



Article

***Cysticercus bovis* at Sohag Province: Prevalence, Morphological and Molecular Characterizations**

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Abstract

From July 2019 to June 2020, the prevalence of *Cysticercus bovis* was conducted to determine the percent of infection and, morphological, histological, and molecular characterizations of *C. bovis* on variant tissues. Results showed that the overall infection rate of *C. bovis* in cattle was (0.47 %) but in buffaloes was (0.04 %). Seasonal prevalence showed that the highest infection rate in cattle was in the spring season (0.56 %) and the lowest infection rate was in autumn (0.41 %), but in buffaloes, the infection rate was (0.05 %) in the summer season and (0.14 %) in the spring season. Organ distribution of *C. bovis* showed that in cattle the highest infection rate (64.43 %) was in heart muscle and the lowest infection rate (35.57 %) was in masseter muscles. In buffaloes, all positive cases were in cardiac muscles. PCR analysis using a primer of 18S rDNA gene produced size of 1600bp band at gel electrophoresis. Histopathological studies showed a cyst cavity formed by the larval stage beside necrosis and atrophy in different examined tissues.

Keywords

Prevalence, *Cysticercus bovis*, Molecular, Sohag province

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1. Introduction

Cattle and buffaloes are considered important food animals which reread to produce meat and milk (Borai *et al.*, 2013). Parasitic infection is recorded as one of the major causes of economic losses worldwide, these losses include partial condemnation of viscera or total condemnation of all carcasses, also reduction of meat and milk production is considered one of the important losses (Hassanin *et al.*, 2013).

Among parasitic zoonotic infestations, bovine Cysticercosis is classified as a major public health disease in both developed and ill-developed countries (Esatgil *et al.*, 2007).

Bovine Cysticercosis (*Cysticercus bovis*) is a parasitic disease caused by the larval stage of *Taenia saginata* in which the final host is human while the intermediate host is cattle. The final host gets infection with *Taenia saginata* through ingestion of meat (raw or undercooked) infected with *C. bovis* while the intermediate host gets infection through ingestion of eggs of *Taenia saginata* (Dupuy et al., 2014).

An intermediate host, striated muscles (Tongue, diaphragm, oesophagus, and masseter muscles), and cardiac muscles are recorded to be the predilection seats for infection with *C. bovis* (Minozzo et al., 2002; Hosseinzadeh et al., 2013; Cueto González et al., 2015).

Bovine Cysticercosis mostly arises in an environment with inadequate post-mortem examination of meat, low level of sanitation, primary rearing practices of animals, and poor management and control policy (Mann, 1983).

Bovine Cysticercosis shows no clinical manifestations in cattle, so detection of disease is based on meat inspection in slaughterhouses (Mirzaei et al., 2016).

Partial or total condemnation of carcass is determined according to the degree of infestation with cysts, total condemnation occurs in case of generalized infestation of carcass while partial condemnation occurs in case of localized infestation of organs by cysts and the rest of carcass will be kept at -7 °C for 3 weeks (Cueto González et al., 2015; Rostami et al., 2015; Mirzaei et al., 2016).

As bovine Cysticercosis is an important zoonotic disease that affects both animal health and the economy of the country, the study aims to identify *C. bovis* and its morphological and histopathological changes in tissue affected by it.

2-Materials and Methods

2.1. Study area

The present study was conducted during the period from July 2019 to June 2020 at seven abattoirs (Akhmim, Sohag city, El-Maragha, Tema, and Tahta) in Sohag Governorate (26°33'N 31°42'E) which located in Upper Egypt. Sohag province has a desert climate as in figure (1).

2.2. Animals

A total of 31610 cattle and 7065 buffaloes were slaughtered during the period of the study according to records at slaughterhouses of the study area. Slaughtered animals were males at age of 1-1.5 years old.

2.3. Sample collection and postmortem examination

Slaughtered animals were examined by a qualified veterinarian for the presence of any abnormal lesions. For the presence of *C. bovis*, meat inspections include heart, diaphragm, esophagus, tongue, masster muscles, and shoulder muscles. In case of positive cases for *C. bovis*, cysts were removed carefully from infected organs and put in plastic bags labeled with locality, sex, age, and date, then send to the Parasitology lab at the Faculty of Veterinary medicine, Sohag University.

