

PREVALENCE OF SARCOCYSTIS SPP. IN SHEEP AND GOATS AND ITS EFFECT ON SOME BLOOD CONSTITUENTS IN SHARKIA PROVINCE

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ABSTRACT

This study was carried out on slaughtered sheep and goats in different slaughter houses in Sharkia Province to determine the prevalence of ovine and caprine Sarcocystis infection and effect on some biochemical clinical pathological parameters.

Parasitological examination of faecal samples revealed presence of eggs of internal parasites in 25(41.67%) lambs(less 9 month) {12(48%) nematodes, 7(28%) trematode } & 6(24%) mixed infection} 29 (48.33%) adult (more 2 year) (sheep {15(51.72%) nematodes,10 (34.48%) trematode & 4(13.79%) mixed infection},19 (47.50%) young goats {11(57.89%) nematodes, 5(26.32%) trematode & 3(15.79%) mixed infection}, 24 (60%) adult goats{14(58.33%) nematodes, 5(20.83%) trematode &5 (20.83 %) mixed infection}.

Serologically, antibodies against Sporocystis spp. infection were observed in sheep serum samples were 47.50% (57) & 40% (32) for goats serum samples. The infection in adult animals were {34 (56.67%) in sheep and 19 (47.50%) in goats} more than in young animals {23 (38.33%) in sheep and13 (32.50 % in goats}. Sarcocystis in sheep distributed as macrocystis (16.67% for young and 11.67% adult), microcystis ovi-cans (10% young, 5% adult) and mixed infection (macrocystis and microcystis) were (56.67% young, 38.33%

adult), meanwhile in goats *Sarcocystis macrocystis* (25% young and 15% adult), *microcystis* (*capricans*) (15% young, 12.5% adult) and mixed infection (*macrocystis* and *microcystis*) 47.50% for young, 32.50% for adult).

Sarcocystis infection in Sheep and goats induced a significant decrease in leukocytic count, monocytes, total protein, albumin, globulin, lymphocytosis, neutrophilia, non significant effect in basophils and eosinophilis beside significant rise AST, ALT & ALP.

It could be concluded that the *Sarcocystis* spp infected both sheep and goat, the infection in adult were more than in young ones. *Sarcocystis* induces changes in biochemical parameters. Therefore, it has of great importance the human to be trained not to feed their dogs and cats with uncooked meat, and abattoir remnants were burned to be effectively broken of infection cycle between the intermediate and the definitive hosts.

INTRODUCTION

Sarcocystis is one of the most prevalent parasites in livestock (**Metwally, et al 2013**). *Sarcocystis* spp. normally develops in 2-host cycles consisting of an intermediate host and the final host; each host may be infected with more than one *Sarcocystis* spp. (**Bhatia 2000**). It is one of the most prevalent protozoan parasites in striated muscles of animals as sheep and goat (**Abo Shepard 1996**). *Sarcocystis* infections of sheep and goats are common throughout the world (**Melinda et al, 2010**). *Sarcocystis* have 4 species were identified from sheep (**Britt and Baker 1990**). Of these, *S.ovicanis* (synonymous=*S.tenella*) and *S. arieticanis*, pathogenic spp form microscopic cysts and are transmitted via *Canids*,

the definitive hosts, whereas *S.ovifelis* (synonymous=*S gigantea*) and *S. medusiformis*, non-pathogenic species, form macroscopic cysts and are transmitted via felids (*Aysen et al 2007*). *Sarcocystis* considered of economic importance, because ruminants will act as an intermediate host for a wide range of species (*Dubey et al.1988*). Control of *Sarcocystis* relied on preventing contamination of pasture and water with feces of dogs, foxes and cats (*Buxton 1998*).

The main objective of the study was to address the prevalence of natural infection of *sheep* and goats in Sharkia province with *Sarcocystis* and its effect on some blood parameters.

