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Serological and Traditional Detection of *Sarcocystis Species* Isolated from Human and Beef Meat in Diyala Province

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Abstract

Sarcocystis is a protozoan, intracellular parasitic disease of the phylum Apicomplexa, it is important disease in Asia, especially the western regions of the continent. It is considered one of the important zoonotic diseases, the infection wide geographic spreading caused by various species. The total Infection rate of traditional methods was 65 % (65\ 100) of sarcocysts isolated from esophagus of 100 cattle were slaughtered at Diyala slaughter house during the period from November 2023 to May 2024. Cattle age ranged from (2-5) years. Different traditional techniques were made to detected sarcocystosis, peptic digestion technique was highly sensitive technique 100% according all other traditional methods. Then Trichnoscopy were 30(30%) while the squeezing less sensitive 26(26%) respectively. The majority of male animals recorded higher infection incidence 75% while the female's cattle rate was 53,8 from the total exanimated animals. The result showed that the highest infection rate was recorded in animal of old age above 4-year-old 100% with significant differences between the age groups. the bradyzoites of *Sarcocystis* parasite were seen by examining the sediment of the digested muscle fluid as banana shape with a spik end of front and rounded rear end and slightly clear nucleus positioned toward the rear end, measurements 13.2× 2.8 µm. In human the total ELISA results of the blood samples (male and female) showed total infected rate (15.28%). the male recorded highly infection rate in 10(34.48%) while the lowest rate in female was (6.34%)The infection rate according to male ages groups showed that (15-25) age highly infected (20.68%) .In females, the highest infection rate recorded in (25-35) age group than other groups.in conclusion the sarcocystis infection in cattle meat and human serum were elevated in Diyala areas and it has become more effected on the health of society due to the consumption of imported or under cocked meat. Therefore, more studies must be conducted to determine the propagate of the disease and attempt control it distribution.

Keywords: macrocystis, ELISA, cattle, Diyala,serology

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INTRODUCTION

The first recorded of *Sarcocystis spp* in cattle was done by (Heydorn *et al* ,2020) were cysts detected in the skeletal muscles of cattle, after that detection the (sexual phases) in man pet animal (dog and cat) correspondingly. The *Sarcocystis* and their species were have structure differ from each other species (Waheeb,2018). Many types are described within Sarcocyctidae family and most of these parasites are found in the muscles (skeletal and vicera) of herbivores, lick poultry and humans being. (*Sarcocystis hominis*) and (S. *suihominis*) use humans being as definitive (final) hosts and are responsible for enteric form (intestinal Sarcocystosis) in the human host. Humans being may be also become (dead-end) hosts for anther host which infected with Sarcocystis spp. after the accidental meat ingestion or prehention oocysts. Sarcocystis is a globally recognized parasite that infects numerous animal species (Roberts and Janovy, 2006). Sarcocystis prevalence in domestic animals varied from 10 to 100 percent. Cattle had the highest incidence, followed by goat and sheep. Felids, Canids and humans are final hosts of Sarcocystis species (Dubey,2015). The economic importance of Sarcocystis in sheep and goats is underrated, as abortion and weight loss are often undiagnosed. In addition to health-related problems, Sarcocystis can result in low wool production (Dessi *et al* ,2022). In human symptoms are reported in 10% of infected individuals, including severe and fatal enteritis. The range of the clinical manifestations depends on the intensity of the infection and the *Sarcocystis* species. *Sarcocystis suihominis* is believed to cause more severe symptoms than *S. hominis* (Latif ,2016). Patients who suffered from acute enteritis had a 3324 of eating raw pork and showed necrotizing or eosinophilic enteritis associated with Sarcocystis on histological examination. Clinical symptoms reported in volunteers who consumed raw pork infested with cysts of S. *suihominis* were bloat, nausea, loss of appetite, abdominal pain, vomiting, and diarrhea. (Fayer ,2004.) Assessed for the Sarcocystis diagnosis by depending on (ELISA). These tests used crude antigens (cystozoites, merozoites.) (Savini,1994) The parasitological data showed a strong correlation with the serological evaluation results. Assessment with the merozoite antigen will give the best

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result and conferm animals infected or not. It was also discovered the (cross-reactivity with heterologous species) of Sarcocystis or T. gondii (Metwally *et al*,2014). Blood serum water buffaloes were taken. were slaughtered at the Ahvaz abattoir, Iran, animals with positive results for *Sarcocystis* bradyzoites recorded 57% Conventional method , while infection rate 54.3% from serum samples was positive for *Sarcocystis* antibodies (Ab) in the ELISA technique . (Masoud *et al*,2007). Microscopy revealed an overall infection rate of 94%. Some Serological test examination of many sera that isolated from the same animals by (ELISA) shows that the infection rate was 98% (Fatma et al, 2008). Another research in Sarcocystis frequency in buffaloes in Egypt's Assiut province, utilizing (90) buffalo meat samples, indicated the frequency of Sarcocystis infection rate was 94.4% from the total animal **(**Metwally *et al*,2014**).**the study design to detection of sarcocystis spp in beaf meat and human by serological diagnosis depending on specific kit to sarcocytis spp, To study the spreading of the disease and detection infection rate and in Diyala Governorate.

