The Incidence of Sarcocystis in Slaughtered Food Animals

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A B S T R A C T

Sarcocystis affects the meat quality, rendering meat unmarketable and unacceptable to consumers. In the present study, total (2000) Slaughtered animals were examined during P.M inspection then the Macroscopic and Microscopic examinations were done for detection of Sarcocystis followed by Polymerase chain reaction (PCR) in El-Basateen automated abattoir in Cairo, Egypt. With special references to species, age, and sex of slaughtered animals, our study revealed that the higher incidence (macroscopic and microscopic) in old buffaloes (48.6%-63.2%) than young ones (41.2-53.8%), respectively followed by cattle, sheep finally camel. In addition to the higher incidence (macroscopic and microscopic) in female buffaloes (25.8%-36.2%) than male ones (37%-49.6%), respectively followed by cattle, camel finally sheep. The PCR confirmed the presence of Sarcocystis in examined samples. SO that, the work recommended that Using of Microscopic examination and PCR for detection and confirmation of Sarcocystosis in abattoirs to avoid animals and human infections with such zoonotic parasite.

Keywords: Sarcocystis, meat, slaughtered food animals, parasites, PCR.

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1. INTRODUCTION

Meat is the most important source of animal protein for a wide range of consumers (FAO, 2013). Nowadays, there has been increasing demand for red meat consumption through the whole world. Consequently, Abattoirs can play the most important role in management and control of many of zoonotic diseases, particularly the sarcocystis which may transmitted to human by consumption of raw or under cooked meat (Mohamed. Sara, 2013).

The pathogenic Sarcocystis was distributed wide world and considered as meat borne pathogens which can enter the food chain any time between farms to fork. Human consumption of raw and under cooked meat can cause (meat- borne illness) either by the parasite itself and/or by their toxins toxico-infection (Dubey et al., 2015).

Meat Sarcocystosis greatly lowers the meat quality, and meat grade rendering the meat and offal unmarketable and unacceptable to consumers., In addition to the great economic losses due to the condemnation of carcasses and /or offal in abattoirs, so that sarcocystosis was an important zoonotic food-borne parasitosis in humans and animals., (Ahmed et al., 2016).

Humans Eating raw or undercooked beef parasitized with zoonotic mature Sarcocysts of *S. hominis* resulted in human intestinal Sarcocystosis, the Symptoms appear within 3 days after raw meat ingestion may due to toxins released from invading bradyzoites in raw meat, Diarrhea and abdominal pain were the most common symptoms (Ortega and Cama, 2018).

There are two types of Human *Sarcocystosis* distributed worldwide human intestinal and human muscular types (Dubey et al., 2015). Human Intestinal Sarcocystosis: takesplaces due to that the humans eat raw or undercooked meat with the sarcocystis cysts. Man becomes the definitive host after the ingestion of raw beef infested with Zoonotic *S. hominis* can cause food- borne illness, symptomized as: gastro enteritis , watery diarrhea, abdominal pain, nausea, vomiting, the infections are asymptomatic and self-limiting, (Fayer et al., 2015).

Zoonotic Sarcocystis *spp*. have been described in domestic cattle, buffaloes, camels , sheep, goats , pigs , deers , birds and wild animals, These zoonotic S. Species have considerable Veterinary, Economic and Public Health

Importance (Ghoneim et al., 2014). *Sarcocystis* have a high specificity for their host Spreading and infestations depend on many factors such as: parasite spp., sex, age, season, virulence of parasite, doses of infestation, immune status of animal, raising conditions, animal spp., pasture and grassing systems, the pollution with sporocysts and their resistance in the environment, other stress factors, etc**,** (Mehlhorn, 2016).

Meat *Sarcocystosis* Considered meat- borne illness during routine veterinary macroscopic &microscopic P.M inspection of carcasses in abattoirs (Mohamed. Sara, 2013).

Nowadays, polymerase chain reaction (PCR) was the common confirmatory test, time/cost effective method used wide world (Gjerde et al., 2016).

Therefore the present study was aimed to detect the incidence of Sarcocystosis in Slaughtered animals at El-Basateen Automated Abattoir, Cairo, Egypt, using macroscopical and microscopical examinations followed by PCR for confirmation. Also, The Effect of Age and Sex on the incidence of Sarcocystis in Slaughtered food animals was discussed.

2. Materials and methods

2.1. Samples collection:

A total of 2000 Slaughtered food animals at El-Basateen Automated abattoir were examined during daily routine P.M inspection of buffaloes, cattles, sheep, and camels(500 each) were with different ages and sex (From January 2015 to february 2018) for detection of *Sarcocystis* using macroscopical, microscopical examination followed by PCR assay.

