

Prevalence of *Sarcocystis* spp. in rodents in Peninsular Malaysia

Stephen Ambu, Evelyn Yin Synn Yeoh, Joon Wah Mak, Srikumar Chakravarthi

The aim of the study was to assess the prevalence of *Sarcocystis* infection in wild and peri-urban rodents in some states in Peninsular Malaysia. The thigh muscle from these rodents were formalin preserved, sectioned and stained with hematoxylin and eosin and examined under light microscopy. Of the 146 muscle tissue examined only 73 were positive for *Sarcocystis* infection. Morphological identification showed the presence of some new morphological types to be present. Different species of *Sarcocystis* were seen in the sections but more extensive studies are needed to identify them to species level.

Keywords: *Sarcocystis*, animal, rodents

Studies have shown the prevalence rate of *Sarcocystis* in Southeast Asia to be high^{1,2}. Rodents as an intermediate host to *Sarcocystis* also pose a public health risk due their close proximity to human habitation. Other animals such as cats, dogs, reptiles, monkeys, camels, llamas, water buffalo, yaks, wild pigs, birds can also act as intermediate hosts². Humans acquire *Sarcocystis* through the ingestion of cysts from undercooked or raw meat of infected livestock³. Human infection with *Sarcocystis* spp. from rodents results in human muscular sarcocystosis, presenting with myalgia, erythematous subcutaneous nodules, fever, bronchospasm, cough, headaches, loss of appetite, weight loss and^{4,5}.

In the current study, a survey of wild and peri-urban rodents was carried out to assess the prevalence of *Sarcocystis* infections in rodents in some states in Peninsular Malaysia. The tissue samples from the trapped rodents were preserved in formalin, subjected to sectioning and histological assessment using hematoxylin and eosin (H&E) stain for identification of *Sarcocystis* infection.

One hundred and forty six rodents belonging to 7 species were examined. The rats were trapped in

the states of Johor, Selangor, Kelantan and Kedah in Peninsular Malaysia. Specimens of brain and thigh muscles were fixed in 10% formalin, paraffin-embedded and sectioned. The histological sections were stained using, H & E. Light microscopy was used to observe the morphology of the cyst structure and the species identified as described in other studies.

Of the 146 thigh muscles examined, 73 (50%) were found to contain *Sarcocystis*. The number of cysts per histological section varied from 1 to 136. Only 3 specimens had more than 100 sarcocysts per section. Generally it was no more than 10 per section. The brain sections were found not to contain any cysts. None of the cysts were macroscopic.

There is evidence of large zoitocysts of *Sarcocystis* containing bradyzoites, in skeletal muscle fibres. The zoitocysts are seen as longitudinal and cross sections of various sizes. Some muscles contained multiple sarcocysts (Figure 1). Varying morphological features were seen in the sarcocysts observed in the sections. In Figure 2 there were numerous cystozoites seen within the sarcocyst and it has a thin cyst wall. In Figure 3 the sarcocyst seen has a thick filamentous cyst wall showing numerous cystozoites within while in Figure 4 the sarcocyst has a striated thick wall showing numerous cystozoites within. In Figure 5 the sarcocyst seen has a thin wall and it is elongate with numerous cystozoites within. All sarcocysts are usually elongated as seen in this longitudinal section.

The measurements of the sarcocysts seen also vary. In Figure 6 the thin-walled sarcocyst measures 206.7 by 65.7 μm ; in Figure 7, a sarcocyst with a striated wall measures 139.6 by 53.2 μm and in Figure 8 the thin-walled sarcocyst measures 247.7 by 53.9 μm . A point to note is that the size of the sarcocyst will depend on the age of the cyst and whether it is cut in cross, oblique or longitudinal sections.

Studies on wild rodents for *Sarcocystis* infection in Singapore, Indonesia and Thailand showed a prevalence

Address for Correspondence:

Professor Stephen Ambu, Department of Postgraduate Studies and Research, International Medical University, No. 126, Jalan Jalil Perkasa 19, Bukit Jalil, 57000 Kuala Lumpur, MALAYSIA.

