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ORIGINAL ARTICLE

Detection of Sarcocyst in Meat from Local Markets in Selangor: Highlighting the Use of Crude Sample in PCR

Azdayanti Muslim, Putri Shafinaz Sharudin, Atiqah Yunus, Norhabsah Omar, Alieya Zakaria, Norshafiqah Mohamad

Faculty of Medicine, Universiti Teknologi MARA, Selangor, Malaysia

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Corresponding author:
Azdayanti Muslim,
Parasitology Discipline,
Faculty of Medicine,
Sungai Buloh Campus,
Universiti Teknologi MARA (UiTM),
Sungai Buloh,
47000 Selangor,
Malaysia.
Tel: +6012-574 0014,
Fax: +6 03-6126 7428
Email: azdayanti@salam.uitm.edu.my

ABSTRACT

Introduction: *Sarcocystis spp.* are obligate intracellular protozoan parasites which cause meat-borne parasitic disease. In Malaysia, sarcocystosis is seen as a potential emerging food-borne zoonosis after a series of large outbreak of human infections. Humans acquire infection either by ingestion of cyst in raw or undercooked infected meat or from sporocysts in contaminated food and water. The goal of this study is to identify the presence of *sarcocystis* parasites in meat of cattle, buffaloes, sheep and goats collected from local markets in Selangor, Malaysia. **Methods:** A total of 64 skeletal muscles samples (57 cattle, 2 buffaloes, 4 goats and 1 sheep) were collected from local markets. The samples were cut randomly into three pieces, squashed firmly between two glass slides and then examined microscopically for the presence of cysts. **Results:** Three samples of meat (4.69 %) from cattle (1), buffalo (1) and sheep (1) were found to be positive for cysts. The cysts were confirmed by PCR as *sarcocystis sp.* **Conclusion:** The results showed low prevalence of *Sarcocystis* infection in meat collected from local markets. However, since there is a transmission among the livestock, extra precaution should be taken in consideration to prevent the spreading of sarcocystosis from animals to human.

KEYWORDS: Sarcocystosis, meat, local markets, Selangor

INTRODUCTION

Sarcocystis spp. are obligate intracellular protozoan parasite which can cause meat-borne parasitic disease alongside with other parasites, *Toxoplasma gondii*, *Taenia spp.* and *Trichinella spp* [1]. This protozoan needs two hosts; definitive (carnivores & omnivores) and intermediate host (herbivores) to complete its life cycle. Humans can serve as both definitive and intermediate host. Therefore, there are two types of sarcocystosis inflicting man; intestinal and muscular sarcocystosis, depending on the type of species [2]. Decades ago, this parasitic infection received less attention. However, the interest on them has re-ignited especially within the past years after a series of large human outbreaks have been reported in Malaysia [3-7].

Intestinal sarcocystosis is acquired when humans eat raw or undercooked infected beef or pork with cyst stage of the parasite [1]. The infected person may present with fever, nausea, abdominal pain, vomiting, diarrhoea and weight loss. However, the infection is often asymptomatic and often self-limited [8]. Studies in livestock in Malaysia have shown that *Sarcocysts* have been identified in cattle [9, 10], sheep [11] and water buffaloes [10, 12]. In addition, recent study has shown high *Sarcocystis* infection with 61.8 % prevalence rate in goat [13]. Most of the infected animals are asymptomatic and identified by incidental finding at necropsy. Nevertheless, some pathological changes such as fever, haemorrhages, low production of milk and meat, abortion and death have been recorded associated with the infection in livestock [2].

Diagnosis of sarcocystosis involves identification of muscular sarcocystosis in tissue samples and is usually made by detection of macroscopic or microscopic cysts in the skeletal muscle, heart, tongue, esophagus, and diaphragm. Squash method is a reliable and easy technique but should be in parallel with other methods such as

histological examination, and digestion technique. Recently molecular detection by PCR is used widely especially for the determination of the species [9, 13, 14].

