



Sarcocystis in carcasses of small ruminant slaughtered in abattoir in Perak, Malaysia

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ABSTRACT

Sarcocystis spp. is a cyst-forming intracellular protozoa with an obligatory two-host life cycle. The prey-predator relationships may result in muscular sarcocystis in the intermediate host and intestinal sarcocystis in the definitive host. The study on the parasite was conducted in abattoir in Perak with the aim to detect the prevalence of sarcocystis in small ruminant from the slaughtered carcasses by digestion method and histological examination. A total of 90 fresh muscle samples from 30 goats were randomly collected commencing from March to August 2016. The result shows 23.33% (7 out of 30) of the animals were positive with *Sarcocystis* microcyst with 20.00% (6 out of 30) and 13.33% (4 out of 30) by digestion method and histological examination, respectively. Most of the positive samples were detected from the thigh muscles by both diagnostic techniques. Among the positive animals, 14.29% (1 out of 7) of the animals were heavily infected with *Sarcocystis* microcyst detected in the heart, esophagus and thigh muscle samples. No gross *Sarcocystis* macrocyst were detected upon observation. Even though sarcocystis in small ruminant in this study was reported less than quarter of the samples, the presence of the parasite could not be ignore as it may infect definitive host which is the carnivorous animals. Provide thoroughly cooked meat to the animals will reduce the potential spread of the infection.

Introduction

Small ruminants are goat and sheep which refer to the cloven-footed animal belongs to the genus of *Capra* and *Ovis*, respectively (Otaru and Iyiola-Tunji, 2015). Some of the goat breeds that available in Malaysia are Boer, Katjang, Jermasia and Jamnapari (Department of Veterinary Services, 2015a), and the sheep breeds are Malin, Dorset, Wiltshire and Suffolk (Department of Veterinary Services, 2015b). Among all, Katjang goat and Malin sheep are recognized as an indigenous breed of Malaysia (Ernie *et al.*, 2010; Mastura *et al.*, 2014). According to Department of Veterinary Services (2018a), the populations of goats and sheep in Perak in 2016 were recorded at 31,074 and 3,902, accordingly. In terms of meat, the small ruminant meat was the least choice of selection compared to beef, carabeef, pork and poultry meat with the per capita consumption for both chevon and mutton in Malaysia were reported at 1.2 kg for the respective year (Kaur, 2010).

Sarcocystis is an emerging zoonotic disease caused by a cyst-forming coccidian from the genus of *Sarcocystis* (Latif and Muslim, 2016). It is an apicomplexan intracellular protozoan with an obligatory two-host life cycle. There are more than 200 species of *Sarcocystis* were recorded worldwide, and the parasite can be found not only in the tissues of mammals including humans, but also reptiles and avian (Dubey, 2015; Fayer, 2004; Fayer *et al.*, 2015). In animals, the prevalence of sarcocystis were reported between 70 to 100% (Oryan *et al.*, 2010; Woldemeskkel and Gebreab, 1996). The life cycle is based on prey-predator relationships and involves herbivore or omnivore as an intermediate host, and carnivore as a definitive host (Singla and Juyal, 2014). The intermediate host is subjected to muscular sarcocystis by ingesting the infective stage (sporocysts), and development of the sarcocyst in the muscle tissue. Whereas, the definitive host consume the infected muscle tissue and developes intestinal

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sarcocystis. The definitive host will then pass the sporocysts to the intermediate host through feces or feces contaminated food by fecal-oral route. As in small ruminants, the infected animals are intermediate host which the sarcocyst either macro- or microcyst that can be found on the muscle tissue (Dong *et al.*, 2018; Dubey, 2015).

