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PREVALENCE OF *SARCOCYSTIS FUSIFORM* IN SLAUGHTERED BUFFALOES IN ASSIUT ABATTOIR, AND STUDY THE EFFECT OF CHILLING ON THEIR VIABILITY AND INFECTIVITY

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ABSTRACT

The high objectives to this study were to determine and evaluate incidence of *Sarcocystis fusiform* infection in slaughtered buffaloes in Assiut abattoir, and additionally study the effect of chilling on their viability and infectivity. Tissue specimens were collected from three hundred and fifty-six buffaloes (337 male buffaloes and 19 female buffaloes) slaughtered at Assiut abattoir during the period from 2/2022 to 1/2023. Samples including esophagus, tongue, and masseter muscles were examined macroscopically and histopathologically. The total prevalence of macroscopic *Sarcocystis* (*S. fusiform*) in examined buffaloes was 8.43%, the infection rate in males (Less than 2 years) and in females buffaloes (4 to 7 years old) were (8.3%) and (10.53%), respectively. The predilection seats of *S. fusiform* in examined buffaloes were; esophagus (90%), followed by tongue (23.3%), throat muscles (20%) and the lowest one skeletal muscle (total body) (3.3%). Concerning seasonal variation the high infection rate was happened and detected in spring (13.9%) while the lowest infection was happened in the autumn season (5.4%). The effect of chilling on the viability of *S. fusiform* cysts was investigated using a vital stain (0.4% trypan blue). Heavily infected muscles were chilled at 4°C for 24, 48 and 72h and was carried out on cats. The reduction rate of *S. fusiformis* bradyzoites after chilling was (0%), (20%) and (54.6%), respectively. However, the parasite lost its infectivity after chilling for three days, where heavily infected muscles cooling at 4°C were rendered non-infective to cats. **Conclusion:** The present study concluded that *S. fusiformis* infection in buffaloes constitutes one of the main causes of economic losses in Assiut slaughterhouses. The incidence of *S. fusiform* was higher in aged female buffaloes and esophagus was the main predilection seat. Additionally, our results showed that cooling heavily infected muscles with *S. fusiformis* at 4 °C leads to a significant reduction that touched as a fact in parasite viability and trypan blue stain effectively measured the viability of *Sarcocystis.* Also, the infectivity of the parasite to cats was lost after chilling for three days**.**

Keywords: Buffaloes, *Sarcocystis fusiformis*, Viability, Trypan blue, Infectivity.

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INTRODUCTION:

In Egypt, buffalos' meat is a major meat source of meat in the market where males only can be slaughtered, but in females there is a strict law prevent it from slaughtering until its end it is production cycle. The prevalence of *Sarcocystis* spp. in buffalo meat of both sexes in Egypt is less examined and investigated (Gerab *et al.,* 2022).

Sarcocystis is an intracellular protozoan parasite belongs to the phylum Apicomplexa and family Sarcocystidae. It is cyst-forming intracellular coccidian parasites with obligate two hosts. *Sarcocystis* needs two obligatory hosts during its life cycle, including a carnivorous as a definitive host and an omnivorous or herbivorous as an intermediate host, so *Sarcocystis* live as intermediate host in as buffaloes. And. It carries parasite as macroscopic or microscopic *Sarcocystis* in their striated muscles (Dubey and Lindsay, 2006). intermediate hosts for *S. fusiformis* are buffalos with felids as the final host (Hilali *et al.,* 2011).

The pathogenic species affect buffalos may lead to severe, fatal disease leading to abortion, reduced milk yield, neurologic signs, and loss of weight. The infection with macroscopic *Sarcocystis* cysts renders the meat unmarketable and leads. Death is a direct harm effect of the parasite infection, particularly in heavy infections animals or untreated weak animals. Also, direct economic losses during inspection at slaughtering abattoir due to carcasses condemnation (e.g., generalized sarcocystosis). Tissue parasites has their zoonotic importance and affect both animal health and / Or production (Abu-Elwafa and Al-Araby, 2008).

