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OPEN LETTER

REVISED The Blastocystis-colorectal cancer hypothesis: correlation is not causation

[version 2; peer review: 2 approved, 1 approved with reservations]

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Abstract

Although superficially persuasive, claims suggesting a causal link between *Blastocystis* and colorectal cancer (CRC) lack robust scientific support. As *Blastocystis* is the most common gut protist found in human populations globally, its detection in CRC patients is unsurprising and does not imply pathogenicity. Current claims championing a causal role for *Blastocystis* in CRC are based on speculative correlations, a single poorly controlled animal study, and inconsistent subtype associations. We argue that linking *Blastocystis* to CRC is premature, misleading, and may give rise to unnecessary concern in patients that are colonised by or test positive for *Blastocystis.* We emphasise the need for rigorously designed investigations to establish causal roles for any microorganism in disease and the importance of conclusions being based on solid evidence, particularly in matters of public health.

Keywords

Blastocystis, colorectal cancer, gut microbiome, correlation, causation

This article is included in the COST Actions gateway.

Open Peer Review

Approval Status ✓ ✓ ?

	1	2	3
version 2 (revision) 22 Sep 2026			
version 1 10 Dec 2025	✓ view	✓ view	? view

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



This article is included in the Blastocystis collection.

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Author roles: Kurt Ö: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Scanlan PD**: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Gentekaki E**: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Robertson LJ**: Writing – Original Draft Preparation, Writing – Review & Editing; **Carmena D** : Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Dogruman-Al F**: Conceptualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Tsaousis AD**: Conceptualization, Funding Acquisition, Writing – Original Draft Preparation, Writing – Review & Editing

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First published: 10 Dec 2025, **5**:379 https://doi.org/10.12688/openreseurope.21693.1

REVISED **Amendments from Version 1**

In our current version we have responded to the useful and constructive feedback of the reviewers by making some edits to the main text. Specifically we acknowledge the important point they raise regarding how the environment may favor the growth of certain microbial species. As our understanding of specific abiotic and biotic factors that may support or inhibit *Blastocystis* growth are relatively limited in both health and disease context we feel that perhaps the most prudent approach here is to emphasise this point a little more strongly in the text and specifically outline the importance of q-PCR to future studies. We also have addressed their point on problems in morphological identification, terminology, and interpretation across studies by including new text highlighting how *Blastocystis* is a highly pleomorphic organism, that links between STs and pathogenicity is simply not known and how this might affect studies and data interpretation.

Any further responses from the reviewers can be found at the end of the article

As active members of the European COST (Cooperation on Science and Technology) Action *Blastocystis under One Health* (CA21105) we wish to express our concerns regarding several statements presented in the review "An Update on *Blastocystis*: Possible Mechanisms of *Blastocystis*-Mediated Colorectal Cancer" by Tocci, Das, and Sayed.[1] Based on our collective expertise in *Blastocystis* research, we wish to note that no comprehensive epidemiological, *in vitro* or *in vivo* data conclusively supports the assertions of Tocci *et al.*[1] who suggest that a direct causal relationship between *Blastocystis* infection or colonisation and the development of colorectal cancer (CRC) has been established. Establishing causation requires carefully designed and integrated mechanistic and epidemiological studies with appropriate controls. Such studies currently do not exist, and the claims in the review of Tocci *et al.*[1] rely heavily on speculative interpretations of limited studies that we discuss in brief below.

Blastocystis is the most common protist in the human gut globally. More than 45 subtypes have been identified; over 10 of these are found in humans, with ST3 the most prevalent globally.[2,3] Despite the first description of *Blastocystis* being published more than 110 years ago,[4] its clinical significance is still under debate.[5–7] According to the available data, when *Blastocystis* is detected in clinical cases it is primarily associated with gastroenterological and dermatological conditions that are related to both the host's immune status and other components of the gut microbiota.[8–11] Although some studies have reported an increased prevalence of *Blastocystis* among cancer patients,[12,13] these studies do not provide any evidence or mechanistic insights that would implicate a causal role for *Blastocystis* in CRC. For example, it could simply be that in CRC, alternations to the local environment, including the tumour microenvironment, which are associated with altered host physiology and immune function, as well as treatment-induced changes in gut ecology and broader dysbiosis, may create conditions that favour the colonisation and/or overgrowth of *Blastocystis.* This may explain an increased prevalence in cancer patients in some studies, however, future work should also include q-PCR assays to assess variation in *Blastocystis* abundance and potential overgrowth across different gut microenvironments and cohorts of interest. An association between *Blastocystis* and carcinogenesis has been shown in a rat model, where urinary oxidative indices correlated with carcinogenesis were shown to be higher in rats infected with *Blastocystis* than in non-infected rats.[14] However, to our knowledge, this is the only *in vivo* study reported in the literature and was conducted in laboratory rodents and not humans. The *Blastocystis* isolate used was ST3, the most common subtype occurring in humans, but not adapted to infect rodents, which typically harbour ST4. Most importantly, this isolate was obtained from a faecal sample of a non-symptomatic individual, concentrated on a density gradient and subsequently used to infect rats with the presence of other microbes (e.g. bacteria, viruses) in the concentrate not excluded.

