Some studies on *Sarcocystis* in sheep and goats in Assiut governorate Egypt.

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Abstract

Sarcocystis **is one of the most important diseases of sheep and goats due to its zoonotic action to a human. The present study aimed to compared the convention method of diagnosis (Macroscopic examination) of *Sarcocystis* with serological test (ELISA) in sheep and goat populations in Assiut, governorate (Egypt), beside the biochemical changes, 480 sheep and 310 goats originating from three settlements in Assiut. Governorate, were examined by macroscopically and serological examination. Macroscopically examination revealed that 205,110 were positive for *Sarcocystis* in sheep and goat respectively in ratio of (42.70% and 35.48% respectively), in opposite of that serological examination revealed 207 and 108 (43.12% and 34.83%) in sheep and goat respectively. There is varity in rate of infection within the three settlements, also sex of animal play important role in rate of infection, where highest percentage was present in females than males. The biochemical studies revealed a significant increase in glucose and significant decrease in albumin level and globulins.**

Eventually, we can conclude that ELISA test have a more sensitivity and specificity technique for diagnosis of *Sarcocystis* and there is a high prevalence of *Sarcocystis* spp. infection managed sheep and goats in Assiut governorate, hence further studies must be carried out to draw a plan for good control programs to avoid human infection. ELISA assay is an efficient diagnostic test for the detection of *Sarcocystis* spp. antibody and biochemical changes are very important in draw therapy protocol.

Keywords: Sheep and goats, Serological, Biochemical studies, Assiut governorate introduction, *Sarcocystis* spp

Introduction

Sheep and goats farm is an important role in the country's economic situation, in Egypt, these animals represent about 4, 47-5, 50 million sheep and 3, 43-4, 55 million goats. Assiut city possesses the biggest population of sheep and goats in the country [1]. Parasitic infection causes serious economic losses; sarcocystosis is one of the parasitic diseases, caused by *Sarcocystis* spp. which has medical importance from its zoonotic effect, a cyst forming coccidian parasite with an obligatory two host life cycle involving carnivorous as definitive hosts and herbivorous or omnivorous as intermediate hosts. Each intermediate and definitive host may harbour more than one *Sarcocystis* spp [2]. *Sarcocystis* spp. is a cyst forming protozoan parasite with a broad host range and is distributed worldwide. The endothelium and muscles and other soft tissues are invaded by apicomplexan protozoans of the genus *Sarcocystis* and form cysts in muscles of various intermediate hosts (people, horses, cattle, sheep, goats, pigs, birds, rodents, camelids, wildlife and reptiles). The cysts vary in size from a few micrometers to centimeters, depending on the host and species. Sarcocystosis infection is commonly observed in sheep, goats and clinically infected animals may present anemia, weight loss and reduced weight gain. Neurological signs (Ataxia, hind limb paralysis and involuntary movement), when the central nervous system is affected [3]. Despite the sporadic outcome of clinical sarcocystosis in small ruminants, there is a major economic impact due to partial or complete condemnation of carcasses containing macroscopic cysts of *Sarcocystis* spp. Previous a study carried out in EL Wade Elgaded revealed that the incidence of infection with *Sarcocystis* spp. was 65.15% and 51.4% of adult sheep and goats respectively; almost of the positive animals, their carcasses were partially or completely condemned, which represent bad economic effect [4]. Sheep are infected by four *Sarcocystis* species: *Sarcocystis tenella* and *Sarcocystis arieticanis*, which have candies as definitive hosts and *Sarcocystis gigantean* (syn. *Sarcocystis ovifelis*) and *Sarcocystis* medusiformis, with cats as definitive hosts [5]. While three *Sarcocystis* species are recorded in goats: *Sarcocystis capracanis* and *Sarcocystis hircicanis*, which have canids as definitive hosts and *Sarcocystis moulei* (syn. *S. hircifelis*) that uses cats as the definitive hosts [6]. The *Sarcocystis* species that infect small ruminants in Egypt and its frequency are poorly known so several studies were conducted for study the diagnosis of *Sarcocystis* spp. by different methods (Macroscopic, microscopic examination and serodiagnostic techniques), were used for study the prevalence of the diseases, serological technique as the Indirect Fluorescent Antibody Technique (IFAT), according to Indirect Haemagglutination test (IHA) and Enzyme Linked Immune Sorbent Assay (ELISA), according to serological tests appeared to be more specific but had low sensitivity in diagnosing acute sarcocystosis [7]. Antigen for these tests is prepared by lysine

