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Review Article

BLASTOCYSTIS HOMINIS: A MYSTERIOUS AND COMMONLY DISREGARDED PARASITE

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Abstract. *Blastocystis hominis (B. hominis) is an anaerobic, single-cell protozoan, commonly present in human and animal stool samples. It can be found in healthy people as well and it still has not been elucidated whether it is a commensal organism or a pathogen. Blastocystosis is a disease caused by the protozoan in humans. The prevalence of the parasitosis varies both between the countries, and between certain population groups within individual countries. Due to poor hygienic conditions, common exposure to animals and intake of contaminated water and food, people in the developing countries have got a higher prevalence of blastocystosis, but economically developed countries have not been spared either. The taxonomy of B. hominis is still a matter of debates. For the reasons of genetic diversity, it has been suggested that the name B. hominis should be replaced with „Blastocystis species". Seventeen subtypes of the species have been so far identified, and a definitive characterization of Blastocystis spp. is possible at the molecular level only. The parasite is transferred by the fecal-oral route. A variety of hosts have been identified, and animal-to-human and vice versa transfers have been documented. The most common manifestations of the infection with the organism are diarrhea, abdominal pain, nausea, and bloating. This infection has also been associated with the irritable bowel syndrome (IBS), non-specific colitis, chronic inflammatory bowel disease (CIBD), and urticaria. The diagnosis can be made using the methods of conventional microscopy (CVM), phase-contrast and electron microscopy, cultivation, serodiagnosis, and by using molecular methods. The infection caused by the parasite does not always require treatment. In symptomatic patients, the first line medical treatment is metronidazole. Further studies are required to resolve all dilemmas regarding the parasite.*

Key words: *Blastocystis hominis, diarrheal syndrome, diagnosis, treatment.*

Introduction

Blastocystis hominis (*B. hominis*) is an ubiquitous parasite spread widely in the tropical climate areas. It is commonly present in human and animal stool specimens (in birds, rodents, reptiles, amphibians, fish, cockroaches). Its role as the cause of an infection has not been fully elucidated. Throughout the literature, the organism has been reported as a commensal organism, but also as a pathogen. In recent years, numerous studies have been published reporting that the infection with *B. hominis* is common in immunocompromised individuals [1,2].

The organism belongs to single cell, anaerobic eukaryotes (protists). Brittain and Swayne, independently of each other, detected the microorganisms studying a cholera epidemic in London in 1849, wrongly identifying them as the cause of cholera [3]. *Blastocystis*, the name of the genus, was given by Alexeieff in 1911, and the following year the name of the species was suggested by Emile Brumpt [4]. Zierdt et al. performed a reclassification and classified the organism among protists, based on its morphological and phenotypic characteristics (one or several nuclei, cellular organelles similar to mitochondria, endoplasmatic reticulum and Golgi apparatus, inability to grow on fungal culture media, resistance to antifungal agents, and sensitivity to antiprotozoal drugs) [5].

Classification

At the end of the XX century, Silberman et al. classified the organism among eukaryotes, of the Heterokontophyta type, based on the molecular analyses of small subunit (SSU) rRNA (SSU-rRNA) and elongation factor 1 (EF-1α) [6]. Although the taxonomy such as this was controversial when related to other studies demonstrating a similarity of *Blastocystis spp*. parasite with protists, the subsequent studies confirmed the assertion presented above [7, 8]. There are over 100.000 members of the Heterokontophyta order, commonly termed heterokonts or stramenopiles, and *Blastocystis spp*. becomes a new member of the complex group of the so-called „botanical protists“ [9,10]. By way of phylogenetic analysis of

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SSUrRNA and HSP70c, a close connection between *Blastocystis spp*. parasite and stramenopiles has been confirmed, in spite of its absence of flagella and tubular elongations [9]. Despite the classification at a molecular level, there is a difference in morphology between blystocystis and other stramenopiles (flagella surrounded by lateral hairlike mastigonemes) [11,12].

