



RESEARCH ARTICLE

Blastocystis subtypes in ruminant livestock from Perak and assessment of zoonotic transmission risks from livestock in Peninsular Malaysia

Rauff-Adedotun, A.A.¹, Lee, I.L.², Bathmanaban, P.³, Yahaya, Z.S.⁴, Farah Haziqah, M.T.^{4*}

¹Department of Public Health, North-Eastern University, PMB 0198, Gombe, Gombe State, Nigeria

²Kulliyah of Medicine & Health Sciences, Universiti Islam Antarabangsa Sultan Abdul Halim Mu'adzam Shah, 09300 Kuala Ketil, Malaysia

³Veterinary Research Institute, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

⁴School of Biological Sciences, Universiti Sains Malaysia, 11800 USM Penang, Malaysia

*Corresponding author: farahhaziqah@usm.my

ARTICLE HISTORY

Received: 3 January 2025

Revised: 19 February 2025

Accepted: 20 February 2025

Published: 30 June 2025

ABSTRACT

Blastocystis is a ubiquitous intestinal protist of humans and animals. It is a genetically diverse organism whose part in the health and disease is still uncertain. This study provides information on *Blastocystis* STs in cattle, goats, and sheep in Perak, Malaysia, and the likely role of livestock animals in *Blastocystis* transmission to humans in Malaysia. Faecal samples from a total of 151 livestock animals consisting of cattle, goats, and sheep from Perak were examined by PCR analysis of the barcode region. *Blastocystis* ST10, ST14, and ST21 were identified in this study, and ST10 was common to all three ruminant livestock animal groups involved. Findings from previously published studies on *Blastocystis* in ruminant and non-ruminant livestock animals in Malaysia support indications that livestock animals may serve as reservoirs of human infections, being as one or more of the following *Blastocystis* subtypes: ST1, ST2, ST3, ST4, ST5, and ST6, have been isolated from both humans and livestock animals within similar regions of the country. Animal handlers are, therefore, advised to exercise proper hygiene to prevent possible transmission of *Blastocystis* from their animals, while further studies on the genetic variants of *Blastocystis* in farm animals and their keepers are required to better understand the role of these animals in human *Blastocystis*.

Keywords: Cattle; goat; Malaysia; sheep; transmission.

INTRODUCTION

Blastocystis is a single-celled Stramenopile inhabiting the intestinal tract of humans and a wide range of animal species (Hublin *et al.* 2021; Popruk *et al.* 2021; Barati *et al.* 2022; Sanggari *et al.* 2022). It is a ubiquitous protist whose transmission is believed to be mainly by the oral-faecal route, either by direct contact with an infected host or indirectly by ingesting faecal-contaminated food or water (Attah *et al.*, 2023; Maloney *et al.*, 2023). The role of *Blastocystis* in its host gut is still widely debated because of its association with gastrointestinal disorders as well as healthy gut microbiota (Stensvold *et al.*, 2020; Higuera *et al.*, 2023). *Blastocystis* exhibits extensive genetic diversity and, at least, 42 genetic variants (subtypes) of the small subunit ribosomal RNA (SSU rRNA) gene have been identified in mammals and birds (Maloney *et al.*, 2023; Satin *et al.*, 2024). Although some *Blastocystis* subtypes (STs) are adapted to humans or specific animal species, some STs have been isolated from both humans and animals, thereby, suggesting the possibility of zoonotic transmission of the protist (Ramirez *et al.*, 2016; Asghari *et al.*, 2021).

The colonization of livestock animals with *Blastocystis* has been detected universally regardless of age, breed, location or breeding system (Hublin *et al.*, 2021; Shams *et al.*, 2021; Salehi *et al.*, 2022). Livestock animals are important for nutritional and economic

purposes and are in constant contact with humans such as farmers and processors. In Malaysia, only a few studies have described *Blastocystis* in ruminant livestock animals (Tan *et al.*, 2013; Noradilah *et al.*, 2017a; Mohammad *et al.*, 2018a, 2018c; Kamaruddin *et al.*, 2020; Rauff-Adedotun *et al.*, 2023), and there has not been any report on *Blastocystis* subtype identification in livestock animals in Perak state. Besides, there is limited data on *Blastocystis* and its subtypes in goats and sheep generally. Information on *Blastocystis* STs in diverse hosts provides a baseline for understanding the distribution and transmission of the gastrointestinal symbiont. Investigation of shared *Blastocystis* variants between humans and other animal species will improve the knowledge of the possible role of such animal species as reservoirs in the transmission of *Blastocystis* to humans. Thus, this study aims to describe the subtypes of *Blastocystis* sp. in cattle, goats, and sheep from the Perak state of Malaysia and to examine the possible role of livestock animals in the zoonotic transmission of *Blastocystis* in Malaysia.

MATERIALS AND METHODS

Ethical approval

Ethical approval for animal use and permission for sampling activities were granted by the Universiti Sains Malaysia Institutional Animal

Care and Use Committee (USM IACUC) and the Department of Veterinary Services (DVS), Ministry of Agriculture and Agro-based Industry Malaysia.

Sample collection

A total of 151 randomly selected faecal samples from cattle, goats, and sheep that were deposited at the Veterinary Research Institute (VRI) in Perak from different parts of the state (Figure 1) were examined in this study. The faecal samples were collected directly from the rectum of the ruminant livestock animals or carefully from the ground after being freshly voided. Samples from each animal were transferred into a labelled stool container and stored at -20°C until molecular analysis.

DNA extraction and DNA barcoding

DNA was extracted from the faecal samples using the Nucleospin DNA Stool Kit (Macherey-Nagel, Germany) following the manufacturer's protocol. Approximately 60-80 mg of each faecal sample was used, with DNA eluted in 30 µL of elution buffer and stored at -20°C until PCR analysis. The extracted DNA underwent DNA barcoding through polymerase chain reaction (PCR) amplification of the barcode region of the small subunit ribosomal RNA (SSU rRNA) gene of *Blastocystis*, using the primers, PCR conditions and gel electrophoresis were performed as previously described by Rauff-Adedotun *et al.* (2023).

Subtyping and phylogenetic analysis

For *Blastocystis* subtype and allele determination, the obtained SSU rDNA sequences were compared with GenBank sequences using the Basic Local Alignment Search Tool (BLAST) (<https://blast.ncbi.nlm.nih.gov/BLAST/>) and the *Blastocystis* (18S) and Sequence Typing (MLST) databases (<https://pubmlst.org/Blastocystis/>) hosted at the University of Oxford (Jolley *et al.*, 2018). Phylogenetic analysis was conducted using Mega 11 software (<http://www.megasoftware.net/>) (Tamura *et al.*, 2021). The nucleotide sequences obtained in this study, along with full-length reference nucleotide sequences for accepted *Blastocystis* sp. subtypes from the reference database

(<http://entamoeba.lshrm.ac.uk/blastorefseqs.htm>) and GenBank were aligned with the complete SSU rRNA gene sequence of *Proteromonas lacerate* (U37108) as the outgroup. Multiple sequence alignment was performed using the Muscle algorithm, and the alignment was trimmed. Phylogenetic trees were reconstructed using the Neighbour-Joining (NJ) and Maximum-Likelihood (ML) methods, with bootstrap analysis (1000 replicates) to assess the reliability of the trees. Newly generated nucleotide sequences of the barcoding region of *Blastocystis* sp. SSU rRNA gene obtained in this survey were deposited in GenBank under the accession numbers ON738373, ON738383, ON738384, ON738405 - ON738408, and ON738419 - ON738422.

