

Review Article

***Blastocystis*: Emerging Protozoan Parasite with High Prevalence in Iran**

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Abstract

Background: *Blastocystis* is a zoonotic protozoan parasite habit in intestinal tract of humans and wide range of animals. Because of the mysterious nature and unknown or less-known aspects of *Blastocystis*, comprehensive information about epidemiology of this parasite is not available. The objective of this study was to investigate the available parasitology studies during the last decade in Iran and determine the prevalence of *Blastocystis* and its position among other intestinal parasites. As well as, investigate the effective factors in its prevalence.

Materials and Methods: All available studies related to the prevalence of intestinal parasites in Iran during the recent decade were collected using information databases. After determinant the mean prevalence of each parasite, the most common parasites, and effective factors on their prevalence were assessed and analyzed.

Results: Different studies showed that the most common intestinal parasite at this period of time was *Blastocystis spp.* with 14.6% prevalence rate. Additionally, in 44.5% of cases *Blastocystis spp.* allocated the first and in 100% of cases, the first to third rank of the most common intestinal parasites in Iran. *Giardia lamblia* and *Entamoeba coli* were in the next category.

Conclusion: To our knowledge, the present study is the first survey in which the *Blastocystis spp.* introduces as one of the most common intestinal parasites in human. Various factors, including the low sensitivity of routine diagnostic methods, hosts multiplicity, easy transportation and unknown impressive factors are effective in the increased prevalence of this parasite. The results of this study could improve the attitude of teachers and researchers towards *Blastocystis spp.*

Keywords: *Blastocystis spp.*, Intestinal parasites, Iran

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Introduction

Blastocystis is a mysterious emerging zoonotic protozoan parasite habit in human intestinal tract, many vertebrates, and even invertebrates hosts¹. This parasite was discovered by a Russian physician, Fedor Aleksandrovich Lesh (Löscher), in 1870. Since the taxonomic status of *Blastocystis* was unknown at

that time, it was ignored². In 1912, *Blastocystis* was first described in intestinal tracts of humans and named *Blastocystis hominis* as a commensal yeast³. Not considering as pathogenic protozoa led to be forgotten for more than ten years⁴. In 1967, it was introduced as eukaryotic protozoa based on morphological criteria. Later, based on SSU-rRNA gene sequence it was placed on new rank, and apart

from other organisms called Stramenopiles that in subsequent revisions named Chromista⁵⁻⁷. Several studies introduced blastocystis as the most common intestinal parasite in the world^{8,9} that could be a result of low-hygiene conditions. In industrialized and developing countries, prevalence of this protozoan is about 10 and 50–60% respectively. Although, in the United States of America (USA) prevalence rate is also reported up to 23%¹⁰⁻¹². Despite it is a decade that *Blastocystis spp.* have been found, except morphological aspect (four major forms; cyst, granulated, amoeboid, and vacuolated (Fig. 1)), other biological aspects including, method of reproduction, life cycle, subspecies, and pathogenicity are not clearly specified and hence it is considered as a mysterious parasite^{13,14}.

The most important factors that led *Blastocystis spp.* to be introduced as a mysterious parasite are as follow; a) In addition to the binary division, as a usual method of reproduction (Fig. 1A), other method of reproduction, like plasmotomy, endodyogeny, schizogony, sporogony and germination have been reported in this protozoa. This variation in proliferation methods is due to the polymorphisms or the genetic origin of parasite¹³⁻¹⁵. b) Although the contaminated food/water with resistant cysts (in two forms of thin wall and causative agent of internal autoinfection; and thick wall with less number and causative agent of transmissions) have been reported as the main routes of transmission of the parasite¹⁶⁻¹⁸. Up to now, five different kinds of life cycle for *Blastocystis spp.* have been suggested that due to the lack of proper animal models, none of them is stated strictly and is attributable^{14,18}. c) In addition to polymorphism, this protozoan has a high-heterogeneity, so that the SSUrRNA gene analysis indicated that genotypes, or subtypes (STs) of this parasite are numerous (Fig. 2)¹⁹. Nine standard subtypes (ST₁₋₉) of the identified genotypes that each tend to a specific host, and have been found as zoonotic in humans are the basis of pathogenicity of the parasites and epidemiological studies (Table 1). For instance, while ST₅ tend to cows and pigs, ST₂ tend to monkeys, ST_{6,7} in the mixed form to birds; and ST₃ is tend more to humans (also called human subtypes), but none of these hosts is specific to the parasite and the parasite can be

