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Parasitology

Prevalence and Molecular Phylogeny of *Ascaridia galli* in Baladi Chickens in Menouf District, Menoufia, Egypt

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ABSTRACT

Native chickens ascaridiosis (*Gallus domesticus*) is a disease caused by obligatory intestinal *Ascaridia galli* (Family: *Ascarididae*) that causes more economic losses in the chicken industry. The current study was conducted in Menouf district, Menoufia, Egypt from January to December 2022 to determine chicken ascaridiosis's prevalence and identify the recovered *Ascaridia galli*. Intestinal samples of 898 randomly selected from Baladi chickens were subjected to parasitological examination. The results revealed that *Ascaridia galli* worms were detected in 12.5 % (112 out of 898). The prevalence was significantly influenced by the area of the examined chickens but not by the season. Morphological description and molecular characterization of recovered *Ascaridia galli* were reported in this study to discriminate *Ascaridia galli* in infected chickens. The PCR of Cox 1 of *Ascaridia galli* produced specific bands of 533 bp. The sequence has an identity percentage of up to 100% with *A. galli* from Brazil, Denmark, and USA. The UPGMA phylogenetic tree showed that the sequence of *A. galli* Cox-1 from Egypt clustered with *A. galli* sequences from Brazil, Denmark, and Ghana. This study provides data on the prevalence and molecular characterization of *A.galli* from the Menouf district, Menoufia, Egypt.

Keywords: *Ascaridia galli*, Cox-1, Molecular identification, Prevalence, Sequences, Baladi chickens, Egypt.

INTRODUCTION

Livestock production was an important economic for the Agriculture sector (Thornton, 2010). *Ascaridia galli* were classified according to (Schrank, 1788), and classified to class Nematoda according to (Griffiths, 1978). *A. galli* was the largest roundworm in chickens (Ashour, 1994). *A. galli* worm inhabited the bird's small intestine lumen (Ackert, 1931). *A. galli* main site was in the small intestine but its tissue site was not understood (Luna-Olivares et al., 2012). *A. galli* was a roundworm present in wild and common domestic birds (Soulsby, 1982). *A. galli* was stated also in brown fowl (Lohmann Brown chicken) and old white (Lohmann LSL chicken) (Gauly et al., 2002). *A. galli* infection caused industry losses in native chickens in new poultry production systems (Permin et al., 2001). *A. galli* was a pathogenic nematode species that was present in high prevalence especially in domestic common native chickens (Griffiths, 1978). *A. galli* infected native chickens of all ages but caused severe damage in the young chick (Jacobs et al., 2003). Ascaridiosis in chickens may be clinical or subclinical (Soulsby, 1982). Clinical ascaridiosis was revealed in the form of a reduction in the production of eggs, death of birds could occur in case of obstruction of lumen by helminths that caused immune suppression and debilitating condition of birds (Simon and Emeritus 2005). Morphological characteristics of *Ascaridia galli* worm were reported by (Soulsby, 1982; Ramadan and Abouznada, 1992; Ashour, 1994; Urquhart et al., 1996 and Anderson, 2000; Piergili Fioretti et al., 2005). *A. galli* was reported in Eygpt (Bazh, 2013), Saudi Arabia (Ramadan and Abouznada, 1992), several European countries (Tarbiat et al., 2015), Peiping-China (Ting, 1936), Nigeria (Dawet et al., 2012; Johnson et al., 2019; Gimba et al., 2019; Laden et al., 2019), Zimbabwe (Percy et al., 2012), North India (Kumar et al., 2015), Rawalpindi-Pakistan (Yousaf et al., 2019), and Thailand (Wuthijaree et al., 2019). Molecular identification of *A. galli* was carried out using the mitochondrial gene of Cytochrome Oxidase subunit 1 (Cox-1) (Nejsum et al., 2008; Biswas et al., 2021; Zhao et al., 2022). We conducted this study to reveal the prevalence of *Ascaridia galli*, related risk factors and molecular characterization by using Cox-1 PCR and sequencing analysis in Baladi chickens from the Menoufia governorate, Egypt.

MATERIALS AND METHODS

1. ***Ethical approval***

The study was included euthanasia of the animal under experments. This was conducted under the specific guidelines of the Animal Care and use Committee in the department of parasitology, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt. All protocols and methodes in this study were compliance with this guidelines and given under an ethical approval number was (IACUG) of VUSC-34-1-23.

