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## Prevalence of *Sarcocystis Fusiformis* and Hydatid Cyst Among Different Ruminants at Menofia Governorate, Egypt

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#### **ABSTRACT**

Sarcocystis fusiformis and Hydatid cysts are considered to be serious problems for both public health and the livestock economy. The current study aimed to determine their prevalence in slaughtered animals at Menofia governorate. Physical examination of 1916 slaughtered animals at different abattoirs of Menofia governorate was carried out from September 2016 to August 2017 to determine the prevalence of recovered parasites. Histopathological examination was performed on the infected tissues. The results reported that 20.5% of the examined animals were infected with *S. fusiformis*. The infection rate of *S. fusiformis was* 85.96% and 0.1% in buffaloes and cattle respectively without any infections recorded in camels, sheep and goats. Sarcocystis infections increased with increasing the age of animals. The highest infection was recorded in winter and spring at 33.3% and 27.13% respectively. The total infection rate of Hydatid cyst was 3.6% with (29.18% in camels and 1.75% in buffaloes). In conclusion *S. fusiformis* and Hydatid cysts prevailed at Menofia governorate with considerable percentages that require more molecular studies to determine their genes and putting strategies to control stray dogs and cats near the abattoirs and efficient condemnation of affected organs.

Key words: Sarcocystis fusiformis, Hydatid cyst, Ruminants and Egypt

#### **INTRODUCTION:**

The most important parasites infesting ruminants and have public health importance are as; Sarcocystis species protozoa which transmitted to human by ingestion of raw or undercooked meat which harboring the infective stage of the parasite. Sarcocystis belongs to the kingdom: protozoa, phylum: Apicomplixa and family Sarcocystidae. They complete their life cycle in two obligate hosts; herbivorous intermediate host and carnivorous definitive host which harbor the sexual stages of the parasite. (Fukuyo *et al.*, 2002)

The parasitic cestode Echinococcus granulosus is a parasite of public health importance and has economic importance, it has also an obligate two-host life cycle including an intermediate host which is mostly ruminant either buffaloes, cattle, camel, sheep or goat. (Altintas *et al.*, 2003 and Kebede *et al.*, 2009).

The intermediate host mostly doesn't show characteristic clinical symptoms so, those

parasitic cysts detected only during PM inspection causing economic losses due to condemnations of affected organs and muscles and have a public health hazard due to its zoonosis (Opara 2006 and Regassa et al., 2009). Because red meat constitutes the main course for daily meals and in Egypt, ruminants are considered the main source for it. Red meat is an important source for zoonotic parasitic diseases due to the role that ruminants play in the life cycle of these parasites so this study aimed to estimate the prevalence of those parasites at different abattoirs at Menofia governorate among different ruminants (cattle, buffaloes, camels, sheep and goat) of different ages and in different seasons and to study the morphological features and Histopathological effects on animal tissues.

#### **MATERIALS AND METHODS**

#### Animals and study period:

A total number of 1916 slaughtered animals (993 cattle, 456 buffalo, 209 camel, 258 sheep and goat) were examined. Samples were collected from different abattoirs at Menofia governorate twice a week from September 2016 to August 2017. The examined animals were divided into three age groups (> 5 years old, between 3 to 5 years and < 3 years).

## Recovery and examination of Sarcocystis fusiformis and Hydatid cyst:

Parasitological examination was conducted during routine carcass examination in different abattoirs through visual inspection of a whole carcass including their organs and muscles for detection of any parasites. Each sample was collected in a separate glass container that labeled with all data (date of collection, site of collection, age of animal, species of animal and sex of animal). The recovered cysts were washed with saline several times and preserved in 70% ethanol for further examinations (Khulbe *et al.*, 1989).

## Identification of recovered Sarcocystis fusiformis and Hydatid cyst:

Morphological description and measurements of the collected cysts were done. These cysts were identified according (Soulsby 1986 and Urquhart *et al.*, 1988).

#### **Histopathological examination:**

Samples were collected from (tongue and esophagus for Sarcocystis fusiformis) and (lung for Hydatid cyst) in 10% neutral buffered formalin and submitted to a pathology laboratory for histopathological examination. Formalin fixed samples were sectioned at 5 mm thickness and stained with Hematoxylin and Eosin (Bancroft 2008).

