

<https://doi.org/10.54500/2790-1203-2023-4-119-4-9>

UDC 616:576.8

IRSTI 76.03.45

Original article

## Clinical-Epidemiological, Immunological, Microbiological Aspects and Prevalence of Blastocystosis among the Population in the Republic of Azerbaijan

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### Abstract

*Blastocystis hominis* is currently one of the most common parasites found in human feces - up to 10% of the population in the developed countries and approximately 50-60% of cases are found particularly in the developing countries. Nevertheless blastocystosis is one of the least studied infectious diseases among intestinal protozoans in the Republic of Azerbaijan.

**The aim of this study** was to assess morbidity of blastocystosis, to determine its clinical picture, its role in the immune system and in the intestinal microflora.

**Methods.** This study involved a total of 8002 patients who applied to the Parasitology Clinic of the Research Institute of Medical Prevention named after V.Yu. Akhundova in Baku, Azerbaijan. As part of the study, the feces of the participants were examined microscopically, using ELISA and PCR methods.

**Results.** This study has presented that course of infection is mostly asymptomatic, although patients could experience abdominal pain, nausea, diarrhea, foul-smelling stool, skin rashes, allergy symptoms, hypersalivation and unpleasant breath. The increase of IL8 was detected among most patients which has indicated negative effect on the immune system, moreover it has caused quantitative and qualitative changes in the normal microflora of the intestines.

**Conclusions.** Based on the results we came to conclusion that blastocystosis has been very widespread among population of Republic of Azerbaijan and it is still vital to continue in-depth study.

**Keywords:** blastocystosis, genotyping, morbidity, opportunistic infection, cytokines, dysbacteriosis, diarrhea.

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2023; 4 (119): 4-9

Received: 17-09-2023

Accepted: 25-10-2023



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## Introduction

Blastocystis is a genus of unicellular protozoan parasite, belonging to Blastocystae class and order of Blastocystida and Blastocystidae family [1]. It causes mostly asymptomatic disease, sometimes with diarrhea. Previously, blastocysts were thought to be yeast cells. They are currently classified as primitive ones [2]. The parasite comprises several species, living in the gastrointestinal tracts of humans and animals including farm animals, birds, rodents, reptiles, amphibians, fish and cockroaches. The ability of blastocyst to live in different organisms makes it to be widespread. It has been found to be more widespread in Colombia, Argentina, USA, Bolivia, Peru, Brazil and Ecuador [3,4,5].

Pathology of the gastrointestinal tract has a great impact on growing body and such infections as Blastocystis Hominis leads not only disruption of normal intestinal microflora, but also can be a cause of complications in liver and pancreas [6]. Many authors have shown that irritable bowel syndrome [7], skin pathologies [8] are associated with blastocystosis invasion. Unfortunately, nowadays doctors do not pay enough attention to this infection and the high resistance of these parasites to traditional antiparasitic drugs creates conditions for its spread. Blastocystis can easily grow and multiply in the gut, and can even remain as a non-pathogenic microorganism in a healthy human intestine [9]. Although the main route of transmission is considered to be the fecal-oral route, other routes of infection are not yet well known [6], and some studies have reported transmission of this parasite through water and animal products. Therefore, measures such as maintaining the cleanliness of water, domestic animals and livestock are believed to be of great importance in preventing the spread of infection [10]. Other study have showed that

## Materials and methods

This retrospective cohort study was conducted from February 2018 until September 2023 based on Parasitology Clinic of National Research Institute of Medical Prevention named after V.Y. Akhundov in the Republic of Azerbaijan.

First of all, the feces of 8002 patients, who applied to our clinic, were inspected microscopically, further positive clinical specimens were examined by ELISA and PCR method. Sequencing of the purified PCR products was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems™ Austin, TX, USA).

The obtained DNA sequences were compared with the homologous sequences of the Blastocystis hominis species in the database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST) program. The age of individuals were up to 78 years. Of the 8002 patients, 3825 were women and 4177 were men.