From Each animal five represented samples were examined esophagus, tongue, diaphragm, heart and Masseter muscles (50 gm of each) collected for the macroscopic and microscopic Sarcocysts examinations and PCR assay at Animal health Research institute, Parasitology Department , Dokky, Giza. The percentage of positive and negative samples microscopically were recorded. The positive Samples were kept at -20 ºC until applling of PCR assays as confirmatory test.

2.2. Macroscopical examination:

According to Ministry of Agriculture, Veterinary Services Authority of Egypt, (1986) the macroscopic sarcocysts were detected by gross inspection, palpation and incisions of the muscular tissues.

2.3. Microscopical examination: (Ahmed et al., 2016)

The Microscopic examination of Sarcocystis (Impression technique), (for both positive and negative macroscopically examined samples): stained with Giemsa stain, and examined by Light microscope (100×) for microscopic Sarcocystis cysts.

2.4. The Polymerase Chain Reaction: Singleplex PCR (Huissien et al., 2017)

2.4.1 The Primer sequences of Sarcocystis species used for PCR:

Application of PCR for identification of *18S rRNA* as species specific gene of Sarcocystis species specific for cattle, camel and sheep was performed essentially by using primers as shown in table (1).

2.4.2. DNA extraction (Silva et al., 2009):

The samples were minced by an electric meat grinder and 25-30 mg of each minced tissue was used following the manufacturer's instructions of a commercial DNA extraction kit (Qiagen,Valencia, CA, USA). The samples were resuspended in 180 µl ATL buffer and 20µl proteinase K (QIAamp DNA Mini Kit), and the protocol recommended for tissue samples was followed. All DNA extracts were stored at -20 ºC until used. This product was used as a template for PCR.

2.4.3. DNA amplification:

2.4.3.1. Amplification of 18S rRNA gene of cattle&buffaloe Sarcocystis (Daptardarkar et al., 2016):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). Accurately, PCR reactions were set up containing the PCR master mix of buffer, deoxynucleotides, DNA polymerase. To each PCR reaction, a 5µl of the genomic DNA (contain ~25 ng) and 10 µm (1.0 µl) of the primer was added. The PCR reaction conditions were as follow: initial denaturation for 2 min at 94 °C; 35 cycles with denaturation at 94 °C for 30 sec, cysts was applied by muscle squeeze method annealing at 52 °C for 30 sec and extension at 72°C for 30 sec; and final extension for10 min at 72 °C.

Extracted DNA samples were elecrophoresed through 1.5% agarose solution in 1x TBE electrophoresis buffer at 80 V for 100 minutes. Finally, the gel was stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

2.4.3.2. Amplification of 18S rRNA gene of camel Sarcocystis (Motamedi et al., 2011):

The PCR was performed (30 µl reactions) using 1 µl (10 pM) of each primer, 0.5 µl Taq polymerase, 0.5 µl dNTP, 2 µl MgCl2, 10 µl DNA, 3 µl buffer 10× and 12 µl distilled water. Reactions were carried out on an Eppendorf Master Cycler Gradient thermal cycler. The PCR required a preliminary denaturation at 94°C for 5 min. The remaining PCR steps were 35 cycles at 94°C (2 min), 57°C (30 sec), 72° C (2 min), with a single terminal step at 72°C (5 min). The amplified DNA fragments were analyzed by 1.5% of agarose gel electrophoresis in 1x TBE buffer stained with ethidium bromide.

2.4.3.3. Amplification of 18S rRNA gene of sheep Sarcocystis (Hamidinejat et al., 2014):

Briefly, amplification of the 18S rRNA gene was carriedout in 50 ll reaction volumes containing 1 ll of DNA template, 5 pmol of reverse and forward primers, 3.0 mM MgCl2, 5.0 ll 109 PCR Buffer, 200 lM of each dNTP and 2.5 U Taq DNA polymerase. The thermal program of PCR was as follows: 94 °C for 5 min, 30 cycles of 94° C for 2 min, annealing at 57 °C for 30 sec, and 72 °C for 2 min, followed by a final extension step at 72°C for 5 min. To verify the results, 10 ll of each PCR product was electrophoresed in a 1 % agarose gel, stained with safe stain and visualized on a UV transilluminator. The PCR products were identified by size using a 100 bp ladder

(Fermentas). The expected PCR product had a length of 609 bp.

2.4.4 PCR Analysis: PCR products were finally analyzed by the agarose gel electrophoresis, banding patterns were documented, as in (Table A) and the positive PCR products were stored at –20 °C till used for the Corresponding DNA sequencing.

2.4.5 DNA Sequencing: three representative samples from positive PCR products were selected for DNA sequencing in one direction using the forward primer (the same used in PCR amplification with primer 1). Sequencing reactions and protocols were performed according to manufacturer procedure using the ABI PRISM® 3100 Genetic Analyzer (Micron-Corp. Korea).

2.5. Statistical analysis:

According to method described by Kirkpatrick & Feeney (2013).

