Journal of Current Veterinary Research

ISSN: 2636-4026

Journal home page: http://www.jcvr.journals.ekb.eg

Food safety and Public health

Prevalence, Economic Losses and Characterization of *Cysticercus Bovis* in Slaughtered Cattle in El-Menofia Province, Egypt

Reyad Shawish[1*], Ahmed Abdel hady[2], Ghada Hadad[3], Zakaria Elbayoumi[1]

(1) Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Sadat City, Sadat City32511, El-Menoufia, Egypt.
(2) Veterinarian at Directorate of Veterinary Medicine, Shebin- El-Kom El-Menoufia, Egypt.
(3) Department of Animal Hygiene and Zoonoses, Faculty of Veterinary Medicine, University of Sadat City, Sadat City 32511, El-Menoufia, Egypt.

**Corresponding author:* reyad.rabea@vet.usc.edu.eg Received: 23/2/2025 Accepted: 30/3/2025

ABSTRACT

The current study aimed to detect the prevalence, economic losses and molecular characterization of *C. bovis* in slaughtered cattle from El-Menofia Province Egypt, through phase starting from January 2022 to December 2022. A total of 9488 slaughtered cattle carcasses (7638 male and 1850 female) were inspected. The findings showed that 0.98% of slaughtered cattle had *C. bovis*. Females were more susceptible than males to infection at 3.2% and 0.44%, respectively. The predilection sites were heart, masseter muscle with a prevalence of 0.67%, 0.31%, respectively. The entire carcass was condemned in one case with heavy infection with prevelance 0.01%. The presence of *C. bovis* was associated with the age of the inspected carcass. The increased prevalence was observed in female cattle older than 5 years. The prevalence was highest in summer and spring at 1.1 % followed by autumn at 0.95% and winter at 0.74%. Concerning the molecular charachterization of *C. bovis the resulting sequences were blasted on the BLAST site (Basic Local Alignment Search Tool)*. The amplified DNA was yielded the expected band size. The sequence of *C. bovis* in this study was highly identical with other sequences of *C. bovis* which was deposited in gene bank. The phylogenetic tree revealed that the current study was founded *C. bovis* in the same genetic clade with the gene bank sequences of *C. bovis.* It is concluded from the current study that *C. bovis.* is a common zoonotic disease between man and animals, so the latent danger increase due to wrong disposal of infected animal bio-waste in addition to unefficient methods of slaughtering and how to deal with this infected organs. Cysticercosis caused significant economic losses, with an estimated total annual loss of 102200 Egyptian Pounds due to the condemnation of organs in this study.

Key words: *C. bovis*; Cattle; Meat inspection; Financial losses; Egypt

INTRODUCTION

Meat present a great source for some parasitic diseases of public health

hazard also, they play a role in the life cycle of those parasites. The human parasitic infection known as taeniasis is brought on by the tapeworm species *Taenia asiatica* (Asian tapeworm), *Taenia saginata* (beef tapeworm), and *Taenia solium* (pork tapeworm). Eating raw or undercooked infected meat exposes humans to the infection. Over the course of two months, the cysticercus transforms into an adult tapeworm that can live for years in the human intestine. The adult tapeworms live in the small intestine after attaching to it with their scolex. Adult *T. saginata* worms are typically 5 m or shorter, while they can grow up to 25 m. (Global Health, Division of Parasitic Diseases and Malaria, 2017) Eating raw beef exposes one to the beef tapeworm, *Taenia saginata*. The larval stages, known as cysticerci, are found in the meat of cattle, which serve as the intermediate host. East Africa has the highest infection rates, and the main economic burden on people is caused by "measly" meat deteriorating. Although they are typically mild, symptoms may include nausea, anorexia, or pain in the abdomen. A proglottid segment passing in the feces is frequently noticed by asymptomatic people. (Daniel and Lamb, 2023) Common locations include the kidney, heart, liver, esophagus, diaphragm, tongue, and masticatory muscles for intermediate-host cysts, *Cysticercus bovis* (Minozzo et al. 2002). *Cysticercus bovis* is a small (pea-sized) oval in shape (FAO/WHO, 2014). *Taenia saginata* is more common in impoverished nations with unsanitary conditions and where people typically consume raw, inadequate, or sun-cured meat (Minozzo, et al., 2002). Compared to dairy, cysticecosis was much more common in feedlots and traditional farming systems. The employment of causal workers and ongoing human-animal contact are hypothesized to be factors that facilitate the spread of *Taenia saginata*. (Dorny, et al., 2000). *Taenia saginata*-caused bovine cysticercosis is a zoonotic illness that calls for regular inspection procedures to find cysticerci (cysts) in beef that is intended for human consumption after slauthtering. Although there is evidence that evaluation of other areas may offer improvements in sensitivity, detection is based on gross examination of typical carcass predilection sites. The heart was the most commonly afflicted site and scored highest overall when only traditional sites (for all animals) were examined. However, it did not differ significantly from the masseter muscle, (Brad et al, 2009).