Email: Stephen_ambu@imu.edu.my

rate of 57–100%^{1,6}, 40%^{2,7} and 33%^{3,8} respectively. A survey of rodents in the wet markets of the Philippines showed sarcocysts in the tongue and diaphragms of rats^{4,9}. Studies conducted in countries outside the Southeast Asian region, such as Lithuania (12.7%) and the Czech Republic (3.49%) showed much lower prevalence rates^{5,6,10,11}. The prevalence rate found in our study (50%) is similar to that found in our neighbouring countries.

Some studies show that human intrusion into the environment is negatively correlated with *Sarcocystis* spp. infection load in rodents^{1,6}. The agricultural industry is one cause of human intervention whereby the prevalence of *Sarcocystis* can be influenced as these parasites are used as biological control for rodents^{7,12}.

The cyst wall in *S. singaporensis* is thick, with tall, stalked, digitiform villi; that of *S. villivillosi* is moderately thin and bears short, stout villi that are covered with microvilli; *S. zamani* cyst wall is thin and bears delicate, branched villi. *Sarcocystis singaporensis* and *S. zamani* apparently have been reported previously from several species of Malaysian wild rodents¹³. In our study we found multiple sarcocysts in the sectioned tissue of some rodents (Figure 1). Sarcocysts with differing morphological features and measurements were seen in the tissue sections suggesting that there were more than one species infecting the rodents. The differing morphological features seen were thin wall (206.7 by 65.7 μm), thick striated wall (139.6 by 53.2 μm), thin wall and elongate (247.7 by 53.9 μm) and the length and width of the sarcocyst. Based solely on these measurements it is not possible to identify the *Sarcocystis* to species level.

Thomas & Dissanaik¹⁴ using serological methods found almost 20% of the subjects in their study in Malaysia to be positive for *Sarcocystis* infection with a high prevalence rate among the Orang Asli and Malay population. Subsequently Kan¹⁵ in her animal studies found 3 distinct *Sarcocystis* spp. that had different morphological characteristics in 4 rodent species in Malaysia. In another study, Beaver et al.⁴ reported

40 cases of *Sarcocystis* spp. (Singapore, India, Africa & Central America) and found 7 morphological types (4 were of monkey origin, 1 of cattle origin and the other 2 of unidentified origin). Arnese et al.⁵, diagnosed 15 US military personnel with service record in Malaysia to be positive for *Sarcocystis* infection while Wong and Pathmanathan¹ found *Sarcocystis* infections in the Malaysian population (21% of 100 specimens) by the examination of biopsy samples from individuals ranging from 16 to 57 years of age. In a global survey, it was found that 2 individuals in Malaysia were infected with *Sarcocystis* sp.² and 19.7% of 243 people tested were found to be positive for infection. Crum-Cianflone³ suggested that *S. hominis* and *S. suihominis* are the species causing enteritis in humans who are accidental hosts due to the consumption of undercooked meat. In a recent study on captive zoo animals in Malaysia, post-mortem analysis found 3 mammals and 5 birds positive for sarcocysts but species identification was not carried out¹⁶. The mean size of the cysts was 254x24.5 μm and the thickness of the wall was 2.5 μm .

Acknowledgements

This study was supported by a research grant from the International Medical University [IMU Project No: BMS 102/2007 (12)].

REFERENCES

1. Wong, K. T., and Pathmanathan R.. High prevalence of human skeletal muscle sarcocystosis in South-east Asia. *Trans. R. Soc. Trop. Med. Hyg.* 1992; 86:631–632.
2. Fayer R. *Sarcocystis* spp. in Human Infections. *Clin Micro Rev* 2004; 17:894–902.
3. Nancy F. Crum-Cianflone. Bacterial, Fungal, Parasitic, and Viral Myositis. *Clin Micro Rev*, 2008; 21 (3):. 473–494
4. Beaver, PC, Gadgil K, and Morera P. *Sarcocystis* in man: a review and report of five cases. *Am. J. Trop. Med. Hyg.* 1979; 28:819–844.
5. Arnese MK, Brown JD, Dubey JP, Neafie RC, Granstrom DE. An outbreak of acute eosinophilic myositis attributed to human *Sarcocystis* parasitism. *Am. J. Trop. Med. Hyg.* 1999; 61: 548-553.
6. Paperna I, Peh KSH, Martelli P, Lian KK., Sodhi NS. Factors affecting *Sarcocystis* infection of rats on small tropical islands. *Ecol. Res.* 2004; 19(5): 475-483.
7. O'Donoghue PJ, Watts, CHS, Dixon BR. Ultrastructure of *Sarcocystis* spp. (Protozoa: Apicomplexa) in rodents from North Sulawesi and West Java, Indonesia. *J. Wild. Dis.* 1987;23: 225-232.

