POTENCIAL DE PERSISTENCIA DOS PROTOZOÁRIOS *BLASTOCYSTIS* SPP.
PERSISTENT POTENTIAL OF PROTOZOA *BLASTOCYSTIS* SPP.
ПЕРСИСТЕНТНЫЙ ПОТЕНЦИАЛ ПРОСТЕЙШИХ *BLASTOCYSTIS* SPP.

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RESUMO

Nos últimos anos, o protozoário parasitário *Blastocystis* spp., que habita o trato gastrointestinal humano, começou a atrair cada vez mais atenção dos parasitologistas. Uma alta incidência de contaminação por blastocisto foi revelada em indivíduos de vários grupos populacionais. Neste artigo, os resultados do estudo mostram altas taxas de invasão de trabalhadores de fundição pelo protozoário mencionado acima, caracterizado por condições perigosas de trabalho, tanto de natureza física quanto química. A capacidade dos protozoários de habitar um nicho ecológico específico depende de suas propriedades biológicas; assim, fatores de persistência são de particular interesse. O artigo descreve o potencial persistente do *Blastocystis* spp., habitando o intestino humano. As propriedades persistentes nos procariontes são um fator fisiológico significativo para a sua existência e determinam o mecanismo de interação dos microrganismos que formam a microflora intestinal. Foi estudado o complexo de fatores de persistência (ALA, ALFA e AHA) do *Blastocystis* spp. Foi encontrada uma correlação direta entre a atividade persistente dos blastocistos e a gravidade dos distúrbios da microbiocenose intestinal. O efeito de fatores desestabilizadores da produção que levam à reestruturação do bioma intestinal foi estabelecido, contribuindo para a diminuição do grupo nativo de microrganismos e para o aumento da flora oportunista. Os dados recebidos permitem utilizar as propriedades biológicas mais importantes da sobrevivência dos microrganismos para estudar os mecanismos de formação de microsimbiocenoses no biótopo do intestino grosso e são um pré-requisito teórico para o desenvolvimento de um método de rastreamento do diagnóstico de disbiose intestinal com base na determinação de ALA, ALFA e AHA de isolados clínicos de blastocistos.

Palavras-chave: *microbiocenose, parasitocenose, distúrbios disbióticos, antiliscito, antilactoferrina.*

ABSTRACT

In recent years, the parasitic protozoan *Blastocystis* spp., inhabiting in the human gastrointestinal tract, has begun to attract more and more attention on the site of parasitologists. A high incidence of blastocyst contamination was revealed in individuals of various population groups. In this paper, the results of the study showing high rates of invasiveness of foundry workers by the above-mentioned protozoan, characterized by hazardous working conditions of both physical and chemical nature. The ability of protozoa to inhabit a particular ecological niche depends on their biological properties; thus, persistence factors are of particular interest. The article describes the persistent potential of *Blastocystis* spp., inhabiting the human intestines. Persistent properties in prokaryotes is a significant physiological factor for their existence and determine the mechanism of interaction of microorganisms that form the intestinal microflora. The complex of persistence factors (ALA, ALFA, and AHA) of *Blastocystis* spp. was studied. A direct correlation between the persistent activity of the blastocysts and the severity of intestinal microbiocenosis disorders was found. The effect of destabilizing production factors leading to the restructuring of the intestinal biome has been established, contributing to a decrease in the indigenous group of microorganisms and an increase of opportunistic flora. The received data allow using the most important biological properties of the survival of

the microorganisms to study the mechanisms of microsymbiocenoses formation of in the biotope of the large intestine and are a theoretical prerequisite for developing a method for screening diagnosis of intestinal dysbiosis based on the determination of ALA, ALFA and AHA of clinical blastocyst isolates.

Keywords: *microbiocenosis, parasitocenosis, dysbiotic disorders, antilysocyme, antilactoferrin.*

АННОТАЦИЯ

В последние годы все большее внимание паразитологов стал привлекать паразитический простейший организм *Blastocystis* spp., обитающий в желудочно-кишечном тракте человека. Выявлены высокие показатели обсемененности бластоцист у лиц различных групп населения. В настоящей работе представлены результаты исследования, регистрирующие высокие показатели инвазированности данными простейшими рабочих литейного производства, характеризующиеся опасными условиями труда как физической, так и химической природы. При этом способность простейших к заселению той или иной экологической ниши зависит от наличия у них биологических свойств, в этом плане несомненный интерес представляют факторы персистенции. В статье представлена характеристика персистентного потенциала простейшего *Blastocystis* spp., населяющего кишечник человека. Наличие персистентных свойств у прокариот является чрезвычайно важным физиологическим аспектом их существования и определяет механизм взаимодействия микроорганизмов формирующих микрофлору кишечного тракта. Изучен комплекс факторов персистенции (АЛА, АЛФА и АГА) простейших *Blastocystis* spp. Обнаружена прямая корреляционная зависимость между персистентной активностью бластоцист и глубиной нарушений микробиоценоза кишечника. Установлено действие дестабилизирующих факторов производства, которые ведут к перестройке кишечного биома, способствуют уменьшению индигенной группы микроорганизмов и увеличению условно-патогенной флоры. Эти материалы позволяют использовать важнейшие биологические свойства выживания микроорганизмов для исследования механизмов формирования микросимбиоценозов в биотопе толстого кишечника и являются теоретической предпосылкой для разработки способа скрининговой диагностики кишечного дисбиоза, основанного на определении АЛА, АЛФА и АГА клинических изолятов бластоцист.

