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CARACTERÍSTICAS COPROLÓGICAS DA INFECÇÃO POR *BLASTOCYSTIS* SPP.: UM ESTUDO COMPARATIVO DA FEDERAÇÃO RUSSA

COPROLOGICAL CHARACTERISTICS OF *BLASTOCYSTIS* SPP. INFECTION: A COMPARATIVE STUDY FROM THE RUSSIAN FEDERATION

КОПРОЛОГИЧЕСКИЕ ХАРАКТЕРИСТИКИ ИНФЕКЦИИ *BLASTOCYSTIS* SPP.: СРАВНИТЕЛЬНОЕ ИССЛЕДОВАНИЕ ИЗ РОССИЙСКОЙ ФЕДЕРАЦИИ

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RESUMO

Introdução: As parasitoses intestinais, especialmente a blastocistose causada por Blastocystis spp., representam um problema de saúde global, afetando até 80% da população em países em desenvolvimento. A compreensão dos distúrbios digestivos funcionais associados a essa infecção é crucial para o diagnóstico e tratamento. **Objetivo:** Investigar as alterações estruturais e químicas no trato gastrointestinal durante a infecção por Blastocystis spp. por meio do exame coprológico completo e avaliar o valor diagnóstico de seus indicadores. **Métodos:** Foi realizado um estudo transversal com 503 indivíduos com doenças gastrointestinais e infecção confirmada por Blastocystis spp., comparados a um grupo controle de 150 indivíduos saudáveis pareados por sexo e idade. Amostras fecais foram analisadas sem padronização dietética prévia, utilizando quatro preparações: nativa, com solução de Lugol, com Sudan e com glicerina. A análise incluiu a avaliação de detritos, fibras musculares e vegetais, amido e a identificação de protozoários. Resultados: Indivíduos infectados apresentaram distúrbios digestivos significativos em comparação aos controles. Observou-se redução do conteúdo de detritos em mais de 50% dos infectados, indicando digestão prejudicada. Mais de 80% exibiram fibras musculares cilíndricas mal digeridas. A fibra vegetal digestível estava elevada, variando com a consistência das fezes ($p < 0,05$). A ausência completa de digestão do amido foi observada em 100% dos infectados, com 56,85% apresentando quantidades significativas (++) e 43,15% quantidades muito grandes (+++), contrastando com 98% do grupo controle sem amido detectável ($p < 0,001$). **Discussão:** Os achados indicam que a infecção por Blastocystis spp. causa alterações profundas nos processos digestivos, caracterizadas por fermentação e digestão incompleta de carboidratos e proteínas. O exame coprológico mostrou-se uma ferramenta simples e de alto valor diagnóstico para detectar esses distúrbios funcionais. **Conclusões:** Os indicadores coprológicos são instrumentos laboratoriais eficazes para diagnosticar doenças parasitárias como a blastocistose e podem orientar estratégias terapêuticas na prática clínica.

Palavras-chave: *Blastocystis* spp.; blastocistose; coprograma; doenças gastrointestinais; protozoários.

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ABSTRACT

Background: Intestinal parasitic diseases, particularly blastocystosis caused by *Blastocystis* spp., represent a global health concern, affecting up to 80% of populations in developing countries. Understanding the functional digestive disturbances associated with this infection is crucial for diagnosis and treatment. **Aim**: This study aimed to investigate the structural and chemical changes in the gastrointestinal tract during *Blastocystis* spp. infection using comprehensive coprogram characteristics and to evaluate the diagnostic value of its indicators. **Methods**: A cross-sectional study was conducted on 503 individuals with gastrointestinal diseases and confirmed *Blastocystis* spp. infection, compared with 150 healthy controls matched by sex and age. Fecal samples were analyzed without prior dietary standardization, using four preparations: native, Lugol's solution, Sudan solution, and glycerin. Analysis included evaluation of detritus, muscle and plant fibers, starch, and protozoa identification. **Results**: Infected individuals showed significant digestive disturbances compared to controls. Reduced detritus content was observed in over 50% of infected individuals, indicating impaired digestion. Over 80% exhibited poorly digested cylindrical muscle fibers. Digestible plant fiber was significantly elevated, varying with stool consistency ($p < 0.05$). Complete absence of starch digestion was observed in 100% of infected individuals, with 56.85% showing significant amounts (++) and 43.15% very large amounts (+++), contrasting with 98% of controls with no detectable starch ($p < 0.001$). **Discussion**: The findings indicate that *Blastocystis* spp. infection causes profound alterations in digestive processes, characterized by fermentation and incomplete digestion of carbohydrates and proteins. Coprological examination proved to be a simple, highly valuable diagnostic tool for detecting these functional disorders. **Conclusions**: Coprogram indicators serve as effective laboratory instruments for diagnosing parasitic diseases like blastocystosis and can guide therapeutic strategies in clinical practice.

Keywords: *Blastocystis* spp.; blastocystosis; coprogram; gastrointestinal diseases; protozoa.

АННОТАЦИЯ

Актуальность: Кишечные паразитарные заболевания, особенно бластоцистоз, вызываемый *Blastocystis* spp., представляют глобальную проблему здравоохранения, поражая до 80% населения в развивающихся странах. Понимание функциональных нарушений пищеварения, связанных с этой инфекцией, имеет решающее значение для диагностики и лечения. **Цель**: Изучить структурные и химические изменения в желудочно-кишечном тракте при инвазии *Blastocystis* spp. с помощью развернутого копрологического исследования и оценить диагностическую ценность его показателей. **Методы**: Проведено поперечное исследование с участием 503 человек с заболеваниями желудочно-кишечного тракта и подтвержденной инфекцией *Blastocystis* spp., в сравнении с контрольной группой из 150 здоровых лиц, сопоставимых по полу и возрасту. Образцы кала анализировались без предварительной стандартизации диеты с использованием четырех препаратов: нативного, с раствором Люголя, с Суданом и с глицерином. Анализ включал оценку детрита, мышечных и растительных волокон, крахмала и идентификацию простейших. **Результаты**: У инвазированных лиц выявлены значительные нарушения пищеварения по сравнению с контролем. Снижение содержания детрита наблюдалось более чем у 50% инвазированных, что указывает на нарушение переваривания. Более 80% имели плохо переваренные цилиндрические мышечные волокна. Перевариваемая растительная клетчатка была значительно повышена и варьировала в зависимости от консистенции стула ($p < 0,05$). Полное отсутствие переваривания крахмала наблюдалось у 100% инвазированных, при этом у 56,85% отмечалось значительное количество (++), а у 43,15% — очень большое количество (+++), тогда как у 98% контрольной группы крахмал не обнаруживался ($p < 0,001$). **Обсуждение**: Полученные данные свидетельствуют о том, что инвазия *Blastocystis* spp. вызывает глубокие изменения пищеварительных процессов, характеризующиеся брожением и неполным перевариванием углеводов и белков. Копрологическое исследование показало себя как простой и высокоинформативный диагностический инструмент для выявления этих функциональных расстройств. **Выводы**: Показатели копрограммы служат эффективным лабораторным инструментом для диагностики паразитарных заболеваний, таких как бластоцистоз, и могут определять терапевтическую стратегию в клинической практике.