MATERIAL AND METHODS

1. Study period and area

To ascertain the presence of *C. bovis* and its economic influence on slaughtered cattle, one-year study was conducted at various abattoirs in EL Menofia governorate, Egypt (Ashmoun, El- shohada, and Quesina abattoirs) from January 2022 to December 2022.

2. Meat inspection for Detection of C. bovis

The current investigation was carried out on 9844 cattle of varied sexes and ages. The presence of *C. bovis* was physically checked on the entire carcasses of slaughtered cattle. Samples from infected organs heart, masseter muscle by make multiple incision in muscles.

3. *PCR analysis of Cysticercus bovis and phylogenic tree*

The whole cyst lesion removed and frozen at -80ºC for molecular characterization. DNA was extracted by a DNA extraction kit (QIAamp

DNA Mini Kit). The extracted DNA was measured using a Spectrophotometer. The primers forward (5´-GGGTGCTGGTATAGGGTGGACT -3´) and reverse (5´-ACGTAAATAAATAAGCCCACAA TATT -3´) were used to amplify 253 nucleotides derived from the *T. saginata* HDP2 253 bp sequence (Chiesa *et al.,* 2010*).* PCR product was sequenced in animal health institute .

The PCR reaction includes 5 µLTemplate DNA, 12.5 µL Emerald Amp GT PCR mastermix (2x premix), 1 µL of Forward primer (20 pmol), 1 µL Reverse primer (20 pmol) and 5.5 µL PCR grade water. The reaction was carried out on a thermocycler (Applied Biosystems, USA). This study used the PCR reaction conditions described by Chiesa *et al., (*2010). The sequences were used to obtain accesstion numbers from Gene Bank . The following sequences of cattle from EI-Menofia Provience and Gene Bank (AB984346 , KY290373, U45988, AB107237, GU097652, AB533172, HQ606075, MIN452862, JX402911, JN986702, JN986703, JN986693, JN986712, MW750280, AB984347, AB984348, AB984350, AB984351, OL459871, OL459872, OL459873, OL459874, OL459875, OL459876, OL459877, OL459878, MK644930, MK644934, MT074049, MT04050) were used to build the phylogenetic tree with the neighbor-joining method(Fig.3). According to Hall, (1999) BioEdit Version 7.0 software ClustalW Multiple alignment algorithm was used to perform a comparative alignment and Sequence identities and divergences were estimated. MEGA 7 software was used to create a phylogenetic tree according to Saitou and Nei (1987).

4. ***Estimation of economic losses***

The following equation was used to calculate the economic losses caused by organ condemnation: The financial loss produced by the condemnation of organs and carcasses was calculated using Ogunrinde and Ogunrinde (1980) formula.

AEL= (acs X ph X ach) + (acs X pr X acr) + (acs X pc X acc)

Where AEL= Annual economic loss expected because of organ or carcass condemnation

Acs =Average sum of cattle slaughtered in Ashmoun ,EI Shohada ,Quesina abattoirs

ph =percent of C. bovis in head. ach = Average cost of head. pr = percent of *C. bovis* in the heart

acr = Average cost of heart. pc = percent of *C. bovis* in carcass.

acc = Average cost of carcass.

RESULTS

3.1 ***Prevalence of C. bovis in slaughtered food animals in El-Menofia governorate***

During one year from january 2022 to Desember 2022. A total of 9488 carcasses (7638 male and 1850 female) of slaughtered cattle were inspected grossly by the naked eye in Ashmoun, El Shohada, and Quesina abattoirs in El-Menofia governorate, Egypt, for presence of C. bovis in it's muscles. In cattle, all examined females were 6-15 years and all the males were 1.5- 3 years. Examination of C. bovis, generally, from cattle infection rate was 0.98% (93/9844) (Table 1). Females were more susceptible than males to infection by C. bovis (59/1850) at 3.2% and (34/7638) at 0.44%, respectively (Table, 1). There was a positive connection between the

age of the examined carcasses and the prevalence of C. bovis. The higher prevalence was reported in female cattle of more than 5 years. The heart and masseter muscles were the highest predilection sites of the cysts (Fig.1). The prevalence of *C. bovis* was higher in the heart 0.67% (64/ 9844) than head 0.31% (29/9844) (Table, 2). The highest prevalence of *C. bovis* in cattle in the four seasons was in summer and spring at 1.1% followed by autumn 0.95% and winter at 0.74%, respectively (Table, 3). Heavy infection with *C. bovis* was observed one case in Quesina abattoir.

