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Study the effect of some processing methods on the viability of *Sarcocystis* cysts by using vital staining techniques

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ABSTRACT

Sarcocystis spp. is one of the most prevalent and pervasive livestock protozoan parasites. Different processing and storage techniques were assessed in this study as control measures for sarcocystosis. In terms of vital stains two techniques trypan blue staining and Acridine orange staining were applied in this study. The overall *Sarcocystis* infection rate among the examined 80 buffaloes in the Assiut Governorate was 70%. Both macroscopic sarcocysts (3.75%) and microscopic sarcocysts (67.5%) differed significantly. The esophagus was the only affected organ exhibiting macroscopic sarcocysts. Besides, microscopic cysts were most prevalent in the esophagus (45%), skeletal muscles (42%) then followed by the diaphragm (36%), heart (33%), masseter muscle (27%) and tongue (25%) without statistical influence. The viability of *Sarcocystis* cysts after 24 hrs. of chilling at 4°C was unaffected, but the survival rate dropped to 48.5% after 48 hrs. of storage with a very high significant difference. Storing esophageal tissue at -18°C for 48 hrs. completely ruined the viability of *Sarcocystis* cysts, whereas at 24 hrs., survival rate was 40.4%, exhibiting a highly significant difference. Microwave treatments employed for (30 sec, 1, 2 and 3 minutes) resulted in 100% mortality of the bradyzoites however for 15 sec. the survival rate was 74.4%, exhibiting a highly significant difference. Treatment I (2% NaCl, 2% pot. Lactate) and Treatment II (2% NaCl, 3% pot. Lactate) showed 40.7% and 21.7% survival rate for *Sarcocystis* cysts, respectively. In conclusion, freezing at -18°C for 48 hours as well as microwave heating for at least 1 minute, are the most effective ways for completely inactivating *Sarcocystis* in buffaloes. While chilling and a mixture of 2% NaCl and 3% pot. Lactate reduces the vitality but does not eliminate it. In addition, Acridine orange is suitable staining method for assessment of *Sarcocystis* viability

Introduction

Meat and meat products are among the most nutritionally dense foods in the human diet. They provide the majority of our body's needs, serving as significant sources of energy and a range of vital nutrients required for numerous metabolic processes (Leroy *et al*., 2023).

Meat and meat products may be infected by *Sarcocystis* spp. that are complex apicomplexan parasites. It causes substantial economic losses in the meat production sector when entire carcasses must be condemned (Peris *et al*., 2024).

Water buffaloes are substantial element of Egyptian animal wealth. FAO estimates that there are currently 3,977,000 water buffaloes in Egypt and that number is expected to grow at a rate of 1.18% annually (Ashmawy *et al*., 2014). *Sarcocystis* sp. is one of the most prevalent and pervasive livestock protozoan parasites (Oryan *et al*., 2010). Both final and intermediate hosts are necessary to complete the parasite's life cycle. The sexual phase occurs inside the final hosts, which are carnivore animals like cats, dogs, wolves, and humans, and the asexual reproduction occurs inside the intermediate hosts, which include herbivores, omnivorous animals, and birds. There are over 100 known species of the genus *Sarcocystis*, which infects mammals, birds and coldblooded animals (Lindsay and Dubey, 2020).

Four species of *Sarcocystis* use buffaloes as intermediate hosts: *S. fusiformis, S. buffalonis, S. levinei, and S. dubeyi. Of these, S. fusiformis* and *S. bulbifalonis* form macroscopic cysts with cats serving as a final host. While *S. levinei* and *S. dubeyi* produce microscopic sarcocysts. Dogs are known to be the definitive hosts for *S. levinei*, how¬ever the definitive host or hosts for *S. dubeyi* are still unknown (Daptardar *et al*., 2016; Lindsay and Dubey, 2020). *Sarcocystis fusiformis* is a protozoan parasite that mainly affects buffalo, causing muscle cysts that may negatively affect the animal's health and reduce the quality of meat for human consumption, resulting in economic losses. It can survive in the host for the duration of its life but may begin to dissipate after three months of infection (JyothiSree *et al*., 2017).

Canid-transmitted species are pathogenic, but felid-transmitted species are not. Fever, anorexia, anemia, emaciation, hair loss, lower milk yields are the noticeable clinical symptoms; some animals may even die in addition pregnant one may abort (Lindsay and Dubey, 2020). Infection with macroscopic *Sarcocystis* leads to a serious economic problem due to condemnation of the infected animal parts or lowering the market profile for the meat (Morsy *et al*., 2018). Expanding herbage contaminated with the parasite's sporocysts makes it more difficult to raise calves free of *Sarcocystis* (Dubey and Rosenthal, 2023). For muscular sarcocystosis, there is no known vaccination or proven effective antiparasitic medication; however, anti-inflammatory medications can mitigate the symptoms (Fayer *et al*., 2015).

Sarcocystis infection is registered as one of the reemerging parasitic zoonotic diseases since human can be infected by eating undercooked meat of infected animal (Oryan *et al*., 2010; Fazly *et al*., 2016).

They represent a public health danger since they can cause serious human intestinal infections in those with impaired immune systems as well as those who have never been exposed to the parasite before (Rosenthal, 2021). So far, meat processors require simple, quick, affordable, and efficient methods to eradicate *Sarcocystis* spp.