## Results

Under those circumstances, novel studies have been conducted in Azerbaijan. Only in recent years, about 8002 people were examined to assess the prevalence of blastocystosis in the population. After all, B.hominis was occurred in 2079 people (25.98±0.49%)

According to prevalence among every age group, blastocystosis was the most common in 12-17-year-old children (31.58±1.63%), 18-40-year-old adults (33.86±1.10%), and the least common in elderly people, 61 years and older (14.21±2.63%;  $p < 0.001$ ).

dientamoeba fragilis and Blastocystis parasites have been found in the intestines of patients with irritable bowel syndrome [11]. Although there was data about asymptomatic and symptomatic courses of infection, it is known that people with weak immunity and prone to diarrheal diseases, including patients with HIV infection and cancer, are more susceptible to it [12]. It should be noted that the indicators of the prevalence of the microorganism are highly dependent on the diagnostic methods used, especially PCR. PCR is considered as a more sensitive screening method for detecting infections caused by Blastocystis [12].

Blastocystosis is a relatively underexplored infectious disease among intestinal protozoans in the Republic of Azerbaijan. Only a handful of research studies have been conducted in this area, and they date back to approximately 30-35 years ago.

The global and local perspectives on blastocystosis, including within the Republic of Azerbaijan, have been somewhat unclear and inconsistent. Therefore, it is imperative to conduct research on the prevalence of B. hominis among various populations in different regions and countries. Furthermore, it is crucial to investigate its role in human pathologies, understand its pathogenesis, and examine the clinical course associated with different species and genotypes, as this knowledge can significantly impact the effectiveness of treatment.

**The aim of our study** was to determine morbidity rate of blastocystosis, its role in the immune system, intestinal microflora and to develop effective treatment schemes using clinical-epidemiological, microbiological, immunological results.

According to statistical analyses, we have used descriptive (Mean, Standard Deviation, Percentages) and inferential statistics (Chi-square Test, Analysis of Variance, Post-hoc Tests, T-Tests, Pearson Correlation, Logistic Regression).

Inclusion criteria has been characterized as the participation of residents of Azerbaijan, both rural and urban ones, men and women have been eligible; participants must have been diagnosed with blastocystosis, confirmed through laboratory testing, with specific attention to the presence of B. hominis and patients with gastrointestinal signs.

Notably, Blastocystosis as diagnosis was significantly detected more among rural (31.88±1.17%) than urban residents (24.52±0.54%;  $p < 0.001$ ). Nevertheless, on average the morbidity of blastocystosis according to the geographic area occurred almost at the same level in the whole country (Kur intermountain region - 24.72±1.44%, Lankaran region - 27.4±3.69%), except Araz province (5.41±3.72%).

In addition, considering the variety of types and genotypes of *B. Hominis*, led us to investigate the genotypes characterization of this parasite among residents of Azerbaijan.

As a result, 66 human fecal samples detected with *B. hominis* were examined by PCR to identify genotypes. ST3 subtype was found in 39 cases (59.1±6.1%), ST2 subtype - 16 (24.2±5.3%) and ST4 subtype – 11 (16.7±4.6). Markedly, only the ST3 subtype in comparison with others is more common in women than men (62.0±7.7% and 55.0±8.0% respectively).

In accordance with clinical picture, complaints of patients with blastocystosis were compared before and after treatment, for this reason 198 people were examined. For example, observations have shown that there was abdominal pain before treatment,

approximately 40.93±3.54%, and after treatment - 12.44±2.37% (p<0.001). In particular, nausea was detected in 38.34±3.50% of patients before treatment and 11.40±2.29% (p<0.001)- after treatment. In the same way, diarrhea was observed in 35.23±3.44% of cases before treatment and it was decreased after treatment - 10.88±3.24% (P<0.001). Such symptoms as foul-smelling stool (47.15±3.59%), skin rash (44.04±3.57%), allergy symptoms (41.45±3.55), hypersalivation (35.23 ±3.44%), unpleasant breath (31.51±3.35%) were revealed among patients before treatment and these symptoms were significantly reduced after treatment (21.24±2.94%, 19.69±2,86%, 15.54±2.61%, 8.29±1.98% and 12.95±2.42% respectively). On the other hand, those indicated symptoms are not pathognomic, consequently, blastocystosis should not be overlooked by a doctor while observing at least one of these clinical signs.

