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Prevalence of Bovine Cysticercosis and *Taenia saginata* in Man

[1]Hanan A. Fahmy, [2]Nashwa O. Khalifa, [3]Reham S. EL-Madawy,
[4]Jehan S.A.Afify, [5]Nagwa S.M. Aly and [6]Omnia M. Kandil

[1]Department of Biotechnology, Animal Health Research Institute (AHRI), Giza,, Egypt
[2]Department of Zoonoses, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt
[3]Department of Parasitology, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt
[4]Department of Food Hygiene, Faculty of Veterinary Medicine, Benha University, Toukh, Egypt
[5]Department of Parasitology, Faculty of Medicine, Benha, Egypt
[6]Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Giza, Egypt

Abstract: The prevalence of bovine cysticercosis was established using routine postmortem inspection of 3450 carcasses of buffaloes slaughtered in 2014 in Kaliouba governorate, among which 313 (9.07%) were detected as harbouring cysticercosis lesions using meat inspection process. The cysts were examined macroscopically for description of their morphology and constituents and classified as viable or degenerating. Viable cysts were microscopically confirmed for demonstration of protoscolex. Out of 100 of patients offered taenicidal drugs examined by microscopic examination through direct and sedimentation of fecal samples, 6 (6%) were positive for *Taenia saginata* (*T. saginata*) eggs. Histological sections of 6 gravid proglottids were identified as *T. saginata*. We used a biomolecular assay targeting the HDP2 gene for developing PCR assay in 20 viable cysts and 6 gravid proglottids. An HDP2 gene-PCR amplification product of the *taeniid* samples of *T. saginata* is approximately 599bp. Partial sequences were generated after gel purification of PCR amplified products of HDP2 gene with sequence analysis and subsequent phylogeny to compare these sequences to those from known strains of *T. saginata* circulating globally and retrieved from GenBank. Most isolates with accession No. KT027580 are closely related to *T. saginata* based on the similarity of nucleotide sequences and phylogenetic relationships. In conclusion, this work indicated high prevalence rate of bovine cysticercosis and *T. saginata*, both morphological examination of the parasite and molecular analysis using bioinformatic tools identified the metacestode and revealed typical *taeniid* features confirmed to *Taenia saginata*.

Key words: Cysticercosis · *Taenia saginata* · Protoscolex · Gravid proglottids · Sequence · Phylogeny

INTRODUCTION

Bovine cysticercosis is one of the most important parasitic diseases caused by the metacestode stage of the human tapeworm *T. saginata*. The public health and economic consequences of this parasite may be considerable due to downgrading and the condemnation of carcasses for food safety importance [1, 2]. The annual losses in cattle feed lots in South Africa due to cysticercosis may reach 330,000 $ per year [1-3]. In Assuit Governorate, the economic losses in cattle and buffaloes feed lots during 1989 – 1992 due to *Cysticercus bovis* (*C.bovis*) were 112,000 LE. [4].

Taenia saginata has a cosmopolitan distribution, but the infection is more important in Africa, Asia and Latin America and in some Mediterranean countries [5]. Habit of eating raw beef dishes, low level of toilet used by human population, backyard slaughter, low availability of taenicides, free access of cattle to surface water and proximity of waste water are important causes for transmission of bovine cysticercosis to a herd of cattle and taeniasi*s* in human population [6]. Live cattle having *C. bovis* shows no symptoms, however, heavy infestation by the larvae may cause myocarditis or heart failure [7]. Human infection that occurs through consuming of infected raw or semi-cooked beef may results in epigastric

Corresponding Author: Omnia Mohamed Kandil, Department of Parasitology and Animal Diseases, National Research Center, El-Behouse Street, Dokki, P.O. Box 12622, Giza, Egypt. E-mail: kandil_om@yahoo.com.

pains, diarrhea, nausea, weakness or loss of appetite [8] Globally, there are 77 million human carriers of *T. saginata* out of which about 40% live in Africa [9].In Eastern African countries like Ethiopia up to 70% of the population reports to have been infected with a tape worm [10].