Evaluating the vitality of parasites is essential for demonstrating the effectiveness of inactivation procedures. One way to do this is by feeding the cats with the sarcocysts and checking for the existence of the *Sarcocystis* oocysts or sporocysts in their excrement. Vital stains can be an effective substitute for utilizing laboratory animals when assessing the vitality of parasites (Peris *et al*., 2024). The dye exclusion test operates on the assumption that live cells have intact cell membranes that exclude definite dyes, such as trypan blue, while dead cells do not (Strober, 2015). The vitality of many parasites, such as *Toxoplasma gondii, Trichomonas vaginalis, Eimeria tenella*, and *Sarcocystis* sp., has been assessed using trypan blue staining (Peris *et al*., 2024).

Acridine orange (AO), a vital dye that can stain both live and dying cells, is another staining method for viability assessment (Giri and Roy 2016). AO can be used to measure chromatin's relative integrity. As DNA fragments, it becomes more accessible to dye stacking, led to increasing the quantity of attached dye particles, green nuclear fluorescence indicates low dye concentration and supercoiled DNA, yellow and yellow orange indicates increasing DNA fragmentation, and red indicates the maximum dye concentration and highest degree of DNA fragmentation. This increase in dye concentration is seen as a change in the wavelength of light emitted (Foley and Cooley, 1998). According to Giri and Roy (2016), living cells always stain green, whereas dying cells vary in color from yellow to orange, based on how much of their membrane integrity has been lost.

So far meat processors need easy, fast, cheap and effective measures for eradication of *Sarcocystis* spp. Therefore, the present study aimed to detect buffaloes' sarcocystosis infection in Assiut Gov. along with its organ distribution. Also, investigate the effect of some inactivation techniques: temperatures (chilling, freezing and microwave) and combination solutions (sodium chloride and potassium lactate mixture) on viability of *Sarcocystis* cysts in esophagus as a meat model. Furthermore, demonstrate of *Sarcocystis* inactivation through using vital stains like Trypan blue and Acridine Orange staining.

Materials and methods

Collection of samples

Between February and December 2023, 80 buffaloes were examined for *Sarcocystis* at various slaughterhouses in Assiut Governorate, Egypt. From each animal that was slaughtered, fresh tissue samples of the diaphragm, skeletal muscle, masseter muscle, tongue, heart, and esophagus were obtained. These samples were then labeled, placed in clean, labeled plastic bags, stored in ice boxes, and quickly brought to the laboratory (Metwally *et al*., 2014).

Diagnosis of Sarcocystis in examined buffaloes

Visual inspection

The muscles were visually examined in the abattoir under good lighting. The specimens were checked for observable abnormalities, particularly in the buffalo's esophageal muscles. Muscle specimens were palpated to detect any firm or odd structures that may suggest the existence of cysts. In the esophageal muscle layer, cysts have the appearance of whitish, elongated (Fig. 1), fusiform objects (El-Sayad *et al*., 2023).

Detection of bradyzoites

To describe bradyzoites, the obtained macroscopic sarcocysts were crushed between two slides, smeared their contents, methanol fixed, stained with Giemsa (Fig. 2) and examined with a light microscope at 1000 x magnifications (Hamidinejat *et al*., 2010).

Microscopic Examination

Muscle pieces (0.5 × 0.5 cm) were squeezed between two slides and microscopically analyzed for microscopic sarcocysts at 100x magnification (Latif *et al*., 2015).

Impact of refrigeration, freezing and heating in a microwave on Sarcocystis vitality

Refrigeration

Several portions of infected samples with macroscopic sarcocysts [50 g each] were subjected to continuous traditional refrigeration (4.0± 2.0°C) for two days. The samples were evaluated for viability after 24 and 48 hours of chilling (González-Fuentes *et al*., 2015).

Freezing

The meat from infected samples containing macroscopic sarcocysts was chopped into many pieces, each weighing an average of 50 g. These portions were then frozen for 24 and 48 hours, respectively, at a temperature of −18°C, the survivability of the sarcocysts was checked at each freezing period (González-Fuentes *et al*., 2015).

Microwave oven

Multiple portions of the samples (50 grams each) were cooked in a Pyrex container using a household microwave oven (Model MW877, KENWOOD ®, 1100W, 2450MHz, China). The meat was heated to maximum power (1100 W at 2450 MHz frequency). Five distinct microwave heating periods of 15 sec., 30 sec., 1, 2, and 3 min. were applied to the samples, each heating period was applied on 5 pieces of meat then the group's interior temperature was recorded via the thermometer (Serrano *et al*., 2007; González-Fuentes *et al*., 2015).

Efficacy of sodium chloride / potassium lactate solution on viability of Sarcocystis

Two concentrations were used: Treatment I was a mixture of 2% sodium chloride and 2% potassium lactate, while treatment II was 2% sod. chloride and 3% pot. lactate. Numerous pieces of *Sarcocystis*-infected meat were soaked in each concentration for 15 minutes and then stored at 4°C for 8 hours before viability was determined using vital stains (Hill *et al*., 2004; Nageib and Mohamed, 2021; Shimaa-Ahmed *et al*., 2022).

Trypan blue exclusion test for the viability of Bradyzoites

Sarcocyst viability was established by the presence of live bradyzoites in the treated meat. In brief, the suspension of bradyzoites was centrifuged for 5 minutes at 100 × g. The supernatant was disposed of and then resuspended in 1 milliliter of PBS. The bradyzoites suspension was combined with an equal volume of 0.4% Trypan Blue stain solution (Diagnostic Biosystems, batch No. RE977), and it was incubated at room temperature for less than three minutes. After adding a drop of this mixture to a hemacytometer, the sample was inspected under a 400X light microscope. The viable bradyzoites (unstained) and the nonviable bradyzoites (stained) were counted independently. The survival rate of the bradyzoites was calculated according to Strober (2015); Murata *et al*. (2018) and Honda *et al*. (2018).