Furthermore, the morphological forms presented in the study[14] appear to be inconsistent with recognised *Blastocystis* morphotypes. Given the marked pleomorphism of *Blastocystis,*[15] morphological variability may complicate reliable microscopic identification and classification,[16] potentially contributing to inconsistencies reported across studies rather than reflecting true biological differences. On this note, Tocci and colleagues[1] outline that certain *Blastocystis* subtypes are pathogenic. However, a detailed understanding of the genetic and phenotypic diversity of *Blastocystis* STs and their biological significance with respect to host health and disease is limited. Robust research data linking the disease-causing potential or pathogenicity of subtypes or morphotypes to CRC is simply not available. Based on the current literature, it is not possible to differentiate *Blastocystis* subtypes and/or strains as being either commensal or pathogenic, as contradictory study findings on *Blastocystis* subtypes and pathogenicity are widespread.[15–18]

Contrary to studies suggesting a role for *Blastocystis* in CRC, a recent study using state-of-the-art metagenomic approaches that presented data from the largest cohort studied to date (involving more than 56,000 individuals from 32 countries) demonstrated a negative correlation between CRC and *Blastocystis.*[19] More generally, a negative correlation between *Blastocystis* and other diseases that involve inflammation of the gastrointestinal tract e.g. ulcerative colitis and Crohn's Disease have been reported in independent studies.[20,21]

Conclusion

The history of microbial oncology, exemplified by *Helicobacter pylori* and gastric cancer[22,23] demonstrates that microbial involvement in cancer is possible. However, in those cases, causation was only established after decades of carefully controlled epidemiological, mechanistic, and clinical studies. For *Blastocystis*, such a body of evidence does not currently exist, and if we critically assess the evidence that is available at present, there is no credible basis for asserting that *Blastocystis* causes CRC in humans. The high prevalence of *Blastocystis* globally makes its detection in cancer patients unsurprising, and current studies provide, at best, speculative correlations. Importantly, premature claims of causation carry the risk of misleading clinicians, alarming patients, and diverting research away from more productive avenues. Furthermore, they undermine the nuanced view emerging from microbiome science,[24–27] where *Blastocystis* is often associated with gut health rather than disease. Future research may yet clarify the potential roles of *Blastocystis*, whether neutral, beneficial, or detrimental, but until such evidence exists, statements linking *Blastocystis* to cancer should be regarded as misleading and premature.

Ethics and consent statement

Ethical approval and consent were not required.

Disclaimer

The views expressed in this article are those of the author(s). Publication in Open.

Research does not imply endorsement by the COST ACTION funding body.

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 06 April 2026

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Major Concerns

1. **Expand Discussion on Reverse Causality**
   While the manuscript suggests that CRC-associated changes may explain *Blastocystis* prevalence, this point could be strengthened by:
   - Elaborating on tumor-associated changes (e.g., hypoxia, immune modulation, mucus alterations)
   - Discussing how these changes may facilitate opportunistic colonization
2. **Clarify Morphological and Subtype Arguments**
   The critique of morphological inconsistencies is important but could be clearer:
   - Specify whether inconsistencies arise from methodological differences or misclassification
   - Clarify whether morphotypes are biologically meaningful or artifacts
   - Avoid overinterpretation given ongoing debates in the field
3. **Address Quantitative vs Qualitative Detection**
   The manuscript focuses on presence/absence data but would benefit from discussing:
   - The importance of *Blastocystis* load (e.g., qPCR-based quantification)
   - Distinguishing colonization from overgrowth
   - Potential threshold effects relevant to disease associations
4. **Integration with Broader Microbiome Context**
   While briefly mentioned, the manuscript could further integrate:
   - Interactions between *Blastocystis* and bacterial communities
   - Whether *Blastocystis* may act as a marker of a "healthy" microbiome rather than a driver

Is the rationale for the Open Letter provided in sufficient detail? (Please consider whether existing challenges in the field are outlined clearly and whether the purpose of the letter is

explained)
Partly

Does the article adequately reference differing views and opinions?
Partly

Are all factual statements correct, and are statements and arguments made adequately supported by citations?
Yes

Is the Open Letter written in accessible language? (Please consider whether all subject-specific terms, concepts and abbreviations are explained)
Yes

Where applicable, are recommendations and next steps explained clearly for others to follow? (Please consider whether others in the research community would be able to implement guidelines or recommendations and/or constructively engage in the debate)
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Microbiome and colorectal cancer

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, however I have significant reservations, as outlined above.