MATERIAL AND METHODS

Animal:

A total number of 200 animals (sheep and goats of both sexes and young animals less than 9 month *and* adult animals more than 2 year), 120 sheep (60 young and 60 adult ones) and 80 goats (40 young and 40 adult ones) were surveyed for the presence of *Sarcocystis* during 2012 from different slaughtered houses in Sharkia province (Zigzag, Abu Hamad, Hehia AND durp Nigm centers).

Blood samples:

Before animal slaughtering 2 blood and 1 Faecal samples were taken from each animal, 1st blood sample was taken in tube contains EDTA for total and differential leukocytic count, 2nd sample was used for obtain a clear serum for estimation of some biochemical parameters and diagnosis of *Sarcocystis* infections by (ELISA).

Faecal samples:

Before animal slaughtering faecal sample was taken from each one for parasitological examination.

Muscle samples collection:

Post animal slaughtering, tissue samples were taken from oesophagus; heart and diaphragm muscles, preserved in ice bags and trans-ported from the slaughter house to the Laboratory for detection of *Sarcocystis* by:

1- Macroscopic examination: Fresh muscle samples were examined macroscopically for presence of macroscopic *Sarcocystis* cysts (*Huong 1999*) and,

2- Microscopic examination: For detection of microscopic *Sarcocystis* cysts:-

A) Small pieces of fresh muscle were compressed between two slides and examined microscopically (*Mowafy, 1993*).

B) Digestion method, 100 mL of digestion medium (2.5 g pepsin 700 and 10 mL HCL in 1L phosphate-buffered saline) was added to 50g (homogenized) and placed in a shaking water bath at 37°C for 30 min. The suspension was then centrifuged for 10 min at 1500 rpm and a precipitate smear was prepared, fixed with absolute methanol stained with Giemsa and examined by light microscopy at x400 and x1000 (*Dubey et al. 1989*).

Serological diagnosis of Sarcocystis (ELISA) Serum samples from all animals were subjected to ELISA for detection of antibody to Sarcocystis (*Voller, et al. 1980*) Antigen was prepared according to *Morsy, etal (1994)*

Determination of leukogram and some biochemical parameters:

Post diagnosis of Sarcocystis infection by macroscopic, microscopic and ELISA beside parasitological examinations of faecal samples, 20 blood samples (adult animals that free from internal and external parasites (10 sheep and 10 goats) were divided into 2 group, 10 animal in each (5 sheep and 5 goats) 1st group blood samples from healthy sheep and goats free from internal and external parasites and *Sarcocystis* (control), 2nd group blood samples from sheep and goats free from internal and external parasites infected with microscopic *Sarcocystis* and +ve ELISA. Total and differential leukocytic count was estimated by using the improved Neubauer chamber and Natt and Herrick,s solution as diluting fluid according to (*Jain, 1993*), serum samples were used for determination of total protein calorimetrically (*Doumas etal 1981*), albumin (*Drupt 1974*) and globulin was calculated as difference between total protein and albumin. (AST &ALT) (*Reitman and Frankel (1957)*), ALP (*John, 1982*).

Statistical analysis:

The obtained data were statistically *Petrie and Watson (1999)*.

RESULT AND DISCUSSION

In the present study, serologically *Sarcocystis* sp. prevalence by ELISA was in ovine 47.50% more than in caprine 40% (table, 2). Same prevalence were reported by **Mahran (2009)** found that the prevalence of ovine *Sarcocystis* was 41.26% and 36.92% in caprine in Red Sea Province. Higher infection rates have also been recorded in Aswan province were 88% (**Bashtar, et al.1990**). Same prevalences rates have also been recorded in other countries that have similar climatic conditions, such as ovine *Sarcocystis* spp. in Turkey were 49% (**Sevinç, 2000**), 55 % in South Australia (**Ford 1987**) 38% in goats in Libya (**Fathi and Abel Haseeb 2006**). This difference in prevalence of *Sarcocystis* infection may be due to different populations of stray dogs which play an important role in infection with *Sarcocystis*. (**Abo-Shehada, 1996**).

