

Subtype Distribution of *Blastocystis* in Türkiye

Türkiye’de *Blastocystis* Alt Tip Dağılımı

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ABSTRACT

Blastocystis is an anaerobic protozoan with global importance because of infecting a variety of hosts and having high prevalence in many countries. *Blastocystis* isolates display remarkable genetic differences, and many subtypes (STs) have currently been defined based on polymorphism in *SSU rRNA* coding gene. Each 25 subtype may have different characteristics such as pathogenicity, host specificity, and structural variations. Most current research on *Blastocystis* has focused on these differences and molecular epidemiology. This review aimed to provide a summary of *Blastocystis* subtype distribution in Türkiye. Regarding human samples, 16 manuscripts were found in the literature, which presented 783 *Blastocystis* isolates from 9 cities in Türkiye. The most common subtype was ST3 (47.9%), the others were ST1 30 (17.5%), ST2 (14.7%), ST4 (4%), and ST5-ST7 (15.9%). There were few studies on animal hosts and environmental samples. The faecal samples from rats, farm, and pet animals were examined for *Blastocystis* subtypes and ST1, ST3, ST4-ST7, ST10, and ST12-ST14 were reported. In addition, two studies reported *Blastocystis* ST1 and ST3 subtypes in environmental water samples. In conclusion, the review of available literature showed that a systematic understanding of the subtype distribution of 35 *Blastocystis* in Türkiye is still lacking. Most of the studies were performed in a limited number of cities, animal hosts, and environmental samples, therefore, more studies from different provinces are needed in forthcoming research. The majority studies were performed in a limited number of provinces, animal species and very few environmental samples, so in the future; there is a need of novel studies that evaluate more samples from different provinces

Keywords: *Blastocystis*, subtypes, Türkiye

ÖZ

Blastocystis, farklı konaklarda enfeksiyon oluşturması ve birçok ülkede yaygın görülmesi nedeniyle küresel öneme sahip anaerobik bir protozondur. *Blastocystis* izolatları arasında yüksek derecede genetik farklılıklar gözlenmekte olup *SSU rRNA* gen bölgesindeki polimorfizmlere dayanan birçok alt tipi (ST) tanımlanmıştır. Bu alt tipler patojenite, konak özgülüğü ve yapısal varyasyonlar gibi farklı fenotipik özellikler sergilemektedir. *Blastocystis* ile ilgili güncel araştırmaların çoğu, bu farklılıklara ve moleküler epidemiyolojiye odaklanmıştır. Bu derleme ile Türkiye’deki *Blastocystis* alt tipi dağılımını konu alan çalışmaların özetlenmesi amaçlanmıştır. İnsan örnekleri ile ilgili olarak Türkiye’nin 9 ilinden toplam 783 *Blastocystis* izolatının yer aldığı 16 makale literatürde yer almaktadır. En sık görülen alt tip ST3 (%47,9) olup bunu sırasıyla ST1 (%17,5), ST2 (%14,7), ST4 (%4) ve ST5-ST7 (%15,9) izlemektedir. Ülkemizde hayvanlar ve çevresel örnekler üzerine az sayıda çalışma yapılmıştır. Sıçan, çiftlik ve evcil hayvanlardan alınan dışkı örnekleri *Blastocystis* alt tipleri açısından incelenmiş olup bu araştırmalarda ST1, ST3, ST4-ST7, ST10 ve ST12-ST14 alt tipleri rapor edilmiştir. Ek olarak, iki çalışmada çevresel su örneklerinde *Blastocystis* ST1 ve ST3 alt tipleri bildirmiştir. Sonuç olarak, mevcut literatür incelendiğinde, *Blastocystis*’in Türkiye’deki alt tip dağılımına ilişkin sistematik ve kapsamlı çalışmaların halen eksik olduğu görülmektedir. Çalışmaların çoğu sınırlı sayıda ilde, sınırlı hayvan türünde ve çok az sayıda çevresel örneklerde yapılmıştır, bu nedenle ilerideki araştırmalarda farklı illerden daha fazla örneğin değerlendirileceği çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Blastocystis*, alt tip, Türkiye

INTRODUCTION

Blastocystis is an intestinal, anaerobic protozoan that infects humans and many other species including primates, canids, swine, rodents, birds, reptiles, and cockroaches (1). It has recently been classified

in stramenopiles (Heteroconta) but differs from others because of colonizing the human intestine and not having a flagellated form. It has a global distribution with an independency of climate and many epidemiological studies reported *Blastocystis* as the most common protozoan in human faecal

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samples (2). *Blastocystis* has a frequency rate of up to 100% in underdeveloped countries, because of poor hygiene habits and socio-economic factors (3). The studies in Türkiye revealed a prevalence of *Blastocystis* varying between 1.4% and 23.5% (4). These studies are highly different from each other in terms of diagnostic methods and also the study population.