Database search

Previous studies reporting *Blastocystis* STs in humans and various livestock animals (both ruminant and non-ruminant) were retrieved from Google Scholar, PubMed, and ScienceDirect. The keywords used included: *Blastocystis*, STs, subtypes, molecular characterization, genetic diversity, poultry, ruminant, livestock, humans, and Malaysia. After removing duplicate articles, a total of 16 articles were obtained, covering Pahang, Penang, Johor, Kedah, Kuala Lumpur, Negeri Sembilan, and Selangor. Information such as the study location, host species, and identified subtypes were extracted from these articles.

Zoonotic risk from livestock animals in Malaysia was assessed by comparing *Blastocystis* subtypes reported in humans with those reported in livestock animals from previous studies.

RESULTS

Blastocystis subtypes and alleles

Of the 151 faecal samples screened by PCR amplification of the barcode region of the SSU rRNA gene, 11 isolates were confirmed to be *Blastocystis* positive based on NCBI BLAST results for the obtained nucleotide sequences.

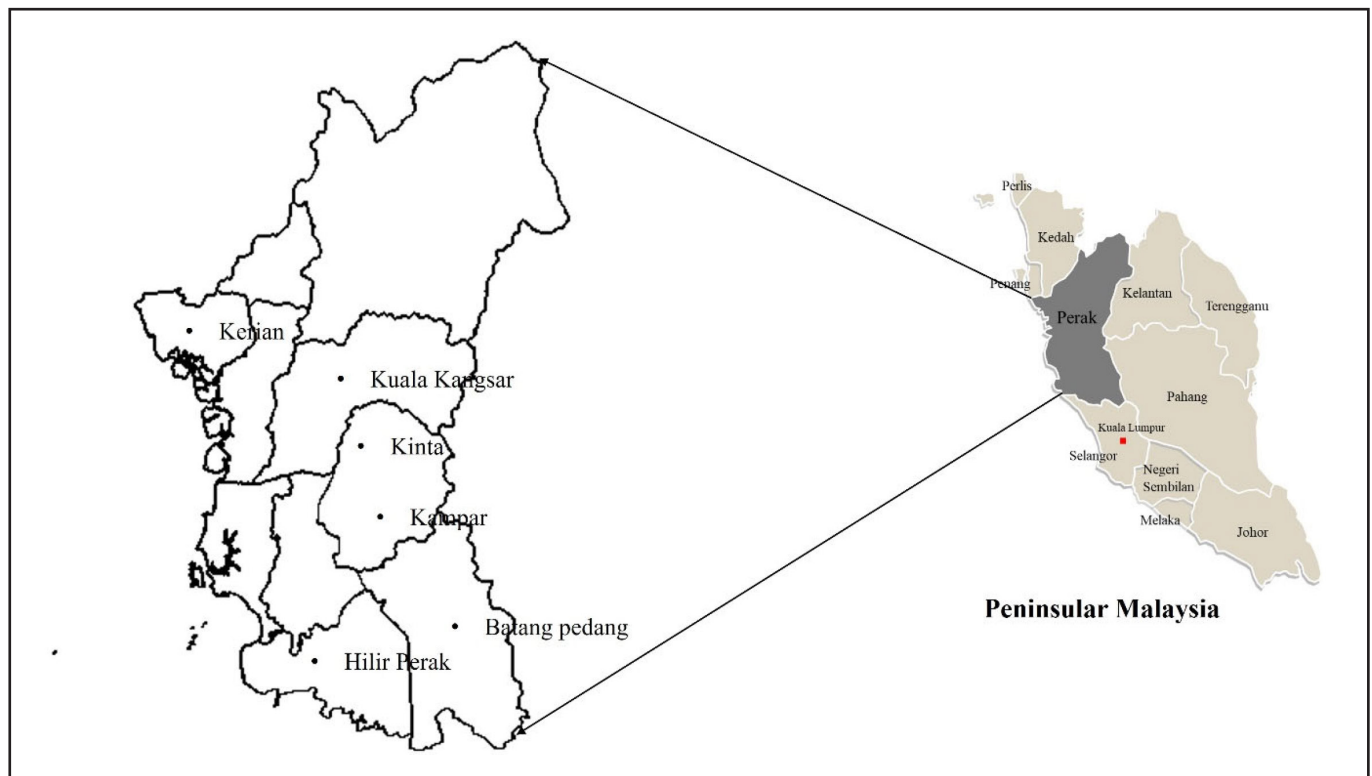


Figure 1. Map of Perak showing sampling locations.

Based on the NCBI nucleotide BLAST results, *Blastocystis* subtypes identified from the three ruminant livestock animal groups involved in this study were ST10 (72.7%; 8/11), ST14 (9.1%, 1/11), and ST21 (18.2%; 2/11). *Blastocystis* ST10 (33.3%; 1/3) and ST21 (66.7%; 2/3) were identified in cattle, and ST10 (75%; 3/4) and ST14 (25%; 1/4) were detected in sheep, while ST10 (100%; 4/4) was observed in goats (Table 1). ST10 was identified in all three ruminant livestock animal groups involved in this study, and was the only subtype observed in goats. *Blastocystis* ST14 was isolated from sheep only, while ST21 was identified in cattle alone. Overall, *Blastocystis* ST10 was predominant in the livestock animals sampled in Perak.

Table 1. Subtype distribution of *Blastocystis* sp. in livestock animals from Perak, Malaysia

Host	<i>Blastocystis</i> subtype		
	ST14	ST10	ST21
Cattle (<i>Bos taurus</i>)	–	1	2
Goat (<i>Capra hircus</i>)	–	4	–
Sheep (<i>Ovis aries</i>)	1	3	–
Total	1	8	2

Blastocystis 18S allele calling for all 11 isolates identified as *Blastocystis* by NCBI nucleotide BLAST was carried out using the *Blastocystis* 18S online database. An exact match was obtained for ST10 isolate from cattle only, which was identified as allele 43 (Figure 2).

Phylogenetic analysis

In the NJ and ML trees constructed (Figures 3 and 4, respectively), the isolates from this study belonging to the same subtype clustered together and with previously recognized subtypes. However, both NJ and ML trees showed that only the ST14 isolate from this study formed a clade with an ST24 reference sequence, with all ST14, ST24, and ST25 sequences nested together. Similarly, the ST23 reference sequence nested with the ST10 sequences.

