

PREVALENCE OF *Blastocystis* sp. IN CATTLE, GOAT AND SHEEP REARED BY DIFFERENT FARM MANAGEMENT SYSTEMS IN PAHANG, MALAYSIA

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ABSTRACT

Blastocystis sp. is a familiar parasite in the gastrointestinal tract causing infection in humans and animals. The purpose of this study is to evaluate the *Blastocystis* sp. prevalence in three sorts of livestock; cattle, goats, and sheep in Pahang, Malaysia, which are reared under two management systems; intensive and semi-intensive farm management system. About 92, 96, and 65 cattle, goat, and sheep fecal samples respectively were collected from different farms around Kuantan, Bera, and Pekan. The fecal samples were cultured in Jones' medium supplemented with 10% heat-inactivated horse serum and incubated at 37 °C for 2 weeks, then observed under light microscopy daily. The total prevalence of *Blastocystis* sp. was 29.34% in cattle (27/92), 29.16% in goats (28/96), and 43.07% in sheep (28/65). Supported the results of this study, *Blastocystis* sp. prevalence was higher in sheep and livestock reared by a semi-intensive farm management system (44.38%). However, further study could be done for *Blastocystis* sp. subtypes identification to determine its genetic diversity. Notwithstanding, this study has provided additional knowledge on the prevalence of each livestock reared in farms around Pahang that serve as important information in understanding host-parasite relationships, besides determining the best farm management system to be applied by farmers.

Key words: *Blastocystis*, cattle, goats, sheep

INTRODUCTION

The livestock industry is an essential and integral component of the agricultural sector; as it provides beneficial animal protein food for the population, employment to the citizen, and raw materials for the agro-based industries. Indeed, the livestock sector is an enormous food industry in Malaysia (Kamarulzaman, 2016). Animal meat is the main protein source of diet for Malaysians. Malaysian livestock industry consists of ruminants and non-ruminants (Mohamed, 2007). However, the ruminant sector is still incapable to meet the local demand for meat production, due to several factors such as poor disease prevention and control, the inadequacy of land resources, expensive feed prices, low-cost import substitutes, and poor private-sector involvement (Shanmuganvelu, 2014), absence of good high-performance breeds, expertise, and workforce (National Agro-Food Policy, 2015). 90% and above of small farm holders still dominated the ruminant sector, causing an underdeveloped ruminant industry, due to improper infrastructure (Kamarulzaman, 2016). Consequently, Malaysia is in needs to import beef mutton and dairy products from outsources including India, Australia, and New

Zealand to fill in the shortage of supply (Rosali & Mohammad Nor, 2015).

Malaysia's cattle population decreased from 703,832 in 2017 to 676,686 in 2018. The cattle populations in Pahang in that year were 126,084; the highest population among other states in Malaysia. Meanwhile, the goat populations decrease from 385,304 in 2017 to 359,200 in 2018. In Pahang, the goat populations were 38,443 in 2017 and decreased to 30,500 in 2018. The sheep populations also reduce from 18,899 in 2017 to 18,831 in 2018 (Department of Veterinary Services, 2018).

Blastocystis sp. is a common anaerobic intestinal parasite of humans and animals, including cattle, goats, and sheep (Vaisusuk *et al.*, 2018). *Blastocystis* sp. is more widespread than *Entamoeba histolytica*-*Entamoeba dispar* complex, *Cryptosporidium*, and *Giardia lamblia* (Boorum *et al.*, 2008; Stensvold *et al.*, 2009; El Safadi, 2014). Its prevalence is higher in developing countries than in developed countries due to poor hygiene and, consumption of contaminated food and water containing cysts (Leelayoova *et al.*, 2004; Yoshikawa *et al.*, 2004; Leelayoova *et al.*, 2008) and close contact with infected animals (Li *et al.*, 2007; Eroglu *et al.*, 2009; Lee *et al.*, 2012). Fecal-oral route is the main mode of transmission for this parasite (Leelayoova *et al.*, 2004; Yoshikawa *et*

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al., 2004; Leelayoova *et al.*, 2008). *Blastocystis* sp. infection is associated with a variety of gastrointestinal disorders, and irritable bowel syndrome and might be linked to the cutaneous lesion (Wawrzyniak *et al.*, 2013). Furthermore, it can also cause abdominal pain, flatulence, nausea, vomiting, and diarrhea (Abdulsalam *et al.*, 2012). *Blastocystis* sp. has several reproductive forms due to several life cycles; including budding, plasmotomy, endodyogeny, and schizogony and its common reproductive form is binary fission (Helena *et al.*, 2017). *Blastocystis* sp. has several morphological forms and is commonly distinguished into four groups which are vacuolar, granular, amoeboid, and cyst. Subtypes, age of culture, and culture conditions could affect the parasite's shape and morphological form (Helena *et al.*, 2014).

