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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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DISTRIBUTION AND FREQUENCY OF BLASTOCYSTIS SP. BY METHODS OF MICROSCOPY AND CULTIVATION IN FAECES OF RESIDENTS OF KHARKOV REGION

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Introduction.

Blastocystis sp. (formerly *Blastocystis hominis*) belongs to the family *Blastocystidae*, a new class *Blastocystea*, subphylum *Opalinata*, infra-kingdom *Heterokonta*, sub-kingdom *Chromobiota*, kingdom *Chromista* and is the most common anaerobic unicellular parasite of the intestinal tract of many animal species, which colonizes 1 to 2 billion people in countries around the world [1,2].

Blastocystis sp. are detected both in fecal samples (FS) of healthy people (asymptomatic individuals) and patients (symptomatic individuals) with non-specific symptoms of gastrointestinal lesions (abdominal pain, diarrhea, flatulence, anorexia, nausea, vomiting, anal itching, uncontrolled weight loss), skin (urticaria), joints (arthralgia) and, less commonly, other systems and organs [3-6]. The results of studies on the prevalence of *Blastocystis sp.* among the people of both cohorts in the world vary widely (from 0.08 to about 90%) depending on the degree of economic development of the country, the level of urbanization of its particular region, the cultural and sanitary and hygienic traditions of communities, the sphere of professional activity of people, and also on laboratory method for the detection of parasites, which was used in epidemiological studies [4,6-13]. In general, the average level of infection with *Blastocystis sp.* population in industrialized countries slightly exceeds 5%, and in developing countries reaches 30-60% [14,15].

To detect *Blastocystis sp.* in FS, microscopic, cultural (cultivation of parasites in vitro), immunological (detection of protozoan antigens using enzyme immunoassay, direct immunofluorescence reaction, immunochromatographic test, etc.) and molecular genetic methods (detection of fragments of the parasite genome using various types of polymerase reaction) are used [4,6,7,9-16]. Each group of detection/identification methods for *Blastocystis sp.* in FS has its advantages and disadvantages [4,7,11,17-19]. Commercial immunological and molecular genetic test systems are quite expensive, and their use requires appropriate laboratory equipment. In addition, to date, such test systems have not undergone state registration in Ukraine and are not available on the national market for practical or scientific use (personal communication of the authors). On the contrary, due to technical availability, traditional methods of light microscopy remain the most commonly used in all countries of the world for laboratory diagnosis of blastocystosis to this day. They are based on detection/identification in FS smears of diagnostic morphological forms (morphoforms) of *Blastocystis sp.*: predominantly vacuolar, granular (and transitional stages), rarely amoeboid, cysts [4,7,9,11,20,21]. Light microscopy of faecal samples remains the most common and accessible method: preparations of wet smears temporarily stained with 1-2% iodine solution; thin fixed smears of FS, stably stained with Heidenhain's iron-hematoxylin stain, Wheatley's modification trichrome stain, Giemsa's stain, modified Field's rapid stain [6,7,18,19,22]. Microscopy of preparations of fecal smears, persistently stained with trichrome and iron hematoxylin, still remains the «gold standard» for routine laboratory diagnosis of many intestinal protozoal diseases, including blastocytosis [16,18,23,24].

Among the shortcomings of the method of direct coproscopy, the most significant are its relatively low sensitivity (on average 55-75%), which is sought to be increased by applying additional procedures for enriching feces (mainly sedimentation), a certain difficulty in identifying *Blastocystis sp.* cells, which are extremely diverse in size and morphology. and their differentiation from other elements that may be present in FS − leukocytes, yeast-like fungi, fat drops, other protozoa (*microsporidia, Cryptosporidium spp. oocysts, Entamoeba spp., Cyclospora sp.*) [3,4,7,18,19].

Cultural methods of diagnostics (short-term cultivation *in vitro*) are an alternative to microscopic methods widely used in practice [6,7,25-30]. They require insignificant material costs and involve the use of equipment typical of bacteriological laboratories with a duration of the detection procedure of 24-72 hours [7,31-32]. Compared to microscopic, culture methods for the detection of *Blastocystis sp.* are characterized by higher levels of sensitivity (about 90% and higher) and specificity (100%) [7,23,24], which allowed the authors of a number of works to propose cultural methods for diagnosing blastocystosis [23-26,32,33].

The goal of the work. To establish the prevalence of *Blastocystis sp.* in fecal samples in different cohorts of residents of the Kharkiv region (clinically healthy and symptomatic people with symptoms of gastrointestinal lesions) by microscopy and cultivation.