The diagnosis of bovine cysticercosis by meat inspection depends very much 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 [6]. In animals, ante mortem diagnosis is routinely performed in bovine, by visual inspection [11]. According to European data on meat inspection, the prevalence ranges from 0.007% to 6.8%, but the real prevalence is considered to be at least 10 times higher [12]. Usually the numbers of cysticerci are low in bovine; hence many cases remain undetected even after conscious postmortem inspection [13].

In human routine diagnosis is based on the microscopic detection of *Taenia* spp., Oncospheres. These methods have a sensitivity of around 38% to 60% [14]. The worms are identified morphologically as *T. saginata* by scolex and/or gravid proglottids [15]. Recently, highly specific PCR-methods have been developed to detect *Taenia* DNA in faeces [16,17] However, these methods have not yet been properly validated in the field. Several molecular techniques have been described for the detection and differentiation of *Taenia* species using Multiplex-PCR [16,18,19]. PCR-Restriction Fragment Length Polymorphism (RFLP) [15, 17,19]. and random amplified polymorphic DNA (RAPD) [20].

In Egypt Epidemiological data are scarce and the available literature are few; prevalence of bovine cysticercosis in cattle, buffaloes and taeniasis in human in Assuit Governorate [4], taeniasis in human in Dakahlia Governorate [21]. Phylogenetic placement of Egyptian *T. saginata* and *Cysticercus bovis* [22], have been carried out.

The main objectives of this study were to update the epidemiological knowledge of the bovine cysticercosis and human taeniasis in Kalioubia governorate and understanding of epidemiology by assessment of infection status using multiple diagnostics tools to this aim.

MATERIALS AND METHODS

Survey and Cyst Collection: Abattoirs survey was conducted from January to December 2014 in collaboration with two official slaughter houses in Kalioubia governorate (Toukh and Benha). Three thousands, four hundred and fifty water buffaloes (*Bubalus bubalis*) aged from 2-6 years old were subjected to postmortem examination and detailed visual inspection measures for *C. bovis*. Inspection of the cyst was done in buffaloes by making deep cuts in relevant organs as tongue, neck, diaphragm, cardiac muscles, skeletal muscles, liver, lung and kidney. The cut surface was inspected visually for *C. bovis* and the cyst counts were recorded. [11, 23].

Sampling from Human: Stools were collected from 100 patients (63 males and 37 females, 20 -55 years old) suffering from gastrointestinal disturbances attended to University of Benha Hospital after offered single oral dose of praziquantel at 10mg/kg b.w. [24].

The fecal samples were taken only in the morning and analyzed macroscopically. About 10grams was collected in a clean labeled plastic container with snap on lids. Samples were kept in a special container transferred to the laboratories and stored at -20°C until examined.

Parasitological Examination

Macroscopic Detection of the Cyst Viability: The cysts were examined macroscopically and classified accordingly as viable or degenerating after pressing by fingers [25]. Fluid-filled, viable cysts were considered mature when they contained a protoscolex. Those without a distinct protoscolex were considered immature. Degenerating cysts were classified as calcified when their contents were solid, as cheesy when smooth, or dull when they contained no contents and were apparently neither viable nor degenerating.

Microscopic Identification of Viable Cysticerci: The viable cyst was 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*. [7].

Detection of *Taeniasis* in Human by Morphological Identification

Morphological Identification of *Taenia saginata* Egg: Direct and sedimentation methods were employed for detection of *T. saginata* viable egg contains onchosphere. Sediments were placed in microscopy slides and examined under the microscope [26].

Histological Identification of *Taenia saginata* Proglottid: Histological sections stained were carried out to count the uterine branches of an intact gravid proglottid. The proglottid was fixed in neutral buffered 10% formalin,

embedded in paraffin and cut into longitudinal sections of 6 mm and stained with Carmine stain and mounted; then uterine branches were counted under a light microscope at a magnification of x40 and identified as *T. saginats* based upon number of uterine branches when 12 or more branches arose to each side from the central uterus branches [15].

