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Molecular study on *Cysticercus bovis* cysts in Menofia abattoirs.

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Abstract

The present study was carried out during 2018 at Shebin El–koom and Menouf abattoirs to determine the prevalence of *Cysticercus bovis* in cattle and buffaloes followed by PCR as a step toward developing a more reliable method for the identification of *C. bovis* in bovine tissues ,gene sequence and phylogentic analysis. The present study was conducted to examine 1470 slaughtered cattle and buffaloes in Shebin El-koom abattoir and 840 slaughtered cattle and buffaloes in Menouf abattoir for the prevalence of *Cysticercus bovis* cysts. In Shebin El-koom, it was found that the infection percentage in slaughtered cattle reached 3.2% and in slaughtered buffaloes reached 3.3%. On the other hand, in Menouf abattoir the prevalence of *C.bovis* cysts was 5.4% in slaughtered cattle and 5% in slaughtered buffaloes. In Shebin El-koom abattoir the percentage of *C.bovis* infection in the examined slaughtered male and female cattle was 2.4% and 5.9% respectively, while for the examined slaughtered male and female buffaloes the percentages were 0.8 and 3.9% respectively. In Menouf abattoir the percentage of *C.bovis* infection in the examined slaughtered male and female cattle were 3.3 and 6.3%, respectively while the infection percentage in the examined slaughtered male and female buffaloes were 1.7 and 6.8%, respectively. The percentage of heart, head (tongue or masseter muscles) and heart and head infections in cattle reached 1.4, 1.9 and 0.8% respectively. *C.bovis* cysts could not be detected in any other parts of the carcass. On the other hand in buffaloes, heart, head (tongue or masseter muscles) and heart and head infections reached 3.2, 0.5 and 0.2% respectively. *C.bovis* cysts could not be detected in any other parts of carcass. PCR showed positive amplification for COI gene segment of *T.saginata* at 253 bp**.** By using gene sequence and phylogenetic analysis *T.saginata* Menofya strain was closely similar to other *T.saginata* strains. The public health hazard of both *C.bovis* and *T.saginata* was discussed**.**

Keywords*: *Cysticercus bovis, T.saginata, public health,

Introduction

Bovine cysticercosis caused by the larval stage of the human tapeworm *Taenia saginata* is a zoonotic muscular disease of great public health significance, especially in developing countries mainly in the African continent where the incidence is high in comparison to other parts of the world (**Mekonnen, 2017).**

The infection is also a problem in developed countries where undercooked beefsteak is consumed. It is important to note that eggs of *T.saginata* have been demonstrated to survive almost all stages of sewage treatment. It is significant that even the high standard of meat inspection in abattoirs of highly developed countries that are expected to identify measly beef carcasses has not succeeded in eliminating this parasite **(Symth, 1994)**. *Taenia saginata* is very long (3-15 meters in length) tapeworm parasite whose adult form is found attached to the small intestinal tracts of human beings. In man it has been known to live for 20 years within single individual. Therefore, it is an intestinal parasite of cattle and humans, causing taeniasis in humans as a result of poor hygiene **(Abilo and Meseret, 2006)**. Taeniasis has debilitating effect on people who already have live of protein deficiency diets suffer from iron deficiency and infested by hook worm **(FAO, 2004).** *T.saginata* is found in small intestine of humans which computed through the absorp-

tion of the digested food and its proglottids migrate to different organs causing different signs **(Kebede *et al*., 2008).** *T. saginata* infection is usually asymptomatic. However, heavy infection often results in weight loss, dizziness, abdominal pain diarrhea, headaches, nausea, constipation or chronic indigestion and loss of appetite. There can be intestinal obstruction in humans. The infection caused by the adult worms in humans give rise to high medical costs **(Fan, 1997)**. The economic losses due to bovine cysticercosis are mainly due to condemnation, refrigeration and downgrading of infected carcasses. Generally, condemnation of meat and organs from infected animals are the causes of reduction of meat production and restriction on import export trade **(Nigatu, 2004)**.

Cysticercosis was significantly more prevalent in feedlots and in traditional farming system than in dairy **(Dorny *et al.,* 2000).** Risk factors for bovine cysticercosis can be increased by the chance of exposure of cattle to infective eggs of *T.saginata* from human faeces/sewage, such as close proximity to public areas, flooding, use of fertilizer that may contain human sewage, use of potentially contaminated feed or water, and employing labour potentially infected with *T. saginata*. The control measures most commonly implemented are based on the organoleptic detection of cysticerci in bovine carcass "predilection" sites during post-mortem inspection. These sites typically include the heart, masseters, tongue, oesophagus, diaphragm and the superficial and cut surfaces of the carcass; the triceps brachii muscle of the forelimb may also be examined in some regions. The heart and masseters consistently rank amongst the most likely sites to detect infection **(Scandrett *et al*., 2009)**. *C. bovis* is small (pea sized) in shape, grayish white, about one centmeter in diameter and filled with fluid in which the scolex is often clearly visible **(Alemneh *et al*., 2017)**. Degenerating cysticerci are more easily detected than viable ones , which are translucent and difficult to differentiate from surrounding host tissue. Since both viable and degenerating cysticerci can co-exist in the same carcass, detection of degenerated cysts does not ensure that viable cysticerci are not present at other sites **(Gajadhar *et al*., 2006)**.

