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Blastocystosis: Epidemiological, clinical, pathogenic, diagnostic, and therapeutic aspects.

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Key words: *Blastocystis* spp.; genotypes; epidemiology; irritable bowel syndrome; urticaria; metronidazole.

Abstract. *Blastocystis* is a nonmoving pleomorphic stramenopile or chromist. Nineteen subtypes of this organism have been identified. It has a worldwide distribution. The prevalence rates in humans are lower than 1% in developed countries and up to 100% in developing countries. It is possible to recognize the fecal-oral transmission, through ingestion of contaminated food and water, and zoonotic spreads. The diagnosis is carried out by direct fecal examination, culture and molecular techniques. *Blastocystis* has virulence factors such as cysteine proteases, serine proteases and legumains, mostly secreted to the pathogen-host interface. This stramenopile has been linked to gastrointestinal symptoms, irritable bowel syndrome, urticarial and arthritis. However, there is not any conclusive evidence of association with the disease. Recently, the hypothesis of the opportunistic pathogen has emerged. Treatment has traditionally been based on metronidazole and other imidazoles. The recent obtaining of the nuclear genome will allow the rational development of new effective drugs. The aim of this review is to highlight the main epidemiological, clinical, pathogenic, diagnostic and therapeutic aspects of the *Blastocystis* infection.

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Blastocistosis: Aspectos epidemiológicos, clínicos, patogénicos, diagnósticos y terapéuticos.

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Palabras clave: *Blastocystis* spp.; genotipos; epidemiología; síndrome de intestino irritable; urticaria; metronidazol.

Resumen. *Blastocystis* es un stramenopile o cromista, pleomórfico, no móvil. Se han identificado diecinueve subtipos (genotipos) de este organismo. Tiene una distribución mundial. Las tasas de prevalencia en humanos son inferiores al 1% en los países desarrollados, y hasta el 100% en los países en desarrollo. Se reconoce la transmisión fecal-oral, a través de la ingestión de alimentos y aguas contaminadas y la transmisión zoonótica. El diagnóstico se lleva a cabo mediante examen fecal directo, cultivo y técnicas moleculares. *Blastocystis* tiene factores de virulencia tales como proteasas de cisteína, proteasas de serina y legumaínas, principalmente secretadas a la interface patógeno-hospedador. Este stramenopile se ha relacionado con síntomas gastrointestinales, síndrome de intestino irritable, urticaria y artritis. Sin embargo, no hay pruebas concluyentes de asociación con la enfermedad. Recientemente, ha surgido la hipótesis de patógeno oportunista. El tratamiento tradicionalmente se ha basado en metronidazol y otros imidazoles relacionados. La reciente obtención del genoma nuclear permitirá el desarrollo racional de nuevas drogas efectivas. El objetivo de esta revisión es destacar los principales aspectos epidemiológicos, clínicos, patogénicos, diagnósticos y terapéuticos de la infección por *Blastocystis*.

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INTRODUCTION

Blastocystis spp. is a polymorphic organism; the vacuolar, granular, amoeboid, and cystic forms are the most frequent. Avacuolar, multivacuolar, and with filamentous inclusions are also recognized forms (1-4). Although some authors consider that the cyst designation is not adequate because it is a chromist, other authors maintain that designation, which will be used in this review (4). However, its life cycle, sources, and transmission mechanisms are not well known. Its transmission occurs through a fecal-oral route, by ingestion of contaminated water and food, and zoonotic spread (5-7). This infectious agent has a worldwide distribution and is the most prevalent stramenopile in humans with prevalence rates from less than 1% in developed countries to 100% in developing countries (8, 9). The relatively recent interest on *Blastocystis*, despite its description a century ago is due to the belief that it causes intestinal disease (10-13).

The taxonomic classification of *Blastocystis* has been very controversial and wrongly considered as plant material, fungus, flagellate and protozoan (14, 15). In 1996, the molecular analysis of the small sub-unit of the rRNA (SSU-rRNA) and elongation factor 1α, showed that *Blastocystis* could be included in the heterokontophyta (stramenopile or chromista) category of eukaryotic phylum, (16, 17). Later studies, using multiple molecular sequences of eight genes of *Blastocystis,* confirmed its taxonomic status as a stramenopile (18). This heterogeneous group includes unicellular and multicellular

organisms, such as brown algae, diatoms, viscous or mucilaginous elements, oomycetes and aquatic molds (17, 18). One of the distinguishing features of stramenopile is the presence of a flagellum, which gives mobility at some stage of their life cycle; contradictorily, *Blastocystis* has no flagellum and is the only stramenopile implicated as a causative agent of human disease (13, 19).

Nineteen genotypes of *Blastocystis* have been identified (2). Denoeud *et al*. in 2011 (20) obtained the nuclear genomic sequence of subtype 7 (ST7). This is the smallest stramenopile genome that has been sequenced, with 18.8 Mb. *Blastocystis* has a total of 6,020 genes, equivalent to 42% of its genome, 24,580 exons or coding regions, 18,560 introns and 2,730 repeat regions. The genome is compact and 25% of the information consists of repetitions. The organism has a manily anaerobic metabolism (20,21).

Blastocystis has been linked to human gastrointestinal disease, known as Blastocistosis or Zierdt-Garavelli´s disease (11, 12). In addition, this infectious agent has been associated with irritable bowel syndrome (IBS), urticaria, ulcerative colitis, cancer and arthritis (13, 22-25). Nevertheless, its pathogenicity is controversial (15, 26-28). In this sense, it is important to highlight that until now, most studies refer to a possible link and not a causal relationship. The statistical analyses are based on statistics of independence and association between variables, without an adequate number of observations; therefore, they are partially conclusive. It is also important to clarify that there are well-designed studies that suggest a pathogenic role of *Blastocystis*. The aim of this review is to highlight the most important epidemiological, clinical, pathogenic, diagnostic, and therapeutic aspects of the *Blastocystis* infection.

EPIDEMIOLOGY

Prevalence in humans

The infection is widespread (7-9, 29, 30). The prevalence is higher in developing countries where water and sewage treatment systems, sanitary facilities, and standard housing developments are insufficient or lacking (31-34). Under these conditions, *Blastocystis* cysts could spread readily through water supplies and distribution systems and food (31-33).

Up to date, few epidemiological studies on the prevalence of *Blastocystis* in humans from developed countries have been conducted. Table I shows some of these reports (8, 30, 35-55) with prevalence values from 0.08% in Germany (8) to 70.3% in the United States (35). In many cases, participants included tourists, immigrants, and refugees. These reports are very interesting because they evaluate the risk of the population of non-endemic areas for infection. In a study of 7,677 patients in Paris, the prevalence of *Blastocystis* varied according to the population group studied: 17.4% in subjects free of any digestive tract disorders, 19.8% in adults with digestive tract disorders, and 13.8% in children ($p<0.01$) (56).

Table II summarizes some of the studies conducted about the prevalence of *Blastocystis* in humans from developing countries from 1990 to 2017. (9, 29, 31, 57-94), these values ranged from 3.4% in Nigeria (60) to 100% in Senegal (9).

Cekin *et al*., conducted a study in 2012 (95) where 2334 patients with gastrointestinal symptoms composed the study group, which included 335 patients with diagnosed inflammatory bowel disease (IBS) and 877 with irritable bowel syndrome (IBS). Patients without any gastrointestinal symptoms or disease ($n = 192$) composed the control group. The mean ± standard deviation age of patients with IBS, patients with IBD, patients with gastrointestinal complaints and the control group were 45.8 ± 16.2, 45.2 ± 13.2, 47.3 ± 16.1, and 45.5 ± 15.2 years old respectively with no statistically significant difference between groups ($p = 0.597$). The groups were also comparable to each other in terms of gender. In patients with gastrointestinal complaints, gender distribution was homogenous in patients with ($n = 134$; 83 (62%) females and 51 (38%)

TABLE I
PREVALENCE OF *BLASTOCYSTIS* IN INDIVIDUALS OF DEVELOPED COUNTRIES

Country	Infected individuals (n/N)	%	References
United States	(581/826)	70.3	(35)
United States	(10/139)	7.2	(36)
United States	(46/1,736)	2.7	(37)
United States	(5623/216,275)	2.6	(38, 39)
Italy	(271/514 [a])	52.7	(40)
Italy	(1,234/8,886)	13.9	(41)
Italy	(19/247)	7.7	(42)
Italy	(155/2,138)	7.2	(43)
Italy	(378/5,351)	7.1	(44)
Italy	(67/1,503)	4.1	(45)
Russia	(108/327 [b, c])	33.0	(46)
Russia	(62/1,273 [c, d])	4.9	(46)
Netherlands	(107/442)	24.2	(47)
France	(143/788 [c])	18.1	(48)
Germany	(69/469 [e])	14.7	(49)
Germany	(900/16,817)	5.4	(50)
Germany	(1/1,230 [f])	0.08	(8)
Spain	(585/8,313 [d])	7.0	(51)
China	(20/420)	4.8	(52)
China	(32/1,020 [g])	3.1	(30)
Sweden	(42/1,054)	4.0	(53)
Japan	(33/3,292)	1.0	(54)
Japan	(30/6,422)	0.5	(55)

n = positive samples, N = examined samples, [a] immigrants, [b] patients with hepatitis C, [c] samples tested by PCR, [d] patients with digestive disorders, [e] tourists returning from tropical countries, [f] refugees, [g] prevalence of *Blastocystis* in diarrhea cases.

males) or without *Blastocystis* spp. (n = 2200; 1260 (57.3%) females and 940 (42.7%) males) (p = 0.366). In Venezuela, Panunzio *et al*., in 2014 (96) observed 406 individuals from two communities of the city of Maracaibo in Venezuela, with the following demographic and socio-economic characteristics: over 18 years old (72.1%) with an age range between 1 and 75, predominance of females (51.4%), a level of education higher or equal to diversified secondary education (70.6%) and with an active occupation (89.2%). Regarding the environmental health characteristics, the inhabitants of these communities under study mostly had adequate conditions; nevertheless, 44.8% lived with more than 3 people per room, which showed overcrowding conditions. 44.3% admitted to consuming untreated water (non-mineral, non-filtered, or chemically treated), 39.9% did not have adequate conditions for the disposal of garbage and a predominance of accumulation and presence of harmful fauna in the home was observed in the majority of cases 70.9%. Mainly flies were identified as vectors, and between reservoirs, rodents and dogs; the only activity of environmental sanitation with an adequate service for the entire population was the disposal and elimination of excreta through the public network. These last two designs are specific to the search for *Blastocystis* spp. Other designs are search-oriented.

There is a marked difference between subtypes of *Blastocystis* reported in Europe and the Unites States, and those from Latin America and Asia (molecular epidemiology). In developed countries, ST4 is predominant and in developing nations, there is a greater diversity of genotypes (ST1, ST2, ST3). Yason and Tan, 2015 (2) indicated the existence of 19 subtypes, of which nine have been found in humans, ST3 being the most common. Yoshikawa *et al*. in 2004 (97) examined *Blastocystis* in five populations from each of these countries: Germany, Japan, Thailand, Pakistan, and Bangladesh. The dominant genotype, excluding four populations from Thailand, was the ST3 (41.7 to 92.3%), followed by ST1 (7.7 to 25%) and ST4 (10 to 22.9%).

Distribution in animals

Blastocystis is also very common in animals. It has been detected in arthropods, annelids, amphibians, reptiles, birds, and mammals. High infection rates (from 50% to 100%) have been observed in rats, pigs, and poultry, particularly in domestic chickens (98).