Ключевые слова: *микробиоценоз, паразитоценоз, дисбиотические нарушения, антилизосимная, антилактоферриновая.*

1. INTRODUCTION

The general transformation of the influence of environmental conditions in modern society is evident. A lot of abiotic and biotic factors, including anthropogenic ones, affect human health (Sharapov, 2012). The effects of industrial environmental factors on the body should not be ignored. Industries characterized by hazardous working conditions can be determined. This entirely concerns foundry. In a foundry, harmful factors of both physical and chemical nature are inherent (Soloviev, 2013). Physical factors include vibration, noise, high temperature, ultrasound, ionizing radiation; chemical factors include the influence of isocyanates, formaldehyde, tertiary amines such as dimethylethylamine, triethylamine, silicon dioxide, metal oxides. All of the above factors produce favorable conditions for different diseases (Lazarenkov and Khoreva, 2016).

Literature analysis showed that vibration causes changes in cardiac activity, in the nervous system, spasms of blood vessels, changes in the

joints, limiting their mobility (Turovets *et al.*, 2015). Studies of the noise effect on the human body also revealed changes in the functioning of the nervous and cardiovascular systems (Afanasova *et al.*, 2010). During the examination, workers exposed to noise, often complain of irritability, headaches, drowsiness, increased fatigue, poor sleep, dizziness. Persons constantly in contact with the noise of varying degrees and duration revealed disturbances of thermoregulation, increased pulse levels, and blood pressure (Izmerova and Denisova, 2003). Chemical factors inhibit hemopoiesis, disturbs metabolism, and causes functional changes in the nervous system and gastrointestinal tract (Denisov *et al.*, 2010).

Studying the influence of environmental factors on the human body is not a new problem. However, the influence of the entire spectrum of foundry factors on the microbiome of the worker's body is still not completely studied. Intestinal flora serves as an indicator of the microorganisms state, and when exposed to destabilizing environmental factors, its qualitative and quantitative change occurs, indigenious flora

decreases, and opportunistic pathogenic group increase (Kuvaeva and Ladodo, 1991).

In the last decade, many authors have shown the role of opportunistic microorganisms in the occurrence of intestinal infections. The epidemiological situation on parasitic diseases is especially acute; more than 1.3 million patients with various parasitoses are officially registered annually in the country. However, the incidence of parasitic infestations and their importance for public health and the country as a whole remains underestimated for a number of reasons. The reasons for the preservation of such a situation are the difficulties of specific diagnostics, lack of a clear system for recording the incidence rate, insufficient knowledge of doctors, and misunderstanding of both doctors and the population in the field of diagnosis and treatment of parasitoses.

Blastocystis spp is the most widespread among the parasitic microorganisms. (Abe, 2004; Dogruman, 2009). According to the classification of microorganisms, this protozoan belongs to the kingdom of Stramenopiles (synonym Chromista, Heterokonta), subdomain Chromobiota, subtype Opalinata, class Blastocystea, order Blastocystida, family Blastocystidae, genus Blastocysts (Hameed, 2011).

The growing interest of scientists and practical parasitologists in this protozoan is explained by its spread in the world. It is found in 30–50% of the inhabitants of developing countries and in 1.5–10% of developed countries (Iguchi, 2007; Jones, 2009; Meloni *et al.*, 2011). Besides, blastocysts are often present in patients with gastroenterological diseases, allergies. For example, due to Horiki *et al.* (1999), in examining a practically healthy population from Tokyo (Japan), blastocysts were found in 7.4% of cases (Horiki, 1999), and in similar studies in Perm, performed by N. M. Koza *et al.* in 1997 - 2001, the blastocystic invasion was revealed in 13.1% of cases (Koza *et al.*, 2002). In Omsk, according to the results of population surveys in 1999 – 2001, the rate of *Blastocystis hominis* detection in healthy persons was within 0.9 – 2.3% (Starostina *et al.*, 2002).

However, in clinical practice, laboratory assistants often do not register blastocysts in the test material. This is explained by the fact that the pathogenic nature of protozoan remains controversial, and, therefore, the registration of the results is not mandatory, and also by inadequate knowledge of laboratory assistants of this object.