bradyzoites released from cysts and is not presently available commercially. Antigens prepared from *Sarcocystis* spp. of one host generally cross react with antibodies against homologous and heterologous *Sarcocystis* spp [8].

Our study aimed to determine the frequency of *Sarcocystosis* infection in sheep and goats, the biochemical changes associated with the infection and evaluated the accuracy of the serological technique for diagnosis.

Materials and methods

Animals

480 sheep and 310 goats of both sexes were followed during slaughter in abattoirs and/or private slaughter and a necropsy was examined for sarcocystosis by the naked eye and blood samples collected from each animal for serological and biochemical examination in Assiut governorate for the sero prevalence of antibodies to *Sarcocystis* spp. 20 positive sheep and goats (10 sheep and 10 goats respectively) for sarcocystosis by necropsy and serological examination and used as a positive control and twenty negative samples of sheep and goats for sarcocystosis by necropsy and serological examination were used as control negative animals (10 sheep and 10 goats respectively) (Table 1) [9].

Table 1. *Total number of animals carried in the present study from different localities in Assiut governorate.*

Animal	**Sheep**			**Goats**		
Location	**No of examined**	**M**	**F**	**No. of examined**	**M**	**F**
Abo-Tege city	140	70	70	82	41	41
Assiut city	200	100	100	125	62	63
El-Khanium city	140	70	70	103	52	51
Total	480	140	140	310	155	155
Note: M=Male, F=Female.						

Macroscopic examination and tissue samples collection

The carcass was examined by the naked eye (macroscopically) in abattoirs for the presence of macroscopic *Sarcocystis* cysts. Fresh tissue samples (esophagus, myocardium, tongue and diaphragm) from sheep and goats were examined by the naked eye for sarcocystosis in an abattoir [10]. At least ten transversal cuts were done using a scalpel in the tongue and heart for macroscopic cyst visualization (naked eye examination). The whole esophagus was longitudinally sectioned to expose the esophageal lumen and its internal and external walls were macroscopically examined (naked eye examination) for *Sarcocystis* spp. cyst with the help of a syringe needle (19 G) and the use of a stereomicroscope, the cysts were individually collected and transferred to the laboratory for the preparation of antigen (Figure 1) [11].

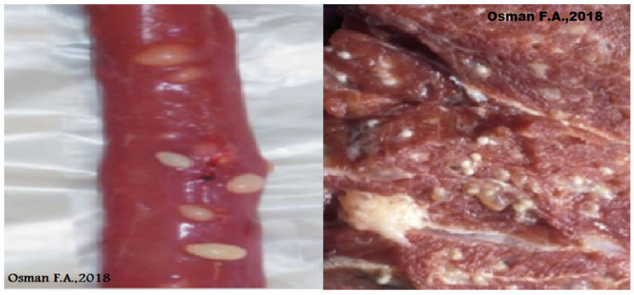

Figure 1. *Sarcocystis in the muscle of goats and esophagus of sheep.*

Microscopic examination of fresh tissue

Microscopic examination for cysts to select the positive tissue and preparation of antigen, according to tissue grinding (Impression): Approximately 50 g of each tissue was ground in a manual meat grinder and mixed with PBS (pH 7.2). After homogenization, it was filtered in gauze and centrifuged (600×g for 10 min). The supernatant was discarded and the sediment was added to 1.5 mL tubes. Three smears were made with a fine layer of the sediment and stained with conventional Hematoxylin Eosin (HE) and visualized through an optical microscope (100X magnifications) (Figure 2) [12].

