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Parasitology

Prevalence of Cestodes in Baladi Chickens and Molecular Characterization of *Raillietina echinobothrida* in Menouf District, Menoufia, Egypt

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ABSTRACT

Native Chickens' tapeworms are enteric obligatory intestinal parasites and cause losses in chicken production. The present study was conducted in Menouf district, Menoufia, Egypt through the year 2022 to determine the prevalence of tapeworms in chickens. A special study on morphology, sizes of recovered *Raillietina echinobothrida,* molecular characterization using ITS-2 rDNA region PCR and sequencing analysis, and P.M lesion was fully discussed. Intestinal samples of 898 randomly selected from Baladi chickens were subjected to parasitological examination. The cestodes had a prevalence of 12.03%. *Raillietina* spp. had a prevalence of 7.3%. The prevalence of each species was 1.7% for *Raillietina tetragona*, 3.6% for *R. echinobothrida*, and 2.1% for *R. cesticellus. Cotugnia digonopora* had a prevalence of 4.7%. The prevalence of infection was influenced also by season but not by locality. The PCR of ITS-2 rDNA of *R. echinobothrida* produced specific bands at 615 bp. The sequence had an identity percentage of 93.69% with the ITS-2 sequences of *R. echinobothrida* from Egypt and 90.96% with the ITS-2 sequence from China. The phylogenetic analysis showed that the sequence of ITS-2 of *R. echinobothrida* from Egypt clustered in the same taxon as the *R. echinobothrida* sequences from Egypt and China. The sequencing analysis using the ITS-2 gene will be a valuable tool for species identification. The current study presented the prevalence of cestodes in Baladi chickens and the molecular

characterization of *R. echinobothrida* in Menouf district, Menoufia, Egypt. The effect of locality and season on the prevalence was considered at $P < 0.05$.

Keywords: *Raillietina echinobothrida*; ITS2; Molecular characterization; histopathology, Chickens.

INTRODUCTION

Parasitic diseases were badly affecting bird production. Tapeworms (phylum Platyhelminthes, class Cestodes) (Belete et al., 2016). The percent of poultry protein consumption compared to total animal production in the world was increased (Alasadiy et al., 2020). Tapeworms had physically segmented body and found in the small intestine or expelled with feces (Leeson and Summer, 2009). Chickens parasitic infection may be clinical or subclinical (Soulsby, 1986). Cestodes are the most common parasites that cause high damage and great losses in the bird industry due to their effects on feed consumption, conversion rate, egg production, and body weight, and cause high mortality, especially in young age birds (Puttalakshmamma et al., 2008). Helminthes parasites cause severe pathological lesions which could lead to severe illness or death to bird in some times (Soulsby, 1986). The cestode worms live over a greater length in the intestine of old chickens than in young (Gray, 1972). *Raillietina echinobothrida* has round rostellum and nearly round suckers (Hofstad et al., 1984). *R. echinobothrida* inhabits the small intestine of infected domestic native chickens (*Gallus domesticus*) (Rahmadani et al., 2022). Of the three *Raillietina* spp., the *R. echinobothrida* was the most pathogenic worm which caused hyperplastic enteritis and nodules may be formed in heavy infections at the site of worm attachment in the intestine, so it was called "Nodular tapeworm disease" (Simon and Emeritus, 2005). In histopathological identification, there was a variable degree of degenerative, necrosis changes and infiltration of lymphocytes, eosinophils, and heterophils (Mir et al., 2010).

Raillietina echinobothrida was reported in different localities such as Ghana (Poulsen et al., 2000), Ethiopia (Eshetu et al., 2001), Bauchi (Yoriyo et al., 2008), Kenya (Maina et al., 2012), Eastern Shewa zone, Ethiopia (Hussen et al., 2012), northwest Himalayan region of India (Dar and Tanveer, 2013), and Iraq (Amin and Kakabwab, 2019). Molecular identification of *R. echinobothrida* was carried out using nuclear gene of internal transcribed spacer 2 (ITS2) and mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 (ND1) was recorded (Littlewood et al., 2008; Ramnath et al., 2014; Butboonchoo et al., 2016; Panich and Chontananarth 2021; Yang et al., 2021). We conducted this study to reveal the prevalence of cestodes and their related risk factors and molecular characterization of *R. echinobothrida* using ITS-2 rDNA region PCR and sequencing analysis in native chickens from the Menoufia governorate, Egypt.

MATERIALS AND METHODS

1. Ethical approval:

Dealing with animals in this study was conducted following the regulations of the Institutional Animal Care and Use Committee (IACUC) at the University of Sadat City, Egypt. The study was given the ethical protocol approvel number (IACUG) OF VUSC-34-1-23.

2. Sample collection and study area:

The present study was conducted in Menouf district, Menoufia, Egypt through the year 2022 from January to December to determine the prevalence of tapeworms in chickens. Chickens were reared at small house keeping system. Live cestodes worms were collected from the small intestine of naturally infected domestic Baladi chickens from four villages in the Menouf district (Gizi, Sirs Elian, Barhim, and Elhamol), Menoufia governorate, Egypt. Samples were transferred to the Laboratory of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt for processing.

3. Sample preparation:

Preparation of the collected cestodes for examination was made according to standard technique (Carleton, 1957; Pritchard and Kruse 1982). The washed relaxed worms (by physiological saline) were compressed between two slides in the (10% formalin) overnight for fixation (Pritchard and Kruse 1982). Acetic acid-alum carmine was used at 1% concentration for staining the worm samples. Examination of worms by light microscope (LM) at Lab of Parasitology, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt. Samples were identified with all data (time, date of collection, and locality). For molecular and sequencing analysis, the worm samples were rinsed well with clean water several times and frozen at -20 ℃ for later DNA extraction.

