Comparative methods for detection of sarcocyst in fresh meat, some meat products and evaluate the quality of infected meat

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Abstract
This study was carried-out on 200 samples of different tissues of freshly slaughtered animals 100 each of buffaloes and cattle that include (heart, tongue oesophagus and skeletal muscle) that were collected from Alexandria abattoir for detection of *Sarcocystis* spp. by macroscopic examination, followed by microscopic examination by muscle squash and peptic digestion for detecting bradyzoites while compression technique and histological technique were applied to detect microscopic *Sarcocystis* spp. It was found that the prevalence of macroscopic cysts was 25% in buffaloes (*S. fusiformis*), while no macroscopic cysts have been detected in cattle . The result for detection of microscopic *Sarcocystis* spp. in buffaloes and cattle were (75% and 80%) respectively. This result was obtained by peptic digestion which is the most accurate method compared with compression technique and muscle squach which are less accurate but simple and rapid. Histopathological technique for detection of the species of microscopic *Sarcocystis* revealed thin wall microscopic *Sarcocystis* (*S.levinei* and *S.cruzi*) for buffaloes and cattle, respectively. Thirty samples of sausage were purchased from different groceries. The result of peptic digestion method in sausage was 20% of examined samples .To study the effect of sarcocyst on quality of meat chemical analysis were applied on the infected meat samples include determination of pH value, TVB-N % and TBA %.

Key words:
Sarcocystis spp., fresh meat, meat products, muscle squash, peptic digestion, chemical analysis (pH value, TVB-N % and TBA %).

Introduction

Cattle and buffaloes represent a multi-source for human including meat, milk. Their proteins are considered the most important category of animal protein used for human consumption. One of the most series problems among animals is the parasitic infection which not affects the general health condition of animals only but also can cause certain disease in human. Presence of *Sarcocystis* in meat can causes a great economic losses in animal production industry in Egypt, through as it causes a down grading of meat quality and causes it unfit for human consumption **(Mostafa and Yasein, 2010).** It is assumed that buffaloes as well as cattle are solely infected with their own species. buffaloes (*Bubalus bubalis*) in Egypt harbour four *Sarcocystis* spp. Two macroscopic *S. fusiformis* and *S. buffalonis (S. bovifelis*) and two microscopic *S.levinei* (*S. bovicanis*) and *S. dubeyi* which with unknown definitive host but thought to be zoonotic, **(Elsayed, 2010 and Hilali *et al.*, 2011)**.

Cattle are mainly infected with three *Sarco-*

cystis spp. Two microscopic *Sarcocystis cruzi* (*S. bovicanis*), *Sarcocystis hominis (S. bovihominis*) and one macroscopic *Sarcocystis hirsute (S.bovifelis*) **(Dubey *et al.,* 1989)**. The mode of transmition of *Sarcocystis* to animals through ingestion of contaminated food and water with fecal matter (sporocysts) of stray dog and cat **(El morsey *et al.,* 2014)**. *Sarcocystosis* is a zoonotic parasitic disease transmitted to man through consumption of infected meat and meat products with well developed tissue cysts **(Juyal and Bhatia, 1989)**. The presence of *Sarcocystis* in muscles produces a toxin (sarcocystin), which affects on the heart and gastrointestinal tract **(Gracey, 1992)**. Heavy infection with macroscopic *Sarcocystis* spp. lead to total condemnation of the infected carcasses, as *Sarcocystis* infection renders meat unacceptable to consumer and causes health hazards.

This study aimed to throw the light on best methods used for detection of *Sarcocystis* spp. in meat animals and sausage as well as its effect on meat quality and its suitability for human consumption.

Materials and Methods

Samlpes. The heart, tongue,oesophagus, and skeletal muscles of 100 cattle and 100 buffaloes were collected from Alexandria abattoir, and 30 fresh sausage samples.

A-Parasitological examination.

1-Macroscopic *Sarcocystis*.

Direct observation of the fresh meat samples for macroscopic *Sarcocystis* spp. by naked eye

2-Microscopic *Sarcocystis* Spp.

