Received: 05 March 2023, Accepted: 14 April 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 3, 352-359

# Morphological and Molecular Studies of Ecto- and Endoparasites Infested Chicken in Ismailia Province, Egypt

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## Abstract

The native breed of chicken is one of the most income-producing species in the poultry sector in the Egyptian governorate of Ismailia. Thus, the objective of the current study was to identify the collected parasites using a light microscope and estimate the prevalence and seasonal dynamics of the collected helminths using the newly introduced molecular biology methods. 120 chickens out of 170 recorded (70.59%) prevalence of helminth infestation during the period from September 2021 until the end of August 2022. Four nematodes with a prevalence of 44.12 %, which were *Ascaridia galli*, *Heterakis gallinarum*, *Subulura brumpti*, *Trichostrongylus tenuis*, and four cestodes with 26.47 %, which were *Raillietina tetragona*, *R. echinobothrida*, *Hymenolepis carioca*, and *Choanotaenia infundibulum*. *Eimeria* spp. infestation (11.18%), which were *E. tenella*, *E. maxima*, *E. mitis*, and *E. brunetti*. Ectoparasites (15.88%) were *Echidnophaga gallinacea*, *Lipeurus caponis*, *Menopon gallinae*, *Columbicola columbae*, and *Dermanyssus gallinae*. The identities of the certainly recovered nematode and cestode species were confirmed by the blast test using DNA sequence data. Thus, it is advised to use the molecular approach as the primary methodology for the accurate identification of helminths, particularly in closely related species.

KEYWORDS
Helminths, Native chicken, Egypt, Prevalence, Molecular Biology.

## INTRODUCTION

One of the domesticated species that is raised most intensively is poultry, which is also one of the most developed and profitable animal production industries (Lambio, 2012). Hence, chicken production is contributing significantly and increasingly to the national economies of most nations (Ferdushy *et al*., 2016). Many intestinal helminth infections can considerably lower the productivity of poultry (Van *et al*., 2020). Gastrointestinal helminths (cestodes and nematodes), as well as *Eimeria* species, belong to the most common internal parasitic infections that affect poultry. These infections result in significant damage and considerable financial losses to the poultry industry because they reduce the feed conversion ratio, cause weight loss, reduce egg production, and kill young birds (Puttalakshmamma *et al*., (2008).

Ectoparasites are thought to be the primary reasons for growth retardation, decreased vitality, and bad conditions in the birds, which therefore reduce the quality and production of meat and eggs (Ruff, 1999). Among the poultry species, the native breed of chicken is one of the most suitable income-generating species in the poultry industry in Ismailia Province, Egypt. Consequently, the present study aimed to identify the collected parasites using a light microscope and estimate the prevalence and seasonal dynamics of the collected helminths using the newly introduced molecular biology methods.

## MATERIALS AND METHODS

### *Ethical approval*

The Suez Canal University's Ethics Committee gave its approval for this work. All animal tests were carried out by the Faculty of Veterinary Medicine, Suez Canal University, and Egypt's Guide for the Care and Use of Laboratory Animals, with approval number (2020013).

### *The Sampling areas*

A total of 170 native breeds of chicken were taken within the restrictions of Ismailia province during the period from the beginning of September 2021 to the end of August 2022. The skins and the feathers of chickens were examined and visually checked carefully for the presence of ectoparasites, and the entire gastrointestinal tract from the esophagus to the rectum was extracted and examined directly in the parasitology laboratory, faculty of veterinary medicine, Suez Canal University.

### *Parasitic identification*

Each part of the alimentary canal was opened separately in a petri dish containing physiological saline, and its contents were


ISSN: 2090-6277/2090-6269/ 

shaken in a jar and left to settle for 30 minutes. Also, an outer cutaneous membrane of the gizzard was removed smoothly to detect if any worms existed or not, then examined under a stereoscope for the presence of parasites.

For nematode specimens, they were treated and cleared with lactophenol, and the glycerol jelly method was used by Fleck and Moody (1988). For cestodes specimens, they were stained by dilute aceto-alum carmine (Fleck and Moody, 1988). The parasites were detected under a light microscope and diagnosed according to the diagnostic keys of Soulsby (1982). A calibrated ocular micrometre was used to measure helminth Before or during mounting, large nematode specimens had their entire length measured directly.

For *Eimeria* spp. identification, the faecal samples were collected in trays with a 2.5% potassium dichromate solution and examined according to Permin and Hansen (1998). All ectoparasites were transported directly to a tube containing 70 % ethanol. Then, the specimens were immersed in 10% sodium hydroxide to melt any debris or feathers, then washed several times with water and examined according to Hassan *et al.* (1958).

*Molecular identification*

A total of 70 specimens (nematodes and cestodes) were utilized, and they were immersed in 70% ethanol until the DNA was separated using the QIAamp DNA Mini Kit (Qiagen) according to the manufacturer's instructions. Genomic DNA was kept at -20 °C until use. For nematodes using the 18S small subunit ribosomal RNA gene, partial sequence PCR, and for cestodes using the rDNA internal transcribed spacer 2 regions. Primers are shown in Table 1. The PCR happened following the cycling situation: initial denaturation at 95 ºC for 5 min. then, 10 cycles of 92 ºC for 1min. 48 ºC for 1 min. and 72 ºC for 90 sec. This step was followed by an extra 32 cycles at 92 ºC for 1 minute, 54 ºC for 35 seconds, and 72 ºC for 90 seconds. This was followed by a final extension at 72 ºC for 7 minutes. The amplification results from these genes were separated on a 1.6% agarose gel containing 0.4 µg/ml of ethidium bromide at 90 volts for 40–60 min.

The purified and sequenced amplified PCR product bands were removed from the gel by Solgent Co. Ltd. (South Korea). Following that, sequences were examined using BLAST® by Johnson *et al.* (2008). A phylogenetic tree was constructed in MEGAX by Kumar *et al.* (2018).

*Statistical analysis*

The data were analyzed using descriptive statistics after being entered as raw data into an Excel spreadsheet. Data were evaluated using the Chi-square test of categorical data as a non-parametric test, according to Snedecor and Cochran (1991).

## RESULTS

The present research showed that 70.59% of the examined chickens were infested by various helminth parasites. The nematode infestation was 32.95%, followed by cestodes (26.47%). There are 2.94 % mixed infestations in Table 2.

The peak helminth infestation was observed during spring (73.07%), whereas the lowest prevalence (67.39%) was recorded in autumn. For nematodes, the maximum infestation rate is in the autumn (43%), and the lowest one is in the winter (21%). For cestodes, the highest infestation rate was in winter (50%) and the lowest one was in summer (15.79%), as shown in Table 3.