#### **RESULTS**

#### **Prevalence:**

Samples were collected during the period from September 2016 to August 2017; and (20.5%) of a total number of 1916 visually inspected carcasses were infested with Sarcocystis fusiformis cysts and (3.6%) were infected with Hydatid cysts. The infection rate of Sarcocystis cysts was (0.1%) in cattle and (85.96%) in buffalo carcasses, while not recorded in camels, sheep and goat. Hydatid cyst was recorded in (29.18%) of camels, and in (1.75%) of buffaloes, while not detected in cattle or in sheep and goat Table (1).

Sarcocystis fusiformis was found to infest 6 of 1109 inspected carcasses (0.54%) that were less than 3 years old, 134 of 457 of inspected carcasses (29.32%) of 3:5 years old, and 323 of 350 (92.28%) of inspected carcasses older than 5 years Table (2).

The highest incidence of Sarcocystis infection was during winter (33.3%) among all ruminants followed by spring (27.13%), autumn (13.37%) and summer (8.19%) Table (3).

Sarcocystis fusiformis was detected in highest incidence in the area of Shebin El-Kom, then Tala and El-Shohadaa with percentages 37.5%, 30.77%, and 18.67% in sequence Table (4).

Hydatid cyst found with an overall incidence of infection was estimated to be 3.6%. The highest incidence of Hydatid cysts was found in animals older than 5 years old (11.43%), then animals from 3:5 years (6.35%), while not detected in animals less than 3 years old (table 2).

Hydatid cyst was detected with the highest incidence in winter and spring then summer and

autumn with percentages 5.56%, 5.4%, 2.16% and 1.23% respectively (table 3). Those animals harbored Hydatid cyst during the hot season were 38 out of 980 examined animals representing about (3.8%), while in cold season they were 31 out of 936 examined animals (3.3%).

Table (1) Infection rate related to animal species.

Animal species	No. examined	+ve	Parasitic cyst found		
			Sarcocyst	Hydatid cyst	
Cattle	993	1	1 (.1%)	0	
Buffalo	456	401	392 (85.96%)	8 (1.75%)	
Camel	209	61	0	61 (29.18%)	
Sheep, Goat	258	0	0	0	
Total	1916	463	393 (20.5%)	69 (3.6%)	

Table (2): Infection rate according to age.

	NO.			Result of examined	
Age	Examine d	NO. +ve	%	Sarcocyst	Hydatid cyst
<3years	1109	6	0.54	6 (.54%)	-
3:5years	456	134	29.32	105 (23.03%)	29 (6.35%)
>5years	350	323	92.28	282 (80.57%)	40 (11.43%)
Total	1916	463	24.17	393 (20.5%)	69 (3.6%)

Table (3): Infection rate according to Season.

Season	NO. Examined	NO. +ve	%	Result of examined	
				Sarcocyst	Hydatid cyst
Summer	464	48	10.34	38 (8.19%)	10 (2.16%)
Autumn	486	72	14.8	65 (13.37%)	6 (1.23%)
Winter	450	175	38.89	150 (33.3%)	25 (5.56%)
Spring	516	168	32.56	140 (27.13%)	28 (5.4%)
Total	1916	463	24.17	393 (20.5%)	69 (3.6%)

**Table (4): Infection rate according to locality.** 

Area	NO.	NO.	%	Result of examined		
	Examined	+ve		Sarcocyst	Hydatid cyst	
Tala	104	33	31.73	32 (30.77%)	1 (0.96%)	
El- Shohadaa	1692	375	22.16	316 (18.68%)	59 (3.48%)	
ShebinEl- Kom	120	55	45.83	45 (37.5%)	9 (7.5%)	
Total	1916	463	24.17	393 (20.5%)	69 (3.6%)	

#### Morphological examination of cysts:

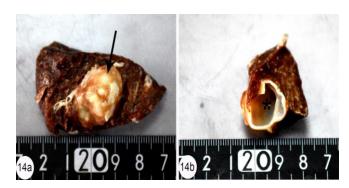
# 1. Morphological characters of Sarcocystis cyst:

Macroscopic and microscopic cysts were recorded and found seated under the serosal membrane of the muscle and embedded between the muscle bundles with the direction of the muscle fibers with different depths. They found to be easily detached from the muscle and removed intact, the cysts appeared spindle-shaped with creamy white color. Macroscopic cysts found in different sizes and measured from 0.1-2.3 cm length and 0.1-0.5 cm width. (fig.1)

# Fig. 1a. Gross photographs. Sarcocysts in buffalo esophageal muscles. Arrow indicating the largest one. Fig. 1b. Left, sarcocyst in buffalo esophageal muscles (arrowhead); Right, sarcocyst in buffalo tongue muscles

## 2. Morphological characters of Hydatid cyst:

The Hydatid cysts found were spherical, found in various sizes from 1cm in diameter to 6 cm and with various densities in organs of different animals from only one cyst to the number of 8 cysts in the organ. The Hydatid cyst found was composed of thick external fibrous capsule attached well to the surrounding tissues and not easily removed and external germinal layer and internal germinal layer. Cyst fluid, containing protoscolices present as floating sand in the fluid. One calcified Hydatid cyst found in heart, it was small sized cyst embedded in the heart tissue and not easily removed. (fig.2)



**Fig. 2.** Gross photographs. Hydatid cysts in camel lung tissues. **Fig.8.14a**, showing intact hydatid cyst (arrow). **Fig.8.14b**, showing opened hydatid cyst (asterisk).

## Histopathological examination of *Sarcocystis* cysts:

Sarcocystis appear in cut section to have a thick cyst wall, the cyst is divided into chambers filled with bradyzoites. (fig.3).

(arrow).

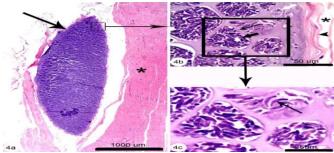
The cyst is surrounded with parasitophrous vacuole by high magnification and the surrounding muscle tissue is healthy with no tissue reaction and no inflammatory cells. (fig.3). Higher magnification power of cut section showing mother cells lining the wall from inside and when mature resides to the center forming chambers filled of bradyzoites. (fig.3b).

#### Histopathological characters of Hydatid cyst:

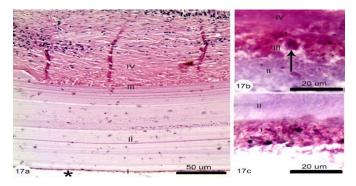
Hydatid cyst is composed of fibrous cyst wall. (fig.4). it's composed of several layers: Inner surface of the wall which have the Hydatid sand. The cyst capsule of the parasite; Fibrous connective tissue layer tightly attached to the surrounding healthy organ tissue. (fig.4.a, b & c).

The tissue reaction against the parasitic capsule appears to be formed of mononuclear cell aggregates, giant cells, fibrocytes, and connective tissue bundles.

Higher magnification with bar 100um showing congestion of interalveolar venules, and aggregation of macrophages in the interalveolar tissues.



**Fig. 3a.** Panoramic view of sarcocyst in buffalo esophageal muscles (arrow) with no inflammatory reaction around (asterisk). Bar 1000 um. **Fig. 3b**. X40 from fig. 4a, showing a parasitophorous vacuole (asterisk), cyst wall (arrowhead), mother cells of cyst chambers (white arrow), bradyzoite in cyst chamber (black-thick arrow), and trabeculae separating cyst chambers (black-thin arrow). Bar 50 um. **Fig. 3c.**X100 from fig. 4a, showing bradyzoite in cyst chamber (arrow). Bar 25 um.



**Fig. 4 a, b and c.** Higher magnification of the hydatid cyst wall in fig. 4. **I**, the inner surface of cyst wall which having the sand (asterisk); **II**, the whitish cyst capsule; **III**, layer of foreign body giant cells (arrows); **IV**, fibrous connective tissue layer. Bars, 23a 100 um; 23b & 23c 20 um.

#### **DISSCUSSION:**

In the recent study, it was recorded that positive cases for meat parasites infestation were 463 out of 1916 carcasses representing about 24.16%. That is a considerable percentage concerning economy and public health. The most parasites found were *Sarcocystis fusiformis*, during days of samples collection with a total percentage of 20.5% and Hydatid cyst was recorded with percentage of 3.6%.