Previous studies on *Blastocystis* in humans and livestock animals in Malaysia and evaluation of zoonotic risk

From a literature search, articles were found documenting the STs of *Blastocystis* in humans and one or more livestock animal groups from Perak, Pahang, and Selangor (Kuala Lumpur included). However, reports on *Blastocystis* STs of humans alone (none on any livestock animal) were obtained from Johor and Kedah; while reports from Penang were from livestock animals only (none on humans).

Livestock animals studied included cattle, goat, sheep, deer, quail, chicken, ostrich, turkey, swan, and duck; and the following subtypes were identified: ST1, ST2, ST3, ST4, ST5, ST6, ST7, ST8, ST10, ST13, ST14, ST15, and ST25. In humans, ST1 - ST6 were the subtypes identified to date. Figure 5 illustrates the *Blastocystis* STs recognized in humans and each livestock animal group from Malaysia, highlighting the shared, therefore, potentially zoonotic subtypes.

In Pahang, *Blastocystis* ST1 - ST5 have been identified in humans; interestingly, ST1, ST3, ST4 and ST5 were reported in cattle, ST4 in goats, while ST1, ST2, ST3, ST6, ST7 and ST9 were detected in several poultry birds in this same state of Malaysia. In Selangor (and Kuala Lumpur), however, there were reports of ST1 - ST6 isolation from human hosts; while ST1, ST3, and ST6 were reported in goats. There was the detection of *Blastocystis* ST1 - ST5 in humans in Perak

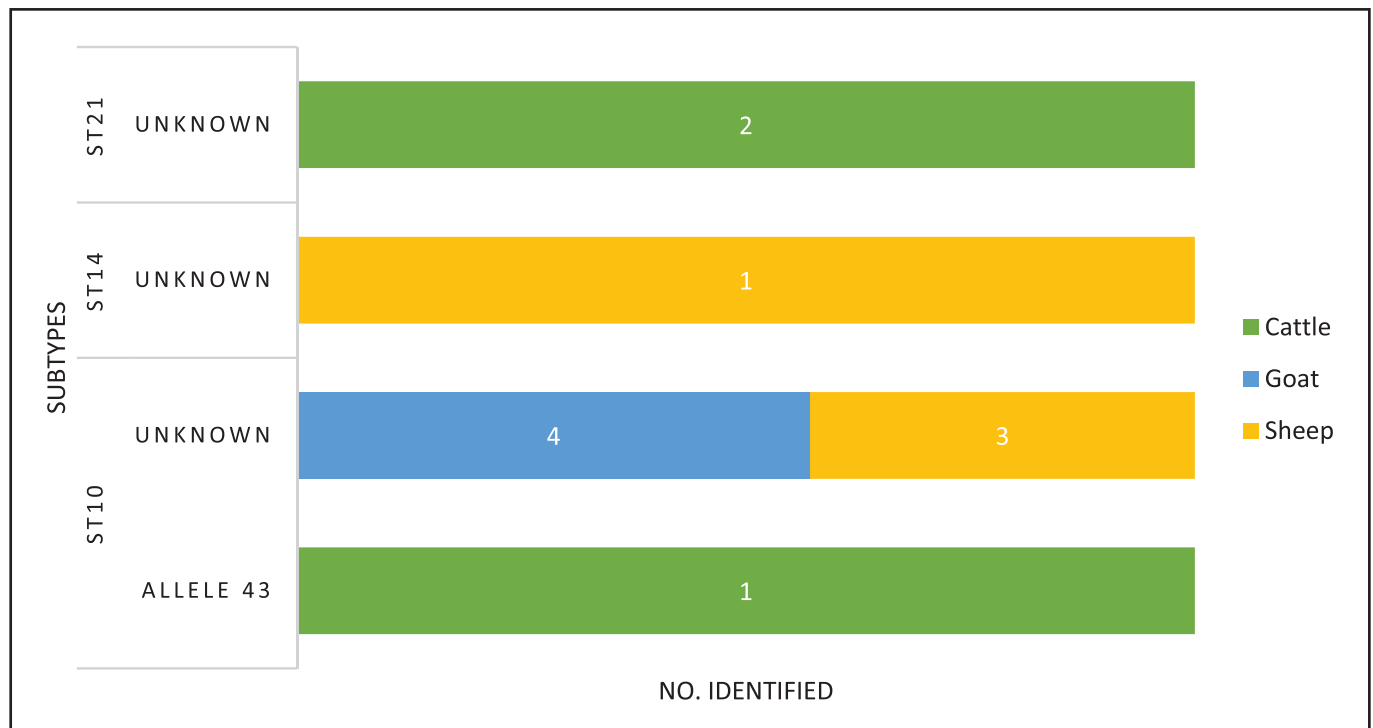


Figure 2. Frequency of *Blastocystis* 18S alleles detected in livestock animals in Perak, Malaysia.