Oesophogeal muscles were selected to be examined in this study, which were considered the predilection site for *Sarcocysts* spp.

a- Compression technique (Thornton and Gracey, 1976): One gram from each fresh sample was divided into small pieces and compressed between the two slides. The prepared samples were examined under the low power the microscope (100 x).

b- Muscle squash: **(Odening *et al.,* 1996)**: About 1 g of muscles was cut into small pieces, approximately 3–5 mm thick, and crushed strongly between two glass slides and after staining with Giemsa examined under the microscope (400×).

c- Peptic digestion: **(Dubey *et al.,* 1989): Approximately** 10 g of muscles were crushed in 50 ml of digestive solution (containing 1.3 g pepsin, 3.5 ml HCl, and 2.5 g NaCl in 500 ml of distilled water), and digested for 30 minutes in water bath at 40°C.The mixture was centrifuged at 1500 rpm for 5 minutes. Smear from the sediment was then stained with Giemsa stain and examined microscopically at 400× magnification for detecting of bradyzoites.

d-Histopathological technique (Avapal *et al.,* 2004): Freshly collected muscle samples were fixed in 10% neutral buffered formalin solution, dehydrated in graded ethyl alcohol, embedded in paraffin, cut at 5-µm thickness and processed routinely for hematoxylin and eosin staining.

4-Meat product (Sausage): 30 samples of fresh sausage were examined by peptic digestion.

B-Chemical examination: The chemical examination was made on 25 muscle samples infected with macroscopic and microscopic *Sarcocystis* spp. cysts for detection their meat quality through:

1- Determination of pH EOS 63- 11/2006

. The pH value was determined by using an electrical pH meter (Bye model 6020, USA)

2- Determination of Total Volatile Nitrogen (T.V.N) was recommended by **(EOS)** 63-9/2006:

TVN/100g =26.88×(2-T2) mg .

Where T2= volume of NaOH consumed in the titration .

3- Determination of Thiobarbituric acid number (TBA):

The method adopted for estimation of TBA by **(EOS)** 63-10/ 2006:

T B A values were recorded as mg malondhyde per 100g of sample.

Calculation:

Concentration of malonaldehyde=

$$\frac{0.016 + 2.875 \text{ X mg \%}}{10}$$

Where, X = The absorbance rate

Results

A- Parasitological examination.

(Table, 1) revealed that macroscopic *Sarcocystis* spp. infection was (25 %) and (0 %) cysts in buffaloes and cattle. While was (75 %)of buffaloes and (80%) of cattle were infected with microscopic one.

The macroscopic cysts (*S. fusiformis*) was 25% appear grossly as spindle shaped white or creamy in color, cysts measured 3-35mm long and often appear as resembling cucumber seeds. (Fig. 1).

(Table, 2) examination of slaughtered buffaloes clarified that oesophagus was the, most organ that frequently found to be infected 22 (22%), followed by the tongue,3 (3%) while the heart showed no macroscopic infection. while macroscopical examination of muscles of 100 cattle organs revealed absence of cysts.

(Table, 3) showed that 69% of fresh oesophogeal muscle samples of slaughtered buffaloes were found to be positive for *Sarcocystis* spp cysts using compression technique, 70% by muscle squash technique, 73% found to be positive for *Sarcocystis* spp. cysts by histopathological techniques and 75% by Peptic digestion. While The result of microscopical examination of cattle muscles revealed that**,** 71% by compression technique, 72% muscle squash, 78% histopathological techniques and 80% by Peptic digestion. (Figs 2 and 3). Histopathological technique revealed microscopic cysts *S. cruzi* and *S. levinei.* in buffaloes and cattle, respectively (Figs. 5 and 6).

(Table, 4) 20 % of examined fresh sausage samples using peptic digestion methods were found positive for bradyzoites. (Fig.4)

B- Chemical examination.

Table (5) indicated that, the mean pH value in infected samples with *sarcocystis* reached to 5.8±0.16 and 5.30±0.33 in uninfected fresh meat. (Table, 6).

