SHORT COMMUNICATION

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

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

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

Sarcosporidiosis, also known as sarcocystosis, is the disease caused by the 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. that have been reported in pigs (*Sus scrofa*) such as *S. meischeriana*, *S. porcifelis* and *S. suihominis* have their final hosts which are the dog (*Canis lupus familiaris*), cat (*Felis catus*) and human (*Homo sapiens*) respectively (Solaymani-Mohammadi and Petri 2006). *Sarcocystis meischeriana* is widely distributed in various regions in 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 Philippines (Claveria et al. 2001). However, there is no report of *S. meischeriana* infection in pigs in Malaysia and other neighboring countries. On the other hand, there are studies that show high prevalence of *S. suihominis* in countries such as India (Saleque and Bhatia 1991), Japan (Saito et al. 1998), China (Li et al. 2007) and USA (Dubey and Powell 1994). To date, no publications have been found on the infection of pigs with *S. porcifelis* and *S. suihominis* in Malaysia and other Southeast Asian countries.
According to Lindsay et al. (1995), *S. meischeriana* is the most prevalent and most pathogenic, while *S. suihominis* is less prevalent and less pathogenic in pigs. However, *S. suihominis* received more attention in medical communities due to its public health impact as it infects humans who are its definitive host (Banerjee et al. 1994; Chhabra and 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 observation of muscle tissue samples. The whitish filamentous, spindle shaped, rice-grain-like presenting macrocyst-forming sarcocyst has been observed in the muscles of heart, tongue, masseter, esophagus, diaphragm, biceps and femoris muscles (Lam et al. 1999). According to Hamidinejat et al. (2010), the gold standard and a commonly applied technique for the detection of the *Sarcocystis* spp. is the pepsin digestion technique. It is considered as one of the most sensitive methods to detect the presence of the bradyzoites in muscle tissues (Dubey et al. 1989). Therefore, the aim of this study is to determine the prevalence of muscular sarcosporidiosis in pigs by using the pepsin digestion technique.

In this study, a total of 150 muscle tissue samples taken from the heart (50 samples), esophagus (50 samples) and thigh (50 samples) of twenty Yorkshire and thirty Landrace pigs (*Sus scrofa domesticus*) that were 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 commenced from May until August 2014. All the samples were grossly checked visually for any presence of macrocysts of *Sarcocystis* spp. *in situ* before being kept in chiller box with 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 mentioned 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 layer covering the muscles were removed. The homogenized sample was then transferred into a 250 ml beaker and was left to settle down for 5 minutes. After discarding the supernatant, the remaining 50 ml sediment was digested with 1.5% hydrochloric acid (HmbG®, Germany) and pepsin (Sigma®, USA) solution. The samples were then incubated for 12 hours at 30°C in a waterbath (Memmert W350, Germany). The digested samples were then sieved through a
nylon-meshed tea strainer and centrifuged for 5 minutes at 1500 rpm (Eppendorf Centrifuge 5804R, Germany). After removing the supernatant, a drop of the sediment was placed on a microscope slide and stained with Giemsa (Sigma®, USA) on the microscope slide. The slide was then examined under a light microscope (Leica DME, USA) at x400 power magnification for detection of the bradyzoites.

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

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) of 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 prevalence rate of pigs infected with Sarcocystis spp. in Uttar Pradesh, India was only 26.89% (32 out of 119). In other countries, sarcocysts in pigs were reported in 27.3% (9 out of 33) in Manila, Philippines (Claveria et al. 2001) and 16.3% (17 out of 104) in East Hokkaido, Japan (Omata et al. 1993). From the studies carried it is apparent that Sarcocystis spp. is ubiquitous in many regions of the world.

Prestwood et al. (1980) have reported that Sarcocystis spp. in pig can be detected using digestion techniques to reveal the zoites. Additionally, Dubey and Powell (1994) reported that Sarcocystis spp. could also be detected in heart muscle of the pig by using the digestion technique. According to Collins et al. (1980), they found that the digestion techniques were more sensitive in detection of Sarcocystis spp. than histological method.

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

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REFERENCES


**Table 1:** The number (n) and percentage (%) of samples with sarcocystis bradyzoites detected in three different type of samples by digestion technique.

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Samples with positive sarcocystis bradyzoites</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>Heart muscle (n = 50)</td>
<td>13</td>
</tr>
<tr>
<td>Esophagus muscle (n = 50)</td>
<td>15</td>
</tr>
<tr>
<td>Thigh muscle (n = 50)</td>
<td>18</td>
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Table 2: Prevalence rate of sarcosporidiosis in pigs.

<table>
<thead>
<tr>
<th>Type of 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>
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Prevalence rate (%) = \( \frac{\text{Total no. of infected animal (n)}}{\text{Total no. of animal (N)}} \times 100 \)