MATERIALS AND METHODS

Animals of study

one hundred esophagus samples of slaughtered cattle of different age groups (less than 1 Year to up 4 year) to macroscopic Examination and Molecular study, blood samples obtained from 92 human of different ages and genders to ELISA techniques, were subjected to current study from September 2023 to the end of August 2024at different area of Diyala city.

Samples collection

Samples of slaughtered cattle were weight between 100-600gm selected from esophagus, then transferred in individual plastic labeled bags and conveyed to Styrofoam box from the different slaughtered area at Diyala, in a timely manner to the Parasitology laboratory in collage of Veterinary medicine, University of Diyala, Iraq.slaughtered cattle and blood serum were kept in 8°C on refrigerator until examination depending on (Narges *et al* fatma(2013**).**

Macroscopic examination

Gross investigation by the naked eye was performed to detect the macroscopic cysts on fresh esophagus samples. Also, the same examination was done on imported beef muscle samples (Faraj and Kawan,2012; Ahmed *et al*,2016).

B- Squeezing method.

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Garlic pressusing in this method by putting 3 gram from each samples inside the presser and crush solution drop transferred to slide with cover slide and examination by light microscope at 10x and 40x (AL-Taee et al,2009**).**

Trichnoscopy examination

Muscles were cuttied to very small pieces, and then pressed between two slides. Smears were examined under the light microscope (10x and 40x) for detection the microscopic tissue cyst **(**Claveri *et al*,2000**,** Waheeb *et al* ,2018**).**

Digestion method

Utilized the muscle of infected animal by classical method of pepsin digestion with some modification, 20gm of slaughtered cattle esophagus, and the same weight for imported beef was scratched then put in flask contain 100ml of digested medium for 12- 18 hours at 25°C (room temperatures). The digested medium composed from (pepsin 1.3gm, 2.5gm of NaCl, and 3.5ml of HCI were dissolved in 500ml of sterile distilled water). Materials filtered by sterile double layer gauze, all materials centrifuge for 5 minutes at 2800 rpm. Finally, the sediment was put in eppendorf tubes (1.5ml) and kept at -20°C until molecular examination. As well as, the slides prepared from sediment drop were stained with Giemsa for bradyzoites identification when examined under microscope at 100x **(**Hamidinej *et al*, 2015**).**

blender technique

This technique first used to concentration of the bradyzoites of Sarcocystis to detect the bradyzoites as one of traditional techniques in Iraq. A total of 50gm of tissue pieces were taken from esophagus and imported meat, thane cut for small pieces as $2cm^2$ and smallest, the pieces putted in blender with 100 ml saline for 15-20 second. The smashed materials filtered by using double layer gauze, thane centrifuged for 5 minutes at 2800 rpm at room temperature. After pouring the supernatant, 10 drops of sediment were used per samples to perpetrated 10 slides by put one drop of sediment on slid and covered by cover-slip thane examine under light microscope at 40x **(**Imre *et al* ,2019**).**

Giemsa Staining

A smear on a laboratory slide prepared from tissue sediment, air dried and fixed in

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methanol for 3 minutes, the slides were impeded in a staining jar filled with 1:10 dilution of Giemsa stain (10ml of Giemsa stain (stock) plus 90ml of buffer solution pH 7.0) for 20 minutes, stain was poured off and rinsed several times with distilled water, the slides were left to dry on the staining rack and examined at ×100 magnification with oil immersion using a light microscope **(**Waheeb,2018**).**