The results which illustrated in Table (5) cleared that, the mean value of T. V. N of examined samples was 13.2±0.69 mg /100g, while12.5±1.2mg/100g in uninfected fresh meat (Table 6).

Table (5) revealed that the mean value of T. B. A of infected meat sample was 0.35 ± 0.022 mg/kg while it was 0.27± 0.002 mg/kg in uninfected fresh meat (table 6)

Table (1). Prevalence of *Sarcocystis* spp. infection in buffaloes and cattle.

Animal species	Number of examined samples	Macroscopic cysts	Microscopic cysts	Total *Sarcocystis spp.*
Buffaloes	100	25 (25 %)	75(75 %)	75 %
Cattle	100	0 (0 %)	80 (80 %)	80 %

Table (2). Prevalence of macroscopic in *Sarcocystis* spp in different examined organs of buffaloes and cattle.

Animal species	Number of positive samples	Oesophagus	Tongue	Heart
Buffaloes	25 %	22 (22 %)	3 (3 %)	0 (0 %)
Cattle	0 %	0 (0 %)	0 (0 %)	0 (0 %)

Table (3). Prevalence of microscopic *Sarcocystis* spp. in buffaloes and cattle using different detection methods.

Animal species	Number	compression technique	Muscle squash	Histopathological techniques	Peptic digestion
Buffaloes	100	69(69%)	70 (70 %)	73 (73 %)	75 (75 %)
Cattle	100	71(71%)	72 (72%)	78 (78 %)	80 (80 %)

Table (4). Prevalence of *Sarcocystis* spp. in sausage using peptic digestion methods.

Total number of	Number of positive	Percentage of positive
	No.	%
30	6	20

Table (5). Statistical analytical results of chemical analysis of examined meat samples (n=25).

Chemical analysis	Min	Max	Mean ± S .E
pH	5.58	5.98	5.8 ±0.016
T.V.B-N	8.3	19.4	13.2±0.69
T.B.A	0.16	0.53	0.35±0.022

Table (6). Some biochemical parameter of uninfected fresh meat.

Parameter of Non infected samples	pH	T V B- N	T B A
	5.3±0.33	12.5±1.2	0.275±0.002

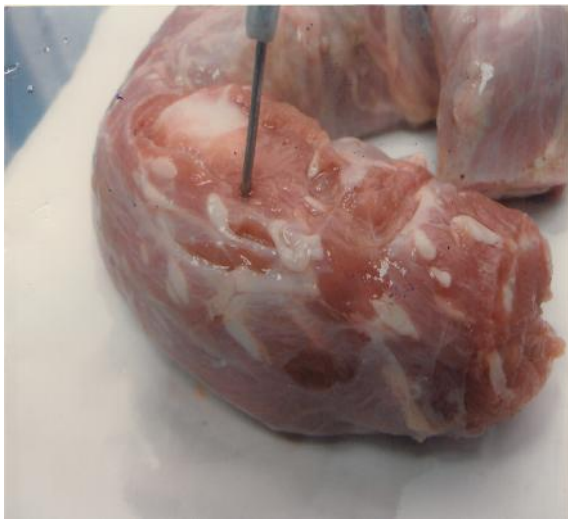

Fig (1). *Sarcocystis fusiformis* in oesphegus of buffaloes

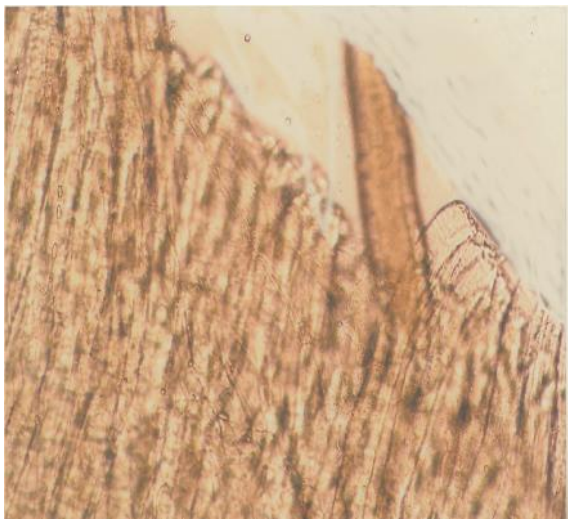

Fig (2). Microscopic cyst of *sarcocystis* bycompression technique (x100)