TABLE II
SELECT REPORTS ON PREVALENCE OF *BLASTOCYSTIS* IN INDIVIDUALS FROM DEVELOPING COUNTRIES

Country	n/N	Prevalence (%)	Reference
Senegal	93/93 [a]	100	(9)
Brazil	149/172	86.6	(57)
Brazil	80/382 [b]	21.0	(58)
Nigeria	167/199 [a]	84.0	(59)
Nigeria	13/384	3.4	(60)
Ecuador	44/55 [a]	81.5	(61)
Argentina	90/115	78.3	(62)
Argentina	80/350	22.9	(63)
Venezuela	150/228	65.8	(64)
Venezuela	28/45	62.2	(31)
Venezuela	16/34	47.0	(65)
Venezuela	46/98	46.9	(66)
Venezuela	42/100	42.0	(67)
Venezuela	42/130	32.3	(68)
Venezuela	133/426	31.2	(33)
Venezuela	32/110	29.1	(69)
Venezuela	87/303	28.7	(68)
Venezuela	3/12	25.0	(70)
Venezuela	775/3,514	22.1	(71)
Venezuela	178/823	21.6	(72)
Venezuela	48/301	16.0	(73)
Venezuela	206/2,009	10.3	(74)
Chile	292/462	63.2	(75)
Chile	240/670	35.8	(76)
Chile	15,807/44,653	35.4	(29)
Chile	1,874/6,162	30.4	(77)
Lebanon	157/249 a	63.0	(78)
Tanzania	106/174 a	61.0	(79)
Côte d'Ivoire	64/110 d	58.2	(80)
United Arabian Emirates	59/133	44.4	(81)
Mexico	48/115	41.7	(82)
Malaysia	43/105 c	41.0	(83)
Malaysia	103/253	40.7	(84)
Malaysia	77/300	25.7	(85)
Malaysia	102/500	20.4	(86)
Malaysia	27/163	17.0	(82)

Continuation: **TABLE II**

Country	n/N	Prevalence (%)	Reference
Malaysia	186/1,760 d	10.6	(87)
Cuba	40/104	38.5	(88)
Peru	728/2,056	35.4	(89)
Peru	9/91	9.9	(90)
Thailand	94/343	27.4	(91)
Bolivia	43/185	23.2	(92)
Karachi	59/339	17.4	(93)
Iran	81/500	16.2	(94)

n = positive samples, N = examined samples, [a] samples tested by PCR, [b] molecular characterization of *Blastocystis* in indigenous communities, [c]*in vitro* culture technique, [d]*in vitro* culture and PCR.

Environmental distribution

Blastocystis has been isolated in developing and developed countries from several environmental water matrices such as surface water, wastewater, and potable water. The prevalence has ranged from 0% (99, 100) to 100% (99). Noradilah *et al*., 2016 (99) in addition to identifying *Blastocystis*, also indicated the genotype, identifying ST3 and ST1 in rainy season and in less proportion to ST1 and ST2 during the dry season, in surface waters of Malaysia (Table III) (1, 5, 31-33, 99-104). The findings presented in this table suggest that the organism is prevalent in several types of water in developing countries and that this vehicle may represent a source of infection in these areas.

Transmission

The sources and transmission mechanisms of *Blastocystis* have not been determined accurately. We know that the cyst is the only transmissible form of the chromists (105). Some studies have observed the same genotypes in infected patients and sewage samples, implying unfiltered or unboiled water from wells as a source of infection (5). Estuaries of rivers and sewage water have also been involved in the transmission, suggesting the fecal-oral route (85, 86, 106). In Argentina, *Blastocystis* was identified in the municipal water system (107). The identification of cysts in water, suggests this vehicle as a potential source of infection. Microorganisms considered as an index of water quality for human consumption, include among others, *Cryptosporidium* and *Giardia* (108). *Blastocystis* is an agent to take into consideration within international standards of microbiological quality of water. Very few studies worldwide relate to this point (Table III).

Some animals have been implicated as sources of zoonotic transmission based on phylogenetic research and other molecular studies (109). The recent determination of the genotypes of *Blastocystis* in different hosts showed a similarity between isolates from humans and animals (98). In another study, the genotypes isolated from humans and pets were equal (6). These findings suggest the possibility of zoonotic potential of the stramenopile. In fact, the wide distribution of *Blastocystis* in the animal kingdom and zoonotic spread among animals, suggests this transmission mechanism in humans (110). It is essential to continue these investigations to elucidate this important aspect.

TABLE III
DISTRIBUTION OF *BLASTOCYSTIS* IN ENVIRONMENTAL WATER MATRICES

Country	Matrix	Contaminated n/N	Contaminated (%)	Subtypes	Reference
Malaysia	Surface water	7/7	100	ST3	(99)
Malaysia	Surface water	3/7	42.9	ST1	(99)
Malaysia and Scotland	Sewage	47/123	38.0	n.a	(5)
Malaysia	Surface water	80/240	33.3	n.a	(32)
Malaysia	Surface water	2/7	28.6	ST2	(99)
Malaysia	DWTP*	22/85	25.9	n.a	(32)
Malaysia	Surface water	53/240	22.1	n.a	(32)
Malaysia	Surface water	0/7	0.0	ST1	(99)
Malaysia	Surface water	0/7	0.0	ST2	(99)
Venezuela	Surface water, wastewater, and drinking water	23/25	92.0	n.a	(31)
Venezuela	Surface water	19/75	25.3	n.a	(33)
Venezuela	Drinking water	0/36	0.0	n.a	(100)
Turkey	Rivers, sea, drinking water	47/228	20.6	n.a	(101)
Thailand	Water reservoirs	1/5	20.0	n.a	(102)
Egypt	Water sources	53/336	15.8	n.a	(1)
Egypt	Water sources	13/1320	1.0	n.a	(103)
Philippines	WWTP &	9/62	15.0	n.a	(104)

* DWTP = Drinking water treatment plants, & WWTP = Wastewater treatment plants, n = positive samples (Blastocystis was observed), N = examined samples, n.a = not available.

CLINICAL MANIFESTATIONS

Symptoms attributed to the chromist include nonspecific gastrointestinal symptoms such as diarrhea, nausea, vomiting, abdominal pain, flatulence, tenesmus, constipation, anorexia, weight loss, fatigue, fever, chills, dehydration, itching, and insomnia (10-13). In some studies, the infection has been associated with IBS (Table IV) (22, 23, 95,111-122), ulcerative colitis, urticaria, cancer, and arthritis (Table V) (24, 25, 123-131). Eosinophilia and fecal leukocytosis have also been observed (10-12). Blastocystosis has been frequently associated with diarrhea in immunosuppressed patients such as those with AIDS and solid organs transplant (132). However, other studies suggest that the stramenopile is not significantly associated with inflammatory processes(28). The differences between haplotypes of the major histocompatibility complex (HCM-I and HCM-II) in different populations, *Blastocystis* strains (genetic variability), virulence factors, nutritional status, environmental factors and even the study design itself, could at least partially explain these discrepancies (133).

PATHOGENICITY

Despite the discovery of *Blastocystis* a century ago, its pathogenic role remains controversial. This aspect of the organism

TABLE IV
RELATIONSHIP BETWEEN *BLASTOCYSTIS* AND IRRITABLE BOWEL SYNDROME

Country	Patients n/N (%)	Control n/N (%)	Subtypes (%)	Reference
Italy	15/81 (18.5%)	23/307 (7.5%)	n.a.	(111 [a])
France	13/56 (23.2%)	9/56 (16.1%)	Patients: ST4 (46.2%), ST3 (23.1%), ST2 (23.1%), ST1 (7.7%), ST5 (7.7) Control: ST4 (100%), ST2 (11.1%)	(112[a])
Denmark	18/124 (15%)	45/204 (22%)	n.a.	(113[b])
India	50/150 (33.3%)	15/100 (15%)	Patients: ST3 and ST1 * & Control: ST3 and ST1 *	(114)
Malaysia	6/35 (17%)	4/75 (5.5%)	Patients: ST3 (50%), ST4 (33.3%), ST5 (16.6%) Control: ST3 (50%), ST2 (25%), ST1 (25%)	(115)
Pakistan	44/95 (46%)	4/55 (7%)	n.a.	(116 [a])
Pakistan	95/158 (60%)	38/157 (24%)	n.a.	(117[a])
Pakistan	90/171 (53%)	25/159 (16%)	n.a.	(118[a])
Turkey	8/21 (38)	5/43 (11.6%)	n.a.	(119 [a])
Turkey	51/877 (5.8%)	6/192 (3.1%)	n.a.	(95)
Mexico	n.a./115 (15.7%)	n.a./209 (12%)	n.a.	(23)
Mexico	14/45 (31.1%)	6/45 (13.3%)	n.a.	(22 [a])
Peru	18/37 (49%)	105/148 (71%)	n.a.	(120[a])
Thailand	8/59 (13.6%)	3/25 (12%)	n.a.	(121)
Thailand	11/66 (16.7%)	6/60 (10%)	n.a.	(122)

[a]Authors concluded that there is an association between *Blastocystis* and IBS, [b] authors that concluded that there is not a relationship, *No statistical differences among *Blastocystis* subtypes, [&] No statistical differences between patients and controls, n.a. not available.

has been widely discussed in the literature during the last two decades, since Phillips and Zierdt, associated it with diarrhea (10). It is observed both in patients with or without gastrointestinal manifestations, and the low number of patients examined in the investigations, preventing conclusions about the causal relationship between the agent and disease. We do not know with certainty if *Blastocystis* is a commensal, pathogenic or opportunistic organism. However, several *in vitro* and *in vivo* studies suggest that the stramenopile is actually a pathogen (13, 116, 119, 134). In recent years, evidence suggests that *Blastocystis* causes gastrointestinal disorders due to its significant association with individuals with diarrhea or IBS (13, 22). The emerging view is that the organism is an opportunistic pathogen by its association with immunosuppressed patients suffering of diarrhea (135). However, Shah *et al*. (136) reported that *Blastocystis* and *Endolimax nana* co-infection resulted in chronic diarrhea in an immunocompetent human male.

TABLE V
RELATIONSHIP BETWEEN *BLASTOCYSTIS* SUBTYPES AND SOME DISEASES

Country	Patients Disease (n)	Control group (n)	Subtypes % (group)	Reference
Argentina	Urticaria [a] and gastrointestinal symptoms 39	28	ST3 71.4% (patients) 28.6% (control).	(24)
Egypt	Urticaria 54 [b]	50	ST3 100% (patients) 100% (control).	(123)
Egypt	Urticaria 54	50	ST3 100% (patients) 100% (control).	(124)
Italy	chronic urticarial 1	None	n.a.	(125)
Turkey	Ulcerative colitis patients 150 [c]	None	ST3 66.7% ST1 16.7% ST2 8.3% ST7 8.3%	(126)
Turkey	Cancer and *Blastocystis* 25	Cancer and no *Blastocystis* 207	ST3 59% (patients) ST1 23% (patients) ST2 18% (patients).	(25)
Netherlands	Ulcerative colitis 45	123	n.a.	(127)
USA	Ulcerative colitis 1	n.a	n.a.	(128)
Spain	Reactive arthritis 1	n.a	n.a	(129)
Germany	Arthritis 1 [d]	n.a	n.a	(130)
Jamaica	Rheumatoid arthritis 1	n.a	n.a	(131)

[a]Allele (a34) significantly associated with urticaria patients, [b] There was no significant difference between the patients with acute and those with chronic urticarial, [c] Low colonization of *Blastocystis* infection in ulcerative colitis patients during active stage, [d] the detection of *Blastocystis* in synovial fluid implicate an infectious rather than a reactive etiology of arthritis.

Experimental studies *in vitro*, using human cell line polarized intestinal epithelial HT-29 and two strains of *Blastocystis* (PL34), suggest pathogenicity of the organism. Although no evidence of entry of the stramenopile into cells was observed, a decrease in transmembrane electrical resistance, ultrastructural changes in the cytoskeleton elements of cells, apoptosis of monolayer cell, and increased production of TNF-α were noted (137, 138).

Genotypes and pathogenicity

Blastocystis presents a large genetic variation and it is proposed that the different genotypes or subtypes may be associated with its pathogenic potential (112). Several studies have tried to differentiate *Blastocystis* isolates from symptomatic and asymptomatic patients, through phenotypic characteristics such as isoenzymes models and proteins profiles (139).