3. RESULTS

3.1. Macroscopic examination of Sarcocystis:

From the present study it was found that the large size Sarcocystis were (10-15 mm long x 0.7 – 6 mm width) and appeared as fusiform, oval , spindle, elongated, cucumber or rice seeds shaped, consists of opaque bodies, milky white in color, in between muscle bundles with the longitudinal axis of the muscle mass. In these organs, macrocysts were seen either just beneath the serosal surface, as in esophagus, or deep in the muscular layer, as in diaphragm and tongue and masseter muscles. The small sized Sarcocystis were (1 – 5 mm long x 0.1 – 0.6 mm width) and appeared as (Threads- like shaped) under perimysial connective tissue along the longitudinal axis of the myocytes as in Fig. (3): Macroscopic fusifomis White Sarcocystis (15m length) in Oesophagus of Buffaloe. Fig. (4): Macroscopic fusiformis shaped Sarcocystis cysts 10mm length in oesophagus of buffaloe. Fig. (5): Macroscopic fusiform shaped Sarcocystis 15 mm length in Tongue of Buffaloes. Fig. (6): Macroscopic Heavy infestation of spindle shaped Creamy White Sarcocystis 7 mm length in esophagus muscle of Cattle, Fig. (7): Macroscopic spiral shaped White Sarcocystis 6 mm length in Masseter muscle of Cattle Fig. (8): Macroscopic White cylindrical shaped Sarcocystis 9 mm length in esophagus of Camel.

3.2 Microscopic examination of Sarcocystis:

It found that mature *Sarcocysts* contained numerous bradyzoites and many peripheral metrocytes; all were (banana or crescent-shaped) with the anterior end more pointed than the posterior one. As in Fig (9): Bradyzoites (b) (banana-shaped) and metrocytes (m) (stumped and less curved) from Sarcocytis cyst of buffaloes (Giemsa stain) (X 1200). Fig. (10): Microscopic Stumped shaped Sarcocystis cyst in esophagus of buffaloes (X100) (300 X 91 µm) .Fig. (11): Microscopic Elongated cyst with rounded ends in diaphragm of camel. (X 100) (950 X 150 µm. Fig. (12): Microscopic Spindle shaped Sarcocystis cyst in esophagus of cattle. (X 100) (900 X 186 µm).Fig. (13): Microscopic Spiral shaped *Sarcocystis* cyst in diaphragm of cattle. (X 100). Outer layer appeared homogenous non-striated (arrows). (870 X 220 µm. Fig. (14): Microscopic Spiral shaped and elongated *Sarcocystis* cyst in masseter muscle of sheep (X 100). Outer layer appeared striated with perpendicular rods (arrows). (1432 X 190).

3.3 Incidence of Sarcocystis depending on Sex, in Table (2) and fig. (1):

Revealed that: The average incidence of both Macroscopic and Microscopic sarcocyts was for male 25.8% & 36.2%, for female 37% & 49.6%. Concerning to cattle the average incidence For male it was: 23.40 % & 28.60 %, for female cattle it was: 32.60 % & 44.60%, In camel the average incidence For male it was 16.60% & 25.80%, for female it

was 22.2% &30%, regarding to sheep the average incidence For male it was: 12% &16.60%, for female it was 15.40% & 24.80%.

3.4. Incidence of Sarcocystis depending to Age, in Table (3) and fig. (2):

It was found that the incidence of sarcocystis achieved for old buffaloes was: 48.6% macroscopic & 63.20% microscopic, for young buffaloes it was: 41.20% &53.80%, regarding to old cattle it was: 32.80 % &43.20 %, for young cattle it was: 23.20 % & 29.60 %, in old camel it was:13.6% & 25.8%, in young camel it was:5.8 &13%., in old sheep it was:13.20 % & 27.20%, in young sheep it was: 7. 80 % & 17.80%.

3.5. PCR Reading results (photograph 15, 16, and 17) & (table 1):

3.5.1. Incidence of Sarcocystis in cattle and buffaloes using PCR:

It was found that three samples out of five confirmed positive for cattle while four samples out of five were confirmed positive for buffaloes as resulted in Photograph (15): Agarose gel electrophoresis of PCR of 18S rRNA gene (570 bp) for characterization of *Sarcocystis s*pecies detected in cattle and buffaloe tissues.

3.5.2. Incidence of Sarcocystis in camels using PCR:

It was achieved that two samples out of five confirmed positive as resulted in Photograph (16): Agarose gel electrophoresis of PCR of 18S rRNA gene (539 bp) for characterization of *Sarcocystis s*pecies detected in camel tissues.

3.5. 3. Incidence of Sarcocystis in sheep using PCR:

It was found that, four samples out of five confirmed positive as resulted in Photograph (17): Agarose gel electrophoresis of PCR of 18S rRNA gene (609 bp) for characterization of *Sarcocystis s*pecies detected in sheep tissues.