8. Jakel T, Khoprasert Y, Sorger I, Klimt D, Seehabutr V, Suasa-ard K, Hongnark SS. Sarcosporidiasis in rodents from Thailand. J. Wild. Dis. 1997; 33(4): 860-867.
9. Claveria FG, Causapin J, Aneceta de Guzman M, Toledo MG, Salibay C. Parasite biodiversity in *Rattus* spp. caught in wet market, SEA J. Trop. Med. Pub. Hlth 2005; 36(4): 146-148.
10. Grikenine J, Mazeikyte R. Investigations of sarcosporidians (*Sarcocystis*) of small mammals in Kamasta Landscape Reserve and its surroundings. A. Zool. Lit. 2000; 10(3): 55-68.
11. Svobodova M, Vorisek P, Votypka J, Weidinger K. Heteroxenous Coocidia (Apicomplexa: Sarcocystidae) in the populations of their final and intermediate hosts, European Buzzard and small mammals. A. Prot. 1994; 43: 251-260.
12. Jakel T, Khoprasert Y, Endepols S, Archer-Baumann C, Suasa-ard K, Promkerd P, Kliemdt D, Boonsong P, Hongnark S. Biological control of rodents using *Sarcocystis singaporensis*. Int. J. Paras. 1999; 29(8): 1321-1330.
13. Beaver P.C. and Maleckar J.R. *Sarcocystis singaporensis* Zaman and Colley. *Sarcocystis Villivilliosi* SP.N., and *Sarcocystis Zamani* SP. N.: Development, morphology, and persistence in the laboratory rat, *Rattus norvegicus*. J. Parasitol, 1981, 67(2): 241-256.
14. Vijayamma Thomas & Dissanaik. Antibodies to *Sarcocystis* in Malaysia. Trans. Roy. Soc. Trop. Med & Hyg. 72 (3), 1978.
15. Kan S.P. Ultrastructure of the cyst wall of *Sarcocystis* spp. from some rodents in Malaysia. Int. J. Parasitol. 1979: 475-480.
16. Baha L, Vellayan S, Omar E, Sulaiman A, Noryatimah MD. Sarcocystosis among wild captive and zoo animals in Malaysia. Kor J Parasitol, 2010, 48 (3): 213 - 217.

Figure 1: Photomicrograph showing multiple sarcocysts amidst muscle fibres. The cysts are elongated and stained darker and are rimmed completely by the muscle fibres. (H&E, 100X).