2.4. Laboratory examination

Cysts were examined firstly macroscopically by the naked eye, then pressed by fingers to be categorized into calcified or viable cysts. Viable cysts were transparent in colour, bladder-like contain one protoscolex and filled with fluid while calcified cysts contain cheesy solid material (Fahmy et al., 2015).

Secondly, cysts were examined microscopically by histopathological protocols using eosin and hamatoxylin stain (Suvarna et al., 2013).

Cysts that intended to be used in molecular identification were washed with normal saline and preserved at 70 % ethyl alcohol.

2.5. PCR analysis

DNA was extracted from the samples by using Quick-gDNA™ Miniprep Kit (ZYMO RESEARCH, USA, 50 Preps) according to the manufacturer's instructions. The used primers for detection of 18S DNA gene were selected according to (Yan et al., (2013)). The selected primers were '5-TCC TGC CAG TAG TCA TAT GC-3' (forward) and '5-CTTGTTACG ACT TTT ACTTCCTCT-3' (reverse). The mixer of Taq polymerase (Thermo Scientific, USA) 12.5µl, primers (Forward and reverse) 1µl 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 sec, 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 7min). The PCR products were stored in the thermal cycler at 4°C until they were collected.

2.6. Statistical analysis

The collected data in the present study were introduced into Excel spreadsheets (Windows 2010), and statistically analyzed using IBM SPSS statistics software, version 22. (Chicago, IL). According to the data distribution, descriptive data was reported as means (M) with standard deviations and ranges. Frequency distributions were used to characterize categorical variables. The Chi-square test was employed. A *P* values of < 0.05 was significant while *P* values of < 0.01 was highly significant.

3. Results

In the present study, macroscopic examination showed that the average size of cyst was 1-1.5cm, whitish in color, oval, filled with transparent fluid, and containing 1 protoscolex.

In the current study, data in the table (1) showed that the overall rate of infection with *C. bovis* in cattle was (0.47 %) while in buffaloes was (0.04 %).

Seasonal prevalence in the table (1) showed that the highest infection rate in cattle was in the spring season (0.56 %) followed by the summer season (0.48 %) then the winter season (0.42 %) and the lowest infection rate was in autumn (0.41 %). In buffaloes, infection with *C. bovis* was in the summer and spring season, the prevalence rate of infection was (0.05 %) and (0.14 %) respectively.

As in table (2), organ distribution of *C. bovis* showed that the highest infection rate in cattle was in heart muscle (64.43 %) and the lowest infection rate was in masseter muscles (including tongue and head muscles) with an overall prevalence (35.57 %). In buffaloes, all positive cases were in heart with overall prevalence (100 %).

Based on PCR analysis, results showed that amplification of the 18S rDNA gene produced a band of 1600bp on gel electrophoresis (Figure2).