Regarding the seasonal dynamics, it has been found that *Ascaridia galli* reached the highest level in spring (25%), the lowest in winter (2.94%), for *Heterakis gallinarum*, the peak of infestation was in autumn (6.52%) and the lowest in spring (1.92%), *Subulura brumpti* showed the highest level in winter (11.76%) and the low-

Table 1. Primers used in PCR.

| Gene | F-Primers | R-Primers | Reference |
|---|---|---|---|
| Nematode 18S small subunit ribosomal RNA gene | CGCGAATRGCTCATTACAACAGC | GGGCGGTATCTGATCGCC | Floyd *et al.* (2005) |
| Cestodes rDNA internal transcribed spacer 2 regions (ITS2) | 3Sforward 5'GGTACCGGTGGATCACTCGGCTCGTG 3' | A28Reverse 5'GGGATCCTGGTTAGTTTCTTTTCCTCCGC3 | Ramnath *et al.* (2014) |

Table 2. Prevalence of helminth parasites detected among the examined chickens.

| No. exam. Chickens | No. infested Chickens | % infestation | Types of Helminths | | | | Mixed infestation | |
|---|---|---|---|---|---|---|---|---|
| | | | Nematodes | | Cestodes | | Nematodes and Cestodes | |
| 170 | 120 | 70.59% | No. infested | % | No. infested | % | No. infested | % |
| | | | 56 (51*+5**) | 32.95 | 45 (40*+5**) | 26.47 | 5 | 2.94 |

Chi-Square=23.839, df= 2, p value = 0.000 (highly significant)

Table 3. Seasonal dynamics of helminth parasites detected among the examined chickens.

| Season | No. exam. | No. infests. | % | Nematodes | | Cestodes | | Mixed infestation | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | No. infested | % | No. infested | % | No. infested | % |
| Autumn | 46 | 31 | 67.39 | 20 | 43% | 11 | 23.91 | 1 | 2.17 |
| Winter | 34 | 24 | 70.59 | 7 | 21% | 17 | 50 | 1 | 2.94 |
| Spring | 52 | 38 | 73.07 | 19 | 37% | 11 | 21.15 | 1 | 1.92 |
| Summer | 38 | 27 | 71.05 | 10 | 26% | 6 | 15.79 | 2 | 5.26 |
| Total | 170 | 120 | 70.59 | 56 | 33% | 45 | 26.47 | 5 | 2.94 |

Pearson Chi-Square= 2750, df= 121, p value = 0.000 (highly significant)

est in spring (3.85%), and *Trichostrongylus tenius* was detected more in autumn (15.22%) than in summer (5.26%) Table 4.

Additionally, the peak of infestation of *Raillietina tetragona* was in winter (26.47%), and the lowest in spring (7.61%). *R. echinobothrida* reached its highest level in winter (23.53%), and the lowest level in summer (5.26%), and *Hymenolepis carioca* and *Choanotaenia infundibulum* were found only in spring (1.92%), as shown in Table 5.

Concerning *Eimeria* spp. encountered in the present study, the infestation of *E. tenella* was 5.88%, *E. mitis* (4.12%), *E. maxima*, and *E. brunetti* infestations recorded (0.59%) for each, with the mixed infection including *E. tenella* and *E. mitis* being (2.35%), as shown in Table 6.

Concerning the ectoparasites encountered in the present study, the infestation of *Echidnophaga gallinacea* was 1.18%. *Menopon gallinae* (5.88%) *Lipeurus caponis* (4.71%), *Columbicola columbae* (1.76%), and *Dermanyssus gallinae* (2.35%), as shown in Table 7.

Morphological characters of the collected nematodes, cestodes, *Eimeria* Sp., and arthropods are shown in Figs. 1, 2, 3 and 4 respectively.


Fig. 1. (A) The anterior side of adult *Ascaridia galli* points to a simple club-shaped oesophagus. Scale bar = 400 µm (x4), and B) *A. galli* adult male posterior side has subequal spicules (s), a slit-like precloacal sucker (pre), and well-developed caudal papillae (p). Scale bar = 400 µm (x4); C) *A. galli* adult female posterior side has a straight and conical posterior end. Scale bar = 400 µm (x4), D) Anterior portion of adult *Heterakis gallinarum* showing three lips with lateral alae and a posterior bulb-shaped oesophagus (x10), E) *H. gallinarum* adult male posterior end showing unequal spicules, a prominent precloacal sucker, large and well-developed caudal alae and papillae (x10), F) *H. gallinarum* adult female posterior portion showing a pointed and tapered posterior end. Scale bar= 170 µm (x10), G) Anterior end of adult *Subulura brumpti*. Showing a double-bulb-shaped oesophagus (x10), H) *S. brumpti* adult male posterior end has two subequal spicules, elongate slit-shaped precloacal sucker. Scale bar= 170 µm (x10), I) *S. brumpti* female posterior end showing less pointed posterior end. Scale bar= 170 µm (x10), J) *Trichostrongylus tenuis* adult female (x4), scale bar = 400 µm. K) *T. tenuis* anterior end female with three inconspicuous lips, the oesophagus was thin and elongated (x40), L) *T. tenuis* posterior end female (x40), posterior end, the digital proses is pointed posteriorly, scale bar = 50 µm.

