SHORT COMMUNICATION

HISTOLOGICAL FINDINGS OF *Sarcocystis spp.* IN EXOTIC MEAT

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Sarcocystis spp. are coccidian parasites that belong to the Phylum *Apicomplexa*, Order *Eucoccidiotorida* and Family *Sarcocystidae*. Members of the Family *Sarcocystidae* are characterised by an alteration of sexual and asexual generations in their respective definitive and intermediate hosts. *Sarcocystis spp.* has an obligatory prey-predator two host cycle (Fayer, 2004). The asexual cycle develops only in the intermediate host, which in nature is often a prey animal. Intermediate hosts become infected when they ingest oocysts or sporocysts. Then sporozoites are released in the intestines and cross into the bloodstream and they multiply asexually in the walls of small blood vessels before invading the skeletal where they form the sarcocyst wall and multiply as merozoites for several generations. The merozoites eventually develop into infective zoites (bradyzoites) within the sarcocysts. Only the bradyzoite stage is infectious. Sexual stages develop only in a definitive host (Dubey *et al.*, 1989). The definitive host becomes infected by eating sarcocysts containing bradyzoites in muscle tissues. Sarcocysts are oval, whitish cysts that vary in size from microscopic to visible, depending on the host on species. The diseases cause by *Sarcocystis spp.* was known as Sarcocystosis or Sarcosporidiosis. In horses, the disease is known as Equine Protozoal Myoencephelitis (EPM) which is a serious neurologic disease of horses (Dubey *et al.*, 2001). Most of the affected animals are asymptomatic and the parasite is discovered only at slaughter. In severely affected animals, they may develop clinical signs which include fever, anorexia, cachexia, diarrhea, muscle spasm, anaemia and weakness. These signs are often seen in immunocompromised animals or animals with heavy infestations (Soulsby, 1982).

In Malaysia, *Sarcocystis spp.* have been reported in domestic and wild animals, including domestic and field rats, moonrats, bandicoots, slow loris, buffalo, monkey and human (Dissanaike and Kan, 1978; Kan and Dissanaike, 1978; Kan and Pathmanathan, 1991; Ambu *et al.*, 2011;

Tappe *et al.,* 2012). Sarcocysts can be detected in meat by direct observation of macroscopic sarcocysts or microscopic examination of histological sections (Fayer, 2004). Macroscopic examination of fresh muscle samples were examined grossly *in situ* for the presence of less than 30 mm whitish filamentous, rice-grain-like any globular appearance presenting macrocyst-forming sarcocyst (USGS National Wildlife Health Center, 2012). On the other hand, microcyst-forming sarcocyst may not be detected by macroscopic examination. Therefore, further observation by microscopic examination has to be carried out. Microscopic examination such as the histopathological examination of tissues can be a great of value as a diagnostic tool because the finding of the sarcocysts is definitive.

In this study, a total of fifty (n=50) samples including meat and organs (heart, liver and intestine) were examined from various species of exotic animals caught in the wild from January 2012 until April 2012. The samples were collected by post mortem of suspected exotic animals. All fresh samples were stored between 4°C to 6°C and sent to the Veterinary Research Institute (VRI) for further examination of the presence of sarcocysts either by macroscopic or microscopic examination. Tissue samples for histological examinations were processed using the routine paraffin technique (Baticados and Baticados, 2012).

Briefly, for histological examination the tissue samples were fixed in 10% formalin solution to arrest the tissue decomposition or degeneration. Thereafter, tissues were dehydrated with increasing concentrations of alcohol. A portion of each sample was individually embedded in paraffin and serially sectioned (3 µm to 10 µm) using rotary microtome (Microm HM315R, Germany), followed by slide mounting and haematoxilin and eosin (H&E) staining. Processed samples were individually examined under low power and high power magnification and oil immersion objective when necessary. A systematic approach was employed in the examination of the entire slide. Photomicrographs were obtained using a microscope (Olympus BX41, Japan) with camera head (Olympus DP20, Japan) and LCD monitor.

As a result, histological examination revealed 8% (n=4) of the samples were positive for the presence of sarcocysts. Sarcocysts were found in a squirrel meat (Negeri Sembilan), a terrapin meat (Perak) and two civet cats meat (Kelantan and Sarawak). Results of histological examination of positive sarcocysts are presented in Figure 1 to Figure 4.

The sarcocysts developed as a result of schizogony after the ingestion of food or water contaminated with sporocyst of *Sarcocyst* spp. by the exotic animal (intermediate host). The time for full maturation of sarcocysts in the muscle of intermediate host varies between the *Sarcocyst* spp. species, and is approximately 85 to 100 days for microcyst-forming sarcocyst to 1 to 4 years for macrocyst-

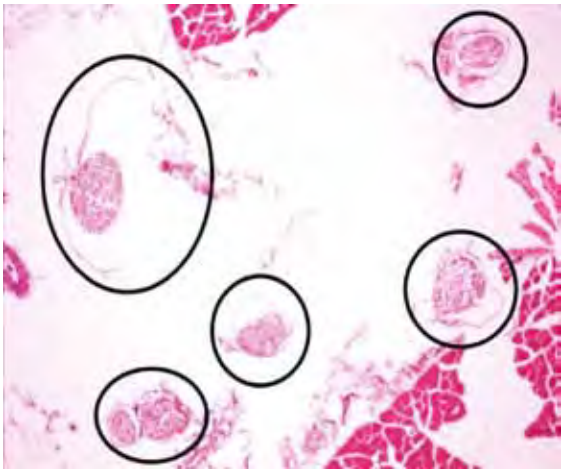

Figure 1. Dark circle indicates sarcocyst in squirrel meat (Negeri Sembilan). H&E stain. Magnification, x400.