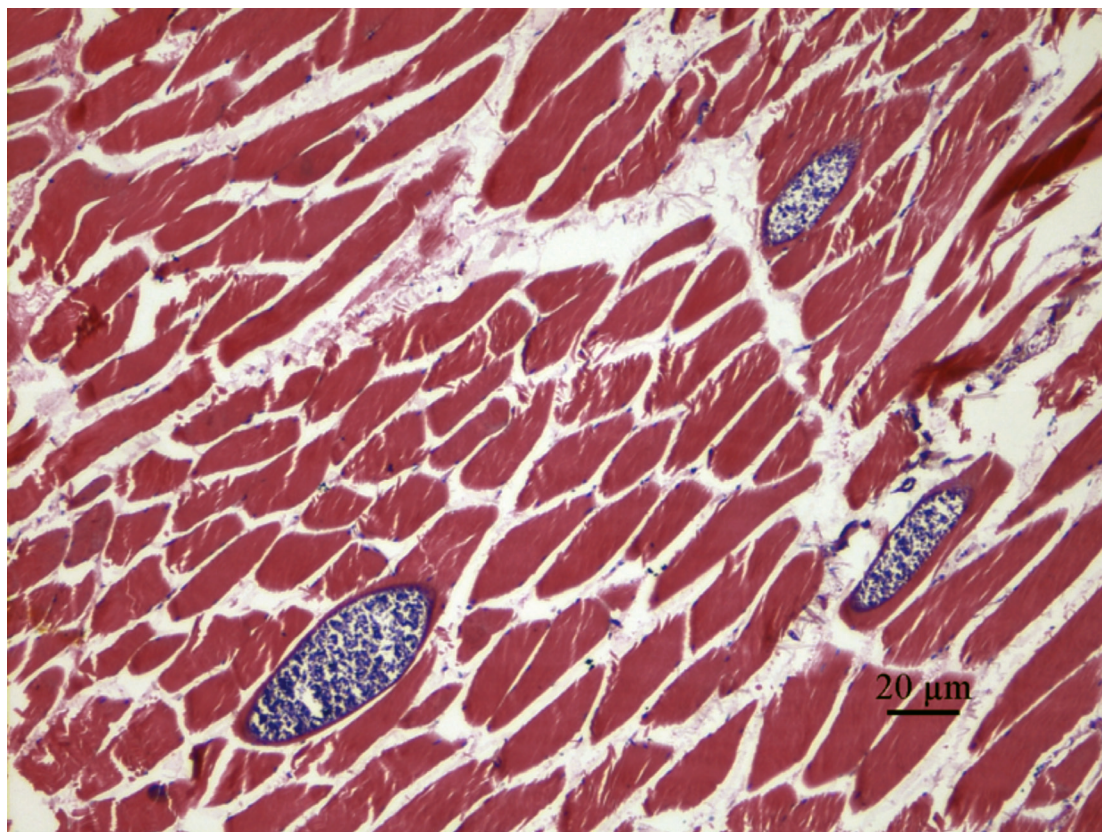


Figure 2: Photomicrograph showing numerous cystozoites within the sarcocyst. (H&E, 1000X).