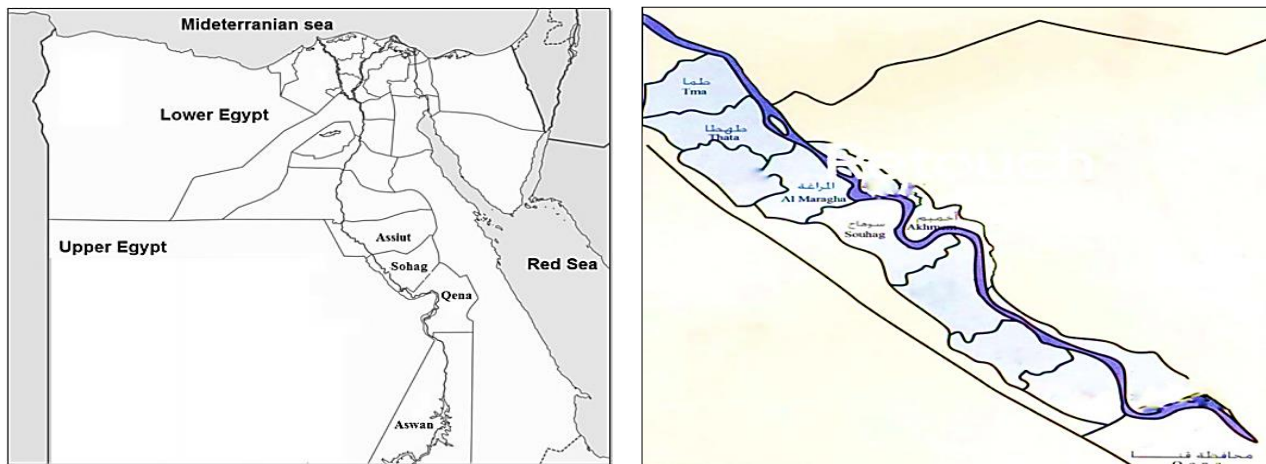


Figure 1. Different districts of the study area in Sohag Governorate represented in Egypt map

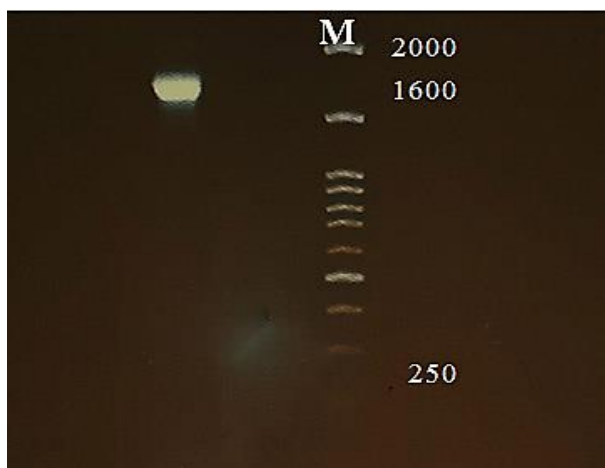


Figure 2. Agarose gel electrophoresis of 18S rDNA gene PCR products of *C. bovis* M= 100bp DNA ladder

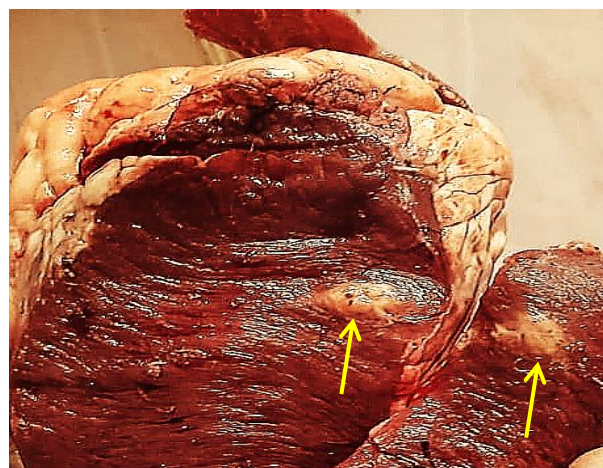


Figure 3. Macroscopic appearance of *C. bovis* in heart



Figure 4. Macroscopic appearance of *C. bovis* in masseter muscles.



Figure 5. Macroscopic appearance of *C. bovis* in tongue.