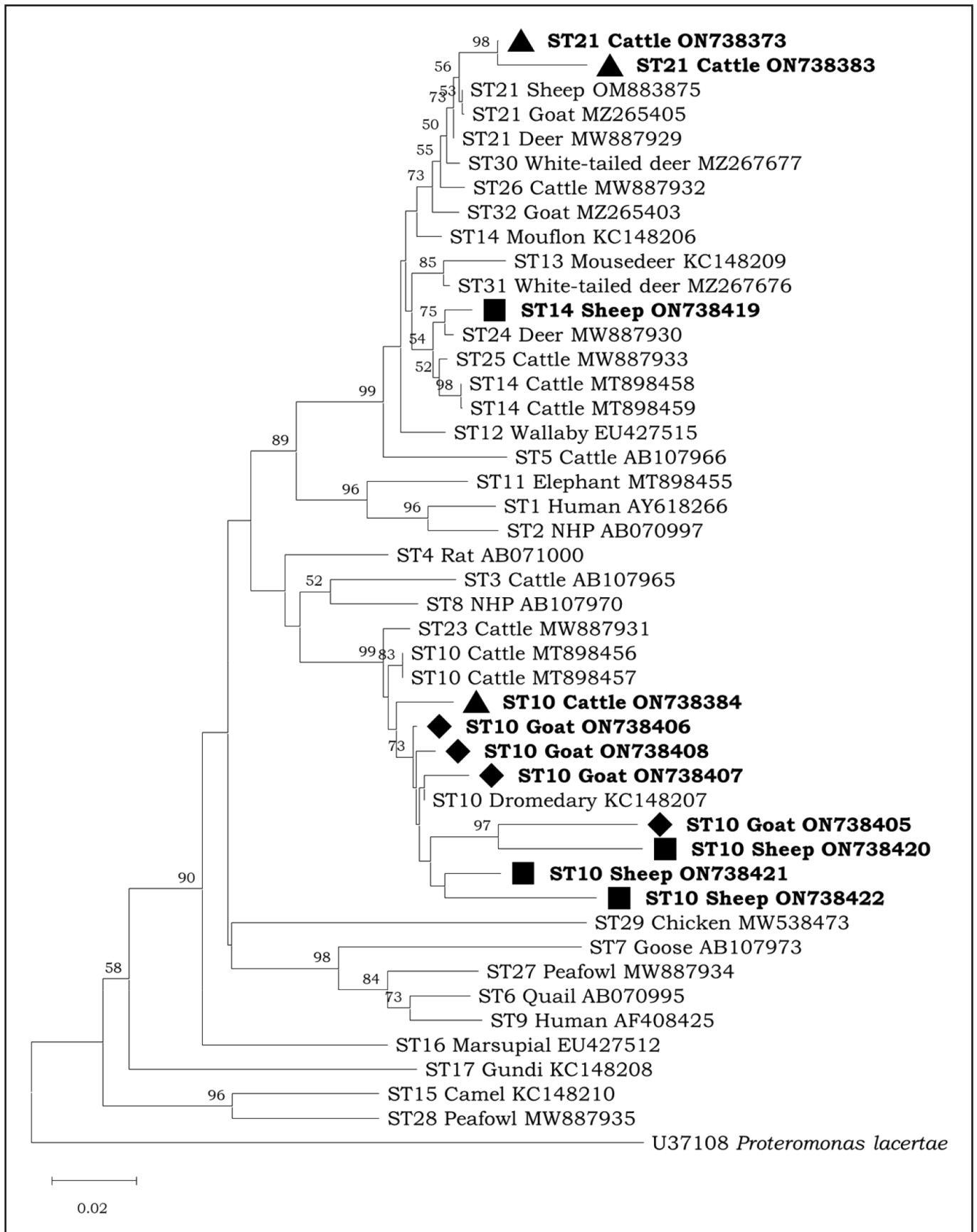


Figure 3. Neighbour-Joining analysis of sequences from this study and reference sequences. The triangle, square, and diamond icons represent sequences from cattle, sheep, and goats from this study, respectively.

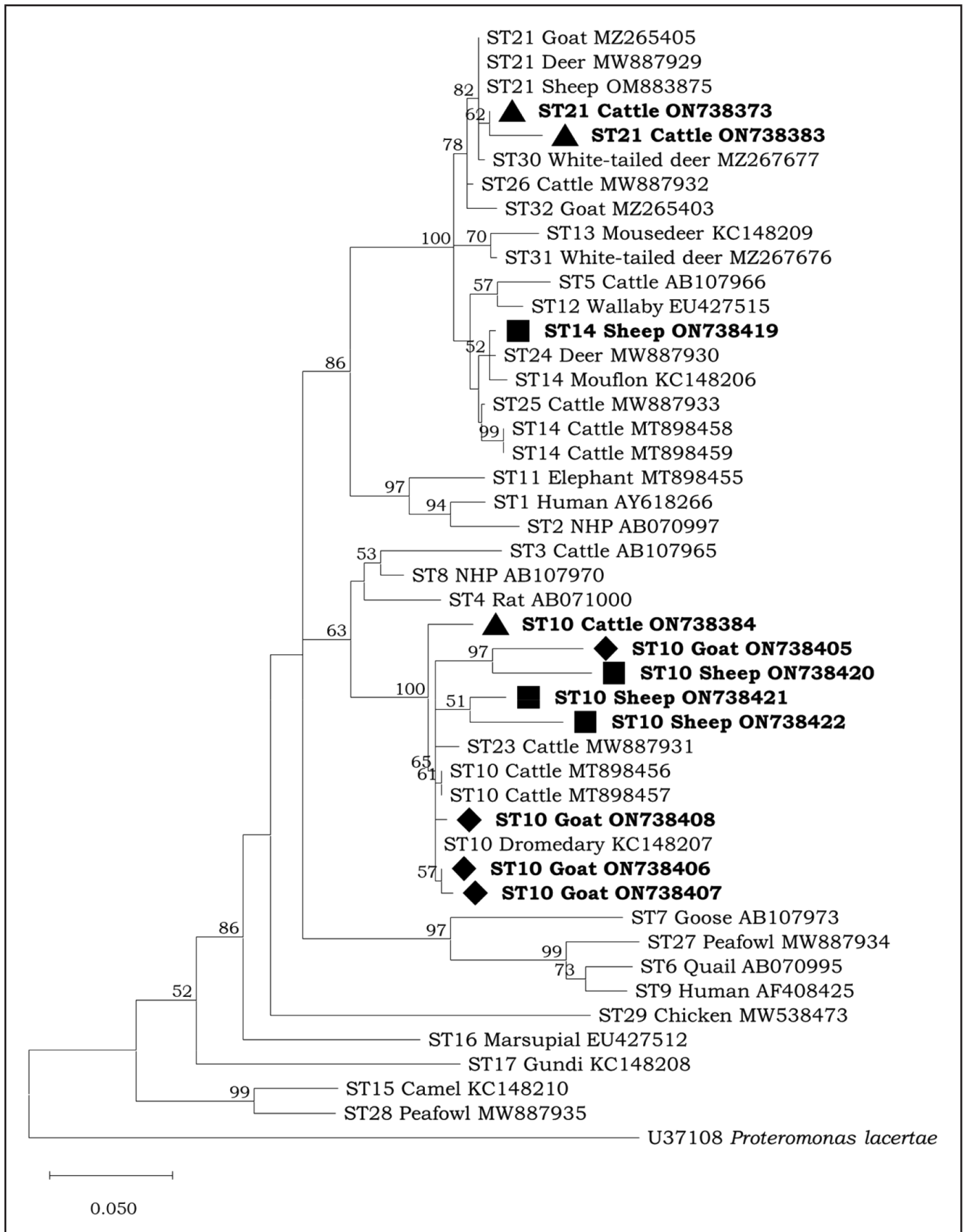


Figure 4. Maximum-Likelihood analysis of sequences from this study and reference sequences. The triangle, square, and diamond icons represent sequences from cattle, sheep, and goats from this study respectively.