4. Morphological observations of the recovered worms:

For light microscope observations, the worms were mounted in Canada balsam or DPX on a slide then a coverslip was applied overnight at 37 °C in the incubator to dry and undergo microscopic identification. The identification of the worm depended on shape, diameter of all parts, scolex breadth, and characters of mature and gravid segments. Helminthes cestodes were identified morphologically according to (Soulsby, 1986). Accurate morphological identification was obtained by colored photographs.

5. DNA extraction:

DNA was isolated from the worms using kit (Easy Pure Genomic DNA Kit®). The reaction volume was 25µL, including 1 µL of the forward and reverse primers, 2 µL of DNA (100 ng), 5 µL of a master mix (Super Mix 2X Easy Taq PCR®), and up to 25 µL of double-distilled water (DDW). The PCR reaction was performed on a thermal cycler (G-STORM). The DNA was analyzed on 1% agarose gel. The isolated DNA was stored at -20 °C.

6. PCR amplification of ITS2 rDNAs region:

The ITS2 rDNAs region was amplified by using the PCR primers, forward. 3S (5' GGT ACC GGT GGA TCA CTC GGC TCG TG-3') and Reverse. A28 (5' GGG ATC CTG GTT AGT TTC TTT TCC TCC GC-3') (Bowles et al., 1995; Ramnath et al., 2014). The PCR reaction conditions were as follows: 5 min initial denaturation at 95 °C, followed by 35 cycles of 30 sec DNA denaturation at 95 °C, 45-sec primer annealing at 55 °C, and extension at 72 °C for 1 min. and the final extension at 72 °C for 10 min. The product of the amplification of DNA was exposed to agarose gel electrophoresis examination by using an ethidium bromide stain.

7. Sequencing and Phylogenetic analysis:

The PCR products were purified and

sequenced at the Animal Health Research Institute, Ministry of Agriculture, Dokki, Egypt. The sequences were blasted with the NCBI BLAST tool. The sequence was deposited in GenBank. The neighbor-joining phylogenetic tree was constructed by the MAFFT program version 7. The ITS-2 rDNA region sequence of *R. echinobothrida* from Menouf, Menoufia, Egypt (LC806100) was used to make the tree with other sequences from GenBank, including *R. echinobothrida* (MH122787, MT908910, MW602635, MW602636, and MW602637), *R. dromaius* (AY382320), *R. australis* (AY382317), *R. beveridgei* (Y382318), *R. chiltoni* (AY382319), and *R. tetragona* (MH122788). *Taenia solium* ITS-2 (KT899311) was used as an outgroup.

8. Histopathological examination:

Intestinal samples were taken and fixed in neutral buffered formalin (10%) for fixation. After 72 hours, samples were dehydrated, embedded all in paraffin wax, and sectioned (3 µm) for staining by using hematoxylin and eosin stain (H&E) (Bancroft and Gamble 2008). Histological photographs were picked with a Leica digital EC3 camera.

9. Statistical analysis:

The effect of locality and season on the prevalence of cestodes was analyzed using the Q-square test by the SPSS program. Statistical significance was considered at $P < 0.05$.

RESULTS

1. Prevalence of recovered cestodes:

The recovered cestodes were detected at a prevalence of 12.03% (108 out of 898). The prevalence of *Raillietina* spp. was 7.3% (66 out of 898), which included *Raillietina tetragona* by 1.7% (15 out of 898), *Raillietina echinobothrida* 3.6% (32 out of 898) and *Raillietina cesticellus* 2.1% (19 out of 898). *Cotugnia digonopora* was detected at 4.7% (42 out of 898). The infection rate of tapeworm was recorded in four localities of Menouf district, Menoufia province, Egypt. The highest prevalence of *R. tetragona* was in Sirs Elaian at 2.8%, but the lowest was in Barhim at 0.5%. The highest prevalence of *R. echinobothrida* was in Elhamol (5.5%), but the lowest was in Barhim (2.5%). The highest infection rate with *R. cesticillus* was 3.2% in Sirs Elaiana, then decline to reach 1.2% in Elhamol village. The highest prevalence of *Cotugnia digonopora* was 8% in Elhamol, then this percentage decreased to 3.5% in both Barhim and Sirs Elaian (Table 1). The locality has a nonsignificant effect on the prevalence of tapeworms (X^2 = 6.5425 and $P < 0.088$) (Table 1). The locality has a nonsignificant effect on the infection prevalence of *R. tetragona* (X^2 = 5.1226 and $P < 0.163039$), *R. echinobothrida* (X^2 = 2.5668 and $P < 0.463345$), *R. cesticillus* (X^2 = 2.9223 and $P < 0.403753$), and *Cotugnia digonopora* (X^2 = 5.6423 and $P < 0.130373$) (Table 1). The highest prevalence was in the summer season (14.6%) and (7.3%) for *Raillietina* spp. and *Cotugnia digonopora*, respectively. while the lowest infection rate was in the spring season at 2.3% and 0.6% for *Raillietina* spp. and *Cotugnia digonopora*, respectively (Table 2). The prevalence of chicken tapeworms was significantly affected by the season (X^2 = 45.6702 and $P < 0.00001$). The season significantly affected the prevalence of *Raillietina* spp. (X^2 = 32.6546 and $P < 0.00001$) and *Cotugnia digonopora* (X^2 = 13.4244 and $P < 0.003803$) (Table 2).

2. The Morphological description of the recovered Raillietina echinobothrida:

R. echinobothrida was recorded as (Fig. 1). *R. echinobothrida* was whitish color its size ranged from 16-23 cm (18 cm) in length. Scolex was round and large. Rostellum had 2 rows of hammer-shaped hooks. Sucker was armed and round. Neck was absent. Genital pore opening was unilateral. Mature segment had one set of genital organs. Gravid segment had 6-12 egg per each egg capsule.