The prevalences of infection with *Sarcocystis* spp in young animals were 38.33% for sheep, 32.50% for goat less than adult age 56.67% for sheep and 32.50% for goat (table 2). Same observation was recorded by **Martinez, et.al. (1989)** and **Abo-Shehada (1996)** stated that prevalence of ovine sarcocystosis was higher in adult sheep than young ones. Prevalence of *Sarcocystis* infection in Czechoslovakia in young sheep were 35.67% and 61% in adult sheep **Svobodova and Nevole (1990)**, 22.6% in meat-producing animals under 6 months of age in Iraq (**Latif et al. 1999**). *Sarcocystis* spp. infection was higher in old camels (>5 year rather than young camels (<2 year) **Lotfi, et. al. (1995)**.

Gross and microscopical examination of muscle samples of 120 sheep and 80 goats slaughtered in Sharkia Province abattoir revealed that the infection rate of Sarcocystis was 47.50% in sheep distributed as (macrocytistis 16.67% for young & 11.67% adult–microcytistis (ovicans) 10% young 5% mature–mixed infection (macrocytistis and microcytistis were 56.67% for young, 38.33 % for adult one) (table 3), meanwhile in goats Sarcocystis was 40% in sheep distributed as (macro-cystis 25% for young and 15% adult – microcytistis (capricans) 15% young, 12.5% adult – mixed infection (macrocytistis and microcytistis were 47.50% for young, 32.50 %for adult one) (table, 3). Same results were recorded by *Al-Sultan, et.al. (2012)* who found microcytistis ovicans in heart muscles of slaughtered sheep in Kelantan (Malaysia). *Al-Taee, et al. (2009)* recorded microcytistis ovicans and microcytistis capricanis in heart muscles of slaughtered sheep and goats in Iraq.

The present study showed that a significant decrease in total leukocytic count and monocyte associated with significant increase in neutrophils and lymphocyte beside isignificant effect in eosinophilis and basophilis in sheep and goats infected with *Sarcocystis spp* (table 4). These changes in leukocytic count may be due to infections which stimulate migration of leukocytes from peripheral blood toward the tissue where parasites was found or to the regional lymphoid tissues, and hence total leukocyte decreased below the normal level (*Okur, et.al.1995*). Decreased monocytes may be due to monocytes that were drawn from the blood toward the infected tissue and transformed into macrophages to attack the parasites which found mainly in tissues (*Melinda et.al 2010*). Increased lymphocytes are responsible for mediating immunity against parasites (*Abdel-Azeem, et.al. 2009*).

Our study revealed a significant reduction in total protein, albumin, globulin beside significant increase in AST, ALT and ALP in sheep and goats infected with *Sarcocystis spp* (table, 5). Similar results were recorded by *Nabih and Abd El- Hamid (1984)* and *Fayer and Lunde (1987)*. This result may be due to the degenerative and necrotic changes accompanied the damage of muscles due to *Sarcocystis spp* infection and its toxins (*Keneko, 1989*). More explanations were presented by (*Gharib, 1989*) who reported that this enzyme is widely distributed allover muscle cells and tissues. Furthermore, its increase reflects an active pathological process in muscles due to *Sarcocystis spp*. *Sarcocystis spp* induce significant decrease in T protein, albumin and globulin in calves (*Dessouky, et al. (1984)*). Reduction in protein picture in animals infected with *Sarcocystis spp* may be due to decrease in gamma globulin (*Gill, et al 1988*). *Sarcocystosis* in calves induced elevation in liver enzymes (*Nevole, et al. 1986*). Elevation in liver enzymes may be due to development muscular cysts lead to degenerative changes, rupture of muscle fibers and sometimes myositis develops in which led to increase liver enzymes (*Nevole et al 1981*). *Sarcocystosis* cause degenerative hepatitis lead to increase liver enzymes (*Prasse and Fayer 1981*).

