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Research Article

IDENTIFICATION OF *Sarcocystis* sp. INFECTION IN CATTLE IN BABYLON PROVINCE, IRAQ

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Abstract

The present research was conducted in different area of Babylon city, Iraq for identification of *Sarcocystis* spp. by using One hundred twenty esophagus samples of slaughtered cattle (84 male and 36 female) of different age groups (less than 1 Year to up 4 year) were subjected to current study from 10 samples imported beef samples randomly collected at were subjected to current study from July 2022 to the end of December 2022 in different area of Babylon city. Samples were examined by traditional methods (Trichnoscopy, squeezing and pepsin digestion). In addition, Result by using traditional methods show 74.6% of samples were positive of *Sarcocystis* infection, and found the pepsin digestion method its best technique used for the detection of *Sarcocystis* in slaughtered cattle imported and beef tissues, followed by Trichnoscopy and squeezing technique were less sensitive. In additionally, we took 10 samples from positive samples in order to make histopathology for them in order to see the cyst and bradyzoites clearly.

Article History

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Key words: *Sarcocystis*, Cattle, Traditional methods and Iraq.

1. Introduction

Sarcocystosis is a zoonotic infection, caused by a specific intracellular protozoan parasite, which under the phylum Apicomplexa and family Sarcocystidae (Abdel-Gaber *et al.*, 2020). It's worldwide distributed (Rosenthal, 2021). Miescher was first reported *Sarcocystis* in 1843 in striated muscle of a house mouse as white thread like cysts with no scientific name (Shnawa and Swar, 2021; Dubey *et al.*, 2016). Life cycle of *Sarcocystis* spp. has based on a prey-predator relationship, which characterized by muscular cyst formation in the muscular tissue of the prey, thus called (Muscular Sarcocystis), and when the cyst ingested by predator host, the sexual stage will developed and the cyst will be colonization in the lamina propria called (Intestinal Sarcocystis), which finally produced the sporulated oocysts (Shnawa and Swar, 2021).

Sarcocystis spp. is mainly a veterinary problem, as more than 50 % of cattle, pigs and sheep are infected (Nimri, 2014). Presence of macrocystis on muscles decreased the quality of meat which leads to rejection of meat by consumers (Fayer, 1976; Huong and Uggla, 1999) The muscular *Sarcocystis* are mainly seen in esophageal, cardiac, skeletal muscle of domestic animals (Aleman *et al.*, 2016).

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2. Materials and Method

Animals of study

One hundred twenty esophagus samples of slaughtered cattle (84 male and 36 female) of different age groups (less than 1 year to up 4 year) were subjected to current study from July 2022 to the end of December 2022 at different area of Babylon city.

Samples collection

Samples of slaughtered cattle were small piece near 10 - 15 cm selected from esophagus, then transferred in labeled clean plastic bags and we put in box from the different slaughtered area at Babylon, then we transferred the samples to laboratory. slaughtered cattle beef specimens were kept in 8 °C on Refrigerator until examination (Abdullah, 2021).

Macroscopic examination

On fresh esophageal sample, gross examination with the unaided eye was used to find the macroscopic cysts (Salam and Salih Mustafa, 2021).

Squeezing method

Garlic pressuring in this method by putt 3-4 gm. from each samples inside the presser and crush solution drop transferred to slide with cover slide and examination by Light microscope at 10 x and 40 x (Mohammed *et al.*, 2022).

Trichnoscopy examination

The Muscles were cut to small pieces, and putting between two slides and examine under the Light microscope (10 x and 40 x) for diagnosis the microscopic tissue cyst (Al-Saadi *et al.*, 2020).

Digestion method

Utilize the meat of infected animal by pepsin digestion as classical method of with some modification, 20 gm from meat slaughtered cattle (esophagus), put in container containing digested medium (100 ml) for 12 - 18 hours at 25 °C room temperatures. The digested medium composed from 3.5ml of HCl and pepsin 1.3 gm, and 2.5 gm of NaCl, and were dissolved in 500 ml of sterile distilled water. Then, the Materials filtered by using sterile double layer gauze, and all materials centrifuge for 5 minutes at 2800 rpm. After the sediment was put in Eppendorf tubes (1.5 ml) and kept at -20 °C until molecular examination. And we put sediment drop in the slides prepared were stained with Giemsa stain for bradyzoites identification and examined under microscope at 100 x (Sarafraz *et al.*, 2020).