Muscular sarcocystis may cause clinical symptoms such as muscle weakness, weight loss, anemia and anorexia in serious cases (Dubey, 2015). However, animals usually will develop mild infection and seldom show any symptoms. The parasite was typically detected upon post mortem examination of the carcasses. Sarcocystis infections of sheep and goats are common throughout the world (Anvari *et al.*, 2020; Swar and Shnawa, 2021). Globally, the common species reported in goats are *S. biricanis*, *S. capracanis*, and *S. caprafelis* (syn. *S. moulei*). In sheep, *S. tenella* (syn. *S. ovis*), *S. arieticanis*, *S. gigantea* (syn. *S. ovifelis*) and *S. medusiformis* were usually reported (Bittencourt *et al.*, 2016; Dong *et al.*, 2017; Hong *et al.*, 2016; Hu *et al.*, 2016; Metwally *et al.*, 2019; Zangana and Hussein, 2017). In Malaysia, Latif *et al.* (2015) has reported the detection of *S. capracanis* and *S. ovis* in slaughtered goats and sheep, respectively, in the state of Selangor.

To date, the occurrences of sarcocystis of small ruminant in the state of Perak were underreported due to the lack of study conducted. Therefore, this study was aimed to detect the prevalence of sarcocystis in small ruminant from the carcasses slaughtered in abattoir in Perak, Malaysia by digestion method and histological examination.

Materials and Methods

Site and time

The study was performed in Tapah abattoir under Department of Veterinary Services (DVS), Malaysia. The sampling was done once every interval weeks commencing from March to August 2016.

Sample collection

A total of 90 fresh muscle samples consisting of 30 heart, esophagus and thigh muscle samples each from 30 mix breed of goats were randomly collected from the abattoir. All the fresh muscle samples were divided into two parts for different laboratory diagnostic detection. The samples for digestion method were kept in sealed plastic bag and stored between 2°C and 8°C in the ice box during transportation. Whereas, samples for histological examination were cut with an estimated size of 2 cm³ and placed inside the glass bottles with 10% formalin at room temperature. Further laboratory examination

was conducted at Zoonotic and Pathology Section of Veterinary Research Institute, Ipoh, Perak within 48 hours of samples arrival.

Gross examination

At the abattoir, the goat carcasses were inspected thoroughly to detect the presence of *Sarcocystis* macrocyst upon post mortem examination. Furthermore, all the fresh muscle samples collected for digestion method were double checked for any presence of the visible cyst. The rice grain-like or any whitish filamentous appearance presenting the *Sarcocystis* macrocyst on the muscle samples were screened thoroughly by naked eyes (Lam *et al.*, 1999). Any suspected samples were also slice and cut into smaller pieces using scissors and scalpel blade to facilitate better visual detection of *Sarcocystis* macrocyst (Fazly Ann *et al.*, 2013).

Digestion method

A 50 g of each fresh heart, esophagus and thigh muscle samples were minced and submerged in 100 mL of distilled water. The samples were then homogenized using blender and settled down for 5 minutes prior removal of the supernatant. The remaining sediment was then digested with 1.5% hydrochloric acid and pepsin (Sigma®, USA) solution. After 12 hours of incubation at 30°C, the digested muscle was sieved through a nylon-meshed tea strainer to remove the indigestible particles. The filtrated solution was then centrifuged at 1500 rpm (Eppendorf Centrifuge 5804R, Germany) for 5 minutes. The supernatant was then discarded and a drop of sediment was dried, methanol fixed and stained with 8% Giemsa (Sigma®, USA). Then, the samples were examined under the light microscope (Leica DME, USA) at X400 magnification for detection of banana-like or crescent shape of *Sarcocystis* bradyzoites.

Histological examination

The formalin fixed heart, esophagus and thigh muscle samples were dehydrated by different concentration of ethanol, embedded with paraffin, sectioned at 4 µm to 5 µm, and prepared for stained with haematoxylin and eosin (H&E) (Bancroft and Stevens, 1999). The processed tissues were then examined under the light microscope (Olympus BX41, Japan) at X100 and X400 magnification for histological detection of the *Sarcocystis* microcyst.

Data analysis

Data were obtained from each sample based on the presence of *Sarcocystis* macrocyst and microcyst observation of the gross examination, digestion method and histological examination. Additionally, the presences of the microcyst were also compared

between digestion method and histological examination used in this study.

