

## Prevalence of *Sarcocystis species* infecting sheep from Egypt

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### ABSTRACT

**Abu El-Wafa, S. A.**

**Al-Araby, M. A.**

**Abbas, I. E.**

**and Elmishmishy, B.**

**M. M.**

Department of Parasitology,

Faculty of Veterinary

Medicine, Mansoura

University, Egypt.

Sheep is considered as a biological guide for the environmental pollution with different parasites because of their grazing habits and rearing patterns. Herein, we examined 540 sheep of different ages and sexes slaughtered at 3 Egyptian Provinces (Dakahlia, Damietta and Cairo), for detection of *Sarcocystis* infection. Esophageal muscle specimens were collected from slaughtered sheep. Samples were examined using both visual inspection and muscle compression technique. Fast 5 minutes 10% Geimsa solution immersion of the fresh samples was perfect for better observation of the microscopic sarcocysts. Formalin-preserved positive samples were subjected to histopathological examination. Overall, 95.37% of the examined sheep were harboring sarcocysts. Two types of sarcocysts were revealed, macroscopic (1% of older sheep) and microscopic (84.43% in smaller and 95.37% in older sheep). The infection was proportionally increased with age, while females were had a higher prevalence than males. Incidences equal to or over than 90% were reported allover the year seasons. Morphologically, macroscopic cysts were ranged from 0.8 – 1 cm in length with thin and smooth walls, while the microscopic ones were ranged from 600 – 700  $\mu$ m in length with thick cyst wall either striated or smooth. Detailed morphological features were given. This report illustrates the wide dispersal of the infective stages of *Sarcocystis species* (sporocysts) in the environment, and could be beneficial for further molecular studies.

**KEY WORDS:** *Sarcocystis*, Sheep, Prevalence, Morphology.

## INTRODUCTION

*Sarcocystis species* represent one of the most prevalent protozoal affections among domestic animals (Dubey et al., 1989). They are obligatory heteroxenous tissue cyst forming coccidial parasites (Odening, 1998). Dogs, cats and man serve as their definitive hosts, while the intermediate host role is played by many herbivores including cattle, buffalo, sheep, goat, camel, pig, rat.....etc. (Dubey et al., 1989 & Dubey, 2015). More than 200 *Sarcocystis species* were recorded (Frenkel and Smith, 2003). Both intermediate and final hosts may harbor one or more *Sarcocystis species*.

Economically, sarcocystosis has a negative impact on the animal industry, through decreasing meat quality with subsequent downgrading or even condemnation of carcasses. In

addition, the disease is incriminated in losses of several million dollars yearly due to abortion, poor growth and acute fatal illness (Dubey, 1976; Dubey et al., 1989).

Sheep are affected by 4 species of *Sarcocystis*; two of them are microscopic cyst forming (*S. tenella* and *S. areticanis*), while the others develop macroscopic cysts (*S. gigantea*, and *S. medusiformis*), Dubey, et al., 2015. In Egypt, two species were recorded, *S. gigantea* and *S. tenella* (El-Saeih, 1998; Mahran, 2009).

Variable studies were carried out in different Egyptian Provinces to estimate the prevalence of sarcoysts in sheep. High rates of infection were recorded in each of Assiut (Ali, 1985), Aswan (Elsayed, 1985), El-Minia (El-Ganiny, 1989), Qena (El-Saieh, 1998), Kalubia (Hassanien, 1992) and Suez Canal

(Basher, 2006) Provinces, while there is no reports from North Delta region.

This study was planned primarily in order to clear out the epidemiological aspects, including the prevalence and different types, of sheep sarcocystosis in Dakahlia, Dameitta and Cairo Provinces, which could offer a beneficial data for future molecular studies.

#### MATERIAL AND METHODS

A total number of 540 slaughtered sheep (*ovis aries*) at the main abattoirs in each of Dakahlia, Damietta and Cairo Provinces, were examined during the period between July 2014 and June 2015. Regular weekly visits were carried out to these abattoirs. Age of the examined animals was assessed through the visual inspection of their teeth. Two age groups were included in this study, the first group was less than 2 years old (140 animals) and the second one

was over than or equal to 2 years of age (400 animals). Both males and females were investigated. Oesophagi (n=540) of all the sheep sample were obtained. Visual inspection along with microscopic examination were used for detection of sarcocysts either macro or micro-scopic. According to **Gut (1982)**, small pieces of meat specimens were cut, compressed between two glass slides and examined under the dissecting microscope using X10 magnification. Fast 5 minutes 10% Geimsa solution immersion of the fresh samples was perfect for better observation of the microscopic sarcocysts (**Fig. 1**).The dimensions of the macro and micro-scopic sarcocysts were measured using a ruler and the ocular micrometer, respectively. Moreover formalin-buffered preserved samples were subjected to histopathological examination (**Bancroft and Stevens, 1996**).