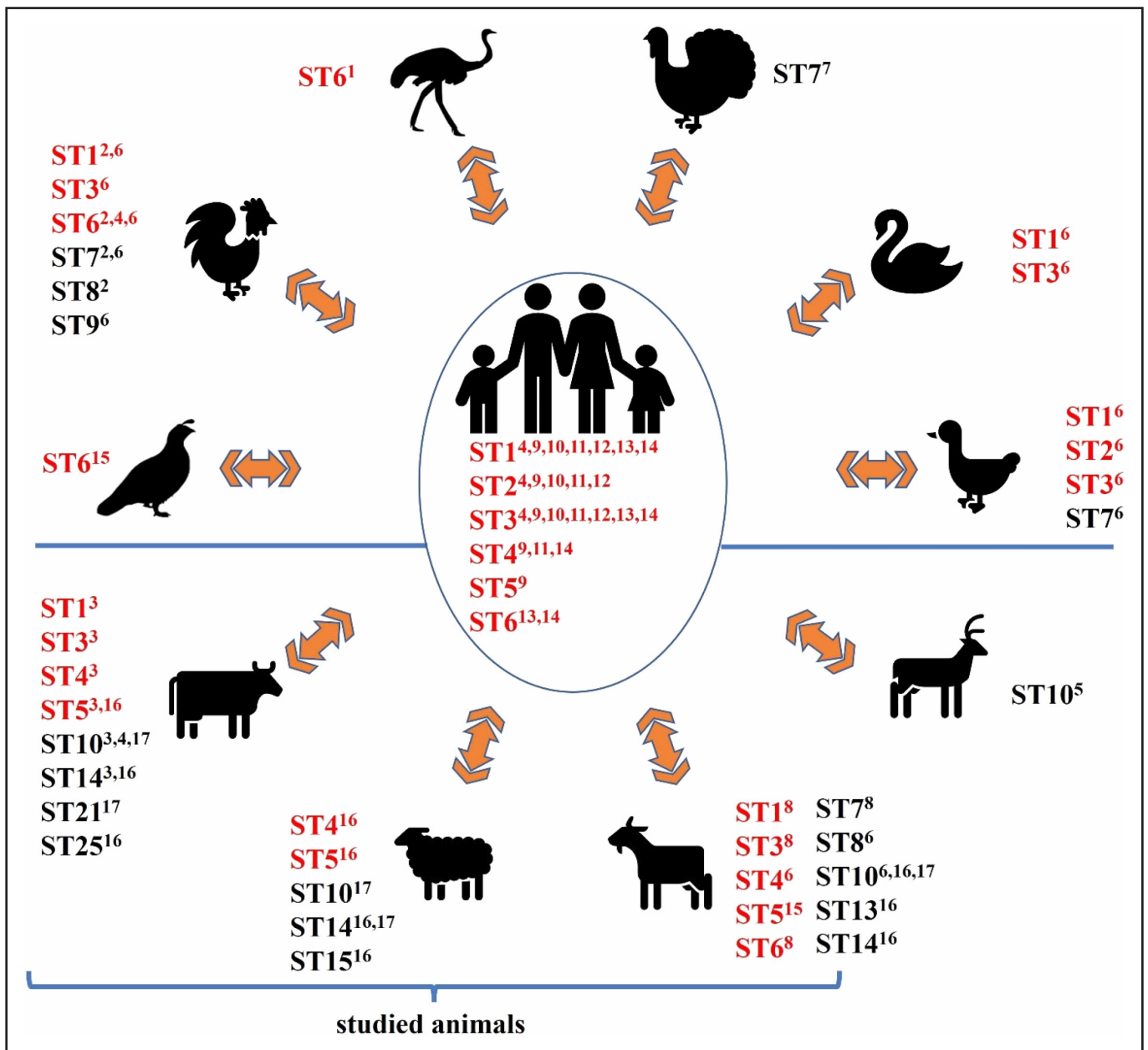


Figure 5. *Blastocystis* sp. subtypes isolated from humans and livestock animals in Malaysia.

Subtypes in red text have been detected in both humans and animals in Malaysia.¹Chandrasekaran *et al.* (2014), ²Farah Haziqah *et al.* (2018), ³Kamaruddin *et al.* (2020), ⁴Mohammad *et al.* (2018a), ⁵Mohammad *et al.* (2018c), ⁶Noradilah *et al.* (2017a), ⁷Siti Alawiyah *et al.* (2021), ⁸Tan *et al.* (2013), ⁹Nithyamathi *et al.* (2016), ¹⁰Sahimin *et al.* (2020), ¹¹Noradilah *et al.* (2017b), ¹²Mohammad *et al.* (2018b), ¹³Angal *et al.* (2015), ¹⁴Theragarajan *et al.* (2019), ¹⁵Rauff-Adedotun *et al.* (2022), ¹⁶Rauff-Adedotun *et al.* (2023), ¹⁷Present study.

from earlier studies, while Farah Haziqah *et al.* (2018) reported the presence of *Blastocystis* ST1 in chicken from Perak. The distribution of *Blastocystis* subtype in humans and livestock according to the states of Peninsular Malaysia are shown in Table 2.

DISCUSSION

Molecular characterization of *Blastocystis* sp. in ruminant livestock animals from Malaysia is sparse. This study represents the first description of *Blastocystis* subtypes in ruminant livestock animals (cattle, goat, sheep) from the Perak state of Malaysia. Altogether, only three *Blastocystis* subtypes, namely ST10, ST14 and ST21, were identified in ruminant livestock animals involved in the present study. ST10 was the dominant subtype and was observed in all three ruminant livestock animal groups, however, ST14 was observed only in sheep while ST21 was identified in cattle alone. *Blastocystis* ST10

is seen to be the most widespread subtype in ruminant livestock animals in Malaysia followed by ST14. Thus, the detection of ST10 and ST14 in this study and others from Malaysia and different parts of the world corroborates the description of hoofed animals as natural hosts to these subtypes (Masuda *et al.*, 2018; Udonsom *et al.*, 2018; Wang *et al.*, 2018; Suwanti *et al.*, 2020; Hublin *et al.*, 2021). Although the identification of ST21 in cattle in this study is the first report of the subtype in livestock in Malaysia, *Blastocystis* ST21 has been reported in cattle from Spain (Abarca *et al.*, 2021), in cattle, sheep, goats and llama from Colombia (Higuera *et al.*, 2021), camels from China (Yang *et al.*, 2021), white-tailed deer from the USA (Maloney *et al.*, 2021) and in sheep and goats from China (Zhang *et al.*, 2023).

Worldwide, a broad range of *Blastocystis* subtypes have been document in cattle (ST1-ST7, ST10, ST12, ST14, ST17, ST21, and ST23-ST26), goats (ST1, ST3-ST7, ST10, ST12, ST13, ST14, and ST21), and sheep (ST1, ST3, ST4, ST5, ST10, ST14, ST15, and ST21) (Higuera *et*

Table 2. *Blastocystis* subtype diversity in humans and livestock in the states of Peninsular Malaysia

Location/State	Host	Subtypes identified															Reference
		ST1	ST2	ST3	ST4	ST5	ST6	ST7	ST8	ST9	ST10	ST13	ST14	ST15	ST21	ST25	
Selangor	Human	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	Angal <i>et al.</i> (2015), Nithyamathi <i>et al.</i> (2016), Thergarajan <i>et al.</i> (2019), Sahimin <i>et al.</i> (2020)
	Livestock	✓	-	✓	-	-	✓	✓	-	-	-	-	-	-	-	-	Tan <i>et al.</i> (2013)
Perak, Selangor	Livestock	✓	-	-	-	-	✓	✓	✓	-	-	-	-	-	-	-	Farah Haziqah <i>et al.</i> (2018)
	Human	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	Nithyamathi <i>et al.</i> (2016)
Perak	Livestock	-	-	-	-	-	-	-	-	✓	-	✓	-	-	✓	-	Present study
	Human	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Penang	Livestock	-	-	-	✓	✓	✓	✓	-	✓	✓	✓	✓	✓	-	✓	Siti Alawiyah <i>et al.</i> (2021), Rauff-Adedotun <i>et al.</i> (2022), Rauff-Adedotun <i>et al.</i> (2023)
	Human	✓	✓	✓	✓	✓	-	-	-	-	-	-	-	-	-	-	Nithyamathi <i>et al.</i> (2016), Noradilah <i>et al.</i> (2017b), Mohammad <i>et al.</i> (2018a), Mohammad <i>et al.</i> (2018b)
Pahang	Livestock	✓	✓	✓	✓	✓	✓	✓	✓	✓	-	-	✓	-	-	-	Noradilah <i>et al.</i> (2017a), Mohammad <i>et al.</i> (2018a), Mohammad <i>et al.</i> (2018c), Kamaruddin <i>et al.</i> (2020)
	Human	✓	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	Nithyamathi <i>et al.</i> (2016)
Kedah	Livestock	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Human	✓	-	✓	✓	-	-	-	-	-	-	-	-	-	-	-	Nithyamathi <i>et al.</i> (2016)
Johor	Livestock	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Poultry	-	-	-	-	-	✓	-	-	-	-	-	-	-	-	-	Chandrasekaran <i>et al.</i> (2014)