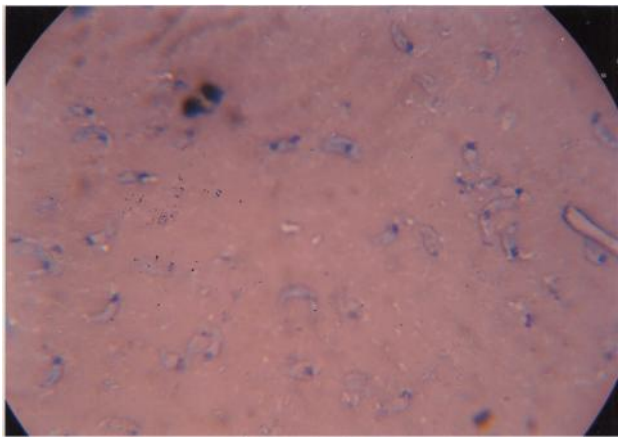

Fig (3). *Sarcocystis* bradyzoites from meat by Muscle sqush x400.

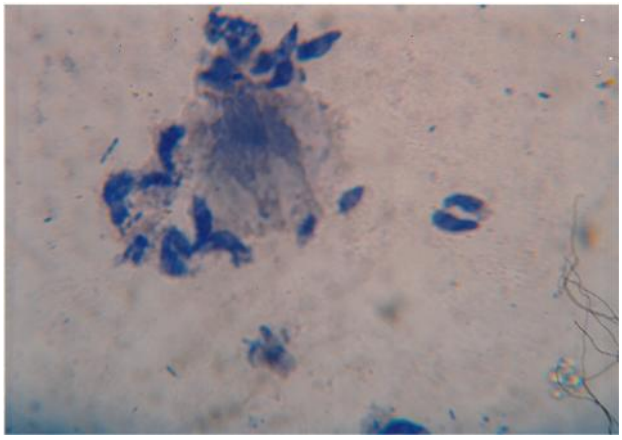

Fig (4) *Sarcocystis* bradyzoites in sausage by Peptic digestion technique (x400).

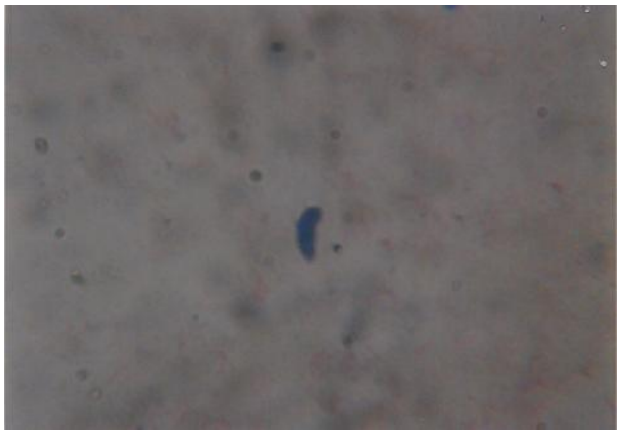

Fig (4). Bradyzoites of *Sarcocystis* by Peptic digestion technique (x400).