3.2 ***Molecular characterization of C. bovis.***

In this study the sample was examined by PCR of *C. bovis* isolated from meat samples from abattoir in El- Menofia Province. The sequence of the primers were used to amplify 253 nucleotides derived from the *T. saginata* HDP2 sequence (Fig.2). The sequence of *C. bovis* in the present study was highly identical with other sequencse of *T. saginata* in gene bank.

3.3. Economic losses of C. bovis in slaughtered cattle.

The financial losses after condemning portions affected with *C. bovis* in slaughtered inspected cattle in the current study were 102200 EGP. Furthermore, the economic costs after condemning heads were 29000 EGP, however, the deficits after condemning hearts were 27200 EGP. Moreover, the economic losses after condemning the two entire carcasses totaled 54,000 EGP. The condemnation of the whole carcass resulted in the highest economic losses (Table, 4).

Table 1. Total number of inspected and infected carcasses by *C. bovis* at El-Menofia abattoirs:

Abattoir	Cattle Carcass								
	Inspected			Infected					
	Male	Female	Total	Male	%	Female	%	Total	%
El-Shohada	2397	966	3363	11	0.46	34	3.5	45	1.3
Quesina	3465	728	4193	14	0.40	19	2.6	33	0.79
Ashmoun	1776	156	1932	9	0.50	6	0.34	15	0.77
Total	7638	1850	9488	34	0.44	59	3.2	93	0.98

Table 2. Organs distribution of the *C. bovis* in cattle carcasses

Infected organ / Sex	Head		Heart		Total	
	Infection	Percent	Infection	Percent	Infection	Percent
Male	11	37.93	23	35.94	34	0.44
Female	18	62.06	41	64.06	59	3.2
Total	29	0.31	64	0.67	93	0.98

Table 3. Total number of inspected and infected carcasses by *C. bovis* at different seasons

Season	Female cattle		Male cattle		Total		
	Inspected	Infected	Inspected	Infected	Inspected	Infected	%
Winter	396	13	2448	8	2844	21	0.74
Spring	688	25	2957	15	3645	40	1.1
Summer	454	13	1296	8	1841	21	1.1
Autumn	221	8	937	3	1158	11	0.95
Total	1850	59	7638	34	9488	93	0.98

Table 4. Economic losses of *C. bovis* in slaughtered cattle

Condemned part	No. of infected animal	Weight (kg)	Total condemned (kg)	Price/ kg (EGP)	Total loss (EGP)
Heart	64	1.7	108.8	250	27200
Head	29	10	290	100	29000
Whole carcass	1	270	270	200	54000
Total		-----		---	102200