3. The measurements of the recovered Raillietina echinobothrida:

The measurements of the recovered *R. echinobothrida* were recorded. Scolex breadth was 0.3 mm x 0.3 mm (0.27 mm). Rostellum breadth was 0.06 mm x 0.06 mm (0.06 mm). Sucker breadth was 0.2 mm x 0.1 mm (0.15 mm). Mature segment was 0.4 mm x 0.7mm (0.55 mm). Gravid segment was 0.5 mm x 1.3 mm (0.9 mm)**.**

4. Molecular characterization of the recovered Raillietina echinobothrida:

Molecular identification of the recovered *R. echinobothrida* was recorded (Fig. 2). A study on four *R. echinobothrida* samples, each of them was collected from each of four areas of study by using specific primer for ITS2 (second internal transcribed spacer gene), the result revealed that two worm samples only give specific band at 615 bp from Gizi and Sirs Elaian. PCR produced specific bands of ITS-2 rDNA of *R. echinobothrida*. The sequence of ITS-2 rDNA region of *R. echinobothrida* from Menouf, Menoufia, Egypt (LC806100) had a size of 628 bp including the 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence. It had an identity percentage of 93.69% with the ITS-2 sequences of *R. echinobothrida* from Egypt (MW602637, MW602636, and MW602635). It had an identity percentage of 90.96% with the ITS-2 sequence of *R. echinobothrida* in chickens from China (MT908910), 90.68% with the ITS-2 sequence of *R. cesticillus* from emus in Australia (AY382321), and 81.19% with the ITS-2 sequence of *R. echinobothrida* in chickens from China (MH122787). The phylogenetic analysis showed that the sequence of ITS-2 of *R. echinobothrida* from Egypt clustered in the same taxon as the *R. echinobothrida* sequences from Egypt and China. Other *Railletina* species in the analysis cluster in another taxon, including *R.* tetragona from China (MH122788) and *R. dromaius* (AY382320), *R. australis* (AY382317), *R. chiltoni* (AY382319), and *R. beveridgei* (AY382318) in *Dromaius novaehollandiae* from Australia (Fig. 3).

5. Histopathological examination of recovered R. echinobothrida:

Histopathological pictures revealed a cross-section of chicken intestine containing the cestode worms in the lumen. There were a slaughed degenerated parts from the intestinal mucosa present inside the lumen due to the mechanical movement of the parasite. There were inflammatory reactions in the mucosae characterized by infiltration of eosinophils, macrophages, plasma cells and lymphocytes beside the degenerated and necrosed enterocytes and epithelium lining of intestinal glands (Fig. 4).

Table 1. Prevalence of chicken tapeworms in different localities in Menouf district.

				Raillietina tetragona		***Raillietina echinobothrida***		***Raillietina cesticillus***		***Cotugnia digonopora***	
Locality	**Total Ex.**	**Total infected**	**%**	**No. Infected**	**%**	**No. Infected**	**%**	**No. Infected**	**%**	**No. Infected**	**%**
Gizi	221	24	10.86	2	0.9	8	3.6	3	1.4	11	5%
Barhim	198	17	8.59	1	0.5	5	2.5	4	2.02	7	3.5 %
Sirs Elaian	316	40	12.66	9	2.8	10	3.2	10	3.2	11	3.5 %
Elhamol	163	27	16.56	3	1.8	9	5.5	2	1.2	13	8%
Total	898	108	12.03	15	1.7	32	3.6	19	2.1	42	4.7 %
X^2		6.5425		5.1226		2.5668		2.9223		5.6423	
P		0.088		0.163039		0.463345		0.403753		0.130373	
***Sig.**		Non		Non		Non		Non		Non	

*Significant at $P < 0.05$.

Table 2. Seasonal prevalence of chicken tapeworms in Menouf district.

	Total cestodes			***Raillietina* spp.**		***Cotugnia digonopora***	
Season	**No. Examined**	**No. Infected**	**%**	**No. Infected**	**%**	**No. Infected**	**%**
Winter	113	5	4.42	3	2.7	2	1.8
Spring	171	5	2.92	4	2.3	1	0.6
Summer	274	60	21.90	40	14.6	20	7.3
Autumn	340	38	11.18	19	5.6	19	5.6
Total	898	108	12.03	66	7.3	42	4.7
X^2		45.6702		32.6546		13.4244	

P	< 0.00001	< 0.00001	< 0.003803
***Significance**	Sig.	Sig.	Sig.

*Significant at P < 0.05.

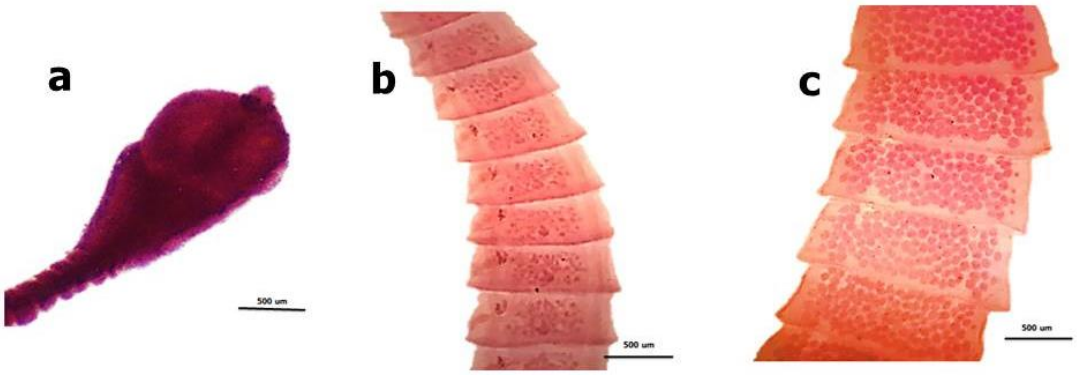

Fig. 1. Photographmicrograph of *Raillietina echinobothrida* in chickens from Menouf district, Menoufia, Egypt. (a) Scolex of *R. echinobothrida*. (b) Mature segment of *R. echinobothrida.* (c) Gravid segment of *R. echinobothrida.* X4. (Scale bar = 500 µm)

