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Original Article

Blastocystis infections in Egypt: An update in cattle from Dakahlia governorate and data meta-analysis

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Abstract

Blastocystis sp. is the most prevalent protozoan parasite in humans and affects a broad range of animals. *Blastocystis* infections are prevalent in humans in Egypt however, there is limited information available on animals. The current study aimed at updating the prevalence in cattle and assessing the prevalence and distribution of *Blastocystis* sp. infections and subtypes in different hosts in Egypt. Faecal samples were collected from 100 cattle as well as faecal contents of intestines of 50 ducks and 90 domestic pigeons. Samples were examined using SSU rRNA gene PCR and sequencing. Studies conducted on humans, domestic animals, and birds in Egypt were reviewed and the random effects models was used to determine the pooled prevalence of infection in humans based on diagnostic methods. One cattle faecal sample was positive (1%), while none of examined pigeons or ducks were infected. ST3 was the identified subtype in the positive isolate which is phylogenetically related to other isolates from humans and animals from different countries. A total of 26 studies on human *Blastocystis* infections in Egypt were used for meta-analysis, resulting in a pooled prevalence of 67.9 %, 47.3%, and 33.3%, based on PCR, culture, and microscopy, respectively. On contrary, limited studies (n = 7) were conducted on diverse animal species. The reported subtypes in humans in Egypt were ST1 - ST4, ST7, ST10, and ST14. Likewise, ST1 – ST7, ST10, and ST14 were reported in animals, and ST2 in water samples from Egypt. This study emphasizes the importance of addressing *Blastocystis* infections in Egypt from a public health and zoonotic perspective in terms of proper diagnosis and control by providing essential data on infections in humans and animals based on current and published data.

Keywords:

Blastocystis, Subtypes, Prevalence, Cattle, Pigeons, Ducks, Egypt

Introduction

Blastocystis sp. is one of the most common intestinal parasites found in the human population worldwide and is considered a persistent component of the gut microbiota (**Andersen and Stensvold, 2016, Stensvold and Clark, 2016**). The most likely mechanism of transmission is the fecal-oral route, involving direct human-to-human contact and the ingestion of water contaminated with environmentally resistant cystic forms produced by both humans and animals (**Tan, 2008, Lee *et al.,* 2012, Parija and Jeremiah, 2013, Roberts *et al.,* 2014**). The high frequency of *Blastocystis* sp. in developing countries is directly linked to poor sanitary and hygiene conditions (**Tan, 2008)**. *Blastocystis* sp. can infect a wide variety of species, including mammals, birds, and amphibians (**Wawrzyniak *et al.,* 2013**). While human infections are sometimes associated with nonspecific gastrointestinal symptoms such as diarrhea, abdominal pain, nausea, and vomiting (**Tan, 2008**, **Roberts *et al.,* 2014**, **Tan *et al.,* 2010**), animal infections are rarely linked to disease (**Hublin *et al.,* 2021**). The pathogenicity of *Blastocystis* is unclear and may vary depending on the parasite subtype (ST) and the patient's immune status (**Tan, 2008, Roberts *et al.,* 2014, Tan *et al.,* 2010, Elwakil and Hewedi, 2010**). *Blastocystis* sp. is also thought to be associated with irritable bowel syndrome (IBS) (**Tan *et al.,* 2010, Poirier *et al.,* 2012**).

The small subunit ribosomal ribonucleic acid gene (SSU rRNA) is often used for diagnosis and subtyping and has been found to have a wide genetic diversity in the genus *Blastocystis* (**Wawrzyniak *et al.,* 2013, Noël *et al.,* 2005, Stensvold *et al.,* 2009, Alfellani *et al.,* 2013**). There are total of 46 genetically distinct lineages known as subtypes (STs) that have been proposed so far (**Wawrzyniak *et al.,* 2013, Alfellani *et al.,* 2013, Stensvold *et al.,* 2012 Maloney *et al.,* 2023, Santin *et al.,* 2024, Koehler *et al.,* 2024**). Some of the recently proposed subtypes, such as STs 18–20 and ST22, are considered invalid (**Stensvold *et al.,* 2020**). ST1–ST4 are the most prevalent of the 14 subtypes observed in humans, while ST5–ST10, ST12, ST14, ST16, and ST23 are rather uncommon to rare (**Maloney *et al.,* 2023**).

Many *Blastocystis* isolates obtained from different animals and birds are assigned to the same STs seen in humans, implying zoonotic potential, however, the impact of animal sources on infection in humans is still to be proven (**Alfellani *et al.,* 2013**). Twenty subtypes (ST1–ST7, ST10, ST12–ST14, ST17, ST21, ST23–26, ST32, ST42, and ST44) have been found in cattle, and the incidence varies from 1.8% to 100% globally (**Hublin *et al.,* 2021, Shams *et al.,* 2021, Figueiredo *et al.,* 2024**). *Blastocystis* infections in Egypt are still poorly understood due to a lack of studies, despite their relevance to public health. Considerable number of reports have focused on detection and subtyping of this protist in humans, while relatively few studies have been conducted to detect *Blastocystis* in animals. The objective of the present study was to

determine the occurrence of *Blastocystis* in cattle, pigeons, and ducsks from Dakahlia governorate, Egypt. Furthermore, this study aimed to provide a comprehensive overview for the epidemiology, distribution and various subtypes of *Blastocystis* infecting animals and humans in Egypt.