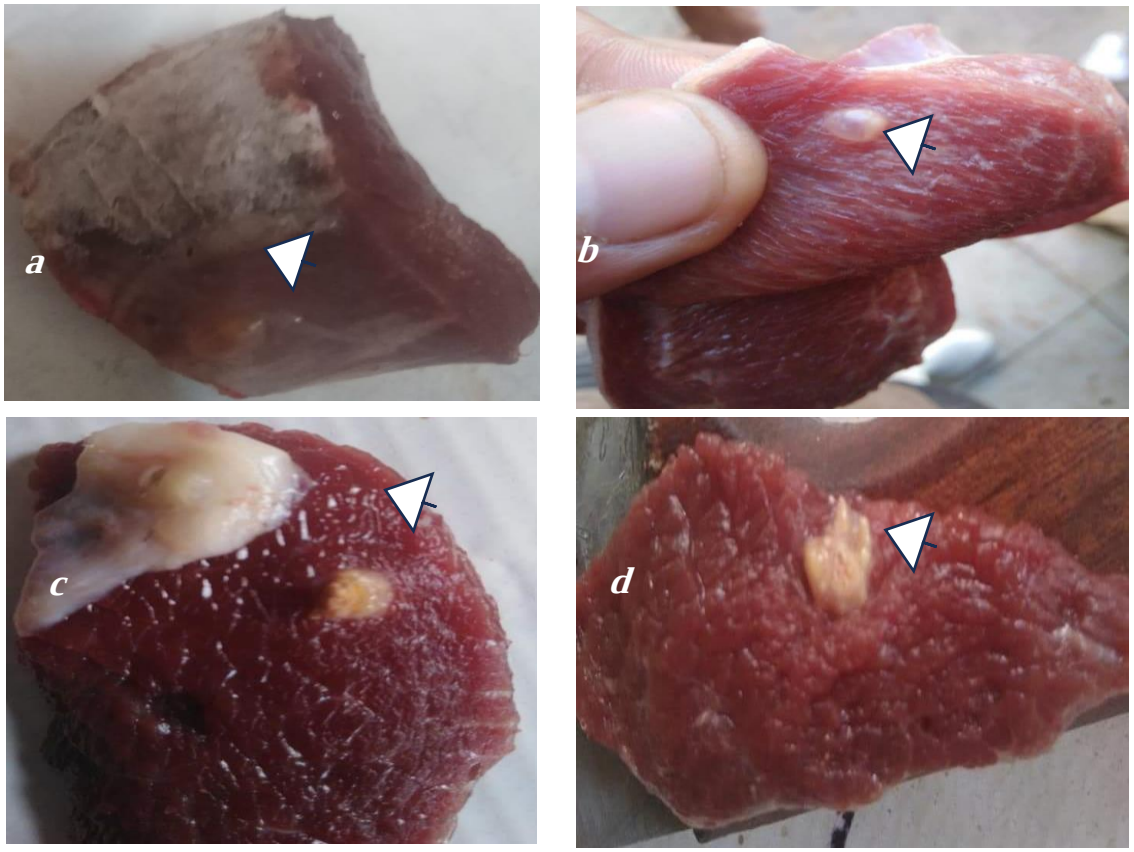


Fig. 1. **(a)**The heart demonstrates that live C. bovis is present in the muscle (arrowhead). **(b)** The masseter muscle showing the presence of live *Cysticercus bovis* showing oval shape fluid filled cyst (arrowhead) . **(c)** A whitish-yellow nodule protruded from the masseter muscle's surface (arrowhead). **(d)** an ancient, calcified lesion visible in the masseter muscle (arrowhead).