Sarcocystosis is one of the zoonotic parasitic scattered all over the world, it is caused by *Sarcocystis* Spp. which are apicomplexan parasites needing to complete its life cycle on a final host and intermediate host which occurs in it , The asexual life cycle with cysts origin called *Sarcocystis ,* while inside the definitive host the sexual life cycle occurred in carnivores and humans are the final host, they can get infection by digestion of raw –non heat treatment- meat containing active *Sarcocystis*, while sporulated oocysts or sporocysts in contaminated water and food cause infection in intermediate hosts by ingestion (Dubey, 2015).

Evaluation to the inactivation techniques of the parasite viability is very important. For example, for assessing and evaluating parasite viability by feeding cats with active cysts and monitoring the presence of oocysts or sporocysts in their faeces, Recently, vital stains are used as a good alternative method for assessing and judgment on parasitic viability (Honda *et al.,* 2018).

To inactivate this parasite Freezing, Heating, marination, and irradiation were all utilized and each had effect, according to the studies (Valizadeh, 2021). But, there were scarce information and little studies about the utilizing chilling to inactivate this parasite (Ref. _)

Therefore, the objectives of this study were to investigate and evaluate the prevalence of macroscopic *Sarcocysts* (*S. fusiformis*) in slaughtered buffaloes at Assiut abattoir. Additionally, determine the efficacy of chilling on the viability and infectivity of *S. fusiformis*.

MATERIALS AND METHODS:

1. Collection of samples:

A total of 356 buffaloes including 337 males (less than 2 years) and 19 females (4 to 7 years old)] were slaughtered at Assiut abattoir during the period from 2/2022 to 1/2023 and examined by routine post-mortem inspection for the prevalence of macroscopic *Sarcocystis* by the naked eye (Valinezhad, *et al.,* 2008).

2. Macroscopic identification:

These specimens were examined carefully by naked eyes for white rice grain-like *Sarcocystis* macrocysts. The revealed cysts were dissected out of the tissue and transported in an Icebox to the laboratory for further examinations and species identification according to Huong (1999). The obtained macroscopic sarcocysts, were measured and crushed between two slides for description of bradyzoites. Slides were fixed with methanol, stained with Giemsa stain and examined with a light microscope (Hamidinejat *et al.,* 2010).

Microscopic examination of muscle tissues:

3. Histopathology:

Tissue specimens from the esophagus infected with macroscopic cysts were fixed in 10% neutral-buffered formalin for 3 days. Then it was processed for paraffin embedding and sectioned into 5-7 µm thick sections. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically (Bancroft and Stevens, 1996).

4. Determination of *Sarcocystis* viability and infectivity during cold storage (chilling):

To evaluate the effects of cold storage on the viability of cysts in meat; the infected meat was cut into same-sized blocks (50 g, 5 × 4 × 3 cm), blocks were kept at 4 °C for 1, 2 and 3 days and then cysts were collected from tissues and bioassay in cats.

A. Purification of bradyzoite:

A pepsin digestion procedure was applied for the purification of bradyzoite according to Bayarri *et al.* (2010). Briefly, 10 macroscopic cysts were crushed with 20 mL of NaCl (0.85%) with a mortar. 20 mL of pepsin solution (pH = 1.1–1.2) was added, and the resulting mixture was incubated at 37 °C for 30 min with agitation. After digestion, the content was filtered and centrifuged for 10 min at 1500 g to separate bradyzoites. Then, 25 mL of $NaHCO_3$ solution (1.2%, pH = 8.3) was added to neutralize the pepsin. Finally, after two PBS washes, the content was reconstituted in 2 mL of PBS. After obtaining isolated bradyzoites through pepsin digestion, a vital stain 0.4 % Trypan blue was performed on each sample to assess their viability after chilling.