Genetic studies have been used to elucidate the pathogenic potential of *Blastocystis*. The ST3 is the most frequently isolated in epidemiological surveys and is probably the only genotype of human origin (7, 140). In one study, ST3 was the most common in symptomatic (59.3%) and asymptomatic (48.5%) patients, followed by ST2 and ST1 (29%). In an analysis of *Blastocystis* isolates by PCR, ST1 (28.6%), ST3 (57.1%) and ST4 (25%) were identified in asymptomatic individuals (6). In another research, the ST1

was the sole dominant in patients with symptoms, while ST3 was the most common in those without symptoms (141, 142). These findings suggest that ST1 and ST3 are associated with pathogenicity and the relationship of *Blastocystis* genotypes with disease (87). The amoeboid form of the stramenopile, which may adhere to the intestinal epithelial cells in symptomatic patients, has been suggested as pathogenic (143). It has been speculated that the amoeboid forms of ST3 contribute to the pathogenicity of *Blastocystis* (127, 144). Other authors consider that the presence of the *Blastocystis* vacuolar form can invade the lamina propia, the submucose, and even the muscle layers in mice infected with *Blastocystis* by oral via (145).

The association of *Blastocystis* with IBS (Table IV) has been suggested. The ST3 was reported as a cause of intestinal disease. Yakoob *et al*. in 2010 (117) studied 330 individuals (171 with diarrhea and IBS and 159 controls) by direct stool examination, culture, and PCR. Regardless of the technique used, they found differences between the symptomatic group and the control group ($p<0,001$), suggesting the stramenopile as a possible cause of IBS. These authors point out *Blastocystis* ST1 as the most frequent in patients with diarrhea and IBS, and ST3 as the most frequent in the group of clinically healthy people; 73% of patients with IBS were infected with one *Blastocystis* genotype (118). It appears that there may be marked differences in the virulence factors among isolates or strains from around the world (146). Rostami *et al*. in 2017 (147) did a systematic review and meta-analysis to examine the possible association of *Blastocystis* and *Dientamoeba fragilis* infections and the development of IBS. In individuals with blastocystosis, the authors found to have a positive association with IBS, while this association was not observed for *D. fragilis* infection.

Some reports associated *Blastocystis* ST2 and ST3 with urticaria (127). A recent study revealed that the allele 34 of *Blastocystis* ST3 was in 85.7% (18/21) of symptomatic urticaria patients as compared with the control group (1/21) ($p<0.0001$) (24). In another study, only ST3 was observed in 61.1% of 54 patients with acute or chronic urticaria, 21 of them had gastrointestinal manifestations. The amoeboid form of the stramenopile was detected in 95.2% of patients with symptoms while in the control group, 8.0% harbored the stramenopile, but not with this form. The symptoms disappeared with the administration of metronidazole (MTZ). The authors concluded that the amoeboid form of this infectious agent is a cause of urticaria (127). The concept of luminal organism, as etiologic agent of skin allergic lesions is very interesting.

Virulence factors

Blastocystis molecules considered factors of virulence include: cysteine-proteases (legumains and cathepsins B); cyclophilin-like protein; serine-proteases; aspactic-proteases; sugar-binding-proteins; metalloprotease; glycosyltransferases; hydrolases type glucide-hydrolase (fucosidase, hexosaminidase, and polygalacturonase); proteases inhibitors (cystatin, type 1-proteinase inhibitor and endopeptidase inhibitor-like protein) (20, 148,149) (Fig. 1). These factors are released by the stramenopile to the pathogen-host interface, and a reactive on digestive enzymes or proteases I is involved in the immune response. Similarly, the presence of proteins with immunoglobulin-like domains into the genome of *Blastocystis* could indicate factors that mediate adherence to host cells. Some proteases of the stramenopile degrade immunoglobulin A. *Blastocystis* has superoxide dismutase (SOD) containing iron, a virulence factor that allows resisting the respiratory crisis, a defense mechanism against pathogens of mammals (20). A type 1 polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS) (Fig. 1) in *Blastocystis* can synthesize metabolites like simple fatty acids and many compounds, such toxins, antibiotics or antimicrobials (20). Despite these findings, the pathogenic role of *Blastocystis* is still controversial.

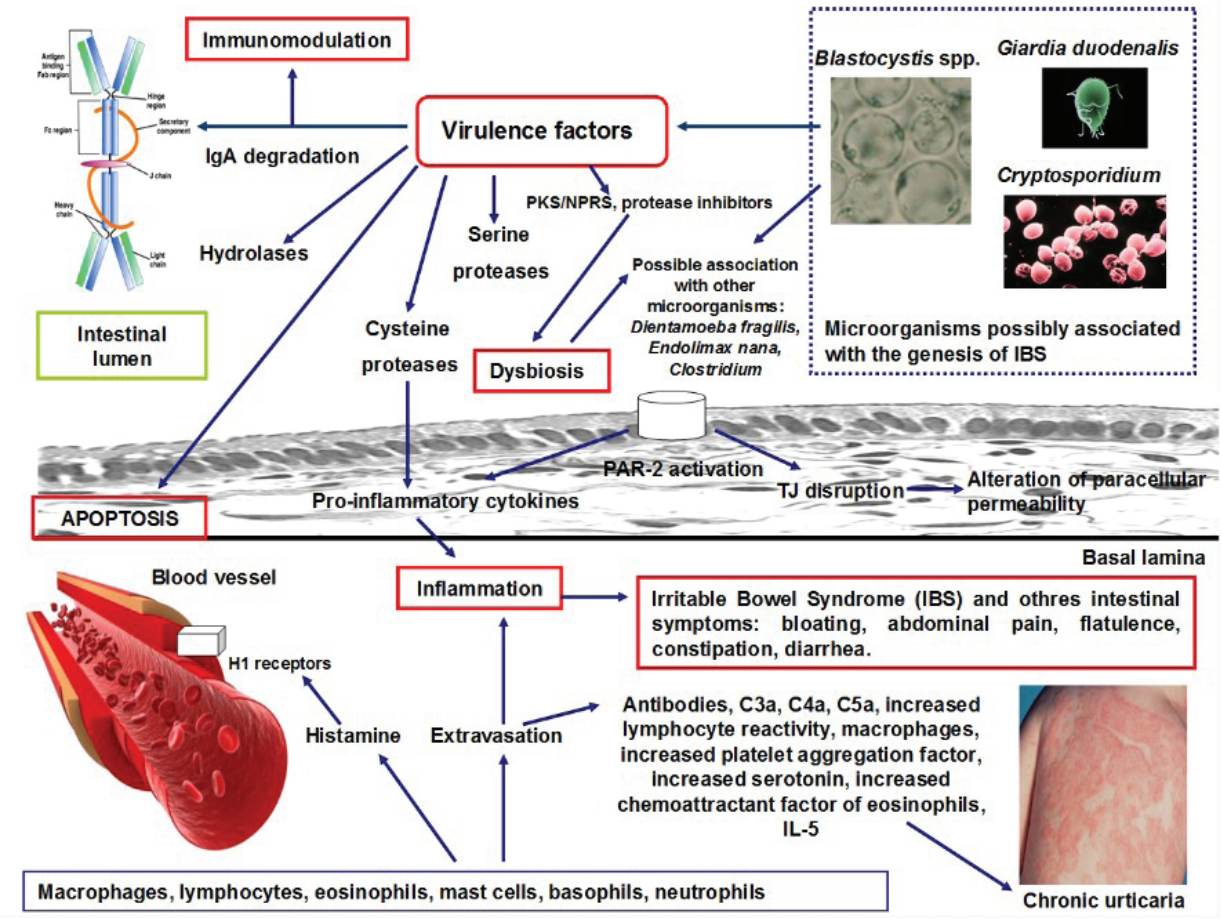

Fig. 1. Immunopathogeny of *Blastocystis infection*. Lepczyńska, Chen and Dzika (149). With permission of the author.

Immunopathogeny

The immunopathogeny of the *Blastocystis* infection depends on factors related to the human host and the infectious agent. In the first group, nutritional and immunological status, age, access to effective drugs (MTZ), and associated infections are included. In the second group, virulence factors, and multiple genotypes or a complex clonal lineage of *Blastocystis* would explain the differences in the prevalence and pathogenicity in different geographic areas (20, 49, 150).

Fig. 1 shows a possible link between the interplay of the chromist and its host, and humoral and cellular factors associated with the genesis of IBS and urticaria. *Blastocystis* is located in the intestinal lumen, particularly in the ileum and colon, where it releases proteases, generates apoptosis, and immunomodulation (by degrade IgA). These events alter paracellular permeability, cause dysbiosis and promote the release of pro-inflammatory cytokine locally (13).The first phase of the inflammatory process involves mast cells, basophils, monocytes, TH2 cells, neutrophils, and eosinophil, mast cells and basophiles appear to be the most important. When a chronic inflammatory process such as urticaria occurs, cells, eosinophils, neutrophils, monocytes, macrophages and T lymphocytes, besides the release of histamine and its interaction with the H1 re-

ceptors in the endothelium of blood vessels, contributes to the chronicity of the disease (149, 151, 152).

After the extravasations of antibodies, macrophages, and anaphylatoxins of the complement system (C3a, C4a, C5a), there is an increase of lymphocyte reactivity, due to the *Blastocystis* infection. The platelet-activating factor stimulates the secretion of serotonin, eosinophils chemotactic factor and IL-5. Stimulated eosinophils release cationic protein (ECP), peroxidase (EPO), and X protein (EPX). These changes lead to hypersensitivity and skin lesions similar to urticaria (124, 149, 153-155).

Several etiologic factors have been associated with chronic urticaria such as thyroid diseases, pseudo-allergens, allergens, *Helicobacter pylori*, other infections/infestations, and autoimmunity/autoreactivity. Patients with chronic urticaria more frequently had seropositivity for fasciolosis, *Anisakis* simplex sensitization, and the presence of *Blastocystis* allele 34 (ST3) as compared with control subjects (155). In 18 independent studies, the reported rate of urticaria in patients with parasitic infections was 1-66.7%. Urticaria, including chronic spontaneous urticaria, might be a quite common symptom of strongyloidiasis and blastocystosis. Pathogenic mechanisms in chronic spontaneous urticaria due to parasite or stramenopile infections may include the participation of specific IgE, Th2 cytokine, eosinophils, activation of the complement and coagulation systems. It has been suggested that urticaria is caused by molecules of the stramenopile that activate certain subsets in Th2 cells, which produce interleukins 3, 4, 5 or 13 (IL-3, IL-4, IL-5, IL13) which mediate allergic responses by IgE (149, 151-155) (Fig. 1).

Panaszek *et al.* in 2016 (156) reported that monomeric IgE may enhance mast cell activity without cross-linking of FcεRI by IgE specific allergen or autoreactive IgG anti-IgE antibodies. Monomeric IgE molecules are heterogeneous concerning their ability to induce survival and activation of mast cells only by binding the IgE to FcεRI, but not affecting degranulation of cells. It was also evident that IgE may react to autoantigens occurring in the blood, not only in chronic spontaneous urticaria, but also in other autoimmune diseases.

LABORATORY DIAGNOSIS

Fresh fecal smear

Diagnosis by direct examination in fresh form with physiological saline solution and diluted lugol solution is widely used in developing countries; however, there is low sensitivity for cysts. In developed countries, access to techniques such as PCR has allowed a better diagnosis in terms of sensitivity. A valuable alternative in developing countries would be the cultivation of *Blastocystis*, which is not necessarily available for reasons of cost and infrastructure (157-160).

Microscopic detection by fresh fecal smears is difficult, mainly due to morphological diversity of the parasite, leading to false negatives. The classic vacuolar form may be infrequent and the rarer cystic, amoeboid, avacuolar and multivacuolar forms may be predominant. The cysts, different in size and appearance with respect to the vacuolar form, may be undetected (161). The variability of the prevalence of *Blastocystis* in the epidemiologic surveys could be due to the difficulty of identifying the organism by examination of fresh fecal smears, especially the cysts because of their small size. Hence, it can be confused with yeasts or detritus. It is likely that surveys underestimate the prevalence of the stramenopile. This technique is not adequate to differentiate *Blastocystis* subtypes.

