

Identification of *Sarcocystis* spp. by Transmission Electron Microscope

Safa T. Whaeeb*, Azhar A. Faraj

Department of Parasitology/College of Veterinary Medicine /University of Baghdad, Iraq

Abstract: This study aimed to provide Identification of *Sarcocystis* spp. by electron microscope macroscopic and microscopic cysts of *Sarcocystis* in sheep. In this investigation, the microscopic cysts of *Sarcocystis* were assessed in slaughtered sheep. The digestion method was used for bradyzoites observation in, esophagus and inter costal muscle samples ($P < 0.05$). Six analysis samples were as conducted on microscopic cysts, Diagnosis two pathogenic species of *Sarcocystis* in Baghdad by electron microscopic (*S. tenella* and *S. arieticanis*).

Keywords: *Sarcocystis*

1. Introduction

Sarcocystosis is caused by species of *Sarcocystis*, an intracellular protozoan parasite in the phylum Apicomplexa(1). These parasites have an indirect life cycle, cycling between a Definitive and an intermediate host (2). Intestinal infections occur in the definitive host, And tissue invasion is seen in the intermediate host, more than ahundred species of *Sarcocystis* are Parasites of domestic and wild animals (3). Ovines can be intermediate hosts for four *Sarcocystis* species (*S. gigantea*, *S. medusiformis*, *S. tenella* and *S. arieticanis*) and can form macroscopic or microscopic cysts in different tissues of production animals (1). These animals can be infected if they ingest sporocysts or sporulated oocysts present in food or water (4). In sheep, the main clinical signs of *Sarcocystis* infection are anorexia, weight loss and hyperthermia. However, depending on the number of sporocysts ingested, nervous signs, premature birth, and abortion are observed (5). *Sarcocystis* spp. has been described primarily on the morphology of mature cyst (cysts containing mainly bradyzoites). Size, shape, and cyst wall ultrastructure of sarcocysts are important in the identification and differentiation of *Sarcocystis* sp., as also are life cycle studies (6, 3). There are numerous detailed investigations on *Sarcocystis* spp. from domestic sheep and goats (7, 8). In the present study described the ultrastructure of *Sarcocystis* spp. from the sheep.

2. Material and Method

Histopathological examination by light microscopic and Transmission electron microscopy (TEM):

This study examined 6 smple were grossly and microscopically for *Sarcocystis* spp. infection. For light microscopy, size 1 -3 μm samples of the esophagus and inter costal muscles, fixed in 10% buffered formalin solution (pH 7.4), and embedded in paraffin (9, 10). Sex sections were stained with hematoxylin and eosin. For electron microscopy, about 1 mm³ pieces of esophagus and 3 inter costal muscles and fixed with 2.5% glutaraldehyde solution in 0.1M phosphate buffer (pH 7.4) for 4 h. After washing with buffer, the specimens were post-fixed with 1% osmium tetroxide at 4 °C for 4 h. Afterwards, the specimens were dehydrated in a

graded ethyl alcohol series and two changes of propylene oxide, and embedded in epon mixture. (11, 12, 13, 14).

3. Result and Discussion

Histopathological finding and Transmission electron microscopy (TEM) of (esophagus, muscle):-

This study taken sex sample (3esophagus and 3 intercostal muscle)to diagnosis the structure of cyst by using histological technique, appear different forms of the cyst including the oval elliptical cylindrical and conical, wall of cyst appear. Figure (1).

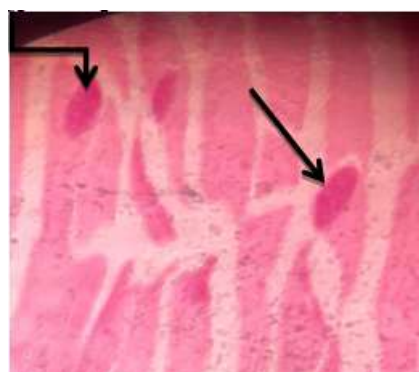


Figure 1: Show cyst of *Sarcocystis* in sheep by Histopathological (H &E)

Sex Fresh samples of muscle tissue from esophagus and intercostal muscle were tested; individual *Sarcocystis* found in the musculature were extracted from the muscle fibers under a dissecting microscope for fresh-state examination or prepared for transmission electron microscopically (TEM) investigations.