Acridine orange (AO) staining

As described by Ravindran *et al*. (2007) and Ghazy *et al*. (2023) the prepared smears were air-dried and fixed in methanol for 2-3 minutes. Acridine orange dye "C.I. 46005; Scharlab, SPAIN. www.scharlab.com" was freshly prepared and applied to the fixed smears at a concentration of 0.01% for a duration of two minutes. The dye was subsequently removed using distilled water.

The slides were examined at 1000X magnification using a fluorescence microscope "Olympus Corporation of America, New Hyde Park, NY" equipped with a 470–490 nm filter. Dead bradyzoites fluoresced red, inactive *Sarcocystis* bradyzoites fluoresced brownish green [Green/red], while living *Sarcocystis* bradyzoites fluoresced green (Khatoon *et al*., 2014; Ghazy*et al*., 2023).

Statistical analysis

Data statistical analysis was performed using "GraphPad Prism, a Windows version 9.5.1" software package (GraphPad-Software, LLC, USA). To compare the categorical variables between the two types, the Chi-square test was employed. The result was defined as highly significant when the P value was less than 0.001(McHugh, 2013).

Results and Discussion

Animals' skeletal and cardiac muscles are impacted by *Sarcocystis*. Eating infected raw or undercooked meat can expose humans to the infection (Dubey *et al*., 2015). This parasite has substantial economic and human health implications. As a result, proper preventative techniques for disabling it are necessary.

As can be shown in Figs. 1, 2, the overall *Sarcocystis* infection rate among the examined 80 buffaloes in the Assiut Governorate was 70% (56/80). Both macroscopic sarcocysts (3.75%) and microscopic sarcocysts (67.5%) differed significantly ($p < 0.0001$, chi2 = 70.8). Likewise, Said (1996) found the prevalence of buffaloes' sarcocystosis in the Assiut Governorate was 76.8%.

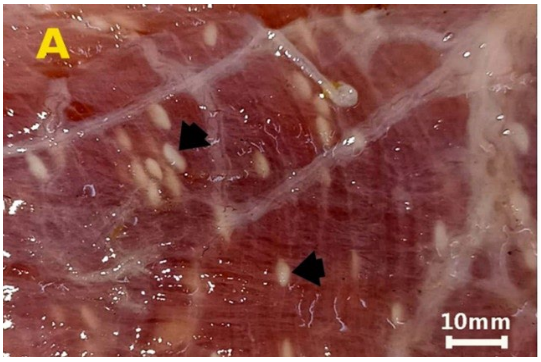

Fig. 1. Macroscopic *Sarcocystis fusiformis* cyst in buffaloes' Esophagus (black arrowhead).

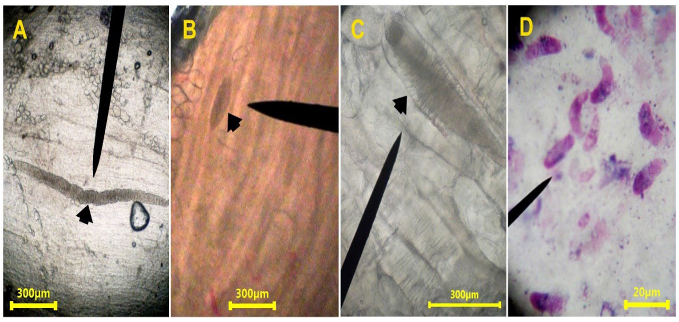

Fig. 2. [A], [B] and [C] Microscopic *Sarcocystis* cyst in muscles of infected buffaloes (black arrow head). [D] bradyzoites stained with Giemsa stain showing banana-shaped *Sarcocystis* bradyzoites (black arrowhead).

Besides El-Dakhly *et al.* (2011) discovered that 78.9% of the buffaloes investigated in Beni-Suef Governorate abattoirs were infected with microscopic *Sarcocystis* spp. and 6.9% with macroscopic *Sarcocystis* spp, furthermore Ashmawy *et al.* (2014) revealed that (67.6 %) of the tested water buffaloes' serum samples at Alexandria province were seropositive to *Sarcocystis* spp by ELISA. Conversely, Khalifa *et al.* (2008) revealed a twenty eight percent infection rate of macroscopic buffaloes' sarcocystosis at Sohag Governorate slaughterhouses. Also in New Valley Governorate, Metwally *et al.* (2014) recorded a high prevalence of sarcocystosis between buffaloes by ELISA testing (94 %) even though the prevalence by macroscopic and microscopical examination were 25.5 % and 27.7%, respectively. Likewise, Ahmed *et al.* (2016) reported 8.33% of buffalo carcasses have macroscopic sarcocystosis at El-Kharga abattoir, whereas 22% of them harbored microcysts.

The size of the sample, the organs being studied, the diagnostic methods employed and -above all- the presence of infectious stages in the surrounding environment could all account for variations in these results. Numerous factors could contribute to the high infection rate, such as the final host's release of millions of already infectious sporocysts over several months, the sporocysts' long-term resistance to the external environment, and the definitive host's little to no immunity to sarcocystosis, which means it constantly sheds sporocysts when it eats infected meat containing sarcocysts (Metwally *et al*., 2014; Dubey *et al*., 2016).

Our results in Table 1 revealed that the esophagus was the only affected organ exhibiting macroscopic sarcocysts (3.75%). Besides, microscopic cysts were most prevalent in both the esophagus (45%) and skeletal muscles (42%) then followed by the diaphragm (36%), heart (33%), masseter muscle (27%) and finally the tongue (25%). Nevertheless, the distribution of sarcocysts among these organs has an insignificant statistical influence.