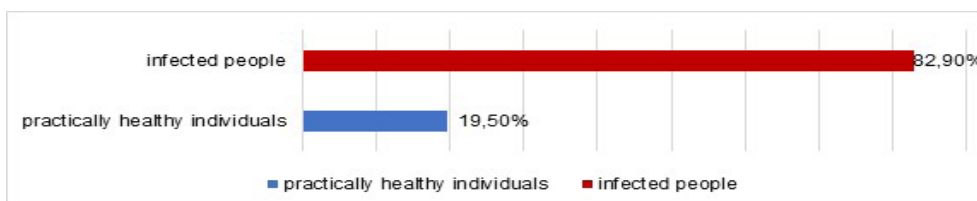
Table 1 - Clinical signs before and after treatment

Sign	Before treatment	After treatment
Abdominal Pain	40.93±3.54%	12.44±2.37% (p<0.001).
Nausea	38.34±3.50%	11.40±2.29% (p<0.001)
Diarrhea	35.23±3.44%	10.88±3.24% (p<0.001)
Strong Fecal Odor	47.15±3.59%	21.24±2.94%
Skin Rashes	44.04±3.57%	19.69±2.86%
Allergy Symptoms	41.45±3.55%	15.54±2.61%
Hypersalivation	35.23 ±3.44%	8.29±1.98%
Unpleasant Breath	31.51±3.35%	12.95±2.42%

Sign Likewise, the level of liver enzymes was examined to detect how blastocystosis affects on liver function. As it was shown that only 12 out of 45 patients with blastocystosis (26.7±1.24%) have had a higher level of ALT. Although the level of AST was not elevated as much as ALT among examined practically healthy individuals and patients with blastocystosis. In the same manner, the level of amylase was increased in 5 patients (8.9±0.6%). As can be seen, blastocystosis has a negative effect on the liver and pancreas altogether.

As it is known that blastocystosis is related to opportunistic infection hence such factors as the state of the immune system and its components affect on occurrence, clinical course, pathogenesis of disease, and the effectiveness of treatment. It was equally important to examine the amount of cytokines IL4 and IL8 in the blood, which showed that the level of IL4 did not exceed the norm among 83 patients with blastocystosis. On the contrary, it was demonstrated that IL8 was higher than normal in 75.9 % of cases.

Moreover, the course of blastocystosis can lead to changes of intestinal microbiota which is very essential. However, this issue has not been adequately studied before in Azerbaijan. For this reason, we have examined the intestinal microflora of 117 patients with blastocystosis and 46 practically healthy individuals. As a result, this study has demonstrated that the normal microflora of the intestine was reduced in 35.9±4.44% of patients with blastocystosis and in 10.9±4.6% of practically healthy individuals (p<0.001). In comparison with practically healthy individuals pathogenic and conditionally pathogenic microflora were found in patients with blastocystosis much more (8.7±4.16%, p<0.001 and 47.0±4.61% respectively). Dysbacteriosis has been also detected significantly more in blastocystosis (82.91±3.48%) in comparison with practically healthy individuals (19.57±5.85%, p<0.001).



Picture 1 - Dysbacteriosis among practically healthy individuals and infected people

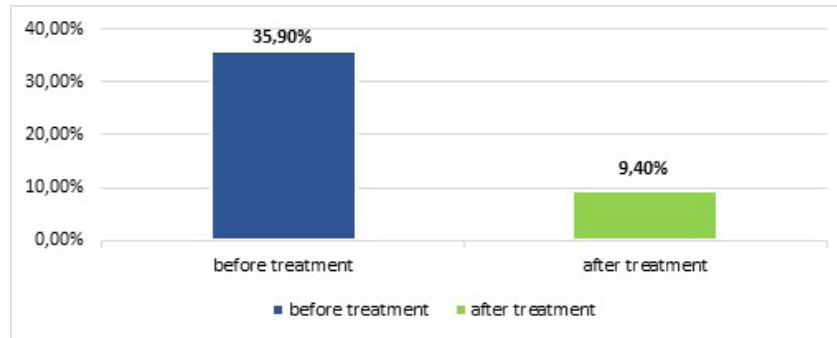
Interestingly, detection of *Candida* fungi was five times more frequent among people with blastocystosis (22.22±3.84%), *Staphylococcus aureus* – by three times, *Hemolytic E.coli* (23.08±3.9%), *lactose negative E.coli* (19.66±3.67%) were four-five times more detectable than