Reviewer Report 27 March 2026

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Sonia H Navia
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The letter addresses the controversial relationship between the gut protist *Blastocystis* and colorectal cancer (CRC). It argues that the current claim of a direct link between both conditions still lacks a solid scientific basis. The authors appropriately highlight the high persistence of *Blastocystis* in healthy populations, which makes its detection in CRC patients not entirely unexpected, but not necessarily evidence of a pathogenic role. In this sense, the manuscript offers a useful and timely counterpoint to premature causal interpretations.
The authors clearly identify the main concern: the publication of a review (Tocci et al.) that

suggests mechanisms of causality without definitive evidence. They also explain well why this can be problematic, as it may unnecessarily alarm colonized patients. Importantly, the manuscript does not ignore studies that support the pathogenicity hypothesis, but instead contrasts them with evidence from large meta-analyses and calls for more rigorous mechanistic and epidemiological studies.

I do, however, have a few suggestions that could strengthen the manuscript:

- First, although CRC clearly changes the intestinal environment, the discussion could expand a bit more on how immunological, physiological, and morphological changes during cancer development may favor opportunistic colonization by *Blastocystis*. This would reinforce the idea that the association may be a consequence of the disease rather than its cause.
- Second, the authors criticize the inconsistent forms described in previous studies. This is an important point, but it would help to clarify whether this refers to problems in morphological identification, terminology, or interpretation across studies. Also, if the authors suggest that different forms may have distinct effects in the colon, that idea should be presented cautiously, since the biological relevance of several *Blastocystis* morphotypes is still debated.
- Finally, I would suggest that future studies should go beyond reporting simple PCR-based presence/absence and also include parasite burden, for example by qPCR. Given that the colon tumor microenvironment changes substantially, it is plausible that cancer may favor increased *Blastocystis* abundance without implying a causal role. In that sense, distinguishing colonization from possible overgrowth could help better frame the association and avoid overinterpretation.

Is the rationale for the Open Letter provided in sufficient detail? (Please consider whether existing challenges in the field are outlined clearly and whether the purpose of the letter is explained)

Yes

Does the article adequately reference differing views and opinions?

Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?

Yes

Is the Open Letter written in accessible language? (Please consider whether all subject-specific terms, concepts and abbreviations are explained)

Yes

Where applicable, are recommendations and next steps explained clearly for others to follow? (Please consider whether others in the research community would be able to implement guidelines or recommendations and/or constructively engage in the debate)

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer immunology, CRC microbiota

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.

Author Response 11 Jun 2026

Pauline Scanlan

Reviewer comments: The letter addresses the controversial relationship between the gut protist *Blastocystis* and colorectal cancer (CRC). It argues that the current claim of a direct link between both conditions still lacks a solid scientific basis. The authors appropriately highlight the high persistence of *Blastocystis* in healthy populations, which makes its detection in CRC patients not entirely unexpected, but not necessarily evidence of a pathogenic role. In this sense, the manuscript offers a useful and timely counterpoint to premature causal interpretations. The authors clearly identify the main concern: the publication of a review (Tocci et al.) that suggests mechanisms of causality without definitive evidence. They also explain well why this can be problematic, as it may unnecessarily alarm colonized patients. Importantly, the manuscript does not ignore studies that support the pathogenicity hypothesis but instead contrasts them with evidence from large meta-analyses and calls for more rigorous mechanistic and epidemiological studies.

Reply: Thank you for you very positive comments on our review and your constructive and important feedback. I do, however, have a few suggestions that could strengthen the manuscript:

1. First, although CRC clearly changes the intestinal environment, the discussion could expand a bit more on how immunological, physiological, and morphological changes during cancer development may favor opportunistic colonization by *Blastocystis*. This would reinforce the idea that the association may be a consequence of the disease rather than its cause.

