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Prevalence and subtype distribution of *Blastocystis* sp. in cattle from Pahang, Malaysia

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Abstract. *Blastocystis* sp. is a common enteric protozoan parasite found in humans and various type of animal worldwide. Recently, genotypic distribution of *Blastocystis* sp. was revealed in insects, rodents, avian and mammals, which exposed its potential of transmiting the infections to human. However, very little information on current level of *Blastocystis* sp. infection were reported in cattle from Malaysia. Herein, a total of 120 stool samples of cattles were collected. While the potential risk of infection such as age, gender, body score, diarrheic condition of the cattle were noted, the management of the farms was also recorded. All stool sample were cultured, but 80 samples were selected for PCR sequencing analysis. The cultivation and microscopic examination revealed only 25% of the cattle (30/120) were infected with *Blastocystis* sp.. But, 43.8% of the cattle (35/80) were found positive upon PCR sequencing. The study also found that age, body score condition, diarrheic condition and certain farm were associated with the infection ($p<0.05$). Six subtypes (STs) that were discovered during the study were ST10 (21.3%;17/35), ST5 (8.8%;7/35), ST3 (7.5%;6/35), ST1 (2.5%;2/35), ST4 (2.5%;2/35) and ST14 (1.3%;1/35). Thus, moderate infections of *Blastocystis* sp. and variants in the genotypic distributions of the cattle suggest its potential for zoonotic transmission. Therefore, this findings could be helpful for further understanding the parasite, which assist studies of its pathogenicity.

INTRODUCTION

Blastocystis sp. is a protozoan parasite that lives in the intestinal tract of diverse animals and human (Alfellani *et al.*, 2013b; Cian *et al.*, 2017). *Blastocystis* sp. has four distinct stages which consist of cyst form, vacuolar form, granular form and amoebic form (Zhang *et al.*, 2007; Zhang *et al.*, 2012). *Blastocystis* sp. has shown high degree of genetic diversity with 17 different genetic subtypes been recognized, which ST3 and ST10 were the most commonly detected in humans (Alfellani *et al.*, 2013b) and cattle respectively (Zhu *et al.*, 2017). Recent molecular studies indicated that *Blastocystis* sp. was detected in human worldwide with detection rates of 22 to 56% in European countries (Bart *et al.*, 2013; Krosgsgaard *et al.*, 2015; Scanlan *et al.*, 2015; Forsell *et al.*, 2016) and 37 to 100% in Asian and African countries (El Safadi *et al.*, 2014; Popruk *et al.*, 2015; Forsell *et al.*, 2016). In developed Asian countries such as Japan and Singapore, a very low 1.1% (Hirata *et al.*, 2007) and 3.3% (Wong *et al.*, 2008) rates of detection were reported respectively. *Blastocystis* sp. has been detected in cattle with low infection rates of 6.7% in Korea (Lee *et al.*, 2018) and the highest rates of infection of 80% in Colombia (Ramirez *et al.*, 2014). While, other countries such as USA, UK, Iran, China and Thailand reported rates of infection of 19 to 50% in cattle (Fayer *et al.*, 2012; Alfellani

et al., 2013; Badparva *et al.*, 2015; Zhu *et al.*, 2017; Wang *et al.*, 2018b; Udonsum *et al.*, 2018).

In human, *Blastocystis* sp. responsible for non specific gastrointestinal symptoms such as headaches, nausea, vomiting, abdominal pain, and persistent diarrhea (El Safadi *et al.*, 2016; Sequi *et al.*, 2017; Ramirez *et al.*, 2017) as well as acute urticaria (Verma and Delfanian, 2013), irritable bowel syndromes (Poirier *et al.*, 2012; Ragavan *et al.*, 2015; El-Badry *et al.*, 2018), ulcerative colitis (Rossen *et al.*, 2015) and could facilitate the proliferation of colorectal cancer (Kumarasamy *et al.*, 2017; Padukone *et al.*, 2017). Unlike human, animal particularly cattle that were infected with *Blastocystis* sp. are commonly healthy carriers and serve as a main reservoir in transmitting the infection to human (Alfellani *et al.*, 2013b; Lee *et al.*, 2018). This was demonstrated by infection in human with close or direct contact with the infected animals (Wang *et al.*, 2014a) as well as experimental infections of chicken and rats with human isolates (Ajjampur *et al.*, 2016). High risk of *Blastocystis* sp. infection were recorded in piggery staff (*Blastocystis* ST5) (Wang *et al.*, 2014a), poultry slaughterhouse workers (*Blastocystis* ST6) (Greige *et al.*, 2018) and primate zoo-keepers (*Blastocystis* ST1) (Parkar *et al.*, 2007; 2010; Stensvold *et al.*, 2009), which reinforcing its zoonotic potential.

In Thailand, recent molecular analysis of *Blastocystis* sp. obtained from cattle revealed ST10, ST12 and ST14 of *Blastocystis* sp. in 42 samples (Udonsom *et al.*, 2018). The study also found similar subtypes in goats which suggests low host specificity due to animal to animal transmission. Whereas, in Colombia, zoonotic genotypes of *Blastocystis hominis* particularly ST1 and ST3 were found in 20 out of 25 (80%) cattles by analysis of the small subunit ribosomal RNA gene (Ramirez *et al.*, 2014). Phylogenetic analysis of this results indicated that *Blastocystis* sp. in cattle can potentially infect human. While, a recent study in China has clearly showed that there is a zoonotic transmission of *Blastocystis* sp. from cattle to human because ST3, ST4 and ST5 genotypes that were found in the cattle is well known as a pathogenic subtype in human (Zhu *et al.*, 2017; Wang *et al.*, 2018b). Nevertheless, no clear pathogenic or non-pathogenic subtype were reported in cattle. Meanwhile in Malaysia, very few studies of *Blastocystis* sp. infection involving a small scale of cattles were conducted in Perak and Zoo Negara Kuala Lumpur, which revealed rate of infection of 34.5% (Hemalata *et al.*, 2014) and 0% (Lim *et al.*, 2008) respectively. Therefore, the present study was conducted to identify the current status of *Blastocystis* sp. infection in domestic cattle from Pahang and its genotypic subtype distribution to evaluate the zoonotic potential of the cattle, which promote further understanding on the distribution of subtypes and burden of infection in cattle populations.

MATERIALS AND METHODS

Ethical Approval

The protocol of this project was approved by the Institutional Animal Care and Use Committee (IACUC) (Ref. no: IIUM/IACUC Approval/2016(12)(88)), International Islamic University of Malaysia (IIUM). Permission from the farm's owners were obtained for the collection of stool samples.

Specimen collection

A total of 120 fresh stools of randomly selected cattle below 2 years old were collected from three local farms in the district of Muazam Shah, Ulu Lepar and Cherok Paloh in Pahang, Malaysia between January 2017 until May 2018. The stool samples were collected directly from the rectum and transferred into sterile stool containers. Other than the farm management system, identification tag, age, gender, date of collection and body score condition (BSC) observation for indication of overall physical and health condition of the cattle (Anon, 1994) were recorded in this study.

