Epidemiology of Blastocystis spp. in primary school students at a central village of Ordu province

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Abstract
Blastocystis spp. is one of the most common intestinal parasites in humans and its pathogenicity is still controversial. The epidemiology of this parasite varies depending on the socio-cultural, socio-economic and geographic circumstances. In this study, Blastocystis spp. was investigated in stool samples of a Primary School students’ in a Central Village in Ordu province who did not have any intestinal complaints. Before starting work, we were granted permission from National Education Directorate and the School Board. Blastocystis spp. was investigated in Primary School Students’ stool samples by direct microscopy and Trichrome staining. The stool samples which showed any form of Blastocystis spp. were evaluated as “microscopically positive” regardless of the number per microscope field. Eighty and 85 of the 165 students participating in working were male and female, respectively. As a result of the microscopic investigation, 45 (27.3%) students were identified as positive. Eighteen of the positive students (40%) were male and 27 (60%) were female. Direct microscopic inspection, sedimentation and permanent staining are routine methods used in diagnosis of Blastocystis spp. This study also evaluated trichrome staining for each stool samples. In our research, this parasite was identified in 27.3% of asymptomatic Primary School students’ stool samples. This suggests the parasite is relatively common in the region. Blastocystis spp. infects via the fecal-oral route. As a result, we presented recommendations for the enhancing personal hygiene, social education and the provision of water treatment for the students to protect themselves from infection with Blastocystis spp.

Keywords: Blastocystis spp., Epidemiology, Ordu, Primary school students

Introduction
Blastocystis spp. which is defined in 1988 by Zierdt can be caused complication in gastrointestinal tract in many animal and human [1]. There are different opinions about pathogenicity, genetic diversity, host spectrum, and treatment options, although the knowledge of parasite morphology for long time [2]. There are many zoonotic genotypes in humans [3]. There are studies in the literature supporting the pathogen of Blastocystis [4-6], whereas, some authors have reported that Blastocystis spp. is not responsible the clinical symptoms of blastocystosis [7,8]. Tan [3] asserts that some populations may be more susceptible to infection with Blastocystis spp. and risk factors of this parasite are immunodeficiency, journey to the tropics, animal low hygiene, contact with contaminated water and food. It is reported that in the rare cases, detection of Blastocystis spp. is only amoeboid form can be shown severe diarrhea and while Blastocystis spp. can lead to protracted and recurrent diarrhea in immunocompromised persons, especially AIDS patients [9]. In addition, Blastocystis infections cause symptoms such as diarrhea, abdominal pain, discomfort, bloating, gas, cramps, vomiting, dehydration, anorexia, insomnia, and nausea, lack of appetite, weight loss, fatigue, itching and tenesmus [10].

Blastocystis spp. has taken in the first row within the gastrointestinal protozoa in epidemiologic study, as well [6,11,12]. The economic developments, increasing human migration, environmental and demographic changes have been reported to be effective in the distribution of parasite. Subtropical climatic zone which is also including Turkey and evolves the proliferation of parasitic disease pathogens provides condition of optimal temperate climate [13]. Nowadays, the prevalence of parasite is different rates in developing countries and developed countries. The prevalence of Blastocystis spp. is the most common gastrointestinal protozoan with 1.5-10% in developed countries, while they are at the rate of 30-50% in developing countries. This situation have been connected to low hygiene, contacting with animals and contaminated water and food

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It has been reported that the prevalence of this parasite was 1% in Japan [15], 40.9% in Brazil [16] and 33.3% in Egypt [17] while, the rate of this parasite ranged from 0.48-44.4% in Turkey [13,18].

Recent studies suggest that it is impossible to explain the relationship between Blastocystis and irritable bowel syndrome when intestinal microbiota is not considered. Researchers have suggested that the environment may become a condition that supports or does not support the multiplication of Blastocystis due to the changing intestinal flora in chronic bowel diseases [19].

Meta-genomic analysis in a retrospective study Blastocystis was detected in 20% of patients with ulcerative colitis (UC), but not in Crohn’s disease [20].

According to our best reached knowledge, many researches were reported regarding as intestinal pathogens in different sites of Turkey. However, this research is the first for the detection on epidemiology of the intestinal parasites in children in Ordu province. In this study, Blastocystis spp. was investigated in stool samples of Primary School students who did not have any intestinal complaints at a Central Village in Ordu province of Black Sea Region in Turkey.

**Materials and Methods**

Before starting work, we were granted permission from National Education Directorate and the School Board. The research was conducted in Department of Medical Parasitology laboratory at School of Medicine in Ordu University by examining 5-14 age group of 165 children’s stool samples collected from Kokenli Elementary School in Ordu province between the periods of February, 2015-March, 2015.

The cover-sealed containers for stools were used for each specimen in research. The class teachers described how to take stool samples for students and it should not be at a rate of no more than 1/3 of the stools container. Fresh stool samples were examined without waiting after reached to the laboratory. The stools were macroscopically evaluated for the color, consistency, whether the presence of blood and mucus and parasitic. The stools slides were examined by direct microscopy and trichrome staining. All slides were screened by the use of direct microscopy under X10 and X40 magnifications and stained slides have been assessed by scanning 100 magnification.

**Results**

In the study, 80 and 85 out of 165 students participating in this research were male and female, respectively. As a result of the microscopic investigation, 45 (27.3%) students were identified as positive. Eighteen of the positive students were found to be male and 27 were female. Blastocystis spp. found over 5 in each field was evaluated as positive. Sixty percent of women and 40% of men has been found to be infecting with parasites. The parasites were found to be in the same samples by Trichrome stain.