It was established that blastocysts have a significant effect on the formation of microbiocenosis of the human intestine, accompanied by a decrease in the seeding rate of indigenous flora (bifidobacteria and lactobacilli), and an increase of opportunistic flora. It was shown that the disturbance of biocenotic relations between pathogenic bacteria and normal intestinal microflora is one of the factors affecting the development of many diseases, especially those with a chronic course. Experimental data confirm a high incidence of blastocysts in individuals with liver diseases, gastric ulcers, and dermatoses (Krasnoperova, 2009).

The discovery of microorganisms belonging to the group of protozoa in the intestinal biome does not always testify to their role in the formation of the studied biotope, and it is absolutely impossible to assess their significance for a macroorganism experiencing constant pressure from a complex of factors of an unfavorable industrial environment, which reduces the protective forces of the human body and makes him vulnerable to various forms of the disease.

Human normoflora provides non-specific resistance of a macroorganism (Tsimmerman, 2014). Microorganisms colonizing the intestine prevent contamination of the mucous membranes of the digestive tract with opportunistic microorganisms, using a large group of secreted bacterial substances as a defense against the pathogen. Conventionally pathogenic flora is present in the intestines of healthy people as additional or transient representatives in certain quantitative and qualitative indicators (Blaser and Falkow, 2014; Kuchumova, 2011).

Thus, in determining the etiological significance of the conventionally pathogenic flora, to which the group of parasitic protozoa *Blastocystis* spp can be attributed, along with their number, factors contributing to persistence must be taken into account, which are considered as a marker that determines the long-term habitation of the pathogen in the host organism. These traits include the ability of microorganisms to degrade lysozyme - antilysozyme activity (ALA), the ability to inactivate lactoferrin - antilactoferrin (ALPA), and antihistamine (AHA) activity.

The purpose of the study was to give a comparative characteristic of the persistent potential of parasitocenosis of the human intestine, under the influence of a complex of unfavorable factors in the working environment.

To achieve this purpose, the following tasks were set:

1. To study microbiocenosis characteristics in human large intestine under the influence of a complex of destabilizing factors of the working environment.
2. To determine the occurrence of protozoa *Blastocystis* spp. in the intestines in norm and in dysbiosis.
3. To study blastocysts biological properties using antilysocyme, antilactoferrin, and antihistone activities as an example.

2. MATERIALS AND METHODS

A series of studies on the intestinal microbiocenosis in persons working in the foundry of the Armature Plant were performed at the clinic of OJSC "Reinforcing Plant", the Helix laboratory and the research laboratory "Diagnostics" in St. Petersburg.

To assess the microbiocenosis, the method of quantitative isolation of types and variants of microorganisms that make up the microbiocenosis according to the order of the Ministry of Health of Russia of 09.06.2003 No. 231 "On approval of the industry standard "Protocol for the management of patients. Intestinal dysbiosis" (OST 91500.11.0004-2003) was used.

Cultures grown on culture media were subjected to group, generic, and species identification.

The inoculation and incubation on solid media for the isolation of lacto- and bifidumbacteria was carried out in anaerostats AE-01 (OJSC NIKI MLT, St. Petersburg) and OXOID (England) using Anaerogaz gas-generating packages. Anaerobic microorganisms were identified using ANAEROTEST 23 (Lachema, Czech Republic). Identification of opportunistic enterobacteria was carried out using general identification schemes. Identification of opportunistic enterobacteria was performed following generally accepted identification schemes using PBDE (Diagnostic Systems, Nizhny Novgorod), ENTEROTEST 24 (Lachema, Czech Republic), NEFERMtest (Lachema, Czech Republic), and Giss mediums. Yeast-like fungi, genus *Candida*, were revealed by their ability to assimilate carbohydrates using the API 20C AUX system (bioMerieux, France).

To detect protozoa, including blastocysts,

both traditional parasitological diagnostic methods (microscopy of fecal smears) and molecular biological methods (PCR) were used. Microscopy of stool smears stained with Lugol solution was carried out in compliance with all requirements for the preparing of preparations (Guidelines 4.2.735-99 "Parasitic methods of helminthism and protozoal diseases laboratory diagnostics" improved by the chief state sanitary doctor of the Russian Federation)

Pavlova and Zierdt media were used to obtain protozoa *Blastocystis* spp. cultures. Persistence factors of microorganisms were studied from 2015 to 2017 at the Institute of Cellular and Intracellular Symbiosis of the Ural Branch of the Russian Academy of Sciences, Orenburg (IKVN Ural Branch of the Russian Academy of Sciences).