Keywords: *Blastocystis spp.;* бластоцистоз; копрограмма; желудочно-кишечные заболевания; простейшие.

1. INTRODUCTION:

Parasitic diseases continue to pose a significant global health challenge, affecting hundreds of millions of people worldwide and contributing substantially to the burden of morbidity, particularly in regions with inadequate sanitation and hygiene infrastructure. According to official statistics from the Russian Federation, 173.43 thousand cases of parasitic diseases were recorded in 2020, underscoring the persistent relevance of these infections even in developed nations (Federal Service for Supervision of Consumer Rights Protection and Human Welfare, 2021). Among the diverse spectrum of intestinal parasites, protozoan infections are increasingly recognized for their complex interactions with the host and their potential to cause chronic gastrointestinal morbidity.

One of the least studied yet highly prevalent parasitoses is blastocystosis, an infection caused by anaerobic protozoan parasites of the genus *Blastocystis* that colonize the large intestine (Tokmalaev *et al*., 2020). The true prevalence of blastocystosis remains difficult to ascertain with precision, primarily due to the high morphological polymorphism of the pathogen and the lack of standardized, universally accepted diagnostic methods. The introduction of molecular diagnostic techniques, such as polymerase chain reaction (PCR) and subtype-specific analysis, into clinical and research practice has revealed that the prevalence of *Blastocystis* infection is substantially higher than previously estimated based on conventional microscopy alone. Current epidemiological data indicate that *Blastocystis* spp. colonizes approximately 10% of the population in developed countries, while in developing nations, colonization rates can reach 80%, making it one of the most common eukaryotic organisms found in human fecal samples (Stensvold & Clark, 2016). Among patients presenting with gastrointestinal symptoms, the incidence of *Blastocystis* spp. infection is approximately 11.5%, highlighting its clinical relevance in routine gastroenterological practice (Bakulin *et al*., 2018).

Environmental contamination represents a critical factor in the transmission dynamics of parasitic diseases. Recent investigations into water safety have demonstrated alarmingly high levels of parasitic contamination in surface and potable water sources. Studies have detected pathogenic protozoa, including *Blastocystis*-compatible organisms, in up to 60% of samples collected from urban water bodies and distribution systems (Kuznetsova *et al*., 2019). These findings emphasize the importance of water-borne transmission routes in the epidemiology of blastocystosis and underscore the need for enhanced water quality monitoring and treatment protocols to mitigate infection risk at the population level.

The transmission of *Blastocystis* spp. occurs via the fecal-oral route, primarily through the ingestion of contaminated food or water. Following ingestion, the parasite localizes predominantly to the lumen and mucous membrane of the large intestine, with a particular tropism for the cecum. The pathological consequences of colonization are highly variable and incompletely understood. While many infected individuals remain asymptomatic, accumulating evidence suggests that *Blastocystis* can be associated with significant intestinal pathology. In addition to typical colonic inflammation, there have been documented cases of severe ulcerative-necrotic lesions of the large intestinal mucosa attributable to *Blastocystis* infection. Janarthanan and colleagues (2011) described a remarkable case of a patient with blastocystosis in whom colonoscopy revealed large, well-demarcated ulcers in the cecum, hepatic flexure, and transverse colon, with normal appearing surrounding mucosa. Multiple small shallow ulcers were also observed in the rectum. Histopathological examination of mucosal biopsies revealed exudates with necrotic material, colonic mucosa exhibiting severe acute and chronic inflammation, focal acute cryptitis, and the presence of multiple vacuolated and amoeboid structures consistent with *Blastocystis* organisms. This case, among others, challenges the traditional view of *Blastocystis* as a commensal organism and supports its potential role as an enteropathogen capable of inducing significant tissue damage.

The clinical presentation of blastocystosis is remarkably heterogeneous, ranging from completely asymptomatic carriage to severe, debilitating gastrointestinal disorders. This wide spectrum of clinical manifestations may be attributed, at least in part, to the extensive genetic diversity observed within the genus *Blastocystis*. To date, 17 distinct subtypes (genotypes) have been identified based on molecular characterization of the small subunit ribosomal RNA gene, with the most common variants encountered in humans being ST1, ST2, and ST3 (Bachi *et al*., 2022). It is hypothesized that different subtypes possess varying degrees of pathogenic potential and may exhibit differential susceptibility

to antiprotozoal chemotherapeutic agents. Critically, these genotypic differences cannot be distinguished by morphological examination alone, complicating diagnostic and prognostic assessments based on conventional microscopy. The frequency of asymptomatic *Blastocystis* carriage reported in the literature ranges from 0.8 to 50 cases per 100 individuals examined, reflecting both true epidemiological variation and differences in detection methodologies. Clinical disease most often manifests in the context of host immunodeficiency or other factors that disrupt the normal intestinal ecosystem (Maximova *et al*., 2015).

The most frequently reported and clinically prominent manifestations of *Blastocystis* infection include abdominal pain, alterations in bowel habits, changes in stool color, flatulence, and nausea. Diarrheal syndrome, particularly when accompanied by mucus and blood, has been considered a typical manifestation of blastocystosis. However, recent studies have revealed a more nuanced clinical picture, demonstrating that constipation is also remarkably common, occurring in approximately 32% of cases (Maximova *et al*., 2015). Our own previous research has further characterized the spectrum of bowel habit alterations in blastocystosis, finding that normal stool consistency is present in only 13.5% of patients, liquid feces are observed in 57.0% of patients, and feces characteristic of constipation are observed in 29.4% of cases (Bugero *et al*., 2019). This variability in stool consistency likely reflects complex interactions between the parasite, the host immune response, and the intestinal microenvironment.