2. Sample collection and study area

The current study was conducted in the Menouf district, Menoufia, Egypt from January to December 2022 to reveal the prevalence of *Ascaridia galli* in chickens. Chickens were reared and collected from the house. Live *A. galli* was collected from the small intestine of infected domestic native chickens from four villages in the Menouf district (Barhim, Gizi, Elhamol, and Sirs Elian), Menoufia governorate, Egypt. Samples were transferred to the Parasitology Lab, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt for further processing. Chickens were ethically euthanized and exposed for postmortem examination according to (Swayne et al., 2020).

3. Sample Preparation

The preparation of collected *Ascaridia galli* for examination was made according to standard technique (Whitlock, 1960; Watson, 1960). The washed relaxed worms (by physiological saline) were mounted in glycerol Jelly (Pritchard and Kruse 1982). The procedure was also previously recorded by (Hansen and Percy, 1990) and the identification keys were used to detect nematode worms (Levine, 1980; Anderson et al., 2009). Examination of worms by light microscope (LM) at Parasitological Lab, Faculty of Veterinary Medicine, University of Sadat City, Menoufia, Egypt. Samples were identified with all data (species of bird, time, date of collection and locality). The specimens were preserved in a mixture of alcohol glycerin (70% alcohol with 5% glycerin). For permanent mounts of worms, they were adapted to lactophenol for clearance (Whitlock, 1960; Watson, 1960). Then was mounted in glycerol Jelly (Pritchard and Kruse, 1982). For molecular and sequencing analysis, the samples were rinsed with water several times and then frozen at -20 °C for further DNA extraction.

4. Morphological observations of the worms recorded region

For light microscope (LM) observations, the worms were mounted on a slide as previously described (Kennedy, 1979). The samples were prepared for permanent slides. The identification of the collected *A. galli* was done by measurements of the diameter and shape of all parts of the worm, their color, head size, esophagus and characteristics of the posterior end for both males and females. Adults of *Ascaridia galli* were identified using the keys and descriptions provided by (Soulsby, 1982) and (Taylor et al., 2007). The accurate morphological descriptions were taken by colored photographs.

5. DNA extraction

DNA was isolated from the *Ascaridia galli* using an extraction kit (Easy Pure Genomic DNA Kit®). The DNA concentration was determined by a spectrophotometer. The reaction volume was 25µL, including; 1 µL from the forward primer and reverse one, 2 µL from selected DNA (100 ng), 5µL from a master mix (Super Mix 2X Easy Taq PCR®), and up top to 25µL from double-distilled water (DDW). The PCR was accomplished on a thermal cycler (G-STORM). The

DNA was evaluated on 1% agarose gel. The secluded DNA was frozen at -20 °C. The PCR products were cleared and sequenced at the Animal Health Research Institute, Ministry of Agriculture, Dokki, Egypt. The sequences were blasted with the NCBI BLAST technique. The sequence was security in GenBank.

6. PCR Amplification of COX-1

The *A. galli* Cox-1 was amplified by using the PCR primers, forward (5' ATT ATT ACT GCT CAT GCT ATT TTG ATG-3') and Reverse (5' CAA AAC AAA TGT TGA TAA ATC AAA GG-3') (Katakam et al., 2010). The PCR reaction was as follows: 15 min initial denaturation at 95℃, followed by 35 cycles of 30 s DNA denaturation at 95℃, 40 -sec primer annealing at 55℃, 60 s extension at 72℃ and a final extension at 72℃ for 10 min. The PCR product of amplification was applied to agarose gel electrophoresis examination by using ethidium bromide.

7. Sequencing and Phylogenetic analysis

The PCR products of *Ascaridia galli* DNA 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 then deposited in GenBank. The UPGMA phylogenetic tree was constructed by the MAFFT program. The Cox-1 sequence of *A. galli* from Menouf, Menoufia, Egypt (LC815276) was used to make the tree with other sequences from GenBank, including *A. galli* (GU138668, GU138669, KP982856, MW243593, and MW243594), *Heterakis gallinarum* (MF066719 and MF066720), *Toxocara cati* (KC200211 and KC200212), *Toxocara vitulorum* (AJ920062), *Toxocara canis* (KC293913 and KC293914), and *Toxascaris leonina* (MK516267). *Ancylostoma caninum* Cox-1 (EU007446) was used as an outgroup.