The prior research indicate that parasite can be rendered inactive by heating, freezing, irradiating, and marinating in acetic acid and Na Cl, each of them had different effect (Franssen *et al*., 2019).

Assessment of parasite vitality is essential to demonstrate the success of inactivation procedures. In terms of vital stains were applied in our study (Fg. 3), trypan blue staining has been commonly used to count alive cells using a transmission microscope without fluorescence (Peris *et al*., 2024). It is a simple, inexpensive, and quick cell viability measurement approach, but it is subject to the problem that viability is evaluated indirectly from cell membrane integrity (Strober, 2015). On the other hand, the Acridine orange staining method yielded well-contrasted images of living, injured, and dead bradyzoites (Ghazy *et al*., 2023). AO is a simple intercalating dye can be used for monitoring the relative integrity of chromatin. The more DNA fragmentation the more increase in the quantity of attached dye particles (Foley and Cooley, 1998).

Instead of cooking, meat processors need to find ways to rid of parasites in meat. Different processing and storage techniques were assessed in this study as control measures for sarcocystosis in esophageal tissue within 24 and 48 hours. Generally, two ways are used to preserve meat: chilling and freezing. The viability of *Sarcocystis* cysts in the esophagus after 24 hours of cold storage at 4°C was unaffected, but the survival rate significantly ($p < 0.0001$, chi 2 = 34.1) dropped to 48.5% after 48 hours of storage, as shown in Table 2. Which may be due to chilling slows down the metabolic processes of parasites, but it does not kill them outright.

This is in approach with the findings of Saleque *et al.* (1990), who established that tissue cysts containing *Sarcocystis* were infective to dogs even they were refrigerated for 7 day. Same results revealed by Honda *et al.* (2018) they found that the parasite withstood for over seven days even after being cooled to 0 and 4°C and acidified to pH 3.0 and 5.0 with

Table 1. Distribution of *Sarcocystis* spp. in different tissue organs of buffaloes.

	Esophagus (n.=80)		Heart (n.=80)		Tongue (n.=80)		Masseter muscle (n.=80)		Skeletal muscle (n.=80)		Diaphragm (n.=80)	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Microscopic *Sarcocystis spp.*	36	45	24	33	27	25	25	29	33	42	31	36
Macroscopic *Sarcocystis spp.*	3	3.75	0	0	0	0	0	0	0	0	0	0

intact the diarrheal toxin.

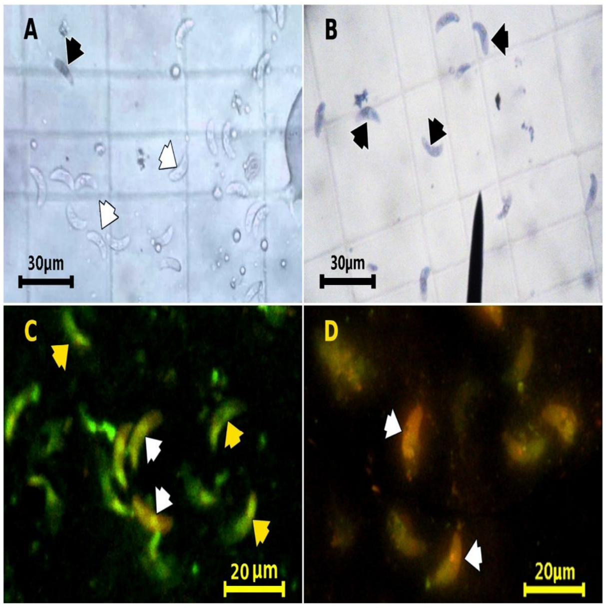

Fig. 3. [A] Bradyzoites stained with trypan blue stain showing live *Sarcocystis* bradyzoites (white arrowhead) and one dead bradyzoite (black arrowhead). [B]bradyzoites stained with trypan blue stain showing dead *Sarcocystis* bradyzoites (black arrowhead). [C] Acridine orange fluorescent staining showing greenish active *Sarcocystis* bradyzoites (yellow arrowhead) and brownish inactive *Sarcocystis* bradyzoites (white arrowhead). [D] Acridine orange fluorescent staining showing brick red died *Sarcocystis* bradyzoites (white arrowhead).

In contrast, in the current study, storing esophageal tissue at -18°C for 48 hours completely ruined the viability of *Sarcocystis* cysts, whereas at 24 hours, survival rate was 40.4%, exhibiting a highly significant difference ($p < 0.0001$, chi 2 = 25), (Table 2). In this instance, Peris *et al*. (2024) discovered that freezing meat at a degree -20 Celsius for 96 to 144 hrs. would effectively destroy *Sarcocystis* spp., with a median viability of 1.5 to 0%, correspondingly. Chen *et al.* (2007) also looked at how *Sarcocystis* structure was affected by prolonged freezing at −20°C. They discovered that by freezing bradyzoites disintegrate and degenerate, most likely leading to losing the bradyzoite activity. It has been proven by additional researchers that inactivating *S. levinei* in buffalo and *S. capracanis* in goats requires only freezing at −20°C for a duration of 24 to 48 hours (Srivastava *et al*., 1986; Singh and Shah, 1990).

Table 2. Effect of different treatments on the viability of bradyzoites of *Sarcocystis fusiformis* in buffaloes.

