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Identification of *Sarcocystis* spp. by Transmission Electron Microscope

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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. ariticanis).***

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 elliptic cylindrical and conical, wall of cyst appear. Figure (1).

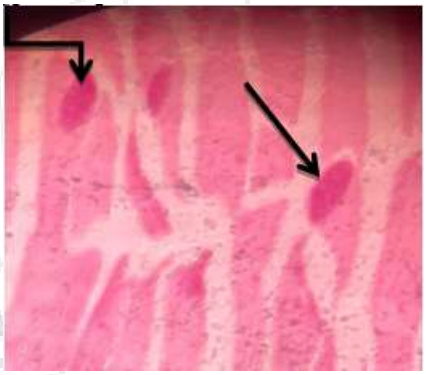

Figure 1: Show cyst of *Sarcocystis* in sheep by Histopathological (H &E)

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).

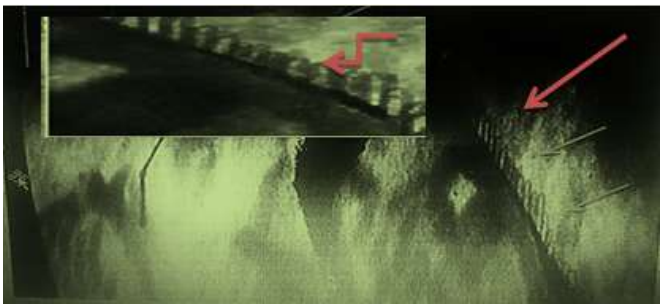

Figure 2: *Sarcocystis tenella* from sheep, fresh state views. mature *sarcocyst* which the finger like protrusions are most prominent.

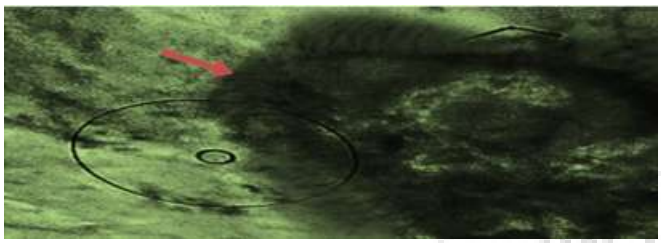

Figure 3: *Sarcocystis arieticanis* from sheep; fresh state view of mature sarcocyst from showing the hair like villar protrusions on the top.

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). S*arcocystis 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 arietcanis* 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).

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