All three farms were located in the rural region. The cattle in Muazam Shah farm were grazing freely in oil palm plantation and mainly dependent on natural sources of rainwater and small river for drinking. Meanwhile, feedlot system were used in the

Ulu Lepar and Cherok Paloh farms. The cattle were grouped in 3 to 5 animals per cage and separated according to age in the Ulu Lepar. Whereas in the Cherok Paloh, the cattle were not separated between ages and held in large cages consisting more than 20 cattles. Cattle in these two farms were lack in sanitation practices and facilities such as poor routine cleaning and disinfection of cages, poor drainage system as well as unprotected water and feed that lead to contamination of sewage disposals.

Three age groups were recorded during the sampling, of which 25 samples were preweaned (<3 months), 51 samples were weaning (4-12 months) and 44 samples were yearlings (13 months-24 months).

The body condition were scored as (1) thin, (2) borderline, (3) optimum, (4) overweight and (5) obese which was described the appearance of five body regions including backbone, hips and shoulder bones, ribs, tail-head area and skeletal body outline. The thin cattle were categorized by sharp backbone, while the hips, shoulder bones and ribs were clearly visible. The tail-head area appeared sunken and hollow with sharp and visible body outline. The optimum cattle condition were reported when the backbones were visible, while the hipbones and ribs were not. The tail head area were not recessed with the body outline appear round and smooth. Obese cattle condition were recorded by the appearance of solid backbones with fat deposit at the hip bones shown. The ribs area seen well rounded, while the tail-head area appeared very lumpy with the body outline bulging well and covered by fat.

Besides, the stool consistency was also recorded based on the following categories (1) none diarrheic stool, and (2) watery stool that was yellow to dark green, malodorous and/or contain blood. The specimens were stored in a polystyrene box at 4°C until further analysis conducted.

***In vitro* cultivation**

Approximately a pea size of each stool sample was inoculated into sterile screw-cap culture tube containing 6 ml of Jones' medium supplemented with heat-inactivated horse serum. Culture was incubated for 5 days in an anaerobic chamber at 37°C and sub-cultured once every three days into fresh sterile medium. Positive culture was defined by the presence of *Blastocystis* sp. forms such as cystis, vacuolar, granular and/or amoeboid forms within 5 days of cultivation upon microscopic examination.

Genomic DNA extraction and PCR sequencing

The genomic DNA was extracted directly from all stool samples using QIAamp DNA stool mini kit (QIAGEN) according to the manufacturer's protocol. A total of 80 DNA samples were subjected to PCR sequencing, which available for only 30 *Blastocystis*-positive isolates. Briefly, 200mg of stool sample was added to 1.4ml ASL lysis buffer, before the mixture was heated at 70°C and centrifuged. Then, the supernatant was collected and enzymatically digested using 15ul of Proteinase K following removal of stool contaminants and impurities. The lysate was added with 200ul of ethanol and centrifuged before subjected to washing buffer and final recollection of the DNA. The DNA yield was stored at -20°C until further used. The purified DNA obtained was in the range of 1.8 to 2.0 absorbance ratio of A260/A280.

Then, *Blastocystis* sp. specific primer pairs, RD5-forward (5'-ATCTGGTTGAT CCTGCCAGT-3') and BhRDr-reverse (5'-GAGCTTTTTAACTGCAACAACG-3') recommended by Scicluna *et al.* (2006) were used in the PCR with conditions of an initial denaturation at 94°C for 1 minute, followed by 30 cycles of 94°C for 1 minute of denaturing, 59°C for 1 minute of annealing and 72°C for 1 minute of extending. Lastly, 72°C for 2 minute of an additional final elongation cycle. The PCR products were stained by fluoroSAFE DNA stain (Invitrogen) and undergone 1.5% agarose gel electrophoresis before been visualized under ultraviolet transilluminator. The approximate 600bp PCR products were then sequenced using ABI PRISMTM 3730 DNA Analyzer (Applied Biosystems, USA), using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster, CA, USA).

Subtype identification and phylogenetic analysis

The DNA chromatograms were checked for quality using Seq scanner2 (Applied Biosystems) and then compared with published sequences in NCBI/BLAST database. The subtype was determined by closest match or similarity based on classification made by Stensvold *et al.* (2007) while the GenBank database sequences with highest percentage identity of approximately 95–100% were considered during analysis. The accession numbers of selected sequences were submitted to GenBank (MK240375 – MK240483) before confirmation of a new subtype was made by phylogenetic analysis.

Briefly, the sequences were assembled using Cluster W and edited in BioEdit v6.0 (Ibis Biosceience, USA), which subsequently been aligned in MUSCLE tool and analysed by Mega 7.1 (The Biodesign Institute, Tempe, AZ, USA). The phylogenetic tree was constructed using neighbouring joined (NL) and maximum likelihood (ML) method of Kimura2 parameter model (Tamura *et al.*, 2013). While, the branch tree was assessed using bootstrap analysis of 1000 replicates, the clusters of trees were considered valid for bootstrap support of above 50% and *Proteromonas lacertae* (U37108), *Developpayella elegans* (U37107) and *Labyrinthuloides haliotidis* (U21338) was used as the out-group.

Statistical Analysis

Statistical data were analysed using SPSS version 20. Descriptive analysis was conducted with mean and percentage of positive *Blastocystis* sp. infection in different groups of potential parameter such as age groups, gender, body score condition (BSC), diarrheic condition and farms. Statistical significant differences between *Blastocystis* sp. infection and the parameters were determined by Kruskal-Wallis test while relationship between *Blastocystis* subtypes and the parameters were determined by Spearman Correlation. Statistical significance was defined as $p<0.05$.

RESULTS

Occurrence of *Blastocystis* sp. in cattle infection

It was found that 30 out of 120 (25%) cattle were infected with *Blastocystis* sp. and vacuolar form was the dominant morphological form observed under 40x microscopic objective during cultivation with the highest growth at day 4 till 6.

Observation of potential risk factors for infection in cattle

The parameters that were considered as risk factor in the analysis such as age, gender, body score condition (BSC), diarrheic condition of the stool and condition of the farms with their corresponding rates of infection were tabulated in Table 1.

The infection was significantly higher ($p<0.001$) in cattle of age group below <3 months old (11.7%) followed by 4 to 12 months old (9.2%) and 13 to 24 months old (4.16%). The study found that gender has no significant association with the occurrence of *Blastocystis* sp. in either females (15.0%) or males (10.0%).

However, cattle with body score of more than 2 (16.7% (20/103)), which were optimum, overweight and obese have significantly ($p<0.05$) higher infection compared to thin and optimum body score of less than 2 (8.3%). The present study also observed that non-diarrheic cattle (17.5% (21/107)) have significant ($p<0.05$) higher infection compared to diarrheic cattle (7.5%).

Out of three cattle farms, 18.3% of the positive samples were detected from farm in Muazam Shah followed by 5% from farm in Ulu Lepar and 2% from farm in Cherok Paloh. Nevertheless, the difference observed in the rate of infection between the farms were significant.

