valuable and well-founded initiative to address a complex and debated issue regarding *Blastocystis*. Given its global distribution and potential clinical relevance, a rigorous and systematic literature assessment is essential.

Therefore, I endorse this study protocol and recommend its approval, with the suggested refinements to further strengthen its methodological robustness.

#### Is the rationale for, and objectives of, the study clearly described?

Yes

#### Is the study design appropriate for the research question?

Yes

#### Are sufficient details of the methods provided to allow replication by others?

Yes

#### Are the datasets clearly presented in a useable and accessible format?

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Infectious diseases, Molecular biology, Microbiome

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 26 February 2025

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**Supaluk Popruk**

Mahidol University, Bangkok, Thailand

This study offers valuable information for conducting a systematic review and meta-analysis. Additionally, the methodology outlined can guide future studies in replicating or expanding upon the results. Furthermore, the study can serve as a foundation for evidence-based data, which can inform future research directions and can be the guidelines for other parasites or pathogens. The

article exhibits a notable weakness in that it lacks a clear delineation of participant types, such as children, villagers, individuals with close contact to animals, and caregivers. The inclusion of these distinct groups may have led to different outcomes.

However, the strengths of this article are guidelines of the related research in systematic review and meta-analysis.

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** I work on parasitology.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 25 February 2025

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**Arutchelvan Rajamanikam** 

University Malaya, Kuala Lumpur, Malaysia

The article is timely to systematically review the literature on the complex and controversial subject of clinical outcome due to *Blastocystis*. This scoping review is concise and all encompassing. However, there could be more evidence on pathogenic characteristics in *Blastocystis*, based on previous studied could have been included. Otherwise, the write-up is clear and serves its purpose.

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Diagnostic Parasitology, Host-parasite interaction

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Reviewer Report 14 February 2025

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**Małgorzata Lepczyńska** 

University of Warmia and Mazury, Żołnierska, Poland

The manuscript is written clearly and is easy to understand. It presents a scoping review protocol, focusing on literature searching strategy for clarifying the current state of knowledge, identifying research gaps, and guiding future research in understanding the relationship between *Blastocystis* presence and clinical outcomes in humans.

The introduction of the manuscript highlights the complexity and analytical challenges of determining *Blastocystis* pathogenicity as well as the sensitivity of detection methods in order to address the conflicting evidence regarding symptomatic and asymptomatic human intestine colonization. The authors underline the high genetic diversity of *Blastocystis*—up to 44 subtypes (STs). Each subtype may have a different, direct or indirect, influence on human health (e.g., through various interactions with the gut microbiota). The interplay between *Blastocystis* subtypes, the host immune response, and the gut microbiota is believed to play a critical role in determining its impact on human health.

According to the available literature, it is impossible to state clearly whether *Blastocystis* is a pathogen or a commensal/beneficial organism to the human gut. In the paper, the authors provided a protocol for conducting a scoping review to assess which data should be taken into consideration in case of performing a review of all accessible studies on human subjects, focusing on the association between *Blastocystis* presence and various clinical outcomes.

The scoping review protocol clearly describes and explains the purpose of the selection process of

published research regarding the evidence on the relationship between *Blastocystis* occurrence and human disease, the performance and accuracy of diagnostic methods used for *Blastocystis* detection, and the treatment and management options for *Blastocystis*. The manuscript presents the assumptions of the proposed protocol in an accessible and concise way, emphasizing the importance of the systematic search methods.

The protocol adequately explained, point by point, defined criteria of inclusion and exclusion of published articles, essential for the given objectives. I consider this protocol extremely important. There are a lot of scientific articles in the recent literature; however, many of them do not provide reliable results—basing on a small study population (or case reports), reporting the patients symptoms without any confirmation of a physician (as *Blastocystis* infection has been associated with non-specific gastrointestinal symptoms such as abdominal pain, diarrhea, nausea, vomiting, bloating, and anorexia, we actually are not able to say for sure if it is the only cause of reported symptoms), or studies where *Blastocystis* is not the main focus, targeting other parasitic infections and accidentally detecting *Blastocystis*, etc. Additionally, the papers containing the methodology of *Blastocystis* detection, which is based on microscopy, culture, or ELISA tests only, in times of developed molecular techniques giving the subtype distribution results, are not enough. The authors rightly highlight the crucial importance of findings that will provide a comprehensive evidence map, providing future study in understanding the nature of *Blastocystis* (helping to unravel what factors determine asymptomatic colonization or symptomatic disease), thereby clearly demonstrating the appropriateness of creating a schedule of literature searching.

The authors pointed to the databases that will be searched and clearly defined the studies selection process (screening and eligibility assessment by two or three reviewers), as well as the eligibility criteria (in the table). At the end, the authors also clarify how the proposed protocol will identify and fill possible gaps in existing knowledge, and according to that, recommendations for future studies will be provided. As a result of the scoping review according to the created protocol, identified and included studies will be presented in two different (tabular and graphical) forms, offering a complete evidence map on the mentioned topic.

**Is the rationale for, and objectives of, the study clearly described?**

Yes

**Is the study design appropriate for the research question?**

Yes

**Are sufficient details of the methods provided to allow replication by others?**

Yes

**Are the datasets clearly presented in a useable and accessible format?**

Not applicable

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** medical parasitology (mainly *Blastocystis*), microbiology, medical biology, medical education

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

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