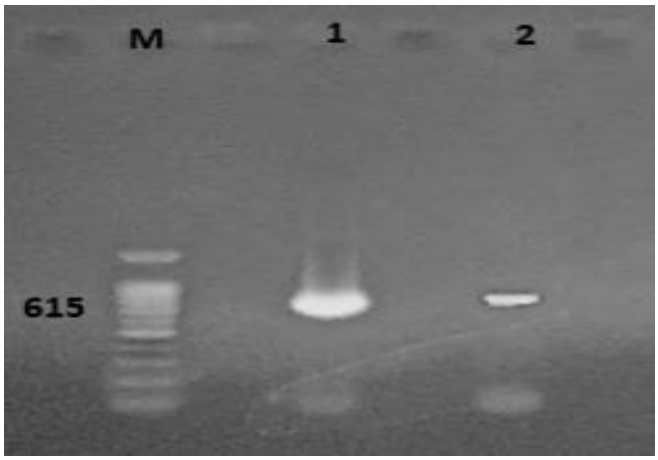

Fig. 2. Gel electrophoresis of the ITS-2 rDNA of *Raillietina echinobothrida.* Lane 1 and Lane 2, two *Raillietina echinobothrida* from Menouf district. M, DNA molecular size marker of 100 bp. The expected PCR product size was 615 bp.

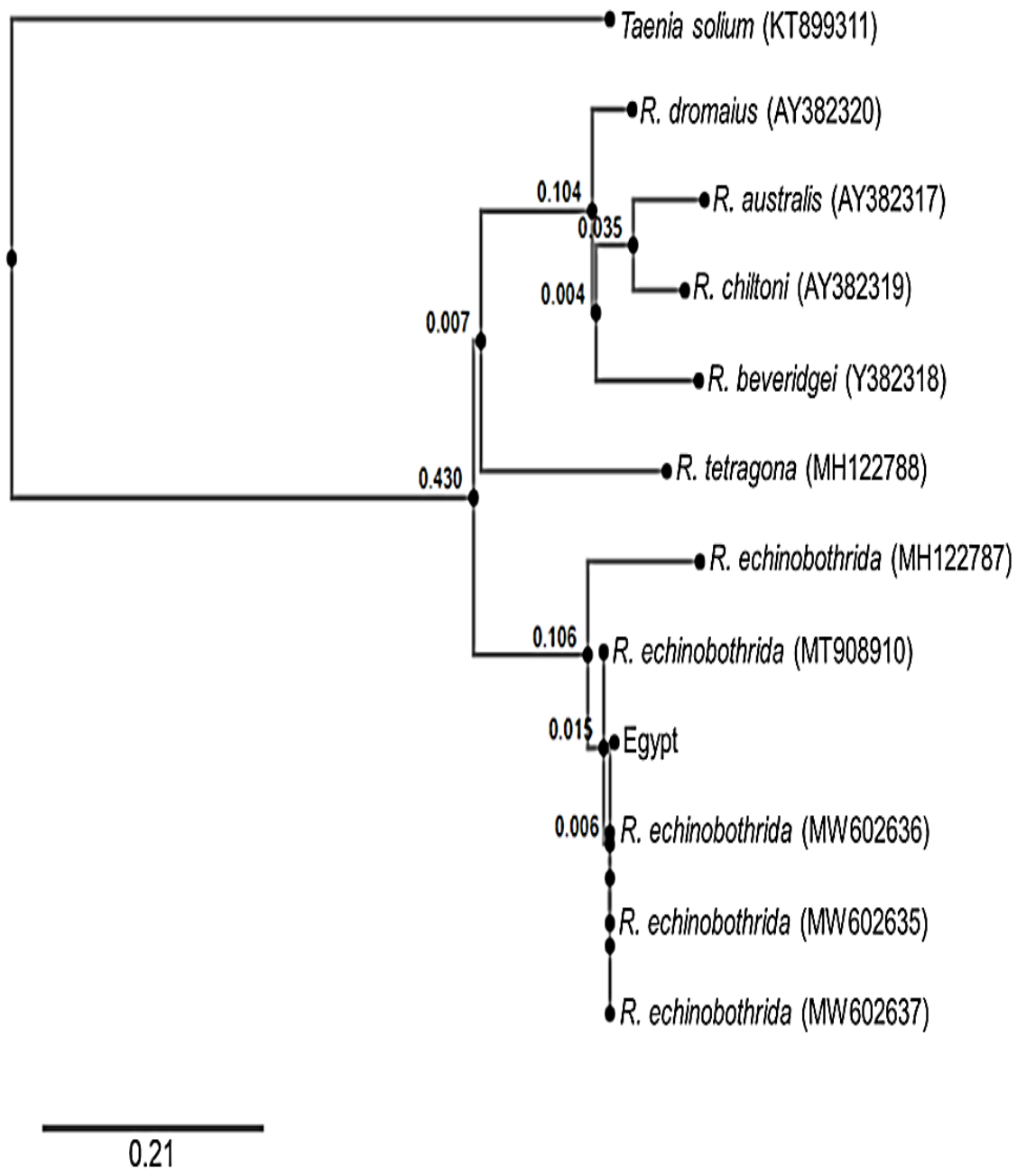

Fig. 3. Neighbor-joining phylogenetic tree of the ITS-2 rDNA region of *Railletina echinobothrida* from chickens in Menouf, Menoufia governorate, Egypt. The tree was drawn to scale and shows the branch lengths.