NA – Not Available. Subtypes with red ticks are those identified in both humans and livestock animals in the different states of Malaysia.

al., 2021; Hublin *et al.*, 2021; Shams *et al.*, 2022; Rauff-Adedotun *et al.*, 2023; Zhang *et al.*, 2023). A limited variety of STs was identified in the present study, and this could be because of the samples were subjected directly to DNA barcoding. DNA barcoding primers may amplify fungal DNA, especially in non-human faecal samples (Stensvold & Clark, 2016).

Intra-subtype variation within *Blastocystis* ST10 was revealed by *Blastocystis* (18S) and sequence typing (MLST) databases (<https://pubmlst.org/Blastocystis/>). Although the other ST10 isolates were of unknown allele(s), allele 43 was detected in cattle in this study as was previously described in cattle from Iran (Salehi *et al.*, 2022).

The phylogenetic trees supported subtypes allocated to the isolates obtained in this study. The nesting of ST24 and ST25 with ST14, and the nesting of ST23 with ST10 observed in the trees were previously explained. Maloney and Santin (2021) observed a 99% sequence similarity between pairs ST24/ST25, ST14/ST24, ST14/ST25, and ST10/ST23 within the barcoding region, while these pairs were all found to share 98% of sequence identity across the full-length SSU rRNA gene.

From previous studies carried out in Malaysia, the six *Blastocystis* subtypes (ST1-ST6) identified, so far, in humans have been detected in several livestock animals in the country as well. *Blastocystis* subtypes common to humans and livestock were observed in Pahang (ST1, ST2, ST3, ST4, and ST5), Selangor (ST1, ST3, and ST6) and Perak (ST1) indicating the possibility of transmission between these two host groups in these states. Interestingly, Noradilah *et al.* (2017a) isolated *Blastocystis* ST9, a typical *Blastocystis* subtype of human, in chicken in Pahang. Nonetheless, there has not been any report on the identification of this subtype in humans in Pahang or in Malaysia till date.

Blastocystis ST10 and ST14, considered to be of bovid origin (Naguib *et al.*, 2024), were identified in our study, and also observed, from other studies, as the dominant and widespread subtypes among ruminant livestock animals in Malaysia. These subtypes have been discovered in humans, albeit sparsely (Khaled *et al.*, 2020, 2021). Despite that the hosts of ST10 and ST14 reported by Khaled *et al.* (2020) and Khaled *et al.* (2021) were neither farmers nor in frequent contact with ruminant livestock animals, these subtypes could still be potentially zoonotic. While there are yet to be studies on *Blastocystis* infections in humans from Penang, *Blastocystis* ST1, ST3, and ST4 have been documented in human hosts from Johor and Kedah (Nithyamathi *et al.*, 2016) with no record obtainable on *Blastocystis* in livestock animals from these states.

Overall, available data of *Blastocystis* subtypes in human and livestock animal hosts from Peninsular Malaysia have indicated the likelihood of animal-to-human and human-to-animal transmission of *Blastocystis* to occur, and the need for farm owners and animal handlers to ensure good hygiene practices to prevent possible zoonotic transmission of *Blastocystis*.

CONCLUSION

The importance of molecular characterization of *Blastocystis* isolates cannot be over-emphasized. Such research provides information on the genetic variants of *Blastocystis*. On the contrary, genetic analyses of *Blastocystis* in humans and potential reservoir hosts such as livestock are insufficient in Malaysia. Further studies on *Blastocystis* and its subtypes in humans and their in-contact animals in different locations are, therefore, required.

Limitation of research

Due to the use of the Barcoding-PCR method for detection of *Blastocystis* in this study, the actual prevalence of *Blastocystis* in the study animals could not be determined. The reason for this limitation is the possibility of false negative result obtainable from the use of this method.

ACKNOWLEDGEMENTS

The authors would like to thank the team members in the Veterinary Parasitology Laboratory, Universiti Sains Malaysia who have helped in carrying out this study. This work was supported by Universiti Sains Malaysia (Postgraduate Research Grant Scheme: 1001.PBIOLOGI.AUPS001 and Short Term Grant 2018: 304/PBIOLOGI/6315156).