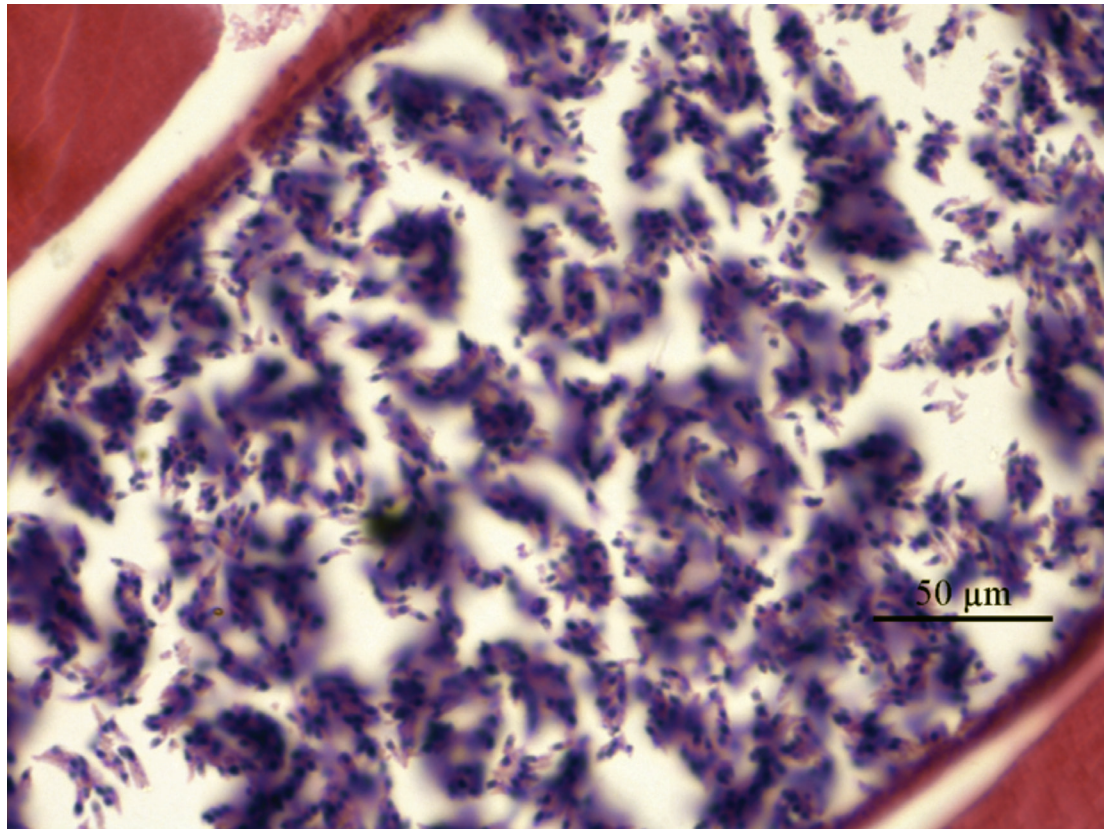


Figure 3: Photomicrograph showing sarcocyst with filamentous cyst wall showing numerous cystozoites within. (H&E, 1000X).

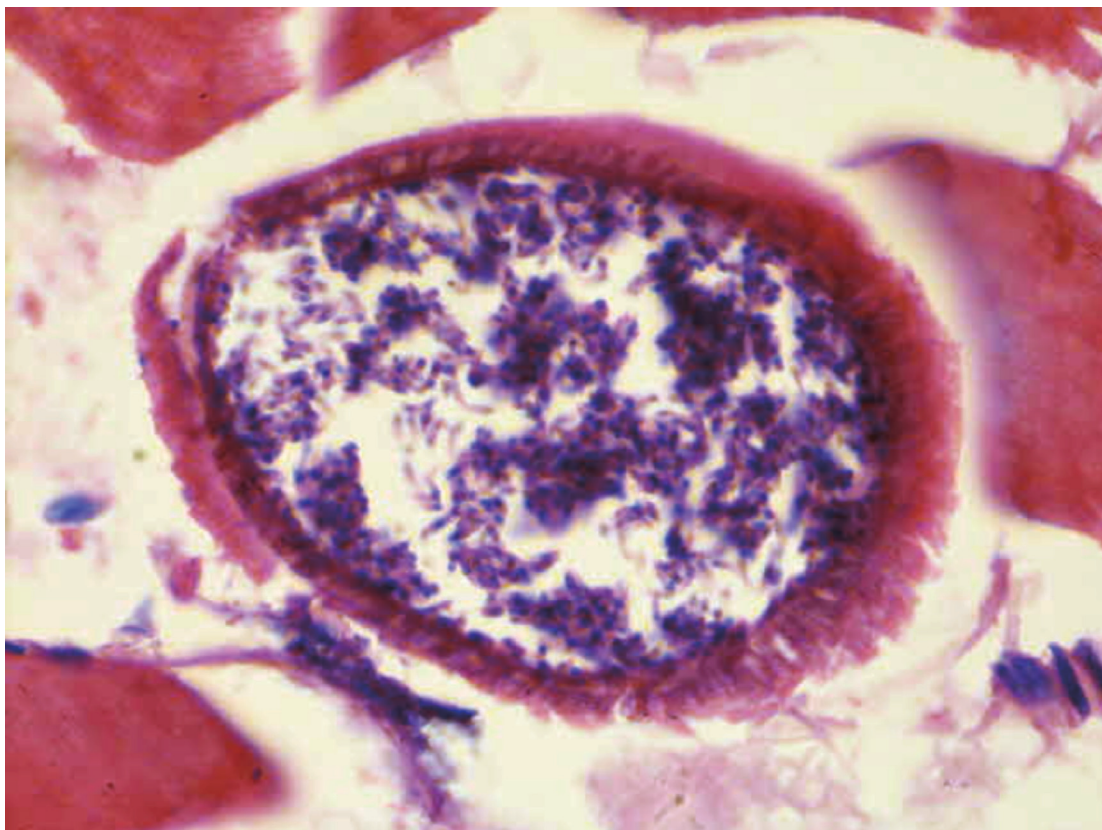


Figure 4: Photomicrograph showing sarcocyst with striated wall showing numerous cystozoites within. (H&E, 1000X).