There is a comparison in *Blastocystis* sp. pathogenicity between animals and humans according to Skotarczak *et al.* (2018). *Blastocystis* sp. infection in humans has a large symptom infection rate as compared to in animals. Humans infected with this parasite may have diverse clinical symptoms including diarrhea, abdominal pain, nausea, bloating, flatulence, and fatigue (Sohail & Fischer, 2005; Zhang *et al.*, 2016). Infected animals may seem healthy without observable disease but can affect the animal's well-being, which results in weight loss and growth retardation in young animals (Maharana *et al.*, 2016). As to Kamaruddin *et al.* (2020) research findings, 16.7% of cattle infected with *Blastocystis* sp. were overweight and obese and 17.5% has a non-diarrheic stool, which is higher than thin cattle and diarrheic stool condition. A tremendous rate of *Blastocystis* sp. infection cause asymptomatic symptoms in animals/individual host (Helena *et al.*, 2017; Ning *et al.*, 2020). Unlike humans, animals were asymptomatic carriers of *Blastocystis* sp., which could cause infection during close contact with humans especially the animal handlers (Kamaruddin *et al.*, 2020; Nigel *et al.*, 2020). In addition, it is proposed that animals possibly were the predominant source of *Blastocystis* sp. potential transmission as they possess low host specificity and zoonotic potential. Despite this, *Blastocystis* sp. pathogenicity would be correlated to its extensive genetic diversity (Ahmed & Karanis, 2018; Adedotun *et al.*, 2020). *Blastocystis* sp. pathogenicity was still under debate as of several factors; the high ratio of the asymptomatic carrier, host susceptibility, colonization of multiple parasites in gut microbiota, and clinical symptoms correlated to different *Blastocystis* sp. subtypes (Jimenez *et al.*, 2019; Nigel *et al.*, 2020).

Risk factors related to gastrointestinal parasite infection in livestock depend on several factors such as age and immunity levels of animals, farm management, and climatic conditions (Tung *et al.*, 2012; Tan *et al.*, 2017). Farm management systems and the environment where the livestock are reared act as important factors in determining livestock

husbandry (Hasan *et al.*, 2014). There are three types of livestock farm management systems applied in Malaysia; intensive, semi-intensive, and extensive system. In the farms where the intensive system is applied, the livestock is kept in a sheltered house for 24 h and provided with food and water. Livestock reared under a semi-intensive system are allowed to graze for 4-6 h a day and housed in the shelter at night (Hashim & Yusof, 2016). Meanwhile, livestock managed under an extensive system is kept free for grazing day and night. Only minimum management was needed for an extensive system (Hasan *et al.*, 2014).

Therefore, the objective of this study was to evaluate the prevalence of *Blastocystis* sp. among cattle, goats, and sheep around Pahang that are reared under two different types of farm management systems. This study will not only provide useful information on the best farm management system to control *Blastocystis* sp. infection among the animals but also provide updated information on the *Blastocystis* sp. prevalence among the livestock in each of the farms studied.

MATERIALS AND METHODS

Fecal samples collection

A total of 253 fresh fecal samples were randomly collected from; cattle ($n=92$), goats ($n=96$), and sheep ($n=65$) aged from two months old to two years old, in farms (two intensive farms and three semi-intensive farms) around Kuantan, Bera, and Pekan, Pahang between November 2018 and February 2021. The fecal samples of each animal were collected per rectum using gloved hands and placed in screw cap containers. All fecal samples were labeled with a date of sampling, corresponding id, type of livestock, age, and gender before being transported to the laboratory in cooler ice packs within 24 h and stored at 4 °C (Wang *et al.*, 2018). The samples were processed and analyzed at Central Research and Animal Facility (CREAM).

Farm management system identification

Two different types of farm management systems were selected namely intensive and semi-intensive systems. For the intensive farm management system, the livestock was enclosed in zero-grazing units and provided with feed and water. The major food resources for the intensive system are the cut grasses or concentrates or a mixture of cut grasses and concentrates. The livestock reared under this system were provided with feed, clean water, and minerals *ad libitum*. On the contrary, livestock reared under a semi-intensive farm management system are brought out to the grazing pasture 4 to 6 h during the day and kept in sheltered houses with supplementary feeding at night (Hashim & Yusof, 2016).

Sample processing and isolation of *Blastocystis* sp. by microscopic observation

The fecal examination was performed by adding 2 g of each sample into a 15 mL screw-capped tube comprising 6 mL Jones medium and supplemented with 10% heat-activation horse serum (Jones, 1946; Suresh & Smith, 2004). All inoculated tubes were closed tightly, placed in a rack, and incubated at 37°C. The presence of *Blastocystis* sp. was monitored daily by their morphological characteristics for 14 days, by placing one drop of cultured sediment onto a glass slide and observed under a light microscope using low (10x) and high (40x) magnification. The parasites were maintained via sub-culturing them to Jones medium and 10% horse serum every 3 to 4 days. Different morphologies of *Blastocystis* sp. in the positive cultures were recorded as vacuolar, granular, ameboid, and cyst forms. The cultures were reported as negative if there was no parasite growth observed until the last day of incubation (Mohd Zain *et al.*, 2017).

Statistical analysis

The data were analyzed by SPSS Version 25.0 (IBM Corp., Armonk, NY, USA). The Spearman Correlation analysis was used to evaluate the significant differences between farm management systems with 3 species of animal hosts that were infected and not infected with *Blastocystis* sp., with a value of $p \leq 0.05$ indicated as significant differences. Descriptive statistics such as frequency and percentage were also calculated using the same software.

