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Research Paper

Sarcocystis infection in Kedah-Kelantan crossbred cattle and Murrah Buffalo slaughtered in abattoir in Perak, Malaysia

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Abstract. Sarcocystis infection, sarcocystosis or sarcosporidiosis is the disease caused by zoonotic intracellular protozoan parasite with an obligatory two-host life cycle namely *Sarcocystis* spp. The affected animals are mostly asymptomatic and the parasite is discovered only at slaughter. The aim of this study is to determine the status of sarcocystis infection in large ruminants slaughtered in four abattoirs in the state of Perak. A total of eighty-six fresh heart muscle and oesophagus samples were collected between February 2013 to October 2013. The samples were examined macroscopically to look for – sarcocyst. Digestion technique was also used to detect the sarcocyst bradyzoites. Part of the samples were preserved in formalin for histological examination. Out of the 86 animals, 19 (22.0%) animals were infected with *Sarcocystis* spp. 22.5% (16 of 71) of cattle and 20.0% (3 of 15) of buffalo were diagnosed with sarcocystis infection. Four animals were detected positive from Ipoh abattoir, followed by 9, 4 and 2 animals from Taiping, Teluk Intan and Tapah abattoir respectively. It is strongly recommended to cook meat thoroughly to reduce the incidence of the disease in humans.

INTRODUCTION

Sarcocystis spp. is an intracellular protozoan parasite with an obligatory two-host life cycle based on prey-predator relationship (Fayer, 2004). Taxonomically the tissue forming coccidian is classified in the family Sarcocystidae and the genus of Sarcocystis. The first description of *Sarcocystis* was reported in mice 150 years ago (Huong, 1999a). According to Narges *et al.*, 2013 there are more than two-hundred species of sarcocystis and the parasite is the most prevalent protozoan among domestic animals. The disease caused by *Sarcocystis* spp. are known as sarcocystis infection, sarcocystosis and sarcosporidiosis.

There are five species of *Sarcocystis* with complete life cycles known in Malaysia. The species are *Sarcocystis fusiformis*, *S. levinei*, *S. villivillosus*, *S. zamani* and *S. singaporensis* (Kan & Pathmanathan, 1991). According to Dubey & Lindsay, 2006), *S. fusiformis* and *S. levinei* have buffaloes as an intermediate host. In cattle, three species of *Sarcocystis* reported are *S. cruzi*, *S. hirsuta* and *S. hominis* (Hamidinejat *et al.*, 2010). Among all, *S. cruzi* is the most common and important species affecting cattle (Mowafy, 2003).

The life cycle of *Sarcocystis* was well described (CDC, 2015; Dubey, 1976; Dubey *et al.*, 1989, 2000; Fayer, 1979, 2004; Lindsay *et al.*, 1995).

The clinical sign of sarcocystis infection in large ruminants are variable. Most of the affected animals are asymptomatic and the parasite is discovered during the slaughtering process in the abattoir. In severely affected animals, they may develop clinical signs which include fever, anorexia, cachexia, diarrhea, anaemia, muscle spasm and weakness. These signs are often seen in animals with heavy infections or in immunocompromised animals (Soulsby, 1982). In infections caused by *S. bovicanis* in cattle, there is usually loss of hair at the end of the tail (Taylor *et al.*, 2007). According to Uggla & Buxton (1990), infection of *Sarcocystis* spp. in cattle may result in abortion and the exact mechanisms for abortions are unknown.

Sarcocystis infection is listed as one of the re-emerging zoonotic parasite meat borne diseases (Dorny *et al.*, 2009). The zoonotic sarcocystis species in cattle that has a public health importance is *S. bovihominis* (cattle-human). A study by Chen *et al.* (2003) mentioned that buffaloes are known to be intermediate host for *S. hominis* which is also found in human. Consumption of undercooked sarcocystis infested meat may encourage transmission of zoonotic infection from large ruminants. The aim of this study is to determine the status of sarcocystis infection in large ruminants slaughtered in four abattoirs in the state of Perak.