B. Trypan blue staining:

By using 0.4 % Trypan blue staining technique applied according to Strober (2015). Briefly, 150 µL of a digestion resulting in 5 × 10^3 bradyzoites / mL and 150 µL of 0.4 % trypan blue were mixed in an Eppendorf tube and incubated for 3 min at room temperature. By using Hemocytometer live and dead bradyzoites were counted under an optical microscope at 400 × magnification. Stained (non-viable) and non-stained (viable) bradyzoites were counted during the next 3–5 min. The reduction rate of bradyzoites was calculated as follows:

$$\text{Reduction rate (\%)} = \frac{\text{Number of dead bradyzoite}}{\text{Number of live bradyzoite+ number of dead bradyzoite}} \times 100$$

5. Evaluate the effect of chilling on the infectivity of *S. fusiform:*

A- Bioassay.

Briefly, ten 10-week-old domestic cats (*Felis catus*) were used, and sarcocysts/muscle tissue was collected from naturally infected adult water buffaloes at Assiut slaughterhouse. Cats were divided into three groups (2 in each). The first one (G1) was fed 15 macroscopic *sarcocysts* of *S. fusiformis* dissected from the esophagus of infected buffaloes after chilling at 4 °C for one day (24 h). The second group (G2) was fed about similar number of macroscopic *sarcocysts* after chilling for 3 days (72 h) from water buffaloes. The last one (G3) served as an un-inoculated control group. Feces from the cats were collected daily during the experimental period (from 2 weeks before infection until 30 days after inoculation) and examined microscopically for the presence *of Sarcocystis* oocysts or sporocysts.

6. Statistical analysis:

The gained data were subjected to analysis of variance (ANOVA). Duncan's multiple range test was used to determine differences among means and the difference was considered 95% significant at (P value ≤ 0.05) (Fleiss 1981).

7. Ethical approval

This study has been approved by the Animal Rights and the Ethical Research Committee of the Faculty of Veterinary Medicine, Assiut University, and all procedures complied with the Egyptian animal welfare regulations. As mentioned by Gjerde *et al.* (2015)

RESULTS

The results of the present study revealed that the total prevalence of *S. fusiformis* in slaughtered buffaloes was 8.43 %, female buffaloes were more susceptible to infection 10.53% than males 8.3% (Table 1).

Table 1: Incidence of *S. fusiformis* in examined buffaloes.

Buffaloes	Examined	Infected	Percentage
Male(1 to 2 years old)	**337**	**28**	**8.3%**
Female (4 to 7 years old)	**19**	**2**	**10.53%**
total	**356**	**30**	**8.3%**

The results in Table 2 demonstrated that the most affected organs with microscopic *Sarcocystis* were the oesophagus (90%) followed by the tongue (23.3%), larynx muscle (20%), and finally skeletal muscles (Total body) (3.3%).

Table 2: Organ distribution of *S. fusiformis* infected buffaloes.

Different tissue parts	*Cysts of S. fusiformis*	
	No. of infected meat	%
Throat muscles	**6**	**20**
Esophagus	**27**	**90**
Tongue	**7**	**23.3**
Skeletal muscle (Total body)1		**3.3**

The results in Table 3 demonstrated the effect of seasonal variation on the infection rate of *S. fusiformis*; the highest infection rate was detected in spring 13.9% followed by summer 9.6 %, winter 7.1 % and the lowest one was in the autumn season 5.4%.

Table 3: Seasonal variation of *S. fusiformis* of examined buffaloes.

seasons	Examined animals	Macroscopic Sarcocystis	
		No. of infected meat	%
Summer	73	7	9.6
Autumn	112	6	5.4
Winter	99	7	7.1
Spring	72	10	13.9
total	356	30	8.43

Concerning age variation; aged buffaloes (4 to 7 years old) were more susceptible to infection than young ones (1 to 2 years old), the infection rate was 10.53% and 7.87%**,** respectively (Table 4).