Serological examination

The Microplate for (ELISA) provided in this specific kit has been (pre-coated) with antibody (Ab) specific to *Sarcocystis*, make it to (solid – phase) antibody. samples are added up to the Microplate wells and then combined to the (specific antibody). And before incubated The a HRP-conjugated antibody (HRP-CON) specificfor *Sarcocystis* is added to Microplate wells to each of it, so the (Ab- Ag) Enzyme labeled (marcking) antibody complex when it formed. unbound reagent will be washed to remove any particles. after that added the TMB substrate solution to each well. Only reacted well which that contain *Sarcocystis* and (HRP- conjugated) of Sarcocystis antibody will be showed the blue color and with time turn to yellow color, the optical density after the addition to the stop solution. is measured by using spectrophotometer. at the wavelength (450 nm.) the *Sarcocystis* result well measured the qualitative determination by comparing with the (CUT OFF) value. the critical value Calculation of (CUT OFF): (Value= the average value of negative control+0.15. Negative judgment): if the (OD value < CUT OFF), the sample is Human *Sarcocystis* negative (-ve). Positive judgment (+ve): if the (OD value $\geq$ CUT OFF), the sample is Human *Sarcocystis* positive.

Serum samples preparation:

The whole blood collected and allow the and in undisturbed room temperature, the blood well be clotted which usually take (10 – 20) minutes and then centrifuged at (2000 – 3000) rpm to remove the clot for 20 minutes,if sediment appear during reservation, we should be cenrifugated again. And the test was do according to the manufacturer's instructions.

Statistical analysis

The statistical was carried by using Ch2 test to detection the significance of the Sarcocystis prevalence in cattle and human (sex, and different tissues samples. The analysis of results was made according to (SAS,2004) Version -5.

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Result

1.Total Infection rate of traditional methods:

The total infection rate showed 65 % (65\ 100) of sarcocysts isolated from esophagus of slaughtered cattle's in diyala provinces (Table1)

Table1: The total infection rate of *Sarcocysts* in slaughtered cattle

samples	No.of meat samples	No.of meat infected	%
Slaughtered cattle meat	100	65	65%

2. The total infection rate of *Sarcocystis* according to different traditional techniques, the table (2) show the peptic digestion technique were highly sensitive technique 100% according all other traditional methods. Then Trichnoscopy were 30(30%) while the squeezing less sensitive 26(26%) respectively.

Table 2: The total infection rate of *Sarcocystis* in slaughtered cattle according to different traditional techniques:

Samples	No. of meat samples examined	Total infection %	Pepsin digestion %	Squeezing %	Trichnoscopy %
Slaughtered cattle meat	100	65(65%)	65(65%)	26(26%)	30(30%)

3. Infection rate of Sarcocystosis according to the sex by traditional techniques

The female economy is linked to milk supply and reproduction, thus females who are slaughtered at old age (the end of reproduction) or if they have health issues have fertile offspring. The majority of male animals killed at abattoirs are calves. Of the 60 positive samples, 35 females were infected, meaning that the overall infection rate was 53,8% due to the high

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number of slaughtered animals, males have a higher infection incidence. of the 40 samples examined, 30 had positive cases; the overall positive case rate was 75%. (Table .3).

Table 3: Sarcocystosis infection rate according to sex:

Sex	**Total number of examinations**	**Number of infected**	**%**
Male	40	30	75%
Female	60	35	53,8%
Total	100	65	65%
X2		**2.93**	
P value		**0.087(NS)**	

NS: No significant difference at P<0.05

4. Infection rate of Sarcocystosis according to age group:

The result showed that the highest infection rate was recorded in animal of old age above 4-year-old 100% with significant differences between the age groups (Table 4).

Age /year	**Total number of examinations**	**Number of infected**	**%**
<1	26	21	80,76%
1 – 2	36	22	66,11%
2 – 3	30	16	53,33%
3 – 4	4	2	50%
> 4	4	4	100%

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Total	100	65	65%
X^2		7.42	
P value		0.115(NS)	

Table 4: Sarcocystosis infection rate according to age group of slaughtered cattle

Morphology of *Sarcocytis* Cyst

Macroscopically examination:

Macroscopically examination of slaughter cattle.The cyst of *Sarcocystis* collected were creamy white in colour, of different shapes, spindle, fusiform and globular and of different sizes varying from 2.0- 18.0 mm x 1.0-5.0 mm.

Characterization of *Sarcocystis* cyst

Microscopic examination of Sarcocystis cyst by using trichnoscopy technique, showed oval, elliptical and conical form divided into compartments were many intercostal with different measurement range (166 × 52.2) µm (40X) (Fig.3)

Figure 3: The micro cyst of *Sarcocystis* spp. in esophagus by trichnoscopy (40 X)

Morphology of bradyzoite by using peptic digestion, muscle blender and squeezing methods.