Materials and methods

1. Ethical considerations

This study was approved by Mansoura University Animal Care and Use Committee (MU-ACUC) according to the ethical principles of animal research, with approval code number: VM.R.24.05.167. No samples were collected from human subjects.

2. Samples collection and parasitological examination

From May to October 2023, rectal faecal samples were collected from 100 cattle calves (3-6 months old) in Dakahlia governorate, Egypt. All sampled cattle were raised in small flocks (10–20 animals/flock) kept in households in rural areas of the governorate. Additionally, intestines of 90 domestic pigeons and 50 ducks were purchased from local markets in Dakahlia. Approximately 5 grams of each faecal sample were crushed, mixed thoroughly with distilled water to form a uniform suspension, and then immediately filtered through two layers of gauze. After that, 14 ml of the suspension was decanted into a centrifuge tube and spun for 10 minutes. The faecal sediments were then washed in PBS by centrifugation (3000 rpm / 5 min), the supernatant was removed, and the sediment was collected in Eppendorf tubes and stored at -20 °C for DNA extraction. DNA extraction was performed using the QIAamp DNA Stool Mini Kit following the manufacturer's protocol.

3. Molecular analysis

Amplification of a fragment (~479 bp) of the SSU rRNA gene was obtained by using the forward primer 5′-GGAGGTAGTGACAATAAATC-3′ and the reverse primer 5′-TGCTTTCGCACTTGTTCATC-3′ [22]. The reactions were carried out in 25 µl final volumes, including 1 µl of each forward and reverse primer, 12.5 µl of PCR green master mix (Emerald Amp GT, Takara, Japan), 3 µl of template DNA, and 7.5 µl of nuclease-free water. The PCR conditions consisted of an initial denaturation step at 94°C for 4 minutes, followed by 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. The amplified PCR products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV fluorescence using a gel documentation system (Bio-Rad). The DNA from the positive PCR product was purified using the DNA Clean & Concentrator®-25 (Zymo Research, USA) and sent for commercial sequencing. The purified product was sequenced in both directions using the same PCR primers with the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems) and the ABI 3730xl DNA sequencer. The nucleotide sequences obtained were aligned and trimmed from both ends using BioEdit and then identified to the subtype level using the National Centre for Biotechnology's (NCBI) BLAST search

(http://www.ncbi.nlm.nih.gov). A phylogenetic tree of SSU rRNA gene sequences of *Blastocystis* spp. was constructed using the neighbor-joining

(NJ) method implemented in MEGA6, and The Jukes and Cantor model was employed.

4. Data collection and analysis

To investigate different patterns of epidemiology and molecular types of *Blastocystis* in Egypt, we conducted electronic searches using various databases such as PubMed, Scopus, Web of Science, and Google Scholar to compile all published data on this protozoon in Egypt. We used the keywords "*Blastocystis*," "humans," "animals," "cattle," "birds," "water," and "Egypt," combined with Boolean operators "AND" and "OR." Additionally, we searched the Egyptian Knowledge Bank's website (http://www.ekb.eg) for relevant reports in local Egyptian journals. Information including the study region, sampling area, number of tested samples, number of test-positive samples, detection technique, and identified subtypes were extracted and tabulated. The gathered data in humans infections were then used for meta-analysis using the OpenMeta[Analyst] software. Pooled estimates were calculated using the random-effects model and the DerSimonian-Laird method, with all estimates determined at a 95% confidence interval.

Results and Discussion

Out of the cattle examined, only one fecal sample tested positive (1%). Meanwhile, all the intestinal contents of the examined pigeons and ducks tested negative. The positive isolate from the calf was successfully sequenced, and the representative nucleotide sequence was deposited in the GenBank database under accession number PP938843. The isolate showed 100% identity with *Blastocystis* subtype 3 (ST3) isolated from humans and animals in other countries. The phylogenetic analysis revealed that this study's isolate clustered with isolates from humans, horses, pigs, and cattle in Mexico, the Philippines, Turkey, Colombia, Spain, Germany, Japan, South Korea, Peru, and Iran (**Fig. 1**). This indicates the possibility of zoonotic transmission through water contamination or direct contact with feces by animal owners and in contact persons.