among practically healthy individuals. *Wheares Proteus*, *Klebsiella*, *Ps.Aeruginosa* were not detected among practically healthy individuals, they were only found in patients with blastocystosis (5.98±2.19%; 3.42±1.68; 6.84±2.33%, respectively). In order to clarify the impact

of blastocystosis on the intestinal microflora, it was also examined after etiological treatment.

Before treatment, reduction of normal microflora was observed in  $35.9\pm 4.44\%$  of patients with blastocystosis and after treatment -  $9.40\pm 2.7\%$  ( $p < 0.001$ ).

Pathogenic and conditionally-pathogenic microflora also occurred significantly less ( $47.0\pm 4.61\%$  and  $10.26\pm 2.81\%$ , respectively,  $p < 0.001$ ) after treatment.



Picture 2 - Reduction of normal microflora before and after treatment

## Discussion

Based on epidemiological data, this infection is mostly registered among young people and people of working age. Moreover, we have discovered that blastocystosis was significantly detected more among rural than urban residents, although the increase of incidents was found in the whole country.

According to data of some researchers, the course of infection mostly passes without any clinical symptoms in humans, however there were some patients in our study who experienced abdominal pain, nausea, diarrhea, foul-smelling stool, skin rashes, allergy symptoms, hypersalivation and unpleasant breath, which were significantly reduced after etiological treatment [13]. In that case, it makes to be difficult for diagnostics and treatment in time. Therefore, it is vital to clarify specific clinical signs of infection.

The high prevalence of blastocysts makes it necessary to study how pathogenic it is for the human body. The fact that this disease passes without symptoms in most cases does not allow us to correctly assess the damage it causes to human health. However, as a result of genome [14] and in vitro and in vivo studies [15], determination of the virulence factor and the damaging effect of the parasite on the intestinal barrier allows a correct assessment of the pathogenesis of blastocystosis. Based on our genotyping study, in comparison with others ST3 subtype was found mostly and more common in women.

## Conclusions

The results of this research has presented that blastocystosis is very widespread among the population in the Republic of Azerbaijan. To summarise, in order to evaluate the role of this disease in human pathologies in Azerbaijan totally, it is essential to continue studying this field deeply.

**Disclosures.** There is no conflict of interest for author.

**Acknowledgements.** The completion of this research would not have been possible without the support and contributions of various individuals and organizations. I express my heartfelt gratitude to: 1) Azerbaijan medical

Likewise, in spite of clinical assessment, we examined functions of liver and pancreas and some changes of enzymes were observed [16,17]. For instance, the level of amylase was increased as well as ALT and AST. As it can be seen, blastocystosis has a negative effect on the liver and pancreas altogether.

In blastocystosis, the increase of IL8 among most patients indicates negative effect on the immune system [18]. One study has shown that blastocystis is able to upregulate IL-8 gene expression in human colonic epithelial cells in vitro, increase epithelial permeability and destroy intestinal IgA. [19] All things considered, it would be recommended to study other cytokines and indicators of immune system as well.

In spite of assessment of enzymes and cytokines, we have detected the difference of normal intestinal microflora before and after treatment. It has presented that quantitative and qualitative disbalance of microflora was significantly reduced after treatment. As study has shown, this infection has influence on the course of disease, the digestive process, metabolism in general and efficacy of treatment among infected patients [19,20].

University and Parasitology Clinic of the V.Y. Akhundova Scientific-Research Institute of Medical Prophylaxis for providing the necessary resources and facilities for conducting this study; 2) my scientific leaders: Doctor of Medical Sciences, A.A. Salehov and Doctor of Sciences in Biology, Professor N.N. Aghayeva.