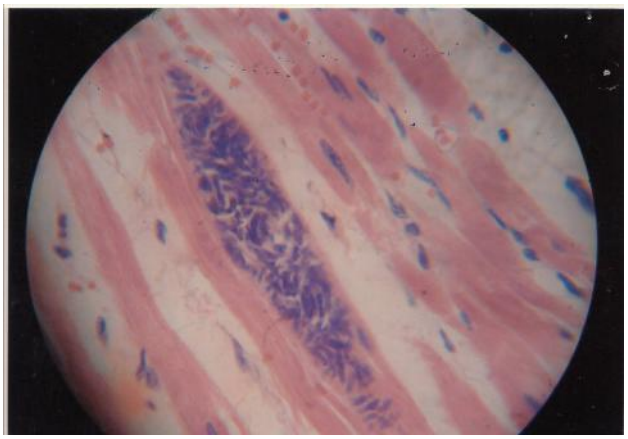

Fig (5) *S. cruzi* in cattle by H & E(x400).

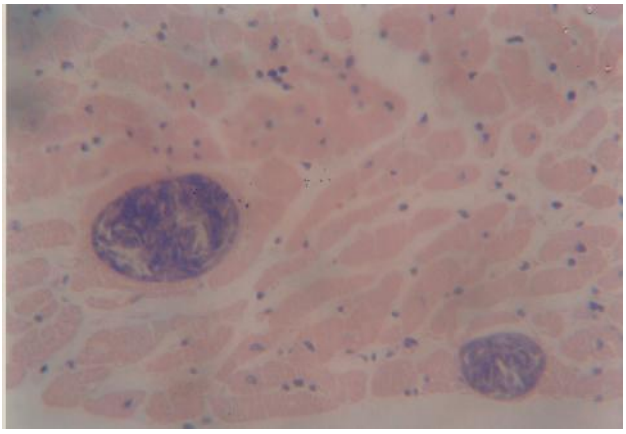

Fig (6). *S. leveni* in buffaloes H & E(x400).

Discussion

The result of macroscopic examination of buffaloes muscles (Table1) revealed that (25%) of examined samples were infected with *S. fusiformis*. Our result agreed with that reported by **(Asmaa *et al.,* 2013)** who found that *S. fusiformis* was (25.5%) in Assiut while in Sohag governorate it was (28%) **(Khalifa *et al.,* 2008)**. Lower incidence (4%) was recorded in Ismailia province by **(Eman and Mahi, 2006)**. **Elsayed *et al.,* (2010)** reported that *S.fusformis* was (17.2 %) in buffaloes aged 2 - 3 years old in Egypt. However, high prevalence rates (50%) was recorded by **(Elkelesh *et al.,* 2011**) in Egypt.

Our result was relatively similar (26.2%) to these isolated in Turkey **(Ozer, 1988)** and (20%) in Iran, **(Ghorbanpoor *et al.,* 2007).** However, high prevalence rate was recorded in other countries (88.2%) in China **(Xiao *et al.,* 1988)** and (86.6%) in India, **(Mohanty *et al.,* 1995)**. The difference in the incidence of *Sarcocystis* infection may be attributed to the difference in ecological conditions and hygiene in the localities were buffaloes are raised also the difference in the age groups.

While the results of macroscopic examination of muscles of 100 cattle showed that no cysts could be detected. This result agreed with the previous investigations by **(Nourollahi *et al.,* 2009; Nourani *et al.,* 2010** in Iran **and Badawy *et al.,* 2012)** in Sharkia Province, Egypt. However few studies found macroscopic cysts in cattle, as the result recorded by **(Fatma *et al.,* 2008)** in Assiut was 4% and **(Baz, *et al.,* 2012)** in Kafr El-Sheikh and Tanta. Macroscopic *Sarcocystis* was identified by 6% in each city. The lack of macroscopic *Sarcocystis* in cattle may be due to the fact that such cysts are feline origin and moreover contact between cattle and cats is not common. The age of cattle may have been the reason because it may take years to develop.