Materials and methods.

The surveyed cohort consisted of residents of the Kharkiv region (n=169), among whom 72 people were with symptoms characteristic of blastocystosis, 97 were asymptomatic, clinically healthy people. FS from each person were taken into 2 sterile disposable containers: with a preservative (10% formalin) in a 1:1 volume ratio for microscopic examination; without preservative (material for culture (in vitro) detection of *Blastocystis sp.*

All 169 FS (their sediments) were subject to microscopic examination after the enrichment (concentration) procedure performed in accordance with the UK. National Standard Method. Staining Procedures/Health Protection Agency. BSOP

TP 39. Is. 1. 2007. 29 p. http://hemltd.ru/publications/sections/Normativ/foreign/samples/medicine/NHS023/article.pdf. [34] with changes as described earlier [3]: in formalin-phosphate-buffered saline (FPBSCS) (pH = 7.4) using a laboratory centrifuge CM-3 «MICROmed» (Ukraine) in the mode of 500 g for 10 minutes.

For the preparation of permanently stained smears, thin smears were prepared from the enriched FS sediment (50 µl), which were stained with Wheatley's modification trichrome stain (mWTS) and Heidenhain's iron-hematoxylin stain (HIHS) [34,35].

Detection of *Blastocystis sp.* in all FS, the culture method was carried out according to the methodology described by Pokhil S.I., Tymchenko O.M., Chigirinskaya N.A. et al. [36]. The presence of parasite cells in the crops was established on the 4th day of incubation by phase-contrast microscopy (total magnification ×600) of 20 µl of a homogenized suspension, and the final identification of detected *Blastocystis sp.* was carried out according to the result of light microscopy (with a total magnification of ×1500) of smears of suspensions permanently stained with mWTS or HIHS [35,36].

Light microscopy of stably stained sediment smears after enrichment with FS, as well as suspensions after cultivation, was performed using a microscope for clinical laboratory diagnostics «MIKMED-2» Yu-33.22.926, with eyepieces with a magnification level of 10×, 15× (total magnification ×1500).

Statistical processing of experimental data was carried out using IBM SPSS Statistics v.19.0 software. The difference in mean values (M±m) was considered statistically significant at $p<0.05$.

Results and Discussion.

The results of a comparative assessment of the detection efficiency of *Blastocystis sp.* methods of microscopy and cultivation in 169 samples (72 from asymptomatic and 97 from symptomatic individuals) of authentic (the same) FS origin are presented in the table 1.

The data in the table indicate a low increase in the frequency of detection/identification of *Blastocystis sp.* culture method (by 4.1%) compared with the frequency of microscopic detection of parasites in all FS (n = 169) ($p > 0.05$). At the same time, the use of the culture method led to an increase in the frequency of detection/identification of *Blastocystis sp.* in FS from asymptomatic individuals (n = 72) by only 2.7%, and in FS from symptomatic individuals (n = 97) by 5.2% ($p > 0.05$).

The analysis of the data obtained leads to the logical conclusion that the use of the culture method made it possible to additionally detect *Blastocystis sp.* and in those FS in which the number of parasite cells was less than the minimum required for their detection by the microscopic method.

Thus, between the total results (negative+positive) of detection/identification of *Blastocystis sp.* microscopic and cultural methods in all FS from residents of the Kharkiv region rf reaches +0.92, and for groups of FS from asymptomatic and symptomatic individuals - rf = + 0.94 and rf = + 0.90, respectively. In the sample of only positive results of detection/identification of *Blastocystis sp.* By microscopic and cultural methods, the value of rf is: + 0.59 for all studied FS, + 0.20 for FS from asymptomatic individuals, and + 0.66 for FS from symptomatic individuals. Therefore, if the level of sensitivity of the used culture method for the detection/identification of *Blastocystis sp.* in all investigated FS to be taken as 100%, then a certain relative sensitivity of the microscopic method will be 79.4%.

In previous years, our colleagues State Institution «Institute of Epidemiology and Infectious Diseases named after L.V. Gromashevsky National Academy of Medical Sciences of Ukraine» for two decades, screening studies of FS (by microscopic method) were carried out from symptomatic and clinically healthy people [37]. The data of our colleagues confirm the opinion of other scientists that *Blastocystis sp.* is the most common protozoan, and the frequency of their detection in FS has been growing over the past 20 years. According to the results of their research, the proportion of infected people increased from 6.5% in 1995-1997. up to 19.5% in 2015-2016 against the background of a decrease in the detection rates of other protozoa. The proportion of *Blastocystis sp.* among found protozoa increased from 57.1% in 1995-1997. up to 97.0% at present [37].

