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ORIGINAL ARTICLE

The prevalence and molecular detection of bovine cysticercosis and its impact on slaughtered cattle in Egypt

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Abstract

Cysticercus bovis is the metacestode of the commonly defined foodborne cestode, *Taenia saginata*. It infects heart, masseter muscles and other muscular sites of cattle causing bovine cysticercosis. So, a retrospective study using archival documents from authorized agencies is done during the period of 2018–2020 with estimating economic losses. Cysts were collected from municipal Beni-Suef and Basatin abattoirs. Molecular approach including cPCR and sequencing analysis is used to prove the species. The prevalence was 0.34% in Beni-Suef abattoir and 5.70% in Basatin abattoir including heads, hearts or all carcasses condemned. Among 27 provinces surveyed, the highest prevalence was recorded in Aswan followed by Cairo, Suez and Red Sea. Moreover, 19 provinces (Cairo, Alexandria, Suez, Dakahlia, Sharkia, Qalyobia, Kafrelsheikh, Gharbia, Menofiya, Ismailia, Giza, Beni-Suef, Assiut, Sohag, Qena, Aswan, Red Sea, South Sinai and El-Wadi El-Gadid) recorded bovine cysticercosis in 2018, 2019 and 2020. On the other hand, Luxor and North Sinai had no infections. The percentages of condemnation among slaughtered cattle were 1.38, 1.49 and 0.87% in 2018, 2019 and 2020, respectively. Condemnations significantly varied among north, middle and south districts of Egypt, and also varied annually. Molecularly, a diagnostic band at an amplicon size 253 bp targeting the COI gene specific for *Cycticercus bovis* was revealed. The obtained sequences showed 100% identity with the different *Taenia saginata* COI GB sequence isolates in many countries worldwide. The phylogenetic analysis method showed that the obtained sequences originating from the same clade of *Taenia saginata* GB isolates globally. Careful meat inspection as well as strict hygienic measures is recommended for both veterinarians and public.

Keywords *Cysticercus bovis* · Egypt · Prevalence · PCR · Sequencing

Introduction

Bovine cysticercosis is an infection by the metacestode of the cyclophyllidean, *Taenia saginata*, with humans as a final host. The method of housing and management of cattle, the qualification of meat inspection in abattoirs, the habit of consuming raw/undercooked meat and animals aging equally contribute to the disease epidemiology. The lack of education as well as inadequate hygiene and sanitary measures facilitate the occurrence of the life cycle and thus the zoonosis of the cestode (Nigatu 2004; WHO 2006; Geinoro and Bedore 2019; Sabuwa et al. 2020).

Uniquely, Africa has a higher prevalence of bovine cysticercosis, posing a high potential of zoonosis. The disease occurs in all districts, both developed and underdeveloped (Abunna et al. 2007). Recently, it has been found that, among estimated 50 million infected persons worldwide, approximately 50,000 human cases yearly deaths occurred (Sabuwa et al. 2020). Meanwhile, 77 million people are infected with adult *Taenia saginata*, with 40.0% in Africa. Moreover, in developing countries, the prevalence of bovine cysticercosis is higher (Fralova 1985; WHO 1995; Tembo 2001). *T. saginata* infections in humans tend to

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occur at a higher rate, with the prevalence over 20.0% (Bowman 1995; Urqhuart et al. 1996; Geinoro and Bedore 2019). In Egypt, the infection with *C. bovis* is prevalent in cattle (6.09%) and buffaloes (9.0%), respectively (Elsayad et al. 2021). In Europe, according to the abattoir inspection data, the prevalence of bovine cysticercosis ranged from 0.01% to 6.8%. (SCVPH 2000), but the actual prevalence is anticipated to be much higher (Geerts et al. 1981; van Knapenand Buys 1985; Onyango-Abuge et al. 1996; Dorny et al. 2000; Abuseir et al. 2006).