transmitted easily between them^{1,17,20,21}. d) Many studies have emphasized on pathogenic potential of blastocystis and found numerous factors affecting its virulence as well. Intestinal clinical symptoms (giardiasis like symptoms) includes diarrhea, abdominal pain, weakness, weight loss, bloating and constipation and extra-intestinal clinical symptoms such as, joint pain and allergy (itching and hives) are attributed to *Blastocystis spp.* Since these protozoa found in patients without any clinical symptoms its pathogenicity is still controversial and doubtful¹. *Blastocystis spp.* introduced by the World Health Organization of the United Nations (WHO) as one of the water-health indices^{22,23}. Specific epidemiological conditions and pathogenicity proof of this parasite made a certain appeal in researchers of parasitology, so that most of the studies over the recent decade were conducted on blastocystis background as it is mentioned as an emerging parasite^{24,25}.

Objective of the present study was to investigate the prevalence of intestinal parasites among studies conducted over the past decade in Iran and identifying the *Blastocystis spp.* position and effective factors on its increasing prevalence.

Methods

We searched all relevant information considering the prevalence of intestinal parasites and *Blastocystis spp.* We referred to the information databases of Medline, PubMed, Scopus, Google scholar, Iranmedex and SID and the used MESH were the combinations of intestinal parasites, blastocystis, prevalence and Iran. After calculating the mean prevalence of each parasite, the most common parasites, and factors affecting their prevalence were evaluated and analyzed.

Results

After investigate and analyzes the results of collected studies, the mean prevalence of intestinal parasites in Iran during the recent decade were determined (Table 2). The most common intestinal parasites were *Blastocystis spp.*, *Giardia lamblia* and *Entamoeba coli* with mean prevalence of 14.6, 12.45 and 7.45% respectively. Other intestinal parasites were in the next category in the rankings. Moreover, in 44.5% of cases *Blastocystis spp.* was in first rank, and in 100% of cases included the first to third of the most common intestinal parasites.

Discussion

Prevalence of *Blastocystis spp.* is differs from country to country, and even in different areas of a

country. Generally, the prevalence of this parasite in developing countries (30–50%) is more than developed countries (1.5–10%), this rate related to several factors including, health standards, disposal of human waste, facing animals, and consuming the contaminated food and water^{9,26}. Other risk factors reported for blastocystis, includes, travel to the tropical areas, abnormal function of the intestinal tract, diabetes mellitus, malignancy, immunosuppression, chemotherapy, and acquired immunodeficiency syndrome (AIDS)^{1,26}.

To our knowledge, the present study is the first survey in Iran that introduces *Blastocystis spp.*, as one of the most-common intestinal parasites in Iran. It proves the position of the prevalence of this parasite in several studies that have been conducted in the past decade. In 44.5% of studies *Blastocystis spp.* had the first rank