In this method the bradyzoites were seen by examining one drop of the sediment of the digested muscle fluid (Fig .2). Bradyzoites of *Sarcocystis* parasite appeared by muscle blender, pepsin digestion and the squeezing technique in slaughtered Cattle meat as banana shape with a spiky

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end of the front and rounded back end, with a little clear nucleus positioned toward the rear end.,measurements 13.2× 2.8 µm (40X).(Fig.4)

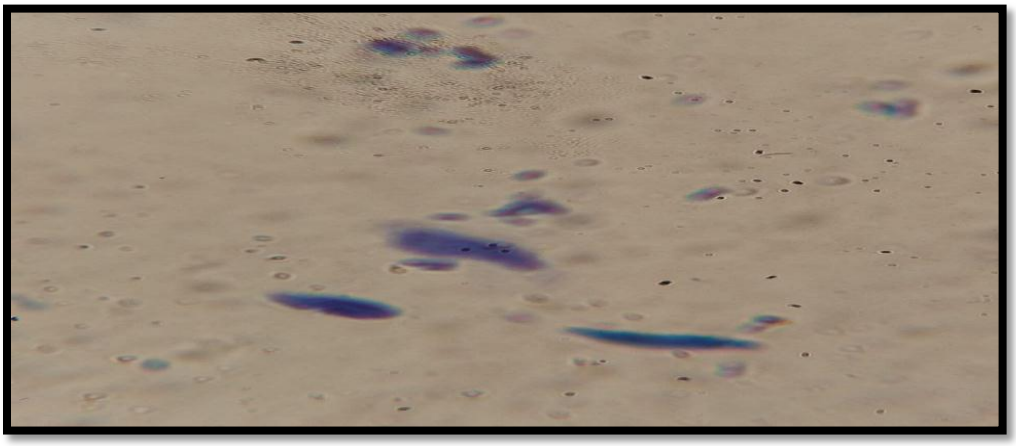

Figure 4: Bradyzoites by using peptic digestion (40 x)

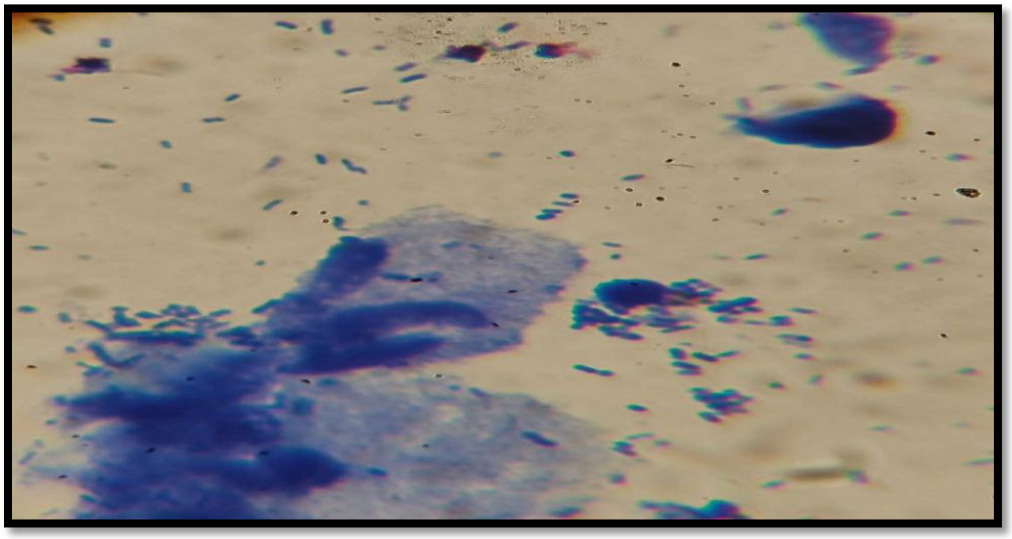

Figure 5: Bradyzoites by using blander(40X)

6.Serlogical diagnosis in human Sarcocystiosis

The total ELISA results of the blood samples collected from the human (male and female) showed infected rate (15.28%). Table (5).

Table 5: The total infection rate of human sarcocystis infection by ELIZA test

No. of sample	No. of positive	No. of negative	Total

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Samples	14	78	92
Percentage	(15.28%)	(84.78%)	100%

6.1The total infection rate of *Sarcocystis* according to different ganders:

Blood samples were taken from different genders, the results recorded highly infection rate in male 10(34.48%) while the lowest rate in female was (6.34%) with highly significant difference at P<0.01,as shown in the table(6).