**Funding.** None.

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### Өзирбайжан Республикасы тұрғындары арасында бластоцистоздың таралуы және аурудың клиникалық-эпидемиологиялық, иммунологиялық, микробиологиялық аспектілері

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### Түйіндіме

*Blastocystis hominis* қазіргі таңда ең көп тараған паразиттердің түрі болып саналып, дамыған елдер тұрғындарының 10%-ының нәжисінде кездессе, ал дамушы елдерде 50-60%-ге дейін жиі кездеседі. Дегенмен, Өзирбайжан Республикасында ішек қарапайымдарының арасында бластоцистоз ең сирек зерттелетін жұқпалы ауру болып саналады.

**Зерттеу жұмысының мақсаты** бластоцистозбен аурушаңдықты бағалап, оның клиникалық көрінісін, иммундық жүйедегі және ішек микрофлорасындағы рөлін саралау болды.

**Әдістері.** Бұл зерттеу жұмысына В.Ю. Ахундова атындағы медициналық профилактика ғылыми зерттеу институтының Паразитология клиникасына (Баку, Өзирбайжан) келген барлығы 8002 науқас қатысты. Қатысушылардың нәжісі микроскопиялық жолмен, ИФА және ПТР әдістері арқылы зерттелді.

**Нәтижесі.** Ауру ағымы негізінен симптомсыз өтті. Алайда науқастардың біршамасында іш ауруы, жүрек айнуы, іш өтуі, нәжістің иісінің тым өткір болуы, терінің бөртуі, аллергия белгілері, гиперсаливация және ауыз қуысынан жағымсыз

иіс шығуы секілді белгілер кездесті. Сондай-ақ, қатысушылардың біраз бөлігінде IL8 деңгейінің жоғарылауы анықталды. Бұл өз кезегінде паразитоздың иммундық жүйеге тигізетін теріс әсерінен көрніс береді. Сонымен қатар, науқастардың ішек микрофлорасында сандық және сапалық өзгерістер анықталды.

**Қорытынды.** Зерттеу нәтижесінде біз бластоцистоздың Әзірбайжан Республикасында өте жиі кездесетіндігін анықтап, осы бағыттағы зерттеу жұмыстарының көлемін ұлғайтудың әлі де болса өзекті болып қала беретінін қорытындыладық.

Түйін сөздер: бластоцистоз, генотиптеу, аурушаңдық, оппортунистік инфекция, цитокиндер, дисбактериоз, іш өтуі.

## Клинико-эпидемиологические, иммунологические, микробиологические аспекты и распространенность бластоцистоза среди населения Азербайджанской Республики

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### Резюме

*Blastocystis hominis* в настоящее время является одним из наиболее распространенных паразитов, обнаруживаемых в фекалиях человека до 10% населения в развитых странах и примерно до 50-60% - в развивающихся странах. Тем не менее, бластоцистоз является одним из наименее изученных инфекционных заболеваний среди кишечных простейших в Азербайджанской Республике.

**Целью исследования** было оценить заболеваемость бластоцистозом, определить его клиническую картину, роль в иммунной системе и микрофлоре кишечника.

**Методы.** В данном исследовании участвовали всего 8002 пациентов, обратившихся в Клинику паразитологии Научно-исследовательского института медицинской профилактики имени В.Ю.Ахундовой в Баку, Азербайджан. В рамках исследования фекалии участвующих были исследованы микроскопически, методами ИФА и ПЦР.

**Результаты.** Это исследование показало, что течение инфекции в основном протекает бессимптомно, хотя у пациентов могут наблюдаться боль в животе, тошнота, диарея, стул с резким запахом, кожная сыпь, симптомы аллергии, гиперсаливация и неприятное дыхание. У большинства исследуемых выявлено повышение уровня IL8, что свидетельствовало о негативном влиянии на иммунную систему, а также были выявлены количественные и качественные изменения в нормальной микрофлоре кишечника.

**Выводы.** На основании полученных результатов мы пришли к выводу, что бластоцистоз очень широко распространен среди населения Азербайджанской Республики и продолжение углубленного исследования в этом направлении по-прежнему остается актуальным.

**Ключевые слова:** бластоцистоз, генотипирование, заболеваемость, оппортунистическая инфекция, цитокины, дисбактериоз, диарея.