Staining techniques

A variety of dyes have been successfully used in identifying *Blastocystis*, even when they are not used routinely in the clinical laboratory. With the acridine orange procedure, the *Blastocystis* DNA in the nucleus stains bright green, the mucus stains green or red, and the RNA stains orange. In a study, this

dye was used to differentiate the cyst from the vacuolar form of *Blastocystis* in cultures with means to promote encystment. The vacuolar form stained yellow and the cyst stained red (1), suggesting that the latter consists of the reproductive granules mentioned by Zierdt (10), who found no evidence that the vacuole plays a role in metabolism and reproduction. This staining technique is simple but its use as a diagnostic procedure requires experience and *Blastocysti* sculture *in vitro* (157).

Concentration techniques

They are a good choice for the diagnosis of the cyst forms of the stramenopile. The density gradient technique method has shown a great sensitivity for the selective concentration of the cysts (19). However, it is not useful in daily diagnostic practice.

Culture

Culture techniques have proved more sensitive than coproparasitoscopic techniques in identifying *Blastocystis* (157). But their use in routine diagnosis is not viable. However, they are the tool of choice for the recovery of viable cysts from drinking water, surface water, and wastewater samples (5, 100).

Serological techniques

There are few studies about *Blastocystis* antibodies and the results are somehow contradictory. In a study, no immune reaction was observed (162). However, other reports showed an IgG anti-*Blastocystis* response with ELISA in 25% of 28 infected patients (163) and a significant increase of IgG against the stramenopile in patients with IBS (13). With the indirect immunofluorescence test, *Blastocystis* antibodies were detected in 70% of asymptomatic infected individuals (164). These studies suggest that the stramenopile causes a humoral immune response in humans. Monoclonal antibodies were characterized against various isolates of *Blastocystis*; the absence of cross-reactivity of these antibodies in humans and animals support the concept that the stramenopile is antigenically heterogeneous.

Molecular techniques

Molecular biological tools have been developed to detect and differentiate *Blastocystis* at the species and genotypes levels but they are not in widespread use since these methods are not viable in third world countries. They have greater sensitivity and specificity than microscopies for detection and diagnosis. Several techniques including PCR have been applied in epidemiological studies of the stramenopile (6, 47, 98, 157).

Mohammad *et al.*, in 2018 (165) did a comparative study of Wheatley's trichrome stain and *In-vitro* culture against PCR assay for the diagnosis of *Blastocystis* spp. in 359 stool samples. The agreement between Wheatley's trichrome stain, *in-vitro* culture and combination of microscopic techniques with PCR assay were statistically significant by Kappa statistics. These authors suggest the use of combination of Wheatley's trichrome stain and *in-vitro* culture as screening tools for detection of *Blastocystis* especially in community laboratories facing financial constraints and that are not equipped with molecular facilities. However, whenever applicable, PCR assay should act as a confirmatory test as it is the only method that can recognize subtypes of *Blastocystis*. Moreover, Skotarczak in 2018 (166) indicates that the development of PCR assays is needed for molecular epidemiology and for mixed infections in health and disease cohorts, and also to help identify sources of *Blastocystis* spp. transmission to humans, as well as to identify potential animal and environmental reservoirs.

A combined study using PCR, as well as 18S mitochondrial sequencing and intermediary metabolism (pyruvate: ferredoxin oxidoreductase, PFOR) genes, in addition to phylogenetic analysis performed in Mexico

on samples derived from 192 children with gastrointestinal symptoms. Taking 21 stool samples from children infected only by *Blastocystis* spp. as a starting point, they found that although the fragment of the PFOR gene analyzed did not allow discrimination between *Blastocystis* STs, this marker grouped the samples in three clades with strengthened support, suggesting that PFOR may be under different selective pressures and evolutionary histories than the 18S gene. Interestingly, the ST3 sequences showed lower variability with probable purifying selection in both markers, meaning that evolutionary forces drive differential processes among *Blastocystis* STs (167). As a future perspective, the development of better and more powerful molecular tools will allow us to obtain better bases to understand the biological and diagnostic aspects in an "enigmatic organism" at present.

TREATMENT

The treatment is another controversial topic, at the beginning *Blastocystis* spp. was considered to be a commensal, and therefore no treatment was required. As the knowledge progressed, an attempt was made to reach consensus on the need for treatment according to the number of organisms present in the patient's fecal sample and the combination of signs and symptoms, when *Blastocystis* was presented as a possible cause of disease. At present, we can reach a consensus regarding its pathogenic potential, therefore it should be treated. Some authors suggest that it is not significantly associated with inflammatory processes or diarrhea in HIV patients (168) and that the presence of the organism does not justify prescribing treatment. Others believe that the stramenopile may be pathogenic according to its genotype and therapy is recommended in cases where *Blastocystis* is involved in gastrointestinal or extra-intestinal diseases (169). In most individuals, without concomitant health problems, diarrhea is often self-limited, suggesting that treatment is unnecessary.

There are few studies on drugs against *Blastocystis*. In 1983, emetine, MTZ, furazolidone, trimethopriminsulfametoxazole (TMP-SMX), 5-chloro-8-hydroxy-7-iodo-quinolone, and pentamidine were reported as the most effective drugs *in vitro* (170). In 1991, one study revealed that 5-nitroimidazoles were effective against the stramenopile (171). In 1996, a study related to traditional Chinese medicine reported the inhibitory effects *in vitro* on *Blastocystis* of *Bruceajavanica* and *Coptis chinensis* extracts, at concentrations of 500 and 100 μg/mL (172).

Batista *et al*. in 2017 (173) demonstrated low efficacy with the use of MTZ in 39 patients, 31 of whom obtained a clinical response (79.5%) but only 15 a microbiological response (48.4%). No dose-effect relationship was observed.

The prevalence of intestinal parasitic infections was evaluated in immunocompromised children with persistent and/or recurrent diarrhea. *Blastocystis* infection was predominant and clinical remission and eradication of the stramenopile was achieved in patients treated with MTZ (174). In another report, six cases of ulcerative colitis associated with *Blastocystis* were cured clinically and parasitologically after the administration of MTZ at a dose of 910 mg bid for 10-14 days (175). In immunocompromised patients, treatment with MTZ for 10-14 days seems to be the best option. According to these clinical studies, the drug of choice for treatment of infection appears to be MTZ, followed by other nitroimidazoles, at doses of 750 mg tid for 5 to 10 days. However, resistance of the vacuolar form and cystic forms in some isolates of *Blastocystis* has been shown, *in vitro*. The cyst is very resistant to drugs that are effective against protozoa; it is resistant to cytotoxic effect of MTZ at the concentration of 5 mg/mL. These findings may explain the ineffectiveness of the drug in the *Blastocystis* clearance in some patients.

Nitazoxanide is a new antiparasitic drug remarkable for its broad spectrum of activity against bacteria, protozoa, and helminths. It could be used as an alternative drug in the treatment of this infection. In a study of 10 children in Mexico, the cure rate was 84% (176). The doses used may be the same used against protozoa; a course of therapy bid for 3 days of 500 mg in adults and adolescents, 200 mg/10 mL in children 4 to 11 years old, and 100 mg/5 mL in children 1 to 3 (177). Studies about drug activity against the stramenopile are very scarce.

Traditionally, MTZ is considered a first-line treatment for *Blastocystis* infection; however, there has been increasing evidence for its lack of efficacy. Treatment failure has been reported in several clinical cases, and recent *in vitro* studies have suggested the occurrence of MTZ-resistant strains. Roberts *et al*. in 2015 (178), tested 12 antimicrobial drugs (MTZ, paromomycin, ornidazole, albendazole, ivermectin, TMP-SMX, furazolidone, nitazoxanide, secnidazole, fluconazole, nystatin, and itraconazole) at 10 different concentrations *in vitro* against 12 *Blastocystis* isolates, ST1, ST3, ST4, and ST8. It was found that each subtype showed little sensitivity to MTZ, paromomycin, and triple therapy (furazolidone, nitazoxanide, and secnidazole) while TMP-SMX and ivermectin were effective. These same authors reported long-term infection and treatment failure in 18 symptomatic individuals infected with *Blastocystis*. Patients were initially treated with MTZ, iodoquinol or triple combination therapy consisting of nitazoxanide, furazolidone, and secnidazole. Following treatment, resolution of clinical symptoms did not occur and follow-up examination revealed ongoing infection with the same subtype. Patients then underwent secondary treatment with a variety of antimicrobial agents but remained infected and symptomatic. Sequencing of the SSU rDNA was completed on all isolates and four subtypes were identified in this group: ST1, ST3, ST4, and ST5 (179).

In the case of HIV patients, the use of highly active antiretroviral therapy in individuals with AIDS has dramatically decreased the prevalence of infectious agents and the duration and severity of the symptoms. If despite this therapy, *Blastocystis* is not eliminated, the patient can be treated again according to clinical criteria (180, 181). The patient responded well to cotrimoxazole and albendazole (182). Studies conducted in Nepal and Iran demonstrate enteric parasitic infections are common in HIV-infected people. The poor immune status as indicated by low CD4 T-cell count may account for higher risk of both opportunistic and non-opportunistic enteric parasitic infection (183). In Iran, the results of genomic analysis of *Blastocystis* isolates in patients with HIV-positive using locus SSU-rDNA indicated a relatively high prevalence of *Blastocystis* in HIV-positive patients. This may also represent that the number reduction of TCD4-positive cells has an effective role in the increased risk of the parasitic infection in HIV-positive patients (184).

PREVENTION AND CONTROL STRATEGIES

Improving personal and environmental sanitation may reduce exposure to feces and contamination of the environment. Proper hygiene habits, food washing, and sanitizing may reduce the risk of acquiring infections (185-187).

In the developing world, where intestinal parasites are prevalent and represent a persistent public health problem (188-191), the most important steps to prevent infection are health education, personal hygiene, adequate hand washing, safe drinking water, proper sanitary infrastructures, and treatment of human sewage. However, these steps represent a difficult challenge for low income-countries.Since water is a potential source of *Blastocystis* cysts, and the organism is resistant to chlorine (192, 193), the quality of drinking water is essential to preventing infection. Water treatment through standard procedures to inactivate cysts by filtration or heating is necessary. For pre-

vention and control of waterborne blastocystosis, specific instructions and regulations developed by international organizations for controlling waterborne protozoa could be used for this organism. From a public health perspective, potential spread of the parasite from water can be avoided only by adequate treatment of household water sources. Studies to assess the quality of stored water and household practices which stimulate post-treatment contamination are highly recommended. Consumers should be aware of risks associated with consumption of raw fruits and vegetables. Boiled or filtered water must be used for washing them and for food preparation (194-196). As *Blastocystis* is very common in many types of animals and there has been speculation that they are a source of zoonotic transmission, steps must be taken to reduce contact with animals.

CONCLUSIONS AND RECOMMENDATIONS

In the post-genome era, investigations have focused on the etiological role of this stramenopile in human disease. Clinical and molecular studies are inconclusive and more causality case-control studies are needed. The equilibrium between investigations that bind or not *Blastocystis* to disease seems to begin to lean in favor of the association with IBD and urticaria. The hypothesis of "opportunistic pathogen" has emerged. The zoonotic potential of *Blastocystis* and its consideration as an index of water quality for human consumption should be addressed and discussed. New chemotherapeutic strategies are needed since resistance to MTZ and related compounds has been reported. Finally, due to its pathogenic potential on cells, the host cells (197), *Blastocystis* infection has become a public health problem that we must address from a global point of view (198).

REFERENCES

1. **Khalifa AM.** Diagnosis of *Blastocystis hominis* by different staining techniques. J Egypt Soc Parasitol 1999; 29: 157-165.
2. **Yason JA, Tan KSW.** Seeing the Whole Elephant: Imaging Flow Cytometry Reveals Extensive Morphological Diversity within *Blastocystis* Isolates. PloS One 2015; 10: e0143974.
3. **MacPherson DW, MacQueen WM**. Morphological diversity of *Blastocystis hominis* in sodium acetate-acetic acid-formalin-preserved stool samples stained with iron hematoxylin. J Clin Microbiol 1994; 32: 267-268.
4. **Devera R.** *Blastocystis* spp. 20 años después. Kasmera 2015; 43: 94-96.
5. **Suresh K, Smith HV, Tan TC.** Viable *Blastocystis* cysts in Scottish and Malaysian sewage samples. Appl Environ Microbiol 2005; 71: 5619-5620.
6. **Eroglu F, Koltas IS.** Evaluation of the transmission mode of *B. hominis* by using PCR method. Parasitol Res 2010; 107: 841-845.
7. **Yoshikawa H, Tokoro M, Nagamoto T, Arayama S, Asih PB, Rozi IE, Syafruddin D.** Molecular survey of *Blastocystis* sp. from humans and associated animals in an Indonesian community with poor hygiene. Parasitol Int 2016; 65: 780-784.
8. **Heudorf U, Karathana M, Krackhardt B, Huber M, Raupp P, Zinn C.** Surveillance for parasites in unaccompanied minor refugees migrating to Germany in 2015. GMS Hyg Infect Control 2016; 11: Doc05.
9. **El Safadi D, Gaayeb L, Meloni D, Cian A, Poirier P, Wawrzyniak I, Delbac F, Dabboussi F, Delhaes L, Seck M, Hamze M, Riveau G, Viscogliosi E.** Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. BMC Infect Dis 2014; 14: 164.
10. **Phillips BP, Zierdt CH.** *Blastocystis hominis*: pathogenic potential in human patients and in gnotobiotes. Exp Parasitol 1976; 39: 358-364.
11. **Garavelli PL.** *Blastocystis hominis* and blastocystosis (Zierdt-Garavelli disease). Ann Ital Med Int 2002; 17: 60-62.

12. **Garavelli PL.** Blastocystosis or Zierdt-Garavelli disease: a clinical pathway. Recenti Prog Med 2006; 97: 397-400.
13. **Poirier P, Wawrzyniak I, Vivarès CP, Delbac F, El Alaoui H.** New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. PLoS Pathog 2012; 8: e1002545.
14. **Johnson AM, Thanou A, Boreham PF, Baverstock PR.** *Blastocystis hominis*: phylogenetic affinities determined by rRNA sequence comparison. Exp Parasitol 1989; 68: 283-288.
15. **Chacín Bonilla L.** Aspectos controversiales de *Blastocystis hominis*: Taxonomía y concepto emergente de patogenicidad. Invest Clin 1991; 32: 147-148.
16. **Silberman JD, Sogin ML, Leipe DD, Clark CG.** Human parasite finds taxonomic home. Nature 1996; 380: 398.
17. **Ho LC, Armiugam A, Jeyaseelan K, Yap EH, Singh M.** *Blastocystis* elongation factor-1alpha: genomic organization, taxonomy and phylogenetic relationships. Parasitology 2000; 121: 135-144.
18. **Arisue N, Hashimoto T, Yoshikawa H, Nakamura Y, Nakamura G, Nakamura F, Yano TA, Hasegawa M.** Phylogenetic position of *Blastocystis hominis* and of stramenopiles inferred from multiple molecular sequence data. J Eukaryot Microbiol 2002; 49: 42-53.
19. **Zaman V.** The diagnosis of *Blastocystis* cysts in human faeces. J Infect 1996; 33: 15-16.
20. **Denoeud F, Roussel M, Noel B, Wawrzyniak I, Da Silva C, Diogon M, Viscogliosi E, Brochier-Armanet C, Couloux A, Poulain J, Segurens B, Anthouard V, Texier C, Blot N, Poirier P, Ng GC, Tan KS, Artiguenave F, Jaillon O, Aury JM, Delbac F, Wincker P, Vivarès CP, El Alaoui H.** Genome sequence of the Stramenopile *Blastocystis*, a human anaerobic parasite. Gen Biol 2011; 12: R29.
21. **Wawrzyniak I, Roussel M, Diogon M, Couloux A, Texier C, Tan KS, Vivarès CP, Delbac F, Wincker P, El Alaoui H.** Complete circular DNA in the mitochondria-like organelles of *Blastocystis hominis*. Int J Parasitol 2008; 38: 1377-1382.
22. **Jiménez-González DE, Martínez-Flores WA, Reyes-Gordillo J, Ramírez-Miranda ME, Arroyo-Escalante S, Romero-Valdovinos M, Stark D, Souza-Saldivar V, Martinez-Hernandez F, Flisser A, Olivo-Diaz A, Maravilla P.** *Blastocystis* infection is associated with irritable bowel syndrome in a Mexican patient population. Parasitol Res 2012; 110: 1269-1275.
23. **Ramírez-Miranda ME, Hernández-Castellanos R, López-Escamilla E, Moncada D, Rodríguez-Magallan A, Pagaza-Melero C, Gonzalez-Angulo A, Flisser A, Kawa-Karasik S, Maravilla P.** Parasites in Mexican patients with irritable bowel syndrome: a case-control study. Parasit Vectors 2010; 3: 96.
24. **Casero RD, Mongi F, Sánchez A, Ramírez JD.** *Blastocystis* and urticaria: Examination of subtypes and morphotypes in an unusual clinical manifestation. Acta Trop 2015; 148: 156-161.
25. **Yersal O, Malatyali E, Ertabaklar H, Oktay E, Barutca S, Ertug S.** *Blastocystis* subtypes in cancer patients: Analysis of possible risk factors and clinical characteristics. Parasitol Int 2016; 65: 792-796.
26. **Tan KS, Singh M, Yap EH.** Recent advances in *Blastocystis hominis* research: hot spots in terra incognita. Int J Parasitol 2002; 32: 789-804.
27. **Tan KSW.** New insights on classification, identification, and clinical relevance of *Blastocystis* spp. Clin Microbiol Rev 2008; 21: 639-665.
28. **Escobedo A, Núñez FA.** *Blastocystis hominis* infection in Cuban AIDS patients. Mem Inst Oswaldo Cruz 1997; 92: 321-322.
29. **Vidal S, Toloza L, Cancino B.** Evolución de la prevalencia de enteroparasitosis en la ciudad de Talca, Región del Maule, Chile. Rev Chilena Infectol 2010; 27: 336-340.
30. **Zhang SX, Zhou YM, Xu W, Tian LG, Chen JX, Chen SH, Dang ZS, Gu WP, Yin JW, Serrano E, Zhou XN.** Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China.Infect Dis Poverty 2016; 5: 64.
31. **Vielma JR, Delgado Y, Bravo YA, Gutiérrez-Peña LV, Villarreal JC.** Enteroparasites and Thermotolerant Coliforms in water and human feces of sectors Juan de Dios González and El Moralito, Colón Municipality, Zulia State. Acta Bioclínica 2016; 6: 25-43.
32. **Ithoi I, Jali A, Mak JW, Wan-Sulaiman WY, Mahmud R.** Ocurrence of *Blastocystis* in

water of two rivers from recreational areas in Malaysia. J Parasitol Res 2011; 123916.

33. **Mora L, Martínez I, Figuera L, Segura M, Del Valle G.** Protozoarios en aguas superficiales y muestras fecales de individuos de poblaciones rurales del municipio Montes, estado Sucre, Venezuela. Invest Clin 2010; 51: 457-466.
34. **Chacín-Bonilla L.** Perfil epidemiológico de las enfermedades infecciosas en Venezuela. Invest Clin 2017; 58: 103-105.
35. **Amin OM.** Seasonal prevalence of intestinal parasites in the United States during 2000. Am J Trop Med Hyg 2002; 66: 799-803.
36. **Scanlan PD, Knight R, Song SJ, Ackermann G, Cotter PD.** Prevalence and genetic diversity of *Blastocystis* in family units living in the United States. Infect Genet Evol 2016; 45: 95-97.
37. **O'Gorman MA, Orenstein SR, Proujansky R, Wadowsky RM, Putnam PE, Kocoshis SA.** Prevalence and characteristics of *Blastocystis hominis* infection in children. Clin Pediatr (Phila) 1993; 32: 91-96.
38. **Kappus KD, Lundgren RG Jr, Juranek DD, Roberts JM, Spencer HC.** Intestinal parasitism in the United States: update on a continuing problem. Am J Trop Med Hyg 1994; 50: 705-713.
39. **Kappus KK, Juranek DD, Roberts JM.** Results of testing for intestinal parasites by state diagnostic laboratories, United States, 1987. MMWR CDC Surveill Summ 1991; 40: 25-45.
40. **Gualdieri L, Rinaldi L, Petrullo L, Morgoglione ME, Maurelli MP, Musella V, Piemonte M, Caravano L, Coppola MG, Cringoli G**. Intestinal parasites in immigrants in the city of Naples (southern Italy). Acta Trop 2011; 117: 196-201.
41. **Calderaro A, Montecchini S, Rossi S, Gorrini C, De Conto F, Medici MC, Chezzi C, Arcangeletti MC.** Intestinal parasitoses in a tertiary-care hospital located in a non-endemic setting during 2006-2010. BMC Infect Dis 2014; 14: 264.
42. **Manganelli L, Berrilli F, Di Cave D, Ercoli L, Capelli G, Otranto D, Giangaspero A**. Intestinal parasite infections in immigrant children in the city of Rome, related risk factors and possible impact on nutritional status. Parasit Vectors 2002; 5: 265.
43. **Pistono PG, Dusi MP, Ronchetto F, Cestonaro G, Guasco C.** *Blastocystis hominis* in Canavese: a retrospective study of samples received for fecal parasitological examination at the Ivrea-Castellamonte Hospital over 42 months. G Batteriol Virol Immunol 1991; 84: 67-76.
44. **Masucci L, Graffeo R, Bani S, Bugli F, Boccia S, Nicolotti N, Fiori B, Fadda G, Spanu T.** Intestinal parasites isolated in a large teaching hospital, Italy, 1 May 2006 to 31 December 2008. Euro Surveill 2011;16: pii 19891.
45. **Guidetti C, Ricci L, Vecchia L.** Prevalence of intestinal parasitosis in Reggio Emilia (Italy) during 2009. Infez Med 2010; 18: 154-161.
46. **Sigidaev AS, Kozlov SS, Tarasova EA, Suvorova MA.** Investigation of the genetic profile of *Blastocystis* species in Saint Petersburg residents with gastrointestinal tract diseases in different age groups. Med Parazitol (Mosk) 2013; 4: 19-23.
47. **Bart A, Wentink-Bonnema EM, Gilis H, Verhaar N, Wassenaar CJ, van Vugt M, Goorhuis A, van Gool T.** Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. BMC Infect Dis 2013; 13: 389.
48. **El Safadi D, Cian A, Nourrisson C, Pereira B, Morelle C, Bastien P, Bellanger AP, Botterel F, Candolfi E, Desoubeaux G, Lachaud L, Morio F, Pomares C, Rabodonirina M, Wawrzyniak I, Delbac F, Gantois N, Certad G, Delhaes L, Poirier P, Viscogliosi E.** Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center study in France. BMC Infect Dis 2016; 16: 451.
49. **Jelinek T, Peyerl G, Löscher T, von Sonnenburg F, Nothdurft HD.** The role of *Blastocystis hominis* as a possible intestinal pathogen in travellers. J Infect 1997; 35: 63-66.
50. **Herbinger KH, Alberer M, Berens-Riha N, Schunk M, Bretzel G, von Sonnenburg F, Nothdurft HD, Löscher T, Beissner M.** Spectrum of imported infectious diseases: a comparative prevalence study of 16,817 German Travelers and 977 Immigrants from the Tropics and Subtropics. Am J Trop Med Hyg 2016; 94:757-766.

51. **González-Moreno O, Domingo L, Teixidor J, Gracenea M.** Prevalence and associated factors of intestinal parasitisation: a cross-sectional study among outpatients with gastrointestinal symptoms in Catalonia, Spain. Parasitol Res 2011; 108: 87-93.
52. **Zhang SX, Yang CL, Gu WP, Ai L, Serrano E, Yang P, Zhou X, Li SZ, Lv S, Dang ZS, Chen JH, Hu W, Tian LG, Chen JX, Zhou XN.** Case-control study of diarrheal disease etiology in individuals over 5 years in southwest China. Gut Pathog 2016; 8: 58.
53. **Svenungsson B, Lagergren A, Ekwall E, Evengård B, Hedlund KO, Kärnell A, Löfdahl S, Svensson L, Weintraub A.** Enteropathogens in adult patients with diarrhea and healthy control subjects: a 1-year prospective study in a Swedish clinic for infectious diseases. Clin Infect Dis 2000; 30: 770-778.
54. **Hirata T, Nakamura H, Kinjo N, Hokama A, Kinjo F, Yamane N, Yamane N, Fujita J.** Prevalence of *Blastocystis hominis* and *Strongyloides stercoralis* infection in Okinawa, Japan. Parasitol Res 2007; 101: 1717-1719.
55. **Horiki N, Maruyama M, Fujita Y, Yonekura T, Minato S, Kaneda Y**. Epidemiologic survey of *Blastocystis hominis* infection in Japan. Am J Trop Med Hyg 1997; 56: 370-374.
56. **Junod C.** *Blastocystis hominis*: a common commensal in the colon. Study of prevalence in different populations of Paris. Presse Med 1995; 24: 1684-1688.
57. **Rebolla MF, Silva EM, Gomes JF, Falcão AX, Rebolla MV, Franco RM.** High prevalence of *Blastocystis* spp. infection in children and staff members attending public urban schools in São Paulo state, Brazil. Rev Inst Med Trop Sao Paulo 2016; 58: 31.
58. **Malheiros AF, Stensvold CR, Clark CG, Braga GB, Shaw JJ.** Short report: Molecular characterization of *Blastocystis* obtained from members of the indigenous Tapirapé ethnic group from the Brazilian Amazon region, Brazil. Am J Trop Med Hyg 2011; 85: 1050-1053.
59. **Poulsen CS, Efunshile AM, Nelson JA, Stensvold CR.** Epidemiological aspects of *Blastocystis* colonization in children in Ilero, Nigeria. Am J Trop Med Hyg 2016; 95: 175-179.
60. **Gyang VP, Chuang TW, Liao CW, Lee YL, Akinwale OP, Orok A, Ajibaye O, Babasola A, Cheng PC, Chou CM, Huang YC, Sonko P, Fan CK.** Intestinal parasitic infections: Current status and associated risk factors among school aged children in an archetypal African urban slum in Nigeria. J Microbiol Immunol Infect 2017; pii: S1684-1182 (17) 30072-30075.
61. **Helenbrook WD, Shields WM, Whipps CM.** Characterization of *Blastocystis* species infection in humans and mantled howler monkeys, *Alouatta palliataaequatorialis*, living in close proximity to one another. Parasitol Res 2015; 114: 2517-2525.
62. **Dib JR, Fernández-Zenoff MV, Oquilla J, Lazarte S, González SN.** Prevalence of intestinal parasitic infection among children from a shanty town in Tucuman, Argentina. Trop Biomed 2015; 32: 210-215.
63. **Minvielle MC, Pezzani BC, Córdoba MA, De Luca MM, Apezteguia MC, Basualdo JA.** Epidemiological survey of *Giardia* spp. and *Blastocystis hominis* in an Argentinian rural community. Korean J Parasitol 2004; 42: 121-127.
64. **Incani RN, Ferrer E, Hoek D, Ramak R, Roelfsema J, Mughini-Gras L, Kortbeek T, Pinelli E.** Diagnosis of intestinal parasites in a rural community of Venezuela: Advantages and disadvantages of using microscopy or RT-PCR. Acta Trop 2017; 167: 64-70.
65. **Velasco J, González F, Díaz T, Peña-Guillén J, Araque M.** Profiles of enteropathogens in asymptomatic children from indigenous communities of Mérida, Venezuela. J Infect Dev Ctries 2011; 5: 278-285.
66. **Velásquez V, Caldera R, Wong W, Cermeño G, Fuentes M, Blanco Y, Aponte M, Devera R.** Elevada prevalencia de blastocistose em pacientes do Centro de Saúde de Soledad, Estado Anzoátegui, Venezuela. Rev Soc Bras Med Trop 2005; 38: 1-2.
67. **Devera R, Azacon B, Jiménez M.** *Blastocystis hominis* en pacientes del Hospital Universitario Ruiz y Páez de Ciudad Bolívar, Venezuela. Bol Chil Parasitol 1998; 53: 65-70.
68. **Chacín-Bonilla L, Sánchez-Chávez Y.** Intestinal parasitic infections, with a special emphasis on cryptosporidiosis, in Amerindians from western Venezuela. Am J Trop Med Hyg 2000; 62: 347-352.

69. **Devera RA, Velasquez VJ, Vasquez MJ.** Blastocistosis en pre-escolares de Ciudad Bolívar, Venezuela. Cad Saude Publica 1998; 14: 401-407.
70. **Chacín-Bonilla L, Guanipa N, Cano G, Raleigh X, Quijada L.** Cryptosporidiosis among patients with acquired immunodeficiency syndrome in Zulia State, Venezuela. Am J Trop Med Hyg 1992; 47: 582-586.
71. **Vielma JR, Pérez I, Villarreal J, Vegas M, Reimi Y, Belisario M, Prieto M, Uzcátegui D, Hernández H, Pineda C, González E, Gutiérrez-Peña L.** *Blastocystis* spp. y enteroparásitos en pacientes que asisten a dos instituciones públicas de atención a la salud, occidente venezolano. Acta Bioclínica 2017; 7: 80-99.
72. **Vielma JR, Pérez IF, Vegas ML, Reimi Y, Díaz S, Gutiérrez-Peña LV.** *Blastocystis* spp. y enteroparásitos en personas que asisten al ambulatorio urbano tipo II IPASME-Barinas. Observador del Conocimiento 2016; 3: 69-74.
73. **Miller SA, Rosario CL, Rojas E, Scorza JV.** Intestinal parasitic infection and associated symptoms in children attending day care centers in Trujillo, Venezuela. Trop Med Int Health 2003; 8: 342-347.
74. **Castrillo de Tirado A, González Mata AJ, Tirado Espinoza E.** Prevalencia de la infección por *Blastocystis hominis*: un año de estudio. GEN 1990; 44: 217-220.
75. **Mejías G.** Las infecciones intestinales parasitarias en los estudiantes rurales del archipiélago de Chiloé, Región X, Chile. Bol Chil Parasitol 1993; 48: 28-29.
76. **Biolley MA, Oberg C.** Infección por *Blastocystis* en pacientes sintomáticos del Hospital Regional de Temuco, Chile. Bol Chil Parasitol 1993; 48: 25-27.
77. **Mercado R, Arias B.** *Blastocystis hominis*: frecuencia de infección en pacientes ambulatorios de la zona norte de Santiago, Chile. Bol Chil Parasitol 1991; 46: 30-32.
78. **Osman M, El Safadi D, Cian A, Benamrouz S, Nourrisson C, Poirier P, Pereira B, Razakandrainibe R, Pinon A, Lambert C, Wawrzyniak I, Dabboussi F, Delbac F, Favennec L, Hamze M, Viscogliosi E, Certad G.** Prevalence and risk factors for intestinal protozoan infections with *Cryptosporidium*, *Giardia*, *Blastocystis* and *Dientamoeba* among school children in Tripoli, Lebanon. PloS Negl Trop Dis 2016; 10: e0004496.
79. **Forsell J, Granlund M, Samuelsson L, Koskiniemi S, Edebro H, Evengård B.** High occurrence of *Blastocystis* sp. subtypes 1-3 and *Giardia intestinalis* assemblage B among patients in Zanzibar, Tanzania. Parasit Vectors 2016; 9: 370.
80. **D'Alfonso R, Santoro M, Essi D, Monsia A, Kaboré Y, Glé C, Di Cave D, Sorge RP, Di Cristanziano V, Berrilli F.** *Blastocystis* in Côte d'Ivoire: molecular identification and epidemiological data. Eur J Clin Microbiol Infect Dis 2017; 36: 2243-2250.
81. **AbuOdeh R, Ezzedine S, Samie A, Stensvold CR, ElBakri A.** Prevalence and subtype distribution of *Blastocystis* in healthy individuals in Sharjah, United Arab Emirates. Infect Genet Evol 2016; 37: 158-162.
82. **Cruz Licea V, Plancarte Crespo A, Morán Alvarez C, Valencia Rojas S, Rodríguez Sásnchez G, Vega Franco L.** *Blastocystis hominis* among food vendors in Xochimilco markets. Rev Latinoam Microbiol 2003; 45: 12-15.
83. **Rajah Salim H, Suresh Kumar G, Vellayan S, Mak JW, Khairul Anuar A, Init I, Vennila GD, Saminathan R, Ramakrishnan K.** *Blastocystis* in animal handlers. Parasitol Res 1999; 85: 1032-1033.
84. 84. **Mohammad NA, Al-Mekhlafi HM, Moktar N, Anuar TS.** Prevalence and risk factors of *Blastocystis* infection among underprivileged communities in rural Malaysia. Asian Pac J Trop Med 2017; 10: 491-497.
85. **Abdulsalam AM, Ithoi I, Al-Mekhlafi HM, Ahmed A, Surin J, Mak JW.** Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary schoolchildren. Parasitology 2012; 139: 1014-1020.
86. **Anuar TS, Ghani MK, Azreen SN, Salleh FM, Moktar N.** *Blastocystis* infection in Malaysia: evidence of waterborne and human-to-human transmissions among the Proto-Malay, Negrito and Senoi tribes of Orang Asli. Parasit Vectors 2013; 6: 40.
87. **Nithyamathi K, Chandramathi S, Kumar S.** Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. PloS One 2016; 11: e0136709.
88. **Cañete R, Díaz MM, Avalos García R, Laúd Martinez PM, Manuel Ponce F.** Intestinal

parasites in children from a day care centre in Matanzas City, Cuba. PLoS One 2012; 7: e51394.

89. **Chincha O, Bernabé-Ortiz A, Samalvides F, Soto L, Gotuzzo E, Terashima A.** Infecciones parasitarias intestinales y factores asociados a la infección por coccidias en pacientes adultos de un hospital público de Lima, Perú. Rev Chilena Infectol 2009; 26: 440-444.
90. **Maco Flores V, Marcos Raymundo LA, Terashima Iwashita A, Samalvides Cuba F, Gotuzzo Herencia E.** Distribución de las enteroparasitosis en el altiplano peruano: estudio realizado en seis comunidades rurales del departamento de Puno, Perú. Rev Gastroenterol Peru 2002; 22: 304-309.
91. **Pipatsatitpong D, Rangsin R, Leelayoova S, Naaglor T, Mungthin M.** Incidence and risk factors of *Blastocystis* infection in an orphanage in Bangkok, Thailand. Parasit Vectors 2012; 5: 37.
92. **Macchioni F, Segundo H, Gabrielli S, Totino V, Gonzales PR, Salazar E, Bozo R, Bartoloni A, Cancrini G.** Dramatic decrease in prevalence of soil-transmitted helminths and new insights into intestinal protozoa in children living in the Chaco region, Bolivia. Am J Trop Med Hyg 2015; 92: 794-796.
93. **Haider SS, Baqai R, Qureshi FM, Boorom K.** *Blastocystis* spp., *Cryptosporidium* spp., and *Entamoeba histolytica* exhibit similar symptomatic and epidemiological patterns in healthcare-seeking patients in Karachi. Parasitol Res 2012; 111: 1357-1368.
94. **Kiani H, Haghighi A, Salehi R, Azargashb E.** Distribution and risk factors associated with intestinal parasite infections among children with gastrointestinal disorders. Gastroenterol Hepatol Bed Bench 2016; 9: S80-S87.
95. **Cekin AH, Cekin Y, Adakan Y, Tasdemir E, Koclar FG, Yolcular BO**. Blastocystosis in patients with gastrointestinal symptoms: a case-control study. BMC Gastroenterol 2012; 12: 122.
96. **Panunzio A, Fuentes B, Villarroel F, Pirela E, Avila Ayari, Molero T, Nuñez M, Parra-Cepeda I**. Prevalencia y epidemiología de *Blastocystis* sp. en dos comunidades del municipio Maracaibo-Estado Zulia. Kasmera 2014; 42: 9-21.
97. **Yoshikawa H, Wu Z, Kimata I, Iseki M, Ali IK, Hossain MB, Zaman V, Haque R, Takahashi** Polymerase chain reaction-based genotype classification among human *Blastocystis hominis* populations isolated from different countries. Parasitol Res 2004; 92: 22-29.
98. **Yoshikawa H, Wu Z, Nagano I, Takahashi Y.** Molecular comparative studies among *Blastocystis* isolates obtained from humans and animals. J Parasitol 2003; 89: 585-594.
99. **Noradilah SA, Lee IL, Anuar TS, Salleh FM, Abdul Manap SN, Mohd Mohtar NS, Azrul SM, Abdullah WO, Moktar N.** Occurrence of *Blastocystis* sp. in water catchments at Malay villages and Aboriginal settlement during wet and dry seasons in Peninsular Malaysia. Peer J 2016; 4: e2541.
100. **Flores-Carrero AD, Peña-Contreras Z, Dávila-Vera D, Colmenares-Sulbarán M, Mendoza-Briceño RV.** Investigación de *Blastocystis* sp. en agua de consumo humano en una población escolar de la zona rural del estado Mérida, Venezuela. Kasmera 2011; 38: 123-129.
101. **Karaman Ü, Kolören Z, Seferoğlu O, Ayaz E, Demirel E.** Presence of parasites in environmental waters in samsun and its districts. Turkiye Parazitol Derg 2017; 41: 19-21.
102. **Leelayoova S, Siripattanapipong S, Thathaisong U, Naaglor T, Taamasri P, Piyaraj P, Mungthin M.** Drinking water: a possible source of *Blastocystis* spp. subtype 1 infection in schoolchildren of a rural community in central Thailand. Am J Trop Med Hyg 2008; 79: 401-406.
103. **Elshazly AM, Elsheikha HM, Soltan DM, Mohammad KA, Morsy TA.** Protozoal pollution of surface water sources in Dakahlia Governorate, Egypt. J Egypt Soc Parasitol 2007; 37: 51-64.
104. **Banaticla JE, Rivera WL.** Detection and subtype identification of *Blastocystis* isolates from wastewater samples in the Philippines. J Water Health 2011; 9: 128-137.
105. **Stenzel DJ, Boreham PFL.** A cyst-like stage of *Blastocystis hominis*. Int J Parasitol 1991; 21: 613-615.
106. **Marshall MM, Naumovitz D, Ortega Y, Sterling CR.** Waterborne protozoan pathogens. Clin Microbiol Rev 1997; 10: 67-85.
107. **Basualdo J, Pezzani B, De Luca M, Córdoba A, Apezteguía M.** Screening of the mu-

nicipal water system of La Plata, Argentina, for human intestinal parasites. Int J Hyg Environ Health 2000; 203: 177-182.

108. Branco N, Leal DA, Franco RM. A parasitological survey of natural water springs and inhabitants of a tourist city in southeastern Brazil. Vector Borne Zoonotic Dis 2012; 12: 410-417.

109. Osman M, Bories J, El-Safadi D, Poirel MT, Gantois N, Benamrouz-Vanneste S, Delhaes L, Hugonnard M, Certad G, Zenner L, Viscogliosi E. Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in France and evaluation of zoonotic transmission risk. Vet Parasitol 2015; 214: 167-170.

110. Noel C, Dufernez F, Gerbod D, Edgcomb VP, Delgado-Viscogliosi P, Ho LC, Singh M, Wintjens R, Sogin ML, Capron M, Pierce R, Zenner L, Viscogliosi E. Molecular phylogenies of *Blastocystis* isolates from different hosts: implications for genetic diversity, identification of species, and zoonosis. J Clin Microbiol 2005; 43: 348-355.

111. Giacometti A, Cirioni O, Fiorentini A, Fortuna M, Scalise G. Irritable bowel syndrome in patients with *Blastocystis hominis* infection. Eur J Clin Microbiol Infect Dis 1999; 18: 436-439.

112. Nourrisson C, Scanzi J, Pereira B, Nkoud-Mongo C, Wawrzyniak I, Cian A, Viscogliosi E, Livrelli V, Delbac F, Dapoigny M, Poirier P. *Blastocystis* is associated with decrease of fecal microbiota protective bacteria: Comparative analysis between patients with irritable bowel syndrome and control subjects. PloS ONE 2014; 9: e111868.

113. Krogsgaard LR, Engsbro AL, Stensvold CR, Nielsen HV, Bytzer P. The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. Clin Gastroenterol Hepatol 2015; 13: 507-513.

114. Das R, Khalil S, Mirdha BR, Makharia GK, Dattagupta S, Chaudhry R. Molecular characterization and subtyping of *Blastocystis* species in irritable bowel syndrome patients from North India. PloS ONE 2016; 11: e0147055.

115. Ragavan ND, Kumar S, Chye TT, Mahadeva S, Shiaw-Hooi H. *Blastocystis* sp. in irritable bowel syndrome (IBS)-detection in stool aspirates during colonoscopy. PloS One 2015; 10: e0121173.

116. Yakoob J, Jafri W, Jafri N, Khan R, Islam M, Beg MA, Zaman V. Irritable bowel syndrome: in search of an etiology: role of *Blastocystis hominis*. Am J Trop Med Hyg 2004; 70: 383-385.

117. Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R. Irritable bowel syndrome: is it associated with genotypes of *Blastocystis hominis*. Parasitol Res 2010; 106: 1033-1038.

118. Yakoob J, Jafri W, Beg MA, Abbas Z, Naz S, Islam M, Khan R. *Blastocystis hominis* and *Dientamoeba fragilis* in patients fulfilling bowel syndrome criteria. Parasitol Res 2010; 107: 679-684.

119. Dogruman-Al F, Simsek Z, Boorom K, Ekici E, Sahin M, Tuncer C, Kustimur S, Altinbas A. Comparison of methods for detection of *Blastocystis* infection in routinely submitted stool samples, and also in IBS/IBD Patients in Ankara, Turkey. PloS ONE 2010; 5: e15484.

120. Vasquez-Rios G, Machicado JD, Gamero MT, Pezua A, Betancourt AB, Terashima A, Marcos LA. Evaluating the role of intestinal parasites in the high rates of irritable bowel syndrome in South America: a pilot study. Folia Parasitol 2015; 62: pii 2015.065.

121. Stensvold CR, Alfellani MA, Nørskov-Lauritsen S, Prip K, Victory EL, Maddox C, Nielsen HV, Clark CG. Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. Int J Parasitol 2009; 39: 473-479.

122. Surangsrirat S, Thamrongwittawatpong L, Piyaniran W, Naaglor T, Khoprasert C, Taamasri P, Mungthin M, Leelayoova S. Assessment of the association between *Blastocystis* infection and irritable bowel syndrome. J Med Assoc Thai 2010; 93: S119-S124.

123. Zuel-Fakkar NM, Abdel-Hameed DM, Hassanin OM. Study of *Blastocystis hominis* isolates in urticaria: a case-control study. Clin Exp Dermatol 2011; 36: 908-910.

124. Hameed DM, Hassanin OM, Zuel-Fakkar NM. Association of *Blastocystis hominis* genetic subtypes with urticaria. Parasitol Res 2011; 108: 553-560.

125. Pasqui AL, Savini E, Saletti M, Guzzo C, Puccetti L, Auteri A. Chronic urticaria and *Blastocystis hominis* infection: a case report. Eur Rev Med Pharmacol Sci 2004; 8: 117-120.

126. Coskun A, Malatyali E, Ertabaklar H, Yasar MB, Karaoglu AO, Ertug S. *Blastocystis* in ulcerative colitis patients: Genetic diversity and analysis of laboratory findings. Asian Pac J Trop Med 2016; 9: 916-919.

127. Rossen NG, Bart A, Verhaar N, van-Nood E, Kootte R, de Groot PF, D'Haens GR, Ponsioen CY, van Gool T. Low prevalence of *Blastocystis* sp. in active ulcerative colitis patients. Eur J Clin Microbiol Infect Dis 2015; 34: 1039-1044.

128. Jeddy TA, Farrington GH. *Blastocystis hominis* complicating ulcerative colitis. J R Soc Med 1991; 84: 623.

129. Tejera B, Grados D, Martinez-Morillo M, Roure S. Reactive arthritis caused by *Blastocystis hominis*. Reumatol Clin 2012; 8: 50-51.

130. Krüger K, Kamilli I, Schattenkirchner M. *Blastocystis hominis* as a rare arthritogenic pathogen. A case report.Z Rheumatol 1994;53: 83-85.

131. Lee MG, Rawlins SC, Didier M, DeCeulaer K. Infective arthritis due to *Blastocystis hominis*. Ann Rheum Dis 1990; 49: 192-193.

132. Silva-Díaz H, Flores-Esqueche L, Llatas-Cancino D, Guevara Vásquez G, Silva-García T. Frequency and *in vitro* susceptibility antiparasitic of *Blastocystis hominis* from patients admitted to the Hospital Regional Lambayeque, Peru. Rev Gastroenterol Peru 2016; 36: 197-202.

133. Skotarczak B. Genetic diversity and pathogenicity of *Blastocystis*. Ann Agric Environ Med 2018; 25: 411-416.

134. Vogelberg C, Stensvold CR, Monecke S, Ditzen A, Stopsack K, Heinrich-Gräfe U, Pöhlmann C. *Blastocystis* sp. subtype 2 detection during recurrence of gastrointestinal and urticarial symptoms. Parasitol Int 2010; 59: 469-471.

135. Rao K, Sekar U, Iraivan KT, Abraham G, Soundararajan P. *Blastocystis hominis*-an emerging cause of diarrhoea in renal transplant recipients. J Assoc Physicians India 2003; 51: 719-721.

136. Shah M, Tan CB, Rajan D, Ahmed S, Subramani K, Rizvon K, Mustacchia P. *Blastocystis hominis* and *Endolimax nana* co-infection resulting in chronic diarrhea in an immunocompetent male. Case Rep Gastroenterol 2012; 6: 358-364.

137. Walderich B, Bernauer S, Renner M, Knobloch J, Burchard GD. Cytopathic effects of *Blastocystis hominis* on Chinese hamster ovary (CHO) and adeno carcinoma HT29 cell cultures. Trop Med Int Health 1998; 3: 385-390.

138. Abdel-Hafeez EH, Ahmad AK, Abdelgelil NH, Abdellatif MZ, Kamal AM, Hassanin KM, Abdel-Razik AR, Abdel-Raheem EM. Immunopathological assessments of human *Blastocystis* spp. in experimentally infected immunocompetent and immunosuppresed mice. Parasitol Res 2016; 115: 2061-2071.

139. Mirza H, Tan KS. *Blastocystis* exhibits inter- and intra-subtype variation in cysteine protease activity. Parasitol Res 2009;104: 355-361.

140. Wong KH, Ng GC, Lin RT, Yoshikawa H, Taylor MB, Tan KS. Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. Parasitol Res 2008; 102: 663-670.

141. Li LH, Zhang XP, Lv S, Zhang L, Yoshikawa H, Wu Z, Steinmann P, Utzinger J, Tong XM, Chen SH, Zhou XN. Cross-sectional surveys and subtype classification of human *Blastocystis* isolates from four epidemiological settings in China. Parasitol Res 2007; 102: 83-90.

142. Eroglu F, Genc A, Elgun G, Koltas IS. Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR. Parasitol Res 2009; 105: 1589-1592.

143. Tan TC, Suresh KG. Amoeboid form of *Blastocystis hominis*-a detailed ultrastructural insight. Parasitol Res 2006; 99: 737-742.

144. Tan TC, Suresh KG, Smith HV. Phenotypic and genotypic characterisation of *Blastocystis hominis* isolates implicates subtype 3 as a subtype with pathogenic potential. Parasitol Res 2008; 104: 85-93.

145. Elwakil HS, Hewedi IH. Pathogenic potential of *Blastocystis hominis* in laboratory mice. Parasitol Res 2010; 107: 685-689.

146. Yan Y, Su S, Lai R, Liao H, Ye J, Li X, Luo X, Chen G. Genetic variability of *Blastocystis hominis* isolates in China. Parasitol Res 2006; 99: 597-601.

147. Rostami A, Riahi SM, Haghighi A, Saber V, Armon B, Seyyedtabaei SJ. The role of *Blastocystis* sp. and *Dientamoeba fragilis* in irritable bowel syndrome: a systematic review and meta-analysis. Parasitol Res 2017; 116: 2361-2371.

148. Puthia MK, Lu J, Tan KS. *Blastocystis ratti* contains cysteine proteases that mediate interleukin-8 response from human intestinal cells in an NF-kappa B-dependent manner. Eukaryot Cell 2008; 7: 435-443.

149. Lepczyńska M, Chen WC, Dzika E. Mysterious chronic urticaria caused by *Blastocystis* spp.? Int J Dermatol 2016; 55: 259-266.

150. Ramírez-Miranda ME, Jiménez-González DE, Rodríguez-Campa ME, González-Angulo A, Hernández-Castellanos R, Sara Arroyo-Escalante A, Romero-Valdovinos M, Martínez-Hernández F, Flisser A, Maravilla P. Irritable bowel syndrome: frequency and phylogenetic relationship of *Blastocystis* sp. from Mexican patients. Rev Gastroenterol Mex 2011; 76: 309-315.

151. Arik Yilmaz E, Karaatmaca B, Sackesen C, Sahiner UM, Cavkaytar O, Sekerel BE, Soyer O. Parasitic infections in children with chronic spontaneous urticaria. Int Arch Allergy Immunol 2016; 171: 130-135.

152. Kolkhir P, Balakirski G, Merk HF, Olisova O, Maurer M. Chronic spontaneous urticaria and internal parasites-a systematic review. Allergy 2016; 71: 308-322.

153. Biedermann T, Hartmann K, Sing A, Przybilla B. Hypersensitivity to non-steroidal anti-inflammatory drugs and chronic urticaria cured by treatment of *Blastocystis hominis* infection. Br J Dermatol 2002; 146: 1113-1114.

154. Gupta R, Parsi K. Chronic urticaria due to *Blastocystis hominis*. Australas J Dermatol 2006; 47: 117-119.

155. Criado PR, Criado RF, Maruta CW, Reis VM. Chronic urticaria in adults: state-of-the-art in the new millennium. An Bras Dermatol 2015; 90:74-89.

156. Panaszek B, Pawłowicz R, Grzegrzółka J, Obojski A. Autoreactive IgE in chronic spontaneous/idiopathic urticaria and basophil/mastocyte priming phenomenon, as a feature of autoimmune nature of the syndrome. Arch Immunol Ther Exp (Warsz) 2016; 65: 137-143.

157. Roberts T, Barratt J, Harkness J, Ellis J, Stak D. Comparison of microscopy, culture, and conventional polymerase chain reaction for detection of *Blastocystis* sp. in clinical stool samples. Am J Trop Med Hyg 2011; 84: 308-312.

158. Chacín-Bonilla L. Diagnóstico microscópico de amibiasis: método obsoleto pero necesario en el mundo en desarrollo. Invest Clin 2011; 52: 291-294.

159. Chacín-Bonilla L, Guanipa N, Cano G, de Young M, Raleigh X, Parra AM. Prevalencia de *Blastocystis hominis* en dos comunidades del Estado Zulia, Venezuela. XLI Convención Anual de ASOVAC. Maracaibo, Venezuela. Acta Cient Venez 1991; 42: 198, 1991.

160. Devera R, Blanco Y, Amaya I, Álvarez E, Rojas J, Tutaya R, Velásquez V. Prevalencia de parásitos intestinales en habitantes de una comunidad rural del estado Bolívar, Venezuela. Kasmera 2014; 42: 22-31.

161. Stensvold CR. Laboratory diagnosis of *Blastocystis* spp. Trop Parasitol 2015; 5: 3-5.

162. Chen JL, Vaudry WL, Kowalewska K, Wenman WM. Lack of serum immune response to *Blastocystis hominis*. Lancet 1987; 2: 1021.

163. Zierdt CH, Zierdt WS, Nagy B. Enzyme-linked immunosorbent assay for detection of serum antibody to *Blastocystis hominis* in symptomatic infections. J Parasitol 1995; 81: 127-129.

164. Kaneda Y, Horiki N, Cheng X, Tachibana H, Tsutsumi Y. Serologic response to *Blastocystis hominis* infection in asymptomatic individuals. Tokai J Exp Clin Med 2000; 25: 51-56.

165. Mohammad NA, Mastuki MF, Al-Mekhlafi HM, Moktar N, Anuar TS. Comparative study of wheatley's trichrome stain and in-vitro culture against PCR assay for the diagnosis of *Blastocystis* sp. in stool samples. Iran J Parasitol 2018; 13: 127-136.

166. Skotarczak B. Genetic diversity and pathogenicity of *Blastocystis*. Ann Agric Environ Med 2018; 25: 411-416.

167. Alarcon-Valdes P, Villalobos G, Martinez-Flores WA, Lopez-Escamilla E, Gonzalez-Arenas NR, Romero-Valdovinos M, Martinez-Hernandez F, Santillan-Benitez JG, Maravilla P. Can the pyruvate: ferredoxin oxidoreductase (PFOR) gene be used as an additional marker to discriminate among *Blastocystis* strains or subtypes? Parasit Vectors 2018; 11: 564.

168. Albrecht H, Stellbrink HJ, Koperski K, Greten H. *Blastocystis hominis* in human immunodeficiency virus-related diarrhea. Scand J Gastroenterol 1995; 30: 909-914.

169. Rajič B, Arapović J, Raguž K, Bošković M, Babić SM, Maslać S. Eradication of *Blastocystis hominis* prevents the development of symptomatic Hashimoto's thyroiditis: a case report. J Infect Dev Ctries 2015; 9: 788-791.

170. Zierdt CH, Swan JC, Hosseini J. *In vitro* response of *Blastocystis hominis* to antiprotozoal drugs. J Protozool 1983; 30: 332-334.

171. Dunn LA, Boreham PF. The *in-vitro* activity of drugs against *Blastocystis hominis*. J Antimicrob Chemother 1991; 27: 507-516.

172. Stenzel DJ and Boreham RE. *Blastocystis*. In: Gillespie S and Pearson RD, editors. Principles and Practice of Clinical Parasitology. Hoboken, New Jersey: John Wiley and Sons 2001. 355-367 p.

173. Batista L, Pérez Jove J, Rosinach M, Gonzalo V, Sainz E, Loras C, Forné M, Esteve M, Fernández-Bañares F. Low efficacy of metronidazole in the eradication of *Blastocystis hominis* in symptomatic patients: Case series and systematic literature review. Gastroenterol Hepatol 2017; 40: 381-387.

174. Idris NS, Dwipoerwantoro PG, Kurniawan A, Said M. Intestinal parasitic infection of immunocompromised children with diarrhoea: clinical profile and therapeutic response. J Infect Dev Ctries 2010; 4: 309-317.

175. Tai WP, Hu PJ, Wu J, Lin XC. Six ulcerative colitis patients with refractory symptoms co-infective with *Blastocystis hominis* in China. Parasitol Res 2011; 108: 1207-1210.

176. Diaz E, Mondragon J, Ramirez E, Bernal R. Epidemiology and control of intestinal parasites with nitazoxanide in children in Mexico. Am J Trop Med Hyg 2003; 68: 384-385.

177. Rossignol JF, Kabil SM, Said M, Samir H, Younis AM. Effect of nitazoxanide in persistent diarrhea and enteritis associated with *Blastocystis hominis*. Clin Gastroenterol Hepatol 2005; 3: 987-991.

178. Roberts T, Bush S, Ellis J, Harkness J, Stark D. *In vitro* antimicrobial susceptibility patterns of *Blastocystis*. Antimicrob Agents Chemother 2015; 59: 4417-4423.

179. Roberts T, Ellis J, Harkness J, Marriott D, Stark D. Treatment failure in patients with chronic *Blastocystis* infection. J Med Microbiol 2014; 63: 252-257.

180. Garavelli PL, Orsi P, Scaglione L. *Blastocystis hominis* infection during AIDS. Lancet 1988; 2: 1364.

181. Wilson KW, Winget D, Wilks S. *Blastocystis hominis* infection: signs and symptoms in patients at Wilford Hall Medical Center. Mil Med 1990; 155: 394-396.

182. Deepika K, Rajkumari N, Liji AS, Parija SC, Hamide A. Multiple parasitic and viral infections in a patient living with HIV/AIDS on antiretroviral therapy. Indian J Med Microbiol 2017; 35: 432-435.

183. Ghimire A, Bhandari S, Tandukar S, Amatya J, Bhandari D, Sherchand JB. Enteric parasitic infection among HIV-infected patients visiting Tribhuvan University Teaching Hospital, Nepal. BMC Res Notes 2016; 9: 204.

184. Piranshahi AR, Tavalla M, Khademvatan S. Genomic analysis of *Blastocystishominis* isolates in patients with HIV-positive using locus SSU-rDNA. J Parasit Dis 2018; 42: 28-33.

185. Chacín-Bonilla L. Las enfermedades parasitarias intestinales como un problema de salud pública global. Invest Clin 2013; 54: 1-4.

186. Mirjalali H, Abbasi MR, Naderi N, Hasani Z, Mirsamadi ES, Stensvold CR, Balaii H, Asadzadeh Aghdaei H, Zali MR. Distribution and phylogenetic analysis of *Blastocystis* sp. subtypes isolated from IBD patients and healthy individuals in Iran. Eur J Clin Microbiol Infect Dis 2017; 36: 2335-2342.

187. Richard RL, Ithoi I, Abd Majid MA, Wan Sulaiman WY, Tan TC, Nissapatorn V, Lim YA. Monitoring of waterborne parasites in

two drinking water treatment plants: a study in Sarawak, Malaysia. Int J Environ Res Public Health 2016; 13: pii E641.171. 174.

188. Chacín-Bonilla L. El problema de las parasitosis intestinales en Venezuela. Invest Clin 1990; 31: 1-2.

189. Chacín-Bonilla L. Geohelmintiasis in Venezuela: Un viejo y grave problema que tiende a persistir. Invest Clin 1985; 26: 1-3.

190. Chacín-Bonilla L, Dikdan Y, Guanipa N, Villalobos R. Prevalencia de *Entamoeba histolytica* y otros parásitos intestinales en un barrio del municipio Mara, estado Zulia, Venezuela. Invest Clin 1990; 31: 3-15.

191. Chacín-Bonilla L, Dikdan Y. Prevalencia de *Entamoeba histolytica* y otros parásitos intestinales en una comunidad suburbana de Maracaibo. Invest Clin 1981; 22: 185-203.

192. Zaki M. Zaman V, Sheikh NA. Resistance of *Blastocystis hominis* cysts to chlorine. J Pak Med Assoc 1996; 46: 178-179.

193. Song JK, Hu RS, Fan XC, Wang SS, Zhang HJ, Zhao GH. Molecular characterization of *Blastocystis* from pigs in Shaanxi province of China. Acta Trop 2017; 173: 130-135.

194. Noradilah SA, Moktar N, Anuar TS, Lee IL, Salleh FM, Manap SNAA, Mohtar NSHM, Azrul SM, Abdullah WO, Nordin A, Abdullah SR. Molecular epidemiology of blastocystosis in Malaysia: does seasonal variation play an important role in determining the distribution and risk factors of *Blastocystis* subtype infections in the Aboriginal community? Parasit Vectors 2017; 10: 360.

195. Kurt Ö, Doğruman-Al F, Tanyüksel M. Eradication of *Blastocystis* in humans: Really necessary for all? Parasitol Int 2016; 65: 797-801.

196. Sekar U, Shanthi M. *Blastocystis*: Consensus of treatment and controversies. Trop Parasitol 2013; 3: 35-39.

197. Wu Z, Mirza H, Teo JD, Tan KS. Strain-dependent induction of human enterocyte apoptosis by *Blastocystis* disrupts epithelial barrier and ZO-1 organization in a caspase 3- and 9-dependent manner. Biomed Res Int 2014; 209163.

198. Stensvold CR, Clark CG. Current status of *Blastocystis*: A personal view. Parasitol Int 2016; 65: 763-771.