The origin of the organism at the species level has not yet been resolved. There are several host-specific species: *B. hominis* in people, and *B. ratti* in rats [13]. Host diversity is well known, and human-to-animal and vice versa transfer is well documented [14]. In humans, any of the isolated species is termed *B. hominis* [4], although, due to its genetic diversity, the suggestions have been put forward that the term *B. hominis* should be replaced with *Blastocystis species* [13].

Because of all these facts, a step towards classification of different species has been made based on their ultrastructural morphological characteristics visualized by way of electron microscopy [15]. Host specificity and pathogenic potential of different isolates correlate with sequence variations in SSU-rRNA [14]. Conserved and variable regions within 18 SSU-rDNAs constitute the basis for identification of phylogenetic relations between the species [13]. Moreover, rRNAs are made use of in diagnostic PCR analyses with high sensitivity [16]. A recent genetic classification of *B. hominis* into subtypes (STs) is the equivalent to earlier species classification [13]. SSU-rDNAs correlate with subtypes and 17 have been reported so far, so that definitive characterization of species is possible at a molecular level only [13].

Host-specificity is determined by STs, where ST1 and ST8 colonize/infect humans (not only them, however, but other hosts as well) [13]. ST9 has been found only in humans, while ST10-17 are present in other hosts as well [14,15].

Biology and Morphology

B. hominis is a strict anaerob with observed intacellular structures similar to mitochondria, but lacking cytochrome enzymes. Intracellular organelles are involved in different metabolic pathways (metabolism of amino acids, biogenesis of iron and sulphur, and tricarboxylic acid cycle). The organism is able to synthesize certain essential cellular phospholipids and to accumulate them most commonly within the storage vacuoles [3]. The average generation time of *Blastocystis* species is 17–22 h, although it depends on the medium used for cultivation. Generation time differs among different STs [17]. The microorganism enters apoptosis after exposure to harsh conditions (exposure to room temperature, air, or antiparasitic agents such as metronidazole). The phenomenon serves as a mechanism aimed to increase the number of viable cells in such conditions [18].

B. hominis has got several different morphological forms. Vacuolar, granular, ameboid, and cystic forms are the ones best described so far. Other morphological forms have also been found on electron microscopy (avacuolar and multivacuolar, of small dimensions and rarely present). In fresh stool samples and culture samples, vacuolar and granular forms are the ones most commonly encountered; they can be visualized using phase-contrast microscopy, with light microscopy of native and stained sample preparations and with electron microscopy [4].

Vacuolar form. This form of *B. hominis* is spherical, containing a central body representing a large vacuole, occupying approximately 90% of the cell, and a thin layer of peripheral cytoplasm situated immediately beneath the cell membrane. The nuclei can be distributed peripherally throughout the cytoplasm. There can be seven nuclei at the most, but there are two nuclei on the average, situated at the opposite ends of the cell [19]. Mitochondrion-related organelles and Golgi apparatus are located peripherally in the cytoplasm. Mitochondria look like roses placed around the nucleus. These structures may protrude within the central body and can have a fiberlike appearance [13]. It has been discovered that the central body is a membrane-enclosed vacuole, containing carbohydrates, fats, and basic proteins. These substances are accumulated within the vacuole by way of the action of the Golgi apparatus and via clathrin-mediated endocytosis [20]. The body most probably has storage and apoptosis-related roles [3,9]. Vacuolar forms can be of different sizes (ranging from 3 μm to 120 μm), but measuring 5 μm to 15 μm on the average [13]. It is generally accepted that this form is most commonly seen in asymptomatic carriers of *B. hominis* [1].

