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Subtype Determination of *Blastocystis* Spp. Isolated from Poultry Tikrit City

Zuhair Mohammed Abed[1], Hayfaa Rajab Alwan[2], Abed Fayyadh Ali[3]

[1]Salah Al-Din Education Directorate, Ministry of Education, Tikrit, Iraq, ORCD 0009-0005-7992-0866

[2]Dept. of Biology, College of Education for pure science, Tikrit University, Tikrit, Iraq, ORCD 0000-0003-4777-0697

[3]Al-imam university college, Tikrit, Iraq, ORCD 0009-0007-3105-5353

ABSTRACT

Blastocystis spp. spread throughout the world, it is one of the intestinal parasites that settle in human's gastrointestinal tract, as well as occurring in other hosts such as Aves, reptiles, cattle and insects. Gross stool samples collected from poultry were 221 samples. *Blastocystis* spp. and subtypes investigated by a PCR reaction which targeting the small subunit of ribosomal RNA (*SSU rRNA*) gene. Microscopic results and sequencing analysis results of *SSU rRNA* gene showed 2 sample were infected with Blastocystis with percentage 0.9% out of 221 poultry fecal samples. The poultry infected with Blastocystis consisted of chicken and turkey, as infected rate in chicken were 0.6% out of 163 chickens while infected rate in turkey were 1.7% out of 58 turkeys. The existence of subtype (ST6) of Blastocystis was confirmed via BLAST analysis

KEYWORDS: *Blastocystis* spp., Fecal samples, PCR, Subtype (ST6), *SSU rRNA* gene

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INTRODUCTION

Blastocystis spp. is a microorganism of one cell inhabits human's intestines and other species of animal, with a wide global distribution, also this micro-eukaryote parasite is the most repeatedly detected in some epidemiological studies [1]. Blastocystis species are intestinal parasites of protozoa which have various life cycle forms including vacuolar, amoeboid, granular and cystic. The main manner of Blastocystis transmission via fecal-oral direct route, and humans may be infected with Blastocystis via consumption food and water which contaminated with Blastocystis cysts [2,3]. In addition, there is supportive evidence that confirm Blastocystis transmission from animals to peoples living in a communal place, may be occur through close contact with shelters of animals [4].

It was noted that isolates taken from chicken, geese and quail have the ability to infect chickens, which proves the occurrence of cross-infection among bird species. Blastocystis transmission can easily occur between birds which belong to same species or different species, this indicates a lack in specificity of the host [5].

Blastocystis possess a wide scope of hosts. The genetic sequencing analysis of *SSU rRNA* gene of Blastocystis that isolated from human, primates, mammals and birds revealed the existence at least 17 subtypes of Blastocystis. Subtype 3 (ST3) is a most common subtype found in humans. Subtypes (STs 1-9) commonly in humans, may be found in animals too, this indicates to possible transmission from animals to humans [6]. In recent years, parasite genetic diversity and host specificity have been highlighted as important research topics. At present, 27 parentage or subtypes of genetically distinct small subunit ribosomal RNA (SSU rRNA) have been characterized in Blastocystis spp. [7,8].

MATERIALS and METHODES

samples collection

A total 221 of Stool sample were randomly collected from domestic poultry (163 chicken and 58 turkey). Samples were collected directly from the rectum in a sterile screw neck vials and transported to the Parasitology Laboratory at Tikrit University. The samples were collected in the summer, from June to October 2022. Feces samples were microscopy directly by used Lugol's solution to investigate the existence of Blastocystis. Positive samples were stored at -20 °C until perform further molecular analysis.

Molecular Method

Blastocystis spp. DNA were extracted by using stool DNA extraction Kit (Manufactured by Korean Bioneer Corporation) according to the instruction of the manufacturer, then purified DNA samples were stored at -20C. In this study,

Polymerase chain reaction (PCR) was used to amplify SSU-rRNA gene, and a primer which consist of forward Blast 505-532: 5'-GAGGTAGTGACAATAAATC-3' and reverse Blast 998-1017: 5'- TGCTTTCGCACTTGTTCATC-3' was used in this reaction [9]. PCR PreMix (manufactured by the Korean company Bioneer) was used to perform amplification of *SSU rRNA* gene. In a PCR reaction 0.5 µl of forward primer, 0.5 µl of revers primer, 5 µl of DNA template and 14 µl of free nuclease water were added to each tube of PreMix to obtain final volume of 20 µl. Temperature conditions of PCR were one cycle at 95 °C for 4 min. to denature the double strands of DNA, then 35 cycles, as each cycle of these consist of (denaturation at 95 °C for 30 s, annealing at 54 °C for 30 s and extension 72 °C for 30 s), then one cycle of a final extension at 72 °C for 5 min. Products of PCR were separated by electrophoresis using agarose gel with concentration 1.5%, then agarose gel was visualized by UV transilluminators.