RESULTS

A total of 253 livestock (cattle, goats, and sheep) were successfully examined from five different farms in

Kuantan, Pekan, and Bera. As summarized in Table 1, the present study summarized that 32.80% (83/253) of overall livestock (cattle, goats, and sheep) were infected with *Blastocystis* sp. The highest prevalence of *Blastocystis* sp. was observed in sheep (43.07%), followed by cattle, (29.34%) and goats (29.16%) under 40x light microscope magnification. As such, day 4 to day 6 of cultivation recorded the highest *Blastocystis* sp. growth.

Out of five farms selected in this study, three farms were located in Kuantan, and one farm in Pekan and Bera respectively. All farms specialized in livestock breeding and trading. Two types of farm management system were selected, which is intensive and semi-intensive farm management system as tabulated in Table 2. Sheep reared under a semi-intensive farm management system recorded the highest prevalence of *Blastocystis* sp. infection (43.07%). The lowest prevalence of *Blastocystis* sp. infection was reported in goats reared under a semi-intensive farm management system (29.16%). Overall, 44.38% (83/187) of livestock reared by the semi-intensive system was being infected by *Blastocystis* sp. In the present study, there is no *Blastocystis* sp. infection reported in livestock animals reared under an intensive system, which is 0% (0/66). The relationship between *Blastocystis* sp. infection with farm management system was analyzed by using Spearman correlation. The study found that the infection in cattle, goats, and sheep with farm management systems was significantly related ($p < 0.05$).

DISCUSSIONS

Blastocystis sp. was discovered in human and vast animals host. Notwithstanding, the studies of this parasite focusing on livestock and its correlation

Table 1. Identification of *Blastocystis* sp. isolated from cattle, goats, and sheep in Pahang (N=253)

Host	Total Number Examined	Number Infected	Prevalence (%)
Cattle	92	27	29.34
Goats	96	28	29.16
Sheep	65	28	43.07
Total	253	83	32.80

Table 2. Comparison of *Blastocystis* sp. infection rate among cattle, goats, and sheep managed under intensive and semi-intensive systems

Animal	Management system								p-value
	Intensive system (N=66)				Semi-intensive (N=187)				
	+	-	Total No.	%	+	-	Total No.	%	
Cattle	0	35	35	0.0	27	30	57	29.34	0.000*
Goat	0	31	31	0.0	28	37	65	29.16	
Sheep	0	0	0	0.0	28	37	65	43.07	
Total	0	66	66	0.0	83	104	187	44.38	

with farm husbandry were insufficiently reported in Malaysia. The present study provided information on *Blastocystis* sp. prevalence in cattle, goats, and sheep from different farm management systems. The study was conducted from November 2018 to February 2021 on five selected farms (two intensive farms and three semi-intensive farms) in Kuantan, Bera, and Pekan, Pahang respectively. The infection rate was 29.34% in cattle, 29.16% in goats and 43.07% in sheep reared by the semi-intensive management system, as of microscopic observation. The differences in the reported prevalence can be due to the difference in animal age, sample size, and geo-climatic factors (Li *et al.*, 2018).

Another factor that could add to the difference in the rate of identification was the detection method. There were several culture methods and growing *Blastocystis* sp., but the xenic in-vitro culture (XIVC) method was commonly used for the cultivation and detection of *Blastocystis* sp. due to its simplicity, ease of application, and cost-saving. XIVC using Jones' medium supplemented with 10% horse serum that was used in this study cultivated the *Blastocystis* sp. isolates with the existence of unknown bacterial flora before it was subjected to microscopic observation. *Blastocystis* sp. cells seemed larger in shape when being cultured in Jones' medium compared to the non-culture samples (Leelayoova *et al.*, 2004; Tan, 2008), which conveniently eased the detection process. XIVC is a sensitive method with the range of 52% to 79% (Stensvold & Clark, 2016) detection method and has been considered the gold standard for *Blastocystis* sp. observation, and diagnosis (Sari *et al.*, 2018).

Blastocystis sp. occurs in a varying range of prevalence as reported by several studies in different geographical areas. However, in livestock, the prevalence rates were reported as low to moderate between 2% to 47% compared to captive animals, which range as low as 8% to 67% (Wang *et al.*, 2018). The prevalence of *Blastocystis* sp. infection reported in the ruminant livestock in Malaysia was 65% in goats, 57.9% in sheep, 34.5% in cattle, 30% in gaurs, and 28.6% in deer (Mohd Zain *et al.*, 2017).

In the present study, the highest prevalence of *Blastocystis* sp. was observed in sheep; (43.07%). The finding was in agreement with a study conducted by Alfellani *et al.* (2013) which revealed a higher prevalence of *Blastocystis* sp. in sheep with a percentage of 23.5% (12/51), as compared to cattle; 22.58% (7/31) in the UK, and 10.52% (4/38) goats infected by *Blastocystis* sp. in Libya. On contrary, the infection rate was only 5.5% (6/109) in north-eastern China Heilongjiang Province among pigs, cattle, goats, and sheep (Wang *et al.*, 2018). Similarly, a relatively low prevalence of *Blastocystis* sp. infection in sheep conducted around the globe was reported. The frequency was 6.0% (50/832) in China (Hasan *et al.*, 2014), 20% (1/5) in Libya (Alfellani *et al.*,

2013), and 0% (0/2) in Italy (Alfellani *et al.*, 2013). Similar to this study, Malaysia (Chandrasekaran *et al.*, 2014) and China (Li *et al.*, 2018) recorded 57.9% (60/104) and 24.0% (18/75) sheep that were positive for *Blastocystis* sp. infection respectively, indicating a high prevalence of *Blastocystis* sp. in sheep. Sheep production is tremendously hindered by gastrointestinal parasites, due to their grazing behavior (Swarnkar & Singh, 2010). Sheep can graze nearly to the ground and pick the lowest grass level as compared to goats, which increases the chance of ingesting the parasite. Also, sheep tend to walk long distances for forage and water (Kochewad *et al.*, 2018). Hence, the risk for sheep getting infected by *Blastocystis* sp. is higher because they were exposed to more areas with contaminated water, pasture, soil, and insufficient nutrition (Chandrasekaran *et al.*, 2014).