In cattle, it is difficult to diagnose in live animals, but if the animal is heavily infested, cysts may be felt on the tongue and face (**Kassaw *et al*, 2017**). Cysticerci in the tissues could cause muscular stiffness, wasting, nervous symptoms and loss of conditions leading to downgrading and condemnation of the affected carcasses **(Ofukwu *et al*., 2009)**. In man the disease is called Taeniasis and is characterized by weakness, nausea, headache, weight loss, abdominal pain , intestinal obstruction, nervous syndromes and epilepsy **(Gracey *et al*.,1999 and Ofukwu *et al*., 2009).**

Polymerase Chain Reaction (PCR) technique has been frequently used in the identification of the species involved in the taeniasis-cysticercosis complex and also in the exclusion of other etiological agents **(Jardim *et al*., 2006 and Flütsch *et al*., 2008)** .The World Organization for Animal Health (OIE) recommends the use of PCR for Taenia species differentiation and suggests that this technique should be applied to the unmistaken identification of the metacestodes larvae **(OIE, 2008).**

The main objective of this study was to examine *C. bovis* and to determine the accuracy of this identification made by the classical methods of meat inspection. The suspected cysticerci were submitted to PCR for confirmation as a step toward developing a more reliable method for the identification of *C. bovis* in bovine tissues followed by gene sequencing and phylogenetic tree to compare it with other genomic sequences in Genbank.

Materials and Methods

The present study was carried out during the period between January and December 2018 at Shebin El –koom and Menouf abattoirs to determine the prevalence of *Cysticercus bovis* in cattle and buffaloes followed by PCR .

The routine inspection procedure for bovine cysticercosis in abattoirs: (Abuseir *et al*., 2006):

It consists of visual inspection of the slaughtered animals, in particular, the inspection of the cut muscles of the split carcass and of sev-

eral specific locations (predilection sites: external and internal masseter muscles, tongue, heart, and diaphragm) after incision.

Macroscopic Detection of the Cysts Maturity: The cysts were examined macroscopically and classified accordingly as viable or degenerating after pressing by fingers **(Minozzo *et al.*, 2002).** Fluid-filled, viable cysts were considered mature as they contained a protoscolex. Degenerating cysts were classified as calcified when their contents were solid, as cheesy when smooth, or dull when they contained no contents.

Microscopic identification of viable cysticerci: The viable cysts were submitted to 30% ox bile solution diluted in normal saline and incubated at 37°C for 1 to 2 hours. A cyst was regarded as viable if the scolex evaginated according to **Gracey *et al*. (2009)**.

Cysticercus bovis were dissected from naturally infected cattle, washed with 0.01 M Tris-HCl (pH 8.0) and then cut to drain out the cyst fluid. The cyst tissue was again washed and stored at 0°C until needed**.**

DNA extraction. DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 25 mg of the sample was incubated with 20 µl of proteinase K and 180 µl of ATL buffer at 56ºC overnight. After incubation, 200 µl of AL buffer was added to the lysate, incubated for 10 min. at 72ºC, then 200 µl of 100% ethanol was added to the lysate. The lysate was then transferred to silica column, centrifugated. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from **Metabion (Germany)** are listed in table (A)

PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products.
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp ladder (Qiagen, Gmbh, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table (A). Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
COI	GGGTGCTG GTATAGGG TGGACT	253	94˚C 5 min.	94˚C 30 sec.	50˚C 30 sec.	72˚C 30 sec.	72˚C 10 min.	**Chiesa *et al.*, (2010)**
	ACGTAAA-TAAATAAG CCCACAAT							

Gene sequence and Phylogenetic Analysis
PCR products were purified using QIAquick PCR product extraction kit. (Qiagen, Valencia). Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) was used for the sequence reaction and then it was purified using Centrisep spin column. DNA sequences were obtained by Applied Biosystems3130 genetic analyzer (HITACHI, Japan), a BLAST® analysis (Basic Local Alignment Search Tool) **(Altschul *et al.*, 1990)** was initially performed to establish sequence identity to GenBank accessions. The phylogenetic tree was created by the Meg Align module of Laser gene DNA Star version 12.1 **Thompson *et al.*, (1994)** and Phylogenetic analyses was done using maximum likelihood, neighbour joining and maximum parsimony in MEGA6 **(Tamura *et al.*, 2013).**

Statistical tools used: chi square(x^2) and P values by using chi-Square on line calculation https://www.socscistatistics.com/tests/chisquare2/default2.aspx). Chi-square test was used to measure the association between qualitative variables. P value was significance if less than 0.05.

Results and Discussion

Table (1). Prevalence of *C.bovis* cysts in the examined cattle and buffaloes in 2 different abattoirs in Menofia governorate.

Abattoirs / species	Shebin El-koom			Menouf		
	No. of examined animals	No. of infected animals	%	No. of examined animals	No. of infected animals	%
Cattle	720	23	3.2	500	27	5.4
Buffaloes	750	25	3.3	340	17	5
Total	1470	48	3.3	840	44	5.2

There is no sig. difference between total infected cattle and buffaloes in both tested abattoirs using chi-square test.