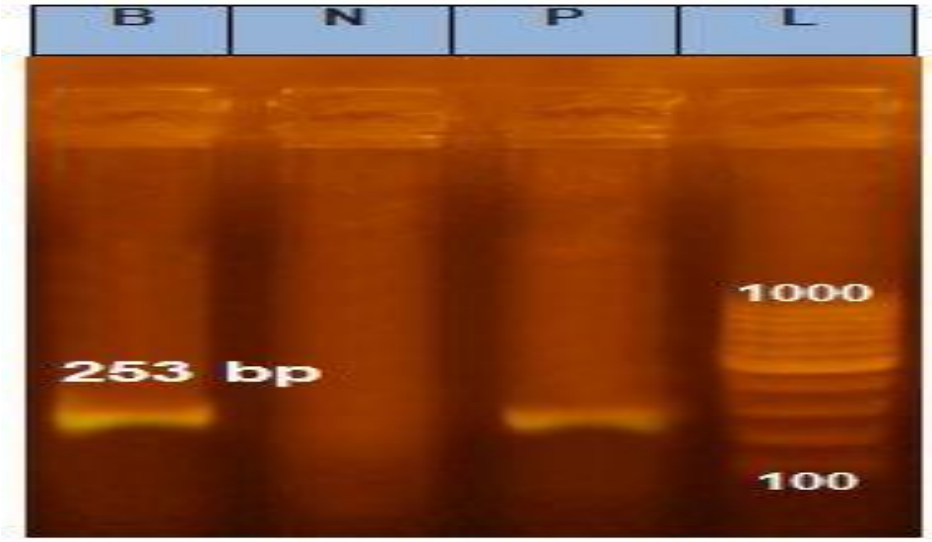
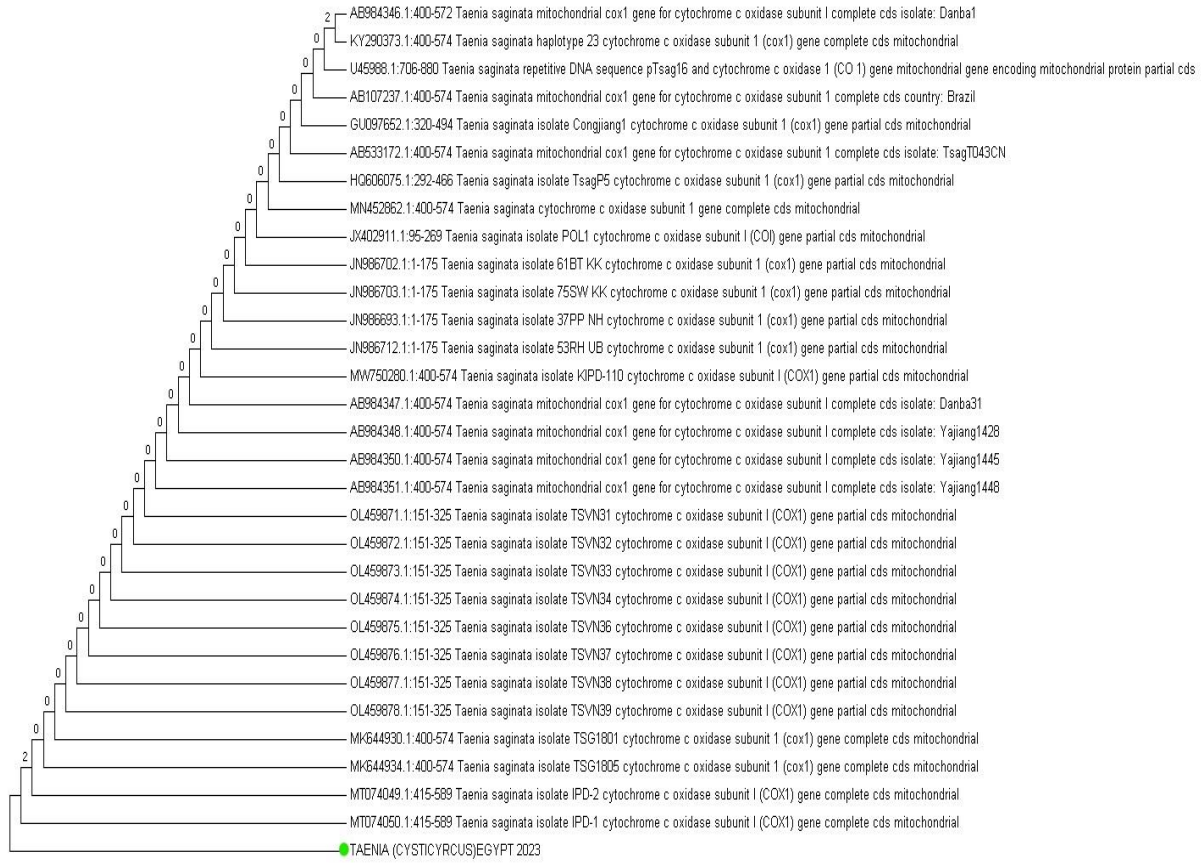


Fig. (2) PCR amplifies the HDP2 gene of *C. bovis* using F and R primers. The expected size of the PCR products was 253 bp. Arrowheads indicate positive.



(Fig. 3) Neighbor-joining (N-J) tree depicting phylogenetic relationships of Cysticercus Egypt 2023 and other recovered from GeneBank; the tree was analyzed by N-J analysis with 1000 bootstrap replicates.

DISCUSSION

A total of 9488 carcass (7638 males and 1850 females) of slaughtered cattle were inspected grossly by naked eye at Ashmoun , Quesina and El-shohda abattoirs at El-MenoFia Province, Egypt during one year starting from January 2022 to Desember (2022) for the presence of *C. bovis* in it's muscles and organs. All examined females were 6-15 years and all the males were 1.5-3 years. The infection rate in cattle generally was (93/9488) 0.98 %. Females were more susceptible than male to infection by *C. bovis* (59/1850) 3.2% and (34/7638) 0.44%, respectively. *C. bovis* infection was found in 0.98% of slaughtered cattle in the El-Menofia Province, Egypt, at various abattoirs. This results were higher than Hashemnia *et al.* (2015) at 0.078%, Geinoro *et al.* (2019) at 0.033%, Allam *et al.* (2022) at 0.24% but lower than Fahmy *et al.* (2015) at 9.07% in the Kalioubia Province, Elkhtam *et al.* (2016) at 6.09% in El-Menofia, at 3%, Dyab *et al.* (2017) at 7.5% in Aswan, El-Sayad *et al.* (2021) at 4.2%, Mazhani *et al.* (2022) at 6.2%, and Fesseha and Asefa (2023) at 4.24%. This difference in this study may be due to poor hygiene and drainage system.