Experiments	Survival rate (%)	
Chilling conditions [at 4°C] **		($p < 0.0001$, chi 2 = 34.1)
For 24h	+ve	-100%
For 48h	+ve	-48.50%
Frozen conditions [at -18°C] **		($p < 0.0001$, chi 2 = 25)
For 24h	+ve	-40.40%
For 48h	-ve	0%
Microwave [High power "1100W, 2450 MHz"] **		($p < 0.0001$, $chi^2 = 208.5$)
For 15sec. [internal temp. 54°C]	+ve	-74.40%
For 30sec. [internal temp. 64°C]	–ve	0%
For 1min. [internal temp. 82°C]	–ve	0%
For 2min. [internal temp. 85°C]	–ve	0%
For 3min. [internal temp. 87°C]	–ve	0%
Nacl / Pot. Lactate Sol.		
2% Nacl/ 2% Pot. Lactate	+ve	-40.70%
2% Nacl/ 3% Pot. Lactate	+ve	-21.70%

Nevertheless, one study, deviates from all others in that it found that *S. wapiti* and *S. sybillensis* could be inactivated in Sika deer meat in just two hours at −20°C (Honda *et al*., 2018).

Parasite killing through freezing depending on various conditions, including the freezing temperature, freezing duration and parasite species (FDA, 2001). The influence rate of home freezers is sluggish so, freezing causes the development of large, sharp ice crystals that tear cell membrane; the subsequent loss of the membrane's stability causes the death of the parasite (Chen *et al*., 2007). Therefore, the longer freezing duration the more formation of crystals that develop and consequently, increase the proportion of cells with damaged membranes (Peris *et al*., 2024). Chilling is generally less efficient than freezing at inactivating *Sarcocystis fusiformis*.

Another approach for avoiding transmission to humans is to inactivate the parasite by thermal treatment (Franssen *et al*., 2019). Heat treatment has been identified by the FAO as one of the most effective methods for controlling parasites. Several studies have shown that the duration of heating is just as important as temperature and should be adjusted so that proper temperatures are achieved, maintained, and dispersed uniformly throughout the meat (Gajadhar, 2015).

Prior studies have demonstrated that, heat inactivation of parasites is an efficient means of removing the danger of parasitosis. This is corroborated by data presented in Table 2, which confirmed that microwave treatment employed for 30 sec. or more resulted in 100% mortality of the bradyzoites, whereas for 15 sec., the survival rate was 74.4%, exhibiting a highly significant difference ($p < 0.0001$, chi2 = 208.5).

These findings are consistent with those of González-Fuentes *et al.* (2015), who found that *Distomummus culorumsuis* (DMS) was not able to withstand heating temperatures above 60°C or microwave heating exposure for six separate times, with a median survival period of 75 s. They added that extended exposure and elevated temperatures often resulted in lower survival periods. Additionally, the US FDA (2017) stated that to inactivate any potential parasites, all the food's contents must reach a temperature of 74°C.

In this context, Collins *et al*. (2005) verified that the infectivity of *Cryptosporidium parvum* oocysts in oysters for neonatal mice was found to be partially, but not significantly, reduced by microwave exposure for 1-3 seconds (43.2-62.5°C). However, heating above 43.2°C resulted in unacceptable changes in oyster meat color and texture.

According to Ali and Al-Mahmoud (2009), the efficiency of heating depends on a number of variables including the type of parasite, the amount of fat, and the thickness of the meat. Microwave microbial destruction involves inactivating and destroying pathogens directly with heat (Heddleson and Doores, 1994). Moreover, dielectric heating occurs when water, fat, and other food ingredients absorb microwave energy. The food's temperature is raised by the energy that is dispersed by molecular spins, vibrations, and/or translations when circular molecules collide with one another and set them in motion (Zitzewitz, 2011). Microwaves generate heat via dielectric heating, resulting in rapid temperature rises that can denature proteins and destroy cellular components.

The process of salting meat is one of these methods. Taste and flavor are not the main reasons for the continuous usage of high salt content cause of adding salt to meals is a traditional method of preserving meat products and food. Salt improves its microbiological stability significantly in addition to its technical benefits (Nageib and Mohamed, 2021). Based on some findings, it was suggested that combinations of NaCl, the primary ingredient in cured products, could inactivate *Toxoplasma* bradyzoites in fresh pork (Hill *et al*., 2018).

The goal of this research was to reduce the amount of salt in meat products. Since earlier researchers found that in order to make tissue cysts nonviable when using sodium chloride alone, the concentration of sodium chloride needs to be increased with a longer exposure period. For this reason, the present study investigated a low concentration of table salt (sodium chloride) in combination with potassium lactate as a trial to render *Sarcocystis* tissue cysts nonviable in meat samples.

Treatment I of the current study demonstrated that there is a 40.7% survival rate for *Sarcocystis* cysts, indicating an impact on their viability in meat. Whereas Treatment II eliminated the *Sarcocystis* tissue cyst in the meat with a survival rate of 21.7% (Table 2), which is consistent with the findings of Hill *et al*. (2006), who found that the tissue cysts in the enhanced pork loin meat became nonviable within 8 hours of exposure to a mixture of 1.4% potassium lactate, 0.10% sod. diacetate and 0.25% sod. tripolyphosphate, or to a mixture of 0.50% sod. tripolyphosphate and 2.0% NaCl at 4°C as an alternative Hill *et al*. (2004). By contrast, Hill *et al*. (2004) demonstrated that exposure to solutions of (≥1.4%) potassium lactate or (≥1.4%) sodium lactate mixed with 2% sodium chloride for a period of seven days altered the survivability of tissue cysts of *T. gondii* in pork. Low levels of sodium chloride and potassium lactate mixture were shown to be effective in rendering *Sarcocystis* tissue cysts nonviable in meat samples. This method provides a possible alternative for meat preservation that preserves quality while improving safety.