Results found in the sex sample of organs sheep *Sarcocystis tenella* and the *Sarcocystis arieticanis* depended diagnosis in *Sarcocystis* shape (figure 2, 3).

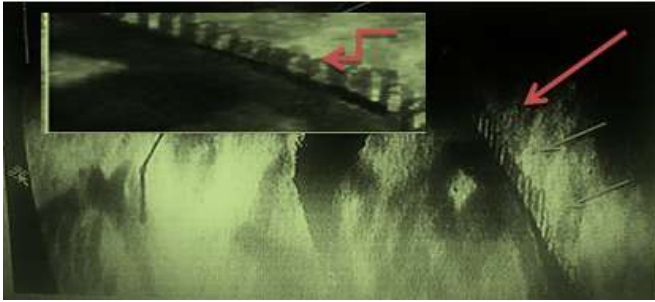


Figure 2: *Sarcocystis tenella* from sheep, fresh state views. mature sarcocyst which the finger like protrusions are most prominent.

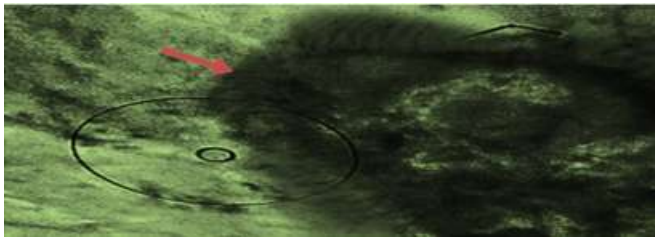


Figure 3: *Sarcocystis arieticanis* from sheep; fresh state view of mature sarcocyst from showing the hair like villar protrusions on the top.

The four *Sarcocystis* species known from domestic sheep are unequivocally defined morphologically and easily well distinguished from each other (14, 15).

They can be distinguished clearly from *S. ferorns* (9) (with flattened mushroom-like protrusions) described from the bighorn sheep (*Ovis canadensis*) as well as from *Sarcocystis* sp. (16) from the American mountain goat (*Oreamnos americanus*) in North America (17). *Sarcocystis* sp. (18) from the chamois (*Rupicapra rupicapra*) in Europe, Both sarcocyst forms from the *Rupicaprinae Rupicapra* sp. and *Oreamnos* sp., appear similar if one compares. By (19), with (20); the protrusions are club shaped, with two longitudinal grooves and a bundle of microtubules reaching into the ground substance. *Sarcocystis tenella* and *S. arieticanis* in sheep and the morphologically very similar species *S. capracanis* and *S. hircicanis* (13), in goats are different species separated by intermediate host specificity, as shown by unsuccessful transmitting experiments with *S. tenella* and *S. arieticanis* to goats (14); (10) and with *S. capracanis* and *S. hircicanis* to sheep (15, 9). On the other hand, cross transmission experiments by (16, 21) provide evidence for a less marked intermediate host specificity. Most *Sarcocystis* spp. described from *Caprinae* and *Rupicaprinae* can be distinguished according to the ultrastructure of the cyst wall and the species of the intermediate host, The species composition of *Sarcocystis* spp. in domestic sheep is well established (9, 11), especially for *S. tenella* and *S. arieticanis* which use canids as definitive hosts. The mature *Sarcocyst* of *S. tenella* and *S. arieticanis* can be distinguished microscopically in the fresh state or also in standard histological preparations. (23, 25). Found in this study the fine structure of the cyst wall of *S. tenella* and *S. arieticanis* were fine structure of the cyst wall were identical domestic sheep similar to that described previously for this parasite from domestic sheep (21).