Molecular characterization of *Blastocystis* sp. isolates

Out of 80 DNA samples, 35 (43.8%) DNA samples that has been successfully

Table 1. The occurrence of *Blastocystis* sp. infection in cattle from different group of categories

Total number of cattle (n)	Categories	Positive samples (n/n)	Occurrence (%)	X^2	p
120	BCS				
	<2	10/17	8.33	11.98	0.00*
	>2	20/103	16.67		
	Gender				
	Female	18/67	15.00	0.28	0.60
	Male	12/53	10.00		
	Age				
	<3 month	14/25	11.67		
	4-12 month	11/51	9.17	14.26	0.00*
	13-24 month	5/44	4.16		
	Diarrheic				
	Yes	9/13	7.50	15.09	0.00*
	No	21/107	17.5		
	Farm				
	Muazam Shah	22/70	18.33		
	Ulu Lepar	6/30	5.00	4.23	0.04*
	Cherok Paloh	2/20	2.00		

x^2: chi-square. If p-value <0.05 was considered significant.

sequenced were positive *Blastocystis* sp. and showed 95-100% similarity identity to the published referenced sequence gene. These samples also had revealed 600bp yield product bands upon gel electrophoresis confirming of its *Blastocystis* sp. isolates (Figure 1). The sequences of positive sample were constructed into genetic tree of neighboring joined (NJ) and maximum likelihood (ML) represented by Figure 2 and Figure 3 respectively. The phylogenetic analysis revealed six subtypes with *Blastocystis* ST10 (21.3%) as the predominant subtype followed by ST5 (8.8%), ST3 (7.5%), ST1 (2.5%), ST4 (2.5%) and ST14 (1.3%) (Table 2). However, no mixed subtype were detected in this study.

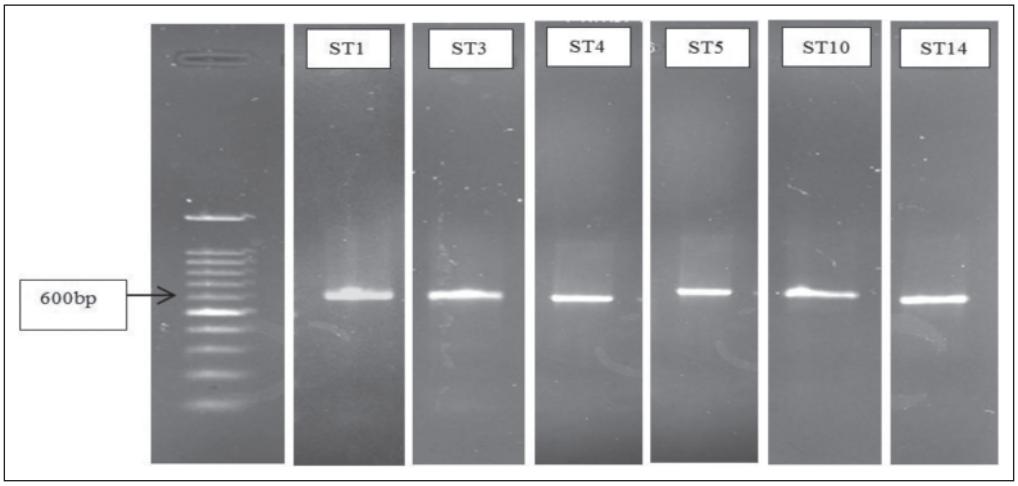

Figure 1. PCR amplication of *Blastocystis* isolates in 1.5% (w/v) gel electrophoresis: Lane 1: 100-bp DNA ladder marker, Lane 2 to 6: 600-bp *Blastocystis* subtype isolate.

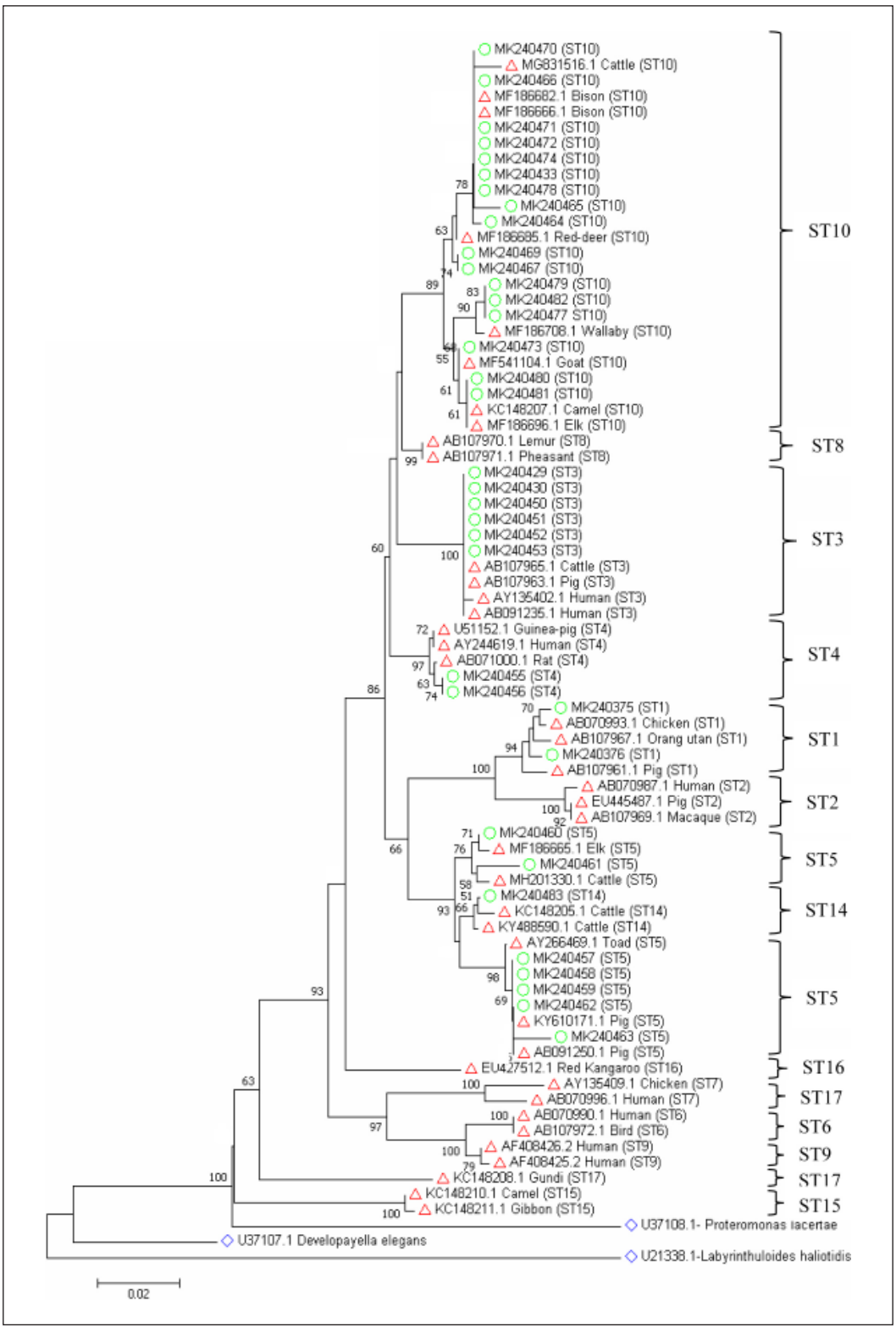

Figure 2. Phylogenetic analysis of *Blastocystis* isolates based on SSU rDNA sequence using neighbour – joining (NJ) method. Bootstraps analysis with 1000 replicates and values more than 50% were shown. O indicate sample genes, Δ indicate reference genes and ◊ indicate outgroups.