It could be concluded that the sporocystis spp in both sheep and goat, infection in adult animals were more than young one. *Sarcocystis* infections induce changes in biochemical parameters. Therefore, it has of great importance that human not feed their dogs and cats with uncooked meat, and the abattoir remnants to be burned, in order to be effectively broken of infection cycle between the intermediate and the definitive hosts.

Table (1): Seroprevalence of sarcocystis spp. infection in sheep and goats sera by ELISA

| parameters age | Mean of -ve control | Cut of value | sheep | | | goats | | |
|-------------------|---------------------------|-----------------|-----------------------|------------|-------|-----------------------|------------|-------|
| | | | T. examined sample | +ve sample | | T. examined sample | +ve sample | |
| | | | | No. | % | | No. | % |
| young | 0.146 | 0.292 | 60 | 23 | 38.33 | 40 | 13 | 32.50 |
| adult | 0.146 | 0.292 | 60 | 34 | 56.67 | 40 | 19 | 47.50 |
| total | 0.146 | 0.292 | 120 | 57 | 47.50 | 80 | 32 | 40 |

Table (2): macro and micro sarcocystis cysts spp. infection in sheep and goats

| Parameters age | | Total | examined sample | | | | | | | |
|-------------------|-------|-------|-----------------|-------|-------|-------|-------|------|-------|-------|
| | | | +ve sample | | | | | | | |
| | | | Macro | | Micro | | mixed | | Total | |
| | | | No. | % | No. | % | No. | % | No. | % |
| sheep | young | 60 | 18 | 30 | 10 | 16.67 | 6 | 10 | 34 | 56.67 |
| | adult | 60 | 13 | 21.67 | 7 | 11.67 | 3 | 5 | 23 | 38.33 |
| | Total | 120 | 31 | 25.83 | 17 | 14.17 | 9 | 7.5 | 57 | 47.50 |
| goat | young | 40 | 10 | 25 | 6 | 15 | 3 | 7.5 | 19 | 47.50 |
| | adult | 40 | 7 | 15 | 5 | 12.5 | 2 | 5 | 13 | 32.50 |
| | Total | 80 | 17 | 27.5 | 10 | 12.5 | 5 | 6.25 | 32 | 40 % |

Table (3): Leukogram of healthy and diseased sheep and goats (n=5)

| Parameters Group | | TLC x10 ³ /ul | differential leukocytic count (x10 ³ /ul) | | | | |
|---------------------|----------|-----------------------------|--|-------------|-------------|------------|------------|
| | | | Neutrophils | Lymphocytes | Eosinophils | Basophilis | Monocytes |
| sheep | control | 8.33±0.14 | 2.98±0.04 | 3.62±0.03 | 0.50±0.03 | 0.58±0.11 | 0.65±0.16 |
| | Diseased | 7.91±0.10* | 3.10±0.03* | 3.73±0.04* | 0.51±0.02 | 0.33±0.08 | 0.24±0.06* |
| goats | control | 8.19±0.11 | 3.30±0.05 | 3.58±0.03 | 0.49±0.09 | 0.24±0.08 | 0.58±0.14 |
| | Diseased | 7.67±0.18* | 3.44±0.04* | 3.36±0.08* | 0.50±0.08 | 0.17±0.04 | 0.20±0.09* |

*P < 0.05

Table (4): Serum protein picture and liver enzymes of healthy and diseased sheep and goats (n=5)