## RESULTS &amp; DISCUSSION

**I- Prevalence of *Sarcocystis species* infection in sheep:**

Examination of 540 sheep esophagus at 3 different Egyptian Provinces revealed a high incidence of *Sarcocystis species* (95.37%), where 515 sheep were found to be infected. Contaminated food, water and pastures by disseminated *Sarcocystis species* sporocysts in the environment are the main source of infection for sheep (**Dubey et al., 1989**).

Variable reasons may be incriminated in initiating such high percent of infection like, the massive contact of dogs and cats (definitive hosts) with sheep herds, the widespread existence of *Sarcocystis species* sporocysts in the environment due to their shedding in large quantities by the predators (**Dubey et al. 2015**), in addition to the ability of sporocysts to face and survive in unsuitable

environmental conditions (**Savini et al. 2006**). Furthermore, dogs can be infected due to their easily accessing to infected sheep offals in the abattoirs surroundings.

Diversified incidences were recorded previously in Egypt, flowing from high (89%) in Suez Canal area (**Basher, 2006**) passing through 68.9% infection rate in Qena Province (**Aly, 2012**) to considerably low (41.26%) in Red Sea area (**Mahran, 2009**). This variation may be due to the difference in number of the examined sheep and dispersal of the definitive hosts.

Globally, high incidences were recorded from Czech Republic (92%), **Gut (1982)**; Turkey (100%), **Dehaghi et al. (2013)** and Iran (100%), **Arshad et al. (2007)**. Although another report (**Daryani et al. 2006**) from another Iranian Province (Arbil) stated a low rate (33.9%). This significant

difference in the infection rate may be attributed to the difference in ambient temperature and humidity within different countries (**Savini et al. 1996**)

Two types of sarcocysts (macroscopic and microscopic) were detected in the present study. A great difference was noted in the prevalence of both types of cysts. Macroscopic cysts were revealed from esophagi of 4 (1%) older sheep only. While, microscopic ones were noted in both age groups in high rates, 84.43% in smaller and 95.37% in older sheep.

It is well known that cats are the definitive hosts of macroscopic cysts, while microscopic cysts are transmitted via dogs (**Dubey et al., 1989**). Therefore, our results support the previous reports (**Basher, 2006; Beyazit et al. 2007; Titilincu et al. 2008; Mahran, 2009; Aly, 2012; Dubey et al., 2015**) which stated the great

role of dogs more than cats in transmission of sheep sarcocystosis. This is might be attributed to the close live of dogs, which kept for protection, with sheep herds (**Abbas, 2011**). Concerning the prevalence of the revealed sarcocysts in different ages, our results showed that sheep over than 2 years had higher prevalence rate (98.50%) than sheep less than 2 years(86.57). This variation could be explained by the time of exposure to the infective stages (sporocysts) that being higher in old ages than younger ones. These results are coincided with **Beyazit, et al., 2007 and Mahran (2009)**.

In addition, the effect of age is also appeared on the relation between animal sex and the infection rate. Results of the present study showed that females were more infected (99%) than males (94.55%). In Egypt, females are

slaughtered at old age due to local restrictions of the official authorities, while males are slaughtered at younger ages. These results are matched with that reported by **Basher (2006); Daryani et al. (2006)** and **Mahran (2009)**. On the other hands, **Dehaghi et al. (2013)** recorded that there was no important difference between males and females.

Approaching the seasonal dynamics of sheep sarcocystosis in the present study, incidences equal to or over than 90% were reported in all seasons of the year. The is observed to increase during Spring (97.71%) to reach its maximum during Summer (98.63%), while being decreased in Autumn (94.74) reaching its lowest rate during Winter (90%). Different results were recorded by **Mahran (2009)** and **Aly (2012)** whom stated that sarcocysts infection in sheep

reaches its peak during Winter and Summer, respectively. A hypothesis might explain the variation in the results of the previously mentioned studies is that, the cysts represent the chronic, longest and persistent stage of *Sarcocystis species* life cycle (**Beyer and Radchenk, 2001**). Therefore, once the cyst is formed, it persists in the tissues for long period and could be noticed all over the year.