Table (2). Prevalence of *C.bovis* cysts in both sexes of the examined cattle and buffaloes.

Species and sex / Abattoirs	Cattle						Buffaloes					
	Male			Female			Male			Female		
	No. of examined animals	No. of infected animals	%	No. of examined animals	No. of infected animals	%	No. of examined animals	No. of infected animals	%	No. of examined animals	No. of infected animals	%
Shebin El-koom	550	13	2.4	170	10	5.9	130	1	0.8	620	24	3.9
Menouf	150	5	3.3	350	22	6.3	120	2	1.7	220	15	6.8
Total	700	18	2.6	520	32	6.2	250	3	1.2	840	39	4.6

There is sig. difference between the infected male and female cattle based on chi-square test.
There is sig. difference between the infected male and female buffaloes based on chi- square test.
There is sig. difference between the infected male (cattle and buffaloes) and the infected female (cattle and buffaloes) based on chi-square test.

Table (3). Distribution of *C.bovis* cysts in different organs of the examined cattle and buffaloes.

Species / Inspected organs	Cattle			Buffaloes		
	Examined	+ ve	%	Examined	+ ve	%
Heart	1220	17	1.4	1090	35	3.2
Head (tongue or masseter muscles)	1220	23	1.9	1090	5	0.5
Heart and Head (tongue or masseter muscle)	1220	10	0.8	1090	2	0.2
Other organs (Diaphragm, esophagus, liver, shoulder muscle and gluteal muscle)	1220	0	0	1090	0	0
Total	1220	50	4.1	1090	42	3.9

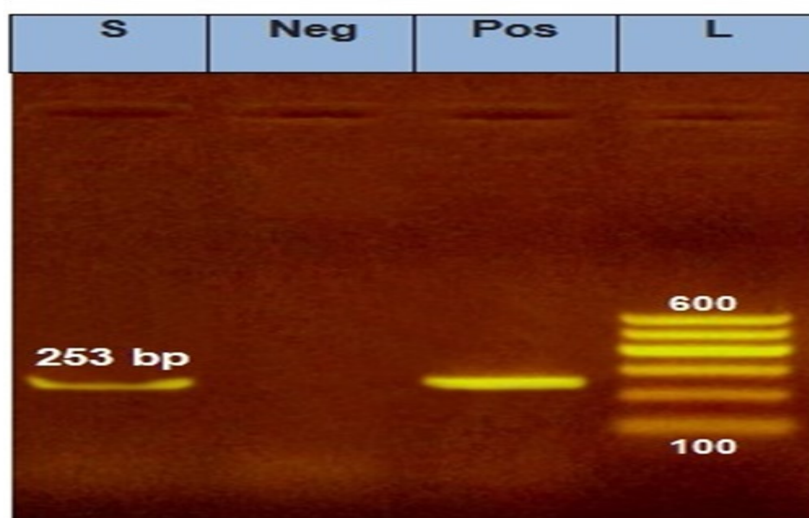

Fig. (1): Amplification of COI gene segment of *T. saginata* by 1.5% agarose gel electrophoresis and stained with ethidium bromide at 253 bp against Lane: (L) 6 00 bp DNA ladder, Lane (Neg): negative control, lane (Pos): positive control.