The adult segmented tapeworm measured a few meters with scolex carrying four cup-shaped muscular suckers, a neck, and strobila, a chain of proglottids. After defecation, following the release of eggs into the environment, cattle might be infected by consuming tainted food or water, grazing on polluted pastures, or both. The oncospheres circulate in blood after hatching and penetrate the intestinal wall, dispersing them throughout the body where they mature into cysticerci where common predilection sites for *T. saginata* cysticerci include the heart and masseter muscles (Dermauw et al. 2018a). In cattle, muscular stiffness, wasting and a loss of conditions resulting in poor quality-carcasses and/or the condemnation of the affected organs resulted. In humans, nausea, headache, increased appetite, weight loss, abdominal pain, intestinal obstruction, nervous syndromes and epilepsy are common (Ofokwu et al. 2009; Sabuwa et al. 2020).

Meanwhile, economic losses are achieved by the occurrence of infected tissues with downgrading carcasses as well as the cost of treatment of infected patients (Dorny et al. 2009; Scandrett et al. 2009; Geinoro and Bedore 2019), thus, potential reduction of the meat business (Kebede 2008; Meku and Tonga 2022). Cysticercosis-related economic losses in the meat sector are highly correlated with the severity of the infection. The carcass with a generalized cysticercosis must be completely rejected. When localized cysticercosis, affected areas must be condemned. Preservation at no more than − 7 °C for duration of three weeks renders the metacestode inactive (Gracey et al. 1999; Elkhtam et al. 2016). The most particular predilection spots for *Cysticercus bovis* are the external and internal masseter pterygoid muscles, heart, tongue, diaphragm, and oesophagus (Elsayad et al. 2021).

In abattoirs, the visual identification is questionable, as cysticerci often misdiagnosed with lesions caused by other pathogens, parasitic like *Sarcocystis* spp., bacterial like *Actinobacillus* or others (Ogunremi et al. 2004; Abuseir et al. 2006). The purpose of the current study is to determine the prevalence and occurrence of *Cysticerus bovis* in slaughtered cattle by the use of meat inspection in abattoirs and molecular detection of cysts as well as estimating the zoonotic potential and economic feedback of their existence.

Materials and methods

Study area and animals

The current retrospective investigation was conducted during the period from 2018 to 2020 at various Egyptian provinces to determine the occurrence of *Cysticercus bovis* and their economic impact in slaughtered cattle. Archival documents of the general veterinary services organization were used to get daily records for those animals adopted to slaughtering. According to the Egyptian laws, in each province, the total number of slaughtered animals as well as the number of heads, hearts, and complete condemned carcasses referred to *Cysticercus bovis* infection were reported. Annually, the prevalence of *Cysticercus bovis* was estimated. The provinces of Egypt were categorized into north (15 provinces), middle (6 provinces) and south (6 provinces). The total number of slaughtered cattle and the number of heads, hearts and the whole carcasses condemned due to *C.bovis* infection were recorded for each province. Estimated heart, head and the whole carcass costs were calculated by multiplying number of condemned head (for example) x average head weight × average price of each kilogram (Yatswako and Alhaji 2017; Abdel-Fatah et al. 2022).

Gathering and identification of *Cysticercus bovis*

A careful routine meat inspection for bovine cysticercosis was done in Beni-Suef municipal abattoir, Beni-Suef and Basatin abattoir, the municipal abattoir in Cairo (Fig. 1). The examination included an overall visual inspection of carcasses followed by a particular inspection of cut muscles inside carcasses in suspected predilection sites like heart, tongue and masseter muscles. As early as possible, specimens were transported to the laboratory of Parasitology, Faculty of Veterinary Medicine, Beni-Suef University for parasitological investigations. Since the scolex of *T. saginata* is unarmed, lacking hooks, with no identifiable criteria, the detection of cysticerci seems to be difficult needing more appropriate tools. At the laboratory, each suspected cyst was carefully dissected, gently compressed between 2 glass slides and examined microscopically under a low power-microscope. The existence of 4 suckers indicates viable cysts. Similar structures of the same size might often caseous, calcified or degenerated masses (Gibson 1959). Collected viable cysts were preserved in 70% ethanol for molecular identifications.