Giemsa Staining

The a smear on a laboratory slide prepared from, after air dried and fixed in methanol for 3 minutes, the slides were putting in a stain jar filled with 1:10 dilution from Giemsa stain (90 ml of buffer solution pH 7.0 and 10 ml of Giemsa stain) for 20 minutes, The slides were placed on the staining rack to dry after the stain was poured off and many times washed with distilled water and examined at 100 x magnification with oil immersion under a light microscope (Choi *et al.*, 2018; Elshahawy *et al.*, 2023).

Histopathological method

Histopathological section was done for confirmed detection of bradyzoites in tissue samples was made for 10 positive samples to observation of 10 % formalin was used for samples fixation. The tissues were processed with ascending concentration degree of ethyl alcohol (70, 80, 90 and 100 % for 1 hour) to prepare thin blocks in paraffin wax, sections were taken at 4 -5 µm the paraffin block was prepared were supply, Hematoxylin and Eosin stained with samples. The tissue cysts were examined with oil at 100 x magnification by Light microscope (Kalantari *et al.*, 2013; Anvari *et al.*, 2020).

3. Results and Discussion

Infection rate by traditional methods

Infection rate of macrocyst and microcyst according all samples

Result show no significant differences ($P<0.05$) of macrocyst and microcyst respectively

in slaughtered cattle beef and Imported meat. Highest prevalence rate of Sarcocystis spp. infected cattle registered at Babylon in our study 75.8 % in slaughtered cattle and 60 % in imported beef which investigated by traditional methods. Jinan Khalid *et al.* (2020) recorded in Baghdad 70.5 % in slaughtered cattle and 64 % in imported beef which investigated by traditional methods. When researchers in Diala province examined the muscular tissue samples of 179 cattle, they discovered the spread of both macrocystis and microcystis was 2.23 % and 81 %, respectively (Al-Taai, 2002) while comparable results have been reported in other nations, with Mongolian cattle accounting for 90 % of the total (Fukoyo *et al.*, 2002), 99.5 % of Argentina's livestock (More *et al.*, 2011), *Sarcocystis* infection in cattle is 66 % in Hungary (Sándor *et al.*, 2015) (Table - 1).

Table - 1: Total infection rate of *cyst.* in slaughtered cattle and imported beef samples by traditional technique examined microscopically

Host	No. of samples examined	Traditional microscopy	
		No. of positive	Percentage (%)
Slaughtered cattle meat	120	91	75.833333
Important meat	10	6	60
Total	130	97	74.615385
X^2	1.221754		
P value	0.269017 NS		

NS: Non-significant differences at (P≤0.05).

Infection rate of *Sarcocystis* according to different traditional techniques

Study identified highly significant differences (P<0.05) between three traditional techniques in both slaughtered cattle and imported beef showed that the pepsin digestion recorded high percentage in diagnosis 75.8 %. Then, the squeezing and Trichnoscopy were less sensitive in diagnosis at 59.2 % and 54.2 % in slaughtered cattle, 20 % and 20 % in imported beef respectively. In our investigation, all traditional procedures (pepsin digestion, squeezing and trichnoscopy) exhibited distinct sensitivities (significant differences at P≤0.05). Similarly, the findings Latif *et al.* (1999) who compared these methods in different animals and concurred with all studies that pepsin digestion is a sensitive test for this reason (Shekarforoush *et al.*, 2005; Al-Hasnawi, 2008). Additionally, Mangas *et al.* (2015) compared two techniques of muscle digestion and scarification for detecting *Sarcocystis* species in beef cattle by collecting 400 samples from slaughtered cattle's tongue and heart and analyzing them using pepsin digestion and scarification and concluded that pepsin digestion was superior to scarification for tongue samples and Scarification of the heart has been proposed as an alternative to digestion for the detection of Sarcocystis infection in cattle, with the benefit of being easy to perform and inexpensive.

Infection rate of *Sarcocystis* spp. in slaughtered cattle according to the sex

Infection showed non-significant difference (P<0.05) was recorded between male and female. The rates of infection were 76.19% in male and 75 % in female. The results of current study showed significant difference between males and females (P<0.05) that disagreement with Savini *et al.* (1992) who demonstrated significant differences between sexes in cattle, mainly significant association between rate of *Sarcocystis* infection and sex of cattle was reported in many studies while the present study were a contrast with Mounika *et al.* (2018) which study of 150 slaughtered cattle over a period of 1 year were examined both macroscopically and microscopically for the presence of *Sarcocystis* infection which found no significant relationship between the prevalence of *Sarcocystis* infection in male (91.76 %) and female (90.76 %) cattle was observed (Table - 3).