Also in Correspond well with ours *Sarcocystis arieticanis* seems to occur less frequently than *S. tenella* (18), but may be more frequent in western Australia (, 24, 22). The present study description is the first report from sheep. There are different data on the size of the *Sarcocystis* of both *Sarcocystis* species described here *Sarcocystis arieticanis* reaches a length of 1.0 mm according to (15), of 0.9 mm (11), of 2.92 mm according to (23), of 1.3 mm (14), and 1.8 mm in our material, *Sarcocystis tenella* is up to 0.58 mm long Boch *et al.* (1979), over 1.0 mm at 105 days post-infection (13), up to 0.7 mm according to (9), up to 1.3 mm according to (16), and 2.4 mm in our samples.

We found *Sarcocystis tenelba* (synonyms: *S. ovicanis*) (11) and *Sarcocystis* sp. (20, 26, 27) in all domestic sheep. The cyst walls were 1.08 to 3.85 μm thick and had a palisade-like texture, with finger-shaped villa are protrusions that were positioned closely side by side, The protrusions were 2.10 to 3.85 μm long and 0.42 to 2.14 μm wide. The distance between the protrusions was 0.2 to 0.5 μm at their base.

The diameters of the compartments in the region of the cyst wall ranged from 11.0 to 49.5 μm in the domestic. Most the domestic sheep also had *Sarcocystis arieticanis*(19). The diameters of the compartments in the region of the cyst wall ranged from 20.0 to 59.9 μm . The diameters of the compartments in direction of the center ranged from 46.6 to 73.3 μm in the domestic (18, 25, 26, 27).

References

- [1] Dubey, J.P. and Lindsay, D.S.(2006). Neosporosis, toxoplasmosis, and sarcocystosis in ruminants. Veterinary Clinics Of North America: Food Animal Practice.,22.,3.,645-671, 2006.
- [2] Stojek, J. K. ; Karamon, J. and Sroka, Cencek, T. (2012).Molecular diagnostics of Sarcocystis spp. Infections. Polish J. Vet. Scie., 15, 3.33-35.
- [3] Chhabra MB, Samantaray S.(2013)Sarcocystis and sarcocystosis in India: status and emerging perspectives., J Parasit Dis. 37: 1– 10.
- [4] Heckerth, A.J. and Tenter, A.M. A (2007). Etiological diagnosis - Sarcocystosis. In: ORTEGA-MORA, L.M Protozoal abortion in farm ruminants: guidelines for diagnosis and control. UK: CAB Internat., Cap.3.3, p.172-231.
- [5] Hong, E.J. ; Sim, C.; Chae, Kim, J.S. H.C. ; Park, J. ; Choi, K.S. ; Yu, D.H. ; Park, C.H.; Yoo, J.G. and Park ;B.K. (2016).Ultrastructural and molecular identification of Sarcocystis tenella (Protozoa, Apicomplexa) in naturally infected Korean native goats. Vet. Med., 61, (7): 374–381
- [6] Bittencourt, M.V.; Meneses, I. D. Ribeiro-Andrade, M.; de Jesus, R.F.; de Araújo, F.R. and Gondim, L.F.(2016) *Sarcocystis* spp. in sheep and goats: frequency of infection and species identification by morphological, ultrastructural, and molecular tests in Bahia, Brazil. Parasitol Res., 115(4):1683-9.
- [7] Santos, R. ; Da Silva, N.; Rodrigues, R.; Pacheco, D.; Araujo, F.; Beck, C. and Olicheski, A.(2002).Detection of bovine Sarcocystis cruzi cysts in cardiac muscles: a