Table 4: Prevalence of *S. fusiformis* in slaughtered buffaloes according to age

Age	Males (♂)		Females (♀)		Total	
	slaughtered (%)	Inf. (%)	slaughtered (%)	Inf. (%)	slaughtered	Inf. (%)
1 to 2 years old	337 (100%)	28 (8.3%)	0 (0%)	0	337	28 (8.3%)
4 to 7 years old	0 (0%)	0 (0%)	19 (100%)	2 (10.53%)	19	2 (10.53%)
Total	337 (94.66%)	28 (8.3%)	19 (5.34%)	2 (10.53%)	356	30 (8.43%)

Fig. (1): Heavy macro sarcocysts of *S. fusiform* on the oesophageal muscle

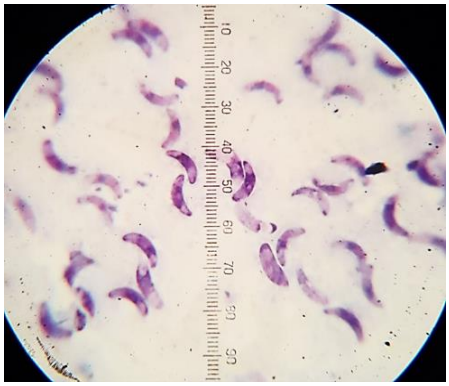

Fig. (2): Banana shaped bradyzoites of *S. fusiform* stained with Gimsa stain x1000. bradyzoites 1000× magnification.

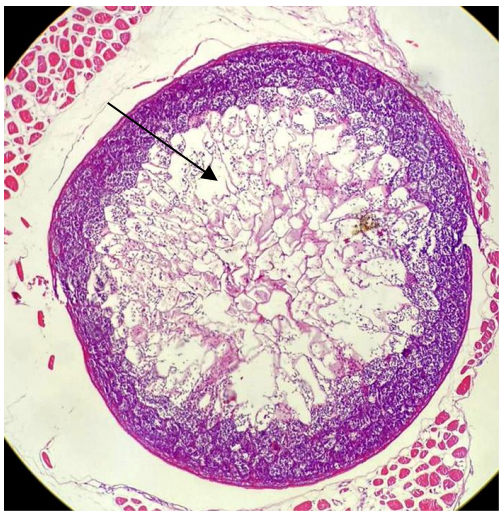

Fig. (3): Cross section of macro sarcocyst of *S. fusiformis* showing internal compartment filled with bradyzoites (arrow) H & E x40.

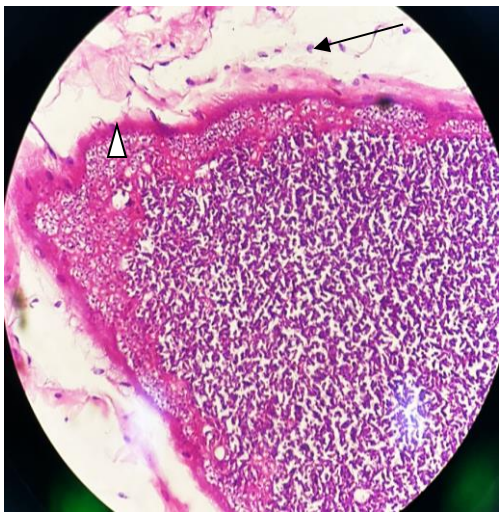

Fig. (4): Longitudinal section of a *S. fusiformis* cyst showing thick cyst wall (head arrow), slight edema and few inflammatory cells infiltration (arrow) H & E x40.

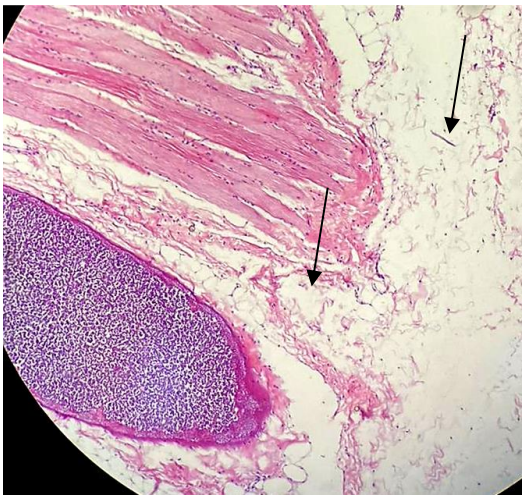

Fig. (5): Longitudinal section of a *S. fusiformis* cyst along the longitudinal axis of the muscular layer which surrounded with slight edema and degeneration (arrows) H & E x40