Although it has been a long-time since it was revealed that *Blastocystis* infects humans, genetic diversity, life cycle, treatment, and pathogenesis of *Blastocystis* have been subjected to considerable discussions in the literature (5-7). It was defined as a pathogen, opportunistic pathogen, or non-pathogenic microorganism. The main problem encountered here was that *Blastocystis* was found in both healthy individuals and symptomatic groups (8-10). *Blastocystis* infections are asymptomatic in a high rate of infected individuals and gastrointestinal symptoms are rarely observed (2,5). It was suggested that *Blastocystis* may be an indicator of healthy intestinal flora (11,12). In addition to non-specific gastrointestinal symptoms such as diarrhoea, abdominal pain, and bloating, dermatological symptoms can be seen in symptomatic *Blastocystis* infections (2,10,13).

Blastocystis has high polymorphic variations in its genome. Following the molecular phylogenetic analysis of *Blastocystis* small subunit ribosomal RNA gene (SSU rRNA), many genotypes or subtypes (STs) have been defined in isolates from humans and non-human hosts (14). Currently, the number of proposed subtypes reached at least to 31; however, some of them are not validated yet and a strong argument is raised (15,16). Recently, many reviews reported *Blastocystis* subtypes in different countries, but not from Türkiye. In this paper, we reviewed the available studies on *Blastocystis* subtypes in humans, animal hosts, and environmental samples.

***Blastocystis* Subtypes in Human Samples**

Türkiye is a transcontinental country that bridges Europe and Asia. It is considered to have suitable conditions for a high prevalence and genetic diversity of *Blastocystis* such as moderate climate, inadequate sanitation in some cities, and migration from other countries. The first available record on *Blastocystis* was published in 1969, a case report (17). There was a large paucity of *Blastocystis* research or papers until the 1990s. Afterwards, studies about the frequencies in renal transplant patients and the treatment of *Blastocystis* were published (18,19). Because of unfamiliar findings on *Blastocystis* pathogenicity, most studies have focused on the clinical properties of *Blastocystis*-infected people. Research on the subject has been mostly limited to the comparisons of patient and control groups before the identification of *Blastocystis* subtypes. The possible clinical consequences of *Blastocystis* infection were studied in different patient groups such as iron deficient, cancer, haemodialysis, irritable bowel syndrome, and inflammatory bowel disease patients (20-23).

Among the *Blastocystis* subtypes, ST1 to ST9 and ST12 have been isolated from human samples (14). The common subtypes in humans are ST1 to ST4, in addition, ST9 has not been reported other than in human samples (16). The studies on *Blastocystis* subtypes in different provinces of Türkiye mostly revealed similar findings in terms of subtype distribution; however, some differences were reported in terms of the number of subtypes. More recently, ST1-

7 has been found in human samples in Türkiye. Most of them revealed the predominance of ST3, followed by either ST2 or ST1. In general, Europeans are mostly colonized by ST1-ST4, in contrast, ST4 is rarely found in other territories including Africa, America, and the Middle East (24). The studies from Türkiye used two methods to identify *Blastocystis* subtypes: Partial sequencing of SSU rDNA coding gene also known as barcoding and sequence-tagged-sites polymerase chain reaction (PCR) (STS PCR). The sequencing of amplified PCR products allows to determine allele differences and intra-subtype genetic variation of *Blastocystis*. In addition, with this method the comparison of other sequences from Genbank or other sources is possible. The detailed data on *Blastocystis* subtypes in human samples is presented in Table 1. *Blastocystis* subtypes from the following cities: Adana, Ankara, Aydın, Erzincan, Eskişehir, İstanbul, İzmir, Manisa, and Muğla. Most studies have been conducted in the Aegean region of Anatolia. Currently, we have no data for human samples in eastern Türkiye. The data from other regions are also very limited, for example, there are only two studies from south of Türkiye, the Mediterranean Region.

***Blastocystis* Subtypes in Animal Samples**

Although relatively most research has been carried out on humans, a very limited number of studies exist that detected *Blastocystis* subtypes in animal samples. The animal hosts of *Blastocystis*, which are previously studied in Türkiye for *Blastocystis* subtypes, are livestock animals, pet animals, and laboratory rats (38-43). The details of these studies are given in Table 2. Host specificity and zoonotic potential of *Blastocystis* have been shown in these studies. The prevalence of *Blastocystis* in animal samples is an important topic for humans, because of the zoonotic potential of *Blastocystis*. It was reported that identified STs in humans are also found in domestic and wild animals (44). Many subtypes including ST1-ST7, ST10, ST12, and ST14 have been reported in cattle samples with higher frequencies of ST10 and ST14 (45,46). Similar to the studies in Türkiye, ST10 is the predominant *Blastocystis* subtype in cattle samples in several countries including the USA (47), China (48,49), the UK, Thailand (50), and Lebanon (51). A study in Türkiye reported that all laboratory animals were infected with ST4, this finding was also consistent with the others that showed the predominance of ST4 in rodents (39,52). A meta-analysis of *Blastocystis* ST found in the cats showed that ST4 (29.5%) was the most common, which was followed by ST10 (22.5%), ST1 (19.8%), and, ST3 (17.6%) (53). In accordance with this data, a study in Türkiye reported that all *Blastocystis* isolates in stray cats were ST4 (38). Finally, birds can be infected with zoonotic subtypes including ST1, ST2, and ST4-ST8, among them ST6 and ST7 are the most common (14,54). The predominant subtypes were ST6 and ST7 in chicken faecal samples in Diyarbakır (42). However, it should be noted that the study groups in animal studies in Türkiye were limited to specific animal groups mostly cattle, sheep, and chicken. *Blastocystis* subtypes in Türkiye are presented in Figure 1.

***Blastocystis* Subtypes in Environmental Samples**

The previous studies reported the presence of *Blastocystis* in water samples, including rivers, recreational waters, and lakes (55,56).

Table 1. *Blastocystis* subtypes in human samples in Türkiye

Province	Study population	Method	ST1	ST2	ST3	Other STs	Ref.
			n (%)	n (%)	n (%)	n (%)	
Manisa	Diagnostic parasitology lab. (n=92)	STS-PCR	17 (18.4)	20 (21.7)	51 (55.4)	ST1+ST3: 2 (2.1) ST2+ST3: 2 (2.1)	(25)
İstanbul	Diagnostic parasitology lab. (n=87)	Sequencing*	8 (9.2)	12 (13.7)	66 (75.8)	ST4: 1 (1.1)	(26)
Ankara	IBD, Chron and diaphoretic patients (n=35)	STS-PCR	1 (2.8)	10 (28.6)	21 (60)	ST2:+ST1: 2 (5.7) ST2+ST3: 1 (2.8)	(20)
Adana	Symptomatic and non-symptomatic cases (n=32)	STS-PCR	20 (62.5)	3 (9.3)	9 (28.1)		(27)
İzmir	Symptomatic patients (n=94)	RT-PCR	13 (13.8)	11 (11.7)	42 (44.7)	ST6: 1 (1.1) ST7 1 (1.1) ST2+ST3: 2 (2.1) ND: 24 (25.5)	(28)
Aydın	Diagnostic parasitology lab. (n=61)	STS PCR	9 (20.5)	13 (29.5)	17 (38.6)	ST1+ST3: 4 (9.1) ST2+ST3: 1 (2.3) ND: 17 (27.9)	(29)
Aydın	Ulcerative colitis patients (n=12)	Sequencing*	2 (16.7)	1 (8.3)	8 (66.7)	ST7: 1 (8.3)	(30)
Aydın	Cancer patients (n=25)	STS PCR	5 (23)	4 (18)	13 (59)	ND: 3 (12)	(31)
İstanbul	Diagnostic parasitology lab. (n=50)	Sequencing*	3 (6)	2 (4)	34 (68)	ST1+ST3: 1 (2) ST2+ST3: 1 (2) ST3+ST5: 3 (6) ST4+ST5: 1 (2) ST1+ND: 1 (2) ST3+ND: 4 (8)	(32)
Muğla	Children (n=35)	Sequencing*	11 (31.4)	9 (25.7)	12 (34.2)	ST7: 1 (2.8)	(33)
Eskişehir	Children (n=46)	RT-PCR	12 (26.1)	4 (8.7)	20 (43.4)	ST4: 5 (10.9) ST1+ST3: 3 (6.5) ST1+ST2: 1 (2.1) ST2+ST3: 1 (2.1)	(34)
Aydın	Diagnostic parasitology lab. (n=95)	Sequencing* and STS PCR	17 (17.9)	21 (22.1)	50 (52.6)	ST7: 4 (4.2) ST2+ST3: 2 (2.1) ST1+ST3: 1 (1.1)	(4)
Erzincan	Urticarial patients and control group (n=18)	STS PCR	4 (22.2)	1 (5.5)	13 (72.2)		(35)
Muğla	Pregnant women (n=14)	Sequencing*	3 (21.4)	2 (14.2)	9 (64.4)		(36)
Adana	Haemodialysis patients and control group (n=44)	STS PCR	1 (12.5)	2 (25)	2 (25)	ST3+ST6: 2 (25) ND:1 (12.5)	(21)
İzmir	Cancer patients (n=43)	Sequencing*	11 (25.6)		8 (18.6)	ST4: 24 (55.8)	(37)

STS PCR: Sequence-tagged-sites PCR, RT-PCR: Real-time PCR, ND: Not detected, * Sanger sequencing, PCR: Polymerase chain reaction

Table 2. <i>Blastocystis</i> subtypes in animal samples in Türkiye				
Province	Animal	Subtypes n (100)	Method	Ref
Aydın	Cattle (n=9)	ST14: 7 (77.7)	Sequencing*	(43)
		ST10: 2 (22.3)		
İzmir	Stray cats (n=7)	ST 4: 7 (100)	Sequencing*	(38)
Kayseri	Cattle (n=32)	ST10: 32 (100)	Sequencing*	(40)
	Sheep (n=84)	ST10: 84 (100)		
	Chicken (n=32)	ST7: 32 (100)		
	Water buffaloes (n=35)	ST14: 35 (100)		
Aydın	Laboratory rats (n=33)	ST4: 33 (100)	Sequencing*	(39)
Kayseri	Cattle (n=88)	ST10: 88 (100)	Sequencing*	(41)
Diyarbakır	Sheep (n=4)	ST5: 4 (100)	Sequencing* and STS PCR	(42)
	Chicken (n=18)	ST6: 6 (33.3%) ST7: 11 (61.1%) ST6 and ST7: 1 (5.5%)		
İzmir	Cattle (n=13)	ST1, ST3, ST13: 1 (7.7%) ST5, ST12: 2 (15.4%) ST10: 6 (46.1%)	Sequencing**	(55)

*Sanger sequencing, ** Not available from the manuscript

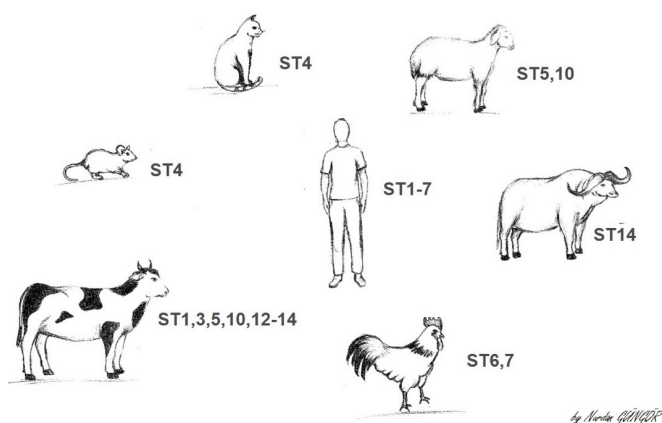


Figure 1. *Blastocystis* subtypes in human and animal hosts in Türkiye. The illustration was originally created by Nurdan Güngör

Lack of appropriate filtration or piped water supplies may be a significant risk factor for the spread of *Blastocystis* infection (57,58). In addition, the consumption of raw or untreated water sources in the environment may be associated with the infection (59,60). Currently, two studies in Türkiye investigated *Blastocystis* subtypes in environmental samples. The first study investigated the *Blastocystis* subtypes in different water samples from Middle Black Sea Region (Sinop, Amasya, and Samsun). They defined subtypes in ten samples and found an equal distribution of ST1 and ST3 (61). The subtypes in this study were as follows: Sinop (ST1, one seawater sample), Samsun (ST1 and ST3, two and one river samples), and Amasya (ST1 and ST3, two and four river samples). Another study in the same region included only river samples and subtyped four *Blastocystis* isolates. They reported that three were ST1 and one was ST3 (62).

CONCLUSION

In different provinces, *Blastocystis* subtypes were investigated in limited numbers and sample sizes. More data is required to have better information on the *Blastocystis* molecular epidemiology in Türkiye. In addition, there is a need for studies dealing with *Blastocystis* subtypes in animal samples, especially wild animal hosts.

* Ethics

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* Authorship Contributions

Concept: E.M., H.E., S.E., Design: E.M., H.E., S.E., Data Collection or Processing: E.M., Analysis or Interpretation: E.M., H.E., S.E., Literature Search: E.M., H.E., S.E., Writing: E.M.

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