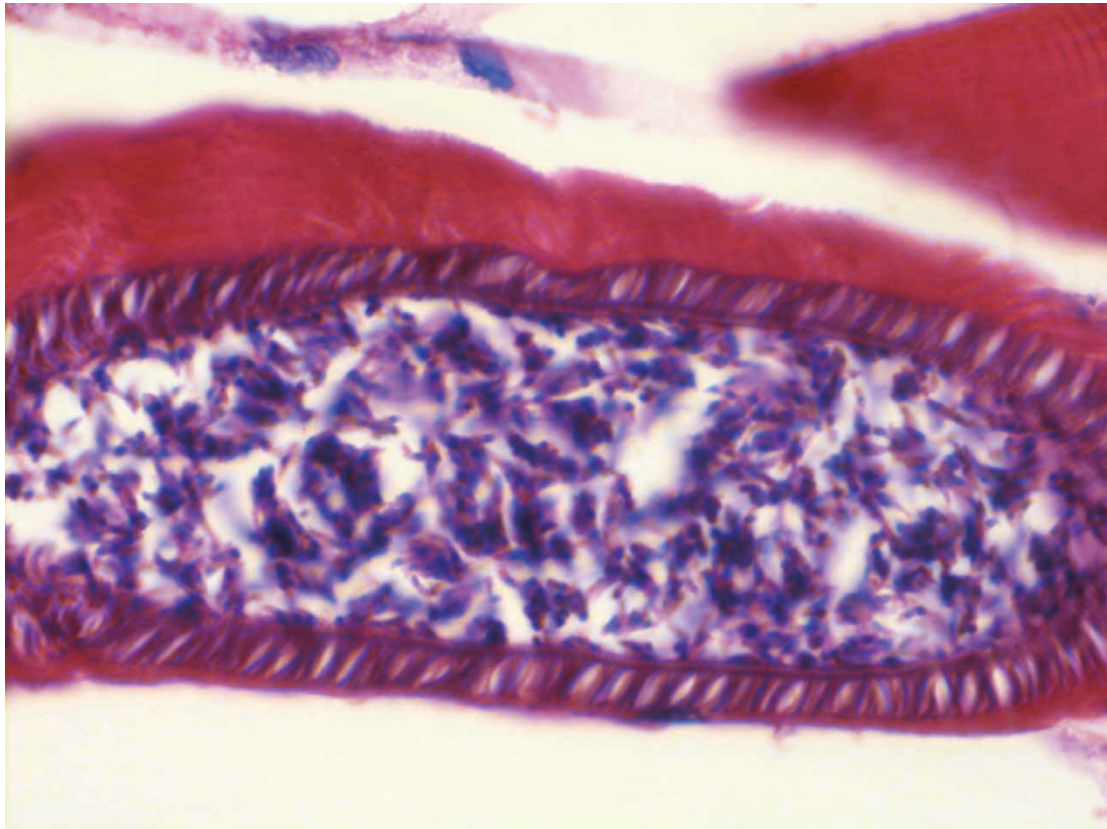


Figure 5: Photomicrograph showing a thin walled elongate sarcocyst with numerous cystozoites within. (H&E, 400X).