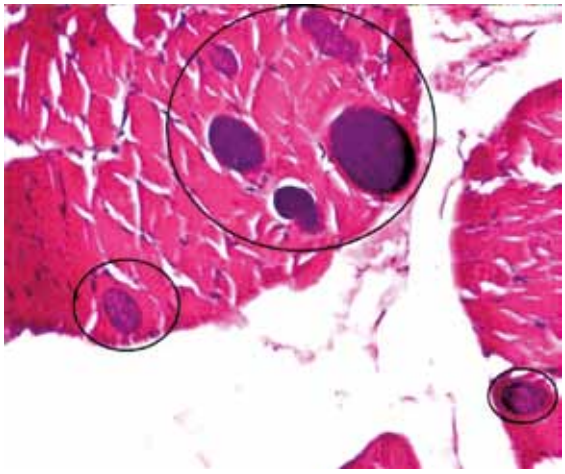

Figure 2. Dark circle indicates sarcocyst in civet cat meat (Kelantan). H&E stain. Magnification, x400.

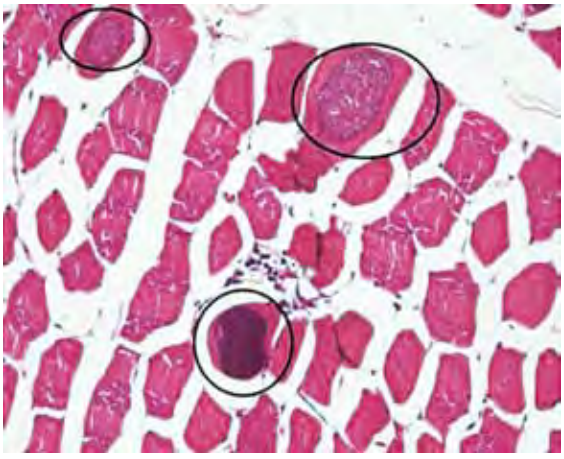

Figure 3. Dark circle indicates sarcocyst in terrapin meat (Perak). H&E stain. Magnification, x400.

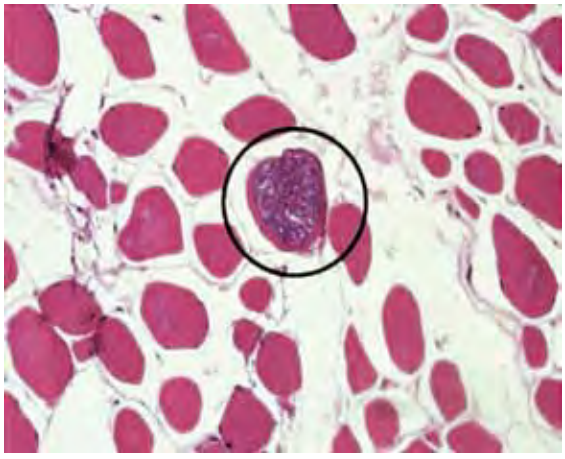

Figure 4. Dark circle indicates sarcocyst in civet cat meat (Sarawak). H&E stain. Magnification, x400.

forming sarcocyst. The sarcocysts may persist for many years in the host, but some may degenerate over time (Dubey *et al.*, 1989). In the context of zoonoses, carnivores and humans (definitive host) may get infected after eating undercooked exotic meat containing sarcocysts. As previously reported, definitive host also can act as both definitive and intermediate host for *Sarcocystis* spp. after ingestion of the infective bradyzoites (Fayer, 2004).

In animal, infection of *Sarcocyst* spp. may result in abortions and neonatal mortality in sheep, goats, canine and

cattle during pregnancy (Uggla and Buxton, 1990). The exact mechanisms behind *Sarcocystis*-induced abortion are unknown. On the other hand, infections of *Sarcocyst spp.* in humans are self-limiting, of short duration and often asymptomatic. As a preventive measure, humans are strongly advised to avoid eating raw or undercooked meat as it may results in infestation of the *Sarcocysts spp.* Consumption of thoroughly cooked meat for at least at 60°C for 20 minutes and drinking boiled or treated water will reduce the occurrence of the *Sarcocyst* spp. transmission to human. Alternatively, freezing the meat at -4°C for 48 hours before ingestion will kill the bradyzoites in the sarcocysts of *Sarcocyst* spp. (Saleque *et al.*, 1990).

Based on the results of this study, the finding of sarcocysts in meat of wild caught animals suggests the presence of *Sarcocyst* spp. in their natural fauna habitat in Malaysia. Due to the increased demand of exotic meats in the market, it is now becoming a public health concern. Although the results reported are fragmentary, it is hoped that the study will stimulate further investigations. As suggested, increasing the sample size with scattered distribution in all states will give a better picture of the *Sarcocyst* spp. infestation in Malaysia.

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