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Zoonotic parasites from exotic meat in Malaysia

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Abstract. Four zoonotic parasites, *Sarcocystis* spp., *Toxoplasma gondii*, *Trichinella* spp. and *Taenia* spp were screened in exotic meats. A total of forty-six (n=46) meat samples from various species of exotic animals were received from all the 14 states in Malaysia from January 2012 to April 2012. All exotic meat samples were examined macroscopically and histologically for the four zoonotic parasites. Results by histological examination of exotic meats showed the presence of *Sarcocystis* and *Toxoplasma* cysts at 8.7% (n=4) and 4.3% (n=2) respectively. No *Trichinella* spp. and *Taenia* spp. were found.

INTRODUCTION

There are four common zoonotic parasites namely *Sarcocystis* spp., *Toxoplasma gondii*, *Trichinella* spp. and *Taenia* spp. which can be transmitted through ingestion of contaminated, undercooked meat with either parasitic cyst or larvae. The probability of humans getting infected by ingestion of exotic meat is much higher than with domestic meat, as inspection of domestic meat is routinely carried out at abattoirs.

Sarcocystis spp. are intracellular protozoan parasite with an obligatory two-host life cycle based on prey-predator relationship (Fayer, 2004). The disease cause by *Sarcocystis* spp. is also known as Sarcocystosis and Sarcosporidiosis. In horses, the disease caused by *Sarcocystis neurona* is known as Equine Protozoal Myoencephalitis. 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 includes fever, anorexia, cachexia, diarrhoea, muscle spasm, anaemia and weakness (Kahn *et al.*, 2005). In Malaysia, *Sarcocystis* cysts have been reported from many domestic and wild animals, including domestic and field rats, moonrats, bandicoots, slow loris, buffalo, monkey and in humans (Kan *et al.*, 1991).

Toxoplasmosis is the disease caused by ubiquitous, obligate intracellular protozoan parasites. The tissue-cyst forming coccidia, *Toxoplasma gondii* infect all warm-blooded animals including mammals and birds, and also humans (Tenter *et al.*, 2000). Toxoplasmosis has also been found in foxes, guinea pigs, rats, voles, hares and rabbits with clinical findings ranging from lethargy, anorexia and inappetence to death (Quinn & McCraw, 1972). In human, toxoplasmosis is a major concern especially in pregnant women because the tachyzoites can migrate transplacentally and cause birth defects in human foetuses. In Malaysia, the chronic form of toxoplasmosis in human is estimated to have prevalence from 10-50% of the population (Tan & Zaman, 1973; Nissapatorn *et al.*, 2003).

Trichinellosis or trichinosis caused by the roundworm nematode, *Trichinella* spp.

is one of the significant, world-wide zoonotic meat parasites. According to Pozio & Murrell (2006), *Trichinella* spp. found in Asia includes both encapsulated species (*Trichinella spiralis*, *Trichinella britovi*, *Trichinella nativa*) and noncapsulated species (*Trichinella pseudospiralis*, *Trichinella papuae*). It can infect most mammals and a few members of others classes of animals. Natural infection occurs in wild carnivores. Humans may get infected through ingestion of undercooked meat with encysted larvae of *T. spiralis* from pigs (Chandrawathani *et al.*, 2010). In animals, most of the clinical symptoms are undiagnosed. A wide range of clinical manifestation in humans has been reported depending on the severity and the species involved. It includes muscle pain, facial oedema, ocular swelling, diarrhoea and abdominal discomfort (Bruschi & Murrell, 2002).

Cysticercosis referring to the presence of metacestode (larval) stages of the tapeworm (*Taenia* spp.) in the various tissues is considered as another zoonotic meat parasite (Yanagida *et al.*, 2012). In humans, *Taenia solium* cysticercosis may lead to epilepsy, intracranial hypertension or meningitis in endemic areas (Noor Azian *et al.*, 2006). It is considered the most important cause of acquired epilepsy in endemic areas. The disease is endemic in several developing countries in Asia, Africa and Latin America. In Asia and the Pacific, *T. solium*, *Taenia saginata* and *Taenia asiatica* were identified as zoonotic *Taenia* spp. (Ito *et al.*, 2004). Ingestion of undercooked infected meat with cysticerci leads to the disease called taeniasis. According to the Center for Food Security and Public Health, Iowa State University (2005), taeniasis is usually asymptomatic but mild abdominal symptoms may occur in some cases including abdominal pain, diarrhoea or constipation, nausea, decreased or increased appetite and weight loss.