Examination of slaughtered animals clarified that oesophagus was the organ that, most frequently found to be infected, followed by the tongue, while the heart showed no macroscopic infection (22%, 3% and 0%) respectively in buffaloes. these results were relatively similar to that previously investigated by **(El-Dakhly *et al.,* 2011)** in Beni-suef Egypt, however **(Dayashanker, 1991)** reported that diaphragm was the most infected tissue followed by oesophagus. The results reported here indicated that all of examined buffaloes were infected with macroscopic *Sarcocystis* as well as infection with the microscopic forms; this implies that these animals were in contact with both felids and canids. The results of comparison between the prevalence of microscopic *Sarcocystis* spp. cysts in buffaloes and cattle were 75% and 80% of examined samples. Our result agreed with that reported by **(Latif *et al.,* 1999)** in Iraq (82.9% in buffaloes and 97.8%in cattle) and disagree with **(Mary, 2006)** in Egypt whose result was 96.5 % of examined buffaloes and 87.6% in cattle by trypsin digestion. Also **(Jehle *et al.,* 2009)** in Northern Vietnam found that the incidence of cattle (63%) while in water buffaloes it was (90%), and **(Latif *et al.,* 2013)** in Malaysia 36.2% in cattle and 66.7% for buffaloes by muscle squach. the differences in the results may be due to the technique used for detection of *Sarcocystis* spp., ecological conditions, age of examined animals and hygiene in the localities. Histopathological technique revealed microscopic cysts *S. levinei.* and *S. cruzi* in buffaloes and cattle. Microscopic cysts (*S. levinei*) was more prevalent than macroscopic cysts, (*S. fusiformis*), our results were agreed with **(El-Dakhly *et al.,* 2011),** Beni suef Egypt. *S. cruzi* is the most prevalent and pathogenic species in cattle where it causes abortion, loss of weight, neurologic sign, fever and death, **(Dubey *et al.,* 1989).**

The comparison between the four techniques for detection of *Sarcocystis* spp. revealed that Peptic digestion is the most accurate method when compared with the histopathological technique. These results agreed with those of **(Florencia and Mary, 2000; Saeid *et al.,* 2009 and El-Dakhly *et al.,* 2011).** Also peptic-digestion is more accurate than muscle squash in detecting *Sarcocystis*, especially the bradyzoit. On the other hand, the results agreed with

those of **(Latif *et al.*, 1999 and Hossein *et al.*, 2010)**. Although compression technique has low sensitivity compared with the previously mentioned methods, it is simple , rapid and can be used in large scale, these results agreed with that reported by **(Wahba, 1994 and Badawy *et al.*, 2012).**

The results of microscopic examination for sausage cleared that, the incidences of *Sarcocystis* bradyzoites were seen in 20 % of examined samples by Peptic digestion. Our results nearly similar to those of **(Yassien *et al.*, 1986)** who found that, the incidences of *Sarcocystis* in examined sausage samples collected from Alexandria provience reached to 23.3 % and higher than that, reported by **(Rahdar and Salehi, 2011)** where they found that the incidences of *Sarcocystis* in sausage reached to 8 % of the examined samples. There are little investigation on meat product contaminated with *Sarcocystis.*

Sarcocystin is a powerful toxin produced by zoitocysts of *Sarcocystis*. This toxin is effective on central nerves, heart, liver and adrenal glands of rabbit. It produces fever at low dose and produces severe diarrhea, collapse and death at high doses. The symptoms of disease such as vomiting, diarrhea, muscle weakness and paralysis are produced by sarcocystin.

However, it should be noted that *Sarcocystis* cysts have sarcocystin and using of infected meat in Sausage may ill a susceptible person. Human can get *Sarcocystis* through consuming under cooked meat and meat products of infected cattle **(Gholam and Eshrat, 2006 and Bobbi *et al.*, 2008).**

In normal state after slaughter the animal the skeletal muscle is subjected to complete rigor mortis which lead to increase muscle acidity (lactic acid) by glycolsis and muscle contraction. In the opposite direction decrease lactic acid production by glycolysis and increase pH value of infected muscle**. Azza *et al.*, (2011)** examined 25 samples of fresh meat chemically and found that the mean value of pH was 5.8 ±o.016 In respect to (TVB-N) and (TBA) were 12.5±1.2 and 0.275±0.02 (table 6) which were in normal level.