Seven earlier studies investigated the prevalence in different animals in Egypt including chicken, ducks, geese, turkeys, pigeons, rabbits, cattle, donkeys, horses, camels, goats, sheep, dogs, and cats (**Sayed *et al.,* 2015, Mokhtar and Youssef, 2018, Abdo *et al.,* 2021, Naguib *et al.,* 2022, Shehab *et al.,* 2021, Elmahallawy *et al.,* 2023, Guyard-Nicodème *et al.,* 2023**) (**Table 1**). Three studies (**Naguib *et al.,* 2022, Mokhtar and Youssef, 2018, Abdo *et al.,* 2021**) estimated the prevalence in cattle from 4 governorates, Dakahlia (27/172, 15.7%), Damietta (4/86, 4.7%), Ismailia (13/18, 72.2%), and Kafr El-Sheikh (8.7-16.3%) based on PCR, culture and microscopy. The lower prevalence in the studied cattle in comparison with earlier reports from Egypt may be attributed to the small sample size, and limited geographic area. However, lower infection rates were also identified in cattle from USA

(73/2539, 2.9%, **Maloney *et al.*, 2019**). It was suggested that age is likely affecting prevalence of this parasite where younger animals showed lower infection rate compared to older ones (**Maloney *et al.*, 2019, Santin *et al.*, 2023**). The subtype ST3 was previously identified in cattle from Kafr El-Sheikh (**Naguib *et al.*, 2022**) and Ismailia (**Mokhtar and Youssef, 2018**). The other detected subtypes from cattle in Egypt were ST4, ST10, ST14 based on sequencing, and ST1 and ST5 based on ST-specific PCR for subtyping.

Chicken were investigated in various governorates (**Sayed *et al.*, 2015, Naguib *et al.*, 2022**, **Mokhtar and Youssef, 2018**, **Guyard-Nicodème *et al.*, 2023**) and a prevalence range of 11.4 - 82.5 % was reported, and ST1, ST6, ST7, ST14 subtypes were identified. Ducks (100%), geese (25%), turkeys (50%), pigeons (46.7%), and rabbits (0%) were studied in one report in Ismailia (**Mokhtar and Youssef, 2018**), and ST1, ST2, ST6, ST7 subtypes were reported. However, pigeons and ducks investigated in this study were free from infection based on SSU rRNA-PCR. Likewise, *Blastocystis* infection was not found in ducks and pigeons from Poland (**Lewicki *et al.*, 2016**) and in pigeons from Iran (**Rostami *et al.*, 2020**). On the other hand, infections in donkeys (1/14, 7.1%), camels (5/20, 25%), and goats (4/14, 28.6%) were reported (**Mokhtar and Youssef, 2018**), and ST1 was reported in three animal species while ST4 was reported in one goat. The prevalence rate was lower in investigated cats (0 - 8%) in Egypt (**Naguib *et al.*, 2022, Mokhtar and Youssef, 2018, Elmahallawy *et al.*, 2023**), meanwhile, there was no evidence of infection in dogs, horses, and sheep up to date.

Water samples of a variety environments, such as Nile river, ground, waste, and tap water were collected from three governorates to estimate the prevalence (**Elshazly *et al.*, 2007, Khalifa *et al.*, 2014, Abd Ellatif *et al.*, 2018, Elseadawy *et al.*, 2023**)
including Dakahlia (0.98%, 2.1%), El-Minia (15.87%) and El-Behera (2.5%) (**Table 2**). One recent study reported the subtype ST2 from water samples in Dakahlia governorate (**Elseadawy *et al.*, 2023**). Although the parasite has been linked to contaminated water, only a few studies have shown that water acts as a route of *Blastocystis* transmission by considering both water and human hosts (**Jinatham *et al.*, 2022**). Nonetheless, the ingestion of water contaminated with environmentally resistant cystic forms produced by both people and animals can be direct source of human infections (**Tan, 2008, Lee *et al.*, 2012, Parija and Jeremiah, 2013, Roberts *et al.*, 2014**).

On the other hand, a total of 27 studies (**Shehab *et al.*, 2021, Abdo *et al.*, 2021, Mokhtar and Youssef, 2018**, **Eassa *et al.*, 2016, EL-Sayad *et al.*, 2019, Salem *et al.*, 2019, El-Taweel *et al.*, 2020, Mossallam *et al.*, 2021, Naguib *et al.*, 2023, El-Badry *et al.*, 2018, Hamdy *et al.*, 2020, Ibrahim *et al.*, 2020, Ali *et al.*, 2022, Souppart *et al.*, 2010, Hassan *et al.*, 2016, El Saftawy *et al.*, 2019, Ahmad and Abdelhameed, 2023, Ibrahim *et al.*, 2024, El-Sayed and Abdel-Wahab, 2011, Mohamed and Khalil, 2023,**

Ahmed *et al.*, 2022, El Saftawy *et al.*, 2023, Gabr *et al.*, 2018, Ahmed *et al.*, 2021, Elnazer *et al.*, 2017, El-Nadi *et al.*, 2017, El-Hady *et al.*, 2018, Zanetti *et al.*, 2020, Javanmard *et al.*, 2018, Kumarasamy *et al.*, 2023, Popruk *et al.*, 2021, Mohamed *et al.*, 2017, Nemati *et al.*, 2021, Jiménez *et al.*, 2022) were conducted in different governorates to estimate the prevalence of *Blastocystis* in humans in Egypt (**Table 3**). Of which 26 studies were used for meta-analysis and resulting in 49 data sets. One study was considered not eligible for meta-analysis as the total number of examined patients were not stated (**Souppart *et al.*, 2010**). Different diagnostic methods were used to estimated the prevalence including microscopy based on direct wet mount and/or trichrome and iodine stained smears, culture and PCR either conventional or quantitive followed by sequencing in some reports. In total, 13 data sets have detected *Blastocystis* DNA in stool samples of 985 humans out of 1588 examined in Egypt, and resulting in a pooled prevalence of 67.9 % (95% CI, 51.7 - 84.1%). This prevalence was considerably higher than that estimated for 1557 humans diagnosed using culturing method in 12 datasets, where, 701 were found positive with a pooled prevalence of 47.3% (95% CI, 33.3 - 61.3%). On the contrary, 23 data sets have screened infection in 4020 humans using microscopy and a comparatively lower pooled prevalence of 33.3 % (95% CI, 26.6 – 39.9%) was detected (**Fig. 2**). However, reports used PCR were either used to screen samples for infection or diagnosis confirmation of positive samples with either microscopy or culture. The PCR was used for screening 1196 samples and 752 were found positive resulting in a similar pooled prevalence of 67.9% (95% CI, 45.9 – 90.0%) (**Fig. 3**).