Results

In this study, the gross examination on the muscle tissue of the goat carcasses shows the absent of *Sarcocystis* macrocyst. However, for *Sarcocystis* microcyst, 7 out of 30 animals (23.33%) were detected positive. Presented in Table 1 is the number of positive animals detected with sarcocyst at the predominant sites according to the diagnostic techniques. The result shows 6 out of 30 (20.00%) animals were positive by digestion method and 4 out of 30 (13.33%) animals positive by histological examination. By digestion method, 2 out of 7 (28.57%) of the positive animals were detected by the present of *Sarcocystis* bradyzoites in the heart and 4 out of 7 (57.14%) from the thigh muscle samples. The detection of *Sarcocystis* microcyst by histological examination shows 1 out of 7 (14.29%) of the positive animals were positive from heart and esophagus each, and 2 out of 7 (28.57%) positive from the thigh muscle samples.

As presented in Table 2, most of the positive samples were detected from thigh muscle sample by both diagnostic techniques. Among the positive animals, 5 out of 7 (71.43%) were detected positive from thigh muscle only, 1 out of 7 (14.29%) from heart muscle only, and another 1 out of 7 (14.29%) was heavily infected which the *Sarcocystis* microcyst were detected in the heart, esophagus and thigh muscle samples.

Table 1. Number of positive animals detected with sarcocyst in heart, esophagus and thigh muscle samples according to the diagnostic techniques.

	Number of positive samples/numbers of animal (%)		
	Macrocyst	Microcyst	
	Gross examination	Digestion method	Histological examination
Heart	0/30 (0.00)	2/30 (6.67)	1/30 (3.33)
Esophagus	0/30 (0.00)	0/30 (0.00)	1/30 (3.33)
Thigh	0/30 (0.00)	4/30 (13.33)	2/30 (6.67)
Total	0/30 (0.00)	6/30 (20.00)	4/30 (13.33)

Table 2. Detection of *Sarcocystis* microcyst in different type of tissue samples by digestion method and histological examination.

	Digestion method			Histological examination		
	Heart	Esophagus	Thigh	Heart	Esophagus	Thigh
Goat 1	+	-	-	-	-	-
Goat 2	-	-	+	-	-	-
Goat 3	+	-	-	+	+	+
Goat 4	-	-	-	-	-	+
Goat 5	-	-	+	-	-	-
Goat 6	-	-	+	-	-	-
Goat 7	-	-	+	-	-	-

Note: (+) detected positive sarcocystis, (-) detected negative sarcocystis

Discussion

In state of Perak there are four available government abattoirs under Department of Veterinary Services (DVS), Malaysia which are located in Ipoh (GPS: 4.569415, 101.0601), Tapah GPS: 4.248245, 101.2341), Teluk Intan (GPS: 3.988708, 100.9528), and Taiping (GPS: 4.836187, 100.7451). Among the abattoirs, Tapah abattoir was selected based on the highest number of small ruminant received for slaughter. Based on observation, Teluk Intan and Taiping abattoir received high number of large ruminants due to the equipped facilities, while Ipoh abattoir received a lot pig to slaughter. During sampling period, only goats were received for slaughter, and no sheep were sent by client on the scheduled sampling days. The number of goats involved in the sampling represents 4.12% (30 out of 728) of the total goats slaughtered in the state of Perak during the sampling months (Department of Veterinary Services, 2018b).

Small ruminants can be infected with the parasite through accidental ingestion of the infective stage which is the sporocyst from the contaminated drinking water and feed. In order to prevent the disease occurrence in the farm, good farm management practice such as consistently provide clean feed and water tray will help to reduce the exposure to the sporocyst. Ingestion of the sporocyst resulted in the release of the sporozoites. The sporozoites will then enter the endothelial cell of the blood vessels and undergo schizogony. The schizonts will then ruptured and release the merozoites that will then penetrate the muscle cells and develop into cyst containing bradyzoites which also known as muscular sarcocystis. The predominant sites of muscular sarcocystis are heart, diaphragm, tongue, esophagus, and skeletal muscle of the intermediate host (Buxton, 1998; Daryani et al., 2006; Dehaghi et al., 2013).