Molecular analyses

DNA extraction and amplification

DNA was extracted from single cyst of each sample using QIAamp DNA Mini Kit Catalogue no.51304 according to

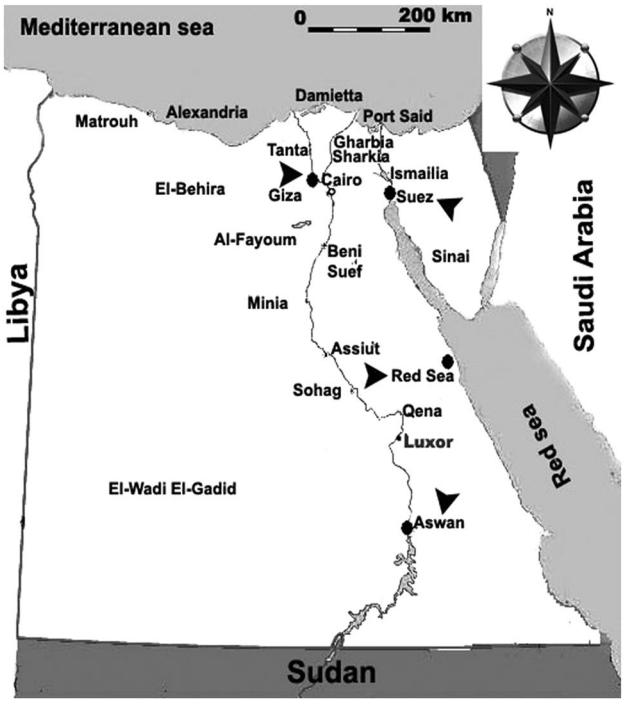

Fig. 1 A map illustrating the occurrence of bovine cysticercosis in animals slaughtered in Beni-Suef and Basatin municipal abattoirs in Egypt during the period of 2018–2020. Arrow heads indicated provinces with highest prevalences

the manufacturer's instructions. The extracted DNA was stored at -20 ◦C till use. The used primers for detection of COI gene were selected according to Chiesa et al. (2010). The selected primers were ′5-GGGTGCTGGTATAGGGTG GACT -3′ (forward) and ′5-ACGTAAATAAATAAGCCC ACAATATT -3′ (reverse). The mixer of Taq polymerase (Thermo Scientific, USA) 12.5 µl, primers (Forward and reverse) 1 µl for each one, dH2O 8.5 µl and DNA template 2 µl was added into the PCR tubes, the final volume of PCR reaction was 25 µl. The mixture was amplified by a thermal cycler under the following conditions (one initial denaturation cycle at 94° C for 4 min, 35 denaturation cycle at 94 °C for 30 s, one annealing cycle at 59 °C for 1 min, one extension cycle at 72 °C for 1 min and one final extension cycle at 72 °C for 7 min. The PCR products were stored in the thermal cycler at 4 °C until they were collected. PCR amplicons were electrophoresed on a 1.5% agarose gel stained with ethidium bromide in tris–acetate EDTA buffer then photographed using UV transilluminator.

Sequencing and phylogenetic analysis

For gene sequencing, PCR products of positive samples were purified with Thermo Scientific GeneJET PCR Purification Kit (#K0701, Lithuania) according to the manufacturer's instructions. Purified PCR products were sequenced on an Applied Biosystems 310 automated DNA sequencer using cycle sequencing ABI prism Big Dye terminator chemistry (Perkin-Elmer/Applied Biosystems, Foster City, CA). Sequences were aligned using Clustal W (Altschul et al. 1990). The obtained sequences were compared using the algorithm BLASTn with those available in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov). Phylogenetic analyses of the data obtained were undertaken by the neighbor-joining (NJ) method (Felsenstein 1985) using MEGA 7 (Saitou and Nei 1987). *Fasciola hepatica* was chosen as the outgroup for rooting trees. Reliabilities for the NJ tree were tested using 1000 bootstrap replications (Kumar et al. 2016).