Infection rate of *Sarcocystis* in slaughtered cattle based on age group by Traditional techniques

The result showed that the significant differences (P<0.05) between age groups. The highest infection rate (88.5) was recorded in animal of more than four years old similar result with Saito *et al.* (2000) found that the prevalence of Sarcocystis infection increased with age. There was a significant relationship between the prevalence of infection among different age groups (P<0.05) The prevalence was found to increase with advancement of age with high infection rate of 95.65 % among the old animals which was consistent with data of *Sarcocystis* infection in cattle from other countries (Hornok *et al.*, 2015) on other hand investigation revealed higher infection rates with both Sarcocystis species in old-aged animals at 5 years and above, than those occurred in younger animals 2 - 3 years (El-Seify *et al.*, 2014) (Table - 4)

Table - 2: Total infection rate of *Sarcocystis* in slaughtered cattle and imported beef samples by different traditional technique.

Host	No. examined sample	Traditional microscopy					
		Pepsin digestion	%	Squeezing	%	Trichoscopy	%
Slaughtered cattle meat	120	91	75.8	71	59.2	65	54.2
Imported meat	10	6	60	2	20	2	20
Total	130	97	74.61	73	56.15	67	51.53
X^2	27.692355						
P value	0.000042**						

NS: significant differences at (P≤0.05).

Table – 3: Sarcocystosis infection rate in slaughtered cattle according to sex by traditional technique.

Gender	No. of examined samples	Traditional microscopy		
		No. of positive	Percentage (%)	Percentage of total (%)
Male	84	64	76.19	53.3
Female	36	27	75	22.5
Total	120	91	75.83	
X^2	0.019488			
P value	0.888977 NS			

NS: Non-significant differences at (P≤0.05).

Table – 4: Sarcocystosis infection rate in Slaughtered cattle according to age group of Slaughtered cattle

Age (year)	No. of the exam. samples	Positive samples		
		No.	%	% of total
≤ 2 years	11	2	18.2	1.7
2 ≤ 3years	12	4	33.3	3.3
3 ≤ 4 years	36	31	86.1	25.8
More than 4 years	61	54	88.5	45
Total	120	91		
X^2	39.213178			
	0.00**			

*Significant differences at (P≤0.05).

Morphology of *Sarcocystis* spp.

Macroscopic examination

The meat samples of slaughtered cattle was examined macroscopically. The cyst of *Sarcocystis* collected were creamy white in color, of different shapes, spindle, fusiform and globular and of different sizes (Figure - 1).

Figure – 1: Photograph of Bovine esophagus showing Macrcystis of *Sarcocystis* spp.

Characterization of Sarcocystis cyst

Microscopic examination of Sarcocystis cyst by using trichnoscopy technique and histopathology, showed oval, elliptical and conical form divided into compartments were many intercostal with different measurement range (Figures - 2 and 3).

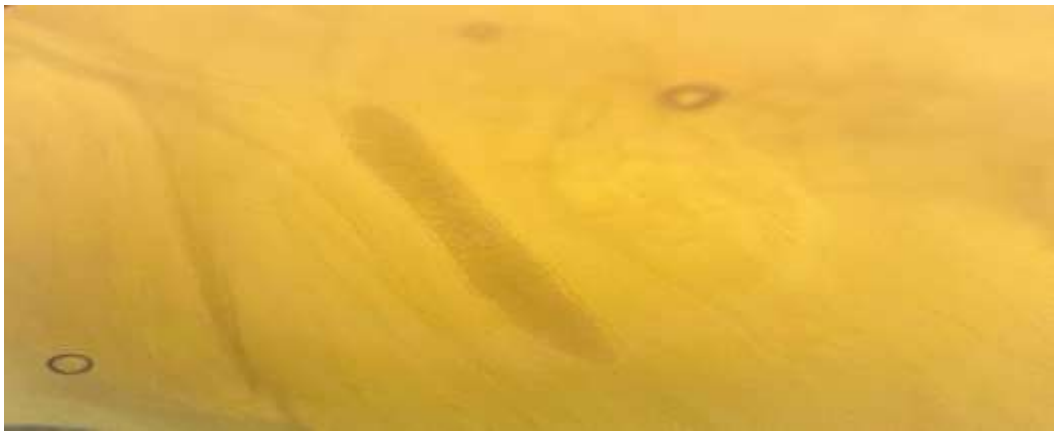

Figure – 2: Microcyst of *Sarcocystis* spp. in Esophagus by Trichnoscopy (40 x)

