SHORT COMMUNICATION

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

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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 meischriana, Sarcocystis porcifelis and Sarcocystis suisominis. 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.

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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 homogenization, all the visible fat layers covering the muscles were removed. The homogenized 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.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Samples with positive sarcocystis bradyzoites</th>
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<tbody>
<tr>
<td>Heart muscle (n = 50)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Oesophagus muscle (n = 50)</td>
<td>15 (30%)</td>
</tr>
<tr>
<td>Thigh muscle (n = 50)</td>
<td>18 (36%)</td>
</tr>
</tbody>
</table>

### Table 2: Prevalence rate of sarcosporidiosis in pigs.

<table>
<thead>
<tr>
<th>Animal breed</th>
<th>No. of animal (n)</th>
<th>No. of infected animal (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yorkshire pig</td>
<td>20</td>
<td>8</td>
</tr>
<tr>
<td>Landrace pig</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>Total (N)</td>
<td>50</td>
<td>29</td>
</tr>
</tbody>
</table>

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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REFERENCES