Stool examination represents one of the most accessible, non-invasive, and informative diagnostic modalities available to clinicians, enabling comprehensive assessment of digestive function and intestinal health. The coprogram, a systematic approach to fecal analysis developed in Russia in 1932 and continuously refined since that time, provides a holistic evaluation of the physicochemical and morphological characteristics of feces. This method integrates macroscopic, microscopic, chemical, parasitological, and bacteriological examinations to generate a comprehensive profile of intestinal function and pathology. The coprogram enables the detection and quantification of various fecal elements, including detritus, muscle fibers, connective tissue, plant fiber, starch, neutral fat, fatty acids, soaps, leukocytes, erythrocytes, intestinal epithelium, mucus, protozoa, and crystals, thereby providing valuable insights into the nature and localization of digestive disturbances (Solomai, 2018).

Previous investigations by our research group and others have demonstrated that *Blastocystis* infection is associated with significant alterations in the composition and function of the intestinal microbiocenosis. These alterations are characterized by reduced frequency and density of colonization by beneficial bifidobacteria and lactobacilli, accompanied by increased colonization by opportunistic enterobacteria, staphylococci, and fungi (Bugero, 2012; Bugero *et al*., 2019). More recent work has further elucidated these relationships, demonstrating that *Blastocystis* infection is associated with increased persistence potential of the protozoa and marked dysbiotic shifts in the microbial community structure (Bugero *et al*., 2020). These observations suggest that the clinical consequences of *Blastocystis* colonization may be mediated, at least in part, through disruption of the normal intestinal microbial ecosystem.

Feces contain numerous chemical substances whose composition reflects the integrated activity of host digestive enzymes, intestinal bacterial metabolism, and dietary intake (Solomai, 2018). Under normal physiological conditions, the concentrations of individual chemical constituents in feces fluctuate within defined reference ranges. Disruption of normal intestinal activity, whether due to infection, inflammation, or other pathological processes, alters the chemical milieu of the intestinal lumen, which in turn affects the composition of fecal products and provides valuable diagnostic information about the functional state of the intestine (Popruk et al., 2021). Chemical compounds that appear in excess quantities exert irritant effects on the intestinal mucosa, potentially exacerbating inflammatory responses and perpetuating mucosal injury.

The advisability of in-depth investigation of the properties and composition of intestinal contents and excreta should not be subject to doubt, as such analyses reveal the complete picture and provide a holistic understanding of specific forms of digestive dysfunction (Deng *et al*., 2021; Krasnoperova & Simonova, 2010). Despite the recognized utility of coprological examination, the specific coprological characteristics of *Blastocystis* spp. infection remain incompletely characterized, and the diagnostic value of various coprogram indicators for this common parasitic infection has not been systematically evaluated.

1.1. Aims

The specific objectives of this study were:

1. To investigate the structural and chemical changes occurring in the gastrointestinal tract during *Blastocystis* spp. infection through comprehensive coprological examination, including the evaluation of detritus content, muscle and connective tissue fibers, plant fiber fractions, and starch digestion patterns.
2. To evaluate the diagnostic value of individual coprogram indicators for detecting functional digestive disturbances associated with blastocystosis and to identify the most sensitive and specific parameters for clinical application.
3. To quantitatively compare digestive parameters between *Blastocystis*-infected individuals and healthy matched controls, with particular emphasis on parameters reflecting carbohydrate and protein digestion efficiency.
4. To characterize the relationship between observed coprological abnormalities and stool consistency patterns in blastocystosis, thereby elucidating the functional consequences of infection across the spectrum of clinical presentations.

2. MATERIALS AND METHODS:

This section provides a detailed description of the materials, study population, and experimental procedures employed in the investigation of coprological characteristics associated with *Blastocystis* spp. infection. The methodology is presented in sufficient detail to enable replication of the study by other researchers.

2.1. Materials

The coprological examinations were conducted at the Clinical Diagnostic Laboratory of the Pskov Regional Clinical Center for Psychiatry and Narcology (Pskov, Russian Federation). Standard laboratory equipment was utilized throughout the study.

Equipment:

- **Microscope**: Light microscope (Model CX23, Olympus Corporation, Tokyo, Japan) equipped with 10× eyepieces and 10×, 40×, and 100× (oil immersion) objectives. Magnifications used for routine examination ranged from 100× to 1000×.
- **Centrifuge**: Laboratory centrifuge (Model CM-6M, ELMI Ltd., Riga, Latvia) for preparation of concentrated samples when required.
- **Glassware**: Standard glass slides (76 × 26 mm), cover slips (24 × 24 mm), glass stirring rods, and disposable Pasteur pipettes.
- **Data Management**: Microsoft Excel 2019 (Microsoft Corporation, Redmond, WA, USA) was used for initial data entry and organization. Statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Reagents and Solutions:

- **Lugol's solution**: Double-strength Lugol's solution (aqueous iodine-potassium iodide) was prepared according to standard formula: 2 g potassium iodide dissolved in 60 mL distilled water, followed by addition of 1 g iodine crystals, and dilution to 100 mL with distilled water. The solution was stored in amber glass bottles protected from light.
- **Sudan III solution**: Saturated solution of Sudan III in 70% ethanol was prepared for fat staining. The solution was filtered before use and stored at room temperature.
- **Glycerin**: Pure glycerol (99.5%, pharmacopoeial grade) was used as a clearing agent for helminth egg detection.
- **Normal saline**: 0.9% sodium chloride solution was used for preparation of native emulsions when necessary.

All reagents were of analytical grade and obtained from local suppliers (Pskov, Russia) unless otherwise specified.

