Molecular subtyping and phylogeny of *Blastocystis* sp. isolated from turkey (*Meleagris gallopavo*) populations in Penang, Malaysia

Siti Alawiyah, J.A.N. 1, Rauff-Adedotun, A.A. 1, Aishah, S. 1, Rusydi Abdul Hafiz, R. 2, Zary Shariman, Y., Farah Haziqah, M.T. 1 *

1School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia
2Department of Veterinary Services, Jalan Bukit Tengah, 14000 Bukit Mertajam, Penang, Malaysia
*Corresponding author: farahhaziqah@usm.my

**ARTICLE HISTORY**

Received: 17 August 2021
Revised: 4 December 2021
Accepted: 4 December 2021
Published: 31 December 2021

**ABSTRACT**

Most poultry farms in Malaysia preferred rearing chickens either for eggs or meat than turkeys. This is due to several challenges such as parasitic load and heat stress in rearing turkey. *Blastocystis* is one of the most common protozoan parasites infecting poultry. As no study was conducted on *Blastocystis* infection in turkey in Malaysia, this study aims to determine the current status, the morphological characteristics and subtyping of *Blastocystis* from turkey reared either in closed house or free-range system in Penang, Malaysia. It was found that the prevalence of *Blastocystis* sp. infection in turkeys were moderately high with 41.6% (25 / 60) in the closed house and 45.0% (45 /100) in free-range system as infection was higher in the female turkeys with no gastrointestinal signs and symptoms. Vacuolar form was the most common form found in the *in vitro* culture ranged between 5 to 20 μm in diameter with a rough surface coat and undulating cell surface viewed under the scanning electron microscope. Meanwhile, the ultrastructure of the cells from turkey isolates were varies with partially expanded electron-opaque vacuoles to electron-dense in fully distended vacuoles. Interestingly, sequence analysis for 30 positive *Blastocystis* isolates from turkeys revealed one subtypes with three alleles namely, ST7 allele 99 (73.4%, n=22), ST7 allele 100 (23.3%, n=7) and ST7 allele 101 (3.3%, n=1). Findings from this study added to our understanding on *Blastocystis* infection in turkey production.

**Keywords:** *Blastocystis*; Malaysia; Penang; protozoan; turkey.

**INTRODUCTION**

Wild turkeys are huge, sexually dimorphic fowls with long feet, wide and curved tails, elongated necks and small heads (Miller, 2018). Wild turkey (*Meleagris gallopavo*) is associated with the other members in the order Galliformes, family Meleagridae and genus *Meleagris*. Wild turkeys are very adjustable in various conditions, capable to live in warm environments as well as to some countries that are frequently blanketed with snow. The adult males, known as tom or gobblers, weigh from 10 to 15 kg throughout their range depends on the type of breeds. The adult females, known as hens, commonly do not surpass 10 kg, with the typical weight from 6 to 9 kg (Cathey et al., 2007). In Malaysia, turkeys are reared for many purposes such as poultry meat as well as a hobby. Turkeys are considered expensive and have a high demand especially during festive season such as Christmas Eve and Deepavali.

The turkey’s usual behaviors are to forage food on soil, therefore, there are numerous types of organisms as well as intermediate hosts that can cause the endoparasites infection in turkeys as they are omnivorous, they have a wide-ranging diet. Mohammad Zarith et al. (2017) stated that studies on the dispersion of parasitic infection in turkeys particularly in Malaysia is still scarce which probably due to Malaysian preference to eat more chicken than turkey, making study on turkey diseases economically insignificant.

Generally, turkeys are having some issues to several parasitic diseases caused by protozoan parasites. Protozoa are single-celled organisms that can be commensals or parasitic in nature. There are certain species of parasitic protozoan which include in the medical importance worldwide. In turkey population, the most common species of parasitic protozoan encountered were *Eimeria* spp. which cause coccidiosis (Sharman et al., 2010) and *Histomonas meleagridis*, the source of Blackhead disease (histosomiasis). Other protozoan which may also infect turkeys include *Hexamita meleagridis* (Hexamitiiasis), *Trichomonas gallinae* (trichomoniasis) and *Cochlosoma anatis* (cochlosomiasis) (Hauck & Hafez, 2012). Apart from that, a neglected zoonotic protozoan known as *Blastocystis* sp. was also been found in...
turbkeys (Lee, 1970; Yamada et al., 1987; Belova & Kostenko, 1990; Belova, 1992a; Mokhtar & Youssef, 2018).