## **II- Morphological description of the revealed sarcocysts:**

Concerning the morphological features of the revealed sarcocysts, Macroscopic cysts were ranged in size from 0.8 – 1 cm in length (mean 0.9 cm, n = 7). They were dull white, oval shaped resembling rice grain, (**Fig. 2**). The cyst wall was thin and smooth ranged from 1.6 – 1.9  $\mu\text{m}$  (mean 1.8  $\mu\text{m}$ ) and often surrounded by connective tissue as a secondary wall in

agreement with **Odening, 1998**. Bradyzoites were located peripherally in the cyst while the center was empty, (**Fig. 3**) and were crescent in shape measuring 11 – 14  $\mu\text{m}$  (mean 12.5  $\mu\text{m}$ , n = 15) in length and 4 – 6  $\mu\text{m}$  (mean 4.5  $\mu\text{m}$ ) width. Nucleus was situated near the blunt end of the body. Obtained morphological parameters of the microscopic cysts were similar to that reported by **Dubey et al. (1989)**; **Odening (1998)** and **Mahran (2009)**.

Microscopic cysts were ranged from 600 – 700  $\mu\text{m}$  (mean 630  $\mu\text{m}$ , n = 12) in length. They were oval or pear shaped and the cyst wall was thick, measured 1 – 3  $\mu\text{m}$  (mean 2.7  $\mu\text{m}$ , n = 12). Their walls appeared either smooth or striated, (**Fig. 4**). Bradyzoites were located peripherally in the cyst while the center was nearly empty and were crescent in shape measuring 6.7 – 7.7  $\mu\text{m}$  (mean 7  $\mu\text{m}$ , n = 15) in

length and 1 – 2.1  $\mu\text{m}$  (mean 1.5  $\mu\text{m}$  n = 15) in width. Nucleus was situated near the blunt end of the body. Obtained morphological parameters of the microscopic cysts were similar to that reported by **Mahran, 2009**.

#### CONCLUSION AND RECOMENDITION.

- High incidences of sarcocysts were reported from sheep which indicates a high environmental pollution by *Sarcocystis species* sporocysts.
- Two types of sarcocysts (macro and micro-scopic) were noted.
- Further molecular studies are needed to characterize each type.

Table (1) Prevalence of macroscopic and microscopic cysts raveled from esophagus according to age.

Age	No. of examined sheep	Macroscopic cyst		Microscopic cyst	
		No. of infected	%	No. of infected	%
<2y	140	0	0	121	86.57
≥2y	400	4	1	394	98.5
Total	540	4	0.74	515	95.37

Table (2) Seasonal prevalence of microscopic cysts in different esophagus samples according to age.

Season	<2y			≥2y			Total		
	No. of examined	No. of infected	%	No. of examined	No. of infected	%	No. of examined	No. of infected	%
Winter	35	24	68.57	95	93	97.89	130	117	90
Spring	34	32	94.12	97	96	98.97	131	128	97.71
Summer	37	36	97.3	109	108	99.08	146	144	98.63
Autumn	34	29	85.29	99	97	97.98	133	126	94.74
Total	140	121	86.43	400	394	98.5	540	515	95.37

Table (3) Prevalence of infection in relation to sex of slaughtered sheep.

Males			Females		
Total No. of males examined	No. of infected males	% of infected males	Total No. of females examined	No. of infected females	% of infected females
440	416	94.55	100	99	99