Sequencing

The sequences of *SSu-rRNA* gene were compared with the sequences found in the national center for Biotechnology Information (NCBI), using standard nucleotide BLAST.

RESULTS

Microscopic result showed 2 sample were infected with Blastocystis with percentage 0.9% out of 221 poultry fecal samples. The poultry infected with Blastocystis consisted of chicken and turkey, as infected rate in chicken were 0.6% out of 163 chickens while infected rate in turkey were 1.7% out of 58 turkeys. (Table1).

Table 1: prevalence of *Blastocystis spp*. and subtype in chicken and turkey

Host	Collected samples	Positive samples %	STs Identified
Chicken	163	0.6 (1\163)	ST6
Turkey	58	1.7 (1\58)	ST6
Total	221	0.9 (2\221)	

The PCR technique was used to examine the samples that containing blastocysts to confirm the microscopic diagnosis. PCR reaction results and agarose gel electrophoresis results showed two bands of size 500bp as in figure1. Sequencing of 2 PCR products was done to determine the subtype of Blastocystis. The consensus sequences that obtained in current study were recorded in GenBank with recording numbers OP796191.1 and OP796192.1. BLAST analysis certain the existence one subtype of Blastocystis ST6.

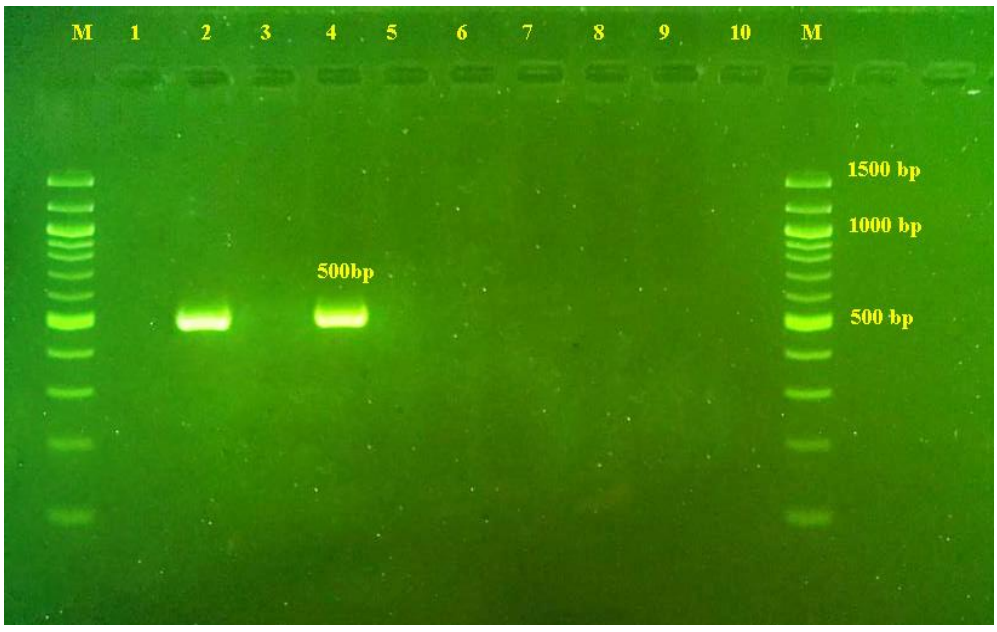

Figure 1: Agarose gel electrophoresis of *SSu-rRNA* gene, The bands in the tracks 2,4 with size of 500 bp is a typical band of the *SSu-rRNA* gene of *Blastocystis spp*. M: DNA ladder (100-1500bp)

DISCUSSION

Although, importance of Blastocystis sp. in public health field is still debated, is one of the evidences that pointing to contamination of the environment with animal feces [10]. In our study, spread of *Blastocystis* in poultry was 1.7%, The similarly, low spread of *Blastocystis spp*. Was 2.1% in chicken [11]. The results of the current study disagree with the results that mentioned by wang *et al*. [12] as they mentioned in their result the prevalence of Blastocyst in chickens was 13%. In a study on the spread of Blastocystis in

birds of French zoos, it was 8.6% [13], while other study in Egypt of prevalence *Blastocystis* spp. among Poultry showed 69.8% of poultry were infected with Blastocystis [14]. In a study conducted by Haziqah *et al*. [15] in Malaysia, it was shown that 80-100% of free chickens were infected with Blastocystis. Prevalence variations maybe due to a variance in the samples number were examined, Various diagnostic methods, diversity of management systems of the farms and stores that owned by producers or variation of animal's adaptation [16].