REFERENCES

- Abarca, N., Santín, M., Ortega, S., Maloney, J.G., George, N.S., Molokin, A., Cardona, G.A., Dashti, A., Köster, P.C., Bailo, B. *et al.* (2021). Molecular detection and characterization of *Blastocystis* sp. and enterocytozoon bieneusi in cattle in northern Spain. *Veterinary Science* **8**: 191. <https://doi.org/10.3390/vetsci8090191>
- Angal, L., Mahmud, R., Samin, S., Yap, N.J., Ngui, R., Amir, A., Ithoi, I., Kamarulzaman, A. & Lim, Y.A.L. (2015). Determining intestinal parasitic infections (IPs) in inmates from Kajang Prison, Selangor, Malaysia for improved prison management. *BMC Infectious Diseases* **15** : 467. <https://doi.org/10.1186/s12879-015-1178-3>
- Asghari, A., Sadrebazzaz, A., Shamsi, L. & Shams, M. (2021). Global prevalence, subtypes distribution, zoonotic potential, and associated risk factors of *Blastocystis* sp. in domestic pigs (*Sus domesticus*) and wild boars (*Sus scrofa*): A systematic review and meta-analysis. *Microbial Pathogenesis* **160**: 105183. <https://doi.org/10.1016/j.micpath.2021.105183>
- Attah, A.O., Sanggari, A., Li, L.I., Nik Him, N.A.I.I., Ismail, A.H. & Farah Haziqah, M.T. (2023). *Blastocystis* occurrence in water sources worldwide from 2005 to 2022: A review. *Parasitology Research* **122**: 1-10. <https://doi.org/10.1007/s00436-022-07731-0>
- Barati, M., KarimiPourSaryazdi, A., Rahmadian, V., Bahadory, S., Abdoli, A., Rezanezhad, H., Solhjoo, K. & Taghipour, A. (2022). Global prevalence and subtype distribution of *Blastocystis* sp. in rodents, birds, and water supplies: A systematic review and meta-analysis. *Preventive Veterinary Medicine* **208**: 105770. <https://doi.org/10.1016/j.prevetmed.2022.105770>
- Chandrasekaran, H., Govind, S.K., Panchadcharam, C., Bathmanaban, P., Raman, K. & Thergarajan, G. (2014). High lipid storage in vacuolar forms of subtype 6 *Blastocystis* sp. in ostrich. *Parasites and Vectors* **7**: 469. <https://doi.org/10.1186/s13071-014-0469-7>
- Farah Haziqah, M.T., Chandrawathani, P., Suresh, K.G., Wilson, J.J., Mohd Khairul Nizam, M.K., Arutchelvan, R., Premaalatha, B. & Mohd Zain, S.N. (2018). Prevalence, ultrastructure and subtypes of *Blastocystis* in chickens (*Gallus gallus*) from Peninsular Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **49**: 921-932.
- Higuera, A., Herrera, G., Jimenez, P., García-Corredor, D., Pulido-Medellín, M., Bulla-Castañeda, D.M., Pinilla, J.C., Moreno-Pérez, D.A., Maloney, J.G., Santín, M. *et al.* (2021). Identification of multiple *Blastocystis* subtypes in domestic animals from Colombia using amplicon-based next generation sequencing. *Frontiers in Veterinary Science* **8**: 732129. <https://doi.org/10.3389/fvets.2021.732129>
- Higuera, A., Leiva, D.E.S., Curtis, B., Patiño, L.H., Zhao, D., Hultqvist, J.J., Dlutek, M., Muñoz, M., Roger, A.J. & Ramírez, J.D. (2023). Draft genomes of *Blastocystis* subtypes from human samples of Colombia. *Parasite & Vectors* **16**: 52. <https://doi.org/10.1186/s13071-022-05619-7>
- Hublin, J.S.Y., Maloney, J.G. & Santin, M. (2021). *Blastocystis* in domesticated and wild mammals and birds. *Research in Veterinary Science* **135**: 260-282. <https://doi.org/10.1016/j.rvsc.2020.09.031>
- Jolley, K.A., Bray, J.E. & Maiden, M.C.J. (2018). Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Research* **3**: 124. <https://doi.org/10.12688/wellcomeopenres.14826.1>
- Kamaruddin, S.K., Mat Yusof, A. & Mohammad, M. (2020). Prevalence and subtype distribution of *Blastocystis* sp. in cattle from Pahang, Malaysia. *Tropical Biomedicine* **37**: 127-141.
- Khaled, S., Gantois, N., Ayoubi, A., Even, G., Sawant, M., El Houmayraa, J., Nabot, M., Benamrouz-Vanneste, S., Chabé, M., Certad, G. *et al.* (2021). *Blastocystis* sp. prevalence and subtypes distribution amongst syrian refugee communities living in North Lebanon. *Microorganisms* **9**: 184. <https://doi.org/10.3390/microorganisms9010184>
- Khaled, S., Gantois, N., Ly, A.T., Senghor, S., Even, G., Dautel, E., Dejager, R., Sawant, M., Baydoun, M., Benamrouz-Vanneste, S., Chabé, M. *et al.* (2020). Prevalence and subtype distribution of *Blastocystis* sp. in senegalese school children. *Microorganisms* **8**: 1408. <https://doi.org/10.3390/microorganisms8091408>