Results

Currently, out of 94,892 slaughtered cattle in Beni-Suef municipal abattoir, 324 (0.34%) parts (heads or hearts)/carcasses condemned due to the occurrence of *C. bovis* during the period of 2018–2020. In Basatin abattoir, out of 392,197 slaughtered animals, 22,344 (5.70%) parts/carcasses condemned during the same period. Meanwhile, among 27 provinces surveyed, the highest prevalence was recorded in Aswan (34.5, 25.0 and 9.1% in 2018, 2019 and 2020, respectively), followed by Cairo (4.2, 8.0 and 4.5%), Suez (0.8, 0.69 and 1.29%), and Red Sea (0.34, 2.3 and 0.5%). Moreover, it has been found that 19 provinces (Cairo, Alexandria, Suez, Dakahlia, Sharkia, Qalyobia, Kafrelsheikh, Gharbia, Menofiya, Ismailia, Giza, Beni-Suef, Assiut, Sohag, Qena, Aswan, Red Sea, South Sinai and El-Wadi El-Gadid) recorded bovine cysticercosis in 2018, 2019 and 2020. On the other hand, Luxor and North Sinai had no infections (Table 1). Cysticerci were predominantly found in hearts and masseters in both Beni-Suef and Basatin abattoirs (Fig. 2).

Currently, it has been found that the total percentages of condemnation among slaughtered cattle were 1.38, 1.49 and 0.87% in 2018, 2019 and 2020, respectively. Condemnations significantly ($P < 0.05$) varied among north, middle and south districts of Egypt, and also varied annually. In the south of Egypt, the percentages were 2.89, 2.14 and 0.91, respectively. Meanwhile, in the north, percentages of condemnations were 0.26, 0.25 and 0.22, respectively. They were 1.81, 2.57 and 1.30 in the middle of Egypt. Concomitantly, in the south Egypt, estimated economic losses induced by condemnation due to *Cysticercus bovis* were 5,846,310, 6,229,968 and 1,287,843 EGP, respectively. In the north of Egypt, 2,566,170, 476,940 and 1,182,102 EGP, while in the middle they were 4,329,657, 7,300,572 and 4,427,640 EGP, respectively (Table 2).

The present study revealed a diagnostic band in all tested specimens at an amplicon size 253 bp targeting the COI gene specific for *Cycticercus bovis*. Moreover, a control

Table 1 Distribution pattern of bovine cysticercosis in Egyptian provinces during the period of 2018–2020

Province	2018	2019	2020
Cairo	4.2	8.0	4.5
Alexandria	0.2	0.16	0.1
Port Said	–	–	0.02
Suez	0.8	0.69	1.29
Damietta	0.09	0.04	–
Dakahlia	0.3	0.53	0.47
Sharkia	0.13	0.04	0.02
Qalyobia	0.04	0.01	0.007
Kafrelsheikh	0.16	0.03	0.116
Gharbia	0.17	0.06	0.08
Menofiya	0.4	0.3	0.3
Behira	0.23	–	0.013
Ismailia	0.08	0.01	0.004
Giza	0.15	0.2	0.19
Beni-Suef	0.2	0.4	0.3
Fayoum	–	0.04	0.01
Minia	0.02	0.01	–
Assiut	0.2	0.1	0.09
Sohag	0.1	0.03	0.02
Qena	0.01	0.002	0.02
Luxor	–	–	–
Aswan	34.5	25.0	9.1
Red Sea	0.34	2.3	0.5
Matrouh	–	0.03	0.01
North Sinai	–	–	–
South Sinai	0.57	0.3	1.3
El-Wadi El-Gadid	0.17	0.2	0.18