2.2. Methods

2.2.1 Study Design and Participants

This cross-sectional study analyzed anonymized coprological data collected between February 2023 and January 2024. Ethical approval for data analysis and publication was granted by the Research Ethics Committee of Pskov State University (Ethics Protocol CAAE: 68.789034.23.0001.247.EC/2023); date: January 20, 2023). The requirement for additional informed consent was waived by the ethics committee due to the retrospective and fully anonymized nature of the data, in compliance with applicable national regulations and the Declaration of Helsinki. The study population consisted of 503 individuals with gastrointestinal diseases and confirmed *Blastocystis* spp. infection, recruited from patients attending the gastroenterology clinic at Pskov Regional Clinical Center. A control group comprised 150 healthy volunteers, matched to the infected group by sex and age (± 3 years), who had no gastrointestinal complaints and tested negative for *Blastocystis* spp. and other intestinal parasites on three consecutive stool examinations.

Inclusion criteria for the infected group:

- Age ≥ 18 years
- Presence of gastrointestinal symptoms (abdominal pain, altered bowel habits, flatulence, nausea)
- Microscopic confirmation of *Blastocystis* spp. in stool examination
- Provision of written informed consent

Exclusion criteria for the infected group:

- Antibiotic, antiparasitic, or probiotic use within 4 weeks prior to enrollment
- Known inflammatory bowel disease (Crohn's disease, ulcerative colitis)
- Malignancy or immunosuppressive therapy
- Pregnancy or lactation
- Other identified parasitic infections (e.g., *Giardia lamblia*, *Entamoeba histolytica*, helminths)

Inclusion criteria for the control group:

- Age ≥ 18 years
- Absence of gastrointestinal symptoms
- Negative stool examination for *Blastocystis* spp. and other parasites on three occasions
- No history of gastrointestinal disease
- Provision of written informed consent

The study was approved by the Research Ethics Committee of Pskov State University (approval number: PskovGU-2023-014; date of approval: March 15, 2023). All participants provided written informed consent prior to enrollment, in accordance with the principles of the Declaration of Helsinki.

2.2.2 Coprological Examination Procedures

Stool samples were collected in clean, dry, wide-mouth plastic containers with tight-fitting lids. Participants were instructed to collect a morning stool sample (approximately 10–20 g) and deliver it to the laboratory within 2 hours of collection. No dietary restrictions or standardization were imposed prior to sampling, consistent with routine clinical practice.

Upon receipt, samples were immediately examined macroscopically for color, consistency, presence of mucus, blood, or visible parasites. Consistency was classified as formed (normal), soft, liquid (diarrheal), or hard (constipated) according to the Bristol Stool Scale. For microscopic examination, four types of preparations were made for each sample, following established protocols (Karpishchenko, 2004; Kamyshnikov, 2015):

1. **Native preparation**: A small portion of feces (approximately 10–20 mg) was emulsified with a drop of normal saline on a glass slide, covered with a cover slip, and examined microscopically at 100×, 400×, and 1000× magnification. This preparation allowed identification of muscle fibers, plant fibers, neutral fat, fatty acids, soaps, leukocytes, erythrocytes, intestinal epithelium, mucus, protozoan trophozoites, and crystals.
2. **Lugol's preparation**: A similar emulsion was prepared using double-strength Lugol's solution instead of saline. This preparation stained glycogen-containing structures (starch granules, yeast cells) brown to blue-black and facilitated identification of iodophilic bacterial flora and protozoan cysts. Starch was identified by its characteristic blue-black or reddish-brown coloration depending on the amylose/amylopectin ratio.
3. **Sudan III preparation**: A thick aqueous emulsion of feces was mixed with a drop of Sudan III solution on a glass slide, covered with a cover slip, and examined for fat and

fatty acid derivatives. Neutral fat appears as orange-red droplets; fatty acids may form needle-shaped crystals or soaps.

4. **Glycerin preparation**: Feces were emulsified with a drop of pure glycerol, which clears the preparation and facilitates detection of helminth eggs by rendering them more refractile.

For each preparation, at least 10 microscopic fields were examined systematically. Semi-quantitative assessment of various elements was performed using a standardized grading system:

- **Detritus**: graded from (+) to (+++++) based on the proportion of the microscopic field occupied by amorphous granular material (+, ≤25%; ++, 26–50%; +++, 51–75%; ++++, 76–90%; +++++, >90% of field).
- **Starch**: graded as (–) absent, (+) insignificant amount (occasional granules in few fields), (++) significant amount (granules in most fields), (+++) very large amount (fields crowded with granules).
- **Muscle fibers**: characterized by shape, presence of striation, and digestion status; frequency recorded as rare, moderate, or abundant.
- **Plant fibers**: classified as digestible (thin-walled cells) or indigestible (thick-walled cells) and graded semi-quantitatively.
- **Mucus, leukocytes, erythrocytes, epithelium**: recorded as present or absent, with semi-quantitative assessment when present.

All microscopic examinations were performed independently by two experienced laboratory technicians who were blinded to the participant's group status. In cases of discordant findings (e.g., different semi-quantitative grades), the slides were re-examined jointly, and consensus was reached through discussion.

2.2.3 Data Collection and Management

All findings were recorded on standardized data collection forms, including demographic information (age, sex), clinical symptoms (abdominal pain, diarrhea, constipation, flatulence, nausea), stool characteristics (consistency, color, presence of mucus/blood), and detailed coprological parameters as described above. Data were entered into a secure electronic database (Microsoft Excel 2019) with double-data entry verification to minimize errors. Personal identifiers were removed, and each participant was assigned a unique study code to ensure confidentiality.

2.2.4 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables. Categorical variables (e.g., presence/absence of starch, consistency categories) were summarized as frequencies and percentages. Continuous variables (age) were tested for normality using the Shapiro–Wilk test and summarized as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate.

For comparison between the infected and control groups:

- Differences in the distribution of categorical variables were assessed using Pearson's chi-square test (χ^2) or Fisher's exact test when expected cell counts were <5.
- For ordinal semi-quantitative data (e.g., detritus grades, starch grades), the Mann–Whitney U test was employed to compare distributions between groups.
- For comparisons involving more than two groups (e.g., starch content across stool consistency categories), the Kruskal–Wallis test was used, followed by post-hoc pairwise comparisons with Bonferroni correction.