Table 1: Target genes and primers used in PCR

Target genes	Primers	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
18S rRNA (Cattle&Buffaloe)	A18S (F)	5′ CGAATGGCTCATTAAAACAG ′3	570	Daptardarkar et al. (2016)
	A18S (R)	5′ CCAACTACGAGCTTTTTAAC ′3		
18S rRNA (Camel)	A18S (F)	5′ GCACTTGATGAATTCTGGCA ′3	539	Motamedi et al. (2011)
	A18S (R)	5′ CACCACCCATAGAATCAAG ′3		
18S rRNA (Sheep)	Sar (F)	5′ GCACTTGATGAATTCTGGCA ′3	609	Paikari et al. (2008)
	Sar (R)	5′ CACCACCCATAGAATCAAG ′3		

Table 2: Incidence of macroscopic and microscopic *Sarcocystis* in slaughtered food animals according to the Sex. (No. =500).

Species	Samples	Male		Female	
		+ve Macroscopic	+ve Microscopic	+ve Macroscopic	+ve Microscopic
Buffalo	Oesophagus	37	52	45	60
	Tongue	30	46	40	56
	Diaphragm	27	33	37	45
	Heart	15	20	26	37
	Masseter Ms.	20	30	37	50
	Average	25.8%	36.2%	37%	49.6%
Cattle	Oesophagus	38	44	41	60
	Tongue	25	30	35	51
	Diaphragm	21	27	33	40
	Heart	13	17	23	30
	Masseter Ms.	20	25	31	42
	Average	23.4%	28.6%	32.6%	44.6%
Camel	Oesophagus	24	35	29	34
	Tongue	17	25	23	30
	Diaphragm	14	21	20	27
	Heart	12	19	18	26
	Masseter Ms.	16	29	21	33
	Average	16.6%	25.8%	22.2%	30%
Sheep	Oesophagus	17	23	25	35
	Tongue	13	17	17	29
	Diaphragm	11	14	12	18
	Heart	7	11	8	13
	Masseter Ms.	12	18	15	29
	Average	12 %	16.6 %	15.4%	24.8 %

Table 3: Incidence of macroscopic and microscopic *Sarcocystis* in slaughtered food animals according to the age (No. =500).

Species	Sampels	Old age		Young age	
		+ve macroscopic	+ve microscopic	+ve macroscopic	+ve microscopic
Buffaloe	Oesophagus	57	76	51	65
	Tongue	50	69	45	60
	Diaphragum	48	68	42	54
	Heart	43	42	29	33
	Masseter ms.	45	61	39	57
	Average	48.6%	63.2%	41.2%	53.8%
Cattle	Oesophagus	44	54	32	35
	Tongue	36	46	29	32
	Diaphragum	33	43	28	32
	Heart	24	35	12	24
	Masseter ms.	27	38	15	25
	Average	32.8%	43.2%	23.2 %	29.60%
Camel	Oesophagus	19	41	9	18
	Tongue	15	29	7	12
	Diaphragum	18	19	6	10
	Heart	8	13	2	9
	Masseter ms.	13	27	5	16
	Average	13.6%	25.8%	5.8%	13%
Sheep	Oesophagus	16	33	12	22
	Tongue	14	30	9	21
	Diaphragum	13	27	8	20
	Heart	8	22	4	12
	Masseter ms.	5	24	6	14
	Average	13.2%	27.2%	7.8%	17.8%

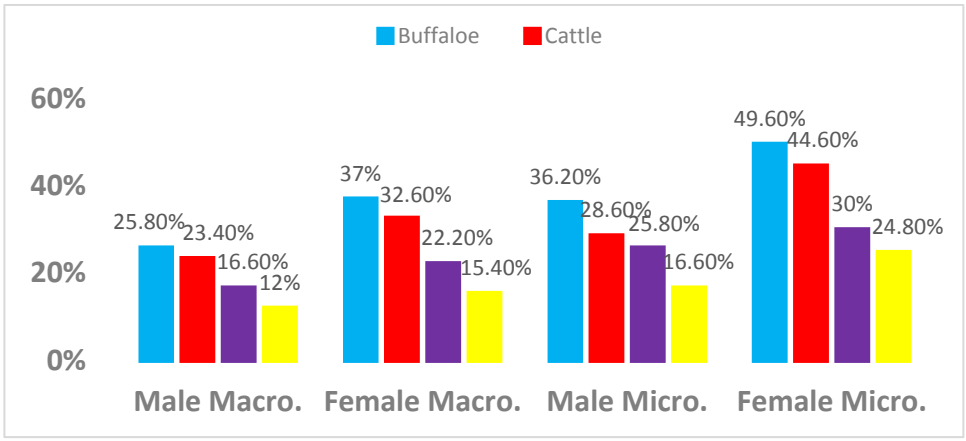

Fig.1. Incidence of macroscopic and microscopic *Sarcocystis* in slaughtered food animals according to the Sex.