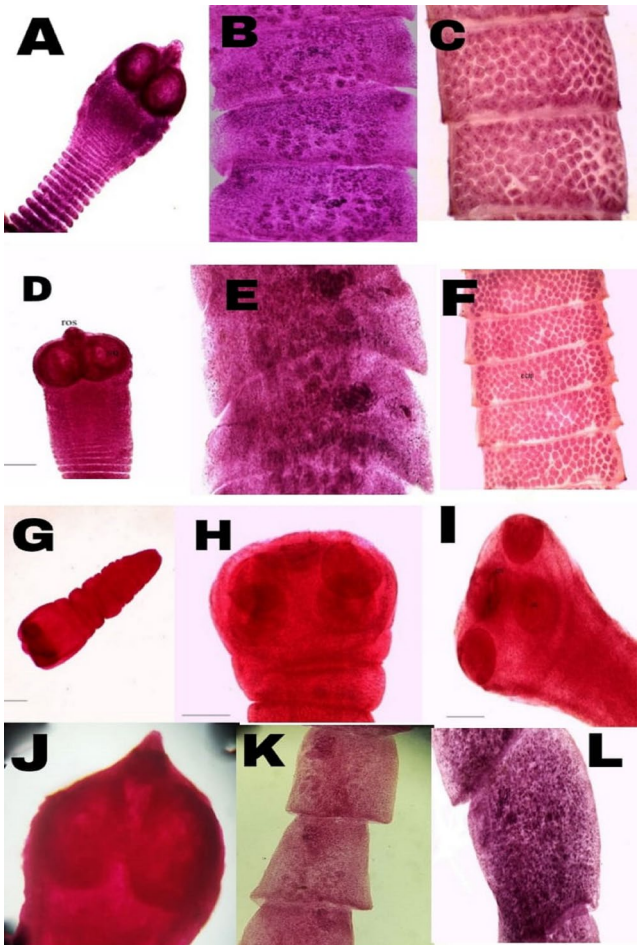


Fig. 2. A) *Raillietina tetragona* scolex has 4 oval-shaped suckers and armed rostellum with hooks (x10), B) *R. tetragona* mature segments with unilateral common genital pores anterior to the middle part (x10), C) *R. tetragona* gravid segments with egg capsules containing several ova (x10), D) *R. echinobothrida* scolex with round-shaped suckers and armed rostellum Scale bar= 170 µm, E) *R. echinobothrida* mature segments with unilateral common genital pores slightly posterior to the middle part (x10), F) *R. echinobothrida* gravid segments with egg capsules containing 6 to 12 ova (x10), G) *Hymenolepis carioca* adult (x4), scale bar = 400 µm (x4), H &I) *Hymenolepis carioca* scolex is globular and carries four rounded unarmed suckers (x10), scale bar = 170 µm , (su): sucker, (ros): rostellum, J) *Choanotaenia infundibulum* scolex were triangular in shape (x40), K) Ch. infundibulum mature segment were bell shape and unilateral, irregular alternating in the anterior third of the lateral margin of each segment. (x10), L) Ch. infundibulum gravid segment (x10) filled with egg.

*Molecular identification of nematode species*

In this study, *Ascaridia galli* samples were investigated as shown in Fig. 5, which were assembled from Ismailia province. They were recognized based on the 18S small subunit ribosomal RNA gene partial sequence PCR; the GenBank accession numbers are (OP476345, OP476346, OP476347, and OP476348).

*Phylogenetic analysis*

Using the MEGA X10.1 program, a phylogenetic analysis based on the 18S rRNA gene was carried out, and neighbor-joining (NJ) techniques were used to build the trees. (Fig. 6). There are significant sequencing differences across the samples used in this investigation; two of them cluster with the same species from the USA and Japan, one sequence is clustered with species from Iraq, and the last one is clustered with species from Egypt and China.

Fig. 7, shows estimates of evolutionary deviation among the present study *Ascaridia* spp. sequences, and sequences of nematode species from the GenBank (18S rRNA).

*Molecular identification of cestodes species*

In this study, *Raillietina* species samples were investigated,

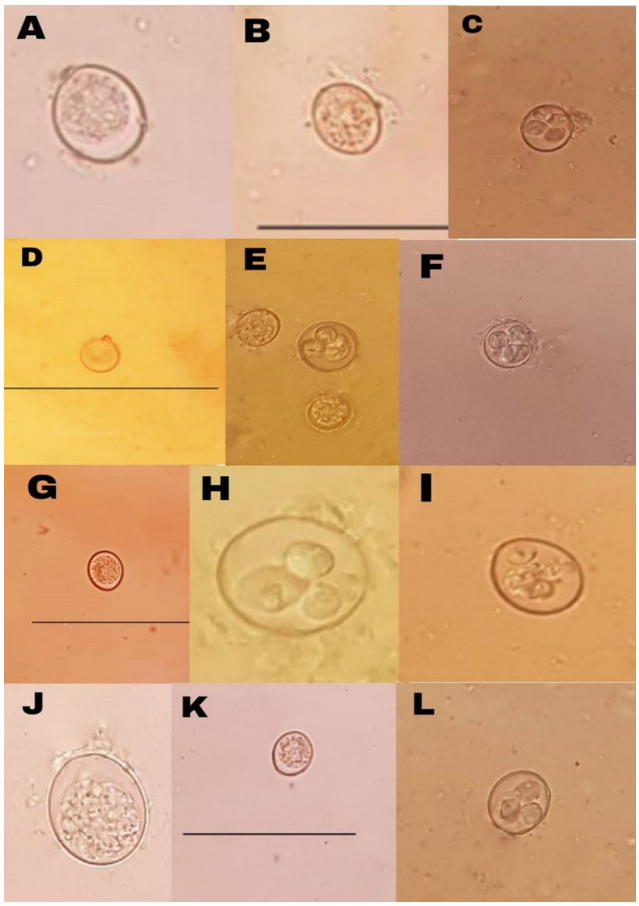


Fig. 3. A) and B) unsporulated *E. tenella* (x 40); scale bar = 100 µm. C) Sporulated *E. tenella* (x40) with 4 sporocysts, each sporocyst containing 2 sausage-shaped sporozoites and a micropyle covered by a micropyle cap is found, D) unsporulated *E. mitis* (x40); scale bar = 100 µm. E) & F) sporulated *E. mitis*, subspherical with a thin, smooth double wall, slight tapering, and small oocyst is a distinctive characteristic of micropyle (x40). G) unsporulated *E. maxima* (x40), scale bar = 100 µm, H) and I) sporulated *E. maxima*, golden yellow in color, large ovoid, thick wall, and no micropyle (x40), J) and K) unsporulated *E. brunetti* no micropyle. (x40), scale bar = 100 µm. L) sporulated *E. brunetti* (x40).

as shown in Fig. 8, which were found in Ismailia province. They were identified based on the rDNA internal transcribed spacer 2 regions.

## DISCUSSION

The present results obtained during a period extending from the beginning of September 2021 to the end of August 2022, (table 2) revealed that There were 120 of the 170 chickens (70.59%) were found infested with endoparasites with similarity to that recorded (72.0%) by Katoch *et al.* (2012) in northwestern India, 61.54% in Ismailia, Egypt (El-Gayer, 1995), on the contrary, higher rates (91.01%) were recorded by Eshetu *et al.* (2001) in Ethiopia, 98.9% in southeastern United States by Yazwinski *et al.* (2013), and lower than that reported (35.5%) by Nnadi and George