- Maloney, J.G., Jang, Y., Molokin, A., George, N.S. & Santin, M. (2021). Wide genetic diversity of *Blastocystis* in white tailed deer (*Odocoileus virginianus*) from Maryland, USA. *Microorganisms* **9**:1343. <https://doi.org/10.3390/microorganisms9061343>
- Maloney, J.G., Molokin, A., Segu, R., Maravilla, P., Mart, F., Villalobos, G., Tsousis, A.D., Gentekaki, E., Muboz-antol, C., Klisiowicz, D.R. *et al.* (2023). Identification and molecular characterization of four new *Blastocystis* subtypes designated ST35-ST38. *Microorganisms* **11**: 46. <https://doi.org/10.3390/microorganisms11010046>
- Maloney, J.G. & Santin, M. (2021). Mind the gap: New full-length sequences of *Blastocystis* subtypes generated via oxford nanopore minion sequencing allow for comparisons between full-length and partial sequences of the small subunit of the ribosomal rna gene. *Microorganisms* **9**: 997. <https://doi.org/10.3390/microorganisms9050997>
- Masuda, A., Sumiyoshi, T., Ohtaki, T. & Matsumoto, J. (2018). Prevalence and molecular subtyping of *Blastocystis* from dairy cattle in Kanagawa, Japan. *Parasitology International* **67**: 702-705. <https://doi.org/10.1016/j.parint.2018.07.005>
- Mohammad, N.A., Al-Mekhlafi, H.M. & Anuar, T.S. (2018a). Subtype distribution of *Blastocystis* isolated from humans and associated animals in an indigenous community with poor hygiene in Peninsular Malaysia. *Tropical Biomedicine* **35**: 849-860.
- Mohammad, N.A., Al-Mekhlafi, H.M. & Anuar, T.S. (2018b). Genetic diversity of *Blastocystis* isolates from symptomatic and asymptomatic orang asli in Pahang, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **49**: 189-197.
- Mohammad, N.A., Al-Mekhlafi, H.M., Mokhtar, N. & Anuar, T.S. (2018c). Molecular detection and subtyping of *Blastocystis* in Javan rusa (*Cervus timorensis*) and sika deer (*Cervus nippon*) from Peninsular Malaysia. *Thai Journal of Veterinary Medicine* **48**: 295-301.
- Naguib, D., Gantois, N., Desramaut, J., Dominguez, R.G., Arafat, N., Atwa, S.M., Even, G., Devos, D.P., Certad, G., Chabi, M. & Viscogliosi, E. (2024). Large-scale molecular epidemiological survey of *Blastocystis* sp. among herbivores in Egypt and assessment of potential zoonotic risk. *Microorganisms* **12**: 1286. <https://doi.org/10.3390/microorganisms12071286>
- Nithyamathi, K., Chandramathi, S. & Kumar, S. (2016). Predominance of *Blastocystis* sp. infection among school children in Peninsular Malaysia. *PLoS One* **11**: e0136709. <https://doi.org/10.1371/journal.pone.0136709>
- Noradilah, S.A., Anuar, T.S., Mokhtar, N., Lee, I.L., Salleh, F.M., Azreen, S.N.A.M., Husnie, N.S.M.M., Azrul, S.M., Abdullah, W.O., Nordin, A. *et al.* (2017a). Molecular epidemiology of *Blastocystis* sp. in animals reared by the aborigines during wet and dry seasons in rural communities, Pahang, Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **48**: 1151-1160.
- Noradilah, S.A., Mokhtar, N., Anuar, T.S., Lee, I.L., Salleh, F.M., Manap, S.N.A.A., Mohtar, N.S.H.M., Azrul, S.M., Abdullah, W.O., Nordin, A. *et al.* (2017b). Molecular epidemiology of *Blastocystis* in Malaysia: Does seasonal variation play an important role in determining the distribution and risk factors of *Blastocystis* subtype infections in the Aboriginal community? *Parasites & Vectors* **10**: 360. <https://doi.org/10.1186/s13071-017-2294-2>
- Popruk, S., Adao, D.E.V. & Rivera, W.L. (2021). Epidemiology and subtype distribution of *Blastocystis* in humans: A review. *Infection, Genetics and Evolution* **95**: 105085. <https://doi.org/10.1016/j.meegid.2021.105085>
- Ramírez, J.D., Sánchez, A., Hernández, C., Flórez, C., Bernal, M.C., Giraldo, J.C., Reyes, P., López, M.C., García, L., Cooper, P.J. *et al.* (2016). Geographic distribution of human *Blastocystis* subtypes in South America. *Infection, Genetics and Evolution* **41**: 32-35. <https://doi.org/10.1016/j.meegid.2016.03.017>
- Rauff-Adedotun, A.A., Douglas, S.J., Rajamanikam, A., Amira Suriaty, Y. & Farah Haziqah, M.T. (2022). Natural infection of *Blastocystis* ST6 among commercial quails (*Coturnix coturnix*) in Penang, Malaysia. *Tropical Biomedicine* **39**: 75-79. <https://doi.org/10.47665/tb.39.1.012>
- Rauff-Adedotun, A.A., Lee, I.L., Abd Talib, N., Shaari, N., Yahaya, Z.S. & Farah Haziqah, M.T. (2023). Prevalence, potential risk factors and genetic diversity of *Blastocystis* in ruminant livestock animals from Penang, Malaysia. *Parasitology Research* **122**: 2193-2205. <https://doi.org/10.1007/s00436-023-07920-5>
- Sahimin, N., Farah Haziqah, M.T., Rajamanikam, A., Mohd Nazri, N.A., Govind, S.K. & Mohd Zain, S.N. (2020). Prevalence and subtypes of *Blastocystis* among migrant workers from different working sectors in Peninsular Malaysia. *Parasitology Research* **119**: 3555-3558. <https://doi.org/10.1007/s00436-020-06865-3>
- Salehi, R., Rostami, A., Mirjalali, H., Stensvold, C.R. & Haghighi, A. (2022). Genetic characterization of *Blastocystis* from poultry, livestock animals and humans in the southwest region of Iran – Zoonotic implications. *Transboundary and Emerging Diseases* **69**: 1178-1185. <https://doi.org/10.1111/tbed.14078>
- Sanggari, A., Komala, T., Rauff-Adedotun, A.A., Awosolu, O.B., Attah, O.A. & Farah Haziqah, M.T. (2022). *Blastocystis* in captivated and free-ranging wild animals worldwide: A review. *Tropical Biomedicine* **39**: 338-372. <https://doi.org/10.47665/tb.39.3.006>
- Santin, M., Figueiredo, A., Molokin, A., George, N.S., Köster, P.C., Dashti, A., González-Barrio, D., Carmenta, D. & Maloney, J.G. (2024). Division of *Blastocystis* ST10 into three new subtypes: ST42-ST44. *Journal of Eukaryotic Microbiology* **71**: e12998. <https://doi.org/10.1111/jeu.12998>
- Shams, M., Shamsi, L., Sadrebazzaz, A., Asghari, A., Badali, R., Omidian, M. & Hassanipour, S. (2021). A systematic review and meta-analysis on the global prevalence and subtypes distribution of *Blastocystis* sp. infection in cattle: A zoonotic concern. *Comparative Immunology, Microbiology & Infectious Diseases* **76**: 101650. <https://doi.org/10.1016/j.cimid.2021.101650>
- Shams, M., Shamsi, L., Yousefi, A., Sadrebazzaz, A., Asghari, A., Mohammadi-Ghalehbin, B., Shahabi, S. & Hatam, G. (2022). Current global status, subtype distribution and zoonotic significance of *Blastocystis* in dogs and cats: A systematic review and meta-analysis. *Parasites and Vectors* **15**: 225. <https://doi.org/10.1186/s13071-022-05351-2>
- Siti Alawiyah, J.A.N., Rauff-Adedotun, A.A., Aishah, S., Rusydi Abdul Hafiz, R., Zary Shariman, Y. & Farah Haziqah, M.T. (2021). Molecular subtyping and phylogeny of *Blastocystis* sp. isolated from turkey (*Meleagris gallopavo*) populations in Penang, Malaysia. *Tropical Biomedicine* **38**: 578-589. <https://doi.org/10.47665/tb.38.4.101>
- Stensvold, C.R. & Clark, C.G. (2016). Molecular identification and subtype analysis of *Blastocystis*. *Current Protocols in Microbiology* **43**: 20A.2.1-20A.2.10. <https://doi.org/10.1002/cpmc.17>
- Stensvold, C.R., Tan, K.S.W. & Clark, C.G. (2020). *Blastocystis*. *Trends in Parasitology* **36**: 315-316. <https://doi.org/10.1016/j.pt.2019.12.008>
- Suwanti, L.T., Susana, Y., Hastutie, P., Suprihati, E. & Lastuti, N.D.R. (2020). *Blastocystis* spp. subtype 10 infected beef cattle in Kamal and Socah, Bangkalan, Madura, Indonesia. *Veterinary World* **13**: 231-237. <https://doi.org/10.14202/vetworld.2020.231-237>
- 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. <https://doi.org/10.1093/molbev/mst197>
- 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**: 85-89. <https://doi.org/10.1007/s00436-012-3107-3>
- Theragarajan, G., Kumar, S., Bhassu, S., Omar, S.F.B.S. & Rampal, S. (2019). Effect of *Blastocystis* sp. in dengue patients - Increase in the treatment cost and exacerbation of symptoms. *PLoS One* **14**: e0211034. <https://doi.org/10.1371/journal.pone.0211034>
- Udonsom, R., Prasertbun, R., Mahittikorn, A., Mori, H., Changbunjong, T., Komalamisra, C., Pintong, A., 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. <https://doi.org/10.1016/j.meegid.2018.07.007>
- Wang, J., Gong, B., Yang, F., Zhang, W., Zheng, Y. & Liu, A. (2018). 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. <https://doi.org/10.1016/j.meegid.2017.11.026>
- Yang, X., Li, Y., Wang, Y., Wang, J., Lai, P., Li, Y., Song, J., Qi, M. & Zhao, G. (2021). Molecular characterization of *Blastocystis* sp. in camelus bactrianus in northwestern China. *Animals* **11**: 3016. <https://doi.org/10.3390/ani11113016>
- Zhang, J., Fu, Y., Bian, X., Han, H., Dong, H., Zhao, G., Li, J., Li, X. & Zhang, L. (2023). Molecular identification and genotyping of *Blastocystis* sp. in sheep and goats from some areas in Inner Mongolia, Northern China. *Parasitology International* **94**: 12-17. <https://doi.org/10.1016/j.parint.2023.102739>