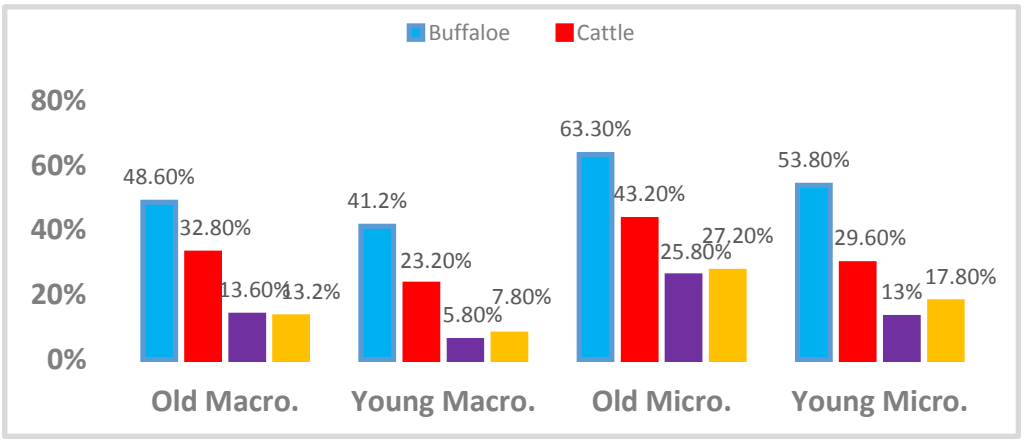

Fig.2. Incidence of macroscopic and microscopic *Sarcocystis* in slaughtered food animals according to the age.

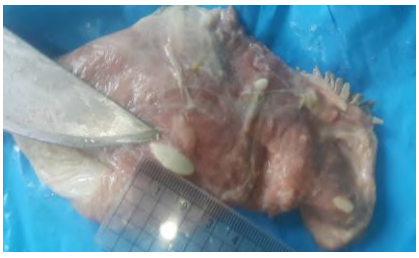

Fig.3. Macroscopic fusifomis White Sarcocystis (15mm length) in esophagus of Buffaloes

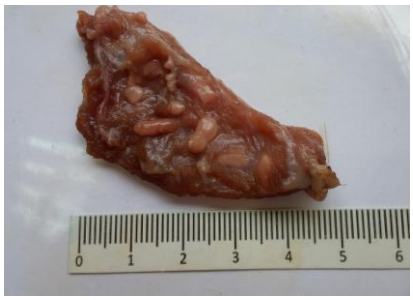

Fig.4. Macroscopic fusiformis shaped Sarcocystis cysts 10mm length in esophagus of buffaloes.

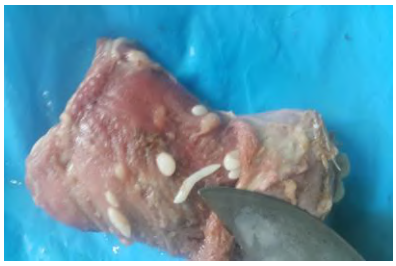

Fig.5. Macroscopic fusiformis shaped Sarcocystis 15 mm length in Tongue of buffaloes.

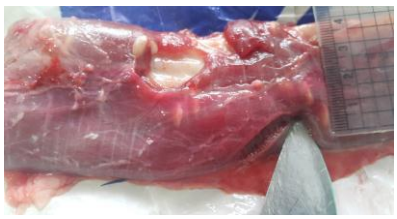

Fig.6. Macroscopic Heavy infestation of spindle shaped Creamy White Sarcocystis 7 mm length in esophagus muscle of Cattle

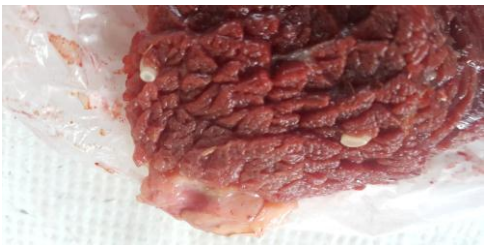

Fig.7. Macroscopic spiral shaped White Sarcocystis 6 mm length in Masseter muscle of Cattle

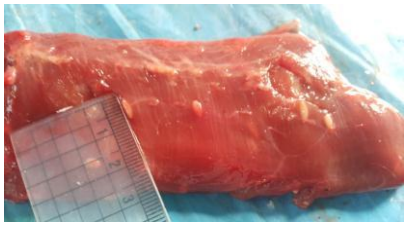

Fig.8. Macroscopic White cylindrical shaped Sarcocystis 9 mm length in esophagus of Camel.