8. Statistical analysis

The effect of locality and season on the prevalence of *Ascaridia galli* was analyzed using the Chi-square by the SPSS program. Statistical significance was considered at $P < 0.05$.

RESULTS

1. Prevalence of recovered Ascaridia galli

The recovered *Ascaridia galli* were detected at a prevalence of 12.5% (112 out of 898). The infection rate of *A. galli* was recorded in four localities of Menouf district, Menoufia province, Egypt. The highest prevalence of *A. galli* was in Gizi at 18.09%, but the lowest was in Barhim at 8.6% (Table 1). The locality has a significant effect on the prevalence of *A. galli* ($X^2 = 10.21$ and $P < 0.0169$) (Table 1). The highest prevalence was in the autumn season (13.8%), while the lowest infection rate was in the summer season at 10.6% (Table 2). The prevalence of chicken ascaridiosis was not significantly affected by the season ($X^2 = 1.536$ and $P < 0.6740$) (Table 2).

2. The Morphological description of the recovered Ascaridia galli

The recovered *A. galli* was recorded as (Fig. 1). *A. galli* was whitish its size ranged in males from 48–78 mm (63 mm) and females 25–118 mm (71.5 mm) in length. The anterior end was cylindrical in shape, smooth cuticle and had 3 large lips. The esophagus was club-shaped. The male posterior end showed two sub-equal or equal spicules, precloacal suckers and 10 pairs of cloacal papillae was presented. The female posterior end was straight from the tail to the cloacal region. The measurements of the recovered *Ascaridia galli* were recorded. Head breadth was 0.2 mm x 0.3 mm (0.26 mm). The lips breadth was 0.13 mm x 0.12 mm (0.13 mm). Esophagus breadth was 1.5 mm x 2.3 mm (1.9 mm). Esophageal width anterior was 0.2mm x 0.3 mm (0.3 mm). Esophageal width posterior club-shape was 0.3mm x 0.8 mm (0.6 mm). Adult male length was 48 mm x 78 mm (63 mm). Pre-cloacal sucker was 0.2 mm x 0.3 mm (0.2 mm). Cloaca was 0.07mm x 0.2 mm (0.2 mm). Spicules (equal) 1.3 mm x1.6 mm (1.45 mm). The male tail region was 0.6 mm x0.8 mm (0.7 mm). The adult female length was 25 mm x 118 mm (71.5 mm). The female tail region was 0.8 mm x1.4 mm (1.1 mm).

3. Molecular characterization of the recovered Ascaridia galli

Molecular identification of the recovered *A. galli* was recorded (Fig. 2). A study of 4 *A. galli* samples, each of them was collected from each of four areas of study by using primers for Cox-1, the resulting adjective that all worms give specific bands at 533 bp but two worm samples only give strong band were from Gizi and Elhamol (Fig. 2) which were subjected for sequencing. The sequence of cox-1 from Menouf, Menoufia, Egypt has an identity percentage of up to 100% with *A. galli* in *Cerdocyon thous* from Brazil (KP982856), *A. galli* larval stages in chickens in Denmark (GU138668 and GU138669), and in chickens in the USA (MT776400). It has an identity percentage of 97.67% with *A. galli* in chickens from China (OQ286042), 97.48% in chickens from China (KT613902, KT613900, KT613899, KT613898, KT613897, KT613892, KT613891, KT613890, KT613889, and NC_021642), in chicken from Bangladesh (OP218593), and *A. galli* larval stages in chickens in Denmark (GU138670), and 97.29% with *A. galli* in chickens from China (MN178641, KT613901, KT613896, KT613894, KT613893, and KT613888). The UPGMA phylogenetic tree showed that the sequence of *A. galli* Cox-1 from Egypt clustered with *A. galli* sequences from Brazil, Denmark, and Ghana and was nearest to *H. gallinarum* taxa while far from the *Toxocara* and *Toxascaris* genera taxa (Fig. 3).

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

Locality	No. examined	No. infected	%
Gizi	221	40	18.09%
Barhim	198	17	8.6%
Sirs Elaian	316	39	12.3%
Elhamol	163	16	9.8%
Total	898	112	12.5%
X^2	10.21		
P	0.0169		
***Sig.**	*		

*Significant at P < 0.05.

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

Season	No. examined	No. infected	% of infection
Winter	113	15	13.3%
Spring	171	21	12.3%
Summer	274	29	10.6%
Autumn	340	47	13.8%
Total	898	112	12.5%
X^2	1.536		
P	0.6740		
***Significance**	Non		

*Significant at P < 0.05.