The pooled prevalence was higher than that reported in some countries such as Brazil (**Zanetti *et al.*, 2020**), Iran (**Javanmard *et al.*, 2018**), and Malaysia (**Kumarasamy *et al.*, 2023**). The high prevalence of human infections in Egypt is concerning and may be linked to poor hygiene, close animals contact, and the consumption of contaminated food or water. Additionally, infection was prevalent in samples obtained from patients with irritable bowel syndrome and those experiencing gastrointestinal symptoms, including diarrhea, abdominal pain, nausea, vomiting, and flatulence (see **Table 3**). Several variables impact the occurrence of *Blastocystis* infection, including the immune system, geographical region, age, and host behaviour (**Popruk *et al.*, 2021**). The reported subtypes in humans in Egypt were ST1 (14 reports), ST2 (13 reports), ST3 (14 reports), ST4 (2 reports), ST14 (3 reports), ST7 (1 report), and ST10 (1 report). All of these subtypes were recorded in animals in Egypt. The subtypes, ST1–ST4, comprise 91.65% of all STs identified in humans globally (**Popruk *et al.*, 2021**). Subtype 3 (ST3) has been extensively documented in global epidemiological studies including human and animal subjects, and it has been proposed that it may be connected to certain clinical symptoms like urticaria and gastrointestinal illnesses (**Popruk *et al.*, 2021, Mohamed *et al.*, 2017,**

Nemati *et al.*, 2021, Jiménez *et al.*, 2022). Mixed and diverse subtypes were reported in humans and animals in Egypt however, their impact on pathogenicity remains unclear.

Conclusions

This study looked into *Blastocystis* infections in cattle, ducks, and domesticated pigeons. Nonetheless, a low infection rate was found in cattle and no infections were detected in ducks and pigeons. All published articles on *Blastocystis* infections in animals, humans, and water samples were reviewed to provide comprehensive information on the infections in the Egyptian population, contact animals, and environmental samples. The study has limitations due to the small number of samples studied animals and restricted geographical areas. Therefore, conducting large-scale epidemiological studies in different governorates on various animal species is recommended to understand the infection dynamics in animals and to clarify the different routes of transmission for human infections. It is necessary to raise public health awareness in Egypt about maintaining personal hygiene and avoiding close contact with animals.

Author contributions

Conceptualization: RE, SS, Sample collection: SE, SS, RE, Experiments, formal analysis, investigation: RE, SS, EE, Literature search: RE, SS, EE, Writing—review and editing: all authors. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Table 1. Prevalence of *Blastocystis* sp. and subtypes identified in animals in Egypt

Governorate	Host	Age range	No. tested	No. positive (%)	Diagnostic method	STs	References
Assuit	Chicken	NS	200	61 (30.5)	Microscopy	-	Sayed et al. 2015
Cairo	Dogs Cats	1 M - 5 Y 1 M - 4 Y	60 32	0 (0) 0 (0)	qPCR- seq.	- -	Naguib et al. 2022
Dakahlia	Cattle Dogs Cats Chicken	> 3 – >6 M 1 M – 5 Y 1 M - 4 Y NS	172 50 57 88	27 (15.7) 0 (0) 1 (1.7) 10 (11.4)	qPCR- seq.	ST10, ST14, MI - ST14 (1) ST7 (10)	Naguib et al. 2022
Damietta	Cattle Chicken	> 3 – >6 M NS	86 47	4 (4.7) 11 (23.4)	qPCR- seq.	ST10, ST14, MI ST7, ST14	Naguib et al. 2022
Gharbia	buffaloes, cows, sheep and goats	N.S.	165	36 (21.8)	Microscopy	-	Shehab et al. 2021
	Dogs Cats Chicken	1 M – 5 Y 1 M - 4 Y NS	34 41 42	0 (0) 1 (2.4) 10 (23.8)	qPCR- seq.	- ST14 ST7	Naguib et al. 2022
Giza	Cats	1 M – 4 Y	25	2 (8.0)	qPCR- seq.	ST3, ST14	Naguib et al. 2022
Kafr El-Sheikh	Cattle	> 24 M	190 31	31 (16.3) 26 (13.7) NS	Culture Microscopy PCR-seq.	 ST14, ST4, ST10	Abdo et al. 2021
	Cattle Chicken	> 3 – >6 M NS	115 40	10 (8.7) 6 (15.0)	qPCR- seq.	ST3, ST4, ST10, ST14, MI ST7	Naguib et al. 2022
Ismailia	Chicken Ducks Geese Turkeys Pigeons Cattle Donkeys Horses Camels Rabbits Goats Sheep Dogs Cats	N.S.	57 25 20 12 15 18 14 15 20 12 14 8 21 8	47 (82.5) 25 (100) 5 (25) 6 (50) 7 (46.7) 13 (72.2) 1 (7.1) 0 (0) 5 (25) 0 (0) 4 (28.6) 0 (0) 0 (0) 0 (0)	Culture PCR using ST-specific primers for subtyping	ST1, ST6, ST7 ST1, ST7 ST1, ST2, ST6, ST7 ST1, ST6, ST7 ST6, ST7 ST1, ST3, ST5 ST1 - ST1 - ST1, ST4 - - -	Mokhtar and Youssef 2018
Dakahlia, Damietta, Kafr El Sheikh, and Gharbia	Chicken		214	39 (18.2)	qPCR		Guyard-Nicodème et al. 2023
Dakahlia, Gharbeya, and Giza	Dogs Cats		218 134	0 0	PCR	-	Elmahallawy et al. 2023