The tow isolates of *Blastocystis* spp., were recorded at NCBI depend on sequencing of subunit ribosomal RNA gene (SSU- rRNA) and became a reference of Iraq and the world with accession numbers OP796191.1 and OP796192.1. The strains that registered at NCBI was identical at ratio 98-99% with Brazilian strain (MN4727), Philippines (EU445485.1), Colombia (ON932544.1), Turkey (MW72873.1), Japan (AB070994.1), China (OK597198.1), Spain (HQ641658.1), Thailand (JQ665850.1), France (AY135411.1), Czech Republic (MW301902.1), Netherlands (KF242016.1) and Iran (MG732909.1) as in table 2. and Fig2. of *Blastocystis* spp. phylogenetic tree.

In the areas which were investigated areas, only subtype ST6 were found in chicken and turkey. In the study which conducted by Maloney *et al*. [17] eight subtype ST5, ST6, ST7, ST10, ST14, ST24 and two new subtype ST27 and ST28 were identified in domestic birds and captive wild birds, while Chen *et al*. [11] found subtype ST7 only in chickens. These results do not mean the subtype ST6 cannot be isolated from other hosts, as Adıyaman *et al*. [18] was able to isolate the subtype ST6 (1/43, 2.3%) from human fecal samples. Molecular studies revealed significant variation in subtypes STs distribution across hosts and geographical regions [19].

It is suggested that ST6 has a wide distribution around the world. In previous studies mentioned species of wild birds and species of domestic birds were reported to harbor subtypes of Blastocystis such as ST1, ST2, ST4, ST5, ST6, ST7, ST8, ST10, and ST20 [13,20]. The ST6 and ST7 subtypes are the most common subtypes in domestic and wild birds, so they are considered a subtypes for birds [21]. Molecular studies of Blastocystis STs in animals showed that the ST7 subtype was the most prevalent with ratio 29%, followed by ST1 with appearance ratio 25% and ST6 16.6% [22].

Besides to birds, the ST6 and ST7 subtypes are occasionally found in some mammals, and the ST6 also present in porkers, cattle, dogs, and goats [21]. Subtypes from ST1 to ST9 and ST12 were reported in humans, suggesting the transmission possibility of these subtypes from animal to human [23]. In a study conducted in Lebanon, it was reported that workers in poultry slaughterhouses were infected with the ST6 subtype, this confirms the transmission of this subtype from chickens to humans [1].

The prevalence of STs in edible animals such as poultry indicates a possible hazard of transmission of *Blastocystis* spp. to humans, also through human dealings with those animals or via animal products consumption.

Table 2: Genetic rapprochement of the subunit ribosomal RNA gene(SSU- rRNA) for isolate of *Blastocystis* spp.

	Accession	Country	Source	Isolation source	Compatibility
1.	**ID: MN472775.1**	**Brazil**	**Blastocystis sp.**	**Phasianus colchicus**	**99%**
2.	**ID: EU445485.1**	**Philippines**	**Blastocystis sp.**	**Chicken**	**99%**
3.	**ID: ON932544.1**	**Colombia**	**Blastocystis sp.**	**Horse**	**99%**
4.	**ID: MW728073.1**	**Turkey**	**Blastocystis sp.**	**Homo sapiens**	**99%**
5.	**ID: AB070994.1**	**Japan**	**Blastocystis sp.**	**Chicken**	**99%**
6.	**ID: OK597198.1**	**China**	**Blastocystis sp.**	**Gallus domesticus**	**99%**
7.	**ID: HQ641658.1**	**Spain**	**Blastocystis sp.**	**Chicken**	**99%**
8.	**ID: JQ665850.1**	**Thailand**	**Blastocystis sp.**	**Homo sapiens**	**99%**
9.	**ID: AY135411.1**	**France**	**Blastocystis sp.**	**turkey**	**99%**
10.	**ID: MW301902.1**	**Czech Republic**	**Blastocystis sp.**	**---------**	**99%**
11.	**ID: KF242016.1**	**Netherlands**	**Blastocystis sp.**	**---------**	**98%**
12.	**ID: MG732909.1**	**Iran**	**Blastocystis sp.**	**Homo sapiens**	**99%**