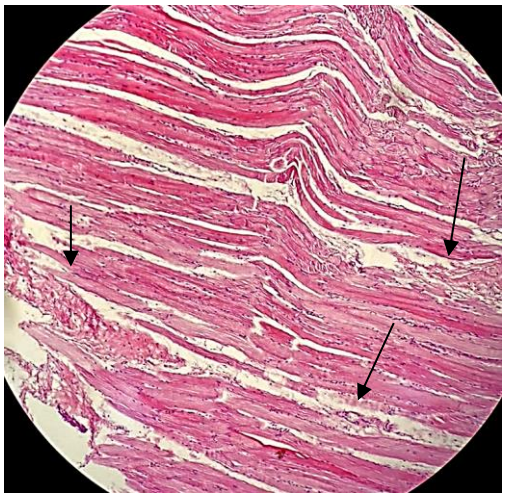

Fig. (6): histopathological section of esophageal skeletal muscle showing degeneration of muscle fibers, associated with edema (arrows) H & Ex40

Morophometric characters of *S. fusiformis:*

Macroscopic cysts of *S. fusiformis* were spindle or fusiform in shape, milky white or creamy in color, with variable sizes (5–15 × 3–7 mm). Stained smears of *S. fusiformis* contents revealed banana-shaped bradyzoites (9- 14 um ×4.5-5.5 um) (Figs. 1, 2).

Histopathology:

Histological examination showed that the thick cyst wall and the interconnecting meshwork divide the cyst into compartments, containing numerous crowded merozoites (Fig. 3 &4).

Degenerative changes of myocytes and hyalinization of muscle fibers with few inflammatory cellular infiltration cells were observed. Atrophy of few myocytes, edema and focal areas of necrosis surrounding the cysts were also seen in examined samples (Figs. 5 & 6).

The effect of chilling on the viability of *S. fusiformis* bradyzoites:

Table 5 shows the effect of chilling on the viability of *S. fusiformis* bradyzoites in blocks of meat kept at 4 °C. There was a significant reduction in the viability of *S. fusiformis* bradyzoites in chilled muscles. The reduction rate in the viability of *S. fusiformis* bradyzoites in chilled infected muscles at 4 °C for 24, 48 and 72 h was 0 %, 20 % and 54.6 %, respectively (figs. 7 & 8)

Table 5: Effect of chilling on the viability and infectivity of *S. fusiformis*.

Time of cold storage (4 °C)	Viability			P-value	Bioassay (infectivity)
	Post-test results	Reduction rate			
		%	Mean±SD		
24h	+ve	0 0 0	0%		+ve
48h	+ve	17.6 15.8 27.6	20.33±6.35 %	0.05	NA
72h	+ ve	49.4 53.7 60.8	54.6±5.76%		-ve

+ve: Live bradyzoites were detected from the sample by microscopic assay, Friedman test was used, Statistically significant difference, P≤0.05, NA: Not analyzed.

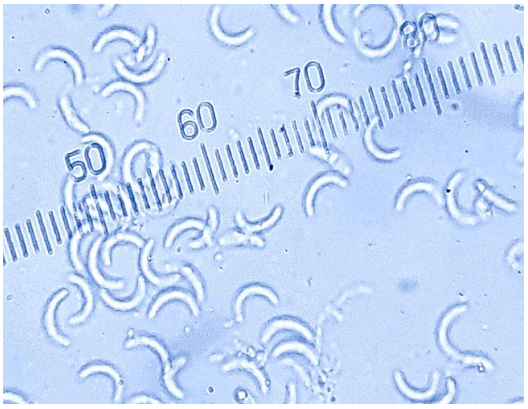

Fig. (7): Trypan blue stain of *S. fusiformis* bradyzoites showing viable bradyzoites (unstained) X400.