Sarcocystis fusiformis prevailed mostly in buffaloes with percentage of 85.96% and in cattle, only 0.1% affected, those results agreed with researches made in Egypt by; El- Dakhly et al., (2011), Mousa et al., (2016), Meshhal (2017). Also, studies made by Jyothisree et al., (2017), More` et al., (2011), Ahmad et al., (2010), Obijiaku (2013), Gjerde (2016), Chiesa et al., (2013). But this didn't agree with the study by Abd El-Hafeez etal., (2015) that he recorded that goats were mostly infected with percentage of 92% then cattle 80% and pigs 68%.

This study also stated that esophagus was the most affected organ with Sarcocystis species (98.98%) then tongue, masseter muscle, diaphragm and skeletal muscle with percentages of 1.78%, 1.02%, 0.76%, and 0.5%, respectively. That agrees with Abd El-Hafeez *et al.*, (2015), Meshhal (2017) and Jyothisree *et al.*, (2017) for macroscopic *Sarcocystis*. But didn't agree with Fukuyo *et al.*, (2002) where the heart was the most organ affected in cattle then tongue

and diaphragm 100%, 90%, and 61.1%, respectively and in sheep, tongue was the mostly affected organ with percentage of 100%. Also, didn't agree with Ahmad *et al.*, (2010) that he stated that masseter muscle was mostly infected with microscopic cysts with percentage 57.1% and then tongue, diaphragm, esophagus, heart, and thigh muscles.

Seasonal infestation in this study revealed that infection rate was higher during the seasons of winter and spring 33.3% and 27.13%, while lower infection rates were recorded during summer and autumn 8.19% and 13.37%.while the studies made by Abd El-Hafeez *et al.*, (2015), Meshhal (2017) and Jyothisree et al, (2017) found no great variation between seasons in infection rate.

The present study showed that the prevalence of Hydatid cyst at Menofia governorate at the area of study was about 11.43% in animals > 5 years and about 6.35% in animals 3-5 years old. Hydatid cyst was recorded in the highest prevalence in camels about 29.19% while in buffaloes were about 1.75% and those results agreed with the studies made in Egypt by (El-Hagin, 2010; Abdel Aaty et al., 2012; Youssef et al., 2013 and Khalifa et al., 2014) except that El-Hagin, 2010 who recorded cysts in both sheep and cattle and Youssef et al., 2013 also recorded cysts in 0.49% of cattle examined while no cysts were recorded in the present study in both animal species and this may be due to the geographical distribution and the contaminated pastures with feline and canine feces shedding the oocysts. However, Abdel Aaty et al., 2012 recorded Hydatid cysts in pigs in about 11.3%, we didn't target pigs in this study.

This study also agreed with studies made in Ethiopia by (Debela *et al.*, 2015) where they recorded Hydatidosis in 23% of examined camels with highest incidence in older females of poor conditions. And studies made in Iran by (Mirzaei *et al.*, 2016) where they found Hydatidosis in camels with percentage 14.64%. But did not agree with those studies also made in Ethiopia by (Berhe, 2009; Kebede, 2009; Amuamuta et a.l, 2012) where they recorded Hydatidosis in cattle with considerable

percentages 32.11%, 16%, 35.7% consequently, and studies in Iran by (Islam *et al.*, 2014; Shahbazi *et al.*, 2016). Studies made in Sudan by (Omer *et al.*, 2010) revealed that camels were the most infested animal with 59.9% then sheep, cattle, and goat with about 11.3%, 6.1%, and 1.9% in sequence. The present study did not agree also with the study made in Iraq by (Kadir and Rasheed 2008) where they found cattle of the highest prevalence of infection then buffalo with percentages 4.38% and 2%.