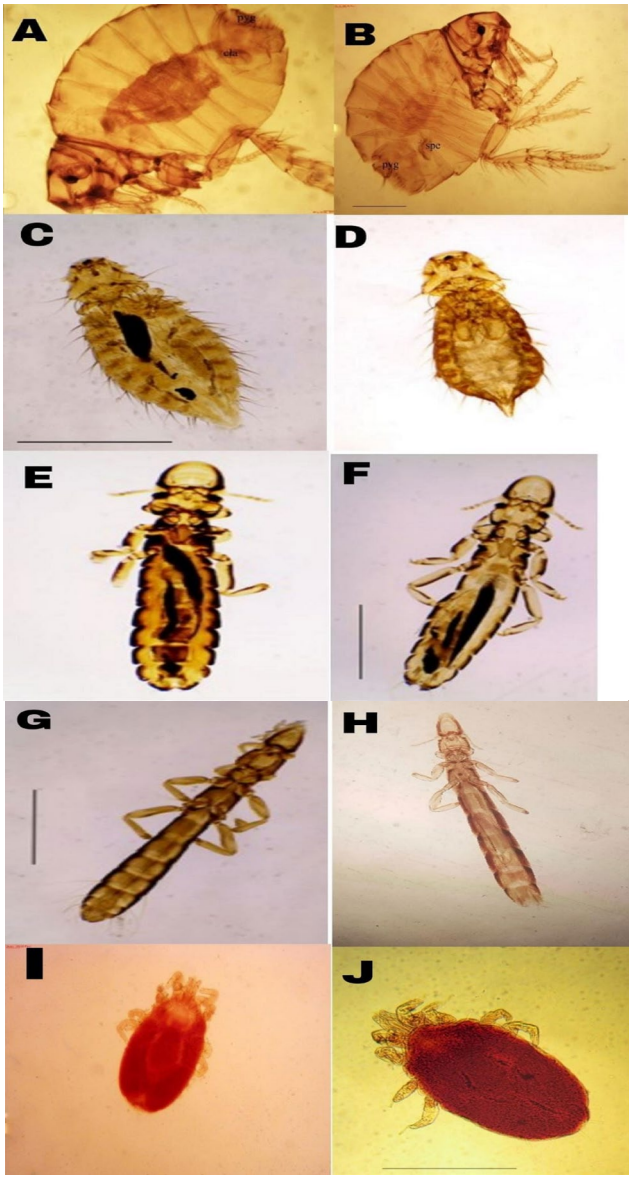


Fig. 4. A) *Echidnophaga gallinacea* male (x4), (cla): claspers, (pyg): pygidium B)*E. gallinacea* female (x4), scale bar = 1 mm, (spe): spermatheca, (pyg) : pygidium. C) Menopon gallinae (x4) female has a round end. D) *M. gallinae* male has a pointed end with a penis posteriorly, scale bar = 1mm. E) and F) *Lipeurus caponis* female (x 4) the cuticle is an irregular outer layer; scale bar = 1 mm. G) *Columbicola columbae* (x4) males have a less rounded end with the penis posteriorly. H) *Columbicola columbae* female has a notched end; scale bar = 1 mm. I) *Dermanyssus gallinae* adult ventral surface, scale bar = 0.5 mm; J) *Dermanyssus gallinae* adult female fully engorged with blood is red in color and has a very prominent single sclerite (dorsal shield) (x4) dorsal surface.

(2010) in Nigeria, and 34.8 % in west Iran (Badparva *et al.*, 2015) The high infection rate is attributed to the lack of application of methods of hygienic measures, poor ventilation, and a lack of attention to using anthelmintic drugs.

In Table 3, the peak of helminth infestation was observed during spring (73.07%), followed by summer and winter (71.05%

Table 4. Seasonal dynamics of nematodes detected among the examined chickens.

| Season | Total No. exam. | Nematodes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Ascaridia galli* | | *Heterakis gallinarum* | | *Subulura brumpti* | | *Trichostrongylus tenuis* | |
| | | No. infested | % | No. infested | % | No. infested | % | No. infested | % |
| Autumn | 46 | 10 | 21.74 | 3 | 6.52 | - | - | 7 | 15.22 |
| Winter | 34 | 1 | 2.94 | - | - | 4 | 11.76 | 2 | 5.88 |
| Spring | 52 | 13 | 25 | 1 | 1.92 | 2 | 3.85 | 3 | 5.77 |
| Summer | 38 | 8 | 21.05 | - | - | - | - | 2 | 5.26 |
| Total | 170 | 32 | 18.82 | 4 | 2.35 | 6 | 3.53 | 14 | 8.24 |

Pearson Chi-Square= 1408, df= 121, p value = 0.000 (highly significant)

and 70.59%, respectively), in agreement with El-Gayer (1995) in Ismailia, Egypt, who noticed that the peak of helminth infestation was in spring (84.62%), with the lowest prevalence in winter (50%). El-Dakhly *et al*. (2019) in Aswan, Upper Egypt, revealed that a higher prevalence of infestation was found in the summer than in the winter. The highest prevalence is attributed to climate factors, especially the warm seasons, which favour egg production and complete the life cycle (Hembram *et al*., 2015).

As shown in Table 3, the prevalence of nematode infestation (32.95%) was nearly similar to that reported (39.87%) by Sreedevi *et al*. (2016) in India and to that reported in Ismailia, Egypt (40.27%) by El-Gayer (1995). This was higher than the results of Javaregowda *et al*. (2016) in India (28.96%), and lower than the results recorded by Mungube *et al*. (2008) in Kenya (74.4%), Tanveer *et al*. (2015) in Kashmir Valley (70 %). Cestodes infestation during this study (26.47%) was similar to El-Gayer (1995) in Ismailia (28.51%), Egypt, and in Nigeria (26.4%) (Idika *et al*. 2016). The results were lower than those reported by Mungube *et al*., (2008) in Kenya (68.1%) and Tanveer *et al*. (2015) in the Kashmir Valley (76.9%). The low rate of infestation with helminths was probably due to the use of anthelmintic drugs and good hygiene measures in housing in the Ismailia governorate.

*Ascaridia galli* had constant counts in their host throughout the rainy season Magwisha *et al*. (2002) in Tanzania, on the contrary, results in Table 4, revealed that the highest percentage of *A. galli* was in spring (25%), and the lowest was in winter (2.94%), which agrees with El-Gayer (1995), who found that that the peak of *A. galli* infestation was in spring (61.54%), and the lowest was in summer (30.77%). The highest infestation rate of *Heterakis gallinarum* was in autumn (6.52%) and the lowest in spring (1.92%), which disagreed with El-Gayer (1995) who observed that the highest level was in summer (4.62%), the lowest was in autumn (3.08%). *Subulura brumpti* was detected at (3.52%) in agreement with El-Dakhly *et al*., (2019) (1.28%) in Aswan, Upper Egypt, but other studies reported higher rate of 15.3% in Morocco (Hassouni and Belghyti, 2006). *Trichostrongylus tenuis* reached 8.23%, which is nearly similar to the finding (12.5%) of Tomza-Marciniak *et al*. (2014) in Germany; the higher percentage was reported by Magwisha *et al*. (2002) (43% grower and 7% adult) in Tanzania; and the lowest one was recorded by Kumar *et al*. (2015) (1.72%) in India. The difference in the percentages is due to the different weather conditions, as the warm season helps the eggs to grow and complete the nematode life cycle of the worms.

In Table 5, the highest percentage of *Raillietina tetragona* was in winter (26.47%) and the lowest one was in spring (7.69%). El-Gayer (1995) recorded that the peak of *R. tetragona* infestation was in autumn (23.08%) and the lowest was in winter (11.54%); the highest infestation rate of *R. echinobothrida* was in winter (23.53%) and the lowest level in summer (5.26%) which disagreed with El-Gayer (1995), who revealed that the highest level of infestation was in spring (20.51%) and the lowest in winter (13.46%), and also disagree with Khater (1993). The variation in the seasonal dynamics may be due to different weather conditions, types of housing, and/or failure to pay attention to the application of hygiene measures.