Reply: This is an important point, and it is likely that changes in the disease intestine relative to a healthy one does favor the growth of certain microbial species. However, our understanding of specific abiotic and biotic factors that may support or inhibit Blastocystis growth are relatively limited in both health and disease contexts. As such we feel that perhaps the most prudent approach here is to emphasize this point a little more strongly perhaps but not go into specific details about what these factors might be as we simply do not have enough information. We have re written the following paragraph to emphasize this a little more and also to address your later point about qPCR and abundance, see paragraph below and main text lines 99-106: "It could simply be that in colorectal cancer (CRC) alterations to the local gut environment (including the tumor microenvironment) that are associated with altered host physiology and immune function, as well as treatment-induced disruption of gut ecology and broader dysbiosis may create conditions that favor the colonization and/or overgrowth of Blastocystis. This could explain the higher prevalence of Blastocystis observed in cancer patients in some studies and in addition to prevalence-based assays, future work should include q-PCR assays to assess Blastocystis abundance and potential overgrowth across different gut microenvironments and cohorts of interest."

1. Second, the authors criticize the inconsistent forms described in previous studies. This is an important point, but it would help to clarify whether this refers to problems

in morphological identification, terminology, or interpretation across studies. Also, if the authors suggest that different forms may have distinct effects in the colon, that idea should be presented cautiously, since the biological relevance of several *Blastocystis* morphotypes is still debated.

Reply: *Blastocystis* is a highly pleomorphic organism, as comprehensively described by Tan 2008 (PMID: 18854485), who outlines the main morphotypes (vacuolar, granular, amoeboid, and cyst), along with less frequently reported variants such as a vacuolar and Mult vacuolar forms. This marked morphological variability can make forms difficult to distinguish (particularly under routine light microscopy examination) and they may not always fit neatly into established categories. As highlighted by Stensvold 2015 (PMID: 25709945), there is also a lack of consensus regarding *Blastocystis* morphology, and some reported forms may represent artifacts. In practical terms, this variability complicates accurate microscopic identification and increases the risk of misclassification, especially in the absence of specialized expertise. Accordingly, when we noted that "the morphological forms presented in the study [14] appear to be inconsistent with recognized *Blastocystis* morphotypes," our intention was not to question specific findings, but rather to suggest that discrepancies across studies may arise from methodological limitations in morphological identification. Therefore, we have revised the manuscript to reflect a more cautious interpretation, emphasizing that (i) *Blastocystis* exhibits substantial pleomorphism, (ii) this variability can hinder reliable microscopic detection and classification, and (iii) these challenges may contribute to inconsistencies reported across studies. This information has been now added in current lines 116-119 of the manuscript.

1. Finally, I would suggest that future studies should go beyond reporting simple PCR-based presence/absence and also include parasite burden, for example by qPCR. Given that the colon tumor microenvironment changes substantially, it is plausible that cancer may favor increased *Blastocystis* abundance without implying a causal role. In that sense, distinguishing colonization from possible overgrowth could help better frame the association and avoid overinterpretation.

Reply: We agree, and we have incorporated the importance of qPCR and quantitative data as outlined above, lines 99-106.

Competing Interests: None

Reviewer Report 04 March 2026

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In this open letter, Kurt et al. emphasize that the implication that *Blastocystis* sp. may act as a causative factor in colorectal cancer (CRC) is not sufficiently supported by the current evidence. They present solid arguments outlining the limitations of the available studies and stress that further experimental and mechanistic research is necessary before any causal relationship can be established.
Importantly, the authors caution against prematurely assigning a pathogenic role to *Blastocystis* sp. in CRC in the absence of robust supporting data. By carefully examining the existing literature, they challenge the assumption that this organism should be regarded as a confirmed etiological factor in colorectal carcinogenesis.
Overall, this commentary is timely and relevant. It provides a balanced perspective on the topic and encourages a more cautious interpretation of the current findings. For these reasons, it is appropriate for Indexing.

Is the rationale for the Open Letter provided in sufficient detail? (Please consider whether existing challenges in the field are outlined clearly and whether the purpose of the letter is explained)
Yes

Does the article adequately reference differing views and opinions?
Yes

Are all factual statements correct, and are statements and arguments made adequately supported by citations?
Yes

Is the Open Letter written in accessible language? (Please consider whether all subject-specific terms, concepts and abbreviations are explained)
Yes

Where applicable, are recommendations and next steps explained clearly for others to follow? (Please consider whether others in the research community would be able to implement guidelines or recommendations and/or constructively engage in the debate)
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Cancer etiology, cancer biology, biomarkers, cancer omics analysis

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.