C. bovis was found in male and female cattle. The increased prevalence was detected in female cattle older than 5 years, where the prevalence in female were 3.2 % and in males were 0.44%. These results were lower than Garedaghi *et al.* (2011) at 8.12% in females and 2.25% in young males, Wondimagegnei and Belete (2015) at 7.45% for females and 3.79% in males, and Figueiredo *et al.* (2019) at 12.8% females and 6.4% males.

This may be due to the long production life of females, but These resulst contradicts Kebede *et al.* (2009) who investigated that adult cattle prevalence for *C. bovis* was 7.6% in males and 6.6% in females. Also, Mekonnen (2017) studied the prevalence of *C. bovis in* males and females infected at rates of 4.03% and 0.91%, respectively. The difference in the present data may be due to males' use in agriculture work and insemination for long periods in these countries. All examined females were over 5 years and all the males were under 3 years, the presence of *C. bovis* was linked with the carcass age. The prevalence was highest in cattle over five years age. This result agree with Abdo *et al.* (2009) who reported that the presence of *C. bovis* was linked with the carcass age, bovine older than 2 years had a higher occurrence than those younger than two years. Similarly, Figueiredo *et al.* (2019) found infection in 21.62 % of adults and 6.60% in older ages. The increased infection in older ages may be due to decreased immunity against the infection and long exposure. This result contradicts Hailu *et al.* (2019), who reported no significant relationship connecting the prevalence of *C. bovis* and the gender or age of the cattle. While Dyab *et al.* (2017) recorde that *C. bovis* in young cattle at 7.5%, which may be due to geographical variations.

According to the organ distribution of cysts in this study the heart and masseter muscles were the highest predilection sites of the carcass. The prevalence of *C. bovis* was higher in the heart 0.67% than head 0.31%. These results consistent with Costa *et al.* (2012) found that *C. bovis* was most prevalent in the heart (1.90%), tailed by the head (1.11%), esophagus (0.08%), carcass (0.07%), diaphragm (0.03%), liver (0.02%), and tongue (0.01%). These results reverses Kebede *et al.* (2009) who showed that the cysts preferred head muscles (the tongue, masseter), cardiac, triceps, and thigh muscles. According to Bedu *et al.* (2011), the tongue had the highest proportion of *C. bovis* (34.4%), tailed by the heart (28.1%), triceps (21.9%), and masseter muscle (15.6%). Belachew and Ibrahim (2012) discovered that *C. bovis* was present in 67.74% of the tongue, 52% of the shoulder, 60% of the heart, and 75% of the masseter muscle.

According to Tesfaye *et al.* (2012), who recorded the prevalence of *C. bovis* was in tongue, masseter muscle, heart, shoulder muscle, and diaphragm at 2.22%, 1.66%, 1.11%, 0.92%, and 0.32%, respectively. Adugna *et al.* (2013) *C. bovis* was found in 4.6% of the carcasses. Distribution was 39.3% in the heart and 30.4% in the tongue which was higher levels than those of the diaphragm and liver. Wondimagegnei and Belete (2015), notice that shoulder muscle showed the highest prevalence of *C. bovis*, tailed by the masseter muscle, tongue, and lastly the heart , Summer and spring had the highest prevalence of *C. bovis* in cattle at 1.1%, followed by autumn at 0.95 % and winter at 0.74 %. These results indicated that seasons did not meaning fully impact to the prevalence of *C. bovis*. In this study the samples were examined by PCR for C. bovis isolated from meat samples collected from EL- Menofia abattoirs.

The primers(5´-GGGTGCTGGTATAGGGTGGACT -3´) and reverse (5´-ACGTAAATAAATAAGCCCACAATA TT -3´) were used to amplify 253 nucleotides derived from the T. saginata HDP2 sequence.

HDP2 primers detected a specific band of 253 bp.

CONCLUSIONS

At El-Menofia slaughterhouses, *Cysticercus bovis*, a disease that is highly common in cattle. The development of *C. bovis* in cattle is linked to a number of risk variables, including sex, age, season, and organ type. Because of the limited incidence of *C. bovis*, it is recommended

that cattle intermediate hosts of *T. saginata* be protected with an effective drainage system to avoid human infection. Medical and veterinary authorities who create effective treatment and prevention strategies to combat these diseases may be concerned about the low frequency of *C. bovis.*

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