*Blastocystis* sp. is a common, non-flagellated, anaerobic stramenopiles (Gentekaki et al., 2017) that inhabits the gastrointestinal tracts in many humans and various animals particularly poultry (Mokhtar & Youssef, 2018). *Blastocystis* exists in four different morphological form namely, vacuolar, granular, amoeboid and cyst form (Tan, 2008). The most common mode of reproduction is binary fission (Adaö & Rivera, 2018) in which cyst is the infective form that accountable in the transmission. The main transmission mode of this protozoan is through the faecal-oral pathway via drinking untreated water and/or poor sanitary conditions.

The occurrence of this organism has been perceived in a wide diversity of species worldwide. It has a great genetic diversity thus the genotypes were assigned using the subtyping nomenclature (ST) (Rauff-Adedotun et al., 2020). Nomenclature *Blastocystis* sp. subtypes (STs) ST1-ST9 was first presented in 2007 (Rauff-Adedotun et al., 2020), after many of subtypes were proposed recently. Starting from the year 2013, new subtypes was recognized which was ST1-ST17 between some hosts (Alfellani et al., 2013; Stensvold & Clark, 2020). Presently, a total of 29 subtypes have been suggested (Rauff-Adedotun et al., 2020). However, four subtypes out of 29 subtypes that have been proposed namely, ST18, ST19, ST20 and ST22 was recently under question due to the probability that they were generated from memento consequently their quixotic emergence (Stensvold & Clark, 2020). The enduring 25 subtypes which include ST1-ST17, ST21, ST23-ST29 have encountered the existing suggested standards for distinctive subtype nominations (Maloney & Santin, 2021). Additionally, ten subtypes, ST1-ST9 and ST12 have been revealed in humans, with fluctuating stages of existence (Greige et al., 2019) later the possibility of zoonotic transmission will occur (Clark et al., 2013; Stensvold et al., 2020).

The most recent study on *Blastocystis* in poultry by Greige et al. (2018) reported that the avian samples specifically from chickens in Lebanon were subtyped and fitted to any ST6 or ST7, with a great majority belongs to ST6. Surprisingly, this subtype also been detected among the chicken handlers which affirmed that there was zoonotic transmission of this ST as those individuals were frequently in a direct contact with the chickens. Meanwhile, Mokhtar & Youssef (2018) reported the occurrence of ST1, the zoonotic subtypes with a prevalence of 7.8% in poultry species among the chicken, ducks, geese and turkeys isolates in Egypt. It was also been reported that they were generated from memento consequently their quixotic emergence (Stensvold & Clark, 2020). The enduring 25 subtypes which include ST1-ST17, ST21, ST23-ST29 have encountered the existing suggested standards for distinctive subtype nominations (Maloney & Santin, 2021). Additionally, ten subtypes, ST1-ST9 and ST12 have been revealed in humans, with fluctuating stages of existence (Greige et al., 2019) later the possibility of zoonotic transmission will occur (Clark et al., 2013; Stensvold et al., 2020).

Most of the previous studies on *Blastocystis* in poultry were concentrated on *Blastocystis* in domestic chickens (Stensvold et al., 2009; Alfellani et al., 2013; Ramirez et al., 2014; Greige et al., 2018; Mokhtar & Youssef, 2018; Wang et al., 2018; Deng et al., 2019; Rauff-Adedotun et al., 2020; Maloney et al., 2021), quails (Maloney et al., 2021), ducks (Maloney et al., 2020; Rauff-Adedotun et al., 2020; Fahim et al., 2021; Maloney et al., 2021) and ostriches (Chandrasekaran et al., 2014; Maloney et al., 2020; Rauff-Adedotun et al., 2020; Deng et al., 2021; Rudzinska et al., 2021; Zhang et al., 2021). As there are very limited study in turkey population worldwide (Lee, 1970; Belova, 1992a; Noel et al., 2003; Sreekumar et al., 2014; Mokhtar & Youssef, 2018; Maloney et al., 2020) and none was conducted in Malaysia, therefore, this study will help to provide a baseline study on this neglected zoonotic protozoan parasite infection in turkey population mainly in the northern region of Peninsular Malaysia.

**MATERIALS AND METHODS**

**Ethical approval**

All animals used in this study were handled according to Animal Ethics and USM Institutional Animal Care and Use Committee (USM IACUC), Universiti Sains Malaysia. Written permission was obtained from the authorities of Department of Veterinary Services as sampling activities were conducted in privately-owned and protected turkey farms.