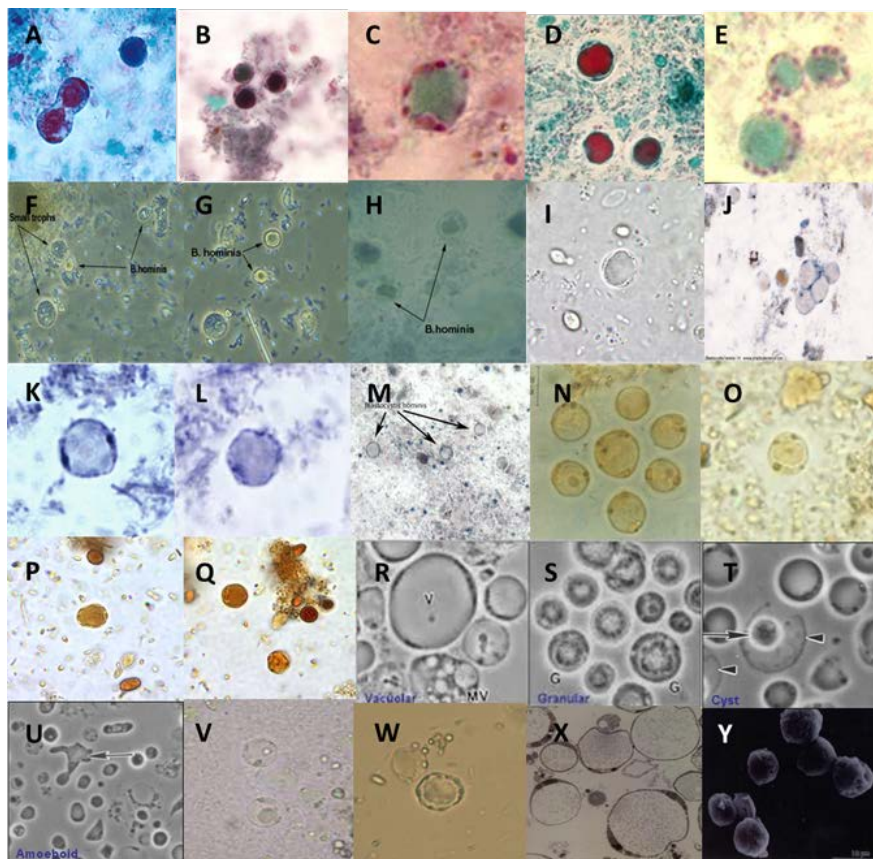


Figure 1. *Blastocystis spp.* different morphological forms stained with various stains under microscopic examination. A; cyst-like forms undergoing binary fission stained with trichrome, B–E; cyst-like forms stained with trichrome, the nuclei in the peripheral cytoplasmic rim are visible, staining purple, F–I; spherical cyst-like stage ranging from 6–35 mm in formalin-fixed wet mount, J–M; vacuolar forms stained with hematoxyline, N–Q; vacuolar forms stained with iodine, R; vacuolar form in normal saline wet mount, S; granular form in normal saline wet mount, T; cyst-like stage in normal saline wet mount, U; amoeboid form in normal saline wet mount, V–W; vacuolar and cyst-like forms in normal saline wet mount, X; vacuolar form has a central vacuole with 1–4 nuclei and 5–20 microns wide on TEM, Y; cyst-like stage on SEM.

Figures are reproduced from Centers for Disease Control and Prevention (CDC) at <http://www.cdc.gov/dpdx/az.html>.

and in 100% of studies allocated the rank of 1st to 3rd of the most common intestinal parasites²⁷⁻⁴⁴. This is in contrast to the common claim that attributes the position to *Giardia lamblia*⁴⁵. The findings are consistent with the result of conducted studies by Lagace-Wiens et al. (2006) in Canada⁸ and Alfellani et al. (2013) in Taiwan⁹ that have reported the *Blastocystis spp.*, as the most common intestinal parasite. Some studies have reported that one billion people in the world are infected with this parasite, and each person maybe infected to the parasite during the lifetime⁴⁶. In addition, unlike other intestinal parasites, some comparative studies³⁷, comparisons of different studies in Iran^{44,47} and even industrial countries like USA,^{12,15} have reported the increase in the prevalence of *Blastocystis spp.*, which is apparently inconsistent with the level of education and health facilities of societies⁴³. However, it is possible that stress or other unknown factors have a

role in this increase^{46,50}.