The confirmed cysts and Tape worms were potted in 70% ethanol and kept at -70°C for later DNA isolation.

Molecular Studies: The tested cyst and proglottid fragments of individual strobila were washed with distilled water to remove any ethanol remaining.

Genomic DNA (gDNA) was extracted from the tested cyst and worm using the DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and used as template DNA for PCR. The design of oligonucleotide primers selected for the detection of HDP2 gene sequences according to published database by Gonza´lez *et al*. [18] and Gonza´lez *et al*. [19] and Harrison *et al*. [27]. by the HDP2F1R1-PCR protocol as the primers are PTs7S35F1 (5-CAGTGGCATAGCAGAGGA-GGAA-3) and PTs7S35R1 (5-GGACGAAGAATGGAGTTGAAGGT-3).

Purified DNA was amplified according to Gonza´lez *et al*. [18]. A total volume of 25µl containing 5µl template of *Taenia sp*. DNA and 20µl of PCR mix using 2X FIREPol® Master Mix (Ready to Load) in Biometra Thermocycler. The cycling conditions of initial denaturation at 94°C for 2min, followed by 35 cycles of denaturation at 94°C for 1min, annealing at 56°C for 1min, extension at 74°C for 1min and final extension at 74°C for 7min. The products were electrophoresed on 1.5% agarose for the detection of expected band amplified gDNA at 599bp stained with ethidium bromide (0.5µg/ml) against GeneRuler 100bp plus DNA ladder (Fermintas). Negative control was included in PCR run.

Sequencing: *T. saginata* gDNA samples were separated by electrophoresis in low-melting-point agarose. Appropriate bands of the amplification products were cut out and purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, Calif.). The amplified fragments were automatically sequenced using the Big-DyeTerminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Langen, Germany) on an automated DNA sequencer (Applied Biosystems). The nucleotide sequences were then aligned with existing sequences of known genotypes from other countries in the GenBank databases using BLAST programs and databases of the NCBI (National Center for Biotechnology Information, Bethesda, MD, USA) (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

Phylogenetic Analysis: Phylogenetic analyses were based on ClustalW computer program as cited by Thompson *et al*. [28]. and the extent of variation was compared by doing pairwise alignment of the nucleotides using MEGA version 5 in FASTA format [29]. Phylogenetic tree were constructed using the neighbour-joining of MegAlign program from LaserGene Biocomputing Software Package (DNASTAR, Madison, WI). The analysis of evolutionary divergence between sequences was conducted using the Maximum Composite Likelihood Model [30]. Evolutionary distance, maximum parsimony methods and evolutionary analyses were conducted in MEGA6 [31].

RESULTS

During 2014, 3450 buffaloes were slaughtered in two officials' abattoirs in Kalioubia governorate. Post mortem examination revealed that out of 3450 slaughtered buffaloes, 9.07% were infected with *C. bovis* and the prevalence of infection was higher in Toukh abattoir 9.49% than Benha abattoir 8.72% (Table, 1). Macroscopic detection of the recovered cysts revealed that out of the total 345 cysticerci collected, 211 (61.15%) were found to be viable while 134 (38.84%) were degenerated. Out of 211 viable cyst examined microscopically, 179 (84.83%) were alive contain protoscolex.

Over a 1-year period we obtained specimens of *Taenia* species from 6 patients after administration anthelminthes. In 6% of the samples, eggs identified microscopically and the prevalence of infection in male was 4 (6.34%) higher than in female 2 (5.40%) (Table, 2). Well-preserved gravid proglottids were recovered and identified by histology stained with Carmine stain identified as *T. saginata* contain more than 12 uterine lateral branches (Figure, 1).

Genomic DNA (gDNA) was extracted from the ethanol-preserved identified 20 viable cyst and 6 proglottids samples. Amplification of DNA of HDP2 gene of *T. saginata* was detected in the accurate size and gave one single band which was detected at approximately 599bp (Figure, 2).