Table 6: The distribution of human Sarcocystosis in different genders:

gender	**Total No.**	**Negative sample%**	**Positive sample%**
Male	29	19(65.5%)	10(34.48%)
Female	63	59(93.65%)	4(6.34%)
Total	92	78(83.13%)	14(15.2%)
X^2		12.18	
P value		<0.0001(HS)	

HS: Highly significant difference at P<0.01

6.2. The total infection rate of *Sarcocystis* according to age groups :

The infection rate according to male ages groups showed that (15-25) age highly infected (20.68%) than other as shown in the table (7).

Table.7: The infection distribution rate in different ages group of males.

Age interval	**Total No.**	**Positive sample%**	**Negative sample %**
15-25 years	18	6(20.68)	12(41.37)
25-35 years	7	4(13.79)	3(10.34)
35-45years	1	0(0)	1(3.44)

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45-55 years	3	0(0)	3(10.34)
X^2		3.70	
P value		0.295(NS)	

In females, the highest infection rate recorded in (25-35) age group than other groups (4.76) %. (Table 8)

Table 8. The infection distribution rate in different age groups of females.

Ages groups	Total No.	Positive sample%	Negative sample %
15-25	20	1(1.58)	19(30.15)
25-35	26	3(4.76)	23(36.50)
35-45	14	0(0)	14(22.22)
45-55	3	0(0)	3(4.76)
X^2		2.39	
P value		0.495(NS)	

Discussion

The disease caused by Sarcocystis is still infects man and several domesticated animals (cattle, sheep, goat, buffalo, etc.) leading to a serious economic losses in Iraq cattle herds (Swar and Shnawa,2021; Kamil and Faraj,2020; Latif *et al*,1999; Swar and Shnawa,2022) The total infection rate of cattle 65 % it is more high of sarcocysts isolated from esophagus of slaughtered cattle's in Diyala provinces due to high consumption of imported meat especially in fast food such as burgers and other types of meat food . male animals recorded high rate 75% while female cattle rate was 53,8% these results differ from many studies (Swar and Shnawa,2022; Idris *et al*,2020)) it may be due to the high number of slaughtered cattle males leading to increase the

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significant rate of infection . the highest infection rate were recorded in animal of old age above 4-year-old 100% with significant differences between the age groups, because the exposure period take long time, which increases the possibility of infection occurring in older ages compared to younger age groups (Hamidineja *et al* ,2015). macroscopically examination of slaughter cattle carcasses showed the cyst of *Sarcocystis* creamy white in color, of different shapes, spindle, fusiform and globular and of different sizes varying from 2.0- 18.0 mm x 1.0-5.0 mm. while the Microscopic examination of Sarcocystis cyst by using trichnoscopy technique , showed oval, elliptical and conical form divided into compartments were many intercostal with different measurement range (166 × 52.2) µm. Bradyzoite of *Sarcocystis* parasite appeared by muscle blender ,pepsin digestion and the squeezing technique in slaughtered Cattle meat as banana shape like with a pointed end of entrior and rounded end posteriorly , the nucleus is clear and located near the end of bradyziot ,measurements 13.2× 2.8 µm (40X) These measurement well be corresponding with (Latif ,2016; Roberts and Janovy, 2006; Savini,1994). In the human the detection of Sarcocystis by serology test (ELISA) is the first time in Iraq, the overall rate was (15.28%). A few serological studies in detection of sarcocystis in the world no previous study in Iraq about human sarcocystosis by serum antibody technique, the infection percentage rate is unique in human population in Diyala province, the results recorded highly infection rate in male 10(34.48%) while the lowest rate in female was (6.34%), it could be related to that male more consuming fast food in restaurants and other tack away shops which may be elevated the infection. The infection rate according to male ages groups showed that (15-25) age highly infected (20.68%) than other, In females, the highest infection rate was recorded in (25-35) age group than other groups (4.76) %. Young and middle-aged age groups are often more likely to fast and frozen foods, unlike older age groups who still adhere to customs and traditions and do not like these meals such as hamburgers, shawarma, sausage and other fast foods that depend on imported frozen meat that is undercooked, which increases the chances of infection with the parasite in our Iraqi society. (Swar and Shnawa,2021; Kamil and Faraj,2020; Latif *et al*,1999; Swar and Shnawa,2022).

Conclusion

Sarcocystosis is still endemic diseases in Iraq , the infection rates was elevated in the in the last ten years, especially in beef meat, which recorded a rate of 65%, and in a serological study, which don in first time in Iraq, human infection was diagnosed at a rate of 15.28% , indicating the seriousness of the spread of the disease and the necessity of conducting more studies to develop control methods to restricted the distribution of the sarcocystosis and trials to control the spreading in diyala areas.

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