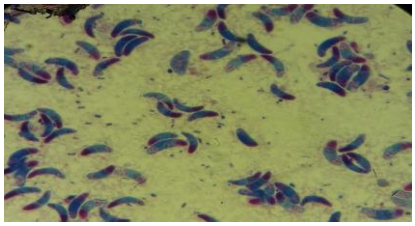

Fig.9. Bradyzoites (b) (banana-shaped) and metrocytes (m) (stumped and less curved) from Sarcocytis cyst of buffaloe (Giemsa stain) (X 1200).

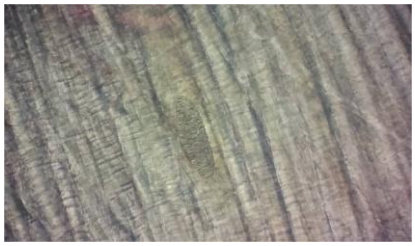

Fig.10. Microscopic Stumped shaped Sarcocystis cyst in oesophagus of buffaloe (X100) (300 X 91 µm) .

Fig.11. Microscopic Elongated cyst with rounded ends in diaphragm of camel. (X 100) (950 X 150 µm.

Fig.12. Microscopic Spindle shaped Sarcocystis cyst in oesophagus of cattle. (X 100) (900 X 186 µm).

Fig.13. Spiral shaped *Sarcocystis* cyst in diaphragm of cattle. (X 100). Outer layer appeared homogenous non-striated (arrows).(870 X 220 µm.

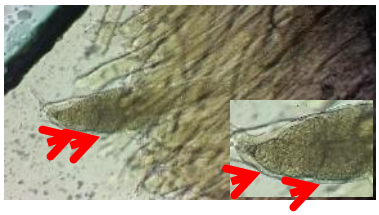

Fig.14. Spiral shaped and elongated *Sarcocystis* cyst in masseter muscle of sheep (X 100). Outer layer appeared striated with perpendicular rods (arrows). (1432 X 190) µm.

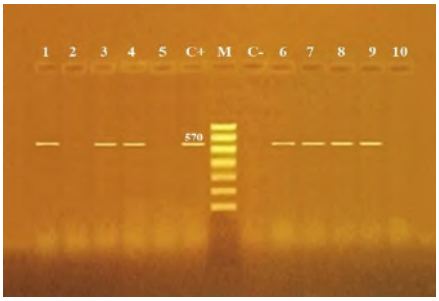

Fig.15. Agarose gel electrophoresis of PCR of 18S rRNA gene (570 bp) for characterization of *Sarcocystis* species detected in cattle and buffaloes tissues:

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive *Sarcocystis* species for 18S rRNA.

Lane C-: Control negative.

Lanes 1, 3 & 4: Positive bands for bovine specimens.

Lanes 2 & 5: Negative bands for bovine specimens.

Lanes 6, 7, 8 & 9: Positive bands for buffalo specimens.
Lane 10: Negative band for buffalo specimens.

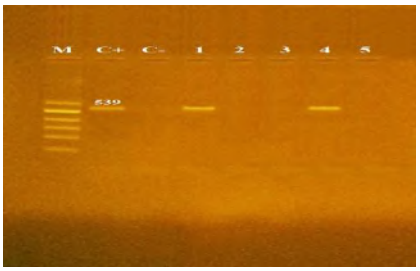

Fig.16. Agarose gel electrophoresis of PCR of 18S rRNA gene (539 bp) for characterization of *Sarcocystis* species detected in camel tissues:

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive *Sarcocystis* species for 18S rRNA.

Lane C-: Control negative.

Lanes 1 & 4: Positive bands for camel specimens.

Lanes 2, 3 & 5: Negative bands for camel specimens.

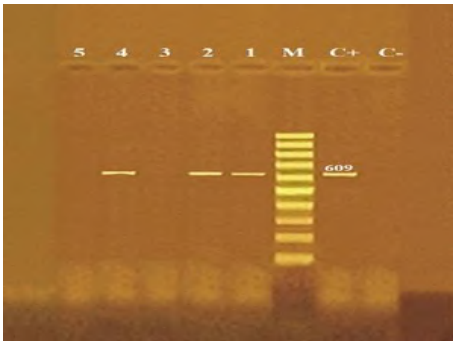

Fig.17. Agarose gel electrophoresis of PCR of 18S rRNA gene (609 bp) for characterization of *Sarcocystis* species detected in sheep tissues:

Lane M: 100 bp ladder as molecular size DNA marker.

Lane C+: Control positive *Sarcocystis* species for 18S rRNA.

Lane C-: Control negative.

Lanes 1, 2 & 4: Positive bands for sheep specimens.

Lanes 3 & 5: Negative bands for sheep specimens.