Table (5) shows that the minimum pH value was 5.58 and maximum 5 98.with a mean value of 5.8±0.016while the mean value of non infected were 5.3±0.33 (table 6) **(Mostafa and Yasin, 2010)**. were found the mean value of PH of infected meat was5.93±0.21 which exceed mean value of non infected samples in their investigation **(Baz *et al.*, 2012)** was found also the PH value of infected samples 5.79±0.02 and the non infected samples was 5.3±0.02.

Sarcocystis lead to esinophilic myositis **(Wouda *et al.*, 2006)**, oedema in affected muscle, high moisture and increase pH value **(Mostafa and Yasein, 2010).**

T.V. B- N is considered as an index of meat freshness and decomposition (**Byun *et al.*, 2003).**

The results which illustrated in table (5) revealed that the mean value of T.V.N-B of infected samples was 13.2 mg /100g ±0.69/100g, with a minimum value as 8.3mg/100gm and maximum was19.4mg /100gm which exceed the normal level as 12.5±1.2mg/100g (table 6).

T B A level has been used successfully to measure lipid oxidation during short- term storage.

Table (5) revealed that the mean value of T.B.A of freshly infected examined meat sample was 0.35±0.022mg/kg but the minimum value was 0.16 mg/kg and the maximum value was 0.53mg/kg while the normal level was 0.275±0.002mg/kg (table 6).

Our results was agreed with **(Baz *et al.*, 2012)** that he was found the mean value of chemical analysis of infected samples were exceed non infected samples.

From the previous results the infection of meat with *Sarcocystis* spp. cause series changes which lead to early incipient spoilage and lower the meat quality.

To safeguard the consumer from being infected adequate cooking by heating to a temperature sufficient to kill parasites inside the meat which is proposed to be 75ºC for at least 15 min **(El-Kelesh *et al.*, 2011).**

Legislation should include the freezing of raw meat for at least 10 days at -10ºC before manu-

facturing the various meat products; special those consumed the raw or under cooked .

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مقارنة طرق أستكشاف طفيل الساركوسيست في اللحوم الطازجة وبعض منتجاتها وتقييم جود تها

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الملخص العربى

أجريت هذه الدراسة على 00 2 عينة من أنسجة مختلفة من الحيوانات المذبوحة الطازجة من كل من الجاموس و الأبقار التي تشمل (القلب واللسان و العضلات المريء) التي تم جمعها من مسلخ الإسكندرية و 30 عينه من السجق تم شراؤها من مختلف محلات البقالة . تم الكشف الكامل على الحيوانات مباشره بعد ذبحها بالعين المجرده لملا حظه المتكيسات العضليه الظاهره وبعد ذلك تم اجراء الاختبارات الميكروسكوبيه بطريقة ضغط قطع من العضلات بقوة بين شريحتين وصبغ العصارة و بطريقه هضم العضلات بالببسين لاكتشاف وجود البرادى ذويت وكذلك بالضغط الخفيف بدون صبغة وا لفحص النسيجى لمعرفة انواع الساركوسيست المجهريه.هذا وقد تبين ان نسبه المتكيسات العضليه العينيه كانت 25% فى عينات الجاموس فقط بينما كانت نسبه العينات المجهريه في كل من الجاموس والأبقار (75 ٪ و 80 ٪) على التوالي بطريقه الهضم التى هى اكثر دقه بالمقارنه بطريقه ضغط العضلات بين شريجتين التى هى ابسط واسرع وبعد فقد تم اجراء وا لفحص النسيجى لاستبيان انواع الساركوسيست المجهريه والتى تميزت بوجود جدار رقيق لكل من الساركوسيست ليفينى وكروزى للجاموس والابقار على التوالى اما نسبه الساركوسيست فى عينات السجق فكانت 20% بطريقة هضم العضلات بالببسين لاكتشاف وجود البرادى ذويت وقد اجريت الاختبارات الكيميائيه (الاس الهيدروجينى ونسبه النيتروجين المتصاعد ونسبة الزناخه) على عينات اللحوم المصابه للكشف عن تاثير الساركوسيست على جودة اللحوم المصابه.