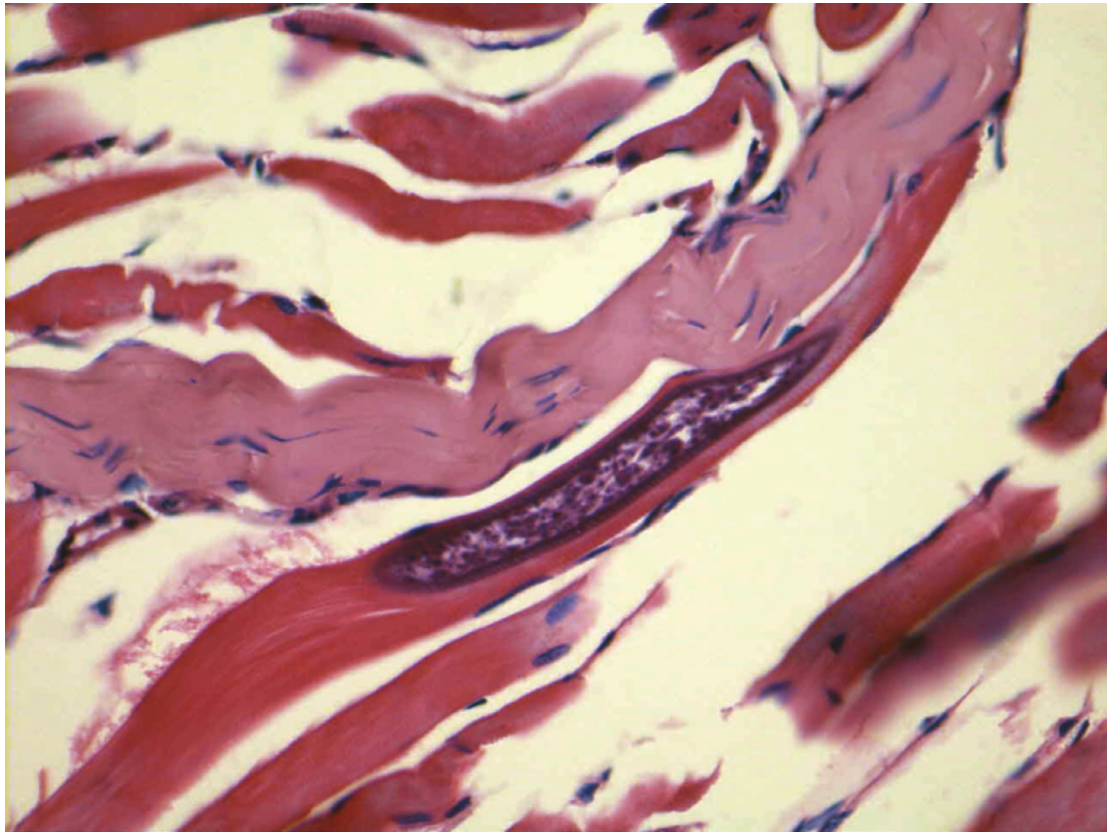


Figure 6: Photomicrograph showing a thin-walled sarcocyst measuring 206.7 by 65.7 μm in the muscle tissue (H&E, 400X).