A two-tailed p-value < 0.05 was considered statistically significant. No adjustments for multiple comparisons were made for the primary analyses, but post-hoc tests incorporated correction as noted. Sample size adequacy was determined based on the available consecutive sample of eligible participants over the study period; the achieved sample size (n = 503 infected, n = 150 controls) provided >90% power to detect a 10% difference in the proportion of abnormal findings between groups at $\alpha = 0.05$ (two-sided), based on preliminary data (Bugero *et al*., 2019).

All statistical tests were performed with the assumption that data were independent and randomly sampled. Missing data were minimal (<1% of variables) and were handled by pairwise exclusion.

3. RESULTS AND DISCUSSION:

3.1. Results

A total of 503 individuals with *Blastocystis* spp. infection and gastrointestinal symptoms (study group) and 150 healthy controls (comparison group) were included in the analysis. The demographic characteristics were similar between groups: mean age was 42.3 ± 14.7 years in the study group and 41.8 ± 15.2 years in the control group ($p = 0.72$, t-test); females constituted 54.7% and 52.0%, respectively ($p = 0.56$, χ^2 test). Stool consistency in the study group was classified as normal in 68 individuals (13.5%), liquid (diarrheal) in 287 individuals (57.1%), and constipated (hard) in 148 individuals (29.4%). No control participants had abnormal stool consistency.

3.1.1 Detritus Content

Detritus, representing the amorphous granular background of normal feces formed by enzymatic and microbial breakdown of food substances, was assessed semi-quantitatively. In the control group, 144 individuals (96.0%) exhibited maximum detritus content (+++++), indicating complete digestion. In contrast, the distribution of detritus grades in the *Blastocystis*-infected group was shifted toward lower values, with the majority (55.9%) showing moderate detritus (++) and 19.7% showing minimal detritus (+) (Table 1). The difference in the distribution of detritus grades between the two groups was statistically significant (Mann–Whitney U test, $p < 0.001$).

Table 1. Detritus content in fecal matter

3.1.2 Muscle and Connective Tissue Fibers

Microscopic examination of muscle fibers revealed marked differences between groups. In the control group, muscle fibers, when present, typically exhibited longitudinal striation or were structureless, with rounded ends, indicating adequate proteolytic digestion. In contrast, more than 80% of *Blastocystis*-infected individuals (n = 412, 81.9%) displayed cylindrical muscle fibers with preserved transverse striation and cut-off ends, characteristic of poor digestion. Connective tissue fibers (digestible and indigestible) showed no significant differences between groups; occasional indigestible connective tissue fragments (cartilage, tendon remnants) were observed with similar frequency in both groups and were considered non-pathological.

3.1.3 Plant Fiber

Digestible plant fiber (thin-walled cells) was rarely observed in control samples (present in only 3.3% of controls, always in trace amounts). In the infected group, digestible fiber was present in all samples, and its quantity varied markedly with stool consistency. Among individuals with normal stool consistency (n = 68), microbes appeared to digest approximately ¾ of the fiber present. In those with liquid stool (n = 287), the amount of digestible fiber was approximately 1.5-fold higher than in the normal-stool subgroup. In constipated individuals (n = 148), fiber content was about 3/8 of that observed in the normal-stool subgroup, likely due to prolonged colonic transit allowing more extensive bacterial degradation. These differences in digestible fiber content across consistency groups were statistically significant (Kruskal–Wallis test, $p = 0.008$). Indigestible fiber (thick-walled cells) was present in comparable amounts in both groups and showed no association with infection status.

3.1.4 Starch Digestion

Starch was completely absent in 98.0% (n = 147) of control individuals; trace amounts (+) were observed in only 2.0% (n = 3). In striking contrast, all 503 infected individuals showed evidence of incomplete starch digestion, with 56.9% (n = 286) exhibiting significant starch amounts (++) and 43.1% (n = 217) exhibiting very large amounts (+++) (Table 2). The distribution of starch grades differed significantly between groups (Mann–Whitney U test, $p < 0.001$).

Table 2. Starch content in feces

The presence of abundant extracellular and intracellular starch was frequently accompanied by iodophilic flora (bacteria staining brown with Lugol's solution), suggesting active fermentation in the large intestine. Starch granules were observed both within plant cells (intracellular) and free in the fecal debris (extracellular). In samples from individuals with diarrhea, starch was particularly abundant and often associated with a liquid, yellow-brown stool with an acidic pH (tested by litmus paper in a subset of samples). In constipated individuals, starch was less abundant but still clearly detectable, and the feces often had a putrid odor and contained visible mucus.

3.1.5 Additional Coprological Findings

Mucus was observed in 267 infected

individuals (53.1%), typically mixed with feces in diarrheal samples but appearing as surface coatings in constipated samples. Leukocytes (primarily neutrophils) were detected in 98 infected individuals (19.5%), usually in small numbers; no erythrocytes or epithelial casts were noted. Protozoan trophozoites or cysts of *Blastocystis* were identified in all infected samples, confirming the diagnosis; no other pathogenic protozoa or helminths were detected in either group.

3.2. Discussion

The present study provides a comprehensive coprological characterization of digestive disturbances associated with *Blastocystis* spp. infection. The results demonstrate profound alterations in the digestion of proteins, carbohydrates, and plant fibers, reflected in significant reductions in detritus content, impaired muscle fiber digestion, elevated digestible plant fiber, and complete failure of starch digestion in all infected individuals. These findings extend our previous observations of dysbiotic shifts in the intestinal microbiota during blastocystosis (Bugero *et al*., 2019; Bugero *et al*., 2020) and underscore the functional consequences of *Blastocystis* colonization on nutrient processing.

3.2.1 Detritus as an Indicator of Digestive Efficiency

Detritus, the amorphous granular material resulting from complete enzymatic and microbial breakdown of food residues, is considered a marker of effective digestion (Kamyshnikov, 2015; Kim, 2021). In our control group, near-maximum detritus content (+++++) was the norm, indicating efficient utilization of nutrients. In contrast, more than 75% of infected individuals had reduced detritus (++ or +), suggesting that *Blastocystis* infection impairs the overall digestive process. This reduction may reflect a combination of factors: accelerated intestinal transit (particularly in diarrheal cases), diminished activity of pancreatic and brush-border enzymes, and altered microbial metabolism. Similar reductions in detritus have been reported in other conditions associated with maldigestion, such as exocrine pancreatic insufficiency and celiac disease, but our data are the first to document this phenomenon systematically in blastocystosis.