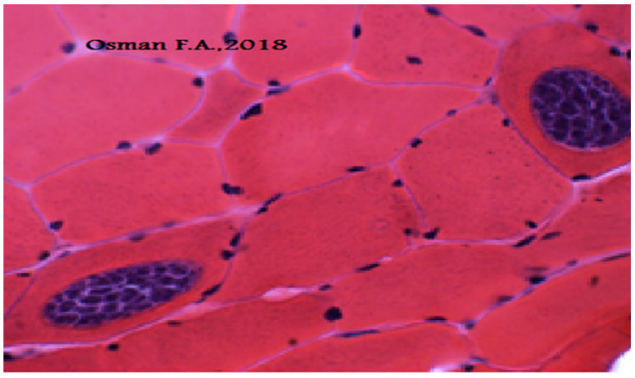

Figure 2. *Sarcocystis of Sarcocystis spp. in muscle goats' tissue, stained with hematoxylin and eosin stain x 100). Notice the bradyzoites within each Sarcocystis.*

Blood samples

Whole blood samples (without anticoagulant) for the separation of serum were collected from the jugular vein in plain tubes and centrifuged at 3,000 rpm for 20 min. The obtained serum samples were transferred to eppendorf tubes and kept at -20°C until used [13].

Antigen preparation

Antigen was prepared from cysts of *Sarcocystis* tissue (positive for macroscopic and microscopic examination) from sheep and goats according to fresh *Sarcocystis* cysts directly removed from infected tissue of slaughtered sheep or goats were digested with trypsin to release bradyzoites [14]. By twelve cycles of freezing at 22°C and thawing at 37°C, bradyzoites were left to leach out soluble material.

Serological analysis

The serological test used for the detection of antibodies to *Sarcocystis* spp. was Enzyme Linked Immune Sorbent Assay (ELISA), according to which is brief after incubation of antigen coated microplate with the tested diluted sera (1:20), *Sarcocystis* spp. specific antibodies were detected by binding the antigen antibodies complex with peroxides labelled anti-ruminant enzyme conjugate for 90 minutes [15]. Both the positive and negative controls were presented, where the Optical Density (OD) of the reaction was read on an ELIZA reader on the wavelength of 450 nm, the results were calculated according to the control serum reading *i.e.* The ratio between the ODs for the sample and the positive control corrected for the ODs of the negative control [16].

Biochemical examination

Blood serum samples were tested spectrophotometric ally for glucose level, total protein, albumin, total globulin (determined by subtracting albumin from serum total protein), creatinine, and Blood Urea Nitrogen (BUN) using available kits (Biomex, France) and following the manufacturer's instruction.

Statistical analysis

Statistical results are expressed in percentages and the incidence of *Sarcocystis* was statistically analysed by the *chi-square test* (×). Considering the variables sex and age, the difference was considered statistically significant at 0.05.

Results

Results of the study were illustrated in Table 2 and summarized as the following, where macroscopically (naked eye) examinations were 205 out of 480 and 110 out of 310 in sheep and goats (42.70%, 35.48%), respectively, while serological examination and revealed that 207 out of 480 sheep and 108 out of 310 goats were positive in percent of 43.12%, 34.83%respectively [17].

Table 2. *Sero-prevalence of Sarcocystis antibodies in the serum of examined animals.*

Animals	Sheep				Goats			
Localities	**Abo-Tege city**	**Assiut city**	**El-Khanium city**	**Total**	**Abo-Tege city**	**Assiut city**	**El-Khanium city**	**Total**
Animals examined	140	200	140	480	82	125	103	310
Naked eye	50	98	57	205	28	32	50	110
Percent%	35.71%	49%	40.71%	42.70%	34.14%	25.60%	48.54%	35.48%
ELISA	50	99	58	207	28	31	49	108
Percent %	35.71%	49.5%	41.42%	43.12%	34.14%	24.80%	47.57%	34.83%
False positive		1	1	2				
False negative						1	1	2

The sensitivity of the ELISA test is 99.04% and 98.18 % in sheep and goats respectively while the specificity of the test is 100% and 100% in sheep and goats respectively. Sensitivity and specificity are calculated as the following.