In case of *Eimeria* infestation in the present work, they were investigated with low prevalence (11.18%) (Table 6) from the examined chickens with mixed infection (2.35%); these conse-

Table 5. Seasonal dynamics of cestodes detected among the examined chickens.

| Season | Total No. exam. | Cestodes | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *Raillietina tetragona* | | *R. echinobothrida* | | *Hymenolepis carioca* | | *Choanotaenia infundibulum* | |
| | | No. infested | % | No. infested | % | No. infested | % | No. infested | % |
| Autumn | 46 | 8 | 17.39 | 3 | 6.52 | - | - | - | - |
| Winter | 34 | 9 | 26.47 | 8 | 23.53 | - | - | - | - |
| Spring | 52 | 4 | 7.69 | 5 | 9.61 | 1 | 1.92 | 1 | 1.92 |
| Summer | 38 | 4 | 10.53 | 2 | 5.26 | - | - | - | - |
| Total | 170 | 25 | 14.7 | 18 | 10.59 | 1 | 0.5 | 1 | 0.5 |

Pearson Chi-Square= 1008, df= 81, p value = 0.000 (highly significant)

Table 6. Prevalence of *Eimeria* species detected among the examined chickens.

| | No. infeststed Chickens | % infestation |
|---|---|---|
| *E. tenella* | 10 | 5.88% |
| *Eimeria mitis* | 7 | 4.12% |
| *Eimeria maxima* | 1 | 0.59% |
| *Eimeria brunetti* | 1 | 0.59% |
| *mixed infection* | 4 | 2.35% |
| Total infested chicken | 19 | 11.18% |
| Total examined chicken | 170 | - |

Pearson Chi-Square= 6.714, df= 4, p value = 0.152 (low significant)

Table 7. Prevalence of arthropods detected among the examined chickens.

| Arthropods | No. infeststed Chickens | % infestation |
|---|---|---|
| *Echidnophaga gallinacea* | 2 | 1.18% |
| *Menopon gallinae* | 10 | 5.88% |
| *Lipeurus caponis* | 8 | 4.71% |
| *Columbicola columbae* | 3 | 1.76% |
| *Dermanyssus gallinae* | 4 | 2.35% |
| Total infested chicken | 27 | 15.88% |
| Total examined chicken | 170 | - |

Pearson Chi-Square= 5.875, df= 4, p value = 0.209 (low significant)

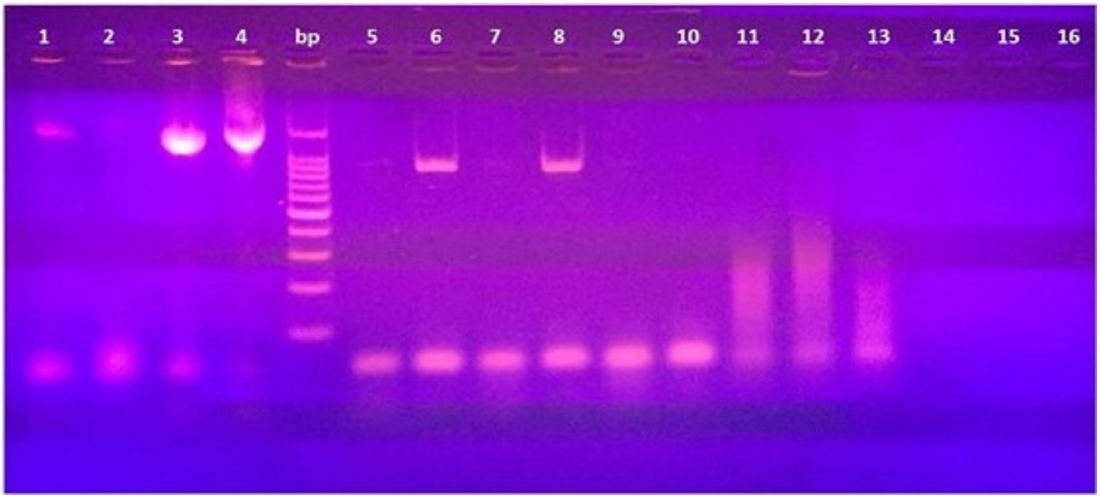


Fig. 5. PCR amplification utilizing *Ascaridia galli* samples using: 18S small subunit ribosomal RNA gene, a DNA ladder placed on the left sides of the gel, fragment sizes characterized in base pairs (bp)..

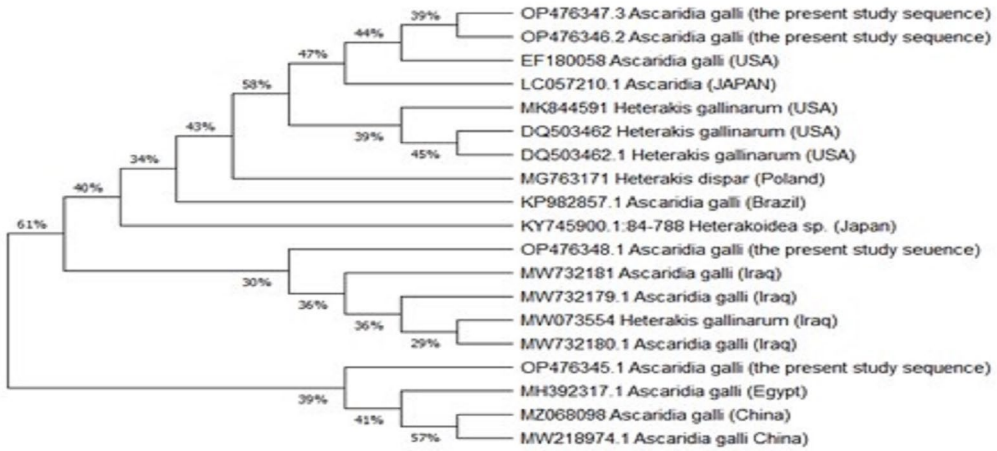


Fig. 6. Phylogenetic relationships of the present study *Ascaridia* spp. sequences from Egypt, and sequences of nematode species from the GenBank based on 18S rRNA.

quences slightly resembled those of Badparva *et al.* (2015) and Mohammed *et al.* (2019) 7.1% and 7.4% respectively, in Nigeria. Results agree with Orunç and Biçek (2009) in Turkey (65%). *E. tenella* was recorded at 5.88%; this result varied from Mungube *et al.* (2008) in Kenya (16.7%), Huang *et al.* (2017) in China (80.67%), and Debbou-Iouknane *et al.* (2018) in Algeria (26.92%). Also, the low infestation revealed in this study for *Eimeria mitis* (4.12%), *Eimeria maxima*, and *Eimeria brunetti* (0.59%) for each, conflicted with the results obtained by Huang *et al.* (2017) for *E. mitis* (55%) and for *E. brunetti* (54%) in China. Where the weather changes in the months over the previous years, it leads to a discrepancy in the proportions.