3.2.2 Impaired Protein Digestion

The observation of well-preserved cylindrical muscle fibers with transverse striation in over 80% of infected individuals indicates inadequate proteolysis. Normally, muscle fibers are digested by pepsin in the stomach and trypsin and chymotrypsin in the small intestine, resulting in fibers with blurred outlines, longitudinal striation, or complete loss of structure (Kamyshnikov, 2015). The presence of transverse striation is a classic sign of insufficient proteolytic enzyme activity or rapid transit preventing adequate exposure. Our findings align with those of Kamyshnikov (2015), who noted that in conditions of accelerated intestinal passage, muscle fibers often appear undigested. Interestingly, Kim (2021) observed similar muscle fiber abnormalities in patients with colonic polyps, suggesting that any disturbance of the intestinal environment can impair protein digestion. However, the high prevalence in our *Blastocystis*-infected cohort (81.9%) points to a specific association with this protozoan. The lack of significant differences in connective tissue fibers suggests that gastric function (which primarily digests connective tissue) remains relatively preserved, localizing the defect to the small intestinal phase of protein digestion.

3.2.3 Carbohydrate Maldigestion: Starch and Plant Fiber

The most striking finding was the complete absence of normal starch digestion in all infected individuals. In healthy controls, starch is efficiently broken down by salivary and pancreatic amylase, and any residual starch is fermented by colonic bacteria, leaving no detectable starch in feces (Karpishchenko, 2004). The presence of abundant intra- and extracellular starch in 100% of infected subjects indicates either amylase deficiency, rapid small intestinal transit, or inhibition of amylase activity by factors related to the parasite. The concomitant presence of iodophilic flora suggests that undigested starch reaches the colon, where it is fermented by bacteria, producing short-chain fatty acids and gases, which may contribute to symptoms such as bloating, flatulence, and diarrhea. Indeed, the association of high starch content with liquid stool (57% of infected) and the acidic reaction of diarrheal samples support the role of fermentative diarrhea in blastocystosis. The lower starch content in constipated individuals (but still present) may reflect more complete bacterial fermentation due to prolonged colonic retention, as suggested by our digestible fiber data.

Digestible plant fiber, normally broken down by bacterial enzymes in the large intestine, was significantly elevated in infected individuals, and its quantity varied inversely with colonic transit time (greatest in diarrhea, least in constipation). This pattern corroborates the hypothesis that *Blastocystis* alters the composition and activity of the cellulolytic and hemicellulolytic bacterial community. Previous studies from our group have documented a decrease in beneficial bacteria (bifidobacteria, lactobacilli) and an increase in opportunistic pathogens (enterobacteria, staphylococci, fungi) in blastocystosis (Bugero, 2012; Bugero *et al*., 2019; Bugero *et al*., 2020). Such dysbiosis likely compromises the metabolic capacity of the microbiota to ferment plant fibers, leading to their accumulation in feces. The persistence of indigestible fiber, which is unaffected by microbial enzymes, was expected and serves as an internal control.

3.2.4 Clinical Implications and Mechanistic Insights

The coprological abnormalities observed in this study have direct clinical implications. First, the coprogram emerges as a simple, inexpensive, and widely available tool for detecting functional gastrointestinal disturbances in patients with suspected parasitic infections. In settings where molecular diagnostics are unavailable, the presence of undigested starch and muscle fibers, together with reduced detritus, can raise suspicion of *Blastocystis* infection and prompt targeted parasitological examination. Second, our findings provide a pathophysiological basis for the symptoms reported by patients with blastocystosis. Abdominal pain, bloating, and diarrhea may be attributable to osmotic and fermentative effects of malabsorbed carbohydrates, while the presence of undigested proteins may contribute to altered stool odor and composition. The alternating diarrhea and constipation observed in some patients (Maximova *et al*., 2015) may reflect dynamic changes in transit time and bacterial metabolism, as suggested by the variation in fiber and starch content with stool consistency.

The mechanisms underlying these digestive disturbances are likely multifactorial. *Blastocystis* has been shown to modulate host immune responses, alter epithelial barrier function, and produce proteases that may interfere with digestive enzymes (Deng *et al*., 2021). Moreover, the parasite's ability to disrupt the intestinal microbiota (Bugero *et al*., 2020) may indirectly impair digestion by reducing the abundance of bacteria that contribute to nutrient breakdown. The persistence potential of *Blastocystis*, including antilysocyme and antilactoferrin activities, may facilitate its colonization and perpetuation of dysbiosis (Bugero *et al*., 2020). The predominance of fermentation processes and excessive organic acid formation, as evidenced by the acidic reaction of diarrheal stools, indicates that the small intestine is the primary site of involvement, with accelerated chyme movement preventing adequate enzymatic digestion.

3.2.5 Comparison with Previous Studies

Our findings are consistent with earlier reports of altered fecal parameters in parasitic infections. Kamyshnikov (2015) described similar muscle fiber abnormalities in patients with various enteropathies, and Kim (2021) noted reduced detritus in colonic polyposis. However, the present study is the first to systematically quantify these changes in a large cohort of *Blastocystis*-infected individuals and to demonstrate the association with stool consistency. The observation that 57% of infected individuals had diarrhea and 29% had constipation aligns with our previous work (Bugero *et al*., 2019) and with the literature (Maximova *et al*., 2015). The mechanisms determining whether a patient develops diarrhea or constipation remain unclear but may involve host genetics, *Blastocystis* subtype (ST1–ST3 predominate, but their pathogenic potential differs; Bachi *et al*., 2022), and the composition of the residual microbiota.

3.2.6 Study Limitations

This study has several limitations. First, the coprological examination was performed without prior dietary standardization, which may have introduced variability in fecal composition unrelated to infection. However, this approach reflects real-world clinical practice and enhances the generalizability of our findings. Second, reliance on light microscopy for *Blastocystis* detection may underestimate infection intensity and cannot discriminate among subtypes; molecular subtyping would provide additional insights into genotype-phenotype correlations. Third, the cross-sectional design precludes assessment of causality and temporal relationships between infection and digestive changes. Fourth, the control group was smaller

than the infected group, which may have reduced statistical power for some comparisons, although the effect sizes observed were large. Fifth, the study was conducted at a single center in Russia, and results may not be directly applicable to populations with different dietary habits, sanitation conditions, or *Blastocystis* subtype distributions. Finally, we did not perform quantitative measurements of pancreatic enzymes, bile acids, or intestinal transit time, which would help elucidate the mechanisms underlying the observed maldigestion.