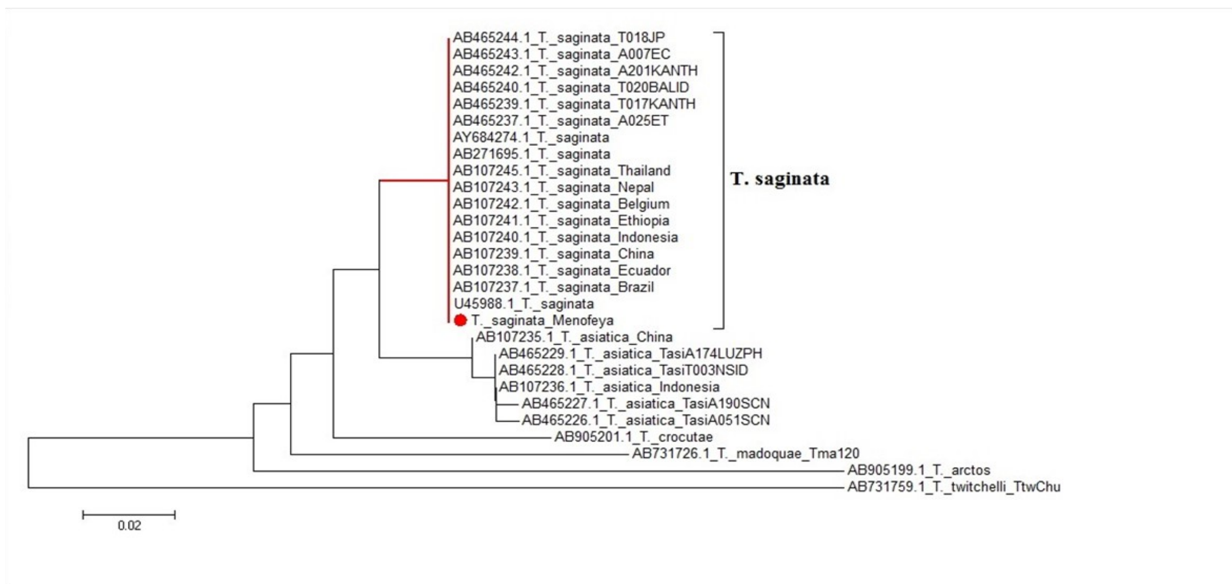

Fig. (2): Phylogenetic relatedness of the COI gene. Maximum-likelihood unrooted tree indicated clustering of the tested strain with *T. saginata* strains apart from other Taenia species.

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		
1		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	1	AB465244.1 T. saginata T018JP
2	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	2	AB465243.1 T. saginata A007EC
3	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	3	AB465242.1 T. saginata A201KANTH
4	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	4	AB465240.1 T. saginata T020BALID
5	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	5	AB465239.1 T. saginata T017KANTH
6	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	6	AB465237.1 T. saginata A025ET
7	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	7	AY684274.1 T. saginata
8	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	8	AB271695.1 T. saginata
9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	9	AB107245.1 T. saginata Thailand
10	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	10	AB107243.1 T. saginata Nepal
11	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	11	AB107242.1 T. saginata Belgium
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	12	AB107241.1 T. saginata Ethiopia
13	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	13	AB107240.1 T. saginata Indonesia
14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	14	AB107239.1 T. saginata China
15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	15	AB107238.1 T. saginata Ecuador
16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	16	AB107237.1 T. saginata Brazil
17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		100.0	96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	17	U45988.1 T. saginata
18	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0		96.7	96.2	96.2	96.2	95.7	95.7	93.4	90.5	86.7	83.9	18	T. saginata Menofeya
19	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4	3.4		99.5	99.5	99.5	99.1	99.1	93.8	91.0	84.8	82.5	19	AB107235.1 T. asiatica China
20	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	0.5		100.0	100.0	99.5	99.5	93.4	90.5	84.4	82.0	20	AB465229.1 T. asiatica TasiA174LUZPH
21	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	0.5	0.0		100.0	99.5	99.5	93.4	90.5	84.4	82.0	21	AB465228.1 T. asiatica TasiT003NSID
22	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	0.5	0.0	0.0		99.5	99.5	93.4	90.5	84.4	82.0	22	AB107236.1 T. asiatica Indonesia
23	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	1.0	0.5	0.5	0.5		99.1	92.9	90.0	84.8	82.5	23	AB465227.1 T. asiatica TasiA190SCN
24	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	4.5	1.0	0.5	0.5	0.5	1.0		92.9	90.0	83.9	82.5	24	AB465226.1 T. asiatica TasiA051SCN
25	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	7.1	6.6	7.1	7.1	7.1	7.7	7.7		90.5	84.8	81.5	25	AB905201.1 T. crocutae
26	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	10.3	9.7	10.3	10.3	10.3	10.9	10.9	10.3		84.4	82.0	26	AB731726.1 T. madoquae Tma120
27	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	13.6	16.1	16.7	16.7	16.7	16.1	17.3	16.1	16.6		80.6	27	AB905199.1 T. arctos
28	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	18.1	20.0	20.6	20.6	20.6	20.0	20.0	21.2	20.6	21.5		28	AB731759.1 T. twitchelli TtwChu
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28		

Fig. (3): Sequence distance of the COI gene of the tested *T. saginata* Menofeya strain (generated by lasergene software) showing identity range of 100% with *T. saginata* strains.

Diagnosis of bovine cysticercosis by inspection depends mainly on the skills and motivation of the meat inspector, which results in important differences in the efficacy of the meat inspection from one slaughter house to the other **(Fahmy *et al.*, 2015).**

During 2018, 1470 slaughtered cattle and buffaloes in Shebin El-koom abattoir and 840 slaughtered cattle and buffaloes in Menouf abattoir were examined for the prevalence of *Cysticercus bovis* cysts in Shebin El-koom. From **table (1)** it was found that 23 infected cases with *C.bovis* cysts out of 720 slaughtered cattle with percentage of 3.2 % and 25 infected cases with *C.bovis* cysts out of 750 slaughtered buffaloes with percentage of 3.3 %. On the other hand in Menouf abattoir the prevalence of *C.bovis* cysts was 27 infected cattle cases out of 500 with percentage of 5.4% and 17 infected cases of buffaloes out of 340 with percentage of 5 %. There was no significant difference in the incidence of infection between total cattle and buffaloes based on the result of chi-square analysis (The chi-square statistic is 0.0905. The p-value is 0.763579. The result is not significant at $p < 0 .05$) but relatively higher percentage of infection was found in the examined slaughtered cattle in comparative to the examined slaughtered buffaloes. This may be explained as a result of high resistance of buffaloes to parasitic infection which agreed with **Abdo *et al.* (2009)** and **Dyab *et al.* (2017) .**