**Sampling sites**

This study was conducted in the Seberang Perai, Penang (Latitude: 5.3700° N and Longitude: 100.4139° E) as almost 50% of farmers reared turkey in this area. Sites were chosen based on types of turkey rearing which was closed house system and free-range system. Sampling activities were conducted on a closed house located at Department of Veterinary Services Penang, Bukit Tengah and several selected backyard farms at Tasek Gelugor, Kubang Menerong and Kepala Batas, Penang.

**Study population**

By adopting convenience sampling method (Dornyei, 2007; Etikan et al., 2016), a total of 160 turkeys consisted of free-range and closed house reared turkeys which involved 90 males and 70 females were examined for *Blastocystis* sp.

The closed house turkeys comprised of commercial broilers that reared specifically for meat. In the closed house, the turkey's reared were the White Holland turkey. They were kept indoors, sealed, retained with controlled temperature and have a good ventilation. Besides, wood shavings were commonly used as deep litter or floor systems with slatted floor. The turkeys were reared by the integrated federal government authorities of Department of Veterinary Services Penang in which the adult female turkey sold to the farmers as an initiative programme from the government. There were 60 faecal turkey samples collected from the closed house involved 20 males and 40 females screened for *Blastocystis* sp.

The turkeys consisting of free-range turkeys were frequently seen in countryside locations where old-style poultry production was practiced. The turkeys reared breed namely, Black turkey and White Holland that were partially confined and allow to scavenge for food freely and return periodically to the homestead or barn for water and food sources such as kitchen waste or feed pallet. The turkeys were kept in a small barn with the build of fenced area to protect from the predator, especially in the night-time. In this study, 100 faecal turkey samples were collected from the sampling sites consists of 50 males and 50 females.

**In vitro cultivation**

A small amount of each faecal sample was inoculated into a sterile screw-top bottle containing 3 ml of modified Jones' medium supplemented with 10% heat-activated horse serum. Each sample was incubated vertically at 37°C for 24 to 48 hours. Later, a drop of the sediment was examined at 400x magnification for *Blastocystis* examination in which positive samples were those with the presence of *Blastocystis* sp. forms. Positive samples were subsequently maintained by sub-culturing every 2 to 3 days and were then stored at -20°C for molecular characterization.

**Microscopy examination**

Smears were carried out from day-3 positive culture samples. Later, these smears were fixed with methanol, stained with 10% Giemsa and then viewed under light microscope at 400x.
7.2. Phylogenetic tree was then constructed with MEGA X. Nucleotide sequences were analysed using BioEdit version 7.2. Phylogenetic tree was then constructed with MEGA X platform x86, x86-64 using neighbour joining p-distance model. The sequences isolate from this study together with other Blastocystis sequences from the GenBank.

The evolutionary history was inferred by using the Maximum Likelihood method and Tamura 3-parameter model (Tamura, 1992). The tree with the highest log likelihood (-11626.88) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbour-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Tamura 3 parameter model, and then selecting the topology with superior log likelihood value. The phylogenetic tree was rooted using Proteomonas lacertae as an outgroup. This analysis involved 49 nucleotide sequences. There were a total of 1922 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

RESULTS

Prevalence of Blastocystis sp. infection
Out of the 160 turkeys, a total of 70 (43.8%) turkey faecal samples that were positive for Blastocystis sp. infection (Table 1) in which none of the study animals showed behavioural signs or indication of Blastocystis sp. infection.

Meanwhile, the prevalence of Blastocystis sp. infection in turkey from closed house was higher in female with 60% (28/50) and 45% (45/100), respectively (Table 1). It was also found that there was no significant difference ($P>0.05$) reported between the type of turkey rearing system and Blastocystis sp. infection ($\chi^2 = 0.169, [df] = 1, P = 0.681$) in this study.

The prevalence of Blastocystis sp. infection in turkeys reared in the closed house and free-range system were 41.6% (25/60) and 45% (45/100), respectively (Table 1). It was also found that there was no significant difference ($P>0.05$) reported between the sex of turkeys and Blastocystis sp. infection ($\chi^2 = 0.681$) in this study.