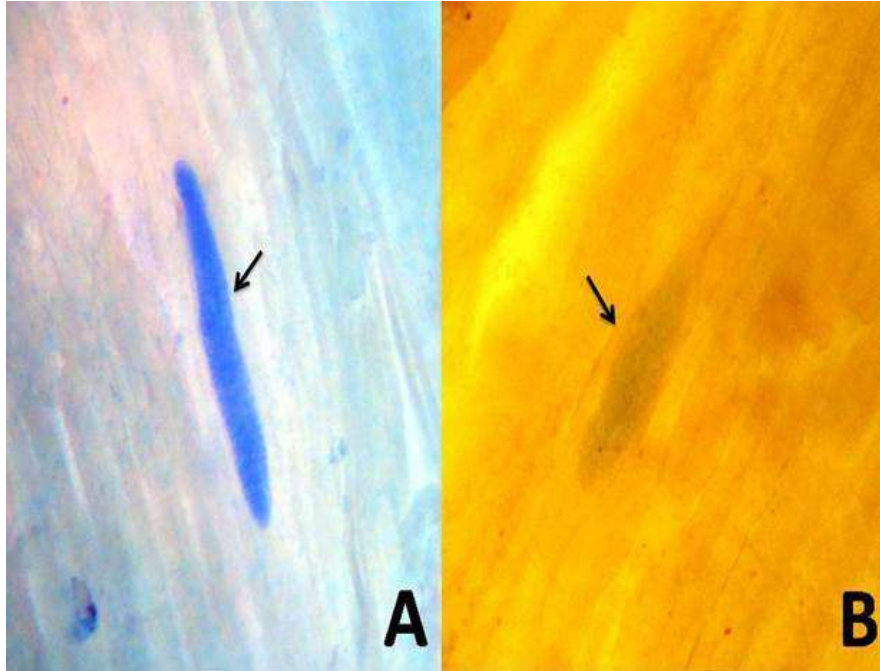


Fig.(1): showing Giemsa stained (A) and non-stained sarcocystis (arrow) in compressed sections of sheep esophagus (X10).



Fig. (2): Showing macroscopic cysts measured with a ruler.

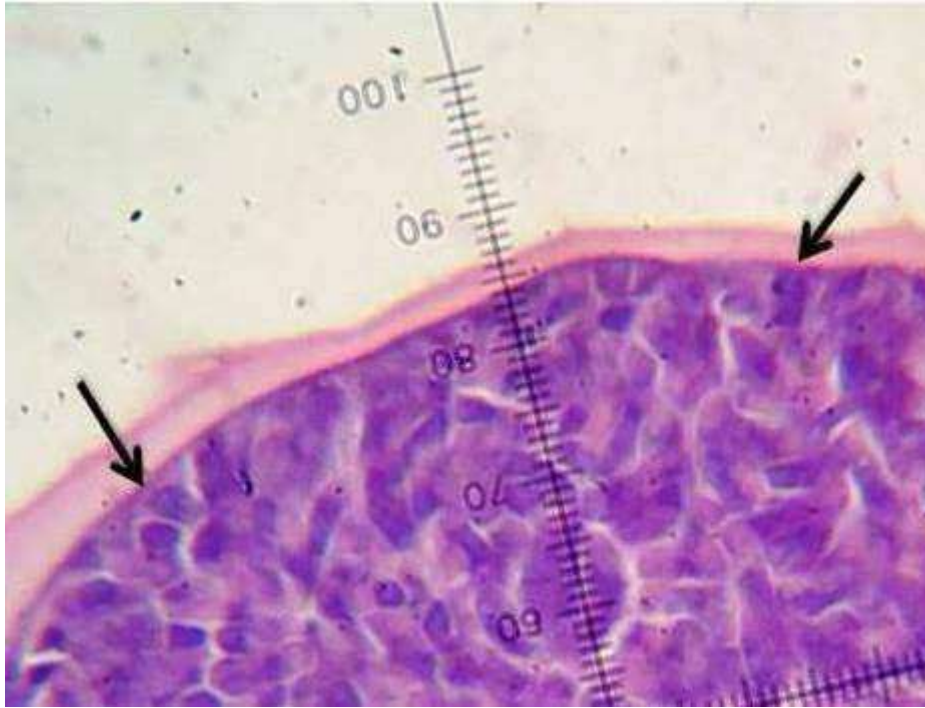


Fig. (3): Showing thin wall of macroscopic cysts (arrow) stained with H & E (X100).