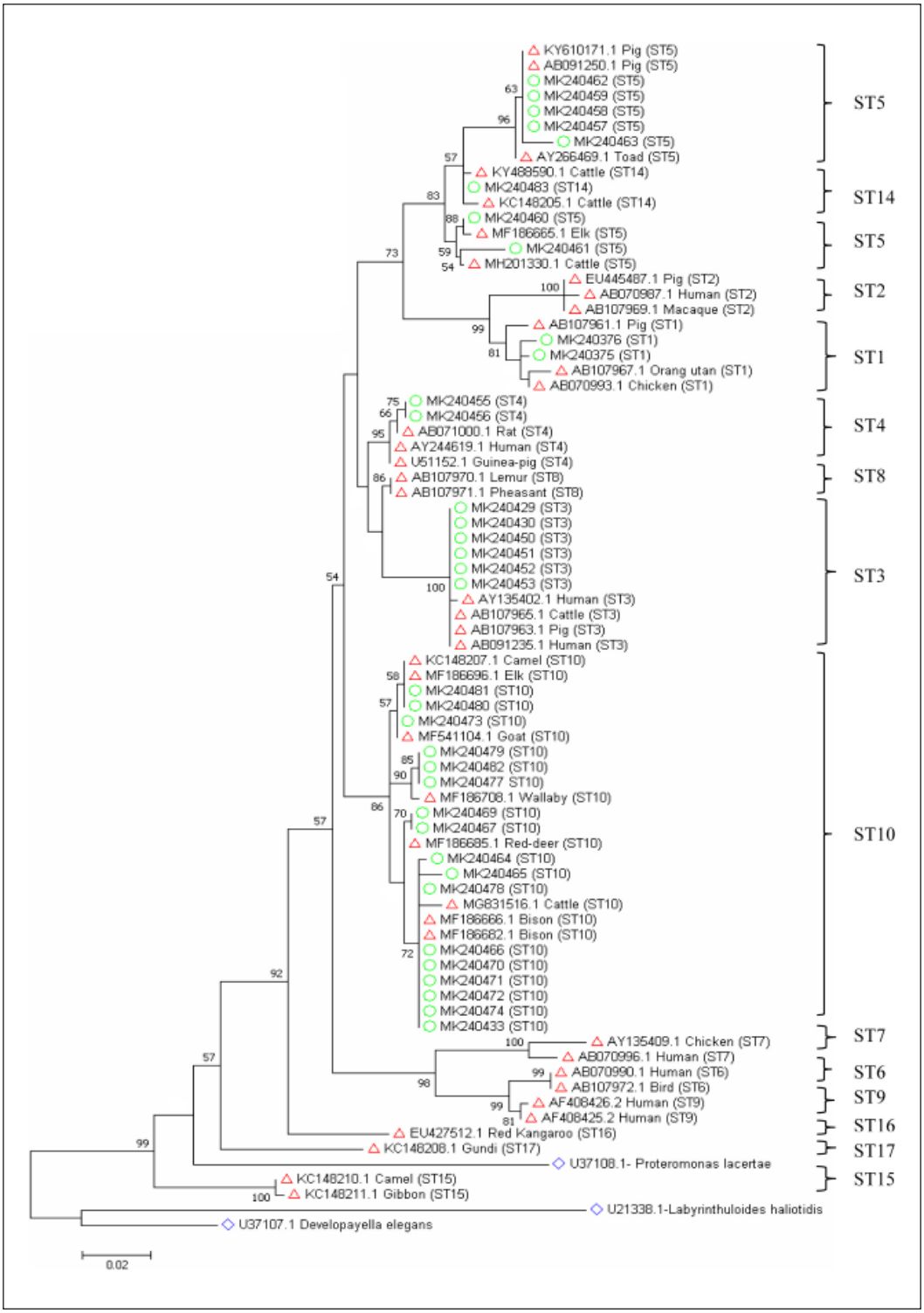

Figure 3. Phylogenetic analysis of *Blastocystis* isolates based on SSU rDNA sequence using maximum likelihood (ML) method. Bootstraps analysis with 1000 replicates and values more than 50% were shown. O indicate sample genes, Δ indicate reference genes and ◊ indicate outgroups.

Table 2. The occurrence of *Blastocystis* sp. in cattle

Total number of cattle (n)	Categories	ST1 n	ST3 n	ST4 n	ST5 n	ST10 n	ST14 n	Total n (%)	p
80	BSC								
	<2	0	1	0	0	2	0	3(3.75)	0.01*
	>2	2	5	2	7	15	1	32(40.00)	
	Gender								
	Female	1	2	0	5	9	1	18(22.50)	0.96
	Male	1	4	2	2	8	0	17(21.25)	
	Age								
	<3 month	2	6	0	1	6	0	15(18.75)	
	4-12 month	0	0	1	5	9	0	15(18.75)	0.00*
	13-24 month	0	0	1	1	2	1	5(6.25)	
	Diarrheic								
	Yes	2	3	0	0	4	0	9(11.25)	0.01*
	No	0	3	2	7	13	1	26(32.50)	
	Farm								
	Muazam Shah	2	6	2	7	7	1	25(31.25)	
	Ulu Lepar	0	0	0	0	4	0	4(5.00)	0.00*
	Cherok Paloh	0	0	0	0	6	0	6(7.50)	
Total occurrence per categories		2(2.50%)	6(7.50%)	2(2.50%)	7(8.75%)	17(21.25%)	1(1.25%)	35(43.75%)	

If p-value <0.05 was considered significant.

Subtype distribution of *Blastocystis* sp. and the potential risk factor

The association between subtype distribution and selected parameter of *Blastocystis* sp. potential risk factor for infection in cattle was presented in Table 2. The study found that the distribution of subtype was significantly related with age groups, body scores, stool consistensy and farm managements ($p<0.05$). However, the difference of subtype distribution in gender was insignificant.

DISCUSSIONS

Blastocystis sp. was discovered in human and variety of animals worldwide. However, the studies of this parasite especially in cattle were rarely been reported in Malaysia. This study presented the occurrence of *Blastocystis* sp. in cattle of below 2 years old for the first time in Pahang, Peninsular of Malaysia. The overall rate of infection was 25% (30/120) based on microscopic observation and 43.8% (35/80) based on molecular analysis, which was considered as moderate rates of *Blastocystis* sp. infection. The difference between the two results was not significant and probably due to convenience sampling of eligible DNA. The overall rate of infection in this study was higher than the recent studies namely in China with 9.5% prevalence (Wang *et al.*, 2018b), Korea with 6.7% prevalence (Lee *et al.*, 2018), USA with 19.2% prevalence (Santin *et al.*, 2011; Fayer *et al.*, 2012), Iran with 9.6% prevalence (Badparva *et al.*, 2015), UK with 22.6% prevalence (Alfellani *et al.*, 2013a) but lower than those from Colombia with 80% prevalence (Ramirez *et al.*, 2014). The differences in the reported occurrences could result from many factors such as variations of age, seasonality, and ecological environments (Zhu *et al.*, 2017). Infection of protozoa in these cattle may affect the productivity of the livestock and quality of the protein products as well as causing weightloss, growth retardation in young animals and zoonosis problems (Thompson and Smith, 2011; Maharana *et al.*, 2016). Therefore, the present study has investigated the subtype distribution of *Blastocystis* sp. and the potential factors of infection in the cattle.