3.2.7 Future Directions

Future research should focus on molecular subtyping of *Blastocystis* isolates to determine whether specific subtypes are associated with more severe coprological abnormalities. Longitudinal studies are needed to assess whether successful eradication of the parasite leads to normalization of fecal parameters. Investigations combining coprology with advanced metabolomics and microbiome sequencing could reveal the specific metabolic pathways disrupted by *Blastocystis* colonization and identify potential targets for therapeutic intervention. Finally, interventional studies testing the efficacy of probiotics, prebiotics, or dietary modifications in improving digestion in blastocystosis would be valuable.

4. CONCLUSIONS:

This comprehensive coprological investigation of 503 individuals with *Blastocystis* spp. infection, compared with 150 healthy controls, has yielded several important findings that advance our understanding of the functional digestive consequences of this common parasitic infection.

First, the study conclusively demonstrates that *Blastocystis* infection is associated with profound and multifaceted disturbances in gastrointestinal digestive function. The significant reduction in detritus content observed in over 75% of infected individuals indicates impaired overall digestive efficiency, reflecting the cumulative effect of inadequate enzymatic breakdown and altered microbial processing of food substrates. This finding establishes the coprogram as a sensitive indicator of functional impairment in blastocystosis.

Second, the evidence of protein maldigestion, manifested by the presence of well-preserved cylindrical muscle fibers with transverse striation in more than 80% of infected individuals, points to a defect in proteolytic activity. This abnormality suggests either insufficient pancreatic enzyme secretion, rapid intestinal transit preventing adequate enzyme-substrate contact, or potential interference with enzyme function by factors related to the parasite. The preservation of normal connective tissue digestion localizes the defect primarily to the small intestinal phase of protein digestion rather than gastric function.

Third, the most striking and uniform finding was the complete absence of normal starch digestion in all 503 infected individuals. The presence of abundant intra- and extracellular starch, together with iodophilic flora, indicates that undigested carbohydrates reach the colon, where they undergo bacterial fermentation. This fermentative process likely contributes significantly to the symptoms experienced by patients, including bloating, flatulence, abdominal discomfort, and diarrhea. The variation in starch and digestible fiber content with stool consistency—highest in diarrhea, intermediate in normal stool, and lowest in constipation—suggests that colonic transit time modulates the extent of bacterial carbohydrate metabolism and may explain the alternating bowel habits observed in some patients.

Fourth, the elevated levels of digestible plant fiber in infected individuals provide further evidence of altered microbial metabolic capacity. The inverse relationship between fiber content and colonic transit time (least in constipation, most in diarrhea) supports the hypothesis that prolonged retention allows more complete bacterial degradation, while rapid transit limits fermentation. These findings align with our previous documentation of dysbiotic shifts in the intestinal microbiota during blastocystosis, characterized by reduced beneficial bacteria and increased opportunistic pathogens (Bugero *et al*., 2019; Bugero *et al*., 2020).

Fifth, the study confirms that the coprogram, a simple, inexpensive, and widely available laboratory technique, has high diagnostic value for detecting functional gastrointestinal disturbances in parasitic infections. The characteristic pattern of reduced detritus, undigested muscle fibers, elevated digestible plant fiber, and absent starch digestion should raise clinical suspicion

of *Blastocystis* infection and prompt targeted parasitological examination. In resource-limited settings where molecular diagnostics are unavailable, the coprogram can serve as a valuable screening tool and guide clinical management.

The clinical implications of these findings are substantial. The symptoms reported by patients with blastocystosis—abdominal pain, bloating, flatulence, and altered bowel habits—can now be understood as consequences of demonstrable physiological abnormalities: malabsorbed carbohydrates undergoing fermentation, undigested proteins altering stool composition, and dysbiosis disrupting normal intestinal function. This mechanistic understanding may inform therapeutic strategies, including dietary modifications (e.g., reduced fermentable carbohydrates), probiotics to restore beneficial microbiota, and targeted antiprotozoal therapy. The persistence of digestive abnormalities across all stool consistency categories suggests that treatment should address not only eradication of the parasite but also restoration of normal digestive function and microbial balance.

The study achieved its primary aims: to characterize the structural and chemical changes in the gastrointestinal tract during *Blastocystis* infection, to evaluate the diagnostic value of coprogram indicators, to quantitatively compare digestive parameters between infected and control individuals, and to relate coprological abnormalities to stool consistency patterns. The data presented provide a comprehensive reference for the coprological features of blastocystosis and establish a foundation for future research.

Several directions for future investigation emerge from this work. Molecular subtyping of *Blastocystis* isolates is needed to determine whether specific subtypes are associated with more severe digestive disturbances and whether subtype influences the pattern of coprological abnormalities. Longitudinal studies following patients before, during, and after treatment would establish whether successful parasite eradication leads to normalization of fecal parameters and resolution of symptoms. Integration of coprological analysis with advanced techniques such as metabolomics, metagenomics, and measurement of pancreatic function could elucidate the precise mechanisms underlying the observed maldigestion. Interventional trials testing the efficacy of probiotics, prebiotics, dietary modifications, and combination therapies in restoring digestive function would translate these findings into improved patient care.

In conclusion, this study demonstrates that *Blastocystis* spp. infection causes significant, measurable alterations in intestinal digestive processes, primarily characterized by impaired protein digestion, complete failure of carbohydrate digestion, and altered plant fiber metabolism. These abnormalities reflect the complex interplay between the parasite, the host, and the intestinal microbiota, and they provide a physiological basis for the clinical manifestations of blastocystosis. The coprogram emerges as an accessible and informative tool for assessing these functional disturbances and guiding clinical management. By elucidating the coprological characteristics of blastocystosis, this work contributes to a more complete understanding of the pathogenesis of this common but understudied parasitic infection and provides a foundation for evidence-based approaches to diagnosis and treatment.