Granular form. This form is very similar to vacuolar forms of *B. hominis*, but contains the granules within the cytoplasm which are often centrally situated. In 1989, Dunn et al. proposed that these structures were similar to myelin-like inclusions, small vesicles, crystal granules, and drops of fat. The granules can be metabolic, reproductive, and lipid ones [13]. It is possible that reproductive granules have a role in schizogony. On the average, there are two nuclei in the cytoplasm (four at the most). They have a slightly smaller diameter compared to vacuolar forms, and measure 9.0 μm to 28.3 μm [19]. They are more frequent in older cultures and the cultures treated with antibiotics, and there has also been the hypothesis that their existence is an indicant of cell death [1].

Ameboid form. This form of *B. hominis* is most rarely encountered. It is irregular in shape, with 1–2 pseudopodia (being stationary nevertheless), with considerable adhesion abilities, enabling its attachment to the bowel mucosa [1]. There is a large vacuole in its cytoplasm, and this form is in fact transformed into cystic form. Since they resemble neutrophils and macrophages, they can easily escape recognition on routine stool sample examinations. Zierdt has suggested Gram staining of unfixed smears to be undertaken for their identification, since these forms undergo lysis when exposed to air, while leukocytes remain intact [4,9]. It is more commonly present in individuals with symptoms of digestive tract infections and in cultures, indicating the pathogenic potential of this form of *B. hominis* [1,21].

Cystic form. These are round or oval, and with smaller dimensions (3–6 μm) [13]. These forms found in certain animals are larger [15]. Cystic forms have a thin, multilayered wall with/without a surface envelope [19]. Their condensed cytoplasm has got several mitochondria and storage vacuoles. The number of nuclei within the cysts varies from 1 to 4. A cyst may survive about a month exposed to air and the temperature of 25°C and enables further spread of the infection – it is a form infectious for humans [1,9].

Vegetative forms are transformed into other vegetative forms with different morphology and can thus escape identification in stool samples [3]. Avacuolar and multivacuolar forms are the most dominant forms *in vivo*, and these also most commonly remain unrecognized on microscopy [22].

Life cycle

Infectious, cystic forms of *B. hominis* are transmitted by the fecal-oral route [23]. The infection may occur after an intake of untreated water or uncooked water plants contaminated with cysts, and also via dirty hands [24, 25]. In an adequate host, the cyst develops via the process of excitation into vegetative forms within the large bowel [9].

Further continuation of the life cycle depends on the subtype compatibility with the host [13]. Other forms can also develop from vacuolar ones. After a period of time after the infection, vacuolar forms form the cysts in the bowel lumen [22]. The encystation occurs during the passage through the large bowel, and the cysts are then excreted via feces. Fecal cysts can be covered with a fiber-like layer which gradually disappears during the cyst development. A thin fibrillar surface layer detected in stool samples plays a significant role in the survival of this parasite *in vivo* [22].

It is thought that different modes of reproduction exist when this organism is concerned (binary fission, budding, plasmotomy, multiple fission, endodyogeny, schizogony). Binary fission is nevertheless the most common mode [26].

Virulence

The studies conducted to establish the pathogenicity of *B. hominis* parasite have been so far unconvincing and disputable. There are some acceptable explanations of pathogenicity related to the species STs and virulence (27, 28). Symptomatic patients are usually infected with ST1 - 4, and 6 (with ST3 being the most common, followed by ST1 and ST2). Subtype-related variations in pathogenicity have been observed as well, which probably can explain the differences between the patogenic and non-pathogenic potentials of the species.

Ameboid *B. hominis* form, which excretes proteases, is the most virulent one. It is predominant in symptomatic patients and these forms should be sought in stool specimens in patient screenings [22,28–30]. In addition to proteases, other hydrolytic enzymes have been identified as well by way of electrophoresis. Lysates lead to cytoskeletal changes and induce apoptosis in epithelial cells, which results in increased bowel permeability. Cystein proteases stimulate mucosal cells to produce interleukin-8. This mechanism is responsible for the loss of fluids and bowel inflammation in the affected. Proteases cleave secretory IgA and help in immune evasion and survival of the parasite [3,31].