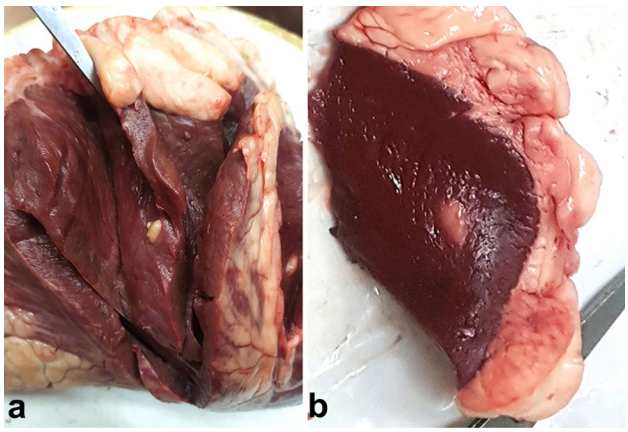

Fig. 2 *Cycticercus bovis* recovered from hearts of slaughtered cattle. **a** A cyst from an animal slaughtered in Beni-Suef municipal abattoir. **b** A cyst from an animal slaughtered in Basatin municipal abattoir

positive sample showed the same specific amplicon size. Meanwhile, negative control samples did not show specific bands (Fig. 3). The obtained sequences showed 100% identity with the different *Taenia saginata* COI GB sequence isolates in many countries worldwide. Sequences obtained from *Cysticercus bovis* cysts showed 100% identity with MT074050 *Taenia saginata* from Cambodia, MK644930 *Taenia saginata* from South Korea, OL459878 *Taenia saginata* from Vietnam, AB984347 *Taenia saginata* from China, JX402911 *Taenia saginata* from Poland, HQ606075 *Taenia saginata* from Thailand and U45988 *Taenia saginata* from USA. Nucleotide sequence alignment of COI gene from the obtained sequences were identical with *T. saginata* isolates either from human stool or the worm itself (Fig. 4). The phylogenetic analysis using neighbor-joining (NJ) method showed that the obtained sequences originating from the same clade of *Taenia saginata* GB isolates of different countries (Figs. 5).

Discussion

Bovine cysticercosis, due to *Taenia saginata*, is often incriminated to induce considerable economic losses to the livestock industry, particularly cattle (Dermauw et al. 2018b). Taeniosis was recorded globally. The determination of the frequency and the distribution pattern might be unclear due to the lack of specific symptoms in carriers (Tembo and Craig 2015; Braae et al. 2018). Abattoir inspection using post mortem examination is a traditional and basic tool to screen bovine cysticercosis depending upon the gross detection of alive/dead/calcified cysticerci in musculature in the head, heart or even in the whole carcass (Rezende et al. 2018).

Underestimating/overestimating cysticercosis often occurs in abattoirs due to the unintended poor/low quality visual inspection by the ill-trained veterinarians who easily fall in misdiagnosis with similar structures induced by pathogens rather than cysticerci, thus posing on the necessity of sanitary conditions as well as the proper visual inspection (SCVPH 2000, 2003; Abuseir et al. 2006).

Currently, the prevalence of bovine cysticercosis was 0.34% in Beni-Suef and 5.70% in Cairo, mainly in hearts and masseters of slaughtered cattle during the period of 2018–2020. Such finding was in accordance with that given by Elmonir et al. (2015) in mid-Delta during January to February 2013 (0.44%) and Abdel Aziz et al. (2022) in Sohag province (0.47%), Egypt. Other literature in Egypt revealed more or less different findings. Among those, Yassien et al. (2013), Abdel-Hafeez et al. (2015), El-Alfy et al. (2017) and Dyab et al. (2017) revealed overall prevalences of 0.58%, 20.0%, 0.51% and 7.5%, respectively. Discrepancies in infection rates might be attributed to

Table 2 Condemnations and estimated economic losses due to bovine cysticercosis of slaughtered cattle in Egypt during 2018–2020