Thus far, subtyping on the basis of the SSU rRNA gene has facilitated the identification of subtype distribution of the *Blastocystis* sp. in these cattle. The current study revealed six subtypes of ST1, ST3, ST4, ST5, ST10 and ST14 as contributors to the infections in farms from Pahang, Malaysia. Among those, ST10 and ST14 were solely found in cattle (Zhu *et al.*, 2017; Lee *et al.*, 2018). The other subtypes have been reported to be found in human (Ramirez *et al.*, 2016) but only ST1 and ST3 were common (Alfellani *et al.*, 2013b). Therefore, cattle was a suitable reservoir for colonization of *Blastocystis* sp. and capable of transmitting the infection to consumers and animal workers through animal products (Song *et al.*, 2017). Therefore, an appropriate measure for proper control and elimination of its distribution in livestock is crucial in order to maintain good quality of the produce.

The study found that ST10 represent the most dominant subtypes in the cattle. It has been reported as predominant in many studies involving cattle worldwide including Denmark (Stensvold *et al.*, 2009), Libya (Alfellani *et al.*, 2013a), UK (Alfellani *et al.*, 2013a) and China (Zhu *et al.*, 2017; Wang *et al.*, 2018b). This subtype were also reported to predominantly found in sheep, goats, camels, yaks, kangaroos, giraffes, bison and onyxes (Alfellani *et al.*, 2013a; Betts *et al.*, 2018; Li *et al.*, 2018) as well as cat and dog (Ruaux *et al.*, 2014; Osman *et al.*, 2015).

The other subtypes found in the study such as ST4, ST5, and ST14 were reported worldwide as commonly appeared in ruminants as well other animals including dog, rodents, birds and non-human primates, which bovine animals possess the highest frequency of infection (Zhu *et al.*, 2017; Wang *et al.*, 2018b; Lee *et al.*, 2018). Although small frequency was detected in the present study, ST3 appeared to have higher frequency than ST1 as observed in Table 2. This subtype has been documented in infections of human in many studies including Malaysia (Nithyamathi *et al.*, 2016; Kumarasamy *et al.*, 2014). The fact that bovine animals are capable of carrying pathogenic *Blastocystis* sp. isolates from human, mammals, ruminants and rodents has reinforced its potential of zoonotic transmission (Cian *et al.*, 2017), thus makes these two subtypes a public health importance.

According to the age data, the highest rate of subtype distribution was observed in cattle of age less than 3 months and the lowest rate were observed in cattle age from 13 to 24 months. Overall, cattle of below 1 year old were infected predominantly by *Blastocystis* ST10 followed by ST5 and ST14 (Table 2). A similar finding was reported by Fayer *et al.* (2012) that cattle in the age of 3 to 5 months were commonly infected with ST10 and ST14. Unlike study on subtypes distribution, studies on prevalence by Zhu *et al.* (2017) and Lee *et al.* (2018), reported low prevalence in cattle below 3 months compared with above 3 months due to maternal immunity action of the young cattle. Immunity status of the animal was apparent in the younger animal compared to older animals, especially in above 2 years old (Tan *et al.*, 2013). In humans, the infection rate is the lowest in young children but increases with age. However the rate would tends to decrease in adulthood (Beyhan *et al.*, 2015). Similarly, study in pigs revealed that the rate is higher in weaned and adult pigs than in piglets, indicating that the age of animals is an important factor (Navarro *et al.*, 2008).

The occurrence and subtype distribution of *Blastocystis* sp. infection was slightly higher in female than in male cattle. This slight difference was also observed in previous reports of *Blastocystis* sp. in cattle (Lee *et al.*, 2018) and in goats (Tan *et al.*, 2013). The difference between gender was not significant and the findings was supported by studies of Maharana *et al.* (2016) and Lee *et al.* (2018) in cattle from India and Korea respectively. However, the unbalanced number of sampling in each gender in the present study could affect the statistical results of the outcome. Nevertheless, many studies have agreed that gender could not be clarified as a factor that contributes to the distribution of infection and permits further investigation to determine the association.

Another factor considered in the study was body condition, with a scoring system was used as indicators for the nutritional need and health condition of the cattle (Matthew *et al.*, 2012). It was also intended to further isolated the animal for target selective treatment. The cattle with a body condition score of below 2 indicated a thin condition, score of 3 indicated an ideal condition while score of above 3 indicated an obese condition. Thin cattle were susceptible to infection while the obese were prone to metabolic problems (Roche *et al.*, 2013). However, the present findings revealed that cattle with ideal and obese conditions had a significantly higher subtypes distribution of *Blastocystis* sp. compared to the thin cattle. Similar results were reported by Adeyemi *et al.* (2017), which found highest infection in goat with fair and good body score condition. These results proved that despite the present of the parasite, the wellbeing of the cattle was not affected and it could be considered as natural host of *Blastocystis*.

In addition, this study found that the *Blastocystis* sp. infection were mainly from non diarrheic cattle. This finding was supported by Lee *et al.* (2018) that recorded a higher rate of *Blastocystis* sp. incidence in the normal stool of a carrier cattle than in diarrheic cattle. These results indicated that *Blastocystis* sp. infection may not certainly lead to diarrhea and therefore symptomatic. In fact, none of the infected cattle had showed signs of illnesses in the study by Fayer *et al.* (2012). Nonetheless, limited studies on clinical sign and symptom of *Blastocystis* sp. infection been reported in livestock animals. Despite the findings in animals, this parasite may induce different level of gastrointestinal symptom in human and lead to disorder or may also be asymptomatic (Ramirez *et al.*, 2014; Nieves-Ramirez *et al.*, 2018). Studies by Wang *et al.* (2014b) indicated that *Blastocystis* sp. colonized in the small and large intestine of the pigs, which showed no evidence of invasion of the epithelium as well as clinical sign of illness. Recent studies explained that this protozoan parasite that colonizes in the gut increased diversity microbiota, which promote a healthy gut of the hosts (Anderson and Stensvold, 2016).

Though the difference in the occurrence and subtype distribution of *Blastocystis* sp. between farms were significant, a higher distribution of subtype in cattle from farm in Muazam Shah was observed. It could be associated with the differences in the management practices or location of the farms because the cattle from Muazam Shah farm that practiced open-grazing harboured many different subtypes of *Blastocystis* ST1, ST3, ST4, ST5, ST10 and ST14 compared to cattles from farm that applied caged-feedlot system that were infected with only ST10 (Table 2). This finding suggest that the outcome may concern with ecological contamination including sharing of abandoned unhygienic water and food which encourage infection among the cattle (Sreekumar *et al.*, 2014; Nithyamathi *et al.*, 2014) as well as transmitted via domestic animal such as cat and dog (Moura *et al.*, 2018; Paulos *et al.*, 2018).

CONCLUSION

The moderate occurrence of *Blastocystis* sp. in cattle below 2 years in Pahang, Malaysia was detected and subtype distribution related to the zoonotic transmission were identified in these cattle. The findings indicated that factors such as age, body score and diarrheic condition of the cattle had influence on the rate of infection. Furthermore, the frequency of infection could be associated with the husbandry farm management. Therefore, future studies should focus on the effect of other potential factors such as domestic host, animal handler and ecological for better understanding of the transmission and managements of the parasite. Meanwhile, health education activities could be enforced by veterinarians, physician, and public health to increase awareness of this parasite among high risk population especially among individuals who work closely with animals.