Whole-genome sequencing has been done for ST7. The genes have been identified which code the proteins that alter bowel homeostasis. The genes responsible for the production of nonribosomal peptides and polyketides (antibacterial and inducing bowel dysbiosis) have also been identified. The target genes coding for hydrolases have been also described (capable of altering the bowel mucous layer and exposing the epithelium for parasite adhesion). Expression of serine proteases and glycosyltransferases disturbs the firm bonds in the bowel mucose epithelium, leading to increased bowel permeability [3,32].

The molecules responsible for extraintestinal manifestations of the infection are relatively unknown. *B. hominis* antigens stimulate T-helper II cells, leading to an IgE-mediated allergic reaction. The organism probably activates the complement cascade, which leads to the release of anaphylatoxin and mast cell activation. Iron deficiency anemia associated with *B. hominis* infection is still awaiting explanation [33,34]. In general, virulent strains are larger, with an uneven, rough surface, they grow slowly and demonstrate an increased affinity to bind to lectins.

The relationship between the severity of infection and clinical manifestations is still unclear [35–38]. One study has proposed that 32kDa proteases of ST3 could be the virulent factors responsible for protein degradation, while another study has found that a *B. hominis* 29 kDa antigen could be used as a pathogenicity marker, enabling differentiation of symptomatic from asymptomatic *B. hominis* infections [39,40]. Increased IgA levels have been described in symptomatic individuals with *B. hominis* infection, compared to healthy asymptomatic carriers of *B. hominis* [41].

A recent study about the impact of *B. hominis* parasite on the expression of gamma interferon and proinflammatory cytokines of the cecal mucosa in rats has shown a significant upregulated transcription of type 1 gene and proinflammatory cytokines IFN-gamma, IL-12, and TNF-α. This suggests that *B. hominis* infection in rats stimulates specific local host responses, involving T cells, monocytes, macrophages, or natural killer cells [42]. Studies on mice inoculated with high doses of *Blastocystis spp*. have shown a loss of weight in mice and onset of diarrhea [43–45]. Studies have also demonstrated that *Blastocystis spp*. can attack the lamina propria, submucose, and muscle layers, and to invade the epithelium of the rat colon in view of the increased levels of hyaluronidase in the urine of rats infected with *Blastocystis spp*., which stil is not a sufficient proof of similar events in people [46,47]. Laboratory rats constitute a good model of the pathogenesis of *Blastocystis spp.* infection, in con-

trast to mice which have not been naturally infected with *Blastocystis spp.* [48].

There have been several reports suggesting that *Blastocystis spp.* could be associated with urticarias in humans [9]. Ameboid forms of ST3 *Blastocystis spp.* have been identified in the cases of acute urticaria, and authors believe that cutaneous symptoms can be caused by a disruption in the immune homeostasis [49]. In another study, *Blastocystis spp.* ST2 has been demonstrated in a patient with severe gastrointestinal complaints and chronic urticaria, in absence of any other infectious agent. The complaints persisted after the initial antibiotic therapy, but were eliminated after combined metronidazole and paromomycin therapy [50].

Clinical Significance and Treatment

In view of the controversies related to the pathogenicity of *B. hominis* in humans, the results of numerous studies confirmed/excluded *B. hominis* as a disease cause. In certain studies, the individuals susceptible to an infection with the parasite have been mentioned: HIV infected individuals, patients with cancer or with other immune difficiency conditions, children from the developing countries, frequent travellers [13].

It seems that clinical manifestations of blastocystosis depend on the subtype of the parasite. ST1 subtype has been found in those with symptoms of the infection [51], while in Columbia it has been documented in asymptomatic examinees [52]. *B. hominis* of ST2 has got a controversial pathogenicity, positively demonstrated in some studies [52–54], and disputed in the others [55,56]. Regarding the most common *B. hominis* subtype ST3 isolated in humans, there have been little evidence that could indicate its pathogenicity. Around 40% of those infected with ST4 of *B. hominis* have got gastrointestinal symptoms, found as well in those with the clinical picture of severe diarrhea [57,58]; ST5 subtype has been found in those with symptoms of the infection [59], in those coming into contact with animals and in animals themselves [60,61]. *B. hominis* subtype ST6 causes diarrhea in a third of the infected, and *B. hominis* subtype ST7 is also associated with the onset of diarrhea [57].

