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. 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

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 (Boorom 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., 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 al., 2008) and close contact with infected animals (Li et al., 2007; Eroglu et al., 2009; Lee et al., 2012).
The prevalence of 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 Skotaraczak 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 37ºC. 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 Superman 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>
<thead>
<tr>
<th>Animal</th>
<th>Total Number Examined</th>
<th>Number Infected</th>
<th>Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>92</td>
<td>27</td>
<td>29.34</td>
</tr>
<tr>
<td>Goats</td>
<td>96</td>
<td>28</td>
<td>29.16</td>
</tr>
<tr>
<td>Sheep</td>
<td>65</td>
<td>28</td>
<td>43.07</td>
</tr>
<tr>
<td>Total</td>
<td>253</td>
<td>83</td>
<td>32.80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total No.</th>
<th>%</th>
<th>Total No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>35</td>
<td>0.0</td>
<td>35</td>
<td>0.0</td>
</tr>
<tr>
<td>Goats</td>
<td>31</td>
<td>0.0</td>
<td>31</td>
<td>0.0</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>0.0</td>
<td>66</td>
<td>0.0</td>
</tr>
</tbody>
</table>

---

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

---

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

<table>
<thead>
<tr>
<th>Animal</th>
<th>Management system</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensive ($N=66$)</td>
<td>Semi-intensive ($N=187$)</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cattle</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>Goat</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>Sheep</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>66</td>
</tr>
</tbody>
</table>
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 gaur, 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 Udomson 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, Udomson 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, dear, and swine (Tan et al., 2014).
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 unhealthy 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 lessons 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.

ACKNOWLEDGEMENTS

This research is fully supported by the FRGS grant, FRGS 15-188-0429. The authors fully acknowledged the Ministry of Higher Education (MOHE) and International Islamic University Malaysia (IIUM) for the approved fund which makes this research feasible. We would also like to acknowledge CREAM, IIUM Kuantan for facilitating the laboratory works. We would also express our gratitude to the farmers for their cooperation throughout the research.
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.

CONFICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES


Inani, R.N.R. & Yusof, M.A. 2018. Seasonal


Navarro, C., Dominguez- Marquez, M.V., Garijo-Toledo, M.M., Vega-Garcia, S., Fernandez Barredo, S., Perez Garcia, M.T., Garcia, A.,
PREVALENCE OF Blastocystis sp.


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.


Wang, J., Gong, B., Yang, F., Zhang, W., Zheng, Y. & Liu, A. 2018. Subtype distribution and genetic characterizations of Blastocystis in pigs,