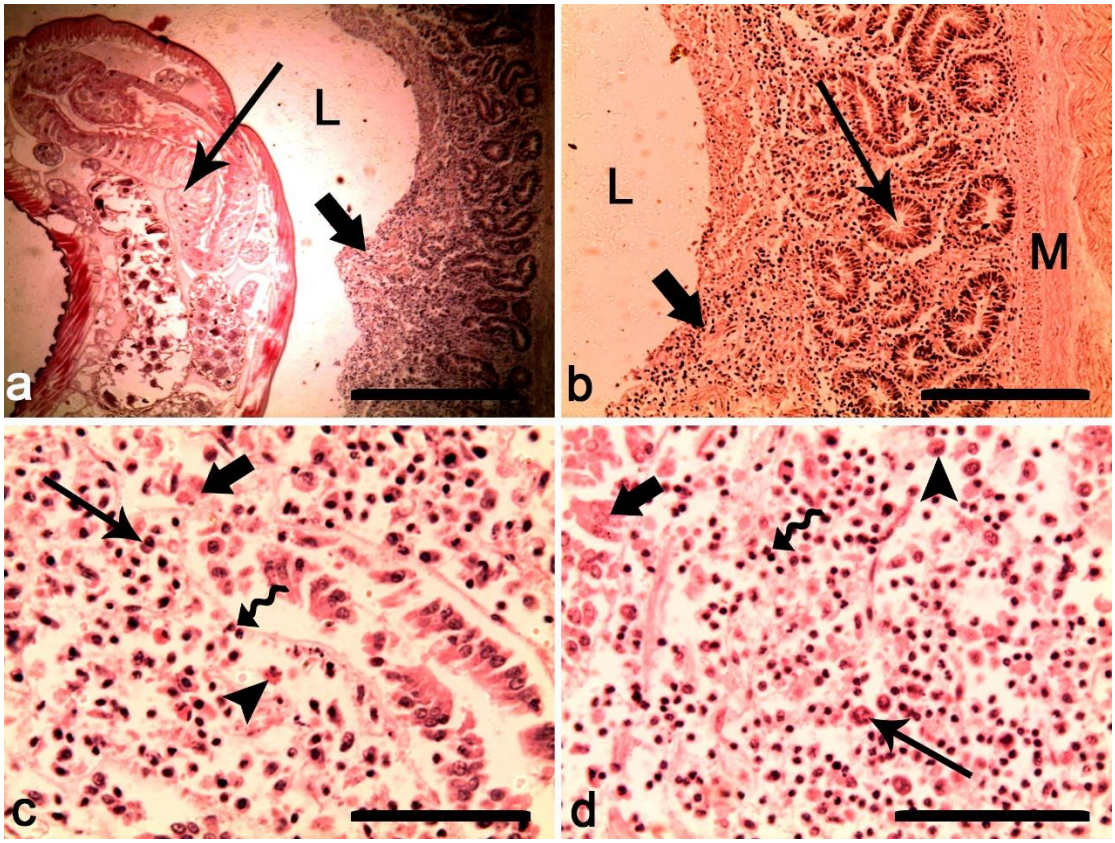

Fig. 4. Intestine, chicken infected with *Railletina echinobothrida*. **a)** showing degenerated intestinal mucosa (thick-arrow) and sagittal section in the cestodes worm (thin-arrow) inside the intestinal lumen (L) (Scale bar = 500 µm). **b)** showing degeneration of the intestinal mucosa with infiltration of inflammatory cells (thick arrow). L) intestinal lumen; Thin arrow) mucosal gland; M) tunica musclosa (Scale bar = 200 µm). **c)** higher magnification of panel (b) showing necrosis of the epithelium linning of mucosal glands (thick arrow) and the intestinal mucosa infiltrated with, plasma cells (thin arrow), lymphocytes (bended arrow) and esinophilis (arrowhead) (Scale bar = 50 µm). **d)** higher magnification of panel (b) showing necrosis and slaughing of the intestinal epithelium (thick arrow) and the intestinal mucosa infiltrated with, macrophages (thin arrow), lymphocytes (bended arrow) and esinophilis (arrowhead) (Scale bar = 50 µm).

DISCUSSION

The present study was carried out on 898 Baladi chickens to determine the prevalence of chicken tapeworms and their related risk factors in Menouf district, Menoufia, Egypt. In the current study, the prevalence of cestode in chicken was 12.03% and this rate was lower than that of Hamza (2009) who reported that the prevalence of cestodes was 88% in Al-Diwaniya region, Maina et al. (2012) recorded cestodes infection at 51.5% in Nairobi, Kenya, Hussen et al. (2012) stated cestodes by (83.0%) in Adamitulu and Ada'a of Eastern Shewa zone, Ethiopia, Yousfi et al. (2013) reported cestodes at 95.61% in North-Western Algeria, Chege (2014) who in Kenya, who recorded the prevalence of cestodes was 87.5%. On the other hand, our finding disagreed with Yadeta et al. (2019) found cestodes at 3.39% and Gunda et al. (2022) found cestodes at 10%. The prevelance of cestodes were lower in this study. This could be attributed to good hygiene in house rearing system and using anthelmintic.

