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SHORT COMMUNICATION

Prevalence of Muscular Sarcosporidiosis in Slaughtered Domestic Pigs in Perak, Peninsular Malaysia

Fazly Ann Zainalabidin*, Muhamad Syamsul Naim Noorazmi, Wan Normaziah Wan Omar Bakri, Geethamalar Sathaya and Mohd Iswadi Ismail

Veterinary Research Institute, Department of Veterinary Services, 59, Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia

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Abstrak: Sarkosporidiosis adalah penyakit yang disebabkan oleh parasit protozoa intraselular iaitu *Sarcocystis* spp. Pada khinzir, tiga spesis *Sarcocystis* spp. telah dikenalpasti antaranya *Sarcocystis meischeriana, Sarcocystis porcifelis* dan *Sarcocystis suihominis*. Tujuan kajian ini adalah untuk menentukan prevalens sarkosporidiosis otot pada khinzir dengan menggunakan teknik penghadaman peptik. Sebanyak 150 sampel segar otot jantung, esofagus dan paha daripada 50 khinzir jenis Yorkshire dan Landrace telah dikumpulkan dari dua buah tempat sembelihan tempatan di Perak mulai bulan Mei hingga Ogos 2014. Kesemua sampel segar tersebut diperiksa secara kasar untuk mengesan pembentukan-makrosis *Sarcocystis* spp. dan seterusnya diproses secara teknik penghadaman peptik bagi mengesan kehadiran bradizoit. Hasil yang diperolehi menunjukkan 58% (29 daripada 50) khinzir adalah positif terhadap *Sarcocystis* spp. Penemuan ini menggambarkan kepentingan untuk melaksanakan langkah-langkah yang ketat dalam pemeriksaan khinzir-khinzir di rumah sembelihan terhadap infeksi *Sarcocystis* spp. kerana kepentingannya terhadap kesihatan awam.

Kata kunci: Sarkosistis, Khinzir, Otot, Penghadaman Peptik, Bradizoit

Abstract: Sarcosporidiosis is a disease caused by intracellular protozoan parasites, namely, *Sarcocystis* spp. In pigs, three species of *Sarcocystis* spp. have been recognised, including *Sarcocystis meischeriana*, *Sarcocystis porcifelis* and *Sarcocystis suihominis*. The aim of this study is to determine the prevalence of muscular sarcosporidiosis in pigs using the pepsin digestion technique. A total of 150 fresh heart, oesophagus and thigh muscle samples from 50 Yorkshire and Landrace pigs were collected from two local abattoirs in Perak from May to August 2014. All the fresh muscle samples were thoroughly examined for macrocyst-forming *Sarcocystis* spp. and processed using the peptic digestion technique to detect bradyzoites. The results from the muscle samples showed that 58% (29 out of 50) of the pigs were positive for *Sarcocystis* spp. These findings highlight the importance of implementing stringent measures for screening pigs in abattoirs for *Sarcocystis* spp. infection because this infection in pigs is a public health concern.

*Corresponding author: fazly@dvs.gov.my

Keywords: Sarcocystis, Pigs, Muscle, Peptic Digestion, Bradyzoites

Sarcosporidiosis, also known as sarcocystosis, is a disease caused by cyst-forming coccidian parasites, namely, *Sarcocystis* spp. There are more than two-hundred species of *Sarcocystis*, and they are the most prevalent protozoan parasites of domestic animals (Kalantari *et al.* 2013). *Sarcocystis* spp. have been reported in pigs (*Sus scrofa*), including *S. meischeriana*, *S. porcifelis* and *S. suihominis*, and dogs (*Canis lupus familiaris*), cats (*Felis catus*) and humans (*Homo sapiens*) serve as their final hosts, respectively (Solaymani-Mohammadi & Petri 2006). *Sarcocystis meischeriana* is widely distributed in various regions of the world, including Southeast Asia. In Southeast Asia, the sarcocysts of *S. meischeriana* have been reported in pigs in Thailand (Bunyaratvej *et al.* 2007) and the Philippines (Claveria *et al.* 2001). However, there is no report of *S. meischeriana* infection in pigs in Malaysia and other neighbouring countries. On the other hand, there are studies that show a high prevalence of *S. suihominis* in countries such as India (Saleque & Bhatia 1991), Japan (Saito *et al.* 1998), China (Li *et al.* 2007) and the US (Dubey & Powell 1994). To date, no publications have been found on the infection of pigs with *S. porcifelis* and *S. suihominis* in Malaysia or other Southeast Asian countries.

According to Lindsay *et al.* (1995), *S. meischeriana* is the most prevalent and most pathogenic species, while *S. suihominis* is less prevalent and less pathogenic in pigs. However, *S. suihominis* has received more attention in medical communities due to its impact on public health because it infects humans who serve as its definitive host (Banerjee *et al.* 1994; Chhabra & Samantaray 2013; Dubey *et al.* 1989; Fayer 2004; Juyal 1991; Tappe *et al.* 2013).