Microorganisms persistence factors were studied using research methods proposed by O.V. Bukharin et al. To study microorganisms antilysocyme activity (ALA) the photometric method was used [23]. *Blastocystis* spp. test culture was grown in a liquid nutrient medium, the supernatant was separated and mixed with lysozyme, while preparing a control. The test sample and control were mixed with a suspension of the *Micrococcus lysodeikticus* test culture (strain No. 2665, L.A. Tarasevich GISK), and antilysocyme activity was determined by the optical density of the resulting mixtures, characterized in that the control used a mixture of nutrient broth with lysozyme, incubated the supernatant with lysozyme, introduced the incubated mixture into a pre-treated Trilon B test culture and measuring the optical density of the experimental sample and control in 2 and 4 hours, then calculated antilysocyme activity according to the formula:

$$K = \frac{V_K - V_L - C_L \cdot (M_{O4} - M_{O2})}{M_{K4} - M_{K2}}$$

where K is the coefficient of blastocysts antilysocyme activity; V_K - the volume of the broth culture of the studied strain; V_L is the volume of lysozyme solution in the initial concentration; C_L - lysozyme concentration; M_{O4} is the average optical density of experimental wells with a broth culture of the studied strain with lysozyme after 4 hours of incubation, units optical density; M_{O2} is the average optical density of experimental wells with a broth culture of the studied strain with lysozyme after 2 hours of incubation, units optical density; M_{K4} - the average value of the optical density of control wells with a broth culture of the studied strain without lysozyme after 4 hours of incubation, units optical density; M_{K2} - the

average optical density of the control wells with a broth culture of the studied strain without lysozyme after 2 hours of incubation, units optical density; 100 - conversion factor.

Microorganisms antilactoferrin activity (ALFA) of was studied according to the method described O.V. Bukharin et al. [24]. The amount of lactoferrin was determined by the method of enzyme-linked immunosorbent assay using sets of "Lactoferrin-strip", CJSC "Vector - Best", Novosibirsk. Test culture *Blastocystis* spp. was grown on solid nutrient medium; a microbial suspension was prepared from the grown agar culture, mixed with lactoferrin, while preparing a control., a liquid nutrient medium was added to the control instead of a microbial suspension. The test sample and control were incubated, the supernatant was separated from the simple blastocyst cells by centrifugation, determining the concentration of lactoferrin in the experimental and control samples by enzyme immunoassay and then calculate ALfa according to the formula: $ALFA = Ck - Co$,

where: ALFA - antilactoferrin activity, ng of inactivated lactoferrin / ml supernatant; Ck is lactoferrin concentration in the control, ng / ml; Co - lactoferrin concentration of in the experiment, ng / ml.

The antihistone activity of the studied microorganisms was determined by the photometric method [25]. To do it histone preparation from bovine thymus manufactured by Sigma (USA) in the form of a lyophilized powder containing a total fraction of histones was used. Test *Blastocystis* spp culture. grown in a liquid nutrient medium, was treated with chloroform, the culture fluid was separated, mixed with histone solution, simultaneously preparing two control samples. Isotonic NaCl solution was added in Control 1, instead of histones solution, and in control 2 liquid nutrient medium was added instead of culture fluid. The experimental and control samples were incubated, after incubation, the meat-peptone broth and the *Micrococcus luteus* test culture (GISK 211001) were added to all samples, re-incubated, the optical density of the suspensions was measured in the experiment and controls, then antihistone activity was calculated be the formular

$$\left(\frac{oDts - oDc1}{oDc1 - oDc2} \right) * 12.84 mcg / ml$$

where: oDts -optical density of the test sample; oDc1 -optical density of control1; oDc2 – optical density of control 2.

3. RESULTS AND DISCUSSION

The study was performed in 129 workers of the enterprise aged from 25 to 55. The control group consisted of 50 healthy m. persons.

Analysis of outpatient cards, record cards with the results of periodic medical examinations for the period from 2013 to 2017, revealed a number of diseases in the studied group. Vibration disease was on the first place ($78.30 \pm 2.7\%$), diseases of the hearing organs were registered in $43.20 \pm 3.2\%$ of workers, diseases of the nervous and cardiovascular systems in $32.40 \pm 3.4\%$ and $28.6 \pm 1.3\%$, respectively, acute respiratory infections in the mean of $19.20 \pm 1.8\%$, diseases of the musculoskeletal system in $8.40 \pm 1.7\%$. A high percentage of digestive organs diseases were registered, their rate was $62.30 \pm 3.6\%$ (80 persons).

The gastrointestinal tract plays a significant role in the human body since the main physiological processes occur there. Therefore, the proper functioning of the human digestive system serves as the basis for normal and full life support. The question of the influence of industrial and technical factors on the microbiome of the human body, which actively performs many functions that provide homeostasis, is still not clear. So monitoring of human health under the influence of unfavorable factors of the working environment and their influence on the digestive system of the body was performed.

In this connection, studied subjects were divided into three groups, depending on the duration of contact with destabilizing industrial factors. The first group consisted of employees working at the enterprise from 1 to 5 years - 26 persons ($32.5 \pm 2.7\%$, $p < 0.03$), the second included those who worked at the enterprise from 5 to 10 years - 32 people ($40.0 \pm 3.9\%$, $p < 0.05$), the third group consisted of employees working at the enterprise 10-15 years and more - 22 persons ($27.5 \pm 1.4\%$, $p < 0.03$).