Globally, lack of awareness about the importance of cooking meat properly and the habit of eating raw meat among some of the societies leads to an increasing prevalence of zoonotic sarcocystosis. In Malaysia, sarcocystosis is being increasingly recognized and consumption of meat is very common. Most of the studies in Malaysia were focusing more on imported or local livestock taken from slaughterhouse. Hence, the study was carried out to identify the presence of *Sarcocystis spp.* in meat from fresh local market in Selangor, hence providing some information regarding the status of sarcocystosis parasitic infections among local breed livestock.

METHODS

In this study, a total of 64 muscle samples were collected randomly from cattle (n=57), buffaloes (n=2), goats (n=4) and sheep (n=1). All samples were acquired from local fresh food market in the vicinity of Selangor from July-August 2015. Each sample, weighing approximately 250g, was immediately placed in plastic bag and transported to the parasitology laboratory at the Faculty of Medicine, Universiti Teknologi MARA (UiTM) and stored at 4°C before further examination. Each sample was cut into three small pieces and was squeezed firmly between two glass slides. The slides were observed under light microscope at x4 and x10 magnifications for the presence of microscopic cysts. Positive meat samples were cut into smaller pieces and were placed into the garlic crushing instrument for juice extraction according to Latif and his colleagues [15]. The juice was then collected into 1.5 ml Eppendorf tube and centrifuged at 5000 rpm for 10 minutes. After discarding the supernatant, the sediments were then smeared on glass slides and were dipped into absolute methanol for 5 minutes then in 1% Giemsa stain for 24 hours. Then the slides were examined under x100 magnification to detect the presence of bradyzoite.

PCR was carried directly using the remaining juice from the three positive meat samples according to manufacturer's protocols for TOYOBO KOD FX Neo from crude sample. An approximately 1µl juice from each sample was pipetted directly onto the prepared TOYOBO KOD FX Neo PCR master mix (total solution: 50 µl) for amplification of D2 region of the 18S rRNA of *Sarcocystis sp.* Forward primer (5'-GGAAGCGATTGGAACC -3') and reverse primer (5' CCTTGGTCCGTGTTTCA -3') [16] were used. PCR was performed using Mastercycler Gradient (Eppendorf, Germany). Reaction mixture consisted of 200 pM of each primer, 25µl of 2x PCR buffer for KOD FX Neo, 10 µl dNTP, 1µl crude sample (juice) and topped with nuclease-free water to a volume of 50µl. The thermal profile for the amplification is shown in Table 1. PCR product was analysed by agarose gel electrophoresis, viewed and analyzed using GelDocXr +3 System (Bio-Rad Laboratories, USA) and MyImage Analysis Software (Thermo Scientific, USA). The expected size of the PCR product was 350bp-450 bp**.**

Table 1 The thermal profile for the amplification of DNA using TOYOBO KOD FX Neo.

	Temperature	Time	Cycle X
Initial denaturation	94 °C	2 min	1
Denaturation	95 °C	30 s	40
Annealing	55 °C	30 s	
Extension	72 °C	1 min	
Final cycle	72 °C	10 min	1

RESULTS

Of 64 meat samples collected, three (3) samples were found to be positive (4.69 %) with microscopic cysts; 1 from cattle (1.75 %), 1 from buffalo and 1 sheep. Of the four (4) goat samples, none of them were found to be infected with *Sarcocystis* parasites. The image of the microscopic cyst is shown in Figure 1.

Figure 1 Microscopic sarcocyst under light microscopy with x10 magnification

Banana shaped with thin cyst wall bradyzoite was seen in the sample of cattle (Figure 2). While a lot of metrocytes (immature bradyzoites) were found from the sample of buffalo and sheep. PCR confirmed the parasite as *Sarcocytis sp*. (Figure 3).