The bovine and human subtypes reported in Iran are similar to other studies in the world but the Iranian birds have shown more resistant to parasite and had different subtype with other bird's subtypes in the world^{48,49}. Generally, increasing prevalence of *Blastocystis spp.* in developing countries, including Iran, would be due to the following reasons:

- 1- Polymorphism of the parasite (Fig. 1) confuse experts in clinical laboratories while diagnosing, because of its four major forms¹³; the only detectable form is the vacuolar form, that means just about 25% of cases of infections are detected with routine diagnostic methods and many of infections are ignored. Parasite is capable to survive up to 11 months and even to the time of proper treatment can survive in colon⁵⁰, this factor can be considered as a resource for the propagation of parasite. The only high-sensitive

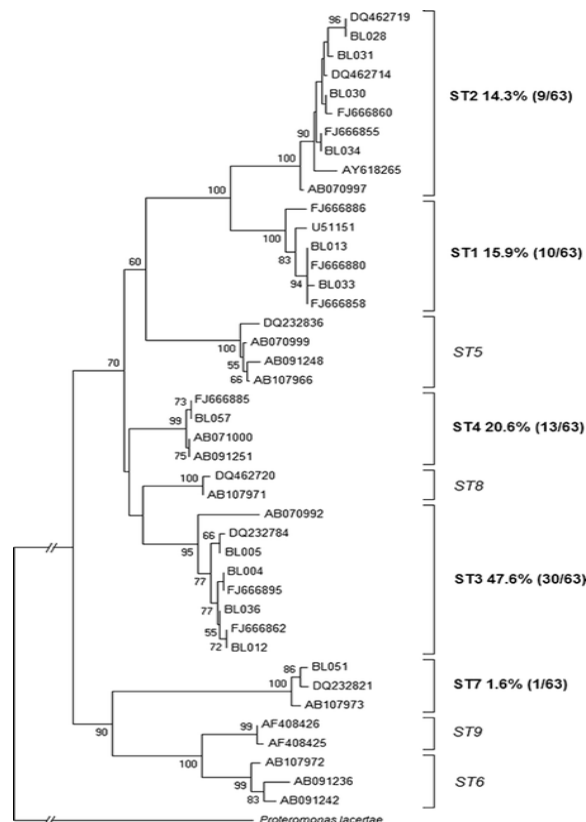


Figure 2. A phylogenetic tree comparing representative *Blastocystis* SSU-rDNA sequences, designated 'BL0-', and reference sequences from GenBank, shown with accession numbers. The tree is inferred, using the neighbor-joining method (with maximum composite likelihood model), based on a hypervariable region at the 5' end of the SSU-rRNA gene. *Proteromonas lacerate* (accession number, U37108) is used as out group. Bootstrap values (%) are indicated at the internal nodes (1,000 replicates). Bootstrap values of <50% are not shown. The subtype prevalence in the 63 samples is shown to the right as a percentage¹⁹. This figure is reproduced directly from the study of Forsell et al., 2012 at <https://researchonline.lshmt.ac.uk/149713/1/149713.pdf>

diagnosis method that may detects all morphological forms of blastocystis, is the polymerase chain reaction (PCR)⁵¹, which due to high cost of equipment is not applicable in all laboratories.

2- Appearance of the clinical signs is the main cause for referring individuals to clinics. Studies

have shown that only some subtypes (ST₁) causes clear symptoms in patient and consequently referred the person to physician⁵². In this case, most people are unaware of their infection and

Table 1: Various geographical distribution of Blastocystis spp. subtype infecting humans⁵⁸.