Diagnosis of the qualitative and quantitative composition of the intestinal microbiocenosis of the examined patients showed disturbances on the part of normoflora. Besides, a decrease in the occurrence of the representatives of obligate microflora was shown: bifidobacteria and propionic bacteria to $85.4 \pm 3.4\%$, $81.3 \pm 3.1\%$, respectively ($p < 0.05$), lactobacilli to $77.4\% \pm 4.3$ ($p < 0.03$), bacteroids up to $87.5 \pm 3.9\%$, ($p < 0.05$), non-hemolytic *E. coli* up to $68.3 \pm 2.9\%$, ($p < 0.05$). In the control group, these figures were within the range of 98-100% ($p < 0.03$). A decrease in the density of

colonization was observed in these groups of microorganisms, for example, for bifidobacteria, it averaged $\log 6.4 \pm 0.4$ CFU/g, for lactobacilli and bacteroids, $\log 7.0 \pm 0.1$ CFU/g and $\log 8.5 \pm 0.1$ CFU/g, respectively ($p < 0.05$).

Depending on the duration of work in the foundry, the frequency and density of colonization of obligate endoflora representatives varied among workers of the 2nd group, reaching maximum deviations from the normocenosis in the examined from the 3rd group. The most visible disorders in the obligate group of microbes were noted among the workers with more than ten years of work experience. The seeding rate in relation to the bifidobacteria group was $\log 5.3 \pm 0.2$ CFU/g, for lactobacilli $\log 6.2 \pm 0.1$ CFU/g, which is significantly lower than in the comparison group. In the control group, the seeding rate for bifidobacteria and lactobacilli was $\log 10.5 \pm 0.3$ CFU/g and $\log 9.9 \pm 0.4$ CFU/g, respectively ($p < 0.05$).

The occurrence of opportunistic flora increased depending on the duration of the technogenic factors of the production environment action. High colonization density was observed for bacteria genus *Enterococcus* spp., *Proteus* spp., *Staphylococcus* spp., and fungi genus *Candida* spp. Their dissemination rates were significantly higher in comparison with the control group and amounted on average to $1g 10.2 \pm 0.3$ CFU/g, $\log 2.6 \pm 0.4$ CFU/g, $\log 5.0 \pm 0.3$ CFU/g and $\log 4.5 \pm 0.4$ CFU/g, respectively ($p < 0.05$). In the control group, these figures were as follows: $\log 2.5 \pm 0.3$ CFU/g, $1g 0.9 \pm 0.4$ CFU/g, $1g 2.3 \pm 0.3$ CFU/g and $1g 2.4 \pm 0.4$ CFU/g, respectively ($p < 0.05$). The occurrence and the density of colonization of opportunistic microbes were directly dependent on the length of service at the enterprise.

A significant increase in the content of *Escherichia coli* with hemolytic activity should be stressed. This indicates their high pathogenicity compared with the control group.

At present, much attention is paid to the importance of colonic dysbiosis in parasitoses. So, besides representatives of the bacterial flora, an assessment of the intestinal parasitocenosis was performed in the subjects studied. *Blastocystis* spp. ($62.00 \pm 5.4\%$), *Lambli* *intestinalis* ($36.72 \pm 3.2\%$) and *Entamoeba coli* ($16.34 \pm 1.3\%$) prevailed as to the frequency of their occurrence.

Studies have shown that, depending on the duration of work in the foundry, the detection of blastocysts in the feces of workers increases

from $56.30 \pm 4.6\%$ in the first group to $85.63 \pm 7.8\%$ in the third group.

A study of the biological properties of *Blastocystis* spp., colonizing the mucous membranes of the large intestine, will allow developing new approaches to assessing the participation of this pathogen in the development of the pathological process, explain the possible reason for the presence of blastocysts in the studied biotope and determine the participation of these protozoa in the formation of the intestinal biome in the persons under study.

Blastocyst strains isolated from the intestines of foundry workers were selected as a material for the study. All the subjects were divided into four groups depending on the severity of dysbiotic disorders (Table 1).

To date, there is no universally accepted classification of intestinal dysbiosis. Classification Intestinal flora dysbiosis, proposed by G. Kuznetsova (1972), was used in this work. It includes 4 degrees of colonic dysbiosis severity: stage I showed a decrease in the number of bifidobacteria and lactobacilli, stage II was characterized by a pronounced increase and subsequent predominance of colibacterial flora, appearance of atypical and enzymatically defective *E. Coli*, in stage III high titers of optional flora associations (staphylococci, streptococci, clostridia, fungi of the genus *Candida*, etc.) were registered while bifidobacteria and lactobacilli decreased on this background and stage IV with a predominance of *Proteus* or *Pseudomonas aeruginosa* prevalence.