In the case of ectoparasite infestation in the present work (Table 7), there was a low prevalence (15.88%); these consequences were lower than the results obtained by Wang *et al.* (2010) in China (88.4 %), Mirzaei *et al.* (2016) in Iran (52.8%), and Salam *et al.* (2009) in Kashmir (100%). The low rate of infestation with ectoparasites in the present survey may be due to the use of insecticides and the great hygienic measurement of insects used in Ismailia Governorate, which affect the intensity and prevalence of ectoparasite infestation.

The prevalence of *Echidnophaga gallinacea* infestation (1.18%) in Table 7; is lower than the results mentioned in Permin *et al.* (2002) (72% young; 74% adult) in Zimbabwe, 76.7% in Kenya (Mungube *et al.*, 2008) (, 8% in Iran (Mirzaei *et al.*, 2016). *Menopon gallinae* was found at 5.88% which is lower than mentioned in Permin *et al.* (2002) (24% of the young; 66% of the adults) in Zimbabwe and Orunç and 22% in Turkey (Biçek, 2009). Regarding the detected *Lipeurus caponis* was recorded in 4.71% of examined fowls, this result was greatly lower than the result of Murillo and Mullens, (2016) in California (20%). The prevalence of *Dermanyssus gallinae* infestation (2.35%) is nearly similar to the results mentioned BY Murillo and Mullens (2016) in California (5%) and is lower than the results mentioned by Mirzaei *et al.* (2016) in Iran (11%), and by Eladl *et al.* (2018) in Egypt (40.9%). The variation in prevalence is due to the different weather conditions, types of housing, and increasing awareness among people.

The confirmation of the identification of *Ascaridia galli* relies heavily on molecular studies, including DNA amplification, sequencing, and phylogenetic analysis. The same kind of study on *A. galli* was also conducted by cohorts Katakam *et al.* (2010), who extracted DNA from the larva of *A. galli* of chicken and amplified it by polymerase chain reaction-linked restriction fragment length polymorphism (PCR-RFLP) assay.

Two samples had a close association and clustered with the same species from the USA and Japan according to the neighbor-joining (NJ) methods used in the trees' construction and phylogenetic analysis, both of which were conducted using the MEGA X10.1 software. One sequence is clustered with species from Iraq, and the last one is clustered with species from Egypt and China. But the phylogenetic analysis by Faizullah *et al.* (2022) showed that the closest strain to *A. galli* was very homologous with *A. galli* GU 138670.199% from the Australian poultry strain. In this research, the 18S small subunit ribosomal RNA gene succeeded in amplification, sequencing, and construction of the phylogenetic analysis of our sample species from the Ismailia governorate, Egypt.

Therefore, this study targeted to create a simple PCR to discover and distinguish cestodes species utilizing specific primers based on the internal transcribed spacer 2 region (ITS2) with 3S

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MW073554_Heterakis_gallinarum_(Iraq) | | | | | | | | | | | | | | | | | | |
| 2 | MG763171_Heterakis_dispar_(Poland) | 1.03 | | | | | | | | | | | | | | | | | |
| 3 | OP476347.3_Ascaridia_galli_(the_present_study_sequence) | 1.06 | 0.01 | | | | | | | | | | | | | | | | |
| 4 | MZ068098_Ascaridia_galli_(China) | 1.42 | 1.30 | 1.36 | | | | | | | | | | | | | | | |
| 5 | MW732181_Ascaridia_galli_(Iraq) | 0.05 | 0.89 | 0.89 | 1.19 | | | | | | | | | | | | | | |
| 6 | KP982857.1_Ascaridia_galli_(Brazil) | 0.96 | 0.01 | 0.00 | 1.19 | 0.88 | | | | | | | | | | | | | |
| 7 | KY745900.1:84-788_Heterakoidea_sp._(Japan) | 0.98 | 0.04 | 0.03 | 1.23 | 0.91 | 0.03 | | | | | | | | | | | | |
| 8 | MK844591_Heterakis_gallinarum_(USA) | 0.98 | 0.01 | 0.01 | 1.22 | 0.90 | 0.01 | 0.04 | | | | | | | | | | | |
| 9 | OP476346.2_Ascaridia_galli_(the_present_study_sequence) | 0.97 | 0.01 | 0.00 | 1.24 | 0.90 | 0.00 | 0.03 | 0.01 | | | | | | | | | | |
| 10 | MW732179.1_Ascaridia_galli_(Iraq) | 0.05 | 0.89 | 0.89 | 1.19 | 0.00 | 0.88 | 0.91 | 0.90 | 0.90 | | | | | | | | | |
| 11 | MW732180.1_Ascaridia_galli_(Iraq) | 0.04 | 0.88 | 0.89 | 1.19 | 0.01 | 0.88 | 0.90 | 0.90 | 0.90 | 0.00 | | | | | | | | |
| 12 | DQ503462_Heterakis_gallinarum_(USA) | 1.03 | 0.01 | 0.01 | 1.29 | 0.90 | 0.01 | 0.04 | 0.00 | 0.01 | 0.90 | 0.90 | | | | | | | |
| 13 | MW218974.1_Ascaridia_galli_China) | 1.42 | 1.31 | 1.37 | 0.00 | 1.19 | 1.19 | 1.24 | 1.23 | 1.25 | 1.19 | 1.19 | 1.30 | | | | | | |
| 14 | MH392317.1_Ascaridia_galli_(Egypt) | 1.49 | 1.35 | 1.39 | 0.61 | 1.35 | 1.33 | 1.31 | 1.30 | 1.32 | 1.33 | 1.34 | 1.35 | 0.61 | | | | | |
| 15 | LC057210.1_Ascaridia_(JAPAN) | 1.12 | 0.01 | 0.02 | 1.39 | 0.91 | 0.00 | 0.04 | 0.02 | 0.01 | 0.91 | 0.90 | 0.01 | 1.40 | 1.39 | | | | |
| 16 | EF180058_Ascaridia_galli_(USA) | 1.03 | 0.01 | 0.00 | 1.30 | 0.89 | 0.00 | 0.03 | 0.01 | 0.00 | 0.89 | 0.89 | 0.01 | 1.31 | 1.35 | 0.01 | | | |
| 17 | DQ503462.1_Heterakis_gallinarum_(USA) | 1.14 | 0.01 | 0.02 | 1.40 | 0.90 | 0.01 | 0.04 | 0.00 | 0.01 | 0.90 | 0.90 | 0.00 | 1.43 | 1.37 | 0.02 | 0.01 | | |
| 18 | OP476345.1_Ascaridia_galli_(the_present_study_sequence) | 1.96 | 1.91 | 1.92 | 1.84 | 1.94 | 1.93 | 1.92 | 1.90 | 1.94 | 1.94 | 1.93 | 1.85 | 1.83 | 1.82 | 1.87 | 1.88 | 1.84 | |
| 19 | OP476348.1_Ascaridia_galli_(the_present_study_seuence) | 1.77 | 1.85 | 1.87 | 1.79 | 1.77 | 1.79 | 1.82 | 1.87 | 1.85 | 1.78 | 1.79 | 1.85 | 1.82 | 1.98 | 1.84 | 1.84 | 1.83 | 1.96 |

Fig. 7. Estimates of evolutionary deviation among the present study *Ascaridia* spp. sequences, and sequences of nematode species from the GenBank (18S rRNA).