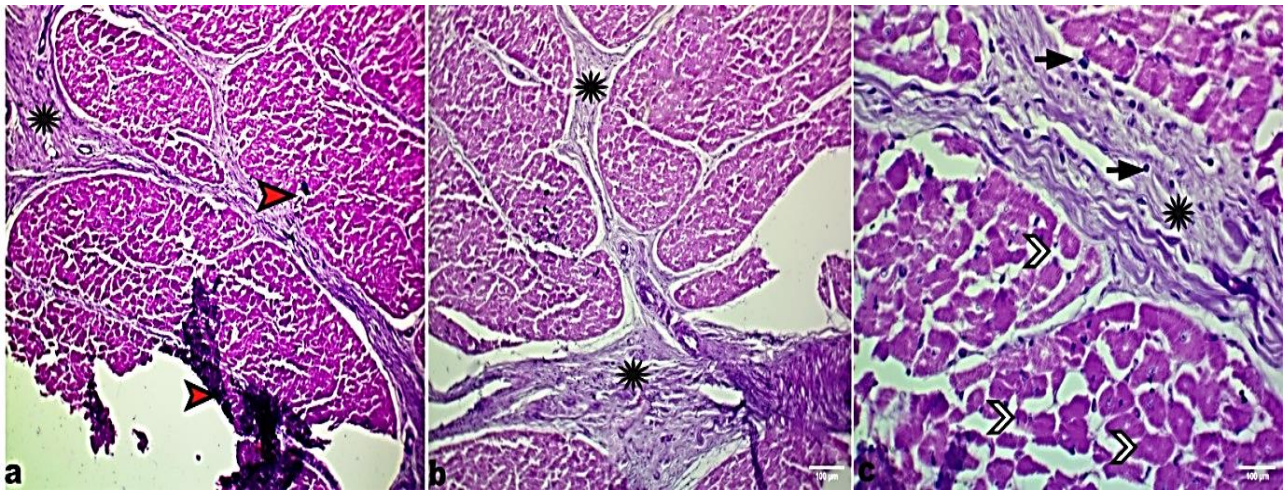


Figure 6. Photomicrograph of heart muscle infected by *C. bovis* sections stained by Hematoxylin and eosin showing: a. parasite cyst (red arrows), b. severe fibrosis between cardiac muscles (stars), c. fibrous tissue (star) infiltrated with mononuclear inflammatory cells (black arrows), necrosis and atrophy at cardiac myocytes (white head arrows)