In this study, cattle have the second highest *Blastocystis* sp. infection compared to sheep and goats, 29.34% (27/92). This finding was closely similar to the study conducted by Kamaruddin *et al.* (2020), in which 25.0% (30/120) of cattle reared on farms around Pahang were infected by *Blastocystis* sp. This also corroborates with the findings of 25.81% of *Blastocystis* sp. infected cattle as reported by Noradilah *et al.* (2017) in Pahang. The study conducted by Udonsom *et al.* (2018) in central and western Thailand showed higher *Blastocystis* sp. infection, which was 50% (21/42) of the total population. In addition, Zhu *et al.* (2017) reported a lower prevalence in comparison to the present study; 10.3% (54/526) in Northeastern China dairy cattle. *Blastocystis* sp. occurrence was reported in cattle worldwide; 10.3% (54/526) in China (Lei *et al.*, 2019), 34.5% (10/29) in Perak, Malaysia (Hemalatha *et al.*, 2014), 50.0% (21/42) in Thailand, 14.4% (72/500) in Indonesia (Adedotun *et al.*, 2020) respectively. Nevertheless, *Blastocystis* sp. infection is relatively higher in Japan is 71% (39/55) (Abe *et al.*, 2002). Interestingly, the latest two studies in Indonesia reported 100% of *Blastocystis* sp. infection in cattle (Susana *et al.*, 2019; Suwanti *et al.*, 2020).

This study shows that goats have the lowest *Blastocystis* sp. infection in comparison to cattle and sheep; 29.16% (28/96) which was closely similar to the result reported by Tan *et al.* (2013) for *Blastocystis* sp. infection among goats in Peninsular Malaysia which is 30.9% (73/236). However, as opposed to the recent study, Udonsom *et al.* (2018) shows that 94.7% (36/38) of goats were positive for *Blastocystis* sp. infection in Central and Western Thailand. Besides, Li *et al.* (2018) show a low prevalence of *Blastocystis* sp. in goats; 2/781 (0.3%). In contrast, the prevalence of *Blastocystis* sp. in Malaysia shows that the goats have the highest *Blastocystis* sp. infection (63.1%) among a total of 118 animals that consists of goats, and cattle, deer, and swine (Tan *et al.*, 2014). In

Northwestern China, *Blastocystis* sp. infections were 54.1% (196/362), 40.4% (78/193), and 78.6% (184/234) among dairy, meat, and cashmere goats (Song *et al.*, 2017). In Malaysia, there was also a low prevalence of *Blastocystis* sp. of 25.81% (8/31) in goats (Noradilah *et al.*, 2017). These findings were supported by Monteiro *et al.* (2017) who stated that goats have high adaptability to the harsh environment; hence it is highly resistant to the disease compared to other ruminants. Due to the browsing behavior of the goats, which does not feed on the grass closest to the ground has lessened the parasitic infection in these goats (Zvinorova *et al.*, 2016).

During the visit to semi-intensive farms in Kuantan and Pekan, the shed was cleaned occasionally per week, causing the accumulation of feces around the shed, creating an unhygienic farm condition. This has increased the risk of gastrointestinal parasite infection due to the contaminated environment and water bodies, which could easily be transmitted through mechanical passage (Khor *et al.*, 2018; Abdullah *et al.*, 2019). This situation could increase the risk of getting an infection due to poor farm sanitation (Hashim & Yusof, 2016). Furthermore, the livestock reared by this system is susceptible because they were exposed to various infections during outdoor grazing (Bandara *et al.*, 2011). Navarro *et al.* (2008) state that the difference in prevalence might be due to the different farm management systems, different adaptations by animals, or different ways of conducting the research in different countries. Different husbandry practices and feeding methods have an important influence on the distribution of *Blastocystis* sp. As a such, infected feed, water, environment, and grazing in contaminated areas could contribute to high numbers of infections, especially in the semi-intensive farm management system (Manyazewal *et al.*, 2018). In addition, the transmission of *Blastocystis* sp. can worsen among animals that are reared in the small space area. All animal fecal samples were collected randomly from different farms that are located apart from each other; hence indicating that the *Blastocystis* sp. prevalence is extensive in farms around Pahang. In conjunction with that, the act of sharing the contaminated food and water is an efficient mode of transmission the lack of awareness among animal handlers about handling zoonoses can worsen the scenario (Navarro *et al.*, 2008).

Livestock farming has an essential role in economic development, which could be affected by gastrointestinal parasitism that lessens the livestock productivity and performance (Zvinorova *et al.*, 2016). Infection of protozoa among livestock may affect the quality of protein products as well as cause zoonosis problems, weight loss, and growth retardation in young animals (Maharana *et al.*, 2016).