Year	Slaughtered cattle	Condemned part			Chi–square	*P*-value
		Number (Heads + hearts + carcasses)	Percentage	Estimated economic losses (EGP)		
2018						
North Egypt	442,146	1159 (421 + 697 + 41)	(0.26)	2,566,170	1.0069	Ns 0.315642
Middle Egypt	304,387	5515 (143 + 5313 + 59)	(1.81)	4,329,657	482.7432	< 0.00001
South Egypt	192,457	6322 (5563 + 757 + 2)	(2.89)	5,846,310	495.7911	< 0.00001
Total 2018	938,990	12,966 (6127 + 6012 + 102)	(1.38)	12,742,137	51.0737	< 0.00001
2019						
North Egypt	499,133	1255 (424 + 782 + 49)	(0.25)	4,769,640	13.1741	0.000284
Middle Egypt	451,940	11,658 (298 + 11,274 + 86)	(2.57)	7,300,572	877.0977	< 0.00001
South Egypt	208,595	4475 (3567 + 857 + 51)	(2.14)	6,229,968	886.1958	< 0.00001
Total 2019	1,159,668	17,388 (4289 + 12,925 + 186)	(1.49)	18,300,180	1829.3855	< 0.00001
2020						
North Egypt	468,121	1009 (194 + 795 + 20)	(0.22)	1,182,102	NA	NA
Middle Egypt	383,633	6280 (205 + 6019 + 56)	(1.3)	4,427,640	NA	NA
South Egypt	167,181	1536 (1012 + 521 + 3)	(0.91)	1,287,843	NA	NA
Total 2020	1,018,935	8825 (1411 + 7385 + 79)	(0.87)	6,897,585	NA	NA

P value considered significant at $P < 0.05$

NA Not applicable

NS non-significant

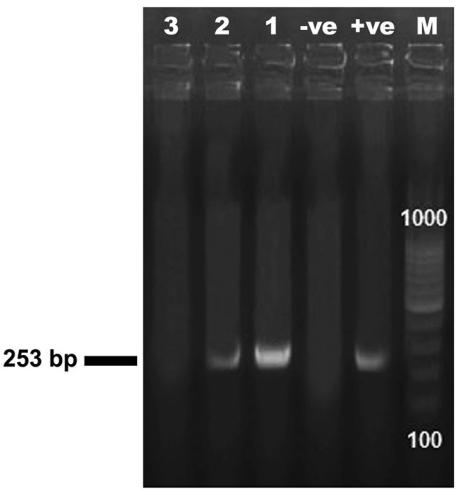

Fig. 3 Amplified COI-based region of DNA products extracted from recovered cystcerci on agarose gel. *Lane* M denoted bp ladder. *Lane* + ve denoted control positive. *Lane* –ve denoted control negative. *Lanes* 1, 2 denoted 2 specimens with cyticerci with clear diagnostic bands at amplicon size 253 bp. *Lane* 3 referred to –ve reaction with another specimen

sampling, expert of veterinarians during abattoir inspection as well as hygienic measures in rearing through which cattle are less exposed to be in contact with *Taenia saginata* eggs.

The present investigation revealed a significant variation in economic losses in north, middle and south Egypt as well as it varied annually. In the south of Egypt, percentages due to economic losses were 2.89, 2.14 and 0.91%, respectively. Meanwhile, in the north, percentages of condemnations were 0.26, 0.25 and 0.22%, respectively. They were 1.81, 2.57 and 1.30% in the middle of Egypt. Concomitantly, in the south Egypt, estimated economic losses induced by condemnation due to *Cysticercus bovis* were 5,846,310, 6,229,968 and 1,287,843 EGP, respectively. In the north of Egypt, 2,566,170, 476,940 and 1,182,102 EGP, while in the middle they were 4,329,657, 7,300,572 and 4,427,640 EGP. Currently, hearts were the most infected organs in addition to masseters. The difference in predilection sites might be referred to the use of animals in various farm activities influencing blood kinetics, thus, the distribution of oncospheres of hatched eggs varies (Dyab et al. 2017; El-Sayed et al. 2021). Those losses estimated by calculating weight of condemned part multiplied by estimated price of kilogram at that time. It is worthy to mention that that price varied according to the economic status of the country including costs of feedstuffs, transportation, workers and others. To the authors' opinion, the discrepancy in economic losses in several abattoirs might be due to the differences in the prevalence of bovine cysticercosis, the prevalence of rejection rate of organs and slaughtering capacity at that period (Birhanu and Abda 2014). Culture and socieconomic factors play