In our study, the prevalence of *R. echinobothrida* in chicken was 3.6%. This finding disagrees with Adamu et al. (2022) recorded *R. echinobothrida* at 20%, Abdo et al. (2022) in Kebele, Ethiopia, who reported *R. echinobothrida* at 5.75%, Malik and Khatoon (2022) reported *R. echinobothrida* at 26.3%, Farah and Khadijah (2020) recorded *R. echinobothrida* by 50%. On the other hand, our findings agree with Belete and Addis (2015) they reported *R. echinobothrida* by 3.6%, and Yadeta et al. (2019) found that *R. echinobothrida* by 1.04%. The infection rate of *R. echinobothrida* were lower in this study. This could be due to difference in area of study and mangment system of birds.

In this study, Chickens *R. echinobothrida* recorded a higher infection rate in summer (14.6%) and a lower in spring (2.3%). This finding disagrees with Chege (2014) who reported *R. echinobothrida* prevalence in the dry season at 54.2% and wet season at 79.2%, Chege et al. (2015) found that cestodes were high in the winter season at 87.5% and low in summer by (83.3%), Sreedevi et al. (2016) recorded a high prevalence in Autmen (43.41 %) and low in summer (38.91 %), Malik and Khatoon (2022) reported that the highest prevalence was at spring (60%), followed by summer (58%), Singh (2016) who reported the highest infection in summer (87.2%), followed by winter (80.9%) and the lowest was in rainy season (77.9%). Variation of the infection rate of the worm could be attributed to variation in the enviromental, geographical, house system and hygenic conditions.

The identification of *Railletina echinobothrida* in chickens was based on the morphological characteristics and measurements of its size, scolex, mature segment, and gravid segment. This finding agrees with Soulsby (1986) who reported that the worm length was up to 25 cm, absent neck, and small round scolex with 100-200 minute hooks, The suckers were round and armed with 8-10 rows of hammer hooks and the egg pouches contain 6-12 eggs. Rahmadani et al. (2022) found *R. echinobothrida* length was from 16-23 cm, with a round scolex (0.27- 0.3 mm in diameter), rostellum armed hooks (0.06x0.07-0.08x0.1 mm), round sucker (0.07x0.08-0.08x0.09 mm in size), short, thick, neck. Hofstad et al. (1984) recorded that *R. echinobothrida* had round suckers rostellum and round rostellum, Also, Simon and Emeritus (2005) reported that *R. echinobothrida* had about 30 cm length and was present in the jejunum.

In the current study, the molecular characterization of *R. echinobothrida* using internal transcribed spacer 2 (ITS-2 rDNAs) produced a specific band at 615 bp, this finding agrees with previous studies with variable size of the reported bands for instance, Ramnath et al. (2014) identified *R. echinobothrida* ITS-2 gene band at 600 bp, Littlewood et al. (2008) and Yang et al. (2021) detected *R. echinobothrida* ITS-2 band at 564 bp, and Panich and Chontananarth (2021) found that the ITS-2 rDNA gene band at 397 bp. PCR produced specific bands of ITS-2 rDNA region of *R. echinobothrida*. The PCR product was sequenced and deposited in GenBank. The sequence of ITS-2 from Menouf, Menoufia, Egypt has a high identity percentage of 93.69% with the ITS-2 rDNA sequences of *R. echinobothrida* from Egypt. It had an identity percentage of 90.96% and 81.19% with ITS-2 of *R. echinobothrida* in chickens from China. The phylogenetic analysis showed that the sequence of ITS-2 of *R. echinobothrida* from Egypt clustered in the same taxon as *R. echinobothrida*

sequences from Egypt and China, while the other *Railletina* species from China and Australia, *R. tetragona*, *R. dromaius*, *R. australis*, *R. chiltoni*, and *R. beveridgei*, clustered in another taxon. Therefore, the detected cestode is *R. echinobothrida*. Our results agree with Ramnath et al. (2014) detected *R. echinobothrida* from chickens in India and detected 600 bp ITS-2 rDNA sequence including regions of 5.8S and 28S region; the annotated product ITS-2 region was found to be 446 bp in length spanning the ITS-2 region. The variable nature of the rDNA ITS-2 region makes it an effective tool for phylogenetic differentiation (Wolf et al., 2005). The ITS-2 region's rapid evolution makes it a popular choice for phylogenetic reconstruction at the species and genus levels (Alvarez and Wendel, 2003; Wolf et al., 2005; Ashokan and Pillai, 2008). Therefore, it will be of value in the molecular characterization of the genus *Raillietena.* The Egyptian sequence of ITS-2 of *R. echinobothrida* was given the same taxon *R. echinobothrida* from Egypt and China but related to the members of different species of the genus *Raillietena* from China and Australia.

Concerning the histopathological results in the current study which revealed a slaughed degenerated parts from the intestinal mucosa present inside the lumen due to the mechanical movement of the parasite. There were inflammatory reactions in the mucosae characterized by infiltration of eosinophils, macrophages, plasma cells and lymphocytes beside the degenerated and necrosed enterocytes and epithelium lining of intestinal glands. our results agree with Simon and Emeritus (2005); Mather and Pandle, (1969) who reported that *R. echinobothrida* was the most pathogenic worm and caused hyperplastic enteritis and may form nodules at its site in the intestine. Also, Mir et al. (2010) detected *R. echinobothrida* P.M in the form of variable degrees of degenerative changes that occurred with a severe infection that was indicated by an increase in the infiltration of lymphocytes and heterophils.

CONCLUSION

Chicken tapeworm prevalence was significantly affected by the season and locality of the examined bird. Further studies should be done on a large scale to investigate chickens' *R. echinobothrida* risk factors. The sequencing analysis using the ITS-2 gene will be a valuable tool for species identification of cestodes of poultry. The current study presented the prevalence of cestodes in natural infected Baladi chickens and the molecular characterization of *R. echinobothrida* in Menouf district, Menoufia, Egypt.