Meanwhile, the prevalence of Blastocystis sp. infection in turkey from closed house was higher in female with 60% (12/20) whereas in male with 32.5% (13/40). As, for the free-range turkeys, the prevalence was also reported higher in female with 56% (28/50) than in male with 34% (17/50) (Table 1). There was a significant difference ($P<0.05$) reported between the sex of turkeys and Blastocystis sp. infection ($\chi^2 = 4.149, [df] = 1, P = 0.042$) in this study.

Morphological forms
From the in vitro cultivation of Blastocystis sp. isolates in the turkey faecal sample, the morphology of the Blastocystis sp. obtained were mostly vacuolar form approximately from 5 to 10 µm in diameter in 1.5% agarose gels and Tris-Acetate-EDTA (TAE) buffer.

<table>
<thead>
<tr>
<th>Study animals</th>
<th>No. of faecal samples</th>
<th>No. of turkeys infected (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Closed-house turkeys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>40</td>
<td>13 (32.5%)</td>
</tr>
<tr>
<td>Female</td>
<td>20</td>
<td>12 (60%)</td>
</tr>
<tr>
<td>Free-range turkeys</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>50</td>
<td>17 (34%)</td>
</tr>
<tr>
<td>Female</td>
<td>50</td>
<td>28 (56%)</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>70 (43.8%)</td>
</tr>
</tbody>
</table>

Table 1. Prevalence of Blastocystis sp. infection in two types of farming practices in turkey population
20 μm in diameter (Figure 1). The granular forms size ranges from 5 to 30 μm in diameter and it was commonly found in the older cultures of isolates (Figure 2).

Mode of reproduction
The morphology of the Blastocystis sp. and different modes of reproduction was observed under light microscopy in the in vitro cultures. Nevertheless, the mode of reproduction commonly observed in this study was binary fission (Figure 3).

Ultrastructure and surface structure
Scanning electron micrographs showed the surface structure of Blastocystis sp. isolated from the selected faecal culture of closed house (B7c) and free-range (FM11) turkeys. The cell surface for both isolates were generally spherical to rounded in shape and had a rough surface coat with undulating cell surface whereas some organisms showed gouges or deep furrows (Figure 4).

Blastocystis cell isolated from the selected faecal culture of closed house (B7c) and free-range (FM11) turkeys were examined by using transmission electron microscopic. It was revealed that Blastocystis cells from the close house turkey isolate showed a central vacuole with partially expanded electron-opaque vacuoles (Figure 5a) whereas Blastocystis cells from the free-range turkey isolate contained a large central vacuole with tiny electron-dense particles in fully distended vacuoles (Figure 5b). Besides, the organisms also possessed a thin wispy surface coat that resembles a slight ruffled appearance of the surface observed under the scanning electron microscope.

Subtype identification, alignment and phylogenetic analysis
According to the sequence analysis of 30 positive Blastocystis isolates, one genotypes and three allele were identified by BLAST queries at Blastocystis Sequence Typing Database (https://www.pubmlst.org/blastocystis): ST7 allele 99 (73.4%, n=22), ST7 allele 100 (23.3%, n=7) and ST7 allele 101 (3.3%, n=1).

In closed house rearing system, allele 99 was the most common allele found with the frequency of 60.0% (6/10), followed by allele 100 with 30.0% (3/10), and allele 101 with 10.0% (1/10). Meanwhile in free range rearing system, allele 99 was also the most common allele with the frequency of 80.0% (16/20), followed by allele 100 with 20.0% (4/20) and none was found for allele 101 in free range turkey rearing system (Figure 6).

Based on the allele distribution in sex of turkey, allele 99 was commonly found in male turkey with the frequency of 54.5% (12/15), followed by allele 100 with the frequency of 42.9% (3/15). In female turkey, it was found that allele 99 was the most common allele with the frequency of 57.1% (10/15), followed by allele 100 with 45.5% (4/15) and allele 101 with 10% (1/15).

The Maximum-likelihood (ML) phylogenetic tree was built to examine the positions of our new sequences against a selection of GenBank reference sequences. It was found that all the sequences obtained form a single clade as indicated in Figure 6.

DISCUSSION
In Malaysia, the broiler chicken, jungle fowl, village chicken and duck are available in numerous places as well as the cost is more affordable than the turkey meat. Turkey meat is typically sold at the average of RM25 to RM30 (Mohammad Zarith et al., 2017) per kg whereas chicken meat is approximately cost for about RM6 to RM10 per kg. In certain countries, market demand for turkey meat is less popular than chicken or even duck meat (Parrott & Walley, 2017). Turkey meat consumption is scarcer particularly in Malaysia rather than the western countries namely, Canada and United States.
Figure 2. Granular form of *Blastocystis* sp. in turkey (arrow).