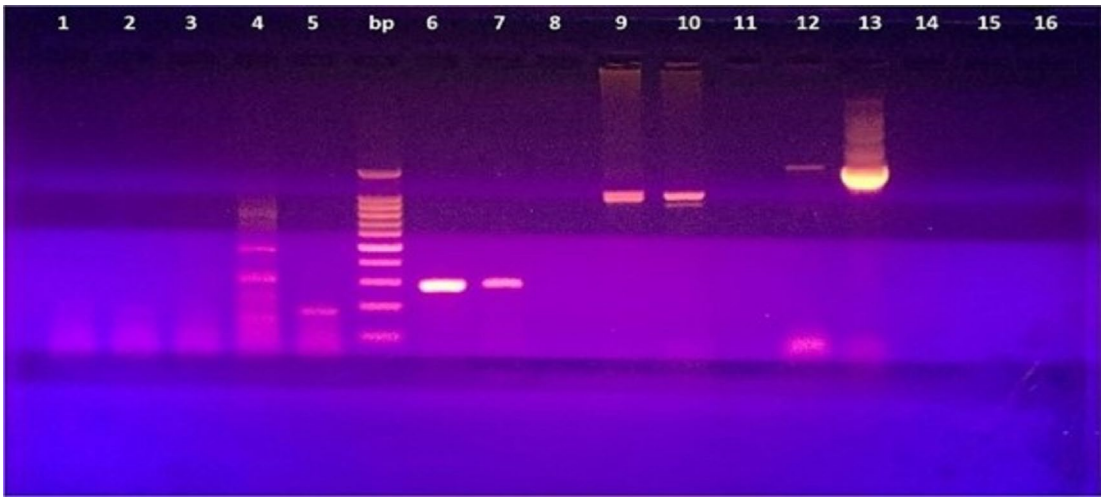


Fig. 8. PCR amplification utilizing *Raillietina* species samples using: the rDNA internal transcribed spacer 2 regions; a DNA ladder placed on the right sides of the gel; fragment sizes characterized in base pairs (bp).

forward (5′ GGTACCGGTGGATCACTCGGCTCGTG3′) and A28 reverse (5′ GGGATCCTGGTTAGTTTTTCCTCCGC3) Ramnath *et al.* (2014), as shown in Table 1. But Panich and Chontananarth (2021) used the internal transcribed spacer 2 (ITS2) region using primers ITS3 (5′- GCATCGATGAAGAACGCAGC3′) and ITS4 (5′- TCCTCCGCTTATTGATATGC-3′) Barber and Erdmann (2000) suggest that the specific primers successfully developed in this study could be useful to designate the epidemic veterinary areas for preventing, managing, and controlling the infection.

## CONCLUSION

Identifying morphologically identical species has shown to be a very successful process when using molecular identification of species. Consequently, it is advised to use the molecular technique as the primary methodological method for the accurate identification of helminths.

## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

## REFERENCES

Badparva, E., Ezatpour, B., Azami, M., Badparva, M., 2015. First report of birds infection by intestinal parasites in Khorramabad, west Iran. Parasit. Dis. 39, 720-724.

Barber, P.H., Erdmann, M.V., 2000. Molecular systematics of the Gonodactylidae (Stomatopoda) using mitochondrial cytochrome oxidase C (subunit 1) DNA sequence data. Journal of Crustacean Biology 20, 20-36.

Debbou-Iouknane, N., Benbarek, H., Ayad, A., 2018. Prevalence and aetiology of coccidiosis in broiler chickens in Bejaia province, Algeria. Onderstepoort Vet. Res. 85, e1-e6.

Eladl, A.H., Hamed, H.R., El-Shafei, R.A., 2018. Prevalence of mites and their impact on laying hen (*Gallus gallus domesticus*) farm facilities in Egypt, with an analysis of deltamethrin residues in eggs and tissue. Avian Pathol. 47, 161-171.

El-Dakhly, K.M., El-Seify, M.A., Mohammed, E.S., Elshahawy, I.S., Fawy, S.A., and Omar, M.A., 2019. Prevalence and distribution pattern of intestinal helminths in chicken and pigeons in Aswan, Upper Egypt. Trop. Anim. Health Prod. 51, 713-718.

El-Gayer, K.A., 1995. Some studies on enteric helminths of chickens in Ismailia province. M.V.Sc. Thesis, Fac. Vet. Med. Suez Canal University, Egypt.

Eshetu, Y., Mulualem, E., Ibrahim, H., Berhanu, A., Aberra, K., 2001. Study of gastro-intestinal helminths of scavenging chickens in four rural districts of Amhara region, Ethiopia. Rev. Sci. Tech. 20, 791-796.

Faizullah, Jan, S.U., Taj, K.,Zia Ud, D., Akbar, M., Sattar, A., Akbar, H. , 2022. Morphological and molecular evidences of *Ascaridia galli* in migratory quail *Coturnix coturnix japonica* from Baluchistan Pakistan. Braz. J. Biol. 82, e258647.

Ferdushy, T., Hasan, M.T., Golam Kadir, A. K. , 2016. Cross sectional epidemiological investigation on the prevalence of gastrointestinal helminths in free range chickens in Narsingdi district, Bangladesh. Parasit. Dis. 40, 818-822.

Fleck, S.L., Moody, A.H., 1988. Diagnostic techniques in medical parasitology: Butterworth Scientific Ltd.

Floyd, R.M., Rogers, A.D., Lambshead, P.J.D., Smith, C.R., 2005. Nematode-specific PCR primers for the 18S small subunit rRNA gene. J. Molecular Ecology Notes. 5, 611-612.