REFERENCES

Abdo, S.; Zakir, S.; Nair, S.K.P. (2022). The Study of the Prevalence of Gastrointestinal Helminths in Chicken, A Study from Bale Zone, South East Ethiopia. Vet. Sci., 1 (1): 1-9, 16-22.

Adamu, M.I.; Adamu, A.H.; Godowoli, I.A.; Ado, N.B. and Goni, K.A. (2022). Helminthes Parasites of Chicken (*Gallus Domesticus*) in Damaturu Metropolis (A Case Study of Locally Sold Chicken in Damaturu Main-Market). bije, FIM Journal, 6 (1): 11-18.

Alasadiy, Y.D.K.; Alhasnawi, A.N. and Eabaid, F.A. (2020). The prevalence of Parasitic Infection in Domestic Chicken. Medico Legal Update, 20 (1): 864- 869.

Alvarez, I. and Wendel, J.F. (2003). Ribosomal ITS Sequences and Plant

Phylogenetic Inference. Mol Phylogenet Evol, 29 (417): 7903.

Amin, O.M. and Kakabwab, S.R. (2019). Prevalence of Gastrointestinal Parasites in Domestic Pigeons and Backyard Chickens in Kalar and Khanaqin districts, Iraq. Journal of Garmian University 6 (1), 1-7: 10-24271.

Ashokan, K.V. and Pillai, M.M. (2008). In Silico Characterization of Silk Fibroin Protein using Computational Tools and Servers. Asian J.Exp. Sci., 22 (3): 265–274.

Bancroft, J.D. and Gamble, M. (2008). Theory and Practice of Histopathological Techniques (6th Ed), Churchill Livingstone, Elsevier Limited, 9 (2): 2023-92003.

Belete, A. and Addis, M. (2015). A Survey of Gastrointestinal Helminthes among Chickens in Bahir Dar town, Ethiopia. Eur. J. Appl. Sci., 7 (2): 64-71.

Belete, A.; Addis, M. and Ayele, M. (2016). Review on Major Gastrointestinal Parasites that Affect Chickens. Journal of Biology, Agriculture and Healthcare, 6 (11): 11-21.

Bowles, J.; Blair, D. and McManus, D.P. (1995). A Molecular Phylogeny of the Genus *Echinococcus*. Parasitology 110 (3): 317–328.

Butboonchoo, P.; Wongsawad, C.; Rojanapaibul, A. and Chai, J.Y. (2016). Morphology and Molecular Phylogeny of *Raillietina* spp., (Cestoda: Cyclophyllidea: Davaineidae) from domestic chickens in Thailand. The Korean journal of parasitology, 54 (6): 777-786.

Carleton, H.M. (1957). Histological Technique for Normal and Pathological Tissues and Identification for Parasites. 3rd ed.London. Oxford Univeristy press. New York, Toronto, 1(1): 343.

Chege, H.W. (2014). Seasonal Parasite Carriage of Village Chicken in Mbeere Subcounty, Antiparasitic Treatments used and Effectiveness of Selected Anthelmintics (Doctoral dissertation, University of Nairobi).

Chege, H.W.; Kemboi, D.C.; Bebora, L.C.; Maingi, N.; Mbuthia, P.G.; Nyaga, P.N. and Githinji, J. (2015). Studies on Seasonal Prevalence of Ecto-and Endo-parasites in Indigenous Chicken of Mbeere Subcounty, Kenya. Livestock Research for Rural Development, 27 (6): 29053-0065.

Dar, J.A. and Tanveer, S. (2013). Prevalence of Cestode Parasites in Free-Range Backyard Chickens (*Gallus domestics*) of Kashmir, India. Agriculture and biology journal of North America, 4 (1): 67-70.

Eshetu, Y.; Mulualem, E.; Ibrahim, H.; Berhanu, A. and Aberra, K. (2001). Study of Gastro-Intestinal Helminths of Scavenging Chickens in Four Rural Districts of Amhara region, Ethiopia. Revue Scient Fique et Technique (International Office of Epizootics), 20 (3): 791-796.

Farah Haziqah, M.T. and Khadijah, S. (2020). Helminthic Parasites in Indigenous Chickens in Penang Island, Malaysia. Tropical biomedicine, 37 (4): 896-902.

Gray, J.S. (1972). The Effect of Host Age on the Course of Infection of *Raillietina cesticillus* (Molin, 1858) in the fowl. Parasitology, 65 (2): 235.

Gunda, S.R.; D'souza, N.M.; Das, S.S.; Cattavarayane, M. and Christopher, A.F.B. (2022). Incidence of Gastrointestinal Helminth Parasites in *Gallus domesticus* (Indigenous fowl) in

a Hot-Humid Tropical Environment. Applied Veterinary Research, 1 (3): 2022013-2022013.

Hamza, H.M. (2009). Prevalence and Distribution of Gastro-Intestinal Helminthes in Local Chickens in Al-Diwaniya region. Journal of Wasit for Science and Medicine, 2 (1): 53-74.

Hofstad, M.S.; Calnek, B.W.; Helmboldt, C.F.; Reid, W.M. and Yoder, H.W. (1984). Diseases of Poultry (12th Ed). Iowa State University, 61 (0): 3.

Hussen, H.; Chaka, H.; Deneke, Y. and Bitew, M. (2012). Gastrointestinal Helminths are Highly Prevalent in Scavenging Chickens of Selected Districts of Eastern Shewa Zone, Ethiopia. Pakistan Journal of Biological Sciences: 15 (6): 284-289.

Leeson, S. and Summer, J.D. (2009). Internal Parasites: Broiler Breeder Production; 1st Edition by Nottingham Journal of Biology, Agriculture and Healthcare. 6 (11): 104-106.