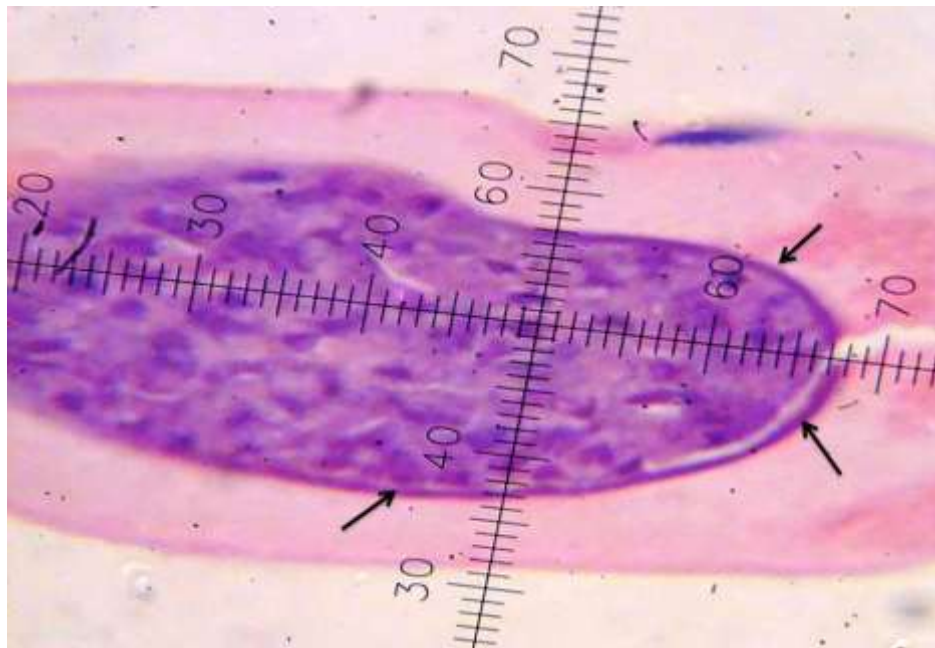


Fig. (4): Showing thick wall of microscopic cysts (arrow) stained with H & E (X100).

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## الملخص العربي

### مدى انتشار المتكيسات العضلية في الاغنام بمصر

صلاح احمد ابو الوفا مصطفى عبد السلام العربي ابراهيم السيد عباس باسم محمد محمد المشمشي

قسم الطفيليات - كلية الطب البيطري- جامعة المنصورة - مصر

أجريت هذه الدراسة على المتكيسات العضلية التي تصيب الاغنام في مصر ، لتحديد مدى انتشار الأنواع المختلفة من هذه الطفيليات في الاغنام المذبوحة بمصر. وكذلك إجراء دراسات مورفولوجية باستخدام الميكروسكوب الضوئي. وقد تم فحص عدد 540 مرئ من ذبائح الاغنام التي ذبحت بمجزر المنصورة بمحافظة الدقهلية، مجزر دمياط بمحافظة دمياط ومجزر البساتين بمحافظة القاهرة في الفترة ما بين يوليو 2014 ويونيو 2015، حيث قسمت الحيوانات المذبوحة إلى مجموعتين عمريتين، الأولى من اقل من سنتان وكان عددها 140 حيوان والثانية أكثر من سنتان وتتكون من 400 حيوان. وقد تم فحص أنسجة المرئ للاغنام، وكان تحديد المتكيسات العضلية الظاهرة للعين يعتمد على فحص الأنسجة بالعين المجردة، بينما تم استخدام طريقة ضغط العضلات وصبغها بصبغة الجسم لتحديد المتكيسات العضلية المجهرية. ووجد أن إصابة الاغنام محل الدراسة بالمتكيسات العضلية الظاهرة في انسجة المرئ للمجموعة العمرية الاكثر من عامين فقط وكانت النسبة (1%)، بينما المتكيسات العضلية المجهرية كانت بنسبة كبيرة (98.5%) ، اما بالنسبة لتباين الاصابة الموسمية فقد وجد ان نسبة الاصابة في فصل الصيف كانت الاكثر علي الاطلاق في كل الانسجة وكل الاعمار موضع الدراسة بينما فصل الشتاء كان الاقل علي الاطلاق في كل الانسجة وكل الاعمار محل الدراسة ويأتي فيما بينهما فصلي الربيع و الخريف بالتتابع. وثبت أن الاغنام المذبوحة موضع الدراسة مصابة بنوعان من أوليات المتكيسات العضلية ، احدهم يرى بالعين المجردة بينما الاخر مجهري وقد تم الوصف الظاهري لهما تحت الميكروسكوب الضوئي فوجد الحويصلات الكبيرة 0.9 سم في الطول بيضاء اللون بيضاوية وتتميز بجدار رفيع سمكة 1.8 ميكروميتر ويحاط بنسيج ضام كجدار ثاني بينما المجهرية 630 ميكرون في الطول بيضاوية الشكل وتتميز بجدار سميك سمكة 2.7 ميكروميتر هذا الجدار محرز في بعض الحويصلات وناعم في البعض الاخر. وقد خلصت الدراسة الي ضرورة عمل دراسة جزيئية متقدمة لتصنيف الانواع التي تم عزلها.