Abbreviation: **NS**, Not stated; **Seq**; Sequencing; **M**, month old; **Y**, year old.

Table 2. Prevalence of *Blastocystis* sp. and subtypes identified in water samples from Egypt.

Governorate	Water type	Diagnostic method	No. samples	No. positive (%)	Subtype	References
Dakahlia	Potable water tanks and river Nile	Microscopy	1320	13 (0.98)	NS	Elshazly et al. 2007
El-Minia	River Nile, waterworks, tap water, water pumps, water tanks, ponds and canal water	Microscopy	336	20 (15.87)	NS	Khalifa et al. 2014
El-Behera	Drinking water	Microscopy	40	1 (2.5)	NS	Abd Ellatif et al. 2018
Dakahlia	Waste water and ground water	Microscopy, and PCR-seq.	190	4 (2.1)	ST2	Elseadawy et al. 2023

NS: Not stated; **Seq**: sequencing.

Table 3. Prevalence of *Blastocystis* sp. and subtypes identified in humans in Egypt

Governorate*	Age range	Clinical conditions	No. tested	No. positive (%)	Diagnostic method	STs	References
Alexanderia	2-14 Y	ALL IC	55 46	30 (54.5) 31 (67.4)	Microscopy	-	Eassa et al. 2016
	NS	NS	100	52 (52) 65 (65) 67 (67)	Microscopy Culture PCR	-	El-Sayed et al. 2019
	Variable	IBS GI Healthy	40 40 40	18 (45) 8 (20) 4 (10)	Microscopy	-	Salem et al. 2019
	5 - 55 Y	Total GI Healthy	100 47	63 (63) 39 (83) 8 (17) 27 (57.6)	Microscopy RFLP PCR - seq.	 ST1, ST2, ST3, ST4	El-Taweel et al. 2020
	NS	GI	120 65 58	65 (54.17) 58 (89.2) 47 (81.03)	Microscopy Culture PCR - seq.	 ST1, ST2, ST3	Mossallam et al. 2021
	1 M – 70 Y	Variable	86	86 (100)	qPCR - seq.	ST1, ST2, ST3, MI	Naguib et al. 2023
Beheira	1 M – 70 Y	Variable	202	154 (76.2)	qPCR - seq.	ST1, ST2, ST3, ST14, MI	Naguib et al. 2023
Beni-Suef	13 - 60 Y	IBS	115	19 (16.5) 22 (19.1)	Microscopy Culture, PCR - seq	 ST1, ST3	El-Badry et al. 2018
	2–12 Y	GI	125	58 (46.4) 67 (53.6)	Microscopy Culture	-	Hamdy et al. 2020