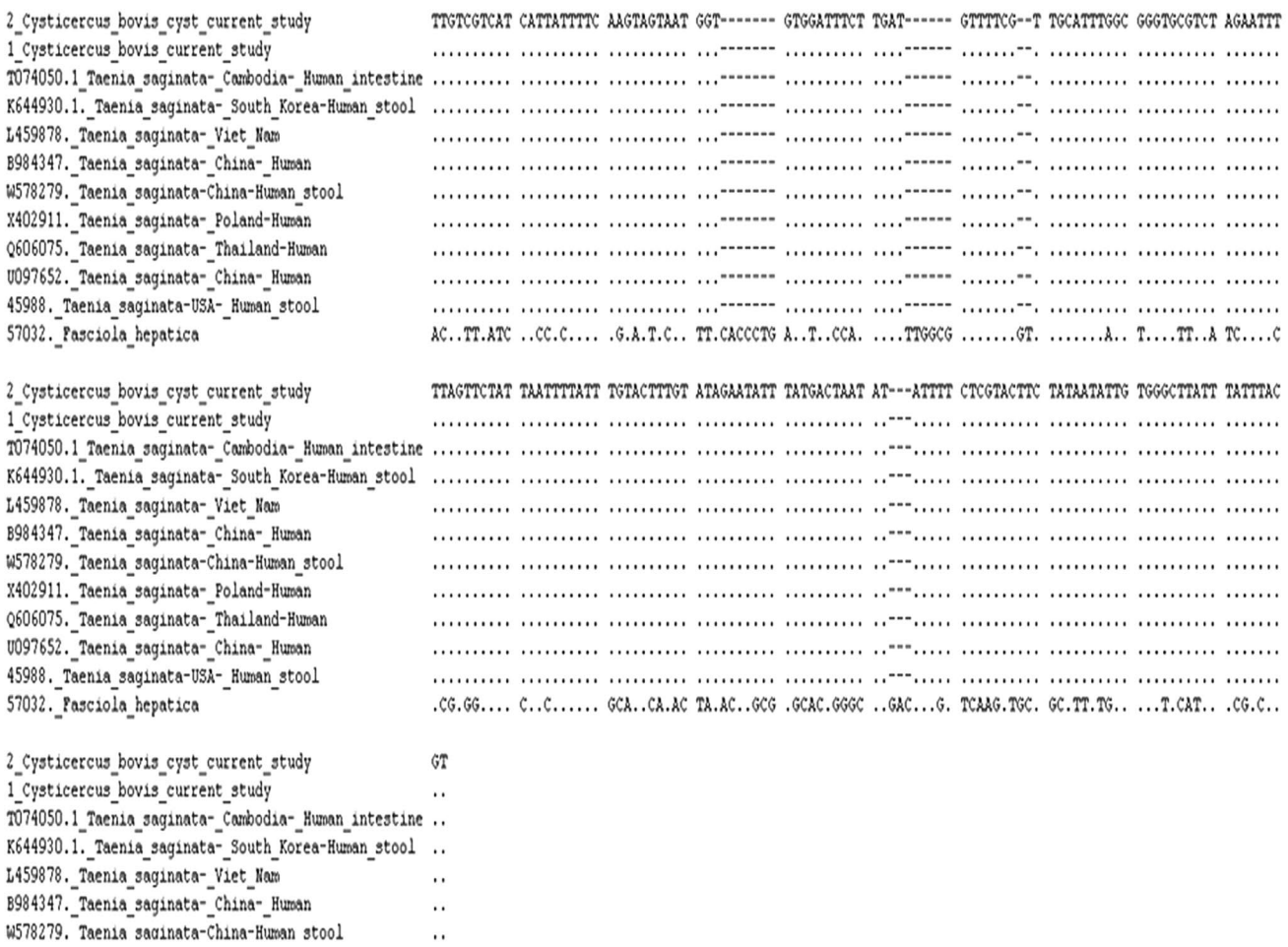

Fig. 4 Nucleotide sequence alignment of COI gene (*Taenia saginata* of South Korea K644930, and Poland JX402911, *Taenia saginata, China* AB984347), *Cysticercus bovis* of the current study

an important role in spreading the disease and so, raising the possibility of condemnation in abattoirs. Among those, consuming raw, semi-raw beef meat, some meat byproducts like basterma encourage the existence of cysts in meat and consequently *Taenia saginata* in humans raised with a continuous source of disseminating eggs. Meanwhile, the close contact of highly potential diseased farmers and workers of abattoirs with cattle permits the occurrence of the disease.