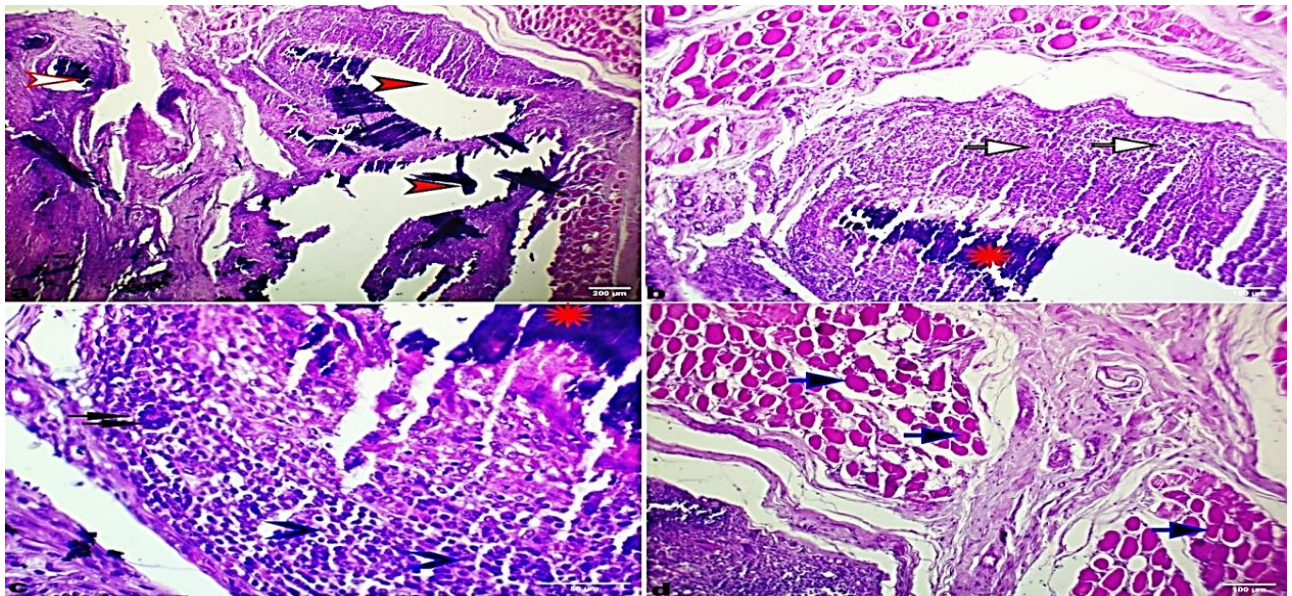


Figure 7. Photomicrograph of muscle infected by *C. bovis* sections stained by Hematoxylin and eosin showing: a. cyst formed by a cavity where the parasite used to be (red arrows), b. a central necrotic and calcified area (star), surrounded by an intense granulomatous wall (white arrows), c. intense inflammatory reaction rich in eosinophilic polymorphonuclear leukocytes (blue head arrows) and Langerhans giant cells (black arrows), d. atrophy of muscle fibers (arrows)

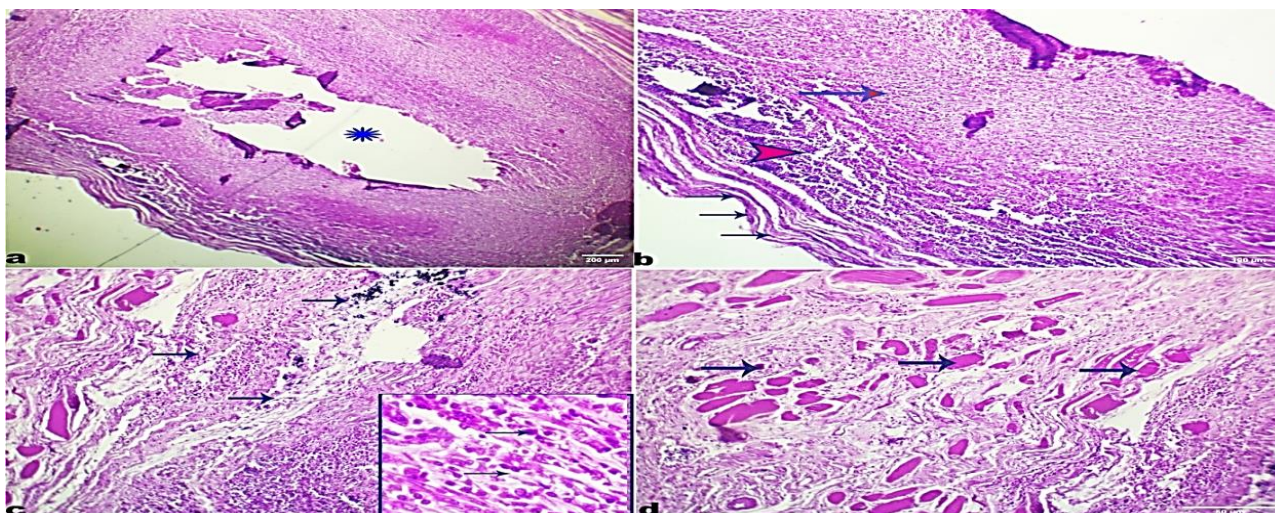


Figure 8. Photomicrograph of tongue infected by *C. bovis* sections stained by Hematoxylin and eosin showing: a: Parasitic granuloma formed by a central cavity containing remnants from the dead calcified parasite (star), b. pyogranulomatous thick wall (thick arrow), necrosis (redhead arrow), all surrounded by a fibrous capsule (thin arrows). c. pyogranulomatous wall showing necrosis (arrows) with an intense inflammatory reaction rich in eosinophilic polymorphonuclear leukocytes (selected square, arrows). d. severe atrophy of muscle fibers (arrows).

Table 1. Prevalence of *C. bovis* infection in Cattle and buffaloes in relation to the season in the study area

Species	Season	Total examined	Positive (N)	Positive ** (%)
Cattle	Summer	8289	40	0.48
	Autumn	6897	28	0.41
	Winter	8101	34	0.42
	Spring	8323	47	0.56
	Total	31.610	149	0.47
Buffaloes	Summer	1921	1	0.05
	Autumn	1759	0	0
	Winter	1916	0	0
	Spring	1469	2	0.14
	Total	7065	3	0.04

** Highly significant

Table 2. Prevalence of *C. bovis* in cattle and buffaloes in relation to organ distribution.