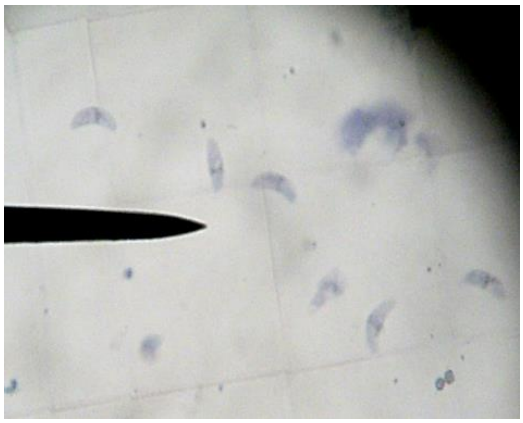

Fig. (8): Trypan blue stain of *S. fusiformis* bradyzoites showing non-viable bradyzoites (blue color) X400.

Feeding cats with chilled *S. fusiformis* cysts in the present work revealed that only cats fed chilled *S. fusiformis* cyst for 24 h (G1) were infected. A parasitological examination of cat's stools (G1) revealed excretion of *Sarcocystis* sporocysts after 3 weeks post-infection. The sporocysts were ellipsoidal measuring 10.5–11.5 × 7 µm, containing four sporozoites and a granular residium (Fig. 9). Cats of (G2) & (G3) were negative for *S. fusiformis* infection during the experiment (30 days).

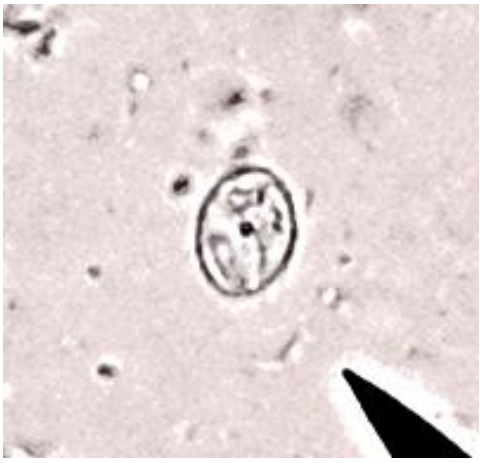

Fig. (9): Sporocyst of *S. fusiformis* X 400

DISCUSSION

According to previous studies, the elimination of *Sarcocystis*-infected corpses costs the meat industry millions of dollars each year. Some *Sarcocystis* species cause digestive difficulties in humans, including nausea, diarrhea, and vomiting (Tenter, 1995).

In this study, the occurrence of *S. fusiformis* detected in the examined total buffaloes was 8.43%. The prevalence of infection in male and female buffaloes was 8.3 % and 10.53 % respectively (table 1).

This result nearly with Dyab *et al.* (2019) who found that the prevalence of *Sarcocyst* in buffaloes was (12 %). This result was lower than that obtained by Abu-Elwafa and AI-Araby (2008) who recorded that the prevalence of *Sarcocystis spp.* cysts was (63.7 %) in examined buffalos and Gerab *et al.* (2022) found that the prevalence of macroscopic sarcocysts was (26.5 %) in slaughtered buffaloes at Tanta abattoir, Egypt. On the other hand, it was higher than that obtained by Felefel *et al.* (2023) who found that sarcocystosis infection was noticed in (3.57 %) of slaughtered large ruminant animals in Alexandria Governorate. The variable degree of infection rate in different localities may attributed to the degree of contamination of the environment with sporocysts, the abundance of definitive host, and the resistance of sporocyst to harsh environmental conditions.

Regarding the organ distribution of *S. fusiformis* in different organs of slaughtered buffaloes; the results revealed that the highest detection rate of *Sarcocystis* lesions was found in the esophagus (90 %) followed by tongue (23.3 %), throat muscles (20 %) and the lowest in skeletal muscles (total body) (3.3 %) as shown in (table 2). This result agreed with Houng (1999) who reported that the most common site for Sarcocystis location was esophagus, followed by the cervical muscle, tongue and heart. Also, El-Dakhly *et al.* (2011), Atif (2012) and Dyab *et al.* (2019) reported that the esophagus was found to be the common site of infestation of *S. fusiformis*.