In this study, there was no infection of *Blastocystis*

sp. occurred among livestock reared in the intensive farm management system (0%). Both intensive farm workers in Kuantan and Bera frequently cleaned and removed the animals' manure (once every two days). In addition, the animals in both intensive farms were given the immunization shoot and anti-parasitic drug twice a year. The intensive farm management system usually implies a large number of animals per area unit and produces a high production, concurrently with the good technology in the feeding process, good sanitary control, and the animals are more sensitive towards the disease (Monteiro *et al.*, 2017). On top of that, although an intensive farm management system requires large costs for the installation and maintenance compared to a semi-intensive system, it provides a balance to the environmental, economic, and social factors of the livestock. *Blastocystis* sp. infection may be regulated or eradicated due to modern farming practices which exhibit a greater emphasis on hygiene compared to the traditional ones (Navarro *et al.*, 2008). Good farm hygiene is the main key to determining a good husbandry system. Without overcrowding on the farm and less contact between the animal, it would reduce parasite transmission (Zvinorova *et al.*, 2016). The animals in intensive farms were segregated according to their health condition, hence preventing overcrowding. As reported by Kamaruddin *et al.* (2020), *Blastocystis* sp. infection in cattle was lower in Ulu Lepar (5%) and Ceruk Paloh (2%) farms which practice intensive management system, as compared to Muazam Shah farm (18.33%) which practices semi-intensive management system.

CONCLUSION

In conclusion, the study shows that *Blastocystis* sp. infection was the highest among sheep reared under a semi-intensive farm management system. No *Blastocystis* sp. was detected in animals reared in an intensive farm management system. As such, *Blastocystis* sp. infection could be related to the farm management system used in livestock rearing. Further studies need to be conducted to determine the relationship between farm management systems and *Blastocystis* sp. genetic diversity.

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ETHICAL STATEMENT

Animal ethical approval for this study was obtained from International Islamic University Malaysia Animal Ethics Committee (IACUC-2018 (25)). Permissions were obtained from the farm managers/owners/animal handlers to have their animals involved.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abdullah, D.A., Ola- Fadunsin, S.D., Ruviniyia, K., Gimba, F.I., Chandrawathani, P., Lim, Y.A. L., Jesse, F.F.A. & Sharma, R.S.K. 2019. Molecular detection and epidemiological risk factors associated with *Cryptosporidium* infection among cattle in Peninsular Malaysia. *Food and Waterborne Parasitology*, **14**: e00035. <https://doi.org/10.1016/j.fawpar.2019.e00035>
- Abdulsalam, A.M., Awatif, M., Ithoi, Init., Hesham, M. & Abdulhamid. 2012. Drinking water is a significant predictor of *Blastocystis* infection among rural Malaysian primary school children. *Parasitology*, **139**(8): 1014-1020. <https://doi.org/10.1017/S0031182012000340>
- Abe, N., Nagoshi, M., Takami, K., Sawano, Y. & Yoshikawa, H. 2002. A survey of *Blastocystis* sp. in livestock, pets and zoo animals in Japan. *Veterinary Parasitology*, **106**(3): 203-212. [https://doi.org/10.1016/S0304-4017\(02\)00050-X](https://doi.org/10.1016/S0304-4017(02)00050-X)
- Adedotun, A.A.R., Mohd Zain, S.N. & Farah Haziqah, M.T. 2020. Current status of *Blastocystis* sp. in animals from Southeast Asia: A review. *Parasitology Research*, **119**: 3559- 3570. <https://doi.org/10.1007/s00436-020-06828-8>
- Ahmed, S.A. & Karanis, P. 2018. *Blastocystis* spp., ubiquitous parasite of human, animals and environment. *Encyclopedia of Environmental Health*, **2**: 1-6. <https://doi.org/10.1016/B978-0-12-409548-9.10947-9>
- Alfellani, M.A., Taner-Mulla, D., Jacob, A.S., Imeede, C.A., Yoshikawa, H., Stensvold, C.R. & Clark, C.G. 2013. Genetic diversity of *Blastocystis* in livestock and zoo animals. *Protist*, **164**(4): 497-509. <https://doi.org/10.1016/j.protis.2013.05.003>
- Badparva, E., Sadraee, J. & Kheirandish, F. 2014. Genetic diversity of *Blastocystis* isolated from cattle in Khorramabad, Iran. *Jundishapur. Journal of Microbiology*, **8**: e14810. <https://doi.org/10.5812/jjm.14810>
- Bandara, D.M.D.S., Premaratne, S. & Dematawewa, C.M.B. 2011. Production and economic characteristics of intensive and semi-intensive dairy cattle management systems in vegetable based farming system in Welimada, Sri Lanka. *Tropical Agricultural Research*, **22**(3): 314-323. <https://doi.org/10.4038/tar.v22i3.3704>
- Boorom, K.F., Smith, H., Nimri, L., Viscogliosi, E., Spanakos, G. & Parkar, U. 2008. Oh my aching gut: Irritable bowel syndrome, *Blastocystis*, and asymptomatic infection. *Parasites & Vectors*, **1**: 40. <https://doi.org/10.1186/1756-3305-1-40>
- Chandrasekaran, H., Govind, S.K., Panchadcharam, C. & Bathmanaban, P. 2014. High lipid storage in vacuolar forms of subtype 6 *Blastocystis* sp. in ostrich. *Parasites & Vectors*, **7**: 469. <https://doi.org/10.1186/s13071-014-0469-7>
- Department of Veterinary Services. 2018. Malaysia: Livestock Population, 2018.
- El Safadi, D. 2014. Children of Senegal River Basin show the highest prevalence of *Blastocystis* sp. ever observed worldwide. *BMC Infectious Disease*, **14**: 164. <https://doi.org/10.1186/1471-2334-14-164>
- Eroglu, F., Genc, A., Elgun, G. & Koltas, I.S. 2009. Identification of *Blastocystis hominis* isolates from asymptomatic and symptomatic patients by PCR. *Parasitology Research*, **105**: 1589-1592. <https://doi.org/10.1007/s00436-009-1595-6>
- Gregório, R., Moura, F. & Oliveira-silva, M.B.De. 2018. Occurrence of *Blastocystis* spp. in domestic animals in Brazil, Triangula Mineira. *Revista da Sociedade Brasileira de Medicina Tropical*, **51**(2): 240-243. <https://doi.org/10.1590/0037-8682-0484-2016>
- Hasan, J., Ahmed, J.U. & Alam, M. 2014. Reproductive performances of Black Bengal goat under semi-intensive and extensive conditions at rural areas in Bangladesh. *Journal of Advanced Veterinary and Animal Research*, **1**(4): 196-200. <https://doi.org/10.5455/javar.2014.a37>
- Hashim, N. & Yusof, A.M. 2016. Rearing systems related to gastrointestinal parasites in goats from selected area in Terengganu. *Jurnal Teknologi*, **10**:133-138. <https://doi.org/10.11113/jt.v78.8018>
- Helena, L.C.S., Carolina, V.B., Renata, C.B. & Heloisa, W.D.M. 2017. *Blastocystis* spp.: Current status and research issues. *EC Gastroenterology and Digestive System*, **3**(1): 48-54.
- Helena, L.C.S., Fernando, C.S. & Heloisa, W.D.M. 2014. *Blastocystis* sp. in splenic cysts: Causative agent or accidental association? A unique case report. *Parasites & Vectors*, **7**: 207. <https://doi.org/10.1186/1756-3305-7-207>
- Hemalatha, C., Chandrawathani, P., Suresh, Kumar, 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.