Clinical characteristics of the disease are non-specific and consist of abdominal pain, acute/chronic diarrhea, nausea, anorexia, bloating, perianal itching (abdominal pain and diarrhea being the most common complaints). The symptoms range from mild and moderate, to severe acute and chronic events. The number of parasites found in stool specimens determines the severity of symptoms and signs of the infection [1,9,22,37,38].

B. hominis is associated with the irritable bowel syndrome (IBS), since the changes in the intestine occur (caused by this disorder) that favor the development of the parasite [13]. It is believed that low-intensity inflammation occurs as the consequence of constant immune activation caused by the parasite and persistent antigen exposure of the host [62]. Moreover, increased levels of IgG2 immunoglobuline against *B. hominis* have been found in the examinees with IBS [63]. Blastocystosis has been associated with non-specific colitis too, as well as with chronic inflammatory bowel disease (HIBD) (including Crohn's disease and ulcerous colitis) [14,64,65].

Eosinophilia and skin changes (primarily urticaria) are rarely encountered in patients [9,13]. There have been several individual cases of *B. hominis* infection in patients with chronic kidney disease [66,67] and arthritis [66, 68-71). A high prevalence of the infection (95.8%) has been described in immunocompromised patients (HIV positive individuals and those with AIDS) [72].

However, not all of the infected develop symptoms and signs of the disease. These are asymptomatic individuals – there are many more asymptomatic cases than those with symptomatic *B. hominis* infection [27,30,41,56,64].

There is also the question whether the infection with this parasite requires treatment. In symptomatic patients with confirmed infection (the finding of *B. hominis* in the stool specimen), it is necessary to examine the presence of other infective agents in the gastrointestinal tract, since there is a real possibility of coinfection with other pathogens as well [13].

Metronidazole is a first choice drug in cases of proven infection. It's effectiveness, however, has been known to vary. It is effective in some patients, but it cannot produce complete eradication of the infection (especially a severe one). There is a possibility that non-responders have been infected with resistant *B. hominis* subtypes. The studies dealing with metronidazole efficacy have not elucidated the association between *B. hominis* subtypes and treatment failure [13].

Trimethoprim – sulfamethoxazole is the second choice drug for those who failed to respond to metronidazole treatment. It has been demonstrated that paromomycin, a wide spectrum antibiotic indicated in acute and chronic intestinal amebiasis, is successful as the treatment of *Blastocystis* infections associated with skin lesions (predominantly urticaria) [73–76].

Yakoob et al. have studied the efficacy of garlic and other dietary herbs *in vitro* in comparison with metronidazole in individuals infected with *B. hominis*. The authors assessed the efficacy of garlic and metronidazole in concentrations of 0.01 and 0.1 mg/ml. They found that garlic and metronidazole were equally effective in both concentrations. *Blastocystis* isolates were not sensitive to other tested herbs such as ginger, black pepper, and white cumin [77]. It has been established that probiotics such as *Saccharomices boulardii* are equally effective as a symptomatic treatment as metronidazole [13,78].

Diagnosis

Conventional microscopy of (CVM) (with/without concentration method):

- **native preparations** (unstained/Lugol stained). If Lugol's solution is added, parasites are stained golden yellow. However, due to *Blastocystis* polymorphic structure, a wrong identification can occur and their misinterpretation as fungi, *Cyclospora spp*., and drops of fat [13]. Classical vacuolar forms do not have to be predominant in fresh stool sample, while smaller forms can be hard to identify [60]. **For the diagnosis of *Blastocystis* infection to be made, several stool samples (at least 3) are required, more than 5 cysts in the visual field without other parasites** [1].
- **stained preparations** (by Giemsa, Gram, Wright, iron hematoxylin) [80]. A common staining in the diagnosis of *Blastocystis* is **trichrome staining**. With this method, the large central body is usually stained green to gray. Inclusion bodies in the cytoplasm stain light to dark red.