| Parameters Group | | protein picture (g/dl) | | | | liver enzymes (μ/L) | | |
|---------------------|----------|------------------------|------------|------------|-----------|---------------------|-------------|-------------|
| | | T.protein | Albumin | Globulin | A/Gratio | AST | ALT | ALP |
| sheep | control | 7.36±0.26 | 3.93±0.22 | 3.43±0.10 | 1.15±0.22 | 54.86 ±1.43 | 39.43 ±1.51 | 58.09±1.74 |
| | Diseased | 6.48±0.19* | 3.47±0.20* | 3.01±0.14* | 1.15±0.18 | 61.54±1.81* | 44.52±1.33* | 66.38±1.94* |
| goats | control | 6.93±0.41 | 3.81±0.19 | 3.12±0.17 | 1.22±0.25 | 48.84 ±1.40 | 43.17±1.42 | 49.81±1.53 |
| | Diseased | 5.61±0.40* | 3.04±0.22 | 2.57±0.16* | 1.18±0.30 | 55.07±1.63* | 48.72±1.63* | 57.17±2.32* |

*P < 0.05

**Fig. (1)****Fig. (2)** sarcocystis ovicanis

A) Macrocystis in oesophagus of goat thick type.

B) Macrocystis in oesophagus of goat thin type.

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معدل الإصابة بالساركوسيسست في الأغنام والماعز وتأثيرها على بعض مكونات الدم بمحافظه الشرقية

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طفيل الساركوسيسستس من الأوليات الطفيلية والتي تسبب خسائر اقتصادية كبيرة في الأغنام والماعز ولتشخيص العدوى بهذاء الطفيل يحتاج إلى اختبارات سيروولوجية مثل اختبار الاليزا بعد تحضير مولد ضد متخصص (Antigen) . لذلك تم فحص عدد 200 عينة سيرم منهم 120 أغنام (60 صغيرة و 60 بالغة) و 80 ماعز (40 صغيرة و 40 بالغة) وتم اخذ عينه براز من كل حيوان لفحصها للطفيليات الداخلية في أماكن مختلفة بمحافظة الشرقية.

تبين من تلك الدراسة أن نسبة الإصابة بالساركوسيسست 47.50 % (57) في الأغنام و 40 % (32) في الماعز . وكانت الإصابة في الحيوانات البالغة 23 (38.33 %) في الأغنام و 34 (56.67 %) في الماعز . والإصابة في الحيوانات الصغيرة غير البالغة كانت 13 (32.50 %) في الأغنام و 19 (47.50 %) في الماعز . وكانت الساركوسيسست موزعه كالاتى (نوع يرى بالعين المجردة في الأغنام 16.67 % الغير بالغه بينما كانت فى البالغة 11.67 % والنوع الذي يرى بالميكروسكوب (ميكروسست أوفي كانز) 10 % في الغير بالغه بينما كانت فى البالغة 56.67 % فى غير البالغه بينما كانت فى البالغة 38.33 % . وكان النوع الذي يرى بالعين المجردة فى الماعز 25 % فى الغير بالغه بينما كانت فى البالغة 15 % والنوع الذي يرى بالميكروسكوب (ميكروسست كابرى كانز) 15 % فى الغير بالغه بينما كانت فى البالغة 12.50 % وعدوى مشتركه من النوعين بنسبه 47.50 % فى غير البالغه بينما كانت فى البالغه 32.50 %

أثبتت هذه الدراسة أن الإصابة بطفيل الساركوسيسست أحدثت نقص معنوي في العدد الكلى لكرات الدم البيضاء، الخلايا الملتهمة الكبيرة، البروتين الكلى، الزلال والجلوبيولين بجانب زيادة معنوية في الخلايا الليمفاوية، والخلايا المتعادلة وزيادة معنوية في الخلايا الحامضية ونقص غير معنوي في الخلايا القاعدية. كذلك الإصابة بالساركوسيسست أدت إلى حدوث زيادة في إنزيمات الكبد (AST، ALT والفوسفاتيز القاعدى).

من تلك الدراسة نستخلص أن طفيل الساركوسيسيتس يصيب الماعز والأغنام سواء كانت تلك الحيوانات بالغه أو صغيرة ومعدل الإصابة في الحيوانات البالغة اكبر من الصغيرة وتلك الإصابة تتسبب في بعض التغيرات في الوظائف البيوكيميائية في الحيوانات.