The shape of sarcocyst in the muscle tissue can be globular, elongated or filamentous. Besides species dependent, according to Hong et al. (2016), the shape and size of sarcocyst is determined by the shape of the host cells and the age of the *Sarcocystis* spp. In small ruminant, the common *Sarcocystis* macrocyst are *S. capraefelis* in goats, and *S. gigantea* and *S. medusiformis* in sheep. While *S. capracanis* and *S. biricanis* in goats, and *S. tenella* and *S. arieticanis* in sheep will be developed as microscopic cyst in the muscle tissue (Dubey et al., 1989). The study by Metwally et al. (2019) on *Sarcocystis* microcyst has reported the size

of *S. capracanis* was at 869 µm × 648 µm to 370 µm × 70 µm and *S. tenella* was at 851 µm × 75 µm to 185 µm × 31 µm by transmission electron microscopy (TEM).

Detection of the *Sarcocystis* bradyzoites of the microcyst in muscular tissue by digestion method is considered as one of the most sensitive and commonly applied technique (Dubey et al., 1989). It is the gold standard for detection of sarcocystis (Hamidinejat et al., 2010). Several digestion methods have been published by using different types of digestion medium namely pepsin, trypsin or hydrochloric acid to release the crescent or banana-like shaped bradyzoites from the sarcocyst upon incubation (Beyazit et al., 2007; Dubey et al., 1989; Huong, 1999; Obijiaku et al., 2013; Rahdar and Salehi, 2011). In this study, the sarcocyst wall was dissolved by peptic digestion as described by Fazly Ann et al. (2014).

Besides digestion method, histological examination is the other commonly used technique for detection and structural determination of *Sarcocystis* microcyst in muscle tissue. The elongated spindle-shaped or oval transection of the microcyst was usually visible located between the muscle bundles. Other available conventional diagnostic techniques for sarcocystis detection that were used in several studies includes; direct scotch cellophane adhesive tape technique, muscle mincing, and squash method, while the advanced techniques are by molecular identification and characterization by polymerase chain reaction (PCR), as well as the electron microscopy techniques such as scanning electron microscopy (SEM) and TEM (Hong et al., 2016; Okita et al., 2017; Rahdar and Kardooni, 2017; Zangana and Hussein, 2017).

The zoonotic impact of sarcocystis has been reported in several studies. One of the outbreaks in Malaysia was reported by Arness et al. (1999) where the United State serviceman was diagnosed with the disease upon muscle biopsy. The occurrence of muscular sarcocystis in the study was probably due to accidental ingestion the infective sporocyst from the infected wildlife in Tioman Island. Human may also get infected by consuming raw or undercooked infected livestock meat. However, in livestock, the zoonotic species of *Sarcocystis* was only reported with *S. hominis* and *S. suis* in beef and pork, respectively (Dubey, 2015). Even though there is no small ruminant zoonotic-related species reported, the muscular parasite may still infect the other animals as it act as an intermediate host. A study by Saleque et al. (1990) have shown that *S. meischeriana* in pork was

safe for dogs after the meat was cooked at 100°C for 5 minutes, 70°C for 15 minutes, and 60°C for 20 minutes, or frozen at -20°C for 24 hours or -4°C for 48 hours. In order to prevent the infection, the precaution by practicing eating well cooked meat should be highly considered. In DVS abattoir, thorough examination of the slaughtered carcasses and the internal edible organs was performed according to the standard operating procedure by trained veterinary personnel. All the suspected infected meats including the macroscopic sarcocyst were removed in order to ensure the safety of the meat supply.

Conclusions

Sarcocystis in small ruminant was detected in almost quarter of the tested animals. The selected conventional method used was qualitatively sensitive in the detection of the sarcocystis microcyst. In order to provide more accurate diagnosis, it is recommended to use advanced molecular technique which enable detection of the species of the parasite. The findings of this study is not only to provide the primary data for the prevalence of sarcocystis in small ruminant in Perak, but it also was intentionally done to alert the public regarding the occurrence of the disease.

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