Table (2) recorded the prevalence of *C.bovis* cysts infection in both sexes of the examined slaughtered cattle and buffaloes in both 2 abattoirs. In Shebin El-koom abattoir the percentage of *C.bovis* infection in the examined slaughtered male cattle was 2.4%. On the other hand, the infection with *C.bovis* in the examined slaughtered female cattle was 5.9 % while for the examined slaughtered male and female buffaloes the percentage was 0.8 and 3.9% , respectively .In Menouf abattoir the percentage of *C.bovis* infection in the examined slaughtered male and female cattle was 3.3 and 6.3 %, respectively while the infection percentage in the examined slaughtered male and female buffaloes was 1.7 and 6.8 % , respectively. There was significant difference between the infected male and female cattle in correlation to non infected male and female cattle based on chi-square analysis(the chi-square statistic is 9.7422, the p-value is 0.001801, significant at $p < 0.05$). At the same time there was significant difference between the infected male and female buffaloes in correlation to the non infected male and female buffaloes based on chi -square analysis(the chi-square statistic is 6.1642, the p-value is 0.013036, significant at $p < 0.05$). Also there was significant difference between the infected male (cattle and buffaloes) and the infected female (cattle and buffaloes) in correlation to the non infected male cattle and buffaloes and the non infected female cattle and buffaloes based on chi-square analysis (the chi-square statistic is 13.2518, the p-value is 0.000272, significant at $p <0 .05$).

In relation to sex, higher percentage of infection was recorded in female cattle and buffaloes (6.2 and 4.6 %) in comparative to male cattle and buffaloes (2.6 and 1.2%). The higher susceptibility of female cattle and buffaloes may be due to the fact that females are presented for slaughter at older age than males after the end of their breeding and milking period, while, males are fattened for a short period indoors and are fed mainly on dry ration until their slaughter which reduce the chance of contracting the infection **(Dorny *et al.,* 2000)**.This agreed with **Abdo *et al.* (2009)** and **Dyab *et al.* (2017)**. However, **Pramanik *et al.* (1984)** and **Okafor (1988)** in their studies found no significant differences in the recorded prevalence between sexes of the animal.

Table (3) declared that out of 50 positive cases of cattle for *C.bovis* cysts, there were 17 cases with heart infection, 23 cases with head infection (tongue or masseter muscles) and 10 cases with heart and head infection (tongue or masseter muscles) with 1.4 , 1.9 and 0.8 % , respectively. *C.bovis* cysts could not be detected in any other parts of the carcass . On the other hand, out of 42 positive cases of buffaloes for *C.bovis* cysts there were 35 cases with heart infection , 5 cases with head infection (tongue or masseter muscles) and 2 cases with heart and head infection (tongue or masseter muscles) with 3.2 , 0.5 and 0.2 % , respectively.

C.bovis cysts could not be detected in any other parts of carcass. The heart muscles were the most predilection site for *C. bovis* in the examined slaughtered buffaloes followed by head. This agreed with (**Abdo *et al,* 2009**; **Cueto González *et al*, 2015 and Abdel-Hafeez *et al*., 2015**). For the examined slaughtered cattle, the most predilection sites for *C.bovis* were head followed by heart that on the same line with **Birhanu and Abda (2014)** who found that tongue was the most predilection site and **ELkhtam *et al.* (2016)** who reported that head (tongue and masseter) was the most infected site than the heart .

In the present study there was variation in distribution of *C. bovis* in tissues that may be attributed to many factors such as species, age and sex . **Pawlowski and Schultz (1992) and Maeda *et al.*(1996)** reported breed, age and the origin of the country of cattle as variation factors in distribution of *C. bovis* in tissues. Also, variation in the distribution of *C. bovis* in different organs and muscles might be due to the blood kinetics and animals daily activities. Any geographical and environmental factors affecting the blood kinetics in the animal affect the distribution of onchosphers as well and hence the predilection sites varies during meat inspection (**Wanzala *et al*., 2003).** Another reason, difference in the skills and motivation of meat inspectors, the speed of the slaughter activities and the meat inspection facilities are among the many other contributory factors (**Kandil *et al*., 2012).**

Out of 92 positive cases of *C.bovis* in slaughtered animals, one viable cyst was taken for accurate and rapid diagnosis of the species using PCR for positive amplification of COI gene segment of *T.saginata* at 253 bp**. Fig (1)**. Phylogenetic studies involving morphological and molecular analyses, which had indicated that *T. asiatica* is indeed a distinct species, but one that is closely related to *T. saginata* **(ITO *et al*., 2003)**. *T.saginata* in Menofya was closely similar to other *T.saginita* strains as (*T.saginata* Brazil Genbank accession AB107237.1, *T.saginata* Ecuador Genbank accession AB107238.1, *T.saginata* China Genbank accession AB107239.1) **Fig (2)**.

DNA sequencing of 213 bp of *T. saginata* COI gene was generated. As shown in sequence distance **(figure 3)**, the sequenced strains showed 100% identity to *T. saginata* strains confirming the clustering of the study strain with *T. saginata.*

The closest identities to other strains were as follow; 96.7% (*T. asiatica* Genbank accession AB107235.1), 96.2% (*T. asiatica* Genbank accessions AB456228.1, AB456229.1, AB107236.1), 95.7% (*T. asiatica* Genbank accessions AB456226.1, AB456227.1).