Partial sequence of HDP2 gene PCR products of *T. saginata* produces a sequence of 535 bp has been submitted to the GenBank with the accession numbers KT027580. The obtained sequence was put to BLAST and

Table 1: Prevalence of cysticercosis in slaughtered buffaloes in two official abattoirs in Kalioubia, Egypt.

		Infected animals			Macroscopic ex. of cysts				Microscopic ex. of viable cysts			
					Viable		Degenerated		Viable		Non-viable	
Abattoirs	No. of slaughtered buffaloes	No.	%	No. of examined cysts	No.	%	No.	%	No.	%	No.	%
Benha	1880	164	8.72	176	114	64.77	62	35.22	94	82.45	20	17.54
Toukh	1570	149	9.49	169	97	57.39	72	42.60	85	87.62	12	12.37
Total	3450	313	9.07	345	211	61.15	134	38.84	179	84.83	32	15.16

Table 2: Prevalence of *taeniasis* in patients administered praziquantel 10mg/kg.

		Infected humans	
Patients	Examined number	Number	%
Female	37	2	5.40
Male	63	4	6.34
Total	100	6	6

Fig. 1: Histological identification of *Taenia saginata* proglottid stained with Carmine stain.

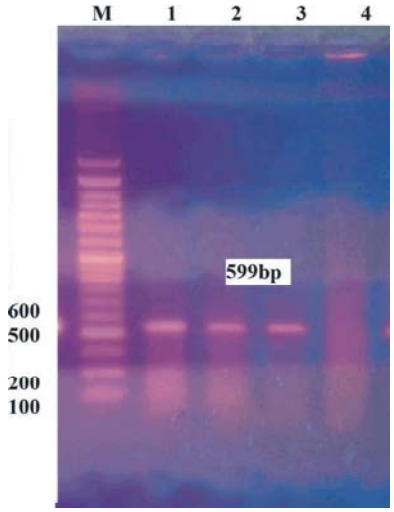

Fig. 2: Amplicon products of Egyptian (Kaluiobia) isolate of *Taenia saginata* of HDP2 gene resolved by 1.5% agarose gel electrophoresis and visualized by ethidium bromide staining at 599bp. The numbers on the left (M) indicate the sizes in bases pairs (bp) of the molecular weight DNA markers of 100bp. (Lanes 1-3): PCR amplicons of Egyptian *T. saginata*, (Lane 4): Negative control.

compared with other available cyclophyllidean cestode sequences from GeneBank. The numbers of base substitutions per site between sequences are shown in the analysis of the new Egyptian isolates and 17 nucleotides sequences. All position containing gaps and missing data were eliminated. There were a total of 535 positions in final dataset. The BLAST hits result shows that the sequences of the Egyptian isolate is closer to those of species of *Taenia*, with maximum similarity to *Taenia saginata* (Figure, 3).

Phylogenetic analysis showed a robust tree clustering all isolates with sequences belonging to the *T. saginata* and *T.asiatica* variant with strong bootstrap values at relevant nodes. Phylogenetic tree shows the evolutionary relationship of the sequences in which the length of the horizontal line was proportional to the estimated genetic distance between the sequences. Such tree indicated that the evolutionary distance between groups is very short (Figure, 4).

The analysis of genetic diversity based on HPD2, DNA sequencing represented the percent of diversion and identity between the new Egyptian isolate and seventeen selected sequences *Taeniid spp.* circulating globally and retrieved from GenBank displayed. Our isolate was phylogenetically compatible (100%) with *T. saginata* from Egypt, JX265977 and 99.4%