Conclusion

The current study's results indicate that buffaloes in the Assiut Government have a high rate of *Sarcocystis* infection, which may pose a risk to the quality and safety of their meat. Freezing at -18°C for 48 hours is the most effective way for completely inactivating *Sarcocystis* in buffaloes. Chilling lowers the viability but not eradicate the parasite. Microwave heating for at least 1 minute, when used correctly, can successfully kill the parasite. Furthermore, a mixture of 2% NaCl and 3% pot. Lactate significantly reduces the vitality of *Sarcocystis* but does not eliminate it, indicating a potential utility in meat processing and preservation. Finally, constant monitoring and adequate meat inspection techniques are needed to ensure the health of livestock and consumer safety. Effective veterinary inspection of meat is essential to provide safe and healthful food to consumers, in parallel with educational health programs about *Sarcocystis* transmission, its source of infection, and control measures that should be followed, especially for high-risk individuals.

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To the soul of our dear friend, Dr. Muhammad S. Tawfik (Food Hygiene Department, AHRI, Assiut Lab). How sad I feel at his departure from our world, and we are also very grateful to him for the effort he put into this scientific research, out of his desire to enrich scientific research and benefit the public. Praying to God Almighty to shower our precious deceased with His vast mercy and to place this knowledge in the balance of His good deeds.

Conflict of interest

The authors have no conflict of interest to declare.

References

Ahmed, A.M., Elshraway, N.T., Youssef, A.I., 2016. Survey on *Sarcocystis* in bovine carcasses slaughtered at the municipal abattoir of El-Kharga, Egypt. Veterinary world 9, 1461-1465. DOI: 10.14202/vetworld.2016.

Ali, H.B., Al. Mahmoud, S.S., 2009. Pathological effect of Cryptosporidium mansonio-ocysts isolated from fish and treated with infra. Red, microwave and ozone in common crap. Iraq J. Vet. Sci. 23, 187-192.

Ashmawy, K.I., Abu-Akkada, S.S., Ghashir, M.B., 2014, Prevalence and molecular characterization of *Sarcocystis* species in water buffaloes (Bubalus bubalus) in Egypt. Tropical animal health and production 46, 1351–1356. DOI:10.1007/s11250-014-0566-9

Chen, L.Y., Zhou, B.J., Yang, Z.Q., Li, C.Y., Attwood, S.W., Wang, W.L., Lei, L., Sun, X.D., Zhang, Z.X., 2007. Effects of frozen storage on the structure of sarcocysts in pig muscle and implications in taxonomic studies. Experimental parasitology 115, 393–398. DOI: 10.1016/j.exppara.2006.10.003

Collins, M.V., Flick, G.J., Smith, S.A., Fayer, R., Rubendall, E., Lindsay, D.S., 2005. The effects of E-beam irradiation and microwave energy on Eastern Oysters (Crassostrea virginica) experimentally infected with Cryptosporidium parvum. The Journal of Eukaryotic Microbiology 52, 484–488. DOI: 10.1111/j.1550-7408.2005.00056.x.

Daptardar, M., Singh, B.B., Aulakh, R.S., Gill, J.P., 2016. Prevalence and first molecular identification of *Sarcocystis* species in cattle and water buffaloes in India. Acta parasitologica 61, 523–528. DOI: 10.1515/ap-2016-0069

Dubey, J.P., Rosenthal, B.M., 2023. Bovine sarcocystosis: *Sarcocystis* species, diagnosis, prevalence, economic and public health considerations, and association of *Sarcocystis* species with eosinophilic myositis in cattle. Internat. J. Para. 53, 663-475. DOI: 10.1016/j.ijpara.2022.09.009

Dubey, J.P., van Wilpe, E., Calero-Bernal, R., Verma, S.K., Fayer, R., 2015. *Sarcocystis* heydorni, n. sp. (Apicomplexa: Sarcocystidae) with cattle (Bostaurus) and human (Homo sapiens) cycle. Parasitology research 114, 4143–4147. DOI:10.1007/s00436-015-4645-2

Dubey, J.P., Calero-Bernal, R., Rosenthal, B.M., Speer, C.A., Fayer, R., 2016. Sarcocystosis of animals and humans, Second Ed. CRC Press, Boca Raton, Florida, USA, pp 1-481.

El-Dakhly, K.M., El-Nesr, K.A., El-Nahass, el-S., Hirata, A., Sakai, H., Yanai, T., 2011. Prevalence and distribution patterns of *Sarcocystis* spp. in buffaloes in Beni-Suef, Egypt. Tropical animal health and production 43, 1549–1554. DOI:10.1007/s11250-011-9840-2

El-Sayad, M., El-Taweel, H., Ahmed, A., Abd El-Latif, N., 2023. Sarcocystosis among buffaloes from slaughterhouses in Nile Delta, Egypt: morphologic assessment and molecular confirmation. Iranian journal of veterinary research 24, 313–319. DOI:10.22099/IJVR.2023.48129.7006

Fayer, R.A., Esposito, D.H.B., Dubey, J.P., 2015. Human infections with *Sarcocystis* species. Clin. Microbiol. Rev. 28, 295-311. DOI:10.1128/CMR.00113-14

Fazly Ann, Z., Muhamad Syamsul Naim, N.A., Niny Fariza, J., Wan Normaziah, W.O.B., 2016. *Sarcocystis* infection in Kedah-Kelantan crossbred cattle and Murrah Buffalo slaughtered in abattoir in Perak, Malaysia. Tropical biomedicine 33, 197–202. PMID: 33579157.