Littlewood, D.T.J.; Waeschenbach, A. and Nikolov, P.N. (2008). In Search of Mitochondrial Marker for Resolving the Phylogeny of Cyclophyllid on Tapeworms (Platyhelminths, Cestode) – A Test Study with Davaineidae. Acta Parasitol., 53 (2): 133–144.

Maina, A.N.; Waruiru, R.M.; Ngatia, T.A.; Mbuthia, P.G. and Munyua, W.K. (2012). Gastrointestinal Parasites and Other Endoparasites of Indigenous Chickens Traded in Nairobi, Kenya.

Malik, R. and Khatoon, N. (2022). Prevalence of Cestode Parasites of *Gallus Domesticus* in Karachi, Sindh, Pakistan. RADS Journal of Biological Research & Applied Sciences, 13 (2): 157-162.

Mathur, S.C. and Pande, B.P. (1969). *Raillietina cestidllus* and *R. tetragona* infections in Chicks Reared on Normal and Deficient Feeds-an Experimental Study. Indian Journal of Animal Sciences, 39 (2): 115-134.

Mir, M.S.; Salam, S.T. and Khan, A.R. (2010). The Prevalence and Pathology of *Raillietina cesticillus* in Indigenous Chicken (*Gallus gallus domesticus*) in The Temperate Himalayan Region of Kashmir-Short Communication. Vet. Arh., 80 (2): 323-328.

Panich, W. and Chontananarth, T. (2021). Molecular Detection of Three Intestinal Cestode Species (*Raillietina echinobothrida, R. tetragona, R. cesticillus*) From Poultry in Thailand. Avian Pathology, 50 (4): 321-326.

Poulsen, J.; Permin, A.; Hindsbo, O.; Yelifari, L.; Nansen, P. and Bloch, P. (2000). Prevalence and Distribution of Gastro-Intestinal Helminths and Hemoparasites in Young Scavenging Chickens in Upper Eastern Region of Ghana, West Africa. Preventive Veterinary Medicine, 45(3-4): 237-245.

Pritchard, M.N. and Kruse, O.W. (1982). Tec. Collection and Preservation of Animal Parasites. Technical Bull. 1 Univ. Nebraska, Lincolon and London, 57 (141): 165-165.

Puttalakshmamma, G.C.; Ananda, K.J.; Prathiush, P.R. Mamatha, G.S. and Suguna, R. (2008). Prevalence of Gastrointestinal Parasites of Poultry in and Around Banglore; India. Veterinary World, 1 (7): 201-202.

Rahmadani, A.; Suprihati, E.; Lastuti, N.D.R.; Warsito, S.H. and Fiorenza, M.P. (2022). Ultrastructural Morphology Comparison of *Raillietina echinobothrida* and *Raillietina tetragona* in Native Chickens at

Sidoarjo Regency Using Scanning Electron Microscope (SEM). Journal of Parasite Science, 6 (2): 232-6.

Ramnath Jyrwa, D.B.; Dutta, A.K. and Tandon, V. (2014). Molecular Characterization of the Indian Poultry Nodular Tapeworm, *Raillietina echinobothrida* (Cestoda: Cyclophyllidea: Davaineidae) Based on rDNA Internal Transcribed Spacer 2 Region. J. Parasit. Dis., 38 (1): 22–26.

Simon, M.S. and Emeritus, M. (2005). Enteric Diseases: ASA Handbook on Poultry Diseases, (2nd Ed), American Soybean Association, 11 (03): 133-143.

Singh, G. (2016). Seasonal Variation of Parasitic Cestode Infection in *Gallus*. International Research Journal of Management Science & Technology, 7 (5): 2250 – 1959.

Soulsby, E.J.L. (1986). Helminths, Arthropods and Protozoa of Domesticated Animals (7th Ed)". Baillière Tindall, London UK. P. 104, 105,166 and 172.

Sreedevi, C.; Jyothisree, C.; Rama Devi, V.; Annapurna, P. and Jeyabal, L. (2016). Seasonal Prevalence of Gastrointestinal Parasites in Desi Fowl (*Gallus gallus domesticus*) in and Around Gannavaram, Andhra Pradesh. Journal of Parasitic Diseases, 40 (3): 656-661.

Wolf, M.; Achtziger, M.; Schultz, J.; Dandekar, T. and Mu¨ller, T. (2005). Homology Modeling Revealed more than 20,000 rRNA Internal Transcribed Spacer 2 (ITS2) Secondary Structures. RNA, 11 (11):1616–1623.

Yadeta, W.; Sori, T.; Debelo, M.; Michael, B.G. and Jilo, K. (2019). Prevalence of Major Gastrointestinal Parasites in Small Scale Commercial Poultry Farms from Jimma Town, Southwest Ethiopia. International Journal of Research Studies in Iosciences, 7 (10): 13-2.

Yang, X.; Zhang, D.; Wu, G.; Tian, W. and Huo, N. (2021). Molecular Phylogenetic Identification and Morphological Characteristics of *Raillietina echinobothrida* (Cestoda: Cyclophyllidea: Davaineidae) in Commercial Chickens in North China. Parasitology Research, 120 (4): 1303-1310.

Yoriyo, K.P.; Adang, K.L.; Fabiyi, J.P. and Adamu, S.U. (2008). Helminthes Parasites of Local Chickens in Bauchi State, Nigeria. Science World Journal, 3(2): 1597-6343 .

Yousfi, F.; Senouci, K.; Medjoual, I.; Djellil, H. and Slimane, T.H. (2013). Gastrointestinal Helminths in the Local Chicken *Gallus gallus domesticus* (Linnaeus, 1758) in Traditional Breeding of North-Western Algeria. Biodiversity Journal, 4 (1): 229-234.