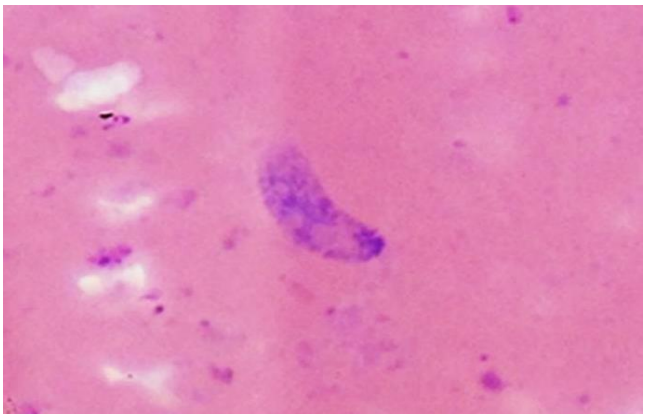

Figure 2 Bradyzoites (banana shaped with nucleus) under light microscopy x100 magnification

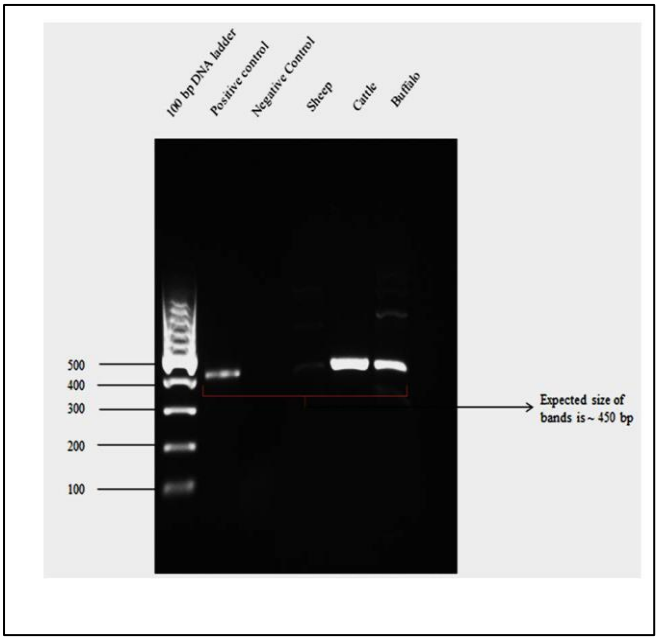

Figure 3 DNA amplification result using TOYOBO KOD FX Neo from crude sample.

DISCUSSION

Our study shows that, the infection rate of *Sarcocystis sp*. is very low with only 4.6 % infection rate in meat samples collected from local market. However, the result was contradictory with the previous studies in meat producing animals in Malaysia using the same method. The most recent study reported the prevalence rate of 86.0 %, 61.0 % and 28.0 % in sheep, goat and cattle respectively [13]. In 2013, 40.8 % cattle and buffalo were found to be infected with sarcocystosis with high infection rate in the esophagus and skeletal muscle [10]. We believed that, the positive rate might be higher if the samples were taken from different parts as well.

In addition, bradyzoites were seen only in cattle sample. Bradyzoite is highly infective and can trigger the infection once consumed. Metrocytes were seen in another two samples and according to Dubey [8], it could be indicated as recently acquired infection. Interestingly, our study confirmed the *Sarcocystis* using TOYOBO KOD FX Neo. This maybe the first study which indicated the successfulness of using PCR from crude samples, without extraction of the DNA. Unfortunately, the PCR was negative in sheep sample, even though it was positive by microscopic examination. The result was not surprising since it could be due to the lack of DNA (only metrocytes were seen) or other technical errors. In 2015, Kutty and team in their comparison study suggested that more than one test were essential for the identification of muscular sarcocystosis [17]. Further comparison study especially on the sensitivity of TOYOBO KOD FX NEO with other molecular techniques need to be done in the future.

Unfortunately sequencing was not done in this study; hence the exact species could not be determined. Nevertheless, studies on meat producing animals in Malaysia and neighboring countries have shown that the predominant species infecting them were *S. bovicanis* [13] and *S. cruzi* [9] in cattle, *S. capracanis* in goat [13], *S. levinei* and *S. fusiformis* [18] in water buffalo and *S. ovicanis* in sheep [13].

CONCLUSIONS

Although the infection rate of sarcocystosis was found low in this study, extra precautions must be taken to prevent the spreading of this infection as it is one of the emerging zoonotic diseases worldwide. Our community needs to be notified about the meat-borne zoonotic diseases, specifically sarcocystosis. Health education to the public is important to prevent its transmission. Consumption of a well-cooked meat is one of the best approaches to prevent sarcocystosis.

Conflict of Interest

Authors declare none.

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