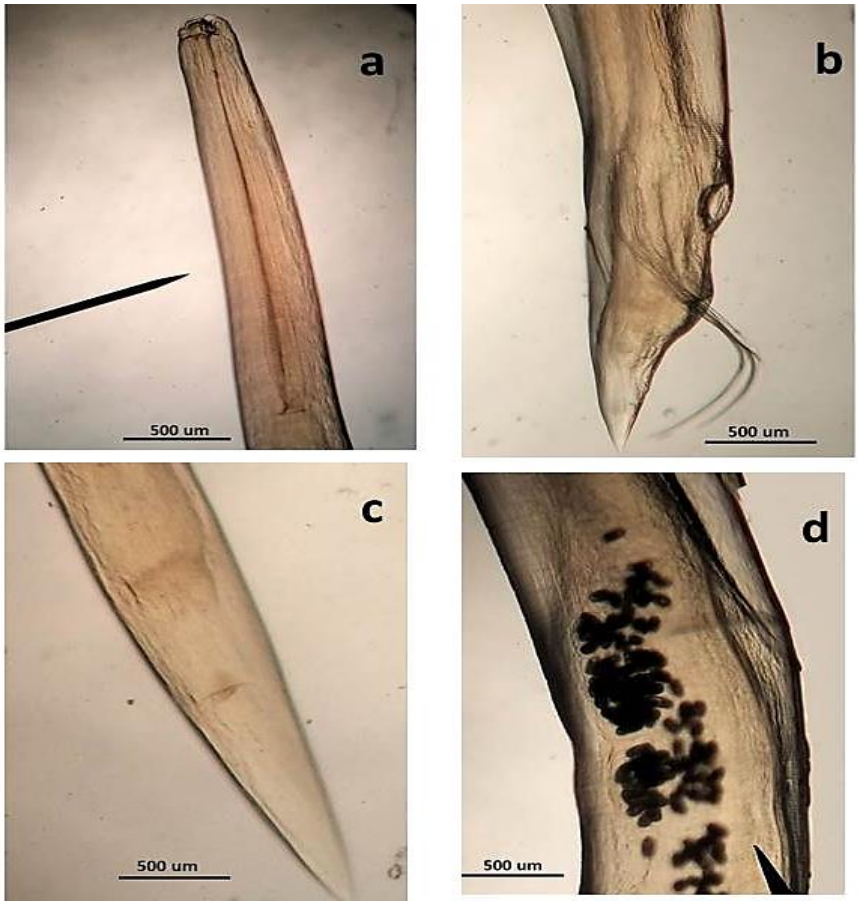

Fig. 1. Photomicrograph of *Ascaridia galli* adult worm. (**a**) Anterior end showing mouth surrounded with three lips and club shaped esophagus **(4×)**, (**b**) Male posterior end with two equal spicules and precloacal sucker **(4×)**, (**c**) Female posterior end **(4×),** and (**d**) Female vulvar region **(4×)**, (Scale bar = 500 µm).