Our results revealed that the infection rate of *Sarcocystis* macrocysts increased with age. These results are similar to the previous reports in Egypt by El–Bahy *et al.* (2019), Gouda *et al.* (2021) and Menshawy *et al.* (2023). This finding may be due to longer exposure periods of aged animals to the sporocysts infection. The frequent contact between ruminant animals and final hosts allows the spreading of infection. Repeat exposure of aged animals to infestations, leads to gradually accumulating cysts in muscle so the infection is easy to diagnose Moreover, the lower number of the examined

aged female bovine carcasses matched with younger steer could affect the infection rate.

Variation of *Sarcocystis fusiformis* associated with season in table 3 shows that the highest infection rate was detected in spring 13.9 % and the lowest one was in the autumn season 5.4 %. While Dong *et al.* (2018) showed that the risk of *Sarcocystis* infection in the autumn was higher than that in other seasons.

Certain inflammatory responses in the tissue around the cysts were detected in the present work in infected samples. This agreed with Jyothi Sree *et al.* (2017) observed degenerative changes such as cloudy swelling of myocytes, and hyalinization of muscle fibers along with infiltration of mononuclear cells, predominantly eosinophils, in all the positive samples. On the contrary, no inflammatory responses in the tissue around the cysts were recorded by Italiano *et al.* (2014) and Gareh *et al.* (2020). Absence of inflammatory reaction may be owing to the cysts' placement within muscle fibers, which are protected by a membrane from host immunity Hidron et al (2010).

Effect of chilling on the viability and infectivity of bradyzoites of *S. fusiformis* (experimental part):

It is critical to implement preventative and control measures such as inactivating or destroying the bradyzoites in contaminated meat, which is one of the phases of infection and the parasite's life cycle (Valizadeh, 2021).

The assessment of parasite viability is necessary to demonstrate the success of inactivation techniques. Vital stains can be a good alternative for assessing parasite viability. Trypan blue staining has been used to evaluate the viability of parasites, including *Toxoplasma gondii* (Shojaee *et al.,* 2019). In the present work, the viability of *Sarcocystis* bradyzoites was evaluated by digestion method using vital stain. Data in table 5, showed a significant reduction in of viability of *S. fusiformis* with chilling at (4 °C), where the reduction rate of *S. fusiformis* bradyzoites for 24, 48 and 72 hours was 0%, 20% and 54.6% respectively. Regarding the vital stain (trypan blue) applied in our study; we concur with Strober (2015) who emphasized that trypan blue staining is a straightforward, rapid, and cost-effective method for counting bradyzoites using optical microscopy.

Animals become naturally infected with *Sarcocystis spp.* by either oral ingestion of sporocysts or transplant-placental transmission in the uterus (Dubey *et al.,* 1989). Concerning the effect of chilling on the infectivity of *S. fusiformis* cysts the present work, found that cats fed on those chilled at 4 °C for 24 h excreted viable sporocysts of *Sarcocystis* after 21 days post-infection. The parasite lost its infectivity after chilling at 4 °C for 72 h, whereas cooling of heavily infected muscles at 4 °C for 72 h rendered non-infective to cats. The results achieved in the current study differ from those obtained by Honda *et al.* (2018) and Valizadeh (2022) who reported that consuming tainted meat that had been held at 4 °C for six or seven days did not affect the infectivity of *Sarcocystis* bradyzoites and can induce disease. Also, El-Kelesh *et al.* (2011) found that cats fed on cysts kept at -2 °C for 1 day shed sporocysts of *Sarcocystis*. These discrepancies may arise from the cysts' varying resistance to chilling of different species of *Sarcocystis* as well as from methodological differences. Or those authors assessed infectivity by feeding cats with cysts and monitoring the presence of oocysts or sporocysts in their faeces only moreover, they did not evaluate viability.

