Parasitic affections of edible offals of slaughtered animals at El-Shohada abattoir, Menofia governorate, Egypt

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A B S T R A C T

A total of 5853 animals slaughtered at El-Shohada abattoir, Menoufia governorate, Egypt represented by 670 camel, 340 cattle, 366 buffalo, 3188 young cattle, 502 young buffalo, 544 sheep and 243 goats were inspected from 21 Dec., 2017 to 20 Dec., 2018 to evaluate the incidence of some parasitic infections (Hydatidosis, Fascioliasis, Cysticercosis and Sarcocystosis) according to seasonal variation. Otherwise, the study aimed to detect the microscopic form of sarcosystic affection in 18 esophageal samples from 6 buffalo (3 suspected and 3 apparently healthy), 3 cattle, 3 sheep, 3 goats and 3 camel by using Polymerase Chain Reaction (PCR). The incidence of hydatidosis was 18%, 0.3% and 0.5% in camel, cattle and buffalo, respectively while it couldn't be detected in young cattle, young buffalo, sheep and goat. The highest total incidence of hydatidosis was recorded in winter (13%). The incidence of fascioliasis was 0.9% in camel, 0.8% in buffalo, 1.2% in cattle and 0.1% in young cattle, but it couldn't be detected in young buffalo, sheep and goat. The cysticercosis couldn't be detected in edible offals of slaughtered animals during the period of the study. *Sarcocyst fusiforms* was detected in edible offals (22.9% in esophagus and 3.7% in tongue) of 62.8% of buffaloes and 0.2% of the young ones with the highest incidence (42%) was recorded in autumn. PCR reading showed that all 3 samples confirmed positive for suspected buffaloes, one of 3 samples confirmed positive for apparently healthy buffaloes, one of three samples confirmed positive for cattle, 2 of 3 samples confirmed positive for sheep, one of 3 samples confirmed positive for goat and three samples were confirmed negative for camel. The present study concluded that there is a need to educate consumers, food handlers and all others who have access to food about the importance of hygiene and it is necessary for cooking food properly.

Keywords: *Incidence, Hydatidosis, Fascioliasis, Cysticercosis, Sarcocystosis.*

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1. INTRODUCTION

Food And Agriculture Organization and also, World Health Organization (FAO/WHO, Codex Alimentarius Commission) defined the edible offal of slaughtered animals as offals that have been passed as fit for human consumption. According to Kenya Standard Offal – Specification, the edible offal are those parts of the carcass that has been passed as fit for human consumption. In the case of food animals, these include red offal, green offal and white offal. Red offal include heart, liver, kidney, spleen, tongue, lungs, pancreas.

Green offal includes the rumen, reticulum, omasum, abomasum, small intestines, large intestines, colon, and gizzards. White offal includes the brain, spine, bone marrow, testicles and teats. Zoonotic parasites can be classified into four categories, direct zoonotic parasites which infect human directly from animals. Metazoonotic parasites as *Fasciola spp*. which can infect humans from invertebrate intermediate hosts. Cyclozoonotic parasites have vertebrate intermediate hosts and include *Echinococcus granulosus* and *Taenia saginata*, finally saprozoonotic parasites can infect human from soil or water (Ahmed, 2014). Hydatidosis is a widespread zoonosis infesting many animals and human. Even though the dog tapeworm (Echinococcus granulosus) is the smallest cestode, It has the largest metacestode. The intermediate hosts are herbivorous animals as cattle, sheep and camels. The final host is dog (Bouree, 2001). Fascioliasis is a foodborne zoonotic disease caused by Fasciola species which is a trematode predominantly found in ruminants (cattle, buffalo, sheep and goats), but can also infect humans (Ashrafi *et al.*, 2014). Fascioliasis reduces animal productivity, weight gain and the production of meat and milk. In addition, it causes moderate icterus, metabolic disorders, and secondary infections due to decrease immunity by chronic fascioliasis and liver condemnation during postmortem inspection in slaughterhouses, while the acute fascioliasis may lead to mortalities (Mason, 2004). Bovine cysticercosis is a disease caused by *Teania saginata* larvae as one of the major parasitic diseases. Cattle act as intermediate host; while man is a definitive host. The disease has worldwide distribution and transmitted to human mainly by consumption of raw or undercooked meat. The lungs, heart, liver, tongue, shoulder muscle, masseter muscle and diaphragm are the predilection sites for this parasite. Eating of cooked meat and proper hygienic practices should be incorporated in the prevention and control of the disease (Alemneh *et al.,* 2017). Sarcocystis infection is a parasitic zoonosis, which may cause acute and fatal clinical diseases in susceptible animal. When raw or undercooked infected beef is consumed by man, it may result in intestinal sarcocystosis (Ifeoma *et al.*, 2013). Sarcocystosis infections in human and animals are caused by single-celled, cyst-forming, intracellular protozoan parasites in the family of Sarcocystidae (Hamidinejat *et al.*, 2015). Among different known species of Sarcocystis in cattle, only *Sarcocystis hominis* is important from the public health viewpoint, because of its zoonotic characteristics (Hajimohammadi *et al.,* 2014). The parasite is transmitted either fecal-orally or by the ingestion of undercooked meat containing sarcocysts (Scott, 2004). The current study focused to evaluate the incidence of hydatidosis, fascioliasis, cysticercosis and sarcocystosis in edible offals of slaughtered animals from 22 Dec., 2017 to 21 Dec., 2018 during detailed post- mortem examination and detection of microsarcocysts by using Polymerase Chain Reaction according to the following objects:

1- Estimation of the incidence of the studied parasitic affections in food slaughtered animals and in its edible offals.
2- Estimation of the incidence of parasitic affections according to seasonal variation.
3- Detection of microsarcocysts by using Polymerase Chain Reaction.

2. Materials and methods

2.1. Collection of samples:

A total of 5853 animals slaughtered at El-Shohada abattoir, Menofia governorate, Egypt represented by 670 camel, 340 cattle, 366

buffalo, 3188 young cattle, 502 young buffalo, 544 sheep and 243 goats were examined from 21 Dec., 2017 to 20 Dec., 2018 for detailed postmortem inspection of parasitic infections (Hydatidosis, Fascioliasis, Cysticercosis and Sarcocystosis).

2.2. Postmortem examination:

Post mortem examination in slaughtered food animals was carried out through visual inspection, palpation and incision of edible offals for detection of hydatidosis, fascioliasis, cysticercosis and macroscopic sarcocysts according to Gracey (1986).

2.3. Detection of microsarcocysts in examined carcasses by using Polymerase Chain Reaction (PCR):

Application of PCR for identification of D2 region in conserved regions *18S rRNA* as species specific gene of Sarcocystis species for buffalo, cow, sheep and goat as well as camel was performed essentially by using primers as shown in the table (A).

Target genes	Primers	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	Reference
18S rRNA (Buffalo, cow, sheep & goat)	SAD 2 (F)	5′ GGAAGCGATTGGAACC ′3	350	Latif et al. (2015)
	SAD 2 (R)	5′ CCTTGGTCCGTGTTTCA ′3		
18S rRNA (Camel)	A18S (F)	5′ GCACTTGATGAATTCTGGCA ′3	539	Motamedi et al. (2011)
	A18S (R)	5′ CACCACCCATAGAATCAAG ′3		

3.1. 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.

3.2. DNA amplification:

3.2.1. Amplification of 18S rRNA gene of buffalo, cow, sheep and goat Sarcocystis (Latif et al., 2015):

The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). Accurately, reaction mixture consisted of 200 pM of each primer, 25µl of 2X PCR Master Mix, 2µl DNA template, and topped with nuclease-free water to a volume of 50µl. The thermal profile consisted of initial denaturation at 94°C for 2minutes, followed by 40 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 55°C, and 1 minute extension at 72°C, with 10 minutes final extension at 72°C. The amplified DNA products 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.

3.2.2. 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.

3. RESULTS

The results revealed that the total incidence of hydatidosis during the period of the study was 2.2%. The incidence of hydatidosis is higher in camel (18%) than cattle (0.3%) and buffalo (0.5%), but couldn't be detected in young cattle, young buffalo, sheep and goat (Table, 1). The incidence in lungs (8.7%) was higher than in livers (0.4%) and hydatid cysts couldn't be detected in kidneys (Table 2). According to seasonal variation, the highest incidence in camels was recorded in winter (21%), while in cattle was recorded in spring (1%), but in buffalo was in summer (1.8%) (Table 3). The results revealed that the total incidence of fascioliasis was 0.26%. The incidence of fascioliasis is 0.9% in camel, 0.8% in buffalo and the incidence was higher in cattle (1.2%) than young one (0.1%). Fascioliasis couldn't be detected in young buffalo, sheep and goat (Table 1). Meanwhile, the highest incidence in buffalo, cattle, young cattle and camel was in spring (1.7%), winter (4.8%), spring (0.2%)and winter (1.8%), respectively (Table 4). Cystecercosis couldn't be detected in edible offals of slaughterd animals during post-mortem inspection (Table 1). The results showed that the total incidence of macroscopic sarcocystosis was 3.9%. The incidence of macroscopic sarcocystosis was higher in buffalo (62.8%) than young buffalo (0.2%) (Table 1). In buffalo, the incidence was higher in esophagus (54%) than in tongue (8.7%), but in young buffalo, the esophagus was only infected (0.2%) (Table 5). The autumn is considered the season of the higher incidence (81%) and (1.5%) of macroscopic sarcocystosis in buffalo and young ones, respectively (Table 6). The results of PCR for detection of microscopic sarcosystosis in 15 esophageal samples in photograph (1) showed that three samples of suspected buffaloes were confirmed positive by PCR, while one sample out of three were confirmed positive for apparently healthy buffaloes. One sample out of three was confirmed positive for cattle. Two samples out of three were confirmed positive for sheep. One sample out of three was confirmed positive for goat. The result of PCR for three samples from camel in photograph (2) showed that all samples were confirmed negative.