Species	Sites of infection	Positive (N)	Positive (%)
Cattle Total=149	Heart	96	64.43
	Masseter muscles (Tongue & head)	53	35.57
Buffaloes Total =3	Heart	3	100
	Masseter muscles (Tongue & head)	0	0

4. Discussion

In the present study, the overall prevalence of *C. bovis* in both cattle and buffaloes was (0.47 %) and (0.04 %) respectively. The present study is higher than the previous study by Elmonir

et al. (2015) in Egypt who found that the overall prevalence of *C. bovis* during January to February 2013 was (0.44 %), other observations are higher than the present one as studies in Egypt by Yassien et al. (2013), Abdel-Hafeez et al. (2015), El-Alfy et al. (2017) and Dyab et al. (2017) who observed that the overall prevalence was (0.58 %), (20 %), (0.51 %) and (7.5 %) respectively. In buffaloes, previous observations are higher than the current study as studies in Egypt by Yassien et al. (2013), Elmonir et al. (2015), and Dyab et al. (2019) who noticed that the overall prevalence was (0.18 %), (0.13 %) and (6 %) respectively. The difference in results might be caused to the difference in sample size, management system, and to what extent the animal is in contact with the parasitic egg of *Taenia saginata*.

Seasonal prevalence showed that the highest infection rate with *C. bovis* in cattle was in the spring season, while in buffaloes, infection was observed only in summer and spring season, the highest infection rate was in the spring season. Elkhtam et al. (2016) in Egypt agree with the present study that the spring season had the highest infection rate, in contrast, El-Alfy et al. (2017) in Egypt found that the winter season had the highest infection rate, and Dyab et al. (2019) in Egypt observed that the highest infection rate was in the summer season. In buffaloes, studies in Egypt by Dyab et al. (2017) and Dyab et al. (2019) found that the highest infection rate with *C. bovis* in buffaloes was in the winter and summer seasons respectively. The variations in infection rate might be due to differences in temperature degree and humidity.

In the present study, organ distribution of *C. bovis* in cattle showed that the highest percentage of infection was in heart muscles while the lowest incidence rate was in masseter muscles (tongue and head). In buffaloes, infection with *C. bovis* in the present study was only in heart muscles with a prevalence of 100 %. Studies by Youssef et al. (2013) and Abdel-Hafeez et al. (2015) in Egypt, Nzeyimana et al. (2015) in Rwanda and Gholami et al. (2019) in Iran confirmed the current observation in cattle that the highest infection rate was in heart, in contrast, Engdaw et al. (2015) in Ethiopia found that the highest infection rate was in triceps muscles. In buffaloes, a study by Dyab et al. (2017) in Egypt showed that infection with *C. bovis* was in cardiac muscles only, also studies by Youssef et al. (2013) and Dyab et al. (2019) in Egypt found that the highest infection rate with *C. bovis* in buffaloes was in heart muscles. The variations depend on the activity of the animal, age and breed, and geographical areas.

5. Conclusion and recommendations

Helminth parasites' diseases remain a worthy cause of the decrease in the productivity of ruminant animals and condemnation of meat in abattoirs in Sohag governorate. Infection with *C. bovis* was higher in cattle (0.47 %) than buffaloes (0.04 %). Cardiac muscles are the most infected site with *C. bovis*. *C. bovis* cyst made more pathological changes in affected tissues.

We recommend further studies to cover all parasitic diseases that affect human health.

References

- Abdel-Hafeez, E. H., Kamal, A. M., Abd Elgelil, N. H. & Abd EL-Fatah, M. M. (2015). Parasites transmitted to human by ingestion of different types of meat, El-Minia city, El-Minia governorate, Egypt. *Journal of the Egyptian Society of Parasitology*, 45(3), 671- 680.
- Borai, M. G., Nagi, A. A., Gab-Allah, M. S., El-Mashad, I. A., & Moustafa, S. A. (2013). Comparative pathological studies on parasitic affections of liver in farm animals. *Benha Veterinary Medical Journal*, 25(2), 284-295.
- Cueto González, S. A., Rodríguez Castillo, J. L., López Valencia, G., Bermúdez Hurtado, R. M., Hernández Robles, E. S., & Monge Navarro, F. J. (2015). Prevalence of *Taenia saginata* larvae