In the course of research, we obtained relatively low rates of increase in the frequency of detection/identification of *Blastocystis sp.* in FS from humans by the culture method (compared to the results of microscopic detection) differ significantly from the significantly higher analogous indicators given by other authors [9,23,24,28]. This can be explained by a number of differences in the design of studies performed by the authors of these works. These differences consist in comparing the frequency of detection of *Blastocystis sp.* culture and light microscopy of wet unstained or temporarily stained smears of unenriched feces, which is generally characterized by a relatively low level of sensitivity.

Table 1. *Frequency of detection of Blastocystis sp. in FS from asymptomatic and symptomatic persons (residents of the Kharkiv region) by microscopy and cultivation methods.*

Source of origin of FS, their number (n)	The number of positive results of detection/identification of *Blastocystis sp*. methods	
	microscopy[1], abs. hours (%)	cultivation[2], abs. hours (%)
FS from asymptomatic individuals, n=72	3 (4,2)	5 (6,9)
FS from symptomatic individuals, n=97	24 (24,7)	29 (29,9)
FS of different origin, Σ n = 169	27 (16,0)	34 (20,1)

Notes:

1-according to the results of the final microscopic identification in permanently stained (mWTS or HIHS) fixed smears of enriched sediments.

2-according to the results of final microscopic identification in permanently stained fixed smears of suspensions of cultures grown on RPMI/IMDMEM medium.

Так, Zhang X., Qiao J., Wu X. et al. [25] studied 398 FS from outpatients, showed an increase in the frequency of detection/identification of *Blastocystis sp.* culture method (on RPMI medium) by 8.9% compared with the efficiency of microscopic detection of these parasites in fecal smears persistently stained with trichrome, and also that the sensitivity level of this microscopic method is 90.8% relative to the sensitivity of the culture method they used.

Hegazy LA, Salama MA, Fawzy EM, et al. [26] studied 72 FS (from asymptomatic and symptomatic individuals), an increase in the frequency of detection/identification of *Blastocystis sp.* culture method varied from 5.6% on Jones's medium up to 16.7% on Boeck and Drbohlav's modified Locke egg diphasic medium (LE) when compared with the rate of microscopic detection of these intestinal parasites in stable trichrome-stained smears; and the relative sensitivity of the microscopic method reached 73.3% of the sensitivity level of the method of primary cultivation of cultures of *Blastocystis sp.* on the LE environment.

Thus, according to the results of our studies, we defined as indicators of an increase in the frequency (by 5.2%) of the detection/identification of *Blastocystis sp.* in FS from symptomatic individuals, both by the culture method and relative sensitivity (79.4%) of a comparable microscopic method for detection/identification of these intestinal parasites are comparable with similar data of a number of foreign scientists [25,26]. Cultural method for detecting *Blastocystis sp.* in FS, in addition to a higher level of sensitivity, it is also characterized by a significantly higher level of specificity (accuracy of identification of parasites), which reaches 100%. This is explained by the fact that the variability of the cell morphology of *Blastocystis sp.* complicates their microscopic identification in fecal smears, even for experienced laboratory personnel.

The vacuolar form of *Blastocystis sp.* easily distinguishable, therefore, with a sufficient number of this type of cells, the possibility of erroneous identification when viewing the preparation is very low. Other forms of *Blastocystis sp.* easily confused with other intestinal protozoa, yeast-like fungi, macrophages, leukocytes, erythrocytes, fragments of intestinal epithelial cells, undigested food elements, plant pollen, mucus lumps and other artifacts (pseudoparasites) of feces. This problem is resolved by using the culture method, since in vitro cultivation of *Blastocystis sp.* is accompanied by a significant increase in the number (in the exponential growth phase by hundreds to thousands of times) of parasite cells and the development of their granular, amoeboid, and cystic forms into a typical easily identifiable vacuolar form.

Conclusion.

1. Based on the results of a parallel study by microscopic and cultural methods of 169 FS from different groups of residents of the Kharkiv region, it was found that the cultural method prevails in terms of the sensitivity of detecting *Blastocystis sp.* in FS (by 20.6%) and is characterized by a significantly higher level of specificity (accuracy of identification of parasites), which reaches 100%.

2. Mastering the cultivation methods of *Blastocystis sp.* in vitro allows them to be used to improve the efficiency of parasite detection in human FS for the purpose of diagnosing blastocystosis, to conduct epidemiological studies to establish the population prevalence of protozoa, to determine the sensitivity of cultures of *Blastocystis sp.* to drugs, monitoring the effectiveness of the etiotropic therapy for blastocystosis, obtaining parasite antigens, studying the pathogenesis of the disease and the virulent potential of pathogen strains of different origin, etc.