Sarcocystis spp. in infected pigs can be detected by macroscopic or microscopic observations of muscle tissue samples. The whitish filamentous, spindle-shaped, rice-grain-like, macrocyst-forming sarcocyst has been observed in the muscles of the heart, tongue, masseter, oesophagus, diaphragm, biceps and femoris (Lam *et al.* 1999). According to Hamidinejat *et al.* (2010), the pepsin digestion technique is the gold standard and is commonly applied for the detection of *Sarcocystis* spp. This technique is considered to be one of the most sensitive methods for the detection of the presence of bradyzoites in muscle tissues (Dubey *et al.* 1989). Therefore, the aim of this study is to determine the prevalence of muscular sarcosporidiosis in pigs using the pepsin digestion technique.

In this study, 150 tissue samples taken from the heart (50 samples), oesophagus (50 samples) and thigh (50 samples) muscles of twenty Yorkshire and thirty Landrace pigs (*Sus scrofa domesticus*), slaughtered at the Ipoh and Taiping abattoirs in Perak, Peninsular Malaysia, were examined for *Sarcocystis* spp. infection. The samples were randomly selected during the slaughtering process from May until August 2014. All the samples were thoroughly examined visually for any presence of macrocysts from *Sarcocystis* spp. *in situ* before being kept in a chiller box at a temperature of 4 to 6°C for transportation. The samples were processed at the biosafety level 2 laboratory (BSL-2) at the Zoonotic Section of the Veterinary Research Institute, Ipoh.

The digestion technique used in this study was previously described by Fazly Ann *et al.* (2014). Fifty (50) grams of each muscle sample was minced and homogenized in 100 ml of distilled water using a blender. Before homogenisation, all the visible fat layers covering the muscles were removed. The homogenised sample was then transferred into a 250-ml beaker and was left to settle for 5 minutes. After discarding the supernatant, the remaining 50-ml sediment was digested with a 1.5% hydrochloric acid (HmbG®, Merck, Darmstadt, Germany) and pepsin (Sigma®, Missouri, USA) solution. The samples were then incubated for 12 hours at 30°C in a water bath (Memmert W350, Schwabach, Germany). The digested samples were then sieved through a nylon-meshed tea strainer and centrifuged for 5 minutes at 1500 rpm (Eppendorf Centrifuge 5804R, Hamburg, Germany). After removing the supernatant, a drop of the sediment was placed on a microscope slide and stained with Giemsa (Sigma®, Missouri, USA). The slide was then examined under a light microscope (Leica DME, Illinois, USA) at 400X power magnification for the detection of bradyzoites.

Macroscopically, all the samples were negative for macrocysts of the *Sarcocystis* spp. Microscopic examination of the heart, oesophagus and thigh muscle samples showed that sarcocysts with bradyzoites were observed in 26% (13 out of 50) of the heart muscle samples, 30% (15 out of 50) of the oesophagus muscle samples and 36% (18 out of 50) of the thigh muscle samples (Table 1). The prevalence rate showed that 58% (29 out of 50) of the pigs slaughtered in both of the local abattoirs in Perak were infected with *Sarcocystis* spp. (Table 2).

Table 1: Number (*n*) and percentage (%) of samples with sarcocystis bradyzoites detected in three different types of samples by the digestion technique.

Type of sample	Samples with positive sarcocystis bradyzoites	
	n	%
Heart muscle (*n* = 50)	13	26
Oesophagus muscle (*n* = 50)	15	30
Thigh muscle (*n* = 50)	18	36

Table 2: Prevalence rate of sarcosporidiosis in pigs.

Animal breed	No. of animal (*n*)	No. of infected animal (*n*)
Yorkshire pig	20	8
Landrace pig	30	21
Total (*N*)	50	29

$$\text{Prevalence rate (\%)} = \frac{\text{Total no. of infected animal } (n)}{\text{Total no. of animals } (N)} \times 100$$

Comparable to our results, using the pepsin digestion technique, Pereira and Bermejo (1988) reported that 43% of the pigs in Spain were infected with *Sarcocystis* spp. Similarly, in India, Saleque and Bhatia (1991) reported a prevalence rate as high as 67.98% (605 out of 890) in pigs infected with *Sarcocystis* spp. In another study in Punjab, India, the prevalence rate of pigs

infected with *Sarcocystis* spp. was reported to be as high as 73.36% (168 out of 229) using the same peptic digestion technique (Avapal *et al.* 2004). However, in contrast, Rout and Saikumar (2015) reported that the prevalence rate of pigs infected with *Sarcocystis* spp. in Uttar Pradesh, India, was only 26.89% (32 out of 119). In other countries, sarcocysts were reported in 27.3% of pigs (9 out of 33) in Manila, Philippines (Claveria *et al.* 2001), and 16.3% of pigs (17 out of 104) in East Hokkaido, Japan (Omata *et al.* 1993). From these studies, it is apparent that *Sarcocystis* spp. is ubiquitous in many regions of the world.

Prestwood *et al.* (1980) have reported that *Sarcocystis* spp. in pigs can be detected using digestion techniques to reveal the zoites. Additionally, Dubey and Powell (1994) reported that *Sarcocystis* spp. could also be detected in the heart muscle of the pig using the digestion technique. According to Collins *et al*. (1980), they found that digestion techniques, and not histological methods, were more sensitive in the detection of *Sarcocystis* spp.

We found that the digestion technique was applicable for use as a first-line method in the detection of *Sarcocystis* spp. in pigs. The use of a combination of the digestion technique with a histological examination or molecular methods, such as a polymerase chain reaction, would be useful for species identification and classification.

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