- (*Cysticercus bovis*) in feedlot cattle slaughtered in a federal inspection type abattoir in northwest Mexico. *Foodborne pathogens and disease*, 12(5), 462-465.
- Dupuy, C., Morlot, C., Gilot-Fromont, E., Mas, M., Grandmontagne, C., Gilli-Dunoyer, P., & Callait-Cardinal, M. P. (2014). Prevalence of *Taenia saginata* cysticercosis in French cattle in 2010. *Veterinary Parasitology*, 203(1-2), 65-72.
- Dyab, A. K., Ahmed, H. A., Hefnawy, Y. A., Abdel Aziz, A. R., & Gomaa, M. A. (2019). Prevalence of Tissue Parasites in Cattle and Buffaloes Slaughtered in El-Minia Governorate Abattoirs, Egypt. *Journal of Veterinary Research*, 4(2), 49-58.
- Dyab, A. K., Marghany, M. A., Othman, R. A., Ahmed, M. A. & Abd- Ella, O. H. (2017). *Taenia Saginata* in Man and Cysticercosis in Cattle and Buffaloes in Aswan Governorate, Egypt. *Journal of the Egyptian Society of Parasitology*, 47(2), 389 - 394.
- El-Alfy, E. N., Al-Kappany, Y. M. & Abu-Elwafa, S. A. (2017). Parasitological and Pathological studies on Tissue parasites among slaughtered animals in Dakahlia province, Egypt. *Egyptian Veterinary Medical Society of Parasitology Journal*, 13, 78-98.
- Elkhtam, A. O., Mostafa, I. A., & Shawish, R. R. (2016). Prevalence and Economic Impact of *Cysticercus bovis* in Slaughtered Cattle in Menofia Province, Egypt. *Alexandria Journal of Veterinary Sciences*, 50 (1), 130-134.
- Elmonir, W., Mousa, W., & Sultan, K. (2015). The Prevalence of Some Parasitic Zoonoses in Different Slaughtered Animal Species at Abattoir in the Mid-Delta of Egypt; with Special Reference to its Economic Implications. *Alexandria Journal of Veterinary Sciences*, 47, 97-103.
- Engdaw, T. A., Alemneh, A. T. & Ambaw, S. T. (2015). Study on the Prevalence of *Cysticercus bovis* in Kombolcha Elfora, North-Eastern Ethiopia. *European Journal of Applied Sciences*, 7 (4), 152-157.
- Esatgil, M. U., & Tüzer, E. (2007). Prevalence of hydatidosis in slaughtered animals in Thrace, Turkey. *Türkiye Parazitoloj Derg*, 31(1), 41-45.
- Fahmy, HA, Khalifa, NO, El-Madawy, RS, Afif, JSA, Aly, NSM, et al, 2015: Prevalence of bovine cysticercosis and *Taenia saginata* in man. *Glob. Vet. J.* 15, 4:372-80.
- Gholami, N., Mosayebi, M., Abadi, P. D. R., Atigh, H. R., Sedaghat, R., Zadeh, M. H. N. & Farahani, M. (2020). Bovine cysticercosis in feedlot cattle in central region of Iran. *Journal of Parasitic Diseases*, 44(1), 25-30.
- Hassanin, F. S., Shaltout, F. A., & Afifi, M. E. (2013). Parasitic affections in edible offal. *Food Control Department, Faculty of Veterinary Med. Moshtohor, Benha University, Egypt*.
- Hosseinzadeh, S., Setayesh, A., Shekarforoush, S. S., & Fariman, S. H. (2013). An epidemiological survey on the determination of *Taenia saginata* cysticercosis in Iran, using a PCR assay. *Veterinary Record*, 172(17), 451-451.
- Mann, I., 1983. Environmental, hygiene and sanitation based on the concept of primary health care as a tool for surveillance, prevention and control of taeniasis/cysticercosis. *Current publication in Health Research in Tropics*, 36: 127-140.
- Minozzo, J. C., Gusso, R. L. F., Castro, E. A. D., Lago, O., & Soccol, V. T. (2002). Experimental bovine infection with *Taenia saginata* eggs: recovery rates and cysticerci location. *Brazilian Archives of Biology and Technology*, 45, 451-455.
- Mirzaei, M., Nematollahi, A., Ashrafihelan, J., & Rezaei, H. (2016). Prevalence of infection with the larval form of the cestode parasite *Taenia saginata* in cattle in northwest Iran and its zoonotic importance. *Türkiye Parazitolojii Dergisi*, 40(4), 190.