Figure 3. Binary fission, the reproduction mode of *Blastocystis* sp. (arrow) observed in turkey.
Turkey population are not frequently been studied probably because they are less economically important to the poultry industry in Malaysia as compared to chicken and duck (Yadav et al., 2021). The only study on parasitic infection in turkey population in Malaysia was conducted by Mohammad Zarith et al. (2017) who reported on the occurrence of endo- and ectoparasites infection in free-range turkey population from Kedah, Malaysia. However, no attempt was made to detect the occurrence of the neglected zoonotic protozoan parasite, Blastocystis sp. infection in the turkey examined.

Studies on Blastocystis sp. infection was widespread and abundant in the animal population particularly in poultry, the avian population (Lee, 1970; Yamada et al., 1987; Belova & Kostenko, 1990; Pakandl & Pecka, 1992; Belova, 1992a, 1992b; Abduljaleel et al., 2012). Generally, cooking and eating turkey meat is associated with several festivities. In America and many parts of Europe, turkey meat will be served for dinner on Christmas Eve and the Thanksgiving Day. However, in Malaysia not only during Christmas Eve, turkey meat will also be being served during Diwali as a fascinating dish known as turkey biryani (Jayaraman et al., 2013).
Yoshikawa et al., 2003; Tanizaki et al., 2005; Stensvold et al., 2007; Tan, 2008; Clark et al., 2013; A Dao & Rivera, 2018; Farah Haziqah et al., 2018; Greige et al., 2018; AbuOdeh et al., 2019; Mohammadpour et al., 2020; Oliveira-Arbex et al., 2020; Boutellis et al., 2021). Similarly, in Malaysia most studies were mainly focusing on Blastocystis infection in chicken population as they are the largest poultry production in the farming system in Malaysia (Farah Haziqah et al., 2018). To date, there are no known study on Blastocystis sp. infection in turkey (Meleagris gallapavo) population in Malaysia.

Therefore, this study was conducted to determine the current status of Blastocystis infection in turkey population in the Northern region in Peninsular Malaysia particularly in Penang, Malaysia as commercial turkey farming was currently increased at the mainland of Penang due to the support and assistance by the DVS Penang. It was found that the prevalence of Blastocystis sp. in turkey population was moderate with the prevalence of 43.8% (70/160) concurrent with Blastocystis infection in turkey from Egypt with 50% (6/12) prevalence (Mokhtar & Youssef, 2018). However, Sreekumar et al. (2014) reported high prevalence of infection in turkey from India with 70% (3/4).

Contrary to previous studies, this study examined a large number of turkeys with 160 animals were examined for Blastocystis infection with none of the positive turkeys showed behavioural signs or indication of infection. Apparently, other birds namely, ostriches infected with this protozoan parasite appeared healthy without any other symptoms as reported by Chandrasekaran et al. (2014). Currently, there is no conclusive evidence suggest the pathogenic role of Blastocystis infection in animals. However, Blastocystis may be a commensal organism that becomes pathogenic when the host is immunosuppressed, malnourished or has other source of infections such as bacterial or viral infection (Ginanjar et al., 2007; Lepczynska et al., 2016).

It was found that Blastocystis infection in free-range turkeys (45%) were slightly higher than the closed house turkeys (41.6%). Besides, based on the statistical analysis there was no significant different between different types

**Figure 6.** Phylogenetic tree of the new Blastocystis sp. sequences from turkey in Penang and reference SSU-rRNA gene sequences from GenBank.
of rearing system and the infection of Blastocystis. Infection among free-range turkey flock was most probably due to the scavenging habits. Thus, the possibility of ingesting the infective stage of Blastocystis sp. in the environment appears to be very high due to the soil floor system in the backyard barns which makes them more susceptible to Blastocystis infection. Although, the closed house turkeys were totally confined in barren windowless enclosed long house with a deep litter system, Blastocystis infection was also reported to be relatively high in the closed house turkeys due to unhygienic practices in the pens such as the infrequent of changing the sawdust material of the floor system.