However, the Egyptian strain showed lower identities to other strains as; 93.4% (*T. crocutae* Gnebank accession AB905201.1), 90.5% (*T. madoquae* Genbank accession AB905201.1), 86.7% (*T. actros* Gnebank accession AB905199.1), 83.9% (*T. twitchilli* Genbank accession AB731759.1).

The isolated strain showed 100% identities with the Japanese *T. saginata* (Genbank accessions AB465237.1, AB465239.1, AB465240.1, AB465242.1 and AB465243.1) detected by **Okamoto *et al.* (2010)** who reported an evidence for hybridization between *T. saginata* and *T. asiatica* strain.

The Egyptian strain also showed 100% identity with the South Korean *T. saginata* (Genbank accession AY684274) detected by **Jeon *et al.* (2007)** who reported the very low divergence with *T. asiatica* and *T. solium*, and that complete sequence of the *T. saginata* mitochondrial genome will serve as a resource for comparative mitochondrial genomics and systematic studies of the parasitic cestodes.

On the other hand, The Egyptian strain also showed 100% identity with different worldwide *T. saginata* strains (Genbank accessions AB107237-AB107245) discussed by **Yamasaki *et al.* (2004)** who differentiated between *T. saginata, T. solium* and *T. asiatica* using PCR based diagnostic markers.

The Egyptian strain also showed 100% identity with the USA *T. saginata* (Genbank accession U45988.1) detected by **Chapman *et al.* (1995)** who reported characterization of species-

specific DNA probes from *Taenia solium* and *Taenia saginata* and their use in an egg detection assay.

However, heavy infection with *T.saginata* in human often results in weight loss, dizziness, abdominal pain diarrhea, headaches, nausea, constipation or chronic indigestion and loss of appetite. There can be intestinal obstruction in humans and this can be alleviated by surgery. The tape worm can also expel antigens that can cause an allergic reaction in the individual. It is also rare cause of pancreatitis, cholecystitis and cholangitis. **WHO (2013)** and **FAO (2004)** stated that the disease can also cause obstruction of the bowel, stomach-ache and migrating proglottids cause inflammation of the appendix, inflammation of the bile duct and unpleasant surprise from the faces. **Teka (1997)** stated that taeniasis in humans causes anal purities due to emerging tapeworm segments but with severe infection humans may experience increased appetite or loss of appetite, abdominal discomfort and digestive upset. Generally, **WHO (2013)** stated that adult *Taenia* parasite is located in the intestinal tracts of humans with variety of problems including: some non-specific signs of intestinal discomfort and pain (e.g. colic signs), vomiting may result, body weakness, headaches, dizziness, irritability and delirium, malnutrition, poor hair quality, intestinal blockage, intestinal perforation and appendicitis.

Economic losses resulting from food borne parasitic zoonoses are difficult to assess. Estimating the global economic impact, prevalence and public health importance of these parasitic zoonoses are handicapped by inadequate information **(Murell, 1991)**. Economic losses from cysticercosis are determined by disease prevalence, grade of animal's infected, potential market price of cattle and treatment cost for detained carcasses (**Feseha, 1995**).

Conclusion and recommendations

In the present study the cysticerci were mainly found at the cardiac and head muscles which indicate that *C. bovis* preference to these locations .Although it showed the existence of lower prevalence of cysticercosis but it needs high attention by the veterinarians in meat inspection. Classical meat inspection techniques relies exclusively on visual examination of the intact and cut surfaces of the carcass (eye and-knife method) cannot detect all of the carcasses infected with cysticerci.

Our recommendations are as follow :

-Public education should be given at all levels to increase public awareness to avoid the consumption of under cooked beef meat and avoid back yard (village) slaughtering of cattle.

-Attention must be given to routine meat inspection. Meat inspectors should be vigilant to detect *C. bovis* in beef carcasses

-Health education to improve personal and environmental hygiene for breaking the life cycle of the disease. People should be made aware of not to defecate in pastures to avoid contamination of environment with proglottids of Taenia eggs where animal graze.

-More studies on immune diagnosis to complete meat inspection procedures.

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References

Abdel-Hafez, H.E.; Kamal, M.A.; Abdel-Gelil, H.N. and Abdel-Fatah, M.M. (2015). Parasites transmitted to human by ingestion of different types of meat , EL-Minia city, EL-Miniagovernorate , Egypt.J. Egypt. Soc. Parasitol. (JESP), 45(3): 671 – 680.

Abdo, B.R.N.; Sayed, A.S.M.; Hussein, A.A.A. and Arafa, M.I. (2009). Occurence of Cysticercosis in cattle and buffaloes and *Taenia saginata* in man in Assiut Governorate, Egypt. Vet. World 2, 5:173-176.

Abilo, T. and Meseret, A. (2006). Medical microbiology, for medical laboratory technology students, 1st ed, University of Gondar: Master printing press, Pp 203-202.