With an increase in work experience from 1 to 15 years or more, the factors of dysbiotic manifestations increased, characterized by quantitative and qualitative values of microorganisms underlying the determination of dysbiosis of varying severity.

The studies performed allowed to determine the quantitative composition of the blastocyst strains isolated from the examined individuals, depending on the degree of intestinal dysbiosis (Table 1).

The greatest number of *Blastocystis* spp. was found in individuals with dysbiotic changes of the II degree of severity - 28 strains ($47.50 \pm 1.7\%$, $p < 0.03$).

The control group (50 people) consisted of persons undergoing outpatient treatment and not contacting the harmful factors of the working environment. The data obtained above were analyzed considering the questionnaire survey, in

which the persons of the comparison group participated. In persons from this group, dysbiosis of varying severity was observed in 4.0 0.7%, $p < 0.03$, blastocyst invasion in 12.0 0.2%, $p < 0.03$ (6 people).

The study of biological properties was carried out on the example of ALA, ALPA, and AGA activities, which are quite widely represented in the group of opportunistic bacteria. However, the study of these traits in *Blastocystis* spp., isolated in individuals exposed to destabilizing factors of foundry and aggravated by gastrointestinal diseases, was not yet carried out.

The role of lysozyme in protecting the macroorganism from infection is well known. Antilysozyme activity is one of the factors that increase the tolerance of microorganisms to the action of human and animal serum lysozyme. Some authors believe that antilysozyme activity contributes to the long-term survival of microorganisms in a macroorganism and can be used in bacteriological laboratories to assess the etiological role of isolated cultures.

Of the 62 studied blastocyst strains, 56 ($89.28 \pm 5.7\%$) possessed the studied trait. To analyze persistent characteristics of *Blastocystis* spp. Three groups of protozoa were chosen: the first included strains with a low ALA level - up to 2 $\mu\text{g} / \text{ml}$ inclusive, the second with a mean ALA level of 3-4 $\mu\text{g} / \text{ml}$, and the third with a high ALA level - 5 $\mu\text{g}/\text{ml}$ or more. The proportion of strains with low ALA values was $33.92 \pm 2.3\%$ (19 strains), with mean ALA - $50.01 \pm 4.6\%$ (28 strains) and $16.07.23 \pm 1.7\%$ (9 strains) with high ALA values (Table 2).

Table 3 shows that half of the blastocyst strains ($50.01 \pm 4.6\%$) isolated from the workers had average antilysozyme activity values within the range of 3-4 $\mu\text{g}/\text{ml}$. The proportion of *Blastocystis* spp. isolates with low and high values of the studied trait were $33.92 \pm 2.3\%$ and $16.07 \pm 1.7\%$, respectively.

The performed studies revealed a direct dependence of the ALA blastocysts manifestation on the severity of dysbiotic changes (Table 3).

Thus, blastocysts isolated in individuals with grade I dysbiosis were characterized to a greater extent by low ALA levels, which amounted to $2.1 \pm 0.02 \text{ mg}/\text{ml}$, while protozoa strains with grade III and IV dysbiosis had completely no low values of the studied trait. Increased ALA levels in blastocysts were observed with more pronounced factors of

intestinal dysbiotic disorders in workers. Among the nine protozoa strains isolated in dysbiosis of the IV degree of severity, two strains were characterized by mean ($2.3 \pm 0.02 \text{ mg}/\text{ml}$) and seven strains ($2.7 \pm 0.05 \text{ mg}/\text{ml}$) high level of the studied property.

Such an increase in the expression of antilysozyme activity in different degrees of dysbiosis is due to the fact that during biocenosis, which is characterized by a high content of opportunistic flora and a sharp decrease in obligate representatives (IV degree of dysbiosis), an inflammatory state of the intestinal mucosa is observed, which is accompanied by the increased concentration of endogenous lysozyme, intensely secreted by exocrinocytes of the colon in response to increased colonization of the digestive tract by optional microorganisms. It can be assumed that assimilating the ecological niche of *Blastocystis* spp. they produce lysozyme, necessary for the formation of biocenosis, which can lead to a compensatory ALA increase in representatives of the obligate group.

The antilactoferrin activity (ALFA) of protozoa was studied. Data on ALFA prevalence and severity in *Blastocystis* spp., isolated in foundry workers, show that the studied protozoa are highly able to inactivate lactoferrin. Antilactoferrin activity was revealed with a frequency of $69.35 \pm 3.7\%$ (43 strains).

Prevalence of ALFA *Blastocystis* spp. ranged from 65 to 289 ng/ml (Table 4). Depending on the parameters of the studied property, all the studied strains were divided into three groups.