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REFERENCES

Adeyemi, M.T., Morenikeji, O.A., Emikpe, B.O. & Jarikre, T.A. (2017). Interactions between gastrointestinal parasitism and pneumonia in nigerian goats. *Journal of Parasitic Diseases : Official Organ of The Indian Society For Parasitology* **41**(3): 726-733.

Ajjampur, S.S., Png, C.W., Chia, W.N., Zhang, Y. & Tan, K.S. (2016). *Ex vivo* and *in vivo* mice models to study *Blastocystis* spp. adhesion, colonization and pathology: closer to proving koch's postulates. *Plos One* **11**(8): e0160458.

Alfellani, M.A., Stensvold, C.R., Vidal-Lapiedra, A., Onuoha, E.S.U., Fagbenro-Beyioku, A.F. & Clark, C.G. (2013a). Variable geographic distribution of *Blastocystis* subtypes and its potential implications. *Acta Tropica* **126**(1): 11-18.

Alfellani, M.A., Taner-Mulla, D., Jacob, A.S., Imeede, C.A., Yoshikawa, H., Stensvold, C.R. & Clark, C.G. (2013b). Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist* **164**(4): 497-509.

Andersen, L.O. & Stensvold, C.R. (2016). *Blastocystis* in health and disease: are we moving from a clinical to a public health perspective? *Journal of Clinical Microbiology* **54**(3): 524-528.

Anon. (1994). Cattle body condition scoring. Available at:http://www.dpi.qld.gov.au/documents/Biosecurity_GeneralAnimal HealthPests And Diseases/Animal-HD-Investigation-Condition-scores.pdf (Accessed: 10th August 2011)

Anuar, T.S., Ghani, M.K., Azreen, S.N., Salleh, F.M. & Moktar, N. (2013). *Blastocystis* infection in Malaysia: evidence of waterborne and human-to-human transmissions among the proto-malay, negrito and senoi tribes of orang asli. *Parasites & Vectors* **6**: 40.

Audebert, C., Even, G., Cian, A., Loywick, A., Merlin, S., Viscogliosi, E. & Chabé, M. (2016). Colonization with the enteric protozoa *Blastocystis* is associated with increased diversity of human gut bacterial microbiota. *Scientific Reports* **6**: 25255.

Badparva, E., Sadraee, J. & Kheirandish, F. (2015). Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. *Jundishapur Journal Microbiology* **8**(3): e14810.

Bart, A., Wentink-Bonnema, E.M., Gilis, H., Verhaar, N., Wassenaar, C.J., van Vugt, M., Goorhuis, A. & van Gool, T. (2013). Diagnosis and subtype analysis of *Blastocystis* sp. in 442 patients in a hospital setting in the Netherlands. *BMC Infectious Diseases* **13**: 389.

Betts, E.L., Gentekaki, E., Thomasz, A., Breakell, V., Carpenter, A.I. & Tsaousis, A.D. (2018). Genetic diversity of *Blastocystis* in non-primate animals. *Parasitology* **145**(9): 1228-1234.

Beyhan, Y.E., Yilmaz, H., Cengiz, Z.T. & Ekici, A. (2015). Clinical significance and prevalence of *Blastocystis hominis* in Van, Turkey. *Saudi Medical Journal* **36**(9): 1118-1121.

Cian, A., El Safadi, D., Osman, M., Moriniere, R., Gantois, N., Benamrouz-Vanneste, S., Delgado-Viscogliosi, P., Guyot, K., Li, L.L., Monchy, S., Noel, C., Poirier, P., Nourrisson, C., Wawrzyniak, I., Delbac, F., Bosc, S., Chabe, M., Petit, T., Certad, G. & Viscogliosi, E. 2017. Molecular epidemiology of *Blastocystis* sp. in various animal groups from two French Zoos and evaluation of potential zoonotic risk. *Plos One* **12**: e0169659.

El Safadi, D., Cian, A., Nourrisson, C., Pereira, B., Morelle, C., Bastien, P., Bellanger, A.P., 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. (2016). Prevalence, risk factors for infection and subtype distribution of the intestinal parasite *Blastocystis* sp. from a large-scale multi-center study in France. *BMC Infectious Diseases* **16**(1): 451.

El Safadi, D., Gaayeb, L., Meloni, D., Cian, A., Poirier, P., Wawrzyniak, I., Delbac, F., Dabboussi, F., Delhaes, L., Seck, M., Hamza, M., Riveau, G. & Viscogliosi, E. (2014). Children of Senegal River Basin show The highest prevalence of *Blastocystis* sp. ever observed worldwide. *BMC Infectious Diseases* **14**: 164.

El-Badry, A.A., Abd El Wahab, W.M., Hamdy, D.A. & Aboud, A. (2018). *Blastocystis* subtypes isolated from irritable bowel syndrome patients and co-infection with *Helicobacter pylori*. *Parasitology Research* **117**(1): 127-137.

Fayer, R., Santin, M. & Macarisin, D. (2012). Detection of concurrent infection of dairy cattle with *Blastocystis*, *Cryptosporidium*, *Giardia*, and *Enterocytozoon* by molecular and microscopic methods. *Parasitology Research* **111**(3): 1349-1355.

Forsell, J., Bengtsson-Palme, J., Angelin, M., Johansson, A., Evengård, B. & Granlund, M. (2017). The relation between *Blastocystis* and the intestinal microbiota in Swedish travellers. *BMC Microbiology* **17**(1): 231.

Forsell, J., Granlund, M., Samuelsson, L., Koskiniemi, S., Edebro, H. & Evengård, B. (2016). High occurrence of *Blastocystis* sp. subtypes 1-3 and *Giardia* intestinalis assemblage B among patients in Zanzibar, Tanzania. *Parasites & Vectors* **9**(1): 370.

Greige, S., El Safadi, D., Bécu, N., Gantois, N., Pereira, B., Chabé, M., Benamrouz-Vanneste, S., Certad, G., El Hage, R., Chemaly, M., Hamze, M. & Viscogliosi, E. (2018). Prevalence and subtype distribution of *Blastocystis* sp. isolates from poultry in Lebanon and evidence of zoonotic potential. *Parasites & Vectors* **11**(1): 389.

Hemalatha, C., Chandrawathani, P., Suresh, K.G., Premaalatha, B., Geethamalar, S., Lily Rozita, M.H., Farah Haziqah, M.T., Sabapathy, D. & Ramlan, M. (2014). The diagnosis of *Blastocystis* sp. from animals-an emerging zoonosis. *Malaysian Journal of Veterinary Research* **5**(2): 15-22.

Hirata, T., Nakamura, H., Kinjo, N., Hokama, A., Kinjo, F., Yamane, N. & Fujita, J. (2007). Prevalence of *Blastocystis hominis* and *Strongyloides stercoralis* infection in Okinawa, Japan. *Parasitology Research* **101**(6): 1717-1719.

Ithoi, I., Jali, A., Mak, J.W., Wan Sulaiman, W.Y. & Mahmud, R. (2011). Occurrence of *Blastocystis* in water of two rivers from recreational areas in Malaysia. *Journal of Parasitology Research* 123916.