Abuseir, S.; Epe ,C.;. Schnieder, T.; Klein, G . and Kühne, M. (2006). V isual diagnosis of *Taenia saginata* cysticercosis during meat inspection: is it unequivocal? Parasitol

Res., 99: 405–409.

Alemneh, T.; Adem, T. and Akeberegn, D. (2017). Mini Review on Bovine Cysticercosis**.** J Healthc Commun. 2017, 2:2.1-8.**1**

Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W. and Lipmanl, D.J. (1990). Basic Local Alignment Search Tool. J. Mol. Biol. 215, 403-410.

Birhanu, T. and Abda, S. (2014). Prevalence, Economic Impact and Public Perception of Hydatid Cyst and *Cysticercus bovis* on Cattle Slaughtered at Adama Municipal Abattoir, South Eastern Ethiopia. American-Eurasian J. Sci. Res. 9,4: 87-97.

Chapman, A.; Vallejo, V.; Mossie, K.G.; Ortiz, D.; Agabian, N. and Flisser A. (1995). Isolation and characterization of species-specific DNA probes from *Taenia solium* and Taenia saginata and their use in an egg detection assay. J Clin Microbiol. ;33 (5):1283-1288**.**

Chiesa, F.; Dalmasso, A.; Bellio, A.; Martinetti, M.; Gili, S. and Civera, T. (2010). Development of a Biomolecular Assay for Postmortem Diagnosis of *Taenia saginata* Cysticercosis. Foodborne Pathogens And Disease, 7(10): 1171-1175.

Cueto González, S.A.; Rodríguez Castillo, J.L. and López Valencia, G. (2015). Prevalence of *Taenia saginata* Larvae (*Cysticercus bovis*) in Feedlot Cattle Slaughtered in a Federal Inspection Type Abattoir in Northwest México. Foodborne Pathog. Dis. 12(5): 462-465.

Dorny, P.; Vercammen, F.; Brandt, J.; Vansteenkiste, W.; Berkvens, D. and Geerts, S. (2000). Serepidemilogical study of *Taenia saginata* Cysticercosis in Belggian cattle. *Vet. Parasitol.* 88: 43-49.

Dyab, A.K.; Marghany, M.E.; Othman, R.A.; Ahmed, M.A. and Abd-ella, O.H. (2017). *Taenia saginata* in man and cysticercosis in cattle and buffaloes in Aswan governorate , Egypt. J. Egyp. Soc. Parasitol., 47 (2):389-394.

ELkhtam, A.O.; Ibrahim, A. ; Mostafa, I.O. and Shawish, R.R. (2016). Prevalence and economic impact of *cysticercus bovis* in slaughter cattle in Menofia province , Egypt. *Research Journal of Applied Biotechnology (RJAB)* Special volume for the first International Conference of Genetic Engineering and Biotechnology, Sharm el Shiekh, Egypt. 26-29 April, 2016.pp101-106.

Fahmy, H.A.; Khalifa, N.O. EL-Madawy, R.S,; Afify, J.S.A.; Aly, N.S.M. and Omnia M. Kandil, O.M. (2015). Prevalence of Bovine Cysticercosis and *Taenia saginata* in Man.Global Veterinaria 15 (4): 372-380.

Fan, P.C. (1997). Annual economic loss caused by *T.Saginata Taeniasis* in East Asia. *Parasitol. Today*, 13: 194-235

FAO (2004). Veterinary Public health disease Fact Sheet; Cysticercosis.

Feseha, G. (1995). Zoonotic disease in Ethiopia. Ethiopia Vet Assoc Proc pp: 22-38.

Flütsch, F.; Heinzmann, D.; Mathis, A.; Hertzberg, H.; Stephan, R. and Deplazes, P. (2008). Case-control study to identify risk factors for bovine cysticercosis on farms in Switzerland. Parasitology 135: 641-646.

Gajadhar, A.A.; Scandrett, W.B. and Forbes, L.B. (2006). Overview of food- and water-borne zoonotic parasites at the farm level. Rev. Sci. Tech. Off. Int. Epiz. (OIE) 25, 595-606.

Gracey, J.F.; Collins, D.S. and J. Hily, J. (2009). Meat .recent. Hygiene. 10th Ed.W.B. Saunders Co. pp: 669-678**.**

Gracey, J.F.; Collins, D.S. and Huey, R.J. (1999). Meat hygiene. 3rd edition. Publisher. W.B. Saunders Co. Ltd / Harcourt Brace and Co. Ltd. London, U.K. Pp 667-680.

ITO, A., Nakao, M. and T. Wandra, T. (2003). Human taeniasis and cysticercosis in Asia. Lancet, 362: 1918-1920.

Jardim, E.A.G.V.; Linhares, G.F.C.; Torres, F.G.; Araújo, J.L.B. and Barbosa, S.M. (2006), Diferenciação específica entre Taenia saginata e Taenia solium por ensaio de PCR e Duplex-PCR. Ciênc Rural 36: 166-172.

Jeon, H.K.; Kim, K.H. and Eom, K.S. (2007). Complete sequence of the mitochondrial genome of Taenia saginata: comparison with T. solium and T. asiatica. Parasitol Int. ;56(3):243-246. Epub 2007 Apr 19.