4. DISCUSSION

4.1. Macroscopic Sarcocysts Morphology:

The present work found that as in fig. (3), (4), (5), (6) and (7), the macroscopic examination of buffaloes and cattle revealed that the large size Sarcocystis were 5-15 mm long x 0.7 – 6 mm width and appeared as fusiformis or oval or cucumber or rice seeds spindle shaped white or creamy colour the small size Sarcocystis were 1 – 5 mm long x 0.1 – 0.6 mm and appeared as threads- like shaped under perimysial connective tissue along the longitudinal axis of the myocytes. Our results was nearly similar to the same macroscopic pictures described by El-Dakhly et al. (2011) in Egypt.

Concerning to camel, the results revealed that as in: Fig. (8): the cylindrical or elongated white Sarcocystis or white creamy Sarcocystis 7 mm length in esophagus muscle of camel. These results are similar to the same results obtained by Motamedi et al. (2011).

Regarding to sheep, the macroscopic Sarcocystis appeared as Oval or Rice- like shaped Sarcocystis 4.5 mm length in esophagus of Sheep. The same results were obtained by EL-Mishmishy (2017) in Dakahlia provence.

4.2. Microscopic Sarcocysts Morphology:

The present study revealed that as in Fig.(9) the presence of abundant of (banana-shaped) Bradyzoites and metrocytes (m) (stumped and less curved) from Sarcocytis cyst of buffaloe (Giemsa stain)(X 1200), Fig. (10): Microscopic Stumped shaped Sarcocystis cyst in esophagus of buffaloes (X100) (300 X 91 µm).The current study results nearly similar to these obtained by El-Dakhly et al., (2011) in Egypt, who found that fusiformis and stumped shaped Sarcocystis in esophagus of buffaloes.

Concerning to cattle, the microscopic cysts appeared as in Fig. (12): Microscopic Spindle shaped Sarcocystis cyst in esophagus of cattle. (X 100) (900 X 186 µm).Fig. (13): Microscopic Stumped shaped Sarcocystis cyst

in heart of cattle. (X 100)(430 X 140 µm).These results are similar to the results obtained by Badawy et al. (2012) at Sharkia Province, Egypt.

Regarding to camel, the study achieved that as in Fig. (11): Microscopic Elongated Sarcocystis cyst with two rounded ends in esophagus of camel. (X 100) (1200 X 180 µm).the present results in camel are similar to the results obtained by Wahba et al. (2014).

In sheep, it was found that the microscopic Sarcocystis as in Fig. (14): Spiral and elongated Sarcocystis cyst in masseter muscle of sheep (X 100).Outer layer appeared striated with perpendicular rods (arrows). (1432 X 190 µm).These results are similar to the same results obtained by EL-Mishmishy (2017).

4.3 Incidence of Sarcocystis depending on Sex of animals in Table (1) and fig. (1):

In the present study, The results achieved that: higher incidence in female buffaloes than in male, for female buffaloes the incidence of macroscopic and microscopic it was: 37% & 49.6%.,while in male buffaloes it was: 27% &48.8%, Nearly the same results agreed with the previous studies obtained by: Abu-Elwafa et al. (2012) with (48 %) incidence in Elsharkia province. high incidence was recorded by Ghonim et al. (2014) with (69%) in Cairo and Giza abattoirs, Egypt. Hanan. (2016) (69%) in female and (62%) in male buffaloes In Beni-Suef provence. and El-Dakhly et al. (2011) (78.9%) in Beni-Suef Governorate, Egypt. On contrary to very low incidence recorded by Ahmed et al. (2016) with incidence of (8.33%) at El-kharga abattoir-New vally province, Egypt.,

In different organs, the highest incidence of Sarcocystis macroscopic and microscopic in buffaloes was recorded in esophagus 60% followed by tongue 56% and, diaphragm50% masseter 45%, finaly heart muscles 37% So that the esophagus was considered the most common sites for sarcocystosis in buffaloes this agreed with previous studies obtained by Ghoneim et al., (2014), who found that, 63% in esophagus, 58%tongue, 51% diaphragm and 44% masseter.

Higher incidence in organs were in esophagus this agreed with previous studies obtained by: Ahmed et al. (2016) with 76.3% oesophagus incidence at Elgharga abattoir-New vally, province And Ghonim et al.(2014) 69% in Egypt.

Concerning to cattle, the present results revealed higher incidence in female than in male, for female cattle it was: 32.60 % and 44.60%., for male cattle were: 23.40 % and 28.60 %. This nearly agreed with previous studies obtained by: Abu-Elwafa et al. (2012) 48.36%., Korany. Hanan, (2016) 50% in female and 45% in male cattle, Ghonim et al. (2014) 60%, and Ali (2013) 68.9% in Qena province. Egypt.