Table1: Incidence of parasitic affections (Hydatidosis, Sarcocystois, Fascioliasis and Cysticercosis) of edible offals in food slaughtered animals during detailed post- mortem examination.

Species	No of examined carcasses	Parasite affections							
		Hydatid cyst		Sarcocyst		Fasciola		**Cysticercus**	
		+ve No.	(%)	+ve No.	(%)	+ve No.	(%)	+ve No.	**(%)**
Camel	670	123	18%	Couldn't be recorded		6	0.9%		
Cattle	340	1	0.3%			4	1.2%		
Buffalo	366	2	0.5%	230	62.8%	3	0.8%		
Young cattle	3188			Couldn't be recorded		2	0.1%	Couldn't be recorded	
Young buffalo	502			1	0.2%				
Sheep	544	Couldn't be recorded		Couldn't be recorded		Couldn't be recorded			
Goat	243								
Total	5853	126	2.2%	231	3.9%	15	0.26%		

Table 2: Incidence of hydatid cyst in edible offals of slaughtered animals during detailed post-mortem examination.

Animal spp.	No. of slaughtered	Affected offals					
		Lung		Liver		Kidney	
		Positive NO.	(%)	Positive NO.	(%)	Positive NO.	(%)
Camel	670	117	17%	6	0.9%		
Cattle	340	1	0.3%	Couldn't be recorded		Couldn't be recorded	
Buffalo	366	2	0.5%				
Total	1376	120	8.7%	6	0.4%		

Table 3: Incidence of hydatid cyst according to seasonal variation from 22 Dec. 2017 to 2018.

Animal Spp.	Season											
	Winter			Spring			Summer			Autumn		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
Camel	223	47	21%	266	51	19%	147	23	15%	34	2	5.9%
Cattle	62	-	-	97	1	1%	47	-	-	134	-	-
Buffalo	65	-	-	115	-	-	112	2	1.8%	74	-	-
Total	350	47	13%	478	52	10.9%	306	25	8%	242	2	0.8%

Table 4: incidence % of Fasciola species in liver of different slaughtered animals according to seasonal variation from 22 Dec., 2017 to 21 Dec., 2018.

	Winter			Spring			Summer			Autumn		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
Buffalo	65	-	-	115	2	1.7%	112	1	0.8%	74	Couldn't be recorded	
Cattle	62	3	4.8%	97	1	1%	47	-	-	134		
Young cattle	762	-	-	980	2	0.2%	831	-	-	615		
Camel	223	4	1.8%	266	2	0.8%	147	-	-	34		
Total	1112	7	0.6%	1458	70	0.5%	1137	1	0.1%	857		

Table 5: Incidence % of Sarcocyst fusiforms in edible offals of old and young slaughtered buffalo from 22 Dec., 2017 to 21 Dec., 2018.

Animal spp.	slaughtered No.	Affected offals					
		Esophagus		Tongue		Total	
		Positive NO.	(%)	Positive NO.	(%)	Positive NO.	(%)
Old buffalo	366	198	54%	32	8.7%	230	62.8%
Young buffalo	502	1	0.2%	Couldn't be recorded		1	0.2%
Total	868	199	22.9%	32	3.7%	231	26.6%

Table 6: Incidence % of Sarcocyst fusiforms on Tounge and oesophagus of old and young buffalo carcasses during detailed post- mortem examination from 22 Dec., 2017 to 21 Dec., 2018.