- Inani, R.N.R. & Yusof, M.A. 2018. Seasonal prevalence of gastrointestinal parasitic infections in goat in a commercial farm, Kuantan, Malaysia. *Asian Journal of Agriculture and Biology*, **6(4)**: 455-460.
- Jimenez, P.A., Jaimes, J.E. & Ramirez, J.D. 2019. A summary of *Blastocystis* subtypes in North and South America. *Parasites & Vectors*, **12**: 376. <https://doi.org/10.1186/s13071-019-3641-2>
- Jones, W.R. 1946. The experimental infection of rats with *Entamoeba histolytica*. *Annals of Tropical Medicine and Parasitology*, **40**: 130-140. <https://doi.org/10.1080/00034983.1946.11685270>
- Kamaruddin, S.K., Mat Yusof, A. & Mohammad, M. 2020. Prevalence and subtype distribution of *Blastocystis* sp. in cattle from Pahang, Malaysia. *Tropical Biomedicine*, **37(1)**: 127-141.
- Kamarulzaman, N.H. 2016. Analysis of Malaysian beef industry in peninsular Malaysia under different importation policies scenarios and rate management systems. *Pertanika Journal of Social Sciences and Humanities*, **21**: 1-16.
- Kochewad, S.A., Meena, L.R., Meena, L.K., Kumar, D., Kumar, S., Meena, L.K. & Singh, S.P. 2018. Livestock based improvement of small and marginal farmers. *Food Technology and Environment*, **3(1)**: 526-532. <https://doi.org/10.46370/sajfte.2017.v03i01.09>
- Khor, S.K., Jamaiyah, M.I., Zulkarnain, M., Suhaimi, A., Shahaza, O., Aishya, H., Syamsul, A. & Saipul, B.A.R. 2018. A survey of gastrointestinal parasites infection on small ruminant farms in Seberang Perai Selatan district, Penang, Malaysia. *Malaysian Journal of Veterinary Research*, **9(1)**: 15-21.
- Lee, L.I., Chye, T.T., Karmacharya, B.M. & Govind, S.K. 2012. *Blastocystis* sp.: Waterborne zoonotic organism, a possibility? *Parasites & Vectors*, **5**: 130. <https://doi.org/10.1186/1756-3305-5-130>
- Leelayoova, S., Rangsin, R., Taamasri, P., Naaglor, T., Thathaisong, U. & Mungthin, M. 2004. Evidence of waterborne transmission of *Blastocystis hominis*. *American Journal of Tropical Medicine and Hygiene*, **70(76)**: 658-662. <https://doi.org/10.4269/ajtmh.2004.70.658>
- Leelayoova, S., Siripattanapipong, S., Thathaisong, U., Taamasri, P., Naaglor, T., Piyaraj, P. & Mungthin, M. 2008. Drinking water: A possible source of *Blastocystis* spp. subtype 1 infection in school children of a rural community in central Thailand. *American Journal of Tropical Medicine and Hygiene*, **79(73)**: 401-406. <https://doi.org/10.4269/ajtmh.2008.79.401>
- Lei, D., Yijun, C., Ziyao, Z., Haifeng, L., Zhijun, Z., Yanchun, H., Hualin, F., Chanjuan, Y. & Guangneng, P. 2019. Epidemiology of *Blastocystis* sp. infection in China: A systematic review. *Parasite*, **26**: 41. <https://doi.org/10.1051/parasite/2019042>
- Li, L.H., Zhou, X.N., Du, Z.W., Wang, X.Z., Wang, L.B. & Jiang, J.Y. 2007. Molecular epidemiology of human *Blastocystis* in a village in Yunnan province, China. *Parasitology International*, **56**: 281-286. <https://doi.org/10.1016/j.parint.2007.06.001>
- Li, W.C., Wang, K. & Gu, Y. 2018. Occurrence of rova *Blastocystis* sp. and *Pentatrichomonas hominis* in sheep and goats in China. *Parasites & Vectors*, **11(1)**: 1-7. <https://doi.org/10.1186/s13071-018-2671-5>
- Maharana, B.R., Kumar, B., Sudhakar, N.R., Behera, S.K. & Patbandha, T.K. 2016. Prevalence of gastrointestinal parasites in bovines in and around Junagadh (Gujarat). *Journal of Parasitic Disease*, **40(4)**: 1174-1178. <https://doi.org/10.1007/s12639-015-0644-6>
- Manyazewal, A., Francesca, S., Pal, M., Gezahegn, M., Tesfave, M., Lucky, M., Teklu, W. & Getachew, T. 2018. Prevalence, risk factors and molecular characterization of *Cryptosporidium* infection in cattle in Addis Ababa and its environs, Ethiopia. *Veterinary Parasitology*, **3**: 79-84. <https://doi.org/10.1016/j.vprsr.2018.03.005>
- Meteorological Department. 2020. Interactive weather in Pahang for 2018 to 2020. URL <https://www.met.gov.my/info/pejabatmeteorologi>
- Mohamed, Z. A. 2007. The livestock industry. In: *50 Years of Malaysian Agriculture: Transformation Issues, Challenges and Direction*. S.A. Idid Fatimah, M.A., Raja Abdullah, N.M., Kaur, B., Abdullah, A.M. (Eds.). Universiti Putra Malaysia Publication, Serdang. pp. 553-584.
- Mohd Zain, S.N., Farah Haziqah, M.T., Woh, P.Y., Fazly Ann, Z., Vickneshwaran, M., Mohd Khalid, M.K.N. & Suresh, K. 2017. Morphological and molecular detection of *Blastocystis* in wildlife from Tioman Island, Malaysia. *Tropical Biomedicine*, **34(1)**: 249-255.
- Monteiro, A., Costa, J.M. & Lima, M.J. 2017. Goat system productions: Advantages and disadvantages to the animal, environment and farmer. In: *Goat Science*. S. Kukovics (Ed.). Intech Open Publication, London. pp. 351-366. <https://doi.org/10.5772/intechopen.70002>
- Noradilah, S.A., Anuar, T.S., Moktar, N., Lee, I.L., Salleh, F.M., Azreen, S.N.A.M. & Abdullah, S.R. 2017. 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(6)**: 1151-1160.
- National Agro-Food Policy. 2015. URL <http://www.moa.gov.my/web/guest/dasar> (accessed 24.7.2019).