- Nzeyimana, P., Habarugira, G., Udahemuka, J. C., Mushonga, B. & Tukei, M. (2015). Prevalence of bovine cysticercosis and age relationship at post-mortem in Nyagatare Slaughterhouse. *World Journal of Agricultural Sciences*, 3 (3), 29-33.
- Rostami, S., Salavati, R., Beech, R. N., Babaei, Z., Sharbatkhori, M., & Harandi, M. F. (2015). Genetic variability of *Taenia saginata* inferred from mitochondrial DNA sequences. *Parasitology research*, 114(4), 1365-1376.
- Suvarna, S. K., Layton, C. & John, D. Bancroft, J. D. (2013). *Bancroft's Theory and Practice of Histological Techniques 7th ed.* , Elsevier Health Sciences, china.
- Yan, H., Lou, Z., Li, L., Ni, X., Guo, A., Li, H., Zheng, Y., Dyachenko, V. & Jia, W. (2013). The nuclear 18S ribosomal RNA gene as a source of phylogenetic information in the genus *Taenia*. *Parasitol Research* ,112, 1343–1347.
- Yassien, M. A., Ahmed, A. M., Soliman S. A. & Youssef, A. I. (2013). Prevalence of Parasitic Infection of Slaughtered Cattle and Buffalo in Municipal Abattoir at Ismailia, Egypt. *Parasitic Zoonoses in Asian-Pacific Region*. Sankeisha Publishing Agent, 19-24.
- Youssef, A.I., Yassien, M.A., Ahmed, A.M. & Soliman, S.A. (2013). Prevalence, Intensity and Viability of Tissue Parasites Infected Bovine Carcasses at Ismailia -Egypt with Special Reference to their Zoonotic implications. *Parasitic Zoonoses in Asian-Pacific Regions*, 40, 1-6.

الملخص العربي
السيستيسيركس بوفيس في محافظة سوهاج: الانتشار والخصائص المورفولوجية والجزيئية

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من يوليو 2019 إلى يونيو 2020، تم إجراء دراسة على مدى انتشار السيستيسيركس بوفيس لتحديد النسبة المئوية للعدوى وكذلك الخصائص المورفولوجية والنسجية والجزيئية على الأنسجة المختلفة. وأظهرت النتائج أن المعدل الإجمالي للإصابة في الأبقار كان (0.47 %) ولكن في الجاموس كان (0.04 %). أظهر الانتشار الموسمي أن أعلى معدل إصابة في الأبقار كان في موسم الربيع (0.56 %) وكان أدنى معدل إصابة في الخريف (0.41 %)، ولكن في الجاموس، كان معدل الإصابة (0.05 %) في موسم الصيف و (0.14 %) في موسم الربيع. أظهر معدل توزيع السيستيسيركس بوفيس أن أعلى معدل إصابة في الأبقار كان في عضلة القلب وكان أقل معدل إصابة (35.57 %) في العضلات، في الجاموس، كانت جميع الحالات الإيجابية في عضلة القلب. تحليل البوليميرز المتسلسل باستخدام البرايمر الخاص بجين 18S rDNA أنتج باندات بحجم 1600. أظهرت الدراسات النسيجية وجود تجويف كيسي تكون بواسطة مرحلة اليرقات إلى جانب النخر والضمور في الأنسجة المختلفة التي تم فحصها.

الكلمات المفتاحية

الانتشار، *Cysticercus bovis*، الجزيئي، محافظة سوهاج