Phase-contrast microscopy

Phase-contrast microscopy is more convenient than light field microscopy when greater magnifications are required and when samples are colorless or their details are so minute that color cannot be discerned well [80]. Phase-contrast microscopy enables the study of morphological features of the cells and of their reproduction via cell division.

The fundamental principle of phase change visualization in phase-contrast microscopy is the separation of background light from the specimen-scattered light, which enables better visualization (highlighting) of the required image details; the phenomenon is based on the property of cells to have a refractive index different than that of the surrounding medium [9].

Transmission electron microscopy (TEM)

In the routine diagnosis of *Blastocystis* TEM is not used; it is used however in the demonstration of atypical forms of the parasite [9].

Cultivation

When CVM specimens are positive to the vacuolar forms of *B. hominis*, cultivation on the Löwenstein-Jensen (LJ) medium is performed: a 48-hour incubation in anaerobic conditions, when white, very bright and mucous colonies grow [81]. A native preparation with physiological solution is made of the suspect colonies and is inspected microscopically, when vacuolar forms of *B. hominis* are seen [82].

Xenic and monoxenic laboratory cultures of *B. hominis* isolates, growing together with non-standardized or individual known types of microorganisms, can be kept alive in the Jones' or Boeck-Drbohlav condensed medium [83,84]. The Jones' medium is the medium of choice for the studies involving cultures for parasite identification from patient specimens [81,85–88].

Axenic cultures, i.e. the cultures without any other living organism(s), demonstrate a rich growth in different media [89,90]. *B. hominis* cells can grow on a solid medium as well, and its colonies macroscopically appear similar to bacterial colonies [91]. The colonies may survive up to 2 weeks and can be preseeded in a liquid or solid medium [92]. It is interesting that the same isolates cultured in a liquid medium reach their maximum cell density around day 4 after inoculation, and enter the dying phase on day 5, so that the growth of their subcultures is made difficult [9]. This indicates that growth characteristics of the same isolates in a solid medium are essentially different from the characteristics of *Blastocystis* isolates grown in a liquid medium.

Axenic cultures of *Blastocystis* isolates are very important for molecular and biochemical research. Axenization can be accomplished by the addition of antibiotic cocktails in order to eliminate bacteria and fungi. Several combinations of antibiotics have been so far described, used with variable success. The procedure is generally a demanding one, lasting for weeks or months, without any guarantee that the bacterial contaminants would be eliminated in the end. It has been supposed that some of the isolates require the presence of bacteria for their survival, so that the removal of all bacteria can result in the death of the parasite [9].

Lanuza et al. have improved the method for *Blastocystis spp*. axenization and succeeded to axenize 25 out of 81 isolates. The time required for axenization was about 3 weeks [93]. In addition to an antibiotic treatment, some authors have pointed out that physical methods can contribute to the success of axenization – separation of parasites from the mass of sprouting bacteria [93–96].

Serodiagnosis

The infections caused by *B. hominis* lead to IgA and IgG immune response, and antibodies can be demonstrated using indirect immunofluorescent (IFA) and enzyme-linked immunosorbent assay (ELISA) tests [97].

An IFA assay involves a highly specific and sensitive method for the confirmation of *B. hominis* parasite. Commercially available immunofluorescent antibodies are used, specific for *B. hominis*. Based on polyclonal antibodies, the subtypes of *B. hominis* ST1-ST3 and ST5 can be determined using the ELISA test [97].

Symptomatic infections are associated with elevated IgG antibody titer. In asymptomatic infections, IgA response is weak or absent. The strongest immune response is reported for chronic infections. Since the knowledge concerning host immune response to *B. hominis* infection is still limited, and since antigen diversity of the parasite is obvious, serologic tests are not routinely used, but their use is of key importance in epidemiological and other scientific research. These methods are far more sensitive and specific

than CM and the tests are nowadays commercially available [9,97].