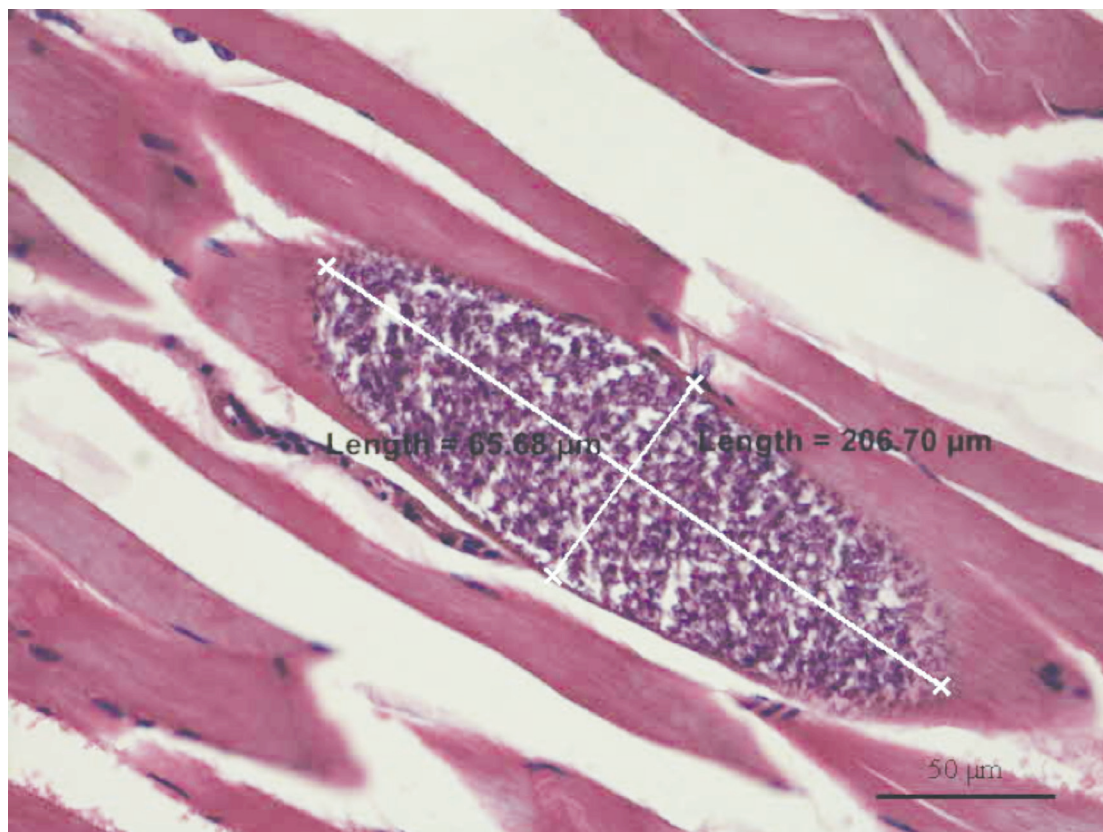


Figure 7: Photomicrograph showing a sarcocyst with a striated wall measuring 139.6 by 53.2 μm . (H&E, 400X).

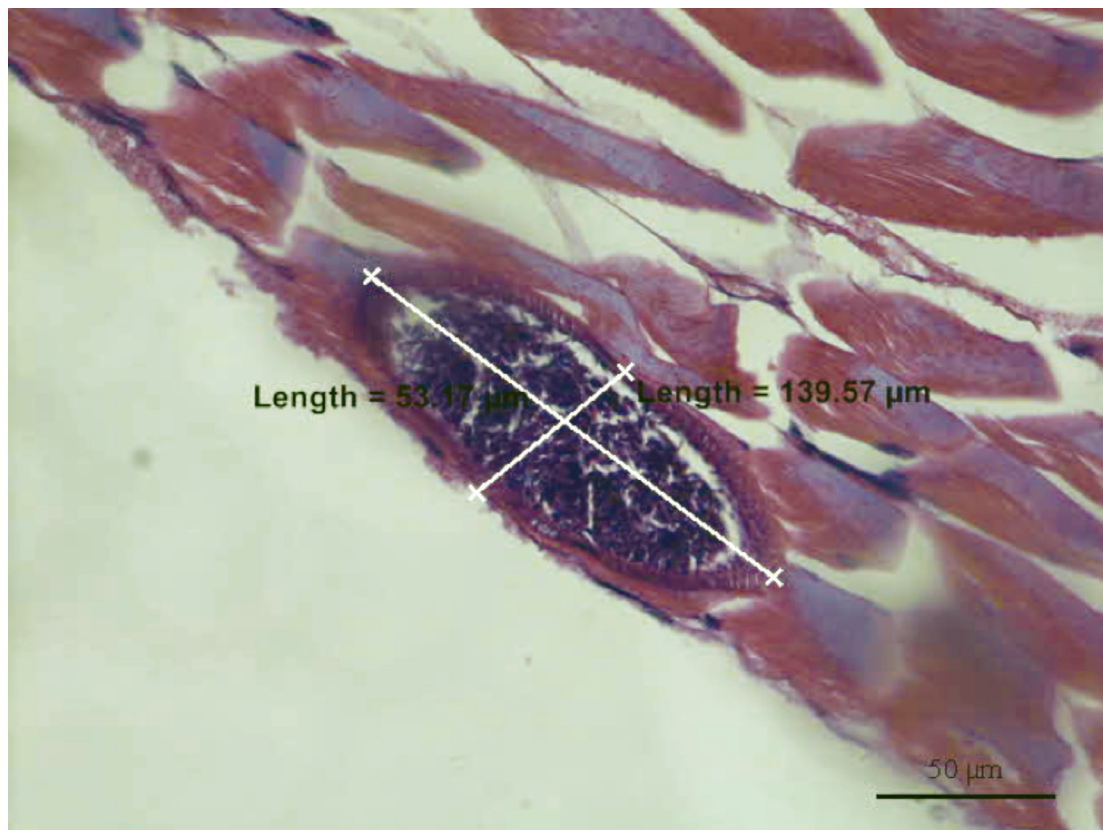


Figure 8: Photomicrograph showing thin-walled sarcocyst measuring 247.7 by 53.9 μm in the muscle tissue (H&E, 400X).