In 1999, seven U.S military personnel who had a history of extensive physical contact with soil, including exposure to the eyes, nose and mouth from mud wrestling and consuming undercooked exotic meat and drank untreated water in Tioman Island, Malaysia, developed clinical signs of *Sarcocystis* infection (Arnes *et al.*, 1999). In relation to the case reported, Ministry of Health, Malaysia received notification from Centers for Disease Control and Prevention (CDC), Atlanta regarding the *Sarcocystis* outbreak in Tioman Island. As a result, a collaborative screening project was planned with the Department of Veterinary Services, Ministry of Agriculture to determine the status of zoonotic parasites in exotic meat in Malaysia.

MATERIALS AND METHODS

Sample Collection

A total of forty-six (n=46) muscle samples including meat and organs such as heart, liver and intestine from various species of exotic animals as in Table 1 were received from all 14 states in Malaysia commencing from January 2012 to April 2012. The samples were collected by post mortem of suspected exotic animals and from several fresh markets. All fresh samples were stored at 4º to 6ºC and sent to the Veterinary Research Institute (VRI), Ipoh for further examination of zoonotic meat parasites within 48 hours after sampling. Samples for histological examinations were stored in 10% formalin.

Table 1. Number of exotic meat samples received according to states in Malaysia

States	No. of samples	No. of positive samples
Johor	5	0
Malacca	1	0
Negeri Sembilan	3	1
Selangor	1	0
Kuala Lumpur	3	0
Perak	4	1
Kedah	2	0
Penang	2	0
Perlis	0	0
Pahang	4	0
Terengganu	1	0
Kelantan	3	1
Sarawak	6	3
Sabah	11	0
Total (%)	46 (100)	6 (12)

Macroscopic Examination

All the fresh muscle samples received were examined grossly *in situ* for the presence of less than 30mm whitish filamentous, rice-grain-like, cucumber seed-like or any globular appearance representing macrocyst-forming *Sarcocystis* spp. (Huong, 1999). The muscle sample were then sliced and cut into smaller pieces to facilitate better visual macroscopic detection of cysts.

Squash Smear Preparation (Stereoscopic Examination)

The suspected cysts of *Sarcocystis* spp. or free bradyzoites can be detected from fresh muscle tissues which were squashed between two slides. The squashed muscles were observed directly under a stereo-microscope at a magnification of 10-60x (Gut, 1982) to confirm the findings.

Impression Smear

A small portion of fresh organ sample of estimated size 2x2cm thickness was cut from both the heart and liver using a pair of scissors. The cut surface was gently pressed onto a clean glass slide and air dried (Christopher *et al.*, 1992). The smears were then fixed with methanol and stained with freshly filtered Giemsa stain at pH 7.2. All the stained smears were then examined under light microscope at 1000x magnification for the presence of any schizont in the endothelium.

Intestinal Scraping

Fresh intestinal sample received was longitudinally cut and the intestinal mucosa was scrapped (Christopher *et al.*, 1992). The scraping was placed on a glass slide and covered with a cover slip (Saudah *et al.*, 2012). The slide was then examined under a microscope with x100 and x400 magnification to qualitatively detect the presence of helminths, helminth ova, helminth larvae or coccidia oocysts.

Histological Examination

All the formalinised tissue samples were cut into pieces measuring 10 to 15mm on the slides and 2 to 3mm in thickness (Talukder, 2007) before being processed for staining with haematoxylin and eosin (H&E). The processed tissues were then examined under the light microscope for histological detection of the protozoan cysts or helminth larvae.

RESULTS

Based on the macroscopic examination, no cysts or rice grain-like structure was grossly visible in the fresh muscle or tissue samples. There were no free bradyzoites seen in squash preparation by stereoscopic examination. There were also no schizonts detected upon microscopic examination of the endothelium of impression smeared samples. The intestinal samples received were negative for any helminth ova and coccidian oocyst.

From histological examination, 13% (n=6) of the samples were positive for zoonotic meat parasites. The number of positive samples according to the states in Malaysia is shown in Table 2. Histologically, sarcocyst and *Toxoplasma* cyst were found in 8.7% (n=4) and 4.3% (n=2) respectively in muscle tissues. Sarcocysts were identified in meats of two civet cats (Kelantan and Sarawak), one in squirrel (N. Sembilan) and one in terrapin (Perak). *Toxoplasma* cyst was found in two exotic meat samples from monkey and squirrel (Sarawak). No *Trichinella* spp. and *Taenia* spp. larvae were observed upon examination. Histological and microscopic examination results of positive *Sarcocystis* and *T.gondii* are presented in Table 3 and Table 4 respectively.