The first group of blastocyst cultures had low values of the studied trait, which were in the range of $65-161 \pm 22.2 \text{ ng}/\text{ml}$, the second - means - $162-223 \pm 30.7 \text{ ng}/\text{ml}$ and the third high - $224-289 \pm 49.3 \text{ ng}/\text{ml}$. An analysis of the quantitative relations of the studied characteristic showed that almost half of the blastocyst strains were characterized by an average ALFA value ($45.51 \pm 2.3\%$). $32.56 \pm 2.7\%$ and $20.93 \pm 3.7\%$ of the studied strains showed, respectively, high and low ALFA values.

As the studies showed, the number of blastocyst strains with high persistent properties had a low percentage of occurrence or was completely absent in dysbiosis of I and II degrees of severity (Table 5). Thus, ALFA analysis showed that protozoa strains isolated in those examined with I degree of dysbiosis had neither mean or high level of the studied trait. In this group, only one strain of blastocysts was

characterized by a low value ($65-161 \pm 22.2$ ng/ml) of the trait.

In the III degree of intestinal dysbiosis, approximately there was the same number of strains, both with mean and low values of the trait. It should be noted that in significant disturbances of the qualitative and quantitative intestinal microflora composition, characterized by an IV degree of dysbiosis, only blastocyst strains were obtained in the experiment that inactivated lactoferrin in high concentrations of the studied persistent property in 11 isolates.

Of the 62 blastocyst strains, 32 *Blastocystis* spp. isolates, isolated from feces of workers, with the pathology of the gastrointestinal tract, had antihistone activity.

The values of antihistone activity ranged from 1.3-12.8 ng/ml, and in mean, were 9.1 ± 1.6 ng/ml. The results obtained in the experiment allowed to divide all the studied blastocyst strains into three groups (Table 6).

The determination of the AHA level revealed in the vast majority of protozoa low ($1.3-5.3 \pm 0.9$) and mean ($5.7-9.1 \pm 4.3$ ng/ml) values of the studied trait. Such cultures accounted for 89.28 3.9% of the studied blastocyst strains. The third group consisted of blastocyst strains with high AHA values ($9.2-12.8 \pm 4.6$ ng/ml) (Table 7).

The number of blastocyst strains isolated from feces of persons in contact with harmful factors of the working environment without obvious dysbiotic disturbances (I degree of dysbiosis) showed the prevalence of protozoa strains with a number of manifested AHA traits. On the contrary, a high level of antihistone activity was observed in blastocyst strains isolated in individuals with dysbiotic changes of moderate and high severity.

Thus, blastocyst strains isolated from feces of individuals exposed to destabilizing factors of the working environment are capable of inactivating certain factors of the natural anti-infection resistance of the microorganism (ALA, ALFA, and AHA). The results of the study allowed not only to reveal them but also to rank according to the degree of their information content (Figure 1).

The antilysozyme trait is the main persistent trait that determines the formation of the intestinal microbiocenosis of a given biotope. It was recorded with a frequency of $89.28 \pm 5.7\%$. Isolated *Blastocystis* spp. strains possess high values of the studied persistent characteristics

(ALA, ALFA, AHA) characterizing their persistent activity.

These traits contribute to the displacement of the dominant symbionts of the intestinal flora by blastocysts, colonization of mucous membranes by the protozoa studied, and leads to the formation of special parasitocenosis.

Since *Blastocystis* spp. possess a wide range of persistent symptoms, the question arises on protozoal infection of the macroorganism cell nucleus, and it is possible to confirm this at the light microscopic level, make an indirect assessment of the morbidity of the population by the occurrence rate of protozoan strains isolated from this contingent of the examined individuals, and also use them to diagnose and predict blastocystosis.

A study of the relationship between the severity of dysbiotic changes and persistent characteristics revealed an increase in the persistent characteristics of blastocysts with an increase in the severity of microecological disturbances in the intestine. The revealed relationship between persistent features of protozoa and the severity of disturbances in the intestinal microbiocenosis made it possible to consider persistence factors as markers of the dysbiotic process.

4. CONCLUSION

The study of the intestinal microbiome in persons working in foundry conditions, characterized by the complex effect of the examined factors of physical and chemical nature on the body, allowed to detect qualitative and quantitative changes in the composition of the normal flora of the large intestine. A decrease in the occurrence of obligate microflora representatives and an increase in the conditionally pathogenic group were registered. Experimental data revealed four severity levels of dysbiotic disorders in individuals of the examined group, which were directly dependent on the length of service at the enterprise. In persons with an experience of 10-15 years or more, in $73.56 \pm 2.3\%$ ($p < 0.05$) of cases, there were changes in intestinal dysbiosis of the 4th degree. In the control group, no visible changes in the composition of the obliterate representatives were found ($p < 0.05$).

The study of intestinal parasitocenosis of the examined biotope showed the prevalence of the protozoan group *Blastocystis* spp. ($62.00 \pm 5.4\%$). The studies showed that, depending on

the duration of work in the foundry, blastocysts in the feces of workers increases from $56.30 \pm 4.6\%$ in the first group, where the duration of work ranged from 1 year to 5 years, up to $85.63 \pm 7.8\%$ in the examined, with experience of 10-15 years or more.