Klous, G., Huss, A., Heederik, D.J.J. & Coutinho, R.A. (2016). Human-livestock contacts and their relationship to transmission of zoonotic pathogens, a systematic review of literature. *One Health*.

Krogsgaard, L.R., Engsbro, A.L., Stensvold, C.R., Nielsen, H.V. & Bytzer, P. (2015). The prevalence of intestinal parasites is not greater among individuals with irritable bowel syndrome: a population-based case-control study. *Clinical Gastroenterology and Hepatology* **13**(3): 507-513.

Kumarasamy, V., Kuppusamy, U.R., Jayalakshmi, P., Samudi, C., Ragavan, N.D. & Kumar, S. (2017). Exacerbation of colon carcinogenesis by *Blastocystis* sp.. *Plos One* **12**(8): e0183097.

Lee, H., Lee, S.H., Seo, M.G., Kim, H.Y., Kim, J.W., Lee, Y.R., Kim, J.H., Kwon, O.D. & Kwak, D. (2018). Occurrence and genetic diversity of *Blastocystis* in Korean cattle. *Veterinary Parasitology* **258**: 70-73.

Li, W.C., Wang, K. & Gu, Y. (2018). Occurrence of *Blastocystis* sp. and *Pentatrichomonas* hominis in sheep and goats in China. *Parasites & Vectors* **11**(1): 93.

Lim, Y.A.L., Ngui, R., Shukri, J., Rohela, M. & Mat Naim, H.R. (2008). Intestinal parasites in various animals at a zoo in Malaysia. *Veterinary Parasitology* **157**(1-2): 154-159.

Maharana, B.R., Kumar, B., Sudhakar, N.R., Behera, S.K. & Patbandha, T.K. (2015). Prevalence of gastrointestinal parasites in bovines in and around Junagadh (Gujarat). *Journal of Parasitic Diseases: Official Organ of The Indian Society for Parasitology* **40**(4): 1174-1178.

Matthews, L.R., Cameron, C., Sheahan, A.J., Kolver, E.S. & Roche, J.R. (2012). associations among dairy cow body condition and welfare-associated behavioral traits. *Journal of Diary Science* **95**(5): 2595-601.

Moura, R.G.F., Oliveira-Silva, M.B., Pedrosa, A.L., Nascentes, G.A.N. & Cabrine-Santos, M. (2018). Occurrence of *Blastocystis* spp. in domestic animals in Triângulo Mineiro area of Brazil. *Revista da Sociedade Brasileira de Medicina Tropical* **51**(2): 240-243.

Mohammad, N.A., Al-Mekhlafi, H.M. & Anuar, T.S. (2018). Subtype distribution of *Blastocystis* isolated from humans and associated animals in an indigenous community with poor hygiene in Peninsular Malaysia. *Tropical Biomedicine* **35**(4): 849-860.

Navarro, C., Domínguez-Márquez, M.V., Garijo-Toledo, M.M., Vega-García, S., Fernández-Barredo, S., Pérez-Gracia, M.T., García, A., Borrás, R. & Gómez-Muñoz, M.T. (2008). High prevalence of *Blastocystis* sp. in pigs reared under intensive growing systems: frequency of ribotypes and associated risk factors. *Veterinary Parasitology* **153**(3-4): 347-358.

Nieves-Ramírez, M.E., Partida-Rodríguez, O., Laforest-Lapointe, I., Reynolds, L.A., Brown, E.M., Valdez-Salazar, A., Morán-Silva, P., Rojas-Velázquez, L., Morien, E., Parfrey, L.W., Jin, M., Walter, J., Torres, J., Arrieta, M.C., Ximénez-García, C. & Finlay, B.B. (2018). Asymptomatic intestinal colonization with protist *Blastocystis* is strongly associated with distinct microbiome ecological patterns. *mSystems* **3**(3): e00007-18.

Nithyamathi, K., Chandramathi, S. & Kumar, S. (2016). Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. *Plos One* **11**(2): e0136709.

Osman, M., Bories, J., El Safadi, D., Poirel, M.T., Gantois, N., Benamrouz-Vanneste, S., Delhaes, L., Hugonnard, M., Certad, G., Zenner, L. & Viscogliosi, E. (2015). Prevalence and genetic diversity of the intestinal parasites *Blastocystis* sp. and *Cryptosporidium* spp. in household dogs in france and evaluation of zoonotic transmission risk. *Veterinary Parasitology* **214**: 167-170.

Padukone, S., Mandal, J. & Parija, S.C. (2017). Severe *Blastocystis* subtype 3 infection in a patient with colorectal cancer. *Tropical Parasitology* **7**: 122-124.

Parkar, U., Traub, R.J., Kumar, S., Mungthin, M., Vitali, S., Leelayoova, S., Morris, K. & Thompson, R.C. (2007). Direct characterization of *Blastocystis* from faeces by PCR and evidence of zoonotic potential. *Parasitology* **134**(Pt 3): 359-367.

Parkar, U., Traub, R.J., Vitali, S., Elliot, A., Levecke, B., Robertson, I., Geurden, T., Steele, J., Drake, B. & Thompson, R.C. (2010). Molecular characterization of *Blastocystis* isolates from zoo animals and their animal-keepers. *Veterinary Parasitology* **169**: 8-17.

Paulos, S., Köster, P.C., de Lucio, A., Hernández-de-Mingo, M., Cardona, G.A., Fernández-Crespo, J.C., Stensvold, C.R. & Carmena, D. (2018). Occurrence and subtype distribution of *Blastocystis* sp. in humans, dogs and cats sharing household in Northern Spain and assessment of zoonotic transmission risk. *Zoonoses Public Health* **65**(8): 993-1002.

Petrasova, J., Uzlikova, M., Kostka, M., Petrzelkova, K.J., Huffman, M.A. & Modry, D. (2011). Diversity and host specificity of *Blastocystis* in Syntopic Primates on Rubondo Island, Tanzania. *International Journal for Parasitology* **41**: 1113-1120.

Poirier, P., Wawrzyniak, I., Vivarès, C.P., Delbac, F. & El Alaoui, H. (2012). New insights into *Blastocystis* spp.: a potential link with irritable bowel syndrome. *Plos Pathogens* **8**(3): 1-4.

Popruk, S., Udonsom, R., Koompapong, K., Mahittikorn, A., Kusolsuk, T., Ruangsittichai, J. & Palasuwan, A. (2015). Subtype distribution of *Blastocystis* in Thai-Myanmar border, Thailand. *The Korean Journal of Parasitology* **53**(1): 13-19.

Ragavan, N.D., Kumar, S., Chye, T.T., Mahadeva, S. & Shiaw-Hooi, H. (2015). *Blastocystis* sp. in irritable bowel syndrome (ibs)-detection in stool aspirates during colonoscopy. *Plos One* **10**(9): e0121173.

Ramírez, J.D., Flórez, C., Olivera, M., Bernal, M.C. & Giraldo, J.C. (2017). *Blastocystis* subtyping and its association with intestinal parasites in children from different geographical regions of Colombia. *Plos One* **12**(2): e0172586.

Ramirez, J.D., Sanchez, A., Hernandez, C., Florez, C., Bernal, M.C., Giraldo, J.C., Reyes, P., Lopez, M.C., Garcia, L., Cooper, P.J., Vicuna, Y., Mongi, F. & Casero, R.D. (2016). Geographic distribution of human *Blastocystis* subtypes in South America. *Infection, Genetics and Evolution* **41**: 32-35.