All recorded cysts in the present study were isolated from lungs, all the previous studies made in Egypt by (El-Hagin, 2010; Abdel Aaty et al., 2012; Youssef et al., 2013 and Khalifa et al., 2014) and studies made in Iran by (Islam et al., 2014, Mirzaei et al., 2016 and Shahbazi et al., 2016) confirmed that lung hydatidosis is the most prevalent in all cases but they also recorded liver hydatidosis in lowest incidence and also spleen hydatidosis was recorded in single case by (Youssef et al., 2013) with percentage 0.94%. However, in Ethiopia lung hydatidosis was of highest prevalence like that in the present study but also liver hydatidosis was of great incidence as that reported in the studies made by (Kebede 2009; Amuamuta et al., 2012 and Debela et al., 2015). Considering that both studies made by (Kebede 2009 and Debela et al., 2015) recorded also that spleen and kidney hydatidosis was of lowest prevalence. But the present study didn't agree completely with the study made in Egypt by (Omar et al., 2013) that they found that liver hydatidosis was higher than lung hydatidosis with percentages 39.3% and 32.5% and found mixed infection 25.8%.

Hydatid cysts were found in higher prevalence during winter and spring then summer and autumn with percentages 5.56%, 5.4%, 2.16%, and 1.23% consequently. However there was no significant difference between hot and cold season as it was 3.8% in hot months and 3.31% in cold months. But this study didn't agree with the studies made in Egypt by (El-Hagin 2010) that he stated that autumn was of highest prevalence for camel hydatidosis 60% while spring was the highest season for cattle and buffalo hydatidosis and in Iran by (Shahbazi *et* 

al., 2016) or in Iraq by (Kadir & Rasheed 2008) and these variations may be due to the change in the source of animals and the area of breeding and the difference in management and handling of the infested organs and the awareness of the farmers.

In this study Sarcocystis were spindle-shape with creamy white color, they were recorded in different sizes 0.1-2.3 cm long and 0.1-0.5 cm wide. Histological examination revealed that the cyst had a thick wall and divided into chambers filled with bradyzoites. Sarcocystis had no tissue reaction or inflammatory cells were detected. This study agreed with the results recorded by Hornok et al., (2015) Gjerede et al., (2016), Mousa et al., (2016), Meshhal (2017); While slight tissue reaction and cells were found in some studies without inflammatory reaction as in the studies done by; Fatma et al., (2008), Valinezhad et al., (2008) that detected slight inflammatory reaction only in heart muscles, and Jyothisree et al., (2017) found that only heavy infestation with microscopic Sarcocystis elicit tissue reaction represented as muscular degenerations, focal leukocytic infiltrations mainly eosinophils, macrophages lymphocytes. But the recent study didn't agree with the study made by Oryan et al., (1996); that they recorded that tissues give reaction with microscopic Sarcocystis represented hemorrhagic foci and leukocytic infiltrations but no reaction with macroscopic Sarcocystis. The study didn't agree either with the studies made by Valinezhad et al., (2008) who recorded inflammatory reactions only in cardiac muscles as hyperemia, polymorph nuclear cells and hemorrhages, nor with Jyothisree et al., (2017) that he recorded sever blood vessels congestion and degenerative changes including; cloudy swelling. hyaline tissue formation infiltration of eosinophils.

Hydatid cysts found were spherical, found in various sizes from 1-6cm in diameter and with various densities in organs of camel and buffaloes from only one cyst to the number of 8 cysts in the same organ. Hydatid cyst is composed of fibrous cyst wall, that's composed of several layers: Inner surface of the wall which

have the Hydatid sand and the cyst capsule of the parasite. Fibrous connective tissue layer tightly attached to the surrounding healthy organ tissue. In the present study, the tissue surrounding the cysts found to elicit a tissue reaction represented as mononuclear cells aggregates, giant cells, fibrocytes, and connective tissue bundles. The present study agreed with the studies made by (Verma and Swamy 2009, Youssef *et al.*, 2013, Al-shabbani 2014, Namjoo and Arzanipour 2014, Abd El-Aziz *et al.*, 2016, Singh *et al.*, 2017, Beigh, *et al.*, 2017, Kumari *et al.*, 2017, Beigh 2018, and Hamrat and Yahia 2018).

#### **CONCLUSION:**

S. fusiformis and Hydatid cysts prevailed at Menofia governorate with considerable percentages that require more molecular studies to determine their genes and putting strategies to control stray dogs and cats near the abattoirs and efficient condemnation of affected organs.

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