	≥ 20 Y	IBS diabetes IBS	100 100 100	65 (65) 87 (87) 25 (25) 42 (42)	Microscopy Culture Microscopy Culture	-	Ibrahim et al. 2020
	23 -79 Y	CRC Healthy	100 100 94	52 (52) 42 (42) 80 (85.1)	Culture PCR and seq.	 ST1, ST2, ST3, ST7	Ali et al. 2022
Cairo	8 to 45 Y	GI	20	20 (100)	Microscopy, PCR-seq	ST1, ST2, ST3	Souppart et al. 2010
	-	GI	50	18 (36)	Culture	-	Hassan et al. 2016
	5.5 Y 8.5 Y	Asth IBS Urticaria Non-Asth IBS Urticaria	150 65 65 150 35 35	65 (43.3) 55 (84.6) 10 (15.4) 35 (23.3) 25 (71.4) 3 (8.6)	Microscopy, PCR-RFLP	ST3, ST4	El Saftawy et al. 2019
	1 M – 70 Y	GI	93	67 (72)	qPCR and seq.	ST1, ST2, ST3, ST14, MI	Naguib et al. 2023
	31.3 ± 17.9 Y	GI	314	89 (28.3) 93 (29.6)	Microscopy PCR	-	Ibrahim et al. 2024
Dakahlia	1 M-70 Y	Variable	301	231 (76.7)	qPCR and seq.	ST1, ST2, ST3, ST10, ST14, MI	Naguib et al. 2023
El-Sharkyia	NS	NS	170	51 (30) 34 (20) 45 (26.5)	Culture Trichrome stain ELISA	-	El-Sayed and Abdel-Wahab 2011
Fayoum	10-19 Y	GI	110 21	21 (19.1) 16 (76.1)	Microscopy PCR and seq.	 ST1, ST2, ST3	Mohamed and Khalil 2023
Gharbia	2-83 Y	NS	300	93 (31)	Microscopy	-	Shehab et al. 2021
Ismailia	NS	NS	157	56 (35.7)	Culture and PCR	ST1, ST2, ST3, ST4, MI	Mokhtar and Youssef 2018
	0.6 -85 Y	 GI Healthy	520 15	80 (15.3) 25 (31.3) 55 (68.75) 11 (73.3)	Microscopy PCR and seq.	 - ST1, ST2, ST3	Ahmed et al. 2022
	1 M – 70 Y	Variable	143	59 (41.3)	qPCR and seq.	ST1, ST2, ST3, MI	Naguib et al. 2023
Kafr El-Sheikh	3–77 Y	-	136	40 (29.4) 36 (26.5) -	Culture Microscopy PCR and seq.	 ST1, ST2, ST3	Abdo et al. 2021
	>20- <50 Y	GI	167 33	33 (19.7) 27 (81.8)	Microscopy PCR	ST3	El Saftawy et al. 2023
Minia	>15 - <30 Y	IBS	100	82 (82) 80 (80) 54 (54)	Culture Microscopy PCR	-	Gabr et al. 2018
	20-67 Y	GI	190	28 (14.7)	Microscopy	-	Ahmed et al. 2021
Sohag	NS	NS	150	25 (16.66)	Microscopy	-	Elnazer et al. 2017

6-12Y	GI	200	21 (10.5)	Microscopy	-	El-Nadi et al. 2017
18-76 Y	NS	100	21 (21)	Microscopy	-	El-Hady et al. 2018

Abbreviation: **ALL,** Acute lymphocytic Leukemia; **Ash**, Asthmatic patients; **CRC,** Colorectal cancers; **IBS**, Irritable Bowel Syndrome; **IC**, Immunocompetent; **MI**, mixed subtypes; **GI**, patients with diarrhoea and other gastrointestinal complaints; **M**, month old; **Y**, year old; **NS**, not stated.

Not included in meta-analysis

*Human samples were mainly collected from hospitals which are visited by patients from different provinces and the mentioned regions are the hospitals location.