CONCLUSION

This study informed that Sarcocystosis constitutes one of the major causes of economic losses among slaughtered animals at Assiut abattoir as a result of organs and/or total carcasses condemnation. Esophagus recorded as the main predilection seat of *S. fusiformis* in buffaloes. Also, the findings of this study cleared a significant reduction in the viability of *Sarcocystis* bradyzoites at 4 ºC

for three days and cannot induce disease in cats experimentally.

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مدى انتشار ساركوسيست فيوزيفورميس في الجاموس المذبوح بمجزر أسيوط ودراسة تأثير التبريد على حيويتها وقابليتها للعدوى

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الهدف من هذه الدراسة هو تحديد مدى الإصابة بالساركوسيست فيوزيفورميس في الجاموس المذبوح في مجزر أسيوط، بالإضافة إلى دراسة تأثير التبريد على حيويته والقدرة على العدوى. تم فحص ثلاثمائة وستة وخمسين رأس من الجاموس (٣٣٧ ذكراً و١٩ أنثى) والتى ذبحت بمجزر أسيوط خلال الفترة من ٢٠٢٢/٢ إلى ٢٠٢٣/١. بلغ إجمالي معدل (الإصابة بالساركوست العياني (فيوزيفورمس) في الجاموس الذي تم فحصه ٨,٤٣٪ ، حيث كان معدل الإصابة في الذكور (أقل من سنة إلى سنتين) وإناث الجاموس (من ٤ إلى ٧ سنوات) ٨,٣٪ و١٠,٥٣% على التوالي. كان توزيع الساركوسيستس في الأعضاء المختلفة للجاموس المصاب هى: في المريء٢٧ (٩٠%)، يليه اللسان٧ (٢٣,٣%)، وعضلات الحلق ٦ (٢٠%)، وأقلها العضلات الهيكلية (عضلات الجسم) ١ (٣,٣%). وفيما يتعلق بالتباين الموسمي فقد سجلت أعلى نسبة إصابة في فصل الربيع ١٣,٩% وأقلها في فصل الخريف ٥,٤%.

تم دراسة تأثير التبريد على حيوية الحويصلات المغزلية وقدرتها على العدوى بعد تبريد العضلات المصابة عند درجة حرارة ٤ درجات مئوية لفترات مختلفة (٢٤ و٤٨ و٧٢ ساعة) حيث تم اختبارها بيولوجيًا بعدوى القطط. تم تحديد حيوية الحويصلات المغزلية عن طريق الفحص المجهري للبراديزويت بعد الصباغة باستخدام صبغة حيوية (التريبان الأزرق ٠,٤٪). وأظهرت النتائج أن انخفاض معنوى فى حيوية الطفيل عند التبريد عند ٤ درجات مئوية لأكثر من ثلاثة أيام (٧٢ ساعة). كانت نسبة موت البراديزويتات من Sarcocystis fusiformis بعد تبريدها عند درجة حرارة (٤ درجات مئوية) لمدة ٢٤، ٤٨ و ٧٢ ساعة هى ٠%، ٢٠% و ٥٤,٥ % على التوالي. كما أن الطفيل فقد قدرته على العدوى فى القطط بعد التبريد لمدة ثلاثة أيام، في حين نجحت العدوى التجريبية فى القطط بالحويصلات التى تم تبريدها عند درجة حرارة ٤ درجات مئوية لمدة ٢٤ساعة فقط.

وخلصت الدراسة إلى أن الإصابة بالساركوسيستس في الجاموس تشكل أحد الأسباب الرئيسية للخسائر الاقتصادية في مجزر أسيوط. وكانت نسبة الإصابة بالساركوسيستس أعلى في إناث الجاموس المسنة. كما أظهرت الدراسة أن تبريد العضلات المصابة باكياس الساركوسيستس عند درجة حرارة ٤ درجات مئوية يؤدي إلى انخفاض معنوى فى حيوية الطفيل كما انه يفقد القدرة على عدوى القطط بعد التبريد لمدة ثلاثة ايام.