Despite the turkeys were reared under a supervision of a veterinary health officer and were treated with antibiotic and anthelmintic medication, both the closed house and free-range turkey population were found to be infected with a high prevalence of Blastocystis. Thus, excellent hygiene and sanitation are vital in avoiding or reducing the infection of Blastocystis because it is the main contributor for the health maintenance of poultry management (Stenzel & Boreham, 1996).

Finding from this study found that there was a significant difference between sex of turkey and Blastocystis infected. The prevalence of Blastocystis sp. infection in female turkeys were higher in both the free-range and closed house turkeys. The higher percentage of infection in the females may be due to the modification in the physiological condition of the animals during the production activity particularly during egg production in female turkeys as reported by Liu & Bacon (2005). Besides, Lloyd (1983) also reported that the advanced level of prolactin and progesterone hormones make the female ruminant more susceptible to any infection. In contrary to a previous study by Azhar et al. (2002), there was no variation in gastrointestinal parasitic infection between the sex of host. The faecal smears with vacuolar and granular forms were stained with Giemsa Stain for confirmative analysis. The size of the Blastocystis forms encountered varied from 5 to 20 µm. The measurements of the vacuolar forms of Blastocystis sp. in chicken were quite varied, with a minimum measurement of 10 µm and a maximum of 30 µm in diameter (Farah Haziqah et al., 2014). According to difference in size and shape, the organism is occasionally difficult to identify by wet mount preparation. Conferring to the study of Zaki et al. (1991), enduring smears seem to be the technique of choice for light microscopic analysis. The staining characteristic of Blastocystis with Giemsa is alike to that defined by Yamada & Yoshikawa (2012) and Sreekumar et al. (2014). With the occurrence of an amorphous and granular substantial in the central vacuole and fluctuating number of nuclei (1-12) in the external rim of the cytoplasm. Morphological characteristics of Blastocystis in turkey isolates were observed. It was found that the most common form of Blastocystis in the in vitro culture was vacuolar. Moreover, granular form was commonly found in the older cultures of isolates.

Meanwhile, reproductive mode commonly observed in the in vitro culture of turkey faeces was binary fission which is characterised by the barrier of the cytoplasm of the mother cell and outcomes in two daughter cells with an identical size and shape. According to several studies on Blastocystis in poultry, there were two types of reproduction mode of the Blastocystis sp. been observed in poultry namely, binary fission and budding (Govind et al., 2002; Yamada & Yoshikawa, 2012; Parija & Jeremiah, 2013; Farah Haziqah et al., 2014).

The surface structure for both isolates of closed house and free-range turkeys were generally spherical to rounded in shape and had a rough surface coat with undulating cell surface whereas some organisms showed gouges or deep furrows similarly indicated in the isolates from diarrhea cattle (Widisuputri et al., 2021). Meanwhile, Cassidy et al. (1994) revealed that the surface structure of chicken isolates appeared to be compact with a smooth and undulating cell surface. It is apparent that the surface structures of Blastocystis sp. from different hosts are variable, and this study notes the surface structure morphology in turkeys as none was reported previously in this bird. Moreover, surface coat may absent in certain forms namely in the vacuolar form and the amoeboid form from human isolates as reported by Dunn et al. (1989) and Stenzel et al. (1991). It has also been suggested that the features of the surface structure of Blastocystis sp. maybe correlated with symptomatic appearance (Widisuputri et al., 2021).

Studies on the ultrastructure of Blastocystis sp. in poultry from Malaysia were previously reported in chickens (Farah Haziqah et al., 2018) and ostriches (Chandrasekaran et al., 2014). This study represented the ultrastructural features of Blastocystis vacuolar form isolated from the close house turkey isolate with a central vacuole contained partially expanded electron-opaque whereas Blastocystis cells from the free-range turkey isolate contained a large central vacuole with tiny electron-dense particles in fully distended vacuoles similarly reported in the barn-reared chicken (Farah Haziqah et al., 2018) and ostrich (Chandrasekaran et al., 2014) cells. Moreover, the ultrastructure features of Blastocystis in turkey was first demonstrated by Lee (1970) who also reported on the occurrence of finely granular material and crystalline inclusions in the central vacuole and dense particles seen in the central vacuole indicating the presence of lipid. Therefore, it can be confirmed that Blastocystis sp. from turkey, chicken as well as the ostrich isolates uses the vacuolar forms to store lipids due to the poultry diets which contains high-fat pellets (Loar & Corzo, 2011; Evans et al., 2015).