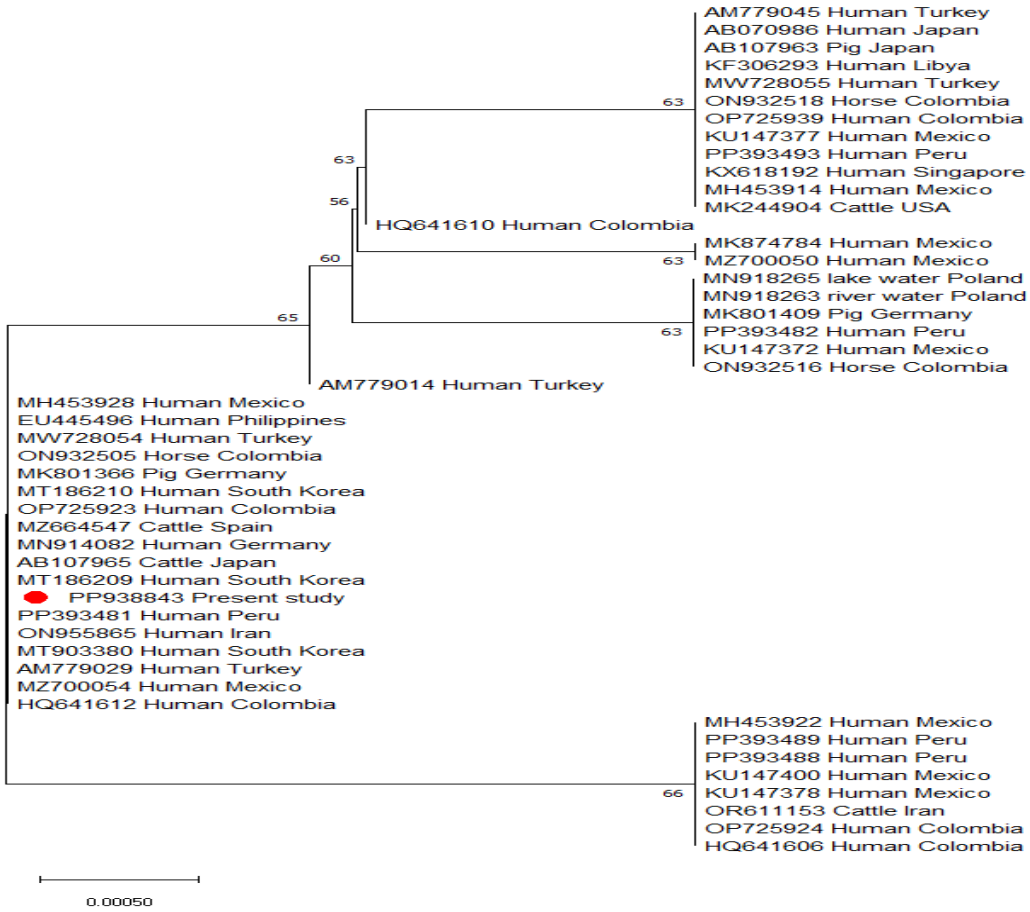

Figure 1. Neighbor-joining (NJ) tree based on the SSU rRNA gene sequences of *Blastocystis* sp. ST3 of isolates. Sequences from this study are labeled. The on Jukes-Cantor method, modeled using gamma distribution was employed. Numbers at the nodes represent the bootstrap values with more than 50% bootstrap support from 1000 pseudoreplicates.

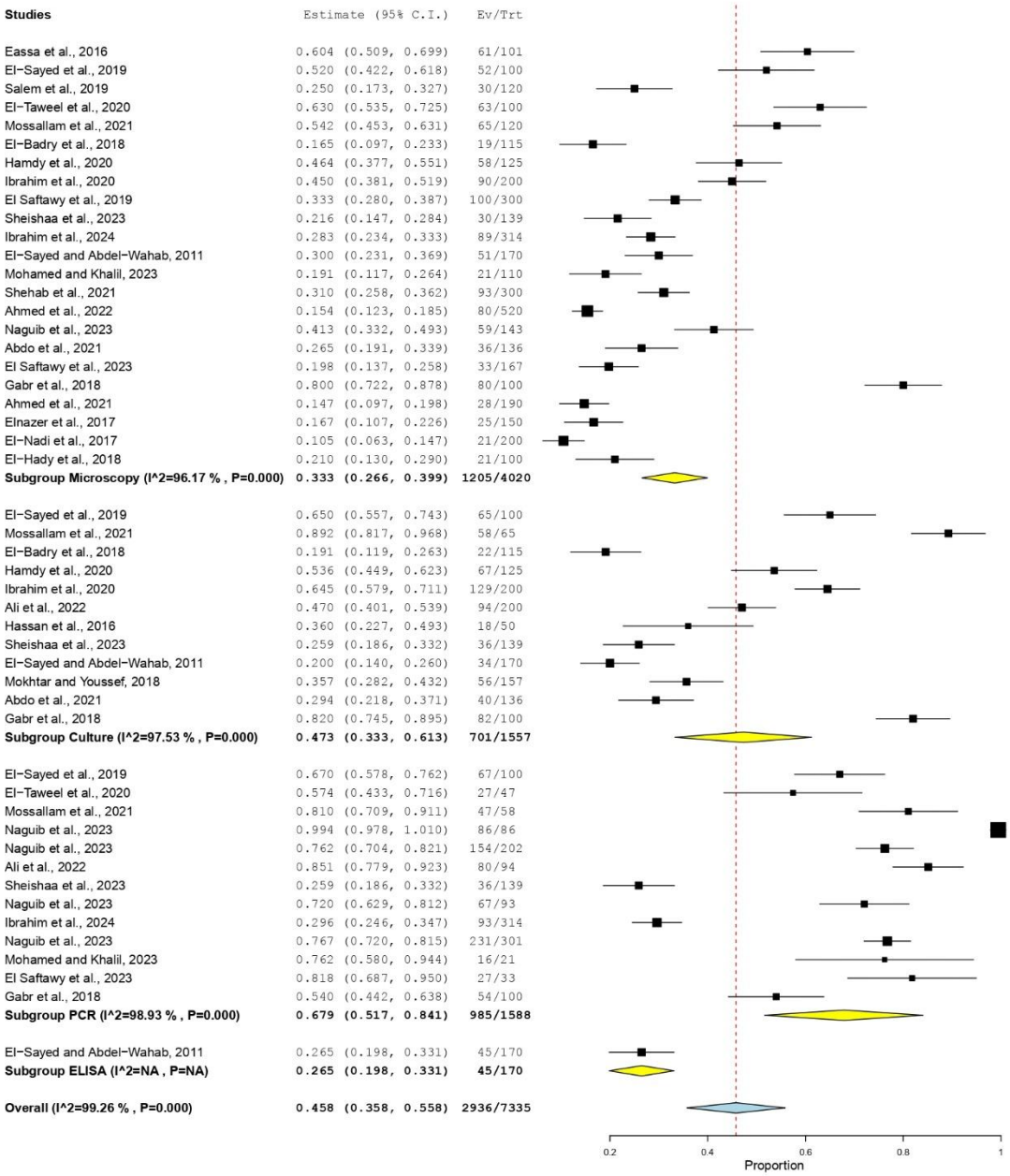

Figure 2. Forest plot diagrams for random effects in the meta-analysis of the prevalence of *Blastocystis* sp. infections in humans in Egypt based on diagnostic methods. The middle point of each line indicates the prevalence, while the length of the line is the 95% confidence interval of each study. Diamonds refers to the prevalence in accordance to detection methods.