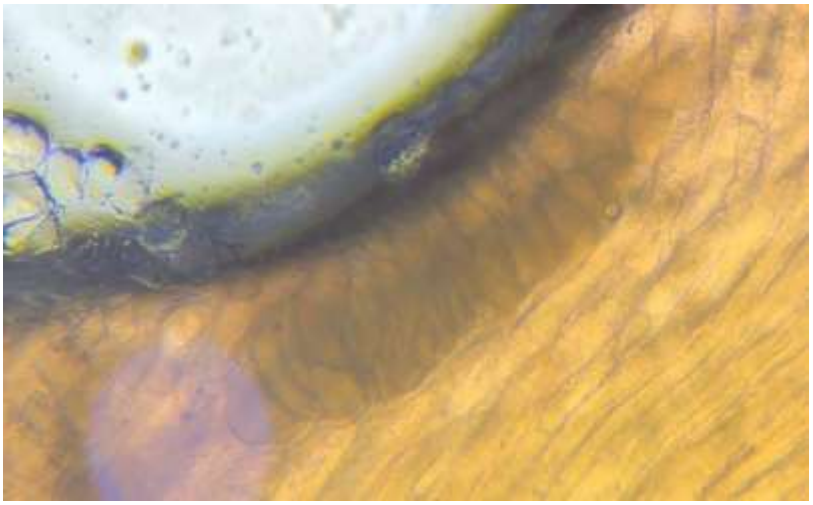

Figure – 3: Microcyst of *Sarcocystis* spp. in Esophagus by Trichnoscopy (40 x)

Morphology of bradyzoite by using pepsin digestion and squeezing methods

This method shows the bradyzoites were seen by examining one drop of the sediment of the digested muscle fluid (Figures 4 and 5). Bradyzoite of Sarcocystis parasite appeared by pepsin digestion and the squeezing technique in slaughtered cattle meat as banana form with a spiked end of front and rounded rear end and slightly clear nucleus located near the rear end.

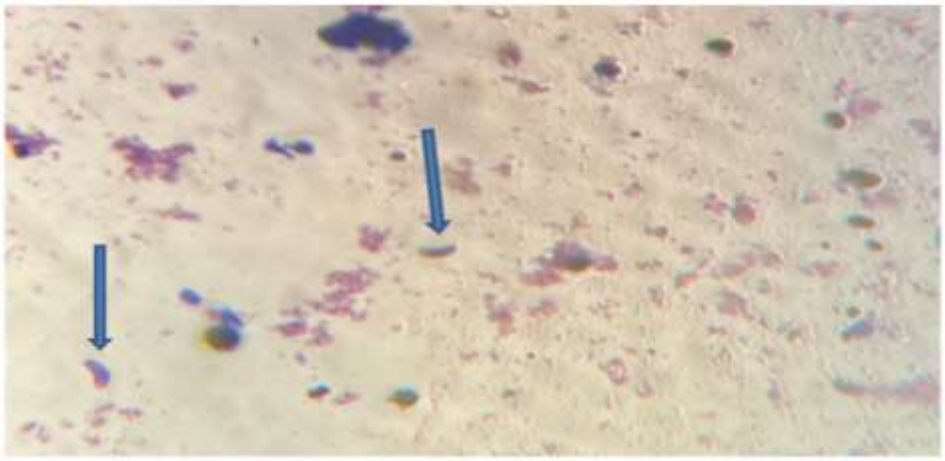

Figure – 4: Bradyzoites by using pepsin digestion in slaughtered cattle stained by Giemsa (100 x)