MATERIALS AND METHODS

Sample Collection

A total of 86 heart muscle and oesophagus samples from 71 Kedah-Kelantan crossbred cattle and 15 Murrah buffalo were collected from four abattoirs in Perak (Ipoh, n=26; Taiping, n=25; Teluk Intan, n=26; Tapah, n=9). The samples were collected randomly collected during the period February 2013 to October 2013. All the samples were stored at 4 to 6°C for digestion technique. The samples for histological examinations were stored in 10% formalin.

Macroscopic Examination

Macroscopic examination was done in situ during the sample collection. The heart muscle and oesophagus were checked for the presence of whitish filamentous, spindle shaped, cucumber seed-like, rice-grain-like or any globular appearance (Lam *et al.*, 1999). The samples were cut into smaller pieces and sliced to facilitate better visual macroscopic detection of macrocysts (Fazly *et al.*, 2013).

Digestion Technique

50 grams of each fresh heart muscle and oesophagus samples were minced and homogenized. The homogenized samples were allowed to settle for 5 minutes before removal of supernatant. The sediment was then digested with 1.5% hydrochloric acid and pepsin solution. After 12 hours of incubation in 30ºC waterbath, the digested samples were sieved through a nylon-meshed tea strainer and centrifuged at 1500 rpm for 5 minutes. After discarding the supernatant, a drop of sediment was stained with Giemsa and examined under the light microscope at X400 magnification for detection of crescent or banana-shape of sarcocystis bradyzoites (Fazly Ann *et al.*, 2014a).

Histological Examination

The formalin stored heart muscle and oesophagus samples were sliced measuring 10 to 15 mm long and 2 to 3 mm in thickness (Talukder, 2007). The samples were then stained with Haematoxilin and Eosin (H&E) (Bancroft and Stevens, 1993). The processed tissues were examined under low power and high power magnification using a microscope for histological detection of the sarcocysts.

RESULTS

Out of 86 animals, 19 (22.0%) (slaughtered in abattoir in Perak) were infected with *Sarcocystis* spp. 22.5% (16 of 71) of the cattle and 20% (3 of 15) of the buffalo were positive. 15.4% (4 of 26) of animals slaughtered in Ipoh

abattoir were positive 13% (3 of 23) cattle and 33.3% (1 of 3 of buffalo were positive) microscopically. 36% (9 of 25) animals 40% (8 of 20) of cattle and 20% (1 of 5) of buffalo slaughtered from Taiping abattoir were positive for sarcocystis infection. 15.4% (4 of 26) and 22.2% (2 of 9) ruminant from Teluk Intan and Tapah abattoir were diagnosed positive *Sarcocystis* spp. with 17.3% (4 of 23) and 20% (1 of 5) of cattle and 0% (0 of 3) and 25% (1 of 4) of buffalo respectively. The results for sarcocystis infection in cattle and buffalo slaughtered in four abattoirs in Perak are shown in Table 1.

On observation, no macroscopic cyst was found upon examination. Microscopically, 10.5% (9 of 86) of heart muscle samples were positive by histological examination (all the samples were from cattle). 14% (12 of 86) of heart muscle samples were diagnosed positive for sarcocystosis by digestion technique with 14.1% (10 of 71) from cattle and 13.3% (2 of 15) from buffalo samples. For oesophagus , 9.3% (8 of 86) were positive with sarcocyst by histological examination with 9.9% (7 of 71) of cattle and 6.7% (1 of 15) of buffalo. 8.1% (7 of 86) of oesophagus was diagnosed positive with the presence of sacocystis bradyzoite by digestion technique with 7% (5 of 71) from cattle samples and 13.3% (2 of 15) from buffalo samples. The total number of positive heart muscle and oesophagus samples are presented in Table 2.

DISCUSSION

Results of our study showed that 22.0% of the animals slaughtered in abattoir in Perak were positive with *Sarcocystis* spp. microscopically. A study by Latif *et al.* (2013) showed that 36.2% of cattle and 66.7% of buffaloes slaughtered in abattoir in Selangor harboured muscular sarcocystis. In comparison with other countries, the highest prevalence was reported in Mongolia with 90% of cattle infected with sarcocystis (Fukuyo *et al.*, 2002). 83% and 79% of water buffaloes were diagnosed positive in Iran (Oryan *et al.*, 2010) and Vietnam (Huong, 1999b) respectively.