- Sensitivity=TP/(TP+FN)
- Sheep=207/(207+2) × 100=99.04%
- Goat=108/(108+2) × 100=98.18%
- Specificity=TN/(TN+FP)
- Sheep=273/(273+0) × 100=100%
- Goat=292/(292+0) × 100=100%

Concerning species of animal

The results revealed that sheep are more susceptible to *Sarcocystis* infection than goats, where 207 out of 480 sheep were infected at a rate of (43.12%), while in goats 108 out of 310 were infected with *Sarcocystis* at rate of (34.83%) [18].

Concerning sexes

As in Table 3, the results of the present study indicated that the incidence of infection in male sheep and goats was lower than the incidence of females infection, where 67 out of 240 (27.91%) and 38 out of 155 (24.51%) were positive for

sarcocytosis in male sheep and goats, respectively, while 140 out of 240 (58.33%) and 70 out of 155 (45.16%) in female sheep and goats were positive, respectively [19].

Table 3. *Sero-prevalence of Sarcocystis antibodies in the serum of examined animals in relation to sexes.*

Animals	**Sheep**			**Goats**		
Sexes of animals	**Males**	**Females**	**Total**	**Males**	**Females**	**Total**
Animals examined	240	240	480	155	155	310
Animals positive	67	140	207	38	70	108
Percent%	27.91%	58.33%	43.13%	24.51%	45.16%	34.838%

Concerning localities

As in Table 4, the results revealed that the higher incidence of infection in sheep was 49.5%, 41.42% and 35.71% in Assiut city, El-khanium city and Abo-Tege city, respectively while in goats was 47.57%, 34.146% and 28.8% in El-khanium city, Abo-Tege city and Assiut city respectively.

Table 4. *Sero-prevalence of Sarcocystis antibodies in the serum of examined animals in different localities.*

Animals	**Sheep**			**Goats**		
Localities	**Examined animals**	**Positive animals**	**Percent%**	**Examined animals**	**Positive animals**	**Percent%**
Assiut city	200	99	49.5%	125	31	28.8%
Abo-Tege city	140	50	35.71%	82	28	34.146%
EL-Khanium city	140	58	41.42%	103	49	47.57%

Biochemical analysis

Biochemical studies result illustrated in Tables 5 and 6 indicated a significant decrease in the level of glucose and albumin level in both hosts (sheep and goats). Significant increase in globulins levels, but a non-significant change in creatinine and urea nitrogen levels in both hosts (sheep and goats) was observed [20].

Table 5. *Some biochemical parameters in sheep infected with Sarcocystis and control sheep.*

Animals/parameters	**Infected sheep**	**Control sheep**
Glucose (mg/dl)	187 ± 15.4**↑	116 ± 16.6
Total protein (g/l)	63 ± 1	70 ± 6.8
Albumin (g/l)	22 ± 0.5**↓	28 ± 0.44
Globulins (g/l)	34 ± 1.3**↓	42 ± 0.8
Creatinine (mmol/l)	111 ± 2.0	114 ± 1.5
Urea nitrogen (mmol/l)	3.9 ± 0.16	4.2 ± 0.15
Note: **↓ indicated a significant decrease in the level of glucose and albumin level in both hosts. **↑ Significant increase in globulins levels.		

Table 6. *Some biochemical parameters in goats infected with Sarcocystis and control goats.*

Animals/parameters	**Infected goats**	**Control goats**
Glucose (mg/dl)	152 ± 15.4**↑	114 ± 16.6
Total protein (g/l)	58 ± 1↓	69 ± 6.8
Albumin (g/l)	21 ± 0.5**↓	29 ± 0.44
Globulins (g/l)	28 ± 1.3**↓	39 ± 0.8
Creatinine (mmol/l)	111 ± 2.0	114 ± 1.5
Urea nitrogen (mmol/l)	5.9 ± 0.16	6.2 ± 0.15
Note: **↓ indicated a significant decrease in the level of glucose and albumin level in both hosts. **↑ Significant increase in globulins levels.		