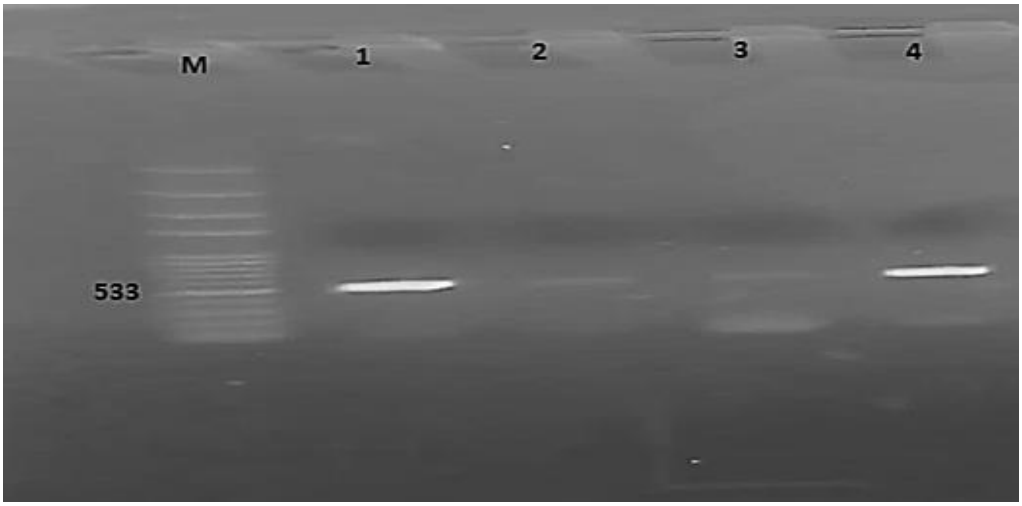

Fig. 2. Gel electrophoresis of the Cox 1 PCR of *Ascaridia galli.* Lane 1. Gizi, lane 2 Sirs Elian, lane 3 Barhim, and lane 4 Elhamol samples of *A. galli*. M. DNA size marker of 100bp.

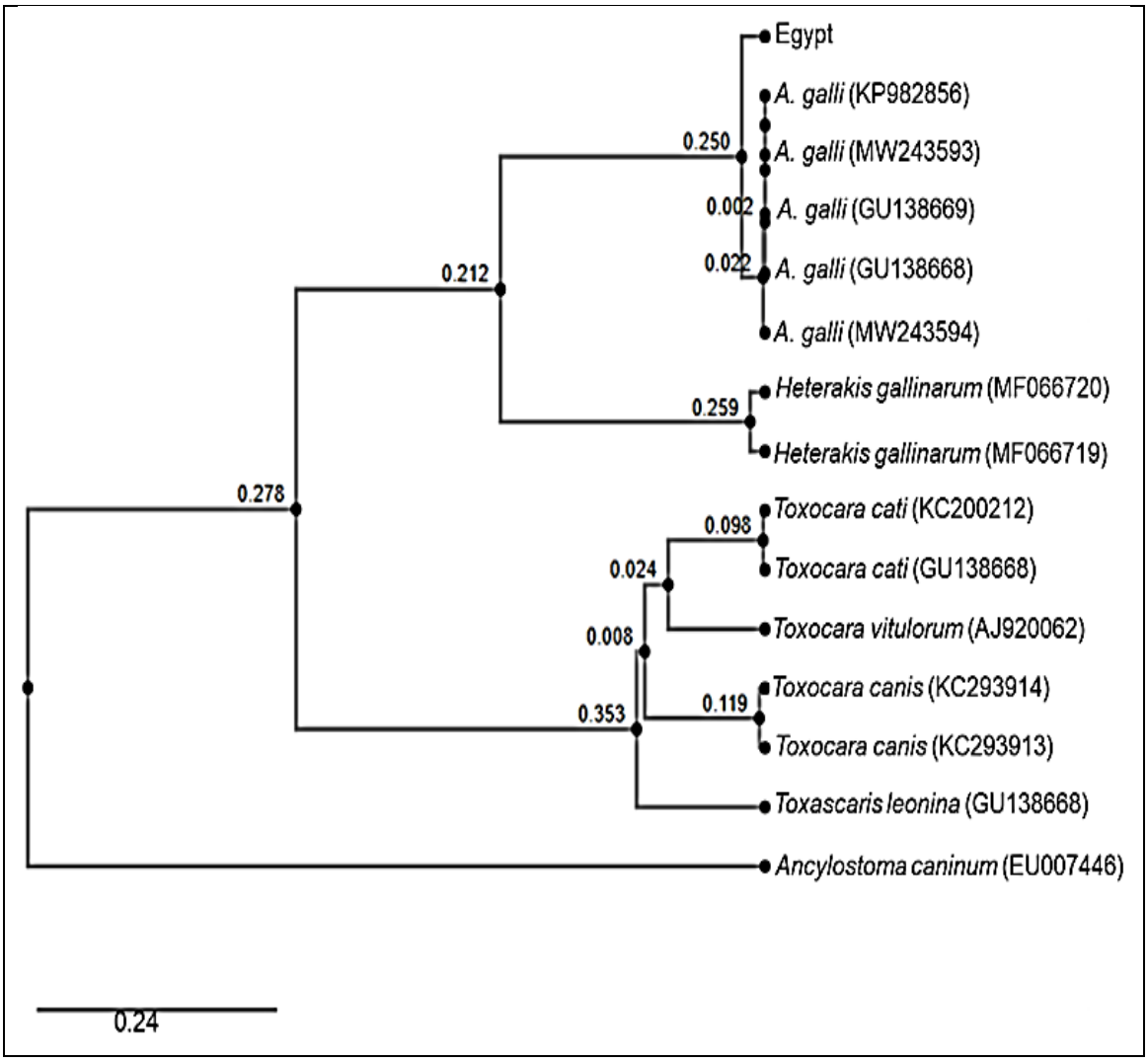

Fig. 3. UPGMA phylogenetic tree of cytochrome oxidase subunit 1 of *Ascaridia galli* from chickens in Menouf, Menoufia Governorate, Egypt. The tree was drawn to a scale and shows the branch lengths.