Regarding to camel the present work recorded higher incidence in female than in male for female it was: 16.80% macroscopic and 30% microscopic. for male The incidence was: 16.60 % macroscopic and 25.80% microscopic., This present results revealed that the higher incidence in female than in male camels in oesophagus this nearly agreed with previous studies recorded by: Ali (2013) 48.7% in Qena province., Wahba et al. (2014) 68.8% in El- Basateen abattoir Egypt.,Abd-Elmalek et al.(2015)55 % in Assuit provence, Egypt. While this results disagreed with these reported by Al-Ani and Amr (2017) 6.8% in Jourdan.

Regarding to sheep the present results achieved that higher incidence in female than in male, for female it was:

11.20%macroscopic and 18.80% microscopic. For male it was:12.09% macroscopic and 16.60% microscopic., These results agreed with the previous studies obtained by Korany. Hanan, (2016) 24% in female and 21 % in male sheep in Beni-Suef provence.

The present work results disagreed with the very higher incidence of sarcocystosis in sheep recorded by Ali (2013) 65.6% in Qena province, (El- Mishmishy 2017) 95.37% microscopic incidence and 0.74% macroscopic incidence in Al-Dakahlia province.

In our study The incidence in female animals was higher than in male ones due to female is mainly with low immunity due to pregnancy and lactation stress which suppressed immune system this agreed with these recorded by Korany .Hanan, (2016) .

4.4 Incidence of Sarcocystis depending on Age in Table (2) and fig. (2):

The results found that higher incidence in old buffaloes than in young, for old buffaloes it was: 48.6% and 63.20%., while in young the incidence of macroscopic and microscopic was: 41.20% and 53.80%.

The results are nearly agreed with these obtained by Hanan (2016) 78% in old & 67 % in young buffaloes, El-Seify et al. (2014) with 68.2% in old & 17.2% in young in El-Gharbia province, Egypt.

Regarding to cattle our results revealed that higher incidence in old cattle than in young. For old the incidence it was: 32.80% & 43.20 %., in young it was: 23.20 % & 29.60 %.

This results agreed with previous studies obtained by Korany. Hanan, (2016) 58% in old & 37% in young cattle In Beni-Suef province.

Concerning to camel our results revealed that higher incidence in old camel than in young. For old camel, it was: 13.6% macroscopic&25.8% microscopic. For young it was: 5.8% &13%. The results agreed with previous studies obtained by: Abd-Elmalek et al. (2015) with incidence 55% in Assiut province, Wahba et al. (2014) 68.8% in El-Basateen abattoir Egypt.

In sheep the results revealed higher incidence in old sheep than in young for old it was: 13.20% macroscopic & 27.20% microscopic. For young it was: 7.80 % & 17.80%. This agreed with the results obtained by Korany. Hanan, (2016) who recorded 32% in old & 23% in young sheep in Beni-Suef province.

The higher incidence in old animals than in young may be due to in old ages the longer and repeated exposure periods to the millions infectious sporocysts , Contaminated the feed, drinking water, and pastures. The continous close -contact between animals and final hosts (dogs and cats) abundantly was keeping the life cycle of Sarcocystis, also, the high resistance of sporocysts to harsh environmental conditions which is the main sources of infestation for animals. So that, the higher incidence in slaughtered food animals, also due to lower immunity in old ages Ahmed et al. (2016).

Moreover El-Basateen abattoir is located in a desert area where stray dogs and cats were abundant Which continouslly shedding millions of infective sporocysts in the environment.,contaminating the food and water of food animals.

4.5. PCR Reading Results in *(photograph (1), (2) and (3) :*

In the present study the PCR amplification of the 18S rRNA gene region was conducted to confirm the positive samples., PCR achieved

that the sarcocystis in buffaloes four samples out of five was confirmed positive followed by cattle three samples out of five was confirmed positive, in sheep three samples out of five was confirmed positive, finally camel two samples out of five were confirmed positive. The present PCR results agreed with The nearly Similar previous results obtained by Abd-ElNaby et al.(2016)70.9% incidence for buffaloe at Alexandria province, EL Seify et al.(2014) 99% incidence in El Mahala - Abattior at El-Gharbia province Egypt., and Ashmawy et al.(2014)67.6%incidence for buffaloes at Alexandria province, Egypt.

5. Conclusion

Our study concluded that the higher incidence of Sarcocystis in El -Basateen abattior were achieved in female than male, also in old than young animals, moreover the microscopic was higher than macroscopic Sarcocystis incidence. Which have puplic health and economic losses.SO that the Routine P.M meat inspections in Abattoirs in Egypt were un-sufficient for detecting Sarcocystosis, this may be due to the presence of more hidden microscopic Sarcocysts, which unapparent macroscopically. Therefore, we strongly recommended that the using of microscopical examination and molecular characterization (PCR) for Sarcocystis diagnosis in abatiors, to avoid human infection of such zoonotic parasites and prevent & control the sarcocystosis.

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