DISCUSSION

Based on the findings, 8.7% of the exotic meat samples were positive for sarcocyst histologically. The cyst developed as a result of schizogony after ingestion by the exotic animal (acts as intermediate host) of food or water contaminated with sporocyst of a suitable *Sarcocystis* spp. The time for full maturation of sarcocyst in the muscle of intermediate host varies in the different *Sarcocystis* spp., and is approximately 85-100 days for microcyst-forming species to 1-4 years for macrocyst-forming species (Fayer

& Johnson, 1973; Rommel *et al.*, 1995). The sarcocyst may persist for many years in the host, but some may degenerate over time. In the zoonotic perspective, carnivores and humans as definitive hosts may get infected after eating undercooked sarcocyst encysted meat. After ingestion, the wall of the sarcocyst is digested, releasing the enclosed bradyzoites. The free bradyzoites will then penetrate the intestinal lamina propria of the definitive host and undergo gametogony to produce oocyst containing two sporocysts, each with four sporozoites. Sporocysts will be released into the lumen of intestine and shed in the faeces of the host. In nature, the definitive host can act as both definitive and intermediate host for *Sarcocystis* after ingestion of the infective stages (Fayer., 2004)

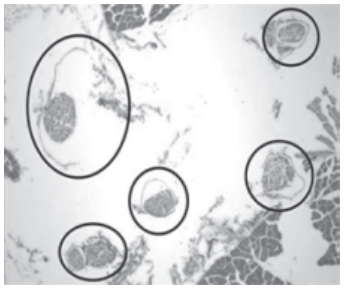
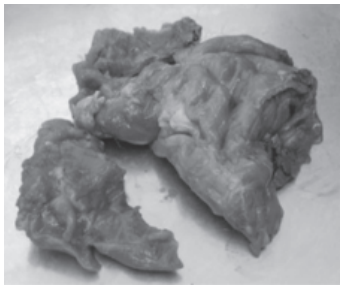
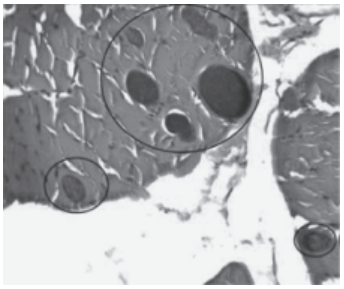
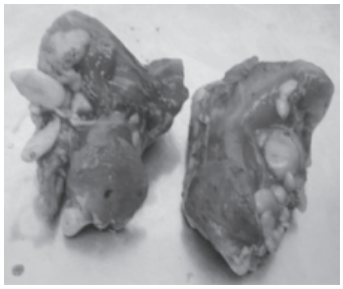
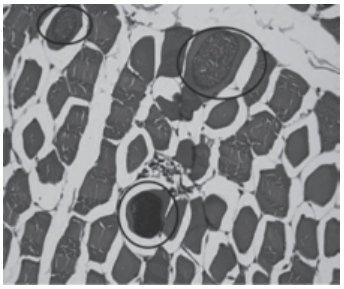
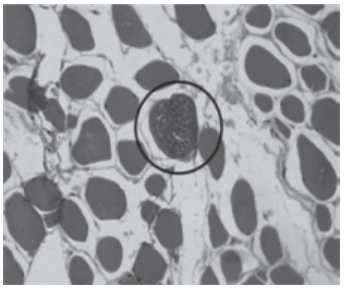
Another common zoonotic meat parasite diagnosed in this study was *T. gondii*. Based on the results, 4.3% of exotic meat samples were positive for *Toxoplasma* cyst upon histological examination. In comparison with *Sarcocystis* spp., *T. gondii* has three infective stages namely tachyzoites, bradyzoites and sporozoites. Accidental ingestion of the sporocyst excreted by the definitive host (cat or other felids) in faeces, soil or herbage, or ingestion of tissue cyst from undercooked infested meat or drinking unpasteurized milk containing infective stage of *T. gondii* are the main routes of transmission (Jittapalapong *et al.*, 2008). Following consumption, the bradyzoites and sporozoites respectively, are released and infect the intestinal epithelium. After several cycles of epithelial invasions, tachyzoites which are the rapidly multiplying trophozoite form of *T. gondii*, emerge, causing tissue destruction and spread the infestation throughout the body. In this stage, transplacental transfer of the tachyzoites from mother to foetus may lead to abortion.