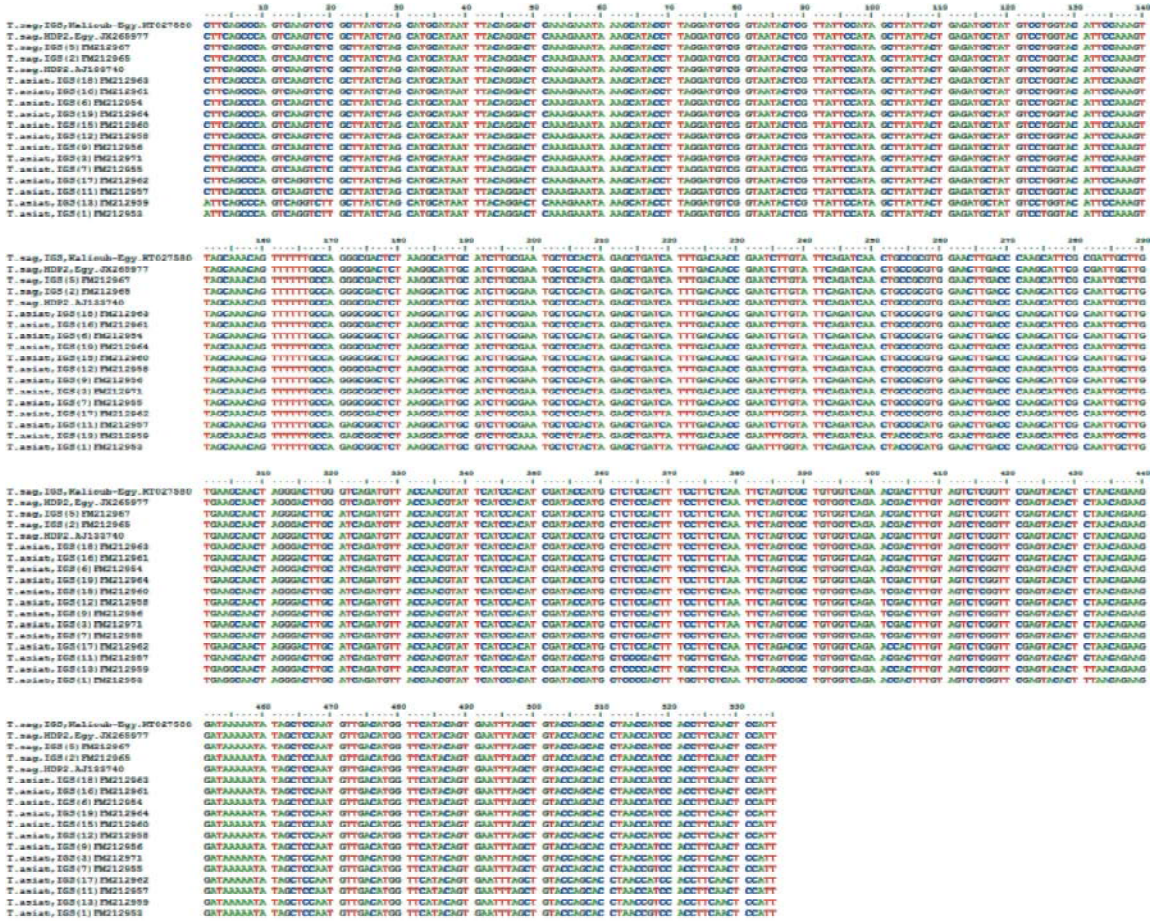

Fig. 3: Nucleotide sequence alignment of *Taenia saginata* Egyptian isolate and compared by doing pairwise alignment of the nucleotides of other isolates in GenBank using ClustalW multiple alignments.