- new technique of concentration for diagnostic. *Acta Scie. Vet.* 30 (2):127–129.
- [8] **Erni, R.; Rossell, M.; Kisielowski, C. and Dahmen, U. (2009).** "Atomic-Resolution Imaging with a Sub-50-pm Electron Probe". *Physi. J.* 102 (9): 96-101.
- [9] **Danilatos, G.D. (1986).** "Colour micrographs for backscattered electron signals in the SEM". *Scanning* 9 (3): 8–18.
- [10] **Dannen, G. (1998).** Leo Szilard the Inventor: A Slideshow (1998, Budapest, conference talk). *American J. of Trop. Med. and Hyg.* 2:99-109.
- [11] **Rudenberg, H Gunther; Rudenberg, Paul G (2010).** "Chapter 6 – Origin and Background of the Invention of the Electron Microscope: Commentary and Expanded Notes on Memoir of Reinhold Rüdénberg". *Advances in Imaging and Electron Physics* 160. Elsevier
- [12] **Luft, J.H. (2000).** "Improvements in epoxy resin embedding methods". *The J. of biophys.and biochem. Cytololol.* 9 (2). p. 409.
- [13] **Von Ardenne, M; Beischer, D (1940).** "Untersuchung von metalloxyd-rauchen mit dem universal-elektronenmikroskop". *Zeitschrift Electrochemie (in German)* 46: 270–277.
- [14] **Baron, J., & Ritov, I. (1994).** Reference points and omission bias. *Organizational Behavior and Human Decision Processes*, 59, 475–498.
- [15] **Smith, J. H., Meter, J.L., Neill, J.G. and Box, E.D. (1987).** Pathogenesis of *Sarcocystis falcatula* in the budgerigar. I. Early pulmonary schizogony. *Laborat. Investigat.* 56: 72-74.
- [16] **Wilber, J.L. (1999).** Pathology of the rabbit. Department of veterinary pathology, Armed Forces Institute of Pathology. Washington, J. Pathol., 16: 10-19.
- [17] **Dubey, J.P. and Bergeron, J.A. (1982).** *Sarcocystis* as a cause of placentitis and abortion in cattle. *Vet. Pathol.* 19: 315-318.
- [18] **Dubey, J.P.; Speer, C.A. and Fayer, R. (1989).** *Sarcocystis of Animals and Man.* CRC Press, Boca raton, Florida. PP. 215.
- [19] **Foreyt, W. J. (1989).** *Sarcocystis* sp. in mountain goats (*Oreamnos americanus*) in Washington: Prevalence and search for the definitive host *Vet. Pathol.* 77:40-67.
- [20] **Collins, G.H.; Atkinson, E. and Charleston, W.A. (1983).** Studies on *Sarcocystis species*. III. The macrocystic species of sheep. *N. Z.Vet. J.* 27, 204-206.
- [21] **Heydorn, A.O.; Gestrich, R.; Mehlhorn, H. and Rommel, M. (1975).** Proposal for a new nomenclature of the Sarcosporidia. *Zeitschrift fur Parasitenkunde-Parasitol. Rese.* 48:73-82.
- [22] **Heydorn, A. (1985).** Zur Entwicklung von *Sarcocystis arleticanis* n. sp. *Berliner und Mnchener Tierrztliche Wochenschrift* 98: 231-241.
- [23] **Balbo, T.; Hossi, P.; Lanfranchi, P. ;Meneguz, C. Meneghi, D. and Canese. M.(1988).** Experimental transmission of a sarcosporidian from alpine ibex to domestic sheep and goats. *Parassito.* 30: 241-247.
- [24] **Boch, J., Hennings, R. and Erber, M. (2012).** Die wirtschaftliche Bedeutung der Sarkosporidiose (*Sarcocystis suicanis*) in der schweinemast. Auswertung eines Feldversuches. *Berliner und Munchener Tierarztliche Wochenschrift*, 93(21):420-423.
- [25] **Bunyaratvej, S.; Bunyawongwiroj, P. and Nitiyanant, P. (2011).** Human intestinal sarcocystiosis: report of six cases. *American J. of Trop. Med. and Hygi.* 31(1):36–41.
- [26] **Schmidtova, D. (1992).** Some characteristics of *Sarcocystis*, *Jap. J. of Vet. Med.Scie.*, 30(5):200–206.
- [27] **Savini, C.; Dunsmore, J.; Hobertson, D. and Seneviratna. P. (1993).** *Sarcocystis* spp in western Australian sheep. *Aust. Vet. J.* 70: 152-154.
- [28] **Nigro, M.; Mancianti, P.; Rossetti, C. and PoLL, A.(1991).** Ultrastructure of the cyst and life cycle of *Sarcocystis* sp. from wild sheep (*Ovis musimon*). *J. of Wildlife Dise.* 27: 217-224