	Winter			Spring			Summer			Autumn		
	No.	+ve	%	No.	+ve	%	No.	+ve	%	No.	+ve	%
Old Buffalo	65	33	50%	115	77	66%	112	60	53%	74	60	81%
Young Buffalo	122	-	-	188	-	-	124	-	-	68	1	1.5%
Total	187	33	17%	303	77	25%	236	60	25%	142	61	42%

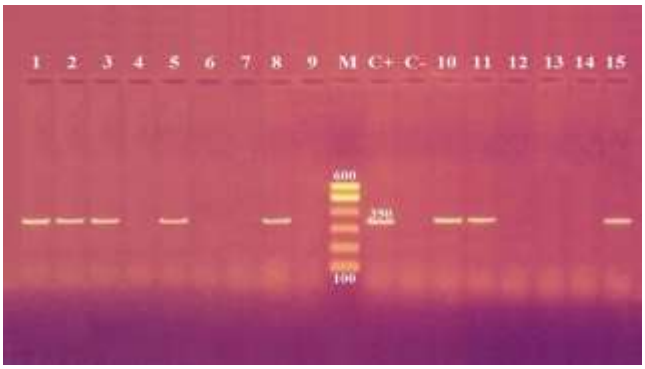

Fig.1. Agarose gel electrophoresis of PCR of 18S rRNA gene (350 bp) for identification of Sarcocystis species in buffalo, cow, sheep and goat tissues.

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 & 3 (suspected buffalo), 5 (apparently healthy buffalo), 8 (cow), 10, 11 (sheep) & 15 (goat): Positive bands for Sarcocystis species. Lanes 2, 6 (apparently healthy buffalo), 7, 9 (cow), 12 (sheep), 13 &14 (goat): Negative bands for Sarcocystis species.

Fig.2. Agarose gel electrophoresis of PCR of 18S rRNA gene (539 bp) for identification of Sarcocystis species 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, 2 & 3: Negative bands for *Sarcocystis* species.

4. DISCUSSION

The regular re-evaluation of the current status of zoonotic parasitic diseases is needed to obtain enough information that will provide inputs in the design and implementation of control strategies (Komba, 2017). The results revealed that the total incidence of hydatidosis was disagreed with high incidence (4.23%) recorded in Tibet Autonomous Region (TAR) and lower incidence (0.24%) recorded in China by Li et al. (2019). The highest incidence of hydatid cyst recorded in camel was nearly similar to the incidence (16%) that was recorded in Mekka-Al-Mukarama by Haroun et al. (2010). This result disagreed with low incidence (2.53%) recorded by Haridy et al. (2006) in Egypt and high incidence (40%) recorded by Khan et al. (2001) in Libya. In addition, the incidence in offals of slaughtered camels agree with Shahat, (2000) and Dyab et al. (2005) in Egypt who mentioned that the lung was the predominant predilection site for *Echinococcus granulosus* larvae. In addition, the highest incidence in camels disagrees with Dyab et al. (2005) who mentioned that the highest incidence in Egypt was recorded in summer. The results revealed that the incidence of hydatid cyst in cattle was nearly similar to the incidence (0.4%) that was recorded in Nigeria by Tijjani et al. (2010). This result disagrees with Dyab et al. (2005) in Egypt who couldn't record any hydatid cyst and Beyene and Hiko (2019) who recorded that the incidence was 33.3% in Ethiopia. Higher result was recorded by Chihai et al. (2016) who recorded that the incidence was 59.3% in the Republic of Moldova. In addition, the incidence of hydatid cysts in offals of slaughtered cattle disagree with Salem et al. (2011) who reported that the most predilection site was the liver and Mitrea et al. (2012) who recorded three cattle had cysts in kidney. In addition, the incidence in slaughtered cattle according to seasonal variation disagrees with Berhe (2009) who reported that the prevalence of hydatidosis was the highest in July and the lowest in April (25.11%). Concerning buffalo, the results revealed that the incidence of hydatid cyst disagree with Dyab et al. (2005) who couldn't record any hydatid cyst in Egypt and Samavation et al. (2009) who recorded that the incidence was (31.87%) in buffalo. In addition, the incidence according to seasonal variation in buffalo disagree with Beyhan and Umur (2010) who could detect hydatid cysts in the liver of slaughtered buffalo. The results revealed the incidence in sheep disagree with very high result (61.9%) recorded by Chihai et al. (2016) in the Republic of Moldova. Low incidence (0.3%) and (1.06%) were recorded by Haridy et al. (2006) in Egypt and Esam Almalki et al. (2017) in Saudi Arabia, respectively. Regarding goats, the results disagree with very high incidence (40%) recorded by Khan et al. (2001) in Libya and low incidence (0.68%) recorded by Haridy et al. (2006) in Egypt. The variation in hydatidosis incidence rate may be due to changes in the environmental condition in different countries, the applied hygienic measures, the methods of control inside abattoirs, the contamination rate in the intermediate hosts, the number of dogs in each area, the slaughtering manner and the feeding status of slaughtered animals. The results showed that the total incidence of fascioliasis disagree with Kouam et al. (2019) who mentioned that the incidence of fascioliasis was 22.62% in Cameroon. The results of the incidence of fascioliasis in slaughtered animals disagree with Abraham and Jude (2014) who found that 36% (126/350) of goat had fascioliasis at Calabar abattoirs in Nigeria, Vreni et al. (2014) who recorded that the prevalence of fascioliasis was 23% in sheep in south-eastern Lake Chad area and Elshraway *et al.* (2017) who mentioned that the incidence of fascioliasis