Discussion

The present study was carried out to investigate the incidence of *Sarcocystis* spp. in naturally infected sheep and goats in Assiut governorate by use of necropsy and serological examination (ELISA test). Sheep and goats antigen for the serological test was prepared from fresh large sized *Sarcocystis* cysts (CZ) of sheep and goats, where their protein content was estimated using the Enzyme Linked Immune Sorbent Assay (ELISA).

Necropsy examinations were carried out after slaughtering (In abattoirs and/or private places) (naked eye), revealed that 205 out of 480 (42.70%) and 110 out of 310 (34.83%) in sheep and goats respectively were positive for *Sarcocystis* cyst, but serological examination (ELISA test) revealed 207 out of 480 (43.12%) and 108 out of 310 (34.83%) in sheep and goat respectively were positive. This result indicated two false positive samples in sheep by ELISA test, which may be attributed to small developed cyst and/or developed antibodies before the cyst formed where the diseased sheep were in the prepatent stage, two false negative samples in goats compose a serious challenge in obtaining conclusive resulted and attributed to disappearing antibody in old and young sheep or absence of antibody in calcified cyst.

The result of the prevalence of sarcocytosis infection (42.70%) in sheep and (34.83%) in goats respectively agreement with a study carried out in new valley governorate, Egypt (65.15%) in sheep and (51.4%) in goats, in Algeria 64.4%, in Turkey 58.9%, in Iran 57.7% and 47% in Slovakia, but disagreement with the study carried in Mongolia, 96.9%, 97.0% in Iraq, 93% in Ethiopia.

Variety of the result in different localities of study, the highest percentage present in Assiut city, El-Khanium and Abo-Tege city in sheep but in goats, the highest percentage present in El-Khanium city, Abo-Tege and Assiut city respectively. The difference in different localities may be attributed to the effect of climate change on sterilization of posture, rearing and habitat management in each locality, rearing of dogs and/or cats with animals and Hygienic condemnation of dead carcasses in each area.

Concerning sex, the prevalence of *Sarcocystis* spp. in this study was higher in females than males of sheep and goats ($p<0.001$), these results agree with disagreement, who mentioned no significant difference about sex. These differences may be attributed to the frequency of pregnancy in females leading to immune compromised and hormonal changes about biochemical results. The result of biochemical studies revealed a significant increase in the level of glucose and that agreement with they mentioned that *Sarcocystis* cause degenerative hepatitis and pancreatic cell lead to increase glucose level. and a significant decrease in albumin and globulin level in both hosts (sheep and goats), that agreement with the result may attribute to degenerative and necrotic effects of muscle, while the non-significant change in creatinine and urea nitrogen levels in both hosts (sheep and goats) were observed and agreement with attributed that to decreased appetite and/or liver degeneration due to sarcocystosis.

The sensitivity was (99.04% and 98.81%) in sheep and goat respectively, while the specificity of the ELISA assay were (100% and 100%) in sheep and goat respectively, in that agreement, they found that the soluble cystozoites extract antigen had a sensitivity of 99% and specificity of 91% when tested by ELISA using naturally infected cattle. Also mentioned a sensitivity of 95% and specificity of 84% using cystozoites extract antigen, but disagreement with who indicated that ELISA had a lower sensitivity and higher specificity for purified soluble antigens extract from cyst wall, cystozoites and fluid of *Sarcocystis* fusiform cysts than crude cyst antigen. Also disagree with who mentioned low sensitivity and specificity were also estimated for the ELISA technique with bradyzoites cysts antigen. It counted to be 80.0% and 53.19% respectively.

Conclusion

Eventually the obtained results of the present study, it was clear that serological diagnosis of *Sarcocystis* spp. in sheep and goats is more sensitive and accurate for diagnostic purposes especially using ELISA which can be used as a reliable test for large numbers of samples.

Conflict of Interest

We declare that there is no potential competing interest or any conflict of interest exists.

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