There are very limited studies on subtype characterization of Blastocystis sp. isolated from turkeys (Noel et al., 2003; Mokhtar & Youssef, 2018). Blastocystis ST6 was reported in the turkey isolates from France (Noel et al., 2003) whereas variety of subtypes was isolated from turkey population in Egypt namely, ST1, ST6 and ST7 (Mokhtar & Youssef, 2018). Blastocystis ST1 was previously detected in variety of animal hosts namely, in chickens (Cian et al., 2017), dogs (Wang et al., 2013), pigs (Valenca-Barbosa et al., 2019), chimpanzees (Roberts et al., 2013), and gorillas (Roberts et al., 2013) as well as humans (Greige et al., 2018). Meanwhile, ST6 and ST7 were previously known as avian subtypes mainly because of its high prevalence in poultry specifically chickens, quails, geese as well as other bird population (Mokhtar & Youssef, 2018). However, ST6 and ST7 is scarce in humans with the prevalence as low as 1% infection of Blastocystis ST6 in the Netherlands (Bart et al., 2013), 3.6% infection of Blastocystis ST6 in Thailand (Jantermort et al., 2013) and 1% infection of Blastocystis ST7 from the American continent (Jiménez et al., 2019).

From this study, ST7 was the only subtype detected from 30 positive isolates with three different alleles namely, allele 99, 100 and 101. Interestingly, ST7 allele 99 was not only found in turkey, it was also been reported in other domestic animals namely, dogs (n=3) (Mohammadpour et al., 2020) and chicken (n=1) from Iran Rahimi et al. (2021). Notably, humans were also found to be infected with ST7 allele 99 as reported in a patient with Clostridium difficile infection (CDI) from Singapore (Deng et al., 2021) and one isolate from patient with diabetes mellitus in Brazil (Melo et al., 2020). Meanwhile, the only available data present to date for ST7 alleles 110 was by Lhotská et al. (2021).
the occurrence of this subtype in the gut-healthy humans from Czech Republic and Deng et al. (2021) reported in one of the patients with CDI from Singapore. As for ST7 allele 101, one isolate was reported from patients with CDI Deng et al. (2021) with 100% identity to those in humans in the Czech Republic (Lhotská et al., 2020). Since these subtypes were previously reported in human populations, animals may serve as reservoir hosts and facilitate transmission to human. Therefore, it can be suggested that transmission may occur between domestic animals to animals or humans.

In this Maximum-likelihood phylogenetic tree, the sequence of Blastocystis generated from this study form a well-supported because Bootstrap proportion and a long branch propagation to monophyletic group. This clade is the sister group of all GenBank sequence. There are low inter-sequences within this clade variability because sequences are quite distinct from their sister group sequences.

CONCLUSION

In this study, it was found that despite being raised in an intensive closed house system, treated with antibiotic and anthelmintic medication under a supervision of veterinary health officer, high prevalence of Blastocystis infection was observed in the closed house turkey population. It can be concluded that establishment with high-quality hygiene and sanitary conditions might result in negative infection as good hygiene practices will contribute to better health maintenance of the birds. Although, these studies have assisted in understanding the morphological characterization of this protozoan parasite in turkey, the morphological characteristics were in accord with the general features of Blastocystis in other bird hosts namely, chickens. Besides, this study has generated a great deal of data on subtype of Blastocystis isolated from turkeys in which zoonotic subtype, ST7 (allele 99, 100 and 101) were identified out of 30 positives isolates from turkey. Thus, zoonotic transmission should be taken into consideration as the animal handlers particularly, turkey farmers or the slaughter workers might have high risk of infection as they are in constant contact with the birds and more susceptible to Blastocystis sp. infection. To date, there is no information on Blastocystis infection in turkey population Malaysia, thus the findings of this study added to our understanding on Blastocystis infection as this is the first study to evaluate the current status, morphology, ultrastructure and genetic characteristics of Blastocystis sp. isolated from free-range and close house turkeys in Malaysia.

ACKNOWLEDGEMENTS

The authors would like to thank Universiti Sains Malaysia (Short Term Grant 2018: 304/PBIOLOGI/6315156) and USM Postgraduate Research Grant Scheme (Siti Alawiyah: 1001. PBIOLOGI.AUPS001) for funding this study. A special thanks to all the staffs at Poultry Unit, Department of Veterinary Services, Penang state and the team from Veterinary Parasitology Laboratory USM for providing tremendous support and assistance on this study.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