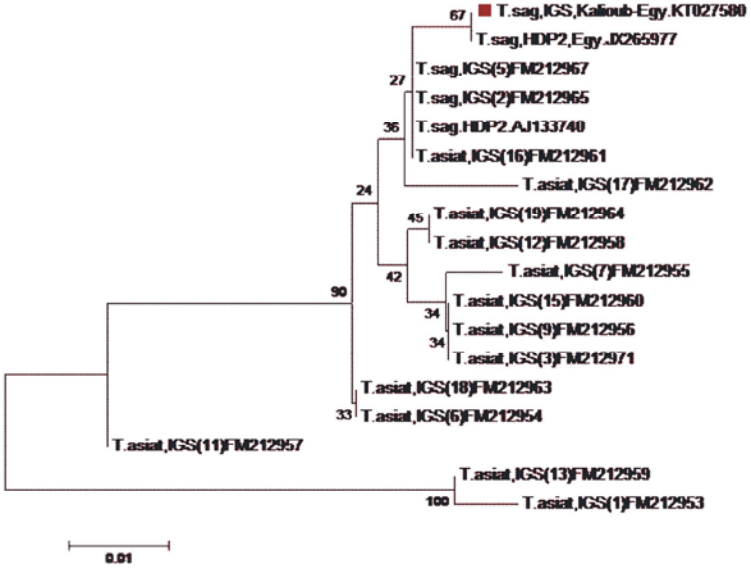

Fig. 4: Phylogenetic tree of T. *saginata* based on nucleotide partial sequences of the HDP2 gene. It is closer to T. *saginata* than T. *asiatica* using the entire nucleotide sequences of *Taenia* species retrieved from GenBank by MEGA5. Genetic distance is indicated below the tree.

Table 3: The percent of diversion and identity between T. *saginata* sample from Egypt and seventeen selected sequences circulating globally from GenBank

Percent Identity

Divergence

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18		
1		100.0	99.4	99.4	99.4	99.3	99.3	99.3	99.1	99.1	99.1	99.1	98.9	98.9	98.3	98.3	95.5	95.3	1	T.sag,IGS,Kalioub-Egy.KT027580
2	0.0		99.4	99.4	99.4	99.3	99.3	99.3	99.1	99.1	99.1	99.1	98.9	98.9	98.3	98.3	95.5	95.3	2	T.sag,HDP2,Egy.JX265977
3	0.6	0.6		100.0	100.0	99.8	99.8	99.8	99.6	99.6	99.6	99.6	99.4	99.4	98.9	98.9	96.1	95.9	3	T.sag,IGS(5)FM212967
4	0.6	0.6	0.0		100.0	99.8	99.8	99.8	99.6	99.6	99.6	99.6	99.4	99.4	98.9	98.9	96.1	95.9	4	T.sag,IGS(2)FM212965
5	0.6	0.6	0.0	0.0		99.8	99.8	99.8	99.6	99.6	99.6	99.6	99.4	99.4	98.9	98.9	96.1	95.9	5	T.sag,HDP2.AJ133740
6	0.8	0.8	0.2	0.2	0.2		99.6	100.0	99.4	99.8	99.4	99.8	99.6	99.6	98.7	99.1	96.3	96.1	6	T.asiat,IGS(18)FM212963
7	0.8	0.8	0.2	0.2	0.2	0.4		99.6	99.8	99.4	99.8	99.4	99.6	99.3	98.7	98.7	95.9	95.7	7	T.asiat,IGS(16)FM212961
8	0.8	0.8	0.2	0.2	0.2	0.0	0.4		99.4	99.8	99.4	99.8	99.6	99.6	98.7	99.1	96.3	96.1	8	T.asiat,IGS(6)FM212954
9	0.9	0.9	0.4	0.4	0.4	0.6	0.2	0.6		99.6	100.0	99.6	99.8	99.4	98.5	98.5	95.7	95.5	9	T.asiat,IGS(19)FM212964
10	0.9	0.9	0.4	0.4	0.4	0.2	0.6	0.2	0.4		99.6	100.0	99.8	99.8	98.5	98.9	96.1	95.9	10	T.asiat,IGS(15)FM212960
11	0.9	0.9	0.4	0.4	0.4	0.6	0.2	0.6	0.0	0.4		99.6	99.8	99.4	98.5	98.5	95.7	95.5	11	T.asiat,IGS(12)FM212958
12	0.9	0.9	0.4	0.4	0.4	0.2	0.6	0.2	0.4	0.0	0.4		99.8	99.8	98.5	98.9	96.1	95.9	12	T.asiat,IGS(9)FM212956
13	1.1	1.1	0.6	0.6	0.6	0.4	0.4	0.4	0.2	0.2	0.2	0.2		99.6	98.3	98.7	95.9	95.7	13	T.asiat,IGS(3)FM212971
14	1.1	1.1	0.6	0.6	0.6	0.4	0.8	0.4	0.6	0.2	0.6	0.2	0.4		98.3	98.7	96.3	96.1	14	T.asiat,IGS(7)FM212955
15	1.7	1.7	1.1	1.1	1.1	1.3	1.3	1.3	1.5	1.5	1.5	1.5	1.7	1.7		97.8	97.0	96.8	15	T.asiat,IGS(17)FM212962
16	1.7	1.7	1.1	1.1	1.1	0.9	1.3	0.9	1.5	1.1	1.5	1.1	1.3	1.3	2.3		97.2	97.0	16	T.asiat,IGS(11)FM212957
17	4.7	4.7	4.1	4.1	4.1	3.9	4.3	3.9	4.5	4.1	4.5	4.1	4.3	3.9	3.1	2.9		99.8	17	T.asiat,IGS(13)FM212959
18	4.9	4.9	4.3	4.3	4.3	4.1	4.5	4.1	4.6	4.2	4.6	4.2	4.4	4.0	3.3	3.1	0.2		18	T.asiat,IGS(1)FM212953