Hassan, A.A., Habib, A Issa, G.I., 1958. The hard ticks of Egypt (Arthropoda;

Acarina; Ixodidae). J. Egypt. Vet. Med. Assoc. 18, 3-20.
Hassouni, T., Belghyti, D., 2006. Distribution of gastrointestinal helminths in chicken farms in the Gharb region--Morocco. Parasitol. Res. 99, 181-183.
Hembram, A., Panda, M.R., Mohanty, B.N., Pradhan, C.R., Dehuri, M., Sahu, A., Behera, M., 2015. Prevalence of gastrointestinal helminths in Banaraja fowls reared in semi-intensive system of management in Mayurbhanj district of Odisha. Vet. World. 8, 723-726.
Huang, Y., Ruan, X., Li, L., Zeng, M., 2017. Prevalence of *Eimeria* species in domestic chickens in Anhui province, China. J. Parasit. Dis. 41, 1014-1019.
Idika, I.K., Obi,C.F., Ezeh,I.O., Iheagwam, C.N., Njoku, I.N., Nwosu, C.O., 2016. Gastrointestinal helminth parasites of local chickens from selected communities in Nsukka region of south eastern Nigeria. J. Parasit. Dis. 40, 1376-1380.
Javaregowda, A.K., Kavitha Rani, B., Revanna, S.P., Udupa, G., 2016. Prevalence of gastro-intestinal parasites of backyard chickens (*Gallus domesticus*) in and around Shimoga. J. Parasit. Dis. 40, 986-990.
Johnson, M., Zaretskaya,I., Raytselis,Y., Merezhuk,Y., McGinnis,S., Madden, T.L., 2008. NCBI BLAST: a better web interface. Nucleic Acids Res. 36, W5-9.
Katakam, K.K., Nejsum, P., Kyvsgaard, N.C., Jørgensen, C.B., Thamsborg, S.M., 2010. Molecular and parasitological tools for the study of *Ascaridia galli* population dynamics in chickens. Avian Pathol. 39, 81-85.
Katoch, R., Yadav, A., Godara, R., Khajuria, J., Borkataki, S., Sodhi, S., 2012. Prevalence and impact of gastrointestinal helminths on body weight gain in backyard chickens in subtropical and humid zone of Jammu, India. J. Journal of Parasitic Diseases 36, 49-52.
Khater, H., 1993. Studies on enteric helminth parasites in domestic birds. M.V.Sc. Thesis, Fac. Vet. Med. (Banha branch) Zagazig University, Egypt.
Kumar, S., Garg, R., Ram, H., Maurya, P.S., Banerjee, P.S., 2015. Gastrointestinal parasitic infections in chickens of upper Gangetic plains of India with special reference to poultry coccidiosis. J. Parasit. Dis. 39, 22-26.
Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 35, 1547-1549.
Lambio, A., 2012. Duck Production. Poultry Production in the Tropics, p. 140.
Magwisha, H.B., Kassuku, A.A., Kyvsgaard, N.C., Permin, A., 2002. A comparison of the prevalence and burdens of helminth infections in growers and adult free-range chickens. Trop. Anim. Health Prod. 34, 205-214.
Mirzaei, M., Ghashghaei, O., Yakhchali, M., 2016. Prevalence of Ectoparasites of Indigenous Chickens From Dalahu Region, Kermanshah Province, Iran. Turkiye Parazitol. Derg. 40, 13-16.
Mohammed, B.R., Ojo, A.A., Opara, M.N., Jegede, O.C., Agbede, R.I.S. , 2019. Haemo- and endoparasites of indigenous chickens reared in Gwagwalada Area Council, Abuja, Nigeria. Ann. Parasitol. 65, 293-296.
Mungube, E.O., Bauni, S.M., Tenhagen, B.A., Wamae, L.W., Nzioka, S.M., Muhammed, L., Nginyi, J.M., 2008. Prevalence of parasites of the local scavenging chickens in a selected semi-arid zone of Eastern Kenya. Trop. Anim. Health. Prod. 40, 101-109.
Murillo, A.C., Mullens, B.A., 2016. Diversity and Prevalence of Ectoparasites on Backyard Chicken Flocks in California. J. Med. Entomol. 53, 707-711.
Nnadi, P., George, S., 2010. A cross-sectional survey on parasites of chickens in selected villages in the subhumid zones of South-Eastern Nigeria. Journal of Parasitology Research, 2010, 141824.
Orunç, O., Biçek, K., 2009. Determination of parasite fauna of chicken in the Van region. Turkiye Parazitol. Derg. 33, 162-164.
Panich, W., Chontananarth, T., 2021. Molecular detection of three intestinal cestode species (*Raillietina echinobothrida*, *R. tetragona*, *R. cesticillus*) from poultry in Thailand. J. Avian Pathology 50, 321-326.
Permin, A., Hansen, J.W., 1998. Epidemiology, diagnosis and control of poultry parasites: FAO. https://www.fao.org/publications/card/es/c/325ee367-8a27-5d3a-add8-396b9ff63769/
Permin, A., Esmann, J.B., Hoj, C.H., Hove,T., Mukaratirwa, S., 2002. Ecto-, endo- and haemoparasites in free-range chickens in the Goromonzi District in Zimbabwe. Prev. Vet. Med. 54, 213-224.
Puttalakshmamma, G., Mamatha, P., Rao, S., 2008. Prevalence of gastrointestinal parasites of poultry in and around Banglore. Veterinary World 1, 201.
Ramnath, Jyrwa, D.B., Dutta, A.K., Das, B., 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, 22-26.
Ruff, M.D., 1999. Important parasites in poultry production systems. J. Veterinary Parasitology. 84, 337-347.
Salam, S.T., Mir, M.S., Khan, A.R., 2009. Prevalence and seasonal variation of ectoparasite load in free-range chicken of Kashmir valley. Trop. Anim. Health Prod. 41, 1371-1376.
Snedecor, G.W., Cochran, W.G., 1991. Statistical Methods, 8th edition. Iowa State University Press., Ames.
Soulsby, E., 1982. Helminths, Arthropods Protozoa of domesticated animals. London: Baillière Tindall, p. 291.
Sreedevi, C., Jyothisree, C., Rama Devi, V., Annapurna, P., Jeyabal, L., 2016. Seasonal prevalence of gastrointestinal parasites in desi fowl (*Gallus gallus domesticus*) in and around Gannavaram, Andhra Pradesh. J. Parasit. Dis. 40, 656-661.
Tanveer, S., Ahad, S., Chishti, M.Z., 2015. Morphological characterization of nematodes of the genera Capillaria, Acuaria, Amidostomum, Streptocara, Heterakis, and *Ascaridia* isolated from intestine and gizzard of domestic birds from different regions of the temperate Kashmir valley. J. Parasit. Dis. 39, 745-760.
Tomza-Marciniak, A.,Pilarczyk, B., Tobiańska, B., Tarasewicz, N., 2014. Gastrointestinal parasites of free-range chickens. Ann. Parasitol. 60, 305-308.
Van, N.T.B., Cuong, N.V., Yen, N.T.P., Nhi, N.T.H., Kiet, B.T., Hoang, N.V., Ribas, A., 2020. Characterisation of gastrointestinal helminths and their impact in commercial small-scale chicken flocks in the Mekong Delta of Vietnam. Trop. Anim. Health Prod. 52, 53-62.
Wang, F.F., Wang, M., Xu, F.R., Liang, D.M., Pan, B.L., 2010. Survey of prevalence and control of ectoparasites in caged poultry in China. Vet. Rec. 167, 934-937.
Yazwinski, T., Tucker, C., Wray, E., Jones, L., Johnson, Z., Steinlage, S., Bridges, J., 2013. A survey on the incidence and magnitude of intestinal helminthiasis in broiler breeders originating from the southeastern United States. Journal of Applied Poultry Research 22, 942-947.