Besides the two described protozoans, *Trichinella* spp. and *Taenia* spp. are listed as common zoonotic meat-borne helminth infections. In this study, all the exotic meat samples were negative for helminth larvae upon examination. Humans may get infected by ingestion of raw or inadequately cooked meat infested with *Trichinella* spp. larvae or cysticercoid meat. For trichinellosis, after ingestion of the contaminated meat, the larvae will develop into adult nematode and undergo reproduction. The new larvae

Table 2. Histological examination results of 46 exotic meat samples from all the states in Malaysia

Source of Exotic Meat	No. of samples	Parasitic Infestation			
		No. of positive samples			
		Sarcocystis	Toxoplasmosis	Trichinellosis	Taeniasis
Frog	10	0	0	0	0
Wild boar	8	0	0	0	0
Squirrel	5	1	1	0	0
Lizard	4	0	0	0	0
Python	3	0	0	0	0
Civet cat	3	2	0	0	0
Terrapin	2	1	0	0	0
Monkey	2	0	1	0	0
Mousedeer	2	0	0	0	0
Tortoise	1	0	0	0	0
Bat	1	0	0	0	0
Deer	1	0	0	0	0
Crocodile	1	0	0	0	0
Dog	1	0	0	0	0
Cobra	1	0	0	0	0
White-Brested Waterhen	1	0	0	0	0
Total (%)	46 (100)	4 (8.7)	2 (4.3)	0	0

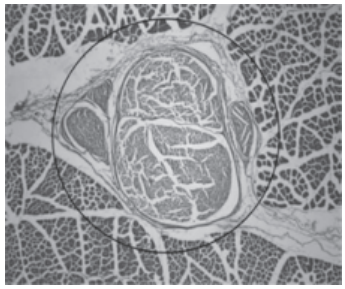
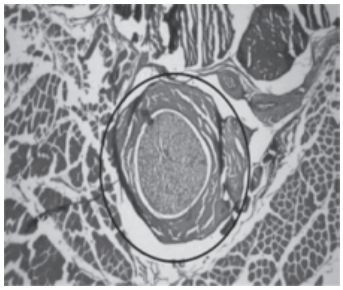
Table 3. Macroscopic and histological examination results of positive *Sarcocystis* spp. in exotic meat samples

Sarcocyst in meat	Macroscopic examinations	Histological examinations
Squirrel meat (Negeri Sembilan)		
Civet cat meat (Kelantan)		
Terrapin meat (Perak)		
Civet cat meat (Sarawak)		

produced will penetrate the intestinal mucosa and travel in capillaries to various organs such as retina, myocardium, lymph node or skeletal muscle of human. In contrast, with taeniasis, after accidental ingestion of the cysticercus in meat, the larvae develope over two months into an adult tapeworm and attaches to the small intestine by the scolex. The adult tapeworm then produces proglottids and releases the eggs in the faeces. An intermediate host may get infected after ingestion of the egg of *Taenia spp*. in contaminated feed or water.

As prevention, humans are strongly advised to avoid eating raw or undercooked meat as it may result in infestation of the

Table 4. Macroscopic and histological examination results of positive *T. gondii* in exotic meat samples

Toxoplasma cyst in meat	Macroscopic examinations	Histological examinations
Squirrel meat (Sarawak)		
Monkey meat (Sarawak)		

zoonotic meat-borne parasites. Consumption of thoroughly cooked meat for at least at 71°C and drinking boiled or treated water will reduce the occurrence of the parasitic transmission to human. For *Sarcocystis*, alternate freezing and thawing the meat at -5°C for 3 to 5 days before ingestion will kill the cyst. Deep-freezing for three weeks is needed to kill *Trichinella* spp. in meat. However, *Trichinella* spp. in bear meat is not effected by freezing even over a long period of time (Dick *et al.*, 2001). Transmission risk will also be minimized by washing hands properly after using the toilet, after outdoor activities, contact with pets, animal faeces or any animal environment and before handling food. Washing and peeling raw fruits and vegetables might reduce the incidence of eating contaminated food with oocyst.

The probabilities of zoonotic infection in exotic animals are much higher compared to livestock animals due to routine meat inspection of livestock meat by licensed personnel in abattoirs to ensure the meat is safe for human consumption. Any suspected infested meat will undergo condemnation. However for exotic meat no inspection is done by any authorized body.

In conclusion, 13% of the exotic meat samples screened were positive for zoonotic meat protozoa which were sarcocystis and toxoplasmosis. Zoonotic helminth larvae such as *Trichinella* spp. and *Taenia* spp. were not detected upon examination of the 46 exotic meat samples. Increasing the sample size of collection from areas all over the country will give a more complete status of the zoonotic meat parasitic infestation in Malaysia. Public education on the dangers of eating raw and undercooked meat will reduce the risk of meat-borne parasitic transmission.

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