No. of reports	Country	Type of Participants	Percentage of Blastocystis sp. single subtype infection									Unknown/mixed	Reference
			1	2	3	4	5	6	7	8	9		
1	Bangladesh	Patient	7.7	-	92.3	-	-	-	-	-	-	-	Yoshikawa et al. 2004
	Germany	Patient	25.0	-	41.7	-	16.7	-	16.7	-	-	-	Yoshikawa et al. 2004
	Japan	Patient	8.0	10.0	52.0	22.0	-	-	4.0	-	-	4.0	Yoshikawa et al. 2004
	Pakistan	Patient	20.0	-	70.0	10.0	-	-	-	-	-	-	Yoshikawa et al. 2004
2	China	Patient	37.1	5.7	40.0	-	-	-	-	-	-	14.3	Yan et al. 2006
3	China	Rural community	-	-	-	-	66.7	-	-	-	-	33.3	Yan et al. 2007
4	China (Eryuan County)	Rural community	8.0	50.7	-	-	-	-	-	-	-	12.0	Li et al. 2007
	China (Menghai County)	Rural community	20.5	1.3	70.5	1.3	-	-	-	-	-	6.5	Li et al. 2007
	China (Shanghai municipality)	Rural community	20.7	3.4	58.6	-	-	3.4	-	-	-	13.8	Li et al. 2007
5	Denmark	Patients	30.0	10.0	60.0	-	-	-	-	-	-	8.0	Li et al. 2007
			3.4	20.7	51.7	24.1	-	-	-	-	-	-	Stensvold et al. 2006
6	Denmark	Patients	17.9	32.1	46.6	3.8	-	-	-	-	-	-	Stensvold et al. 2007
7	Denmark	general population	8.3	12.5	25	37.5	-	-	-	-	12.5	-	Stensvold et al. 2009
			20.7	20.7	16.3	12.0	-	-	5.4	-	-	25.1	Stensvold et al. 2009
8	Denmark	Patients	17.4	13	33.9	13.9	-	8.7	-	8.7	-	-	Rene et al. 2009
9	Egypt	Patient	18.2	-	54.5	-	-	18.2	9.1	-	-	-	Hussein et al. 2008
10	Egypt	Patient	19.1	19.1	61.8	-	-	-	-	-	-	1.0	Souppart et al. 2010
11	France	Patient	16.7	13.3	50.0	10.0	-	-	3.3	-	-	6.7	Souppart et al. 2009
12	Germany	Not available	21.0	1.0	66.0	7.0	-	-	-	-	-	5.0	Böhm-Glönig et al. 1997
13	Greece	Patient	20.0	13.3	60.0	2.2	-	2.2	2.2	-	-	-	Menounos et al. 2008
14	Greece	Patient	14.0	9.8	62.7	1.9	-	1.9	9.8	-	-	-	Vassalos et al. 2010
15	Japan	Patient	3.1	-	93.8	3.1	-	-	-	-	-	-	Yoshikawa et al. 2000
16	Japan	Patient	20.0	21.8	43.6	10.9	-	-	-	-	-	3.6	Kaneda et al. 2001
17	Malaysia	Patient	45.0	5.0	50.0	-	-	-	-	-	-	-	Tan et al. 2008
18	Malaysia	Patient	12.5	5.0	50.0	27.5	-	-	-	-	-	2.0	Tan et al. 2009
19	Nepal	Patient	20.0	20.0	60.0	-	-	-	-	-	-	-	Yoshikawa et al. 2009

20	Pakistan	Patient-IBS	86.0	60.0	47.0	75.0	43.0	50.0	50.0	-	-	2.0	Yakoob et al. 2010
		Healthy persons	14.0	40.0	53.0	25.0	57.0	50.0	50.0	-	-	-	Yakoob et al. 2010
21	Singapore	Patient	22.0	-	78.0	-	-	-	-	-	-	-	Wong et al. 2008
22	Spain	Patient	2.0	3.9	-	94.1	-	-	-	-	-	-	Domínguez-Márquez et al. 2009
23	Thailand	Army personnel	90.2	-	4.6	-	-	-	1.3	-	-	3.9	Thathaisong et al. 2003
24	Thailand	School children	77.9	22.1	-	-	-	-	-	-	-	-	Leelayoova et al. 2008
25	Turkey	Patient - children	21.6	19.6	52.9	-	-	-	-	-	-	5.9	Dogruman-AI et al. 2008
		Patient-adult	14.6	24.4	58.5	-	-	-	-	-	-	2.4	Dogruman-AI et al. 2008
26	Turkey	Patient	9.2	13.8	75.9	-	-	-	1.1	-	-	-	Ozyurt et al. 2008
27	Turkey	Patient	2.9	28.6	60.0	-	-	-	-	-	-	8.6	Dogruman-AI et al. 2009
28	Turkey	Patient	36.0	24.0	10.0	-	-	-	-	-	-	-	Eroglu and Koltas 2010
29	USA	Patient	11.1	-	66.7	-	-	-	-	-	-	22.2	Jones et al. 2009