- Navarro, C., Dominguez- Marquez, M.V., Garijo-Toledo, M.M., Vega-Garcia, S., Fernandez Barredo, S., Perez Garcia, M.T., Garcia, A., Borrás, R. & Gomez-Munoz, 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**: 347-358. <https://doi.org/10.1016/j.vetpar.2008.02.003>
- Ning, C.Q., Hu, Z., Chen, J., Ai, L. & Tian, L.G. 2020. Epidemiology of *Blastocystis* infection from 1990 to 2019 in China. *Infectious Disease Of Poverty*, **9**: 168. <https://doi.org/10.1186/s40249-020-00779-z>
- Rosali, M.H. & Mohammad Nor, N.A.A. 2015. The development and future direction of Malaysia's livestock industry. Food and Fertilizer Technology Center for the Asean and Pacific Region. Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia.
- Shanmuganvelu, S. 2014. Decision Support System in Livestock Production. Research Inaugural Lecture. Malaysian Agricultural Research and Development Institute (MARDI), Serdang, Malaysia.
- Skotarczak, B. 2018. Genetic diversity and pathogenicity of *Blastocystis*. *Annals of Agricultural and Environmental Medicine*, **25(3)**: 411-416. <https://doi.org/10.26444/aaem/81315>
- Sohail, M.R. & Fischer, P.R. 2005. *Blastocystis* hominis and travelers. *Travel Medicine and Infectious Disease*, **3**: 33. <https://doi.org/10.1016/j.tmaid.2004.06.001>
- Song, J.K., Yin, Y.L., Yuan, Y.J., Tang, H., Ren, G.J. & Zhang, H.J. 2017. First genotyping of *Blastocystis* sp. in dairy, meat and cashmere goats in Northwestern China. *Acta Tropica*, **176**: 277-282. <https://doi.org/10.1016/j.actatropica.2017.08.028>
- Stensvold, C.R., Alfellani, M.A., Norkov-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 of Parasitology*, **39**: 473-479. <https://doi.org/10.1016/j.ijpara.2008.07.006>
- Stenzel, D. & Boreham, P. 1996. *Blastocystis* hominis revisited. *Clinical Microbiology Reviews*, **9**: 563-584. <https://doi.org/10.1128/CMR.9.4.563>
- Suresh, K. & Smith, H. 2004. Comparison of methods for detecting *Blastocystis hominis*. *European Journal of Clinical Microbiology and Infectious Diseases*, **23(6)**: 509-511. <https://doi.org/10.1007/s10096-004-1123-7>
- Susana, Y., Suwanti, L.T. & Suprihati, E. 2019. Identification and prevalence of gastrointestinal parasites in beef cattle in Siak Sri Indrapura, Riau, Indonesia. *Indonesian Journal of Tropical and Infectious Disease*, **7**: 155-160. <https://doi.org/10.20473/ijtid.v7i6.10392>
- Suwanti, L.T., Susana, Y., Hastutiek, P., Suprihati, E., Dyah, N. & Latuti, R. 2020. *Blastocystis* spp. subtype 10 infected beef cattle in Kamal and Socah. *Veterinary World*, **13(2)**: 231-237. Swarnkar, C.P. & Singh, D. 2010. Questionnaire survey on sheep husbandry and worm management practices adopted by farmers in Rajasthan. *Indian Journal of Small Ruminants*, **16(2)**: 199-209. <https://doi.org/10.14202/vetworld.2020.231-237>
- Tan, K.S. 2004. *Blastocystis* in humans and animals: New insights using modern methodologies. *Veterinary Parasitology*, **126(1)**: 121-144. <https://doi.org/10.1016/j.vetpar.2004.09.017>
- Tan, K.S., 2008. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clinical Microbiology Reviews*, **21**: 639-665. <https://doi.org/10.1128/CMR.00022-08>
- Tan, T.K., Panchadcharam, C., Low, V.L., Bathmanaban, P., Lee, S.C., Chua, K.H., Sharma, R.S.K., Ngui, R., Tay, S.T., Quaza, N.H.N. & Lim, Y.A.L. 2017. Occurrence of gastrointestinal parasites among small ruminants in Malaysia: Highlighting *Dicrocoelium* infection in goats. *Tropical Biomedicine*, **34(4)**: 963-969.
- Tan, T.K., Panchadcharam, C., Low, V.L., Lee, S.C., Ngui, R., Shama, R.S. K. & Lim, Y.A. L. 2014. Coinfection of *Haemonchus contortus* and *Trichostrongylus* spp. among livestock in Malaysia as Transcriber Spacer II DNA Region. *BMC Veterinary Research*, **10(1)**: 1-7. <https://doi.org/10.1186/1746-6148-10-38>
- 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)**: 85-89. <https://doi.org/10.1007/s00436-012-3107-3>
- Tung, K.C., Huang, C.C., Pan, C.H., Yang, C.H. & Lai, C.H. 2012. Prevalence of gastrointestinal parasites in yellow cattle between Taiwan and its Offshore Islands. *The Thai Journal of Veterinary Medicine*, **42(2)**: 219-224.
- Udonsom, R., Prasertbun, R., Mahittikorn, A., Mori, H., Changbunjong, T., Komalamisra, C. & 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>
- Vaisusuk, K., Saijuntha, W., Sedlak, S., Thanchomngang, T., Pilap, W., Suksavate, W., Stensvold, C.R. & Tantrawatpan, C. 2018. *Blastocystis* subtypes detected in long-tailed macaques in Thailand-further evidence of cryptic host specificity. *Acta Tropica*, **184**: 78-82. <https://doi.org/10.1016/j.actatropica.2017.09.002>