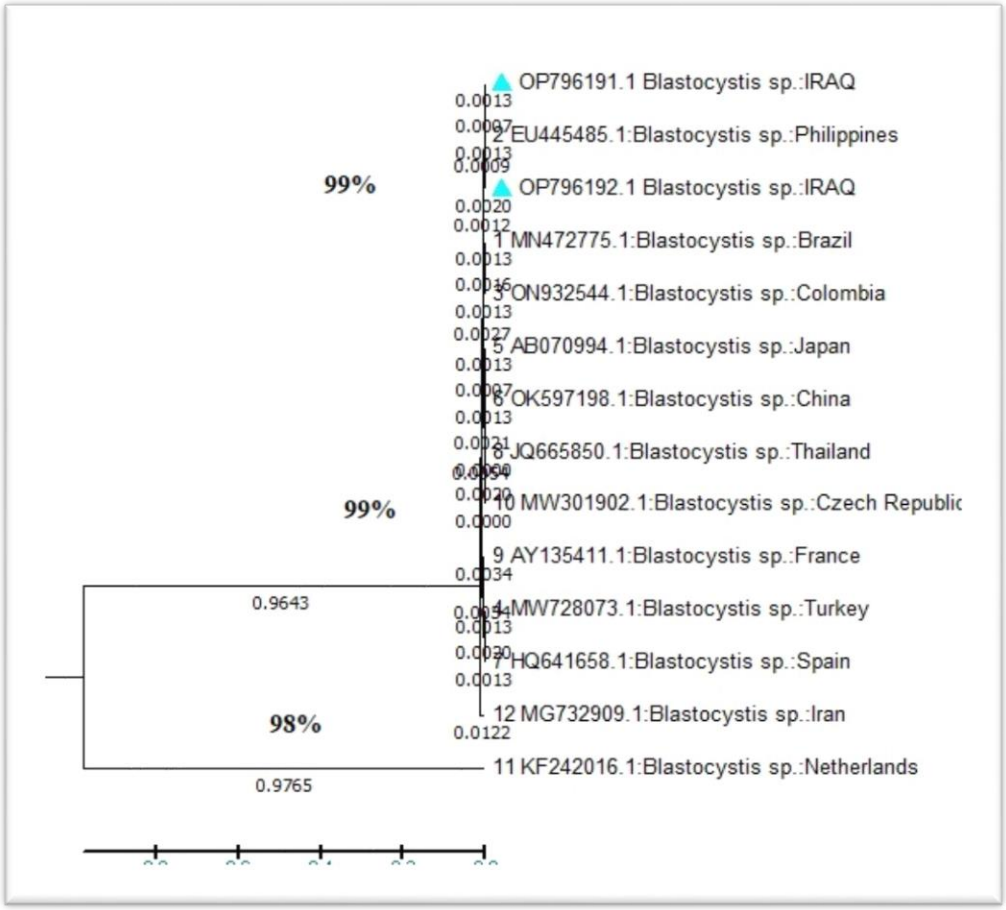

Figure 2: Phylogenetic tree of the *Blastocystis* spp., and reference sequences from Gen Bank

CONCLUSION

Blastocystis are common parasites in humans and animals that live in the small intestine, some subtypes are transmitted from animals to humans through contamination water and food with cysts, in the study area, subtype ST6was identified in chicken and turkey which is predominated in birds, suggesting potential zoonotic transmission.

CONFLICTS OF INTEREST

Intellectual rights of all results, figures and tables mentioned in this manuscript are our ownership.

ETHICS APPROVAL

The research was agreed by the local ethical committee in Tikrit University.

AUTHORS CONTRIBUTION

Z.M. Abed designed the study, collected samples, and diagnosed Blastocystis microscopically and drafted the manuscript and reviewed it. H.R. Alwan Haifa extracted DNA from the Blastocystis samples, detected *SSU rRNA* gene using PCR and analyzed the results of the sequencing of *SSU rRNA* gene. H.A. Ali recording isolates in NCBI. All authors have read and agreed to manuscript version.

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