this led to stability of the parasite, and becomes patients to a source of infection propagation. Further, despite the pathogenicity of parasite²² many Iranian physicians still do not believe that and do not prescribe the proper treatment. In addition, the rising resistance of *Blastocystis spp.* against the only drug of choice, metronidazole⁵³ will also be an important factor affecting its incidence. Comparison of three different time-interval studies using routine and molecular diagnostic methods in Lorestan

province is proving for this claim^{12,24,25,43,47,49}. 3- *Blastocystis* is a heterogeneous zoonotic parasite with multiple subtypes (Figure 2). A wide range of hosts including primates, pigs, rodents, reptiles, amphibians, and insects have been reported^{18,54}. Since, fecal-oral is the main route of transmission among humans and other hosts^{1,16,17}, health care is important in preventing the infection¹, and high incidence of parasite in developing countries may be due to the crowded population, poor personal and environmental

Table 2: Frequency of gastrointestinal parasites in conducted studies in different provinces of Iran during the ten recent years and ranking of *Blastocystis spp.* among them.

Province	Chaharmahal 2013	Lorestan 2014	Zahedan 2008	Shahre kord 2012	Shiraz 2006	Northwest of Iran 2006	Ardebil 2013	Azarbaijane gharbi 2011	Gorgan 2012	HIV-Positive of Iran 2004	Khuzestam 2007	Tehran 2009	Karaj 2009	Mazandaran 2008	Shahreray 2008	Ebol 2005	Yard 2005	Bandar abbas 2011	Total	
Type of intestinal parasite																				
<i>Giardia lamblia</i>	1.6	6.5	10.1	12	8	142	83	205	9.9	7.3	10.9	2.5	3.8	10.2	25.8	142	41.6	172	12.45	
<i>Entamoeba coli</i>	1.7	1.9	10	...	15	...	11.6	14.6	11.6	3.9	9.4	4.8	0.4	7.5	27.8	15.9	7.56	
<i>Iodamoeba butylia</i>	...	1	0.5	2.5	6.6	1.6	0.7	
<i>Entamoeba histolytica</i>	1	0.01	12	0.6	5.8	0.45	
<i>Blastocystis spp</i>	0.7	5	2.2	23	25.4	28.2	19	133	152	44	2.4	12.8	0.08	9.8	54.5	9.6	15.7	24.6	14.6	
Other protozoa	0.15	...	2.8	...	11	2.8	2.9	5.4	0.4	1.5	3.2	9.9	2.8	...	2	
Helminthic parasites	...	0.4	10.8	3.6	1.4	...	0.9	
Total prevalence of intestinal parasites	5.15	16.5	27.3	36.25	59.4	52	44	425	288	18.4	25.6	25.5	4.7	25	10.7	31.5	8.6	48.8	28.4	
Rank of <i>Blastocystis</i>	3 rd	2 nd	3 rd	1 st	1 st	1 st	1 st	3 rd	1 st	2 nd	3 rd	1 st	3 rd	2 nd	1 st	3 rd	3 rd	1 st	...	

hygiene, lack of safe water supplies, socioeconomic status, and inappropriate sewage disposal systems.

4- High prevalence of *Blastocystis* spp. in people who are dealing with animals⁵⁵⁻⁵⁷ or those who travel on weekends to recreational rivers¹⁵, represents the various routes of parasites transition. Although, as mysterious parasite may be transmitted in other ways as well. In Iran, it was found that there is a relation between ST₅ of human strains with bovine source²¹ that would be an effective indicator for the increasing prevalence.

Despite extensive studies on blastocystis throughout the world, Iran is still at the beginning of considering the pathogenicity, epidemiological aspects and prominence of the prevalence of this parasite. Further studies especially in the parasite genome in different hosts are recommended. It seems that disregarding blastocystis is unfair and helps its increasing prevalence.

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