to T. sag IGS(5)FM212967, T. sag IGS(5)FM212965 and T.sag HDP2AJ133740 with diversion 0.6%, while the percentage of identity reached its lowest degree 95.3% to T.asiat IGS(1)FM212953 (Table, 3).

DISCUSSION

Bovine cysticercosis is an infection of bovine caused by the larval stage, *C. bovis*, of the human intestinal cestode, *T. saginata*. This parasite is universally distributed in developing as well as in developed countries [32]. Post mortem inspection is the most common method in use to detect bovine cysticercosis. *C. bovis* is round or oval in shape and when fully developed consists of scolex invaginated into small fluid filled vesicle [32,23]. Meat inspection remains the cornerstone for the control of *T. saginata* [33].

Cysticercosis was carried out in slaughtered buffaloes in Benha and Touhk abattoirs in Kalioubia, Egypt in order to determine the infection rate during a one-year period, 2014**.** Buffaloes were examined by routine meat inspection. The results showed that 313 (9.07%) were infected with *Cysticercus bovis* and the prevalence of infection is (8.72%) in the first abattoir lower than (9.49%) in the second abattoir (Table, 1).

The prevalence of *Cysticercosis* in buffaloes in this study is higher than those recorded by Abdo *et al.* [4]. (0.8%) in Assuit, Egypt. The available literatures of *Cysticercosis* in buffaloe are rare so we obliged to compare the obtained result with *C. bovis* in slaughterd cattle.

Our findings are higher than *C. bovis* recorded in African countries 0.2% in North West Province of South Africa from 2000 to 2010 [34]. 19 (3.6%) in Addis Ababa municipal abattoir [35], 0.25%) [36], 315 (2.67%) in Nigeria [37], 96 (2.09%) [38] and 24 (5.6%) at Elfora abattoir, Bishoftu, Ethiopia [39]. On the contrary, the occurrence of *Cysticercosis* in this study was lower than reordered bovine cysticercosis in Ethiopia 13.3% [10]. in Hawassa Municipal 22.9% [40] and 26% [41]. The variation in the reported prevalence rates may be due to several factors such as climate change, variation in sanitation habit between localities, number of the collected sample as well as control measures and eradication programs [38].

Out of the 345 cysticerci collected and submitted to macroscopic examination, 211 (61.15%) were found to be viable (mature) while 134 (38.84%) were degenerated (immature). Table (1) declared that the prevalence of the viable cysts higher than the degenerated. A notice disagree with those previously observed by Abdo *et al.* [4], who detected most of the cysticerci (90%) were degenerated.