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SUMMARY

DISTRIBUTION AND FREQUENCY OF *BLASTOCYSTIS SP.* BY METHODS OF MICROSCOPY AND CULTIVATION IN FAECES OF RESIDENTS OF KHARKOV REGION

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Introduction. *Blastocystis sp.* – is the most prevalent anaerobic intestinal protozoan parasite in humans and many animals; from 1 to 2 billion people in the world are colonized by this pathogen. *Blastocystis sp.* is found both in faecal samples (FS) of healthy people (asymptomatic persons) and - patients (symptomatic persons) with nonspecific symptoms of gastrointestinal tract, skin, joints and other organs lesions. The prevalence of people affected by *Blastocystis sp.* of both cohorts in the world vary widely (from 0.08% to about 90%) depending on the degree of the country's economic development, sanitary and hygienic conditions, cultural values, etc. Currently, microscopic, cultural, immunological and molecular genetic methods are used for *Blastocystis sp.* detection in stool samples. Each group of methods of *Blastocystis sp.* detection/identification in FS has its advantages and disadvantages.

The goal of this study was to determine the prevalence of *Blastocystis sp.* in faecal samples in different cohorts of people (clinically healthy and symptomatic people with symptoms of gastrointestinal lesions) in Kharkiv by microscopic and cultural methods.

Materials and Methods. Cohort of surveyed residents of Kharkiv (n=169) included 72 clinically healthy individuals and 97 symptomatic individuals with gastrointestinal tract diseases. All 169 FSs (their precipitates) were subjected to microscopic examination after the formalin-phosphate-salt buffer (FPBSCS) enrichment (concentration) procedure (pH=7.4) at 500 g for

10 minutes. *Blastocystis sp.* identification was carried out by means of microscopy of the faecal smears, which were stained by Wheatley's modification trichrome stain (mWTS) and by Heidenhain's iron-hematoxylin stain (HIHS). The inoculated material was a filtered suspension of native FS (200 µl) which was inoculated in 5 ml of liquid media RPMI/IMDMEM (mixture of equal volumes of RPMI and IMDMEM media) with antibiotics and serum. *Blastocystis sp.* culture growth was carried out under anaerobic conditions at 37 oC for 5 days. The blastocysts final identification was carried out by means of light microscopy of suspensions smears stably stained with mWTS HIHS.

Results & Discussion. It was carried out a comparative evaluation of the effectiveness *Blastocystis sp.* detection methods as microscopy (smears of enriched faecal material stained with mWTS or HIHS) and cultivation (on RPMI/IMDMEM medium) based on the results of parallel studies of 169 FS from different groups of people by both methods. An insignificant increase (4.1%) of the *Blastocystis sp.* frequency detection/identification by means of cultural method in comparison with the frequency of microscopic parasites detection in all FS was determined: in FS from asymptomatic individuals (n = 72) only by 2.7%, and in FS from symptomatic individuals (n = 97) - by 5.2% ($p > 0.05$). From all FS in which *Blastocystis sp.* was detected microscopically, the growth of these parasite primary cultures was obtained. Among the total results (negative + positive) *Blastocystis sp.* detection / identification by microscopic and cultural methods in all FS from humans rф reaches +0.92, and for groups FS from asymptomatic and symptomatic individuals - rф=+0.94 and rф=+0.90, respectively. In the sample of only positive results detection / identification of *Blastocystis sp.* by microscopic and cultural methods, the value of rф is: + 0.59 for all studied FS from humans, + 0.20 - for FS from asymptomatic individuals and + 0.66 - for FS from symptomatic individuals.

Conclusion. According to the results of a parallel study of microscopic and cultural methods of 169 FS from different groups of people it was found that the cultural method dominates over microscopic in sensitivity of *Blastocystis sp.* detection in FS (20.6%) and is characterized by a much higher level of specificity (accuracy of parasite identification), which reaches 100%. The method of *in vitro* diagnostics helps to increase the efficiency of parasites detection in human FS, can be used for epidemiological studies to establish the population prevalence of protozoa, to determine the sensitivity of *Blastocystis sp.* cultures to drugs, control of the etiotropic blastocystosis therapy effectiveness, obtaining parasites antigens, study the disease pathogenesis and the virulence potential of pathogen strains of different origin, etc.

Keywords. *Blastocystis sp*., diagnostics, *in vitro*. microscopic examination.