- 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>
- Wawrzyniak, I., Poirier, P., Viscogliosi, E., Dionigia, M., Texier, C., Delbac, F. & Aloui, H. E. 2013. *Blastocystis*, an unrecognized parasite: An overview of pathogenesis and diagnosis. *Therapeutic Advances in Infectious Disease*, **1**: 167-168. <https://doi.org/10.1177/2049936113504754>
- Yoshikawa, H., Wu, Z., Kimata, I., Isek, M., Ali, I.K.M. & Hossain, M.B. 2004. Polymerase chain reaction based genotype classification among human *Blastocystis hominis* populations isolated from different countries. *Parasitology Research*, **92**: 22-29. <https://doi.org/10.1007/s00436-003-0995-2>
- Zhang, S.X., Zhou, Y.M., Xu, W., Tian, L.G., Chen, J.X., Chen, S.H. 2016. Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in Southwest China. *Infectious Disease of Poverty*, **27**: 64. <https://doi.org/10.1186/s40249-016-0157-2>
- Zhu, W., Tao, W., Gong, B., Yang, H., Li, Y., Song, M. & Li, W. 2017. First report of *Blastocystis* infection in cattle in China. *Veterinary Parasitology*, **246**: 38-42. <https://doi.org/10.1016/j.vetpar.2017.09.001>
- Zvinorova, P.I., Halimani, T.E., Muchadeyi, F.C., Matika, O., Riggio, V. & Dzama, K. 2016. Prevalence and risk factors of gastrointestinal parasitic infections in goats in low-output farming systems in Zimbabwe. *Small Ruminant Research*, **143**: 75-83. <https://doi.org/10.1016/j.smallrumres.2016.09.005>