Concerning microscopic detection of the viability of the cysticerci, out of the 211 cysts microscopically examined, 179 (84.83%) were alive contain protoscolex. A result in agreement with Emiru *et al*. [39] and Belachew and Ibrahim [40].

Cysticerci can remain alive in cattle anywhere from weeks to years and such infection in cattle is a public health problem as the infected raw or undercooked beef causes taeniasis in human. It has economic significance as well as the economic losses accruing from the condemned and downgraded carcasses and due to treatment of carcasses before human consumption is substantial [42].

The occurrence of *taeniasis* among the 100 examined patients in this study shows 6 out of 100 (6%) infections. In Egypt our result is higher when compared with those [4] 0.6% and El-Shazly *et al*. [21],0.1% in Assiut and Dakahlia respectively.

Taenia saginata infections were determined microscopically through direct and sedimentation of fecal samples. Infection rate was 6.34% in male higher than 5.40 % in females (Table, 2). A finding was differ with Usip *et al*.[38]. diagnosed *T. saginata* eggs with 2.47% infection rate in males lower than 3.07% infection rate in females in Uyo, capital city of Akwa Ibom State, Nigeria. Moreover [4], did not detect infection in female**.** The differences may be due to differences in location of study as well as personal hygiene, educational level, control and eradication programmes in such localities [38].

Praziquantel treatment resulted in recuperation of adult tapeworms from 6 hosts. Gravid proglottids examined histologically and stained with Carmine stain were identified as *T. saginata* (Figure, 1). The morphological examination of the parasite revealed typical *taeniid* features of gravid segment based upon number of uterine lateral branches [15].

Bovine cysticercosis and taeniasis are common where hygienic conditions are poor and the inhabitants traditionally eat raw or insufficiently cooked or sun-cured meat [25] Preventing animal to have contact with human feces by construction of latrines, detection and treatment of *Taenia* carriers were recorded [6].

A PCR- protocol was developed from the HDP2 sequence-based specific oligonucleotoid primers to establish a *T. saginata* DNA band of approximately 599bp. In the present study the identified 20 viable cysts and 6 proglottids were confirmed by PCR. The genomic DNA of the parasite was isolated and amplified based on a sequence of *T. saginata* 599bp DNA fragment generated from the parasite gDNA using HDP2F1R1 (Figure, 2). The similar resulting in amplification of DNA fragment specific for *T. saginata* was performed by Gonza´lez *et al.*[18] and Nunes *et al*. [17] with oligonucleotide primers of HDP2 gene.

The nucleotide sequence data of HDP2 PCR products of *T. saginata* has been submitted to the GenBank with the accession numbers KT027580 (Figure, 3). Phylogenetic analysis showed that our isolates clustered with *Taeniid spp.* and revealed that KT027580 Egypt put in the same category with JX265977, T. sag IGS(5)FM212967, T. sag IGS(5)FM212965 and T.sagHDP2AJ133740 Figure (4). Phylogenetic tree indicated that the evolutionary distance between groups is very short, suggesting that the genetic divergence is recent.

The percent of diversion and identity between *T. saginata* sample from Egypt and seventeen selected sequences circulating globally from GenBank (Table, 3), our isolate was phylogenetically compatible (100%) with *T. saginata* from Egypt, JX265977 and 99.4% to T. sag IGS(5)FM212967, T. sag IGS(5)FM212965 and T.sag HDP2AJ133740 with diversion 0.6%, while the percentage of identity reached its lowest degree 95.3% to T.asiat IGS(1)FM212953.

CONCLUSION

The result obtained in this study confirmed the present of high percent 9.1% of cysticercosis in slaughtered buffaloes accompanied with overall infestation rate of 6% of taeniasis in human in Kalioubia, Egypt. Therefore, to reduce the transmission of taeniasis/ bovine cysticercosis, public education to avoid consumption of raw meat, use of latrines and improved standards of human hygiene were recommended [10]. Eradication of infection requires co-operation between the Public Health and Veterinary authorities.

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