was 30.88% (695/2251) in cattle and bovine carcasses in Egypt. In addition, the incidence in cattle, buffalo and camel according to seasonal variation disagree with Kasim et al. (2018) who mentioned that high incidence was in March and April in slaughtered cattle, sheep and goats. The transmission of fascioliasis is depending on the presence of intermediate "lymnaea snail" host and final host. This snail host commonly presents in high density during rainfall period annually and/or in highly moist pastures soil (Abraham and Jude 2014). The results showed that the incidence of cysticercosis was nearly similar to the results recorded by Oryan et al. (2012) who mentioned that only one *C. ovis* cyst was detected in sheep and three *C. bovis* cysts in cattle. These results agreed with Kouam et al. (2019) who mentioned that cysticerci was absent in Cameroon. These results disagree with Beyene and Hiko (2019) who recoded that the incidence of *C. bovis* in 384 randomly selected cattle was 8.6% in Ethiopia. Also, these results disagree with Abebe et al. (2015) who recorded that 8.43% of 510 randomly selected slaughtered sheep and 8.5% of 420 randomly selected slaughtered goats were infected with *C. ovis*. The results of the current work revealed that the incidence of macroscopic *Sarcocyst* agree with Saeid et al. (2015) who couldn't detect any macroscopic cysts in cattle and disagree with Mounika et al. (2018) who observed macroscopic sarcocysts in nine cattle mainly in esophagus. The incidence in sheep disagrees with Aysen et al. (2007) who found macrocysts of *Sarcocyst* ovifelis in (24.5%) of sheep mainly in esophagus. In addition, the incidence in camel agrees with Hamidinejat et al. (2013) in Iran who mentioned that no macroscopic cyst was observed in the animals at naked eye inspection. Regarding buffalo, the results were nearly similar to the incidence (66.42%) obtained by Jyothisree et al. (2016). Polymerase Chain Reaction (PCR) is an effective method used worldwide for confirmation of sarcocysts (Daptardar et al. 2016). DNA extraction and PCR amplification of 18S rRNA gene region was conducted to identify sarcocysts in positive samples in different hosts by using special primers. Other authors detected Sarcocyst species by using PCR as Hornok et al. (2015) in cattle and buffalo in Hungary, Kutty et al. (2015) in goat in Selangor, Malaysia and Safa et al. (2016) in Tunisian cattle in North-West Tunisia.

5. Conclusion

The abattoir survey reports still provide good overview of the trend and status of the parasitic zoonoses and can therefore guide in planning future coordinated researches and control programs. The problems of parasitic infestation usually lead to direct or indirect losses in all species of food animals. The direct losses include growth retardation and increased susceptibility to disease, which increase the mortality rate leading to high economic losses. The indirect losses include partial or total condemnation of carcasses after slaughtering inside abattoirs. The incidence of fascioliasis and cysticercosis through the current study gives good impression about their control programs. Hydatidosis stills of high incidence in camel especially in winter and sarcocystosis stills of high incidence in buffalo especially in autumn. Therefore, it is strongly recommended to avoid slaughtering of animals outside abattoirs to prevent zoonosis to human, accurate P.M examination of carcasses in abattoir, sanitary elimination of condemned offals or carcasses of positive parasitic affections with hygienic measures, precautions should be taken in abattoirs to prevent entrance of dogs and cats, avoid eating under-cooked offals, thourlly heat treatment of offals involved in dog and cat diets, finally, using diagnostic methods as PCR for confirmation of microscopic

sarcocystosis and thoughrly cooking of offals " predilection for microsarcocyst" to prevent zoonotic sarcocystosis.

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