Ramirez, J.D., Sanchez, L.V., Bautista, D.C., Corredor, A.F., Florez, A.C. & Stensvold, C.R. (2014). *Blastocystis* subtypes detected in humans and animals from Colombia. *Infection, Genetics and Evolution* **22**: 223-228.

Roche, J.R., Kay, J.K., Friggens, N.C., Loor, J.J. & Berry, D.P. (2013). Assessing and managing body condition score for the prevention of metabolic disease in dairy cows. *Vetetrinary Clinics of North America: Food Animal Practice* **29**(2): 323-36.

Rossen, N.G., Bart, A., Verhaar, N., van Nood, E., Kootte, R., de Groot, P.F., D' Haens, G.R. Ponsioen, C.Y. & van Gool, T. (2015). Low prevalence of *Blastocystis* sp. in active ulcerative colitis patients. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of The European Society of Clinical Microbiology* **34**(5): 1039-1044.

Ruaux, C.G. & Stang, B.V. (2014). Prevalence of *Blastocystis* in shelter-resident and client-owned companion animals in the US Pacific Northwest. *Plos One* **9**: e107496.

Santin, M., Gomez-Munoz, M.T., Solano-Aguilar, G. & Fayer, R. (2011). Development of a new PCR protocol to detect and subtype *Blastocystis* spp. from humans and animals. *Parasitology Research* **109**: 205-212.

Scanlan, P.D., Stensvold, C.R. & Cotter, P.D. (2015). Development and application of a *Blastocystis* subtype-specific PCR assay reveals that mixed-subtype infections are common in a healthy human population. *Applied and Environmental Microbiology* **81**(12): 4071-4076.

Scicluna, S.M., Tawari, B. & Clark, C.G. (2006). DNA barcoding of *Blastocystis*. *Protist* **157**(1): 77-85.

Seguí, R., Klisiowicz, D., Oishi, C.Y., Toledo, R., Esteban, J.G. & Muñoz-Antoli, C. (2017). Intestinal symptoms and *Blastocystis* load in schoolchildren of Paranaguá Bay, Paraná, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo* **59**: e86.

Song, J.K., Yin, Y.L., Yuan, Y.J., Tang, H., Ren, G.J., Zhang, H.J., Li, Z.X., Zhang, Y.M. & Zhao, G.H. (2017). First genotyping of *Blastocystis* sp. in dairy, meat, and cashmere goats in Northwestern China. *Acta Tropica* **176**: 277-282.

Sreekumar, C., Selvaraj, J., Gomathinayagam, S., Thangapandiyan, M., Ravikumar, G., Roy, P. & Balachandran, C. (2013). *Blastocystis* sp. from food animals in India. *Journal of Parasitic Diseases: Official Organ of The Indian Society for Parasitology* **38**(4): 440-443.

Stensvold, C.R., Arendrup, M.C., Jespersgaard, C., Mølbak, K. & Nielsen, H.V. (2007). Detecting *Blastocystis* using parasitologic and DNA-based methods: a comparative study. *Diagnostic Microbiology and Infectious Disease* **59**(3): 303-307.

Stensvold, C.R., Alfellani, M.A., Norskov-Lauritsen, S., Prip, K., Victory, E.L., Maddox, C., Nielsen, H.V. & Clark, C.G. (2009). Subtype distribution of *Blastocystis* isolates from synanthropic and zoo animals and identification of a new subtype. *International Journal for Parasitology* **39**(4): 473-479.

Stensvold, C.R., Christiansen, D.B., Olsen, K.E. & Nielsen, H.V. (2011). *Blastocystis* sp. subtype 4 is common in Danish *Blastocystis*-positive patients pre-

senting with acute diarrhea. *The American Journal of Tropical Medicine and Hygiene* **84**(6): 883-885.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**: 2731-2739.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* **30**: 2725-2729.

Tan, T.C., Tan, P.C., Sharma, R., Sugnaseelan, S. & Suresh, K.G. (2013). Genetic diversity of caprine *Blastocystis* from Peninsular Malaysia. *Parasitology Research* **112**(1): 85-859.

Thompson, R.C.A. & Smith, A. (2011). Zoonotic enteric Protozoa. *Veterinary Parasitology* **182**(1): 70-78.

Udonsom, R., Prasertbun, R., Mahittikorn, A., Mori, H., Changbunjong, T., Komalamisra, C., Pintong, A.R., Sukthana, Y. & Popruk, S. (2018). *Blastocystis* infection and subtype distribution in humans, cattle, goats, and pigs in Central and Western Thailand. *Infection, Genetics and Evolution* **65**: 107-111.

Verma, R. & Delfanian, K. (2013). *Blastocystis hominis* associated acute urticaria. *The American of Journal of The Medical Sciences* **346**(1): 80-1.

Wang, J., Gong, B., Liu, X., Zhao, W., Bu, T., Zhang, W., Liu, A. & Yang, F. (2018a). Distribution and genetic diversity of *Blastocystis* subtypes in various mammal and bird species in Northeastern China. *Parasites & Vectors* **11**(1): 522.

Wang, J., Gong, B., Yang, F., Zhang, W., Zheng, Y. & Liu, A. (2018b). Subtype distribution and genetic characterizations of *Blastocystis* in pigs, cattle, sheep and goats in Northeastern China's Heilongjiang Province. *Infection, Genetics and Evolution* **57**: 171-176.

Wang, W., Bielefeldt-Ohmann, H., Traub, R.J., Cuttell, L. & Owen, H. (2014b). Location and pathogenic potentially of *Blastocystis* in the porcine intestine. *Plos One* **9**(8): e103962.

Wang, W., Cuttell, L., Traub, R.J., Owen, H. & Bielefeldt-Ohmann, H. (2014c). Characterization of the *Blastocystis*-specific faecal IgA immune response in pigs. *Parasite Immunology* **36**(10): 503-508.

Wang, W., Owen, H., Traub, R.J., Cuttell, L., Inpankaew, T. & Bielefeldt-Ohmann, H. (2014a). Molecular epidemiology of *Blastocystis* in pigs and their in-contact humans in Southeast Queensland, Australia, and Cambodia. *Veterinary Parasitology* **203**(3-4): 264-269.

Wong, K.H.S., Ng, G.C., Lin, R.T.P., Yoshikawa, H., Taylor, M.B. & Tan, K.S.W. (2008). Predominance of subtype 3 among *Blastocystis* isolates from a major hospital in Singapore. *Parasitology Research* **102**(4): 663-670.

Zhang, X., Qiao, J.Y., Zhou, X.J., Yao, F.R. & Wei, Z.C. 2007. Morphology and reproductive mode of *Blastocystis hominis* in diarrhea and *in vitro*. *Parasitology Research* **101**: 43-51.

Zhang, X., Zhang, S., Qiao, J., Wu, X., Zhao, L., Liu, Y. & Fan, X. (2012). Ultrastructural insights into morphology and reproductive mode of *Blastocystis* hominis. *Parasitology Research* **110**(3): 1165-1172.

Zhu, W., Tao, W., Gong, B., Yang, H., Li, Y., Song, M., Lu, Y. & Li, W. (2016). First report of *Blastocystis* infections in cattle in China. *Veterinary Parasitology* **246**: 38-42.