DISCUSSION

The present study was carried out on 898 native chickens to determine the prevalence of chicken ascaridiosis in the Menouf district, Menoufia, Egypt and its associated risk factors. In the present study, the prevalence of *Ascaridia galli* in chickens was (12.5%) and this rate was lower than that of Dawet et al. (2012) who reported that the prevalence of *A. galli* in chickens was 19.21 % in Plateau State, Nigeria, Khadijah (2015) who reported *A. galli* 81.3% in Alor Setar, Kedah, Yousaf et al. (2019) who recorded *A. galli* by 28.64% in Rawalpindi, Gimba et al. (2019) who found *Ascaridia galli* by 16.7% in Taraba State, northeastern Nigeria, Johnson et al. (2019) who reported *Ascaridia galli* by 42% in Akwa Ibom State University and Akwa Ibom State, Nigeria, Mubarokah et al. (2019) who reported *Ascaridia galli* by 92% in Indonesia, Wuthijaree et al. (2019)

who reported *Ascaridia galli* by 60.2% in Northern Thailand. On the other hand, our finding disagreed with Kumar et al. (2015) who found *Ascaridia galli* at 3.45 %, and Ameji et al. (2022) who found *Ascaridia galli* at 8.3 %. The prevalence of *A. galli* was lower in this study. This could be attributed to good rearing, periodical anthelmintic use and hygiene conditions in the house-rearing.

In our study, chicken ascariasis recorded a higher infection rate in Autumn (13.8%) and lower in Summer (10.6%). This finding disagrees with Percy et al. (2012) who reported that Summer has a higher infection rate with *A. galli*, Yousaf et al. (2019) who found that the Summer season had the highest infection rate of 36.71% followed by the Rainy season at 27.02% and decline in the Winter season 16.89%, Ameji et al. *(*2022) who reported that the highest rate of infection was in Summer season. Variations in the worm's seasonal prevalence may be due to variations in the geographical, and environmental conditions between the area of study and house system and hygenic conditions.

The identification of *Ascaridia galli* in chickens is based on the morphological characteristics of its size and shape of the anterior and posterior end of both males and females. This finding agrees with Soulsby (1982) who reported that the anterior end has 3 small lips, the male was 50-76 mm and the female was 27-116 mm long, the spicules were sub-equal about 1-4 mm long and the vulva was present anterior to the middle. Ramadan and Abouznada (1992) who found that the females were longer than males which were 58 mm - 60 mm in length. The male end has 2 equal or semi-equal spicules at its end, the right one about 1.30 - 1.70 mm in length and the left one 1.10 -1.50 mm. The male has a pre-cloacal sucker oval shape and there were anal papillae. The male posterior end was curly with a pointed tip that was expanded partially at this base.

In the current study, the molecular characterization of *Ascaridia galli* by using primers of Cox-1 gave a band at 533 base pair, this finding agrees with Katakam et al. (2010); Anane (2021); Anane et al. (2022) who found that the cytochrome oxidase subunit one of the mtDNA was targeted 533-bp. The sequence identity of *A. galli* Cox-1 from Menouf, Menoufia, Egypt is very high with sequences from Denmark, the USA, China, Bangladesh, and Denmark. Moreover, the UPGMA phylogenetic tree showed that the sequence of *A. galli* Cox-1 from Menouf, Menoufia, Egypt clustered in the same taxa with *A. galli* sequences from Brazil, Denmark, and Ghana. Indicating the parasite is *A. galli.*

CONCLUSION

Chicken Ascaridiosis prevalence was significantly influenced by the area of study of the examined fowl but not by the season. Further investigations should be done on a large scale to investigate chicken *Ascaridia galli* risk factors. The sequencing analysis using the Cox 1 gene will be a valuable tool for the identification of the nematodes in poultry. The current study presented

the infection rate of *A. galli* in naturally infected native chickens and the molecular identification of *A. galli* in Menouf district, Menoufia, Egypt.

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