Table 1. Total number of positive sarcocystis infection in cattle and buffalo slaughtered in four abattoirs in Perak

Type of animal	No. of animal (n)	No. of animal with sarcocystis infection (n)	Percentage (%)
Cattle	71	16	22.5
Buffalo	15	3	20.0
Total	86	19	22.0

Table 2. Total number (n) and percentage (%) of positive heart and oesophagus samples according to the type of diagnostic techniques

Type of animals	Type of samples	Diagnostic Techniques	
		Histological Examination, n (%)	Digestion Technique, n (%)
Cattle, n=71	Heart	9 (12.6)	10 (14.1)
	Oesophagus	7 (9.9)	5 (7.0)
Buffalo, n=15	Heart	0 (0)	2 (13.3)
	Oesophagus	1 (6.7)	2 (13.3)

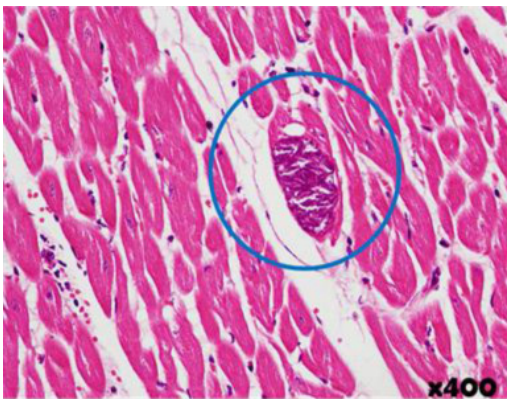

Figure 1. Sarcocystis cyst from cattle heart muscle sample by histological examination.

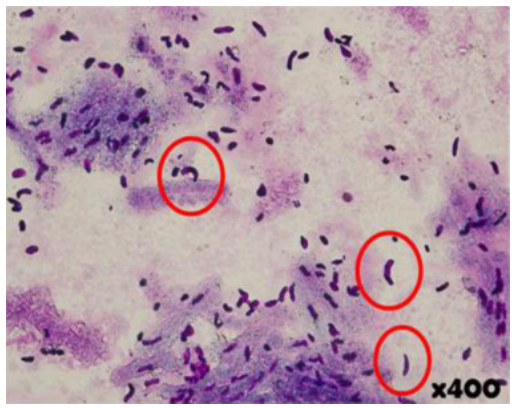

Figure 2. Sarcocystis bradyzoites from buffalo oesophagus sample by digestion technique.

In this study, no gross or macroscopic cyst was diagnosed. Besides oesophagus and heart muscle, as mentioned by Fayer (2004), Fukuyo *et al.* (2002), Huong (1999b), Nourani *et al.* (2010), Oryan *et al.* (2010) & Khalifa *et al.* (2008), the other sites for sarcocystis infections in large ruminants also includes tongue, diaphragm and intercostal muscles. According to Ghoshal *et al.* (1988), oesophageal tissues is the predilection site for *S. levinei* in buffalo. Study by Lindsay *et al.* (1995) stated that some species of *Sarcocystis* avoid certain muscle organ such as *S. hirsuta* in cattle that do not develop in the heart.

According to Kan & Pathmanathan (1991), sarcocystis infection was seen as an emerging food-borne disease in Malaysia because of high seroprevalence results as shown in autopsy cases. Ingestion of undercooked contaminated encysted meat with zoonotic sarcocyst may infect human (Rosenthal *et al.*, 2012). For safe consumption, it is recommended to cook the meat at 70ºC for 15 minutes or by freezing the meat at -4ºC for 2 days or -20ºC for 1 day (Kahn *et al.*, 2005).