**Discussion**

Blastocystis spp. is one of the most common intestinal protozoa in humans. In nature there are many genotypes and human is the host for many zoonotic genotypes [3,21]. The pleomorphic structure of parasite and the lack of standardized diagnostic methods lead to misinterpretation in data about Blastocystis spp. The complexity about the pathogenesis of this parasite have originated from the presence of pathogenic and nonpathogenic genotypes [22]. Epidemiological, in vivo and in vitro studies related with parasite reinforce its the pathogen data [23].

The methods used in the routine diagnosis of Blastocystis spp. have been reported as directly screen, condensation and permanent staining. When directly screen comparing with condensation and Trichrome staining, these methods are reported to be more sensitive than directly screen [6].

Korkmaz et al [12] reported that the difficulty can be experienced in direct microscopic examination of blastocystosis because of different sizes and different morphological types of them and confusable with yeast and leukocytes by inexperienced persons. Even though, the authors found that the presence of the parasite
with trichrome staining higher than native-lugols, this method is also time consuming, laborious, and requires experience. In this study, both directly screen and trichrome staining method were used together.

Mumcuoğlu [24] reported that Blastocystis is found to be positive in 16 patients (29.1%) with irritable bowel syndrome were treated with direct microscopy, 18 (32.7%) with trichrome staining and culture method. Control group 2 without gastrointestinal system complaints; Blastocystis spp. was detected in 1 person (2.0%) by direct microscopy and Trichrome staining and in 3 persons (6%) by culture method. In control group 1, no other pathogens were found in all six patients who were detected 5 and up Blastocystis spp. on direct microscope. In addition to this situation, because of the absence of post-treatment complaints, reported that Blastocystis spp. Is a pathogen that needs to be evaluated by complaints and microscopy adherence?

Korkmaz et al [12] reported the occurrence of Blastocystis spp. in a total of 350 stool samples belonging to patients with diarrhea (n=157) and non-diarrhea (n=193) by native-lugol examination, tricrome staining and direct fluorescent antibody (DFA) methods. Researchers; Blastocystis spp. were found in culture of a total 66 (19%) fecal specimens which were 26 of diarrhea patients (16.6%) and 40 of non-diarrhea patients (21%). Blastocystis spp. was found to be positive in samples with12% (42/350) Native-lugol, 17% (58/350) Trichrome staining and 19% (66/350) DFA method.

In our study, Native-lugol and Trichrome staining were also used for diagnostic purposes of Blastocystis spp.

It has been reported that parasites may be opportunistic pathogens in children and those with immunosuppression and should be monitored more carefully [25]. In people with Blastocystis infection, clinical findings are considered to be due to many factors such as Blastocystis genotype, microbiota, host immune response, Blastocystis concentration and other accompanying infection [19]. Blastocystis infection has been associated with numerous gastrointestinal and some dermatological symptoms (abdominal pain, diarrhea, constipation, weakness, vomiting, headache, dermatological manifestations, joint pain and psychiatric findings) in studies that have identified Blastocystis as pathogenic [26].

In this study, according to anamnesis, it was determined that the parasitized children have digestive system disorders. However, they have reported that they did not go to the doctor with the reasons for their complaints. This can be explained by the fact that the treatment of children should be taken into consideration.

Mumcuoğlu [24], Blastocystis spp. has found to be with 6 (7.5%), 8 (10.0%) and 15 (18.8%) of patients in the control group 1 with acute gastroenteritis complaints by direct microscopy, Trichrome staining and culture method, respectively. In addition, Blastocystis spp. has found to be with 1 (2.0%) and 3 (6%) of patients in the control group 2 with non-acute gastroenteritis complaints by direct microscopy, Trichrome staining and culture method, respectively. Blastocystis spp. has been identified in all of 6 patients in the control group 1 by direct microscopy in each field with the number of its 5 and up. But, no other pathogens were found in direct microscopy. Blastocystis spp. should be assessed by taking into consideration with microscope data and patient’s complaints.

In the resource information reached with parasite epidemiology, the prevalence of this parasite was found to be with 1.5-10% in developed countries, while it was between 30-50% in the developing countries [25]. In America (USA?) and Brazil, the prevalence of Blastocystis was found to be with 18% and 26.5%, respectively [26,27], while, Blastocystis infection rate in Vietnamese pregnant women is reported as 20.4% [28].

In our country, the prevalence of Blastocystis spp. had been reported at ranging from 0.48-44.4% [13,18,29,30]. The prevalence of Blastocystis spp. was found to be with 0.48% in Kütahya [30], 2.1% in Istanbul [31], 22% in Ankara [32], 4.38% in Izmir [11], 7% in Eskisehir [33], 7.64% in Manisa [34], 23.2% in Kocaeli [35], 23.57% in Bursa [36]. According to Korkmaz et al. [12], Blastocystis spp. isolation have been reported in a total of 19% of the stool sample culture with 16% of patients with diarrhea and 21% of non-with diarrhea. Twelve percent of the stool samples are positive by native-lugols, 17% and 19% of them were positive by trichrome staining and DFA method, respectively. In this study, parasite was detected in children who not to go to the doctor because of any complaints.

Conclusion

This study represented that this parasite is relatively common in this region. Enhancing personal hygiene and public education and the provision of water treatment are necessary to protect themselves from Blastocystis infection.

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Competing interests

The authors declare that they have no competing interest

Financial Disclosure

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Ethical approval

Before the study, permissions were obtained from local ethical committee.

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