Molecular methods

Amplification of *B. hominis* DNA obtained from fresh stool samples or from culture is convenient for the purpose of epidemiological and screening studies, and genotyping should be included in the analysis as well [9]. The development of a real time polymerase chain reaction (PCR) method for sufficiently sensitive and rapid detection of *Blastocistis spp*. and the ability to differentiate between the genotypes present in the specimen would be equally useful in screening and in epidemiological studies [9].

Epidemiology

B. hominis is a ubiquitous parasite. Its prevalence varies between the countries and from one population group to another. People in the developing countries have got a higher prevalence of blastocystosis due to poor hygienic conditions and intake of contaminated water and food [51].

However, *B. hominis* has got a wide geographical distribution and can be found in economically developed countries as well [97,98]. A study involving the whole territory of the USA, conducted by the Center for Disease Control and Prevention and using the 1987 data, reports the prevalence of *B. hominis* infection of 2.6% in the general population [99], while a study published in 2000, performed in private laboratories in 48 states of the USA, shows the prevalence of blastocystosis of 23% [100]. In Canada, *B. hominis* was the most common cause of protozoal infections in 2005 (101).

B. hominis was the parasite most commonly isolated in Indonesia, in the HIV infected and those with AIDS tested before the administration of antiretroviral therapy [102] and in Turkey in cancer patients [103]. An increased prevalence of the parasite in individuals in contact with animals suggests the possibility of a zoonosis [27].

The distribution of genotypes established using the PCR method has been reported in several countries: Bangladesh, Germany, Pakistan, Japan, Singapore, Greece, Turkey, China. The ST3 subtype established in China is the most predominant one, while ST1 has been found in a lesser degree in Singapore, Greece, and Germany [13]. There are also mixed type infections with different subtypes, most commonly with ST1 and ST3 [51,55,104,105].

A recent study in our country has shown that children with blastocystosis have colitis as the most common large bowel pathology, without any significant difference between non-specific colitis and HIBD. The infection is most commonly found in children aged 2 to 3 years, followed by those 16 to 18 years of age. Significantly higher number of the infected live in houses compared to flats, and possess domestic animals and/or pets. A positive fecal occult blood test, iron-deficiency anemia, elevated erythrocyte sedimentation and CRP are characteristic of those with *B. hominis* infection and HIBD who have a larger number of parasites in their stool samples. Mesenteric lymphadenitis and splenomegaly are the most commonly described pathologic changes on the abdominal ultrasound of children infected with *B. hominis* hospitalized for the complaints of abdominal pain and/or diarrhea. The author concludes that the establishment of a pathogenic significance of *B. hominis* contributes to the recognition of this protozoan as a pathogen and stresses the necessity of a treatment for the condition [106].

The first studies in the region of Niš concerning the prevalence of blastocystosis in patients with/without infection symptoms have been published about a decade ago; the prevalence of 4.05% was then established among the healthy, and 0.36% among those with symptoms of the infection [107]. Based on still unpublished data of the Parasitology Laboratory, Public Health Institute, the total prevalence of *B. hominis* has been reduced in the last decade (2.7%) in asymptomatic individuals.

Conclusion

Blastocystis hominis is still a mysterious and perhaps scientifically disregarded parasite in human pathology. Differentiation among *Blastocystis* species is not possible using the routine methods. The use of DNA methods enables detection of genetic variations in these parasites with still uncertain taxonomy. Epidemiological molecular studies are especially useful in the establishment of transmission patterns, host specificity, and in the surveillance of chemotherapeutic resistance.

Distribution and genotypic diversity of *Blastocistis spp*. have not been studied so far in Serbia, which necessitates a systematic research of the parasite and filling in of the epidemiological map, as well as the establishment of significance of the organism in human pathology.

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