Kandil, O.M.; Nasr, S.M.; Mahmoud, M.S.; Nassar, S.A.; El-Metanawey, T.; El-Aziz, M.A. and El Ezz, N.M.A. (2012). Serological and Biochemical Studies on Cattle Naturally Infested With Cysticercosis. 87 (8): 877 -981.

Kassaw, M.; Belay, W. and Wale Tesfaye, W. (2017). Prevalence of *Cysticercus Bovis*

in Cattle Slaughterd at Kombolcha ELFORA Meat Processing factory, Northern Ethiopia. Int. J. Curr. Res. Biol. Med. 2(2): 1-6.

Kebede, N.; Tilahun, G. and Hailu, A. (2008). Prevalence of *Taenia saginata* cysticercosis in cattle slaughtered for meat in Addis Ababa abattoir. *J. Trop. Anim. Hlth. and Prod.,* 41: 291-294.

Maeda, G.E.; Kyvsgaard, N.C. and Nansen, P. (1996). Distribution of *Taenia saginata* cysts by muscle group in naturally infected cattle in Tanzania. Prev. Vet. Med. 34:2881-2889

Mekonnen, K. (2017). Study on Prevalence of Cysticercus Bovis in Cattle at Municipal Abbatoir of Kofale District, West Arsi Zone, Oromia Regional. Journal of Biology, Agriculture and Healthcare, 7(17): 61-74.

Minozzo, J.C.; Gusso, R.L.F.; Castro, E.A.; Lago, O. and V.T. Soccol, V.T. (2002). Experimental bovine infection with *Taenia saginata* eggs: recovery rates and cysticerci location. Braz. Arch. Biol. Technol., 45: 451-455.

Murell, B. (1991). Economic losses resulting from food born parasitic zoonoses. In: Hailu D (ed.) Prevalence and risk factor for *Teania saginata* cystycercosis in three selected areas of Eastern Shoa. South Asian Journal of Tropical Medicine 22: 268-270.

Nigatu, K. (2004). *Cystcercus bovis*: Development and Evaluation of serological Tests and prevalence at Addis Ababa Abattoir, Ethiopia.

Ofukwu, R.A.; Akwuobu, C.A. and Okwori, A.I. (2009). Epidemiology and public health importance of bovine cysticercosis in Makurdi, North – Central Nigeria. Tanzania Veterinary Journal. 26(1): 37-42.

OIE (2008). World Organization for Animal Health. OIE Terrestrial Manual 2008 http://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/2008/pdf/2.09.05_CYSTICERCOSIS.pdf Acesso em: 01/09/2009

Okafor, F.C. (1988). Epizootiology of *cysticercus bovis* in Imo state, Nigeria. Angewandte Parasitologie*,* 29(1): 25-30.

Okamoto, M.; Nakao, M.; Blair, D.; Anantaphruti, M.T.; Waikagul, J. and Ito, A. (2010). Evidence of hybridization between *Taenia saginata* and *Taenia asiatica*. Parasitol Int. ;59(1):70-4. doi: 10.1016/j.parint.2009.10.007. Epub 2009 Oct 27.

Pawlowski, Z.S. and Schultz, M.G. (1992). Taeniasis and cysticercosis (*Taenia saginata*). Adv. Parasitol.10:269-273.

Pramanik, A.K.; Bhattacharyya, H.M. and Sengupta, D.W. (1984). Occurrence of *C. bovis* in slaughtered cattle and buffaloes in Calcutta and its public health significance. *Indian J. of Anim. Hlth.* 23 (2): 141.

Scandrett, B.; Parker, S.; Forbes, L.; Gajadhar, A.; Dekumyoy, P.; Waikagul, J.and Haines D. (2009). Distribution of *Taenia saginata* cysticerci in tissues of experimentally infected cattle. Vet. Parasitol., 14: 223-231.

Symth, J.D. (1994). Introduction to Animal Parasitology, 3rd ed. Cambridge University Press, London, UK, Pp 334-340.

Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A. and Kumar, S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.

Teka, G. (1997). Food Hygiene Principles and Food bore Disease Control with Special Reference to Ethiopia, 1st ed. Faculty of Medicine, Department of Community health, Addis Ababa University, Pp 40-62.

Thompson, J.D.; Higgins, D.G. and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22(22): 4673-4680.

Wanzala, W.; Onyango-Abuje, J.A.; kang'ethe, E.K. Zessin, N.M.; Kyule, M.P.O.; Baamaan, H. Och'anda and Harrisa, L.J.S. (2003). Analysis of post mortem diagnosis of bovine cysticercosis in Kenya cattle. Onl. J. vet. Res., pp: 1-9, 28-31.

WHO (2013). Taeniasis/ Cysticercosis. WHO Fact sheet No. 376.

Yamasaki, H.; Allan, J.C.; Sato, M.O.; Nakao, M.; Sako, Y.; Nakaya, K.; Qiu, D.; Mamuti, W.; Craig, P.S. and Ito, A. (2004). DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. J Clin Microbiol. 2004 Feb; 42(2): 548-53.