In conclusion, due to the high prevalence of sarcocystis infection reported from the abattoirs it is strongly recommended to consume thoroughly cooked meat to avoid the incidence of infection in human. Increasing the sample size of collection and using more accurate, efficient and sensitive advanced molecular method such as PCR-RFLP will give a more complete status of the sarcocystis infection in Kedah-Kelantan crossbred cattle and Murrah buffalo in Perak.

REFERENCES

Bancroft, J.D. & Stevens, A. (1993). Theory and practice of histological techniques. 3rd Ed. Churchill Livingstone, Edinburgh.

CDC (Centers for Disease Control and Prevention). (2015). Sarcocystosis. Available at: http://www.cdc.gov/dpdx/sarcocystosis. Date accessed: 15 October 2015.

Chen, X.W., Zuo, Y.X. & Hu, J.J. (2003). Experimental *Sarcocystis hominis* infection in a water buffalo (*Bubalus bubalis*). *Journal of Parasitology* **89**: 393-394.

Dorny, P., Praet, N. & Gabriel, S. (2009). Emerging food-borne parasites. *Veterinary Parasitology* **163**: 196-206.

Dubey, J.P. (1976). A review of *Sarcocystis* of domestic animals and of other coccidian of cats and dogs. *Journal of the American Veterinary Medical Association* **169**: 1061-1078.

Dubey, J.P. & Lindsay, D.S. (2006). Neosporosis, toxoplasmosis and sarcocystosis in ruminants. *Veterinary Clinics of North America: Food Animal Practice* **22**: 645-671.

Dubey, J.P., Saville, W.J.A., Lindsay, D.S., Stich, R.W., Stanek, J.F., Speer, C.A., Rosenthal, B.M., Njoku, C.J., Kwok, O.C.H., Shen, S.K. & Reed, S.M. (2000). Completion of the life cycle of *Sarcocystis neurona*. *Journal of Parasitology* **86**: 1276-1280.

Dubey, J.P., Speer, C.A. & Fayer, R. (1989). Sarcocystosis of animals and man. CRC Press, Boca Raton, Florida. pp. 1-215.

Fayer, R. (1979). Multiplication of *Sarcocystis bovicanis* in the bovine bloodstream. *Journal of Parasitology* **65**: 980-982.

Fayer, R. (2004). *Sarcocystis* spp. in human infections. *Clinical Microbiology Review* **17**: 894-902.

Fazly Ann, Z., Muhamad Syamsul Naim, N.A., Wan Normaziah, W.O.B., Geethamalar, S., Iswadi, M.I. & Ramlan, M. (2014a). Modified digestion technique for detection of sarcocystis bradyzoites. *1st ARCAP & 35th MSAP Annual Conference*, 4th-6th June 2014, Riverside Majestic Hotel Kuching, Sarawak, Malaysia, pp.189-190.

Fazly Ann, Z., Niny Fariza, J., Wan Normaziah, W.O.B., Geethamalar, S., Muhamad Syamsul Naim, N.A., Khebir, V. & Ramlan, M. (2014b). Screening of sarcocystis in frozen meat supplied to Tioman Island, Malaysia. *26th VAM Congress*, 20th-22nd June 2014, Palm Garden Hotel, Putrajaya, Malaysia, pp. 66.

Fazly Ann, Z., Nurulaini, R., Shafarin, M.S., Fariza, N.J., Zawida, Z., Muhamad, H.Y., Adnan, M., Premaalatha, B., Erwanas, A.I., Zaini, C.M., Ong, C.C. & Chandrawathani, P. (2013). Zoonotic parasites from exotic meat in Malaysia. *Tropical Biomedicine* **30**: 535-542.

Fukuyo, M., Battsetseq, G. & Byambaa, B. (2002). Prevalence of sarcocystis infection in meat-producing animal in Mongolia. *Southeast Asian Journal Tropical Medicine and Public Health* **33**: 490-495.

Ghoshal, S.B., Joshi, S.C. & Shah, H.L. (1988). Sporocyst output in dogs fed sporocyst of *S. levinei* of the buffalo (*Bulbalus bulbalis*). *Veterinary Parasitology* **28**: 173-174.