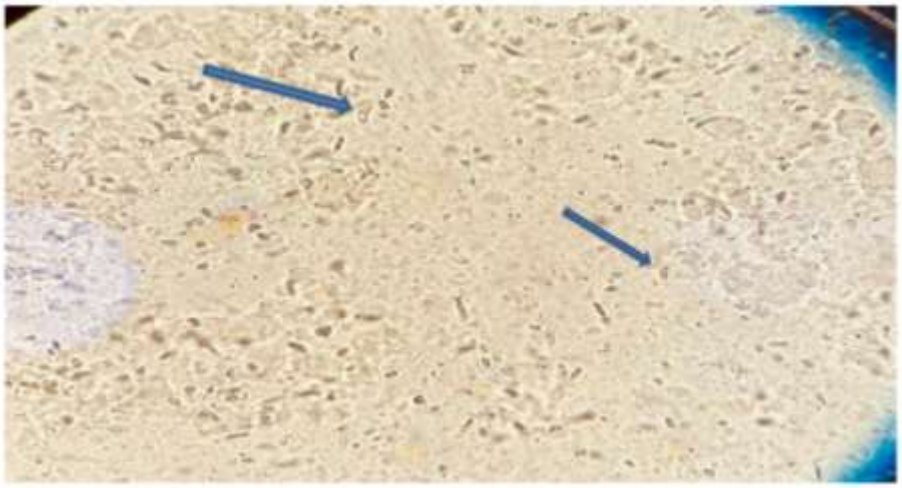

Figure - 5: Bradyzoites by using pepsin digestion in Slaughtered cattle without any stained (100 x)

Histopathological method

For the verified discovery of bradyzoites in tissue samples, histopathological sections were performed on 10 positive samples for inspection. The samples were fixed using 10 % formalin. The tissues were prepared by processing them with ethyl alcohol at increasing concentrations (70, 80, 90, and 100 % for 1 hour) to create thin blocks of paraffin wax. Sections were taken at 4 - 5 µm, and after the paraffin block was ready, it was stained with ematoxylin and Eosin. The tissue cysts were examined with oil at 100 x magnification by light microscope (Kalantari *et al.*, 2013, Anvari *et al.*, 2020).

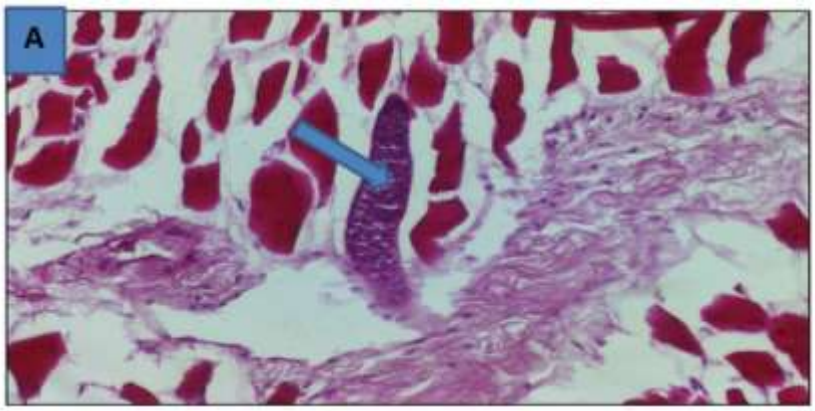

Figure – 6: Photomicrograph of muscular layer of bovine esophagus. Longitudinal section of *Sarcocystis spp.* cyst (arrow) that filled with bradyzoite was observed in myofiber. Note the Sarcocystis cyst occupied most of esophageal myofiber areas (H & E stain 100 x)

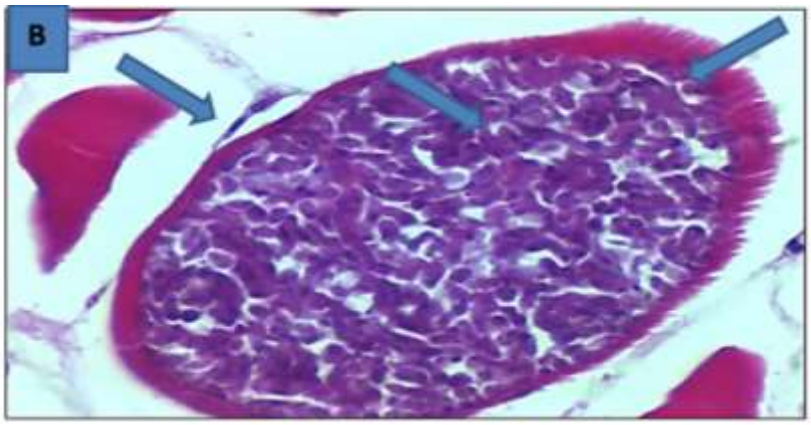

Figure - 7: Photomicrograph of muscular layer of bovine esophagus. Large cross section of *Sarcocystis* spp. cyst (black arrow) that filled with bradyzoite (orange arrow) was observed between myofibers, where the cyst was larger than surrounded myofibers. Note the Sarcocystis cyst wall consist of strait hair like morphology cells (red arrow) (H&E stain 400 x)

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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