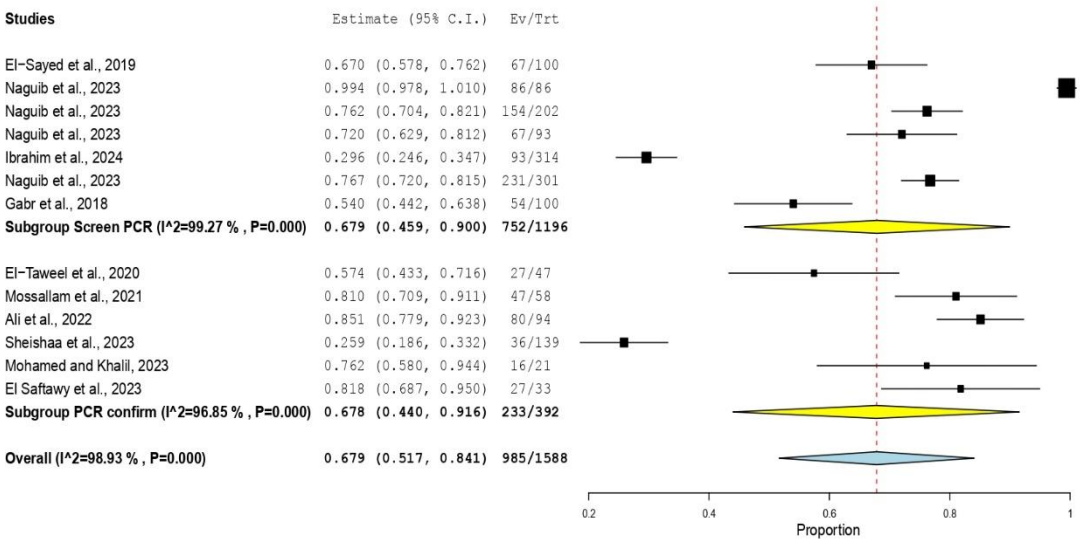

Figure 3. Forest plot diagrams for random effects in the meta-analysis of the prevalence of *Blastocystis* sp. infections in humans in Egypt based on PCR.

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الملخص العربي

عدوى البلاستوسيستس في مصر: تحديث في الأبقار من محافظة الدقهلية مع تحليل التجميعي (التلوي) للدراسات السابقة

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المتبرعمة الكيسية (Blastocystis sp.)هو الطفيلي الأولي الأكثر انتشارًا في البشر ويؤثر على مجموعة واسعة من الحيوانات. تنتشر عدوى Blastocystis بين البشر في مصر، ومع ذلك، هناك معلومات محدودة متاحة عن الحيوانات. تهدف الدراسة الحالية إلى تحديث معدل انتشار العدوى في الأبقار وتقييم معدل انتشار وتوزيع عدوى .Blastocystis sp والأنواع الفرعية في مختلف العوائل في مصر. تم جمع عينات البراز من 100 رأس من الماشية بالإضافة إلى محتويات البراز من أمعاء 50 بطة و90 حمامة منزلية. تم فحص العينات باستخدام تفاعل البوليميراز المتسلسل لجين SSU rRNA . تمت مراجعة الدراسات التي أجريت على البشر والحيوانات المنزلية والطيور في مصر وتم استخدام نماذج التأثيرات العشوائية لتحديد معدل انتشار العدوى بين البشر بناءً على طرق التشخيص. كانت عينة براز واحدة من الماشية إيجابية (1٪)، في حين لم يكن أي من الحمام أو البط المفحوص مصابًا. كان ST3 هو النوع الفرعي المحدد في العزلة الإيجابية والذي يرتبط من الناحية التطورية بعزلات أخرى من البشر والحيوانات من بلدان مختلفة. تم استخدام ما مجموعه 26 دراسة حول عدوى Blastocystis البشرية في مصر للتحليل التلوي، مما أدى إلى انتشار مجمع بنسبة 67.9٪ و 47.3٪ و 33.3٪، بناءً على تفاعل البوليميراز المتسلسل والثقافة والمجهر، على التوالي. على العكس من ذلك، أجريت دراسات محدودة (ن = 7) على أنواع حيوانية متنوعة. كانت الأنواع الفرعية المبلغ عنها في البشر في مصر هي ST1 - ST4 و ST7 و ST10 و .ST14وبالمثل، تم الإبلاغ عن ST1 - ST7 و ST10 و ST14 في الحيوانات، و ST2 في عينات المياه من مصر. تؤكد هذه الدراسة على أهمية معالجة عدوى Blastocystis في مصر من منظور الصحة العامة والحيوانية من حيث التشخيص والسيطرة المناسبين من خلال توفير البيانات الأساسية عن العدوى في البشر والحيوانات بناءً على البيانات الحالية والمنشورة.

الكلمات الدالة:

المتبرعمة الكيسية، الأنواع الفرعية، الانتشار، الأبقار، الحمام، البط، مصر