Based on the traditional visual meat inspection in abattoir, as well as the personnel mistakes in cysts diagnosis, areas of known low percentage, it could be mentioned that it is insufficient and less efficient for surveys and questionnaires of *C. bovis*. Alternatively, more advanced tools, despite of higher costs, are raised. Among those, cPCR and sequencing analysis are demanded (Abusier et al. 2006; El-Sayed et al. 2021). In the current work, cPCR revealed *C. bovis*-specific band of an amplicon size 253 bp targeting COI gene. In Egypt, other literature targeted other genes. Among those, El-Sayed et al. (2021) targeted *HDP2* gene and Abdel Aziz et al. (2022) targeted 18S rDNA gene produced size of 1600 bp. Currently, the used primers worked on CO1 gene of the extracted DNA of *Cysticercus bovis* cysts and recorded 100% sensitivity and specificity. Similarly, the same gene was used to detect the larval stages of *Taenia solium* (Vargas-Calla et al. 2018).

Interestingly, the identity of the obtained sequences of the collected cysts was 100% with many *Taenia saginata* GB sequences worldwide. The obtained sequences showed 100% identity with MK644930 *Taenia saginata* from South Korea (Won et al. 2019). Similarly, it showed 100% identity with many unpublished gene bank isolates worldwide. It is worthy to mention that the obtained sequences showed 100% identity with *Taenia saginata* samples of human origin either adult worms or eggs recovered from stool of infected persons. Findings of the molecular analysis confirmed the data of cysticercosis prevalence that based on the observation of the infected carcasses. Moreover, the sequence analysis of the cysts from animals matches with the adult worms and eggs of human samples comes in agreement with their zoonotic importance.

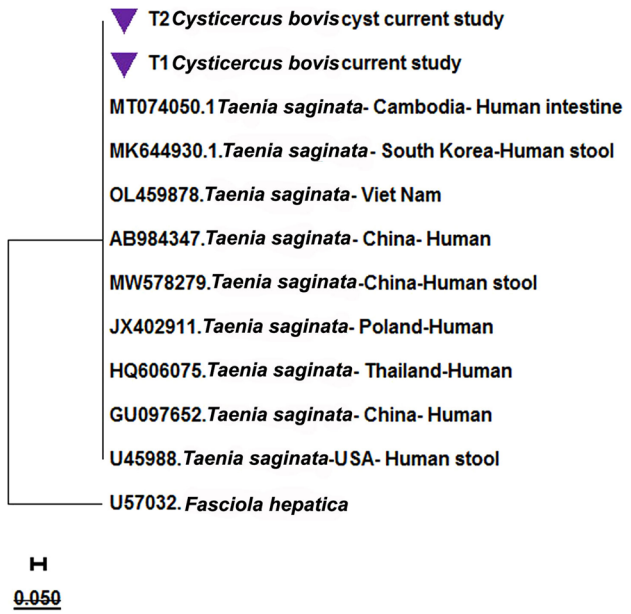

Fig. 5 Phylogenetic relationships between *Cysticercus bovis* based on CO1 DNA fragment sequences of the current study with other of *Taenia saginata* in GenBank using neighbour joining tree. *Cysticercus bovis* of the current study were violet triangular-labelled. *Fasciola hepatica* was used to root the current tree

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Author contributions SAH; samples collection, draft manuscript. ORA lab work, finding analysis. WMA; designing, data analysis. SE; photo editing, statistical analysis. NSA; drafting manuscript, KME; designing, sampling, data analysis, drafting manuscript, revising, photo editing.

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Data availability The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Conflict of interest Authors declare that there is no conflict of interest.

Ethical approval Not applicable, as no specimens were taken from condemned parts in abattoirs with any experiments done for animals or humans.

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