Hamidinejat, H., Jalali, M.H.R. & Nabavi, L. (2010). Survey on sarcocystis infection in slaughtered cattle in South-West of Iran, emphasized on evaluation of muscle squash in comparison with digestion method. *Journal of Animal and Veterinary Advances* **9**: 1724-1726.

Huong, L.T.T. (1999a). Sarcocystis infections of the water buffalo in Vietnam. Doctoral Thesis Swedish University of Agricultural Sciences, Uppsala, Sweden.

Huong, L.T.T. (1999b). Prevalence of *Sarcocystis* spp.in water buffaloes in Vietnam. *Veterinary Parasitology* **86**: 33-39.

Kan, S.P. & Pathmanathan, R. (1991). Review of sarcocystosis in Malaysia. *Southeast Asian Journal of Tropical Medicine and Public Health* **22**: 129-134.

Kahn, C.M., Line, S., Allen, D.G., Anderson, D.P., Jeffcott, L.B., Quesenberry, K.E., Radostits, O.M., Reeves, P.T. & Wolf, A.M. (2005). *Sarcocystosis*. In The Merck Veterinary Manual 9th Edition. Merck & Co. Inc., Whitehouse Station, New Jersey, USA. pp: 974-976.

Khalifa, R.M., El-Nadi, N.A., Sayed, F.G. & Omran, E.K. (2008). Comparative morphological studies on three *Sarcocystis* spp. in Sohag. *Egyption Society of Parasitology* **38**: 599-608.

Latif, B., Vellayan, S., Heo, C.C., Kannan, K.M., Omar, E., Abdullah, S. & Tappe, D. (2013). High prevalence of muscular sarcocystosis in cattle and water buffaloes from Selangor, Malaysia. *Tropical Biomedicine* **30**: 699-705.

Lam, K.K.H., Watkins, K.L. & Chan, C.W. (1999). First report of equine protozoal myeloencephalitis in Hong Kong. *Equine Veterinary Education* **11**: 54-56.

Lindsay, D.S., Blagburn, B.L. & Braund, K.G. (1995). *Sarcocystis* spp. and sarcocystosis. *European Journal of Translational Mycology* **5**: 249-254.

Mowafy, N.M. (2003). Sarcocystis of cattle in El-Minia, Upper Egypt and ultrastructure of sarcocystis hominis cyst. *El-Minia Medicine Bulletin* **14**: 74-87.

Narges, K., Masomeh, B. & Salman, G. (2013). Sarcocystis cruzi: First molecular identification from cattle in Iran. *International Journal of Molecular and Cellular Medicine* **2**: 125-130.

Nourani, H., Matin, S., Nouri, A. & Azizi, H. (2010). Prevalence of thin-walled *S. cruzi* and thick-walled *S. hirsuta* or *S. hominis* from cattle in Iran. *Tropical Animal Health and Production* **42**: 1225-1227.

Oryan, A., Ahmadi, N. & Mousavi, S.M.M. (2010). Prevalence, biology and distribution pattern of *Sarcocystis* infection in water buffalo *(Bubalus bubalis)* in Iran. *Tropical Animal Health Production* **42**: 1513-1518.

Rosenthal, B., Yang, Z. & Yuan, L. (2012). *Sarcocystis* spp. In: Foodborne protozoan parasites. Nova Scientific Publishers, Inc., pp. 151-166.

Soulsby, E.J. (1982). Helminths, arthropods and protozoa of domestic animals. 7th Ed. Lea and Febringer, London, UK. pp. 40-51.

Talukder, M.S.I. (2007). *Histopathology techniques: Tissue processing and stainning* http://pdfooz.org/k-1895188.html. Accessed on 1st April 2015.

Taylor, M.A., Coop, R.L. & Wall, R.L. (2007). Veterinary parasitology. 3rd Ed. Blackwell Publishing, Oxford, UK.

Uggla, A. & Buxton, D. (1990). Immune response against toxoplasma and sarcocystis infections in ruminants: diagnosis and prospects for vaccination. *Revue Scientifique et Technique de l'Office International des Epizooties* **9**: 441-462.