FDA, 2001. CFR - Code of Federal Regulations Title 21 CFR120 (Federal Register Final Rule).

Foley, K., Cooley, L., 1998. Apoptosis in late stage Drosophila nurse cells does not require genes within the H99 deficiency. Development (Cambridge, England) 125, 1075–1082. DOI:10.1242/dev.125.6.1075

Franssen F., Gerard C., Cozma-Petrut A., Vieira-Pinto M., Jambrak A.R., Rowan N., Paulsen P., Rozycki M., Tysnes K., Rodriguez-Lazaro D., Robertson L., 2019. Inactivation of parasite transmission stages: efficacy of treatments on food of animal origin. Trends in Food Science and Technology 83:114-128. DOI:10.1016/j.tifs.2018.11.009

Gajadhar A.A.E., 2015. Foodborne parasites in the food supply web: Occurrence and control. Woodhead Publishing, Cambridge, CB22 3HJ, UK, pp.1-481.

Ghazy, T.A., Sayed, G.M., Farghaly, D.S., Arafa, M.I., Abou-El-Nour, B.M., Sadek, A.S.M., 2023. In vitro antiprotozoal effect of alcoholic extract of hemolymph of *Galleria mellonella* larva against Trichomonas gallinae. Int J Vet Sci. 12, 302-308. DOI:10.47278/journal.ijvs/2022.192

Giri, B.R., Roy, B., 2016. *Cysticercus fasciolaris* infection induced oxidative stress and apoptosis in rat liver: a strategy for host-parasite cross talk. Parasitology research 115, 2617–2624. DOI:10.1007/s00436-016-5008-3

González-Fuentes, H., Hamedy, A., Koethe, M., von Borell, E., Luecker, E., Riehn, K., 2015. Effect of temperature on the survival of *Alariaalata* mesocercariae. Parasitology research 114, 1179–1187. DOI:10.1007/s00436-014-4301-2

Hamidinejat, H., Razi Jalali, MH., Nabavi, L., 2010. Survey on *Sarcocystis* infection in slaughtered cattle in south-west of Iran, emphasized on evaluation of muscle squash in comparison with digestion method. J Animal Vet Advances 9, 1724-1726. DOI:10.3923/javaa.2010.1724.1726

Heddleson, R.A., Doores, S., 1994. Factors Affecting Microwave Heating of Foods and Microwave Induced Destruction of Foodborne Pathogens - A Review. Journal of food protection 57, 1025–1037. DOI:10.4315/0362-028X-57.11.1025

Hill, D.E., Sreekumar, C., Gamble, H.R., Dubey, J.P., 2004. Effect of commonly used enhancement solutions on the viability of *Toxoplasma gondii* tissue cysts in pork loin. Journal of Food Protection 67, 2230-2233. DOI:10.4315/0362-028x-67.10.2230

Hill, D.E., Benedetto, S.M., Coss, C., McCrary, J.L., Fournet, V.M., Dubey, J.P., 2006. Effects of time and temperature on the viability of *Toxoplasma gondii* tissue cysts in enhanced pork loin. Journal of food protection 69, 1961–1965. DOI:10.4315/0362-028x-69.8.1961

Hill, D.E., Luchansky, J., Porto-Fett, A., Gamble, H.R., Fournet, V.M., Hawkins-Cooper, D.S., Urban, J.F., Gajadhar, A.A., Holley, R., Juneja, V.K., Dubey, J.P., 2018. Rapid inactivation of *Toxoplasma gondii* bradyzoites during formulation of dry cured ready-to-eat pork sausage. Food and waterborne parasitology, 12, e00029. DOI:10.1016/j.fawpar.2018.e00029

Honda, M., Sawaya, M., Taira, K., Yamazaki, A., Kamata, Y., Shimizu, H., Kobayashi, N., Sakata, R., Asakura, H., Sugita-Konishi, Y., 2018. Effects of temperature, pH and curing on the viability of *Sarcocystis*, a Japanese sika deer (*Cervus Nippon centralis*) parasite, and the inactivation of their diarrheal toxin. The Journal of veterinary medical science 80, 1337–1344. DOI:10.1292/jvms.18-0123

JyothiSree, C., Venu, R., Samatha, V., Malakondaiah, P., Rayulu, V.C., 2017. Prevalence and microscopic studies of *Sarcocystis* infection in naturally infected water buffaloes (*Bubalus bubalis*) of Andhra Pradesh. Journal of parasitic diseases: official organ of the Indian Society for Parasitology 41, 476–482. DOI:10.1007/s12639-016-0832-z

Khalifa, R.M., El-Nadi, N.A., Sayed, F.G., Omran, E.K., 2008. Comparative morphological studies on three *Sarcocystis* species in Sohag, Egypt. Journal of the Egyptian Society of Parasitology 38, 599–608. PMID: 18853631

Khatoon, R., Jahan, N., Khan, H.M., Rabbani, T., Ahmad, S., 2014. Evaluation of Different Staining Techniques in the Diagnosis of *Trichomonas vaginalis* Infection in Females of Reproductive Age Group. Journal of clinical and diagnostic research: JCDR. 8, DC05–DC8. DOI:10.7860/JCDR/2014/9765.5261

Latif, B., Kannan Kutty, M., Muslim, A., Hussaini, J., Omar, E., Heo, C.C., Rossle, N.F., Abdullah, S., Kamarudin, M.A., Zulkarnain, M.A., 2015. Light microscopy and molecular identification of *Sarcocystis* spp. in meat producing animals in Selangor, Malaysia. Tropical Biomedicine 32, 444-452. PMID: 26695204

Leroy, F., Smith, N.W., Adesogan, A.T., Beal, T., Iannotti, L., Moughan, P.J., Mann, N., 2023. The role of meat in the human diet: evolutionary aspects and nutritional value. Animal frontiers: the review magazine of animal agriculture 13, 11–18.

