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Prevalence and scanning electron microscope of some parasites infecting domesticated and migratory quails from Edko and Rashid districts, El-Behera governorate, Egypt

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A B S T R A C T

Quails have many advantages over other poultry species. Its meat has achieved great popularity as an excellent source of protein and other important nutrients. However, there are some limitations to quails production. One of them is the susceptibility to parasitic diseases that cause severe economic losses. Therefore, this work aimed to determine the infection rate and morphology of parasites infecting quails in El-Behera governorate, Egypt. 100 quails (50 migratory, *Coturnix coturnix japonica* and 50 domesticated quails) were collected. The gastrointestinal tracts of each bird were examined to collect helminths. Fecal materials were examined by direct and flotation methods to detect any coccidian species. The results showed that the total percentage of infection with parasites was 55%. The prevalence of parasitic infection in migratory and domesticated quails was 40% and 70%, respectively. Two species of helminths were recorded, *Raillitina tetragona* and *Heterakis gallinarum* with a prevalence of 87.5% and 22.22%, respectively. The morphology of helminths was described using a scanning electron microscope. *Eimeria bateri*, *Eimeria tsunodai*, and *Eimeria uzura* were among the protozoa identified. The histopathological changes in infected tissue with *Eimeria* species were recorded. In conclusion, this study presented the parasites' prevalence, morphology, and histopathological changes in infected tissue with *Eimeria* species in examined domesticated and migratory quails.

Keywords: Prevalence; *H. gallinarum; R. titragona*; SEM; Edko; Rashid; Quail; *Eimeria* species

1. Introduction

Quail (*Coturnix coturnix*) is one of the smallest poultry birds that provide more advantages such as its resistance to many poultry diseases, its greater capacity to benefit from food, high production proportions, low feed intake, low mortality rate, and their egg and meat are highly valuable. They are characterized by primary low costs, which do not require a wide area for farming, so it represented a modern poultry industry trend (Bashtar et al., 2010; Bahar et al., 2014). Migratory quail, known as common quail (*Coturnix coturnix japonica*) was one of the most migratory birds which migrate from Europe to Egypt during the Autumn season and act as biological and or mechanical vectors playing a role in the ecology and circulation of some zoonotic pathogen threatening human health and domestic animals (Benskin et al., 2009).

There are many parasitic organisms that infect quail's vital systems, digestive, circulatory, and respiratory systems. The common parasites that are infective in the digestive tract of quails are worms (Sheire 2008; Koroglu and Tasan1996), as well as protozoa such as *Eimeria* spp. (Otify1988), *Cryptosporidium* spp. (Zahid et al., 2018), *Isospora* spp., and *Trichomonas* spp. (Hassan et al., 2020), are infective in the digestive tract of quails, while parasites targeting blood include *Plasmodium*, *Haemoproteus* spp., *Aegyptianella* spp., and *Leucocytozoon* spp. (Garcia, 2001; Peterson, 2007).

The prevalence of parasitic infection among domesticated quails was reported in the United States (Duszynski and Gutierrez 1981), China (Wang et al., 2012), and Egypt (El-Madawy 2001). In migratory quail, infection was recorded in Matrouh governorate, Egypt (El Shabrawy et al., 2016), Brazil (Daugschies et al., 1999), and the USA (Ruff et al., 1985). This study is the first one in El-Behera governorate, Egypt.

The reason for this investigation was to study the infection rate of parasitic infection in domesticated and migratory quails and also the description of the morphology of some collected parasites by light and electron microscopes.

2. Material and methods

2.1. Collection of birds

A total number of 100 live quails (50 domesticated and 50 migratory) were collected from Edko and Rashid districts, El-Behera governorate, Egypt.

2.2. Examination of birds

After the collection, the sex of birds was recorded; birds were examined carefully using a hand lens to collect ectoparasites manually. Ectoparasites were collected in alcohol glycerin 70% and were identified according to identification keys (Soulsby 1982). Organs were examined using a dissecting microscope for internal parasites, which were identified according to Soulsby (1982).

2.3. Direct fecal smear

A pinhead drop of fecal material was put on a microscopic slide, was mixed well with a drop of saline 0.9% and covered with a coverslip, and was examined under a light microscope for detection of any oocysts or eggs in feces (Levine, 1985).

2.4. Simple flotation method

Intestinal contents were thoroughly emulsified with a flotation fluid in a tube. More flotation fluid was added to the upper menisci of the fluid till at the brim of the tube. A cover glass was applied to the surface of