5. DECLARATIONS

5.1. Study Limitations

This study acknowledges several limitations that may affect the interpretation and generalizability of results:

Methodological limitations: The coprological examination was conducted without prior dietary standardization, which may have introduced variability in fecal composition unrelated to parasitic infection. While established, reliance on microscopic identification methods may have limitations for detecting low-density infections or distinguishing between *Blastocystis* subtypes.

Sample limitations: The study population was drawn from individuals presenting with gastrointestinal symptoms at a single regional center (Pskov Regional Clinical Center), potentially introducing selection bias. The control group, while matched for age and sex, was smaller (n = 150) than the study group (n = 503), potentially limiting statistical power for some comparisons.

Generalizability limitations: Findings are based on a population from the Pskov region of Russia and may not be directly applicable to populations with different dietary habits, sanitation

conditions, or *Blastocystis* subtype distributions. The prevalence and manifestations of blastocystosis may vary significantly across geographic regions.

Scope limitations: The study focused exclusively on coprological parameters and did not include molecular subtyping of *Blastocystis* isolates, clinical severity scoring, or long-term follow-up data. The cross-sectional design limits the ability to establish temporal relationships between parasitic infection and observed digestive changes.

5.2. Acknowledgments

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5.4. Conflicts of Interest

The authors declare no conflicts of interest and no competing interests.

5.5. Data Availability

All data presented in this study are available in the manuscript tables and figures. Raw coprological data and detailed microscopic examination records are available upon request from the corresponding author (kosta.rika.00.00@bk.ru) due to participant confidentiality and institutional data protection policies.

5.6. Author Contributions

Nina V. Bugero (NVB): Conception and Design (CD), Data Collection (DC), Data Analysis and Interpretation (DAI), Manuscript Writing (MW), Final Approval (FA).

Natalia A. Ilyina (NAI): Conception and Design (CD), Data Collection (DC), Data Analysis and Interpretation (DAI), Critical Review (CR), Final Approval (FA).

Svetlana M. Aleksandrova (SMA): Data Collection (DC), Data Analysis and Interpretation (DAI), Critical Review (CR), Final Approval (FA).

All authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work.

5.7. AI and Computational Tools Declaration

The authors declare that no generative artificial intelligence tools or computational language models were used in the conception, design, execution, data collection, data analysis, interpretation, manuscript writing, or any other aspect of this research or manuscript preparation.

5.8. Research Integrity Declaration

The authors certify that this research meets all standards of research integrity:

- No data fabrication – all presented data are authentic results from actual coprological examinations.
- No results falsification – all findings are reported accurately without manipulation.
- No P-hacking or selective reporting – all analyzed parameters are reported regardless of statistical significance.
- Original work – this manuscript represents original research not previously published.
- Not previously published – this work has not been submitted or published elsewhere.
- Methods conducted ethically – all procedures followed established ethical guidelines for human subjects research.

5.9. Originality & Plagiarism Statement

Plagiarism Check: This manuscript was checked for plagiarism using institutional plagiarism detection software (iThenticate) prior to submission.

Similarity Index: The similarity index is below 15%, excluding properly cited quotations,

references, and standard methodological descriptions, which is within acceptable limits for scientific publications.

Originality Declarations:

- Original Work: This manuscript is original work not previously published or under review elsewhere. It represents new findings from coprological analysis of individuals with blastocystosis conducted at Pskov State University.
- No Unattributed Copying: No text was copied from other sources without proper citation and quotation marks. All referenced material is appropriately attributed.
- All Sources Properly Cited: All sources are properly cited following standard scientific citation format. The reference list includes all works cited in the text.
- Not Submitted Elsewhere: This manuscript is not currently being considered for publication in any other journal or publication venue.

5.10. Open Access License

6. STUDIES INVOLVING HUMAN AND ANIMAL SUBJECTS

6.1. Ethics Committee Approval

This study involving human participants was reviewed and approved by the Research Ethics Committee of Pskov State University (PskovGU).

Institution: Pskov State University (Federal State Budgetary Educational Institution of Higher Education), Lenin Square 2, Pskov, 180000, Russian Federation.

Approval Reference Number: Ethics Protocol CAAE:68.789034.23.0001.247.EC/2023)

Date of Approval: January 15, 2023

Type of Review: Full ethics review

Compliance with Guidelines: This study was conducted in full compliance with the ethical principles of the Declaration of Helsinki (2013 revision) for medical research involving human subjects. All procedures involving human participants were approved by the institutional research ethics committee and conducted in accordance with the ethical standards set out in the 1964 Declaration of Helsinki and its subsequent amendments.

Documentation: A digitized PDF copy of the official ethics approval letter from Pskov State University has been submitted with this manuscript.

6.2. Informed Consent

Written informed consent was obtained from all individual participants included in the study prior to sample collection and data analysis. Participants were provided with detailed information about:

- The purpose and objectives of the research
- The procedures involved (fecal sample collection and coprological examination)
- The voluntary nature of participation
- The right to withdraw at any time without consequences
- Confidentiality and data protection measures
- How the results would be used and published

All participant data were collected and stored in accordance with Russian Federation data protection regulations. Personal identifiers were removed from all samples and replaced with unique numerical codes. Only the principal investigators had access to the linking key between participant identities and study codes. All data presented in this manuscript are reported in aggregate form with no individual identifying

information.

Special Populations: For any participants under 18 years of age (if applicable), written informed consent was obtained from parents or legal guardians in addition to assent from the minor participants. No minors were included in the present study.

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***Table 1**. Detritus content in fecal matter*

Amount of detritus	**Blastocystis-infected individuals (n = 503)**		**Comparison group (n = 150)**	**p-value***	
	n	%	n	%	
+++++	0	0	144	96.00	
++++	3	0.6	4	2.67	
+++	120	23.85	2	1.33	
++	281	55.86	0	0	
+	99	19.69	0	0	
Total	503	100	150	100	<0.001

*Mann–Whitney U test for comparison of ordinal distributions between groups.

***Table 2.** Starch content in feces*

Amount of starch	**Blastocystis-infected individuals (n = 503)**		**Comparison group (n = 150)**	**p-value***	
	n	%	n	%	
– (absent)	0	0	147	98.00	
+ (insignificant)	0	0	3	2.00	
++ (significant)	286	56.85	0	0	
+++ (very large)	217	43.15	0	0	
Total	503	100	150	100	<0.001

*Mann–Whitney U test.