DOI:10.1093/af/vfac093

Lindsay, D.S., Dubey, J.P., 2020. Neosporosis, toxoplasmosis, and sarcocystosis in ruminants: an update. Veterinary Clinics: Food Animal Practice 36, 205-222. DOI:10.1016/j.cvfa.2019.11.004

McHugh M.L., 2013. The chi-square test of independence. Biochemia medica 23, 143–149. DOI:10.11613/bm.2013.018

Metwally, A.M., Abd Ellah, M.R., Al-Hosary, A.A., Omar, M.A., 2014. Microscopical and serological studies on *Sarcocystis* infection with first report of S. cruzi in buffaloes (*Bubalus bubalis*) in Assiut, Egypt. Journal of parasitic diseases: official organ of the Indian Society for Parasitology 38, 378–382. DOI:10.1007/s12639-013-0257-x

Morsy, K., Abdel-Ghaffar, F., Bin Dajem, S., Abdel-Gaber, R., El Gazar, F., 2018. First molecular characterization and morphological aspects of *Sarcocystis fusiformis* infecting water buffalo *Bubalus bubalis* in Egypt. Acta parasitologica 63, 333–345. DOI:10.1515/ap-2018-0038

Murata, R., Suzuki, J., Hyuga, A., Shinkai, T., Sadamasu, K., 2018. Molecular identification and characterization of *Sarcocystis* spp. in horsemeat and beef marketed in Japan. Parasite (Paris, France) 25, 27. DOI:10.1051/parasite/2018026

Nageib, B.R., Mohamed M.H., 2021. Effect of mixture of sodium chloride and potassium lactate on the viability of *Toxoplasma gondii* in meat. Assiut Vet. Med. J. 67, 37-53. DOI:10.21608/avmj.2021.188816

Oryan, A., Ahmadi, N., Mousavi, S.M., 2010. Prevalence, biology, and distribution pattern of *Sarcocystis* infection in water buffalo (*Bubalus bubalis*) in Iran. Tropical animal health and production 42, 1513–1518. DOI:10.1007/s11250-010-9601-7

Peris, M.P., Gracia, M.J., Moreno, B., Juan-Puente, P., Morales, M., Serrano, M., Manzano, M.D., Halaihel, N., Badiola, J., Castillo, J.A., 2024. Identification of *Sarcocystis* spp. in Slaughtered Sheep from Spain and Evaluation of Bradyzoite Viability after Freezing. Veterinary sciences 11, 103. DOI:10.3390/vetsci11030103

Ravindran, R., Lakshmanan, B., Sreekumar, C., John, L., Gomathinayagam, S., Mishra, A.K., Tewari, A.K., Rao, J.R., 2007. Acridine orange staining for quick detection of blood parasites. Journal of Veterinary Parasitology 21, 85-86.

Rosenthal, B.M., 2021. Zoonotic *Sarcocystis*. Res. Vet. Sci. 136, 151-157 DOI:10.1016/j.rvsc.2021.02.008

Said, M.S., 1996. Muscular parasites in slaughtered animals in Assiut Governorate. Thesis PhD, Faculty of Veterinary Medicine Assiut University.

Saleque, A., Juyal, P.D., Bhatia, B.B., 1990. Effect of temperature on the infectivity of *Sarcocystis miescheriana* cysts in pork. Veterinary parasitology 36, 343–346. DOI:10.1016/0304-4017(90)90047-f

Serrano, A., Librelotto, J., Cofrades, S., Sánchez-Muniz, F.J., Jiménez-Colmenero, F., 2007. Composition and physicochemical characteristics of restructured beef-steaks containing walnuts as affected by cooking method. Meat science 77, 304–313. DOI:10.1016/j.meatsci.2007.03.017

Shimaa-Ahmed, M., Hefnawy, Y.A.Arafa, M.I., Abd-El-Malek, A.M., 2022. Effect of Some Processing Methods on the Viability of Metacercariae Infecting Wild and Cultured Fish in El-Menia Governorate. CPQ Medicine 13, 01-13.

Singh, K.P., Shah, H.L., 1990. Viability and infectivity of Sarcocysts of *Sarcocystis capracanis* of the goat after maintaining them at different temperatures. Indian J. Anim. Sci. 60, 429–430.

Srivastava, P.S., Saha, A.K., Sinha, S.R., 1986. Effects of heating and freezing on the viability of sarcocysts of *Sarcocystis* levinei from cardiac tissues of buffaloes. Veterinary parasitology 19, 329-332. DOI:10.1016/0304-4017(86)90080-4

Strober W., 2015. Trypan Blue Exclusion Test of Cell Viability. Current protocols in immunology,111, A3.B.1–A3.B.3. DOI:10.1002/0471142735.ima03bs111

US FDA (U.S. Food and Drug Administration), 2017. Food code, section 3–401.12. In 2017 recommendations of the United States Public Health Service Food and Drug Administration. U.S. Food and Drug Administration, Washington, D.C. https://www.fda.gov/food/fda-food-code/food-code-2017 (accessed 25 April 2024)

Zitzewitz, W.P., 2011. The Handy Physics Answer Book. Visible Ink, 2nd ed., Brownstowen, MI, USA.