The studies performed allowed to determine the quantitative composition of the blastocyst strains isolated from the examined individuals, depending on the degree of intestinal dysbiosis.

The largest number of protozoa is *Blastocystis* spp. found in the feces of individuals with dysbiotic changes of the II degree of severity - 28 strains ($47.50 \pm 1.7\%$, $p < 0.03$). The data obtained can be interpreted in the context of the fact that the simplest blastocysts, together with a community of other microorganisms, participate in the formation of a special microbiocenosis of the studied biotope, characterized by a decrease in representatives of the obligate flora and a significant increase in the optional one.

The data on a high level of severity of persistent properties of strains of *Blastocystis* spp. were obtained using ALA, ALFA, and AHA as an example. The results of the study allowed not only to detect them but also to rank according to the degree of their information content. The leading persistent trait that determines the formation of the intestinal microbiocenosis of a given biotope is their antilysocyme trait.

An increase in the persistent characteristics of blastocysts with an increase in the depth of microecological disturbances in the intestine was established. The revealed relationship between persistent features of protozoa and the depth of disturbances in the intestinal microbiocenosis made it possible to consider persistence factors as markers of the dysbiotic process.

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Fig. 1. Occurrence rate of ALA, ALFA, AHA in blastocyte strains

Table 1. Factors of dysbiotic disorders of the large intestine of workers invaded by *Blastocystis* spp.

Degree of intestinal dysbiosis	Number of examined (n)	Occurrence frequency of intestinal dysbiosis (%)	Number of <i>Blastocystis</i> spp. strains
I	24	$30.00 \pm 2.2\%$	18
II	38	$47.50 \pm 1.7\%$	28
III	11	$13.75 \pm 1.3\%$	9
IV	7	$8.75 \pm 3.2\%$	7
Total	80	100	62

Table 2. Indices of antilysocyme activity (ALA) in *Blastocystis* spp.

Groups	Number of strains with ALA (abs.)	Occurrence rate (%)
1 group (low ALA values)	19	33.92 ± 2.3
2 group (mean ALA values)	28	50.01 ± 4.6
3 group (high ALA values)	9	16.07 ± 1.7
Total	56	100

Table 3. The relationship of the degree of intestinal dysbiosis with the severity of the *Blastocystis* spp. antilysocyme activity

Degree of intestinal dysbiosis	Number of strains with ALA (abs)	Low ALA level (mg/ml)	Mean ALA level (mg/ml)	High ALA level (mg/ml)
		2,1±0,02	2,3±0,02	2,7±0,05
I	10	7	3	-
II	26	10	9	7
III	11	-	6	5
IV	9	-	2	7
Total	56	17	20	19
%	100	30.36 ± 2.8%	35.71 ± 4.2%	33.93 ± 5.6%

Table 4. Indicators of antilactoferrin activity (ALFA) in *Blastocystis* spp.

Groups	Number of strains with ALFA (abs)	Occurrence rate (%)
1 group (low ALFA levels)	9	20.93±3.7
2 group (mean ALFA levels)	20	45.51±2.3
3 group (high ALFA levels)	14	32.56±2.7
Total	43	100

Table 5. The relationship of intestinal dysbiosis degree with the severity of *Blastocystis* spp. antilactoferrin

Degree of intestinal dysbiosis	Number of strains with ALFA (abs.)	Low ALFA level (ng/ml)	Mean ALFA level (mg/nl)	High ALFA level (ng/ml)
		65-161±22.2	162-223±30.7	224-289±49.3
I	-	1	-	-
II	19	9	2	7
III	13	-	6	7
IV	11	-	-	11
Bcero	43	10	8	25
%	100	23.25 ± 2.4	18.60 ± 3.6	58.15 ± 5.3

Table 6. Parameters of antihistone activity (AHA) in *Blastocystis* spp.

Groups	Number of strains with AHA (abs.)	Occurrence rate (%)
1 group (low ALFA levels)	20	62.50±2.1
2 group (mean ALFA levels)	8	25.00±1.2
3 group (high ALFA levels)	4	12.50±3.7
total	32	100

Table 7. The relationship of the intestinal dysbiosis degree with the manifestation of *Blastocystis* spp. antihistone activity

Intestinal dysbiosis degree	Number of strains with AHA (abs)	Low AHA level (ng/ml)	Mean AHA level (ng/ml)	High AHA level (ng/ml)
		1.3–5.3±0.9	5.7-9.1 ± 4.3	9.2-12.8 ± 4.6
I	15	12	3	-
II	9	-	9	-
III	4	-	3	1
VI	4	-	-	4
Bcero	32	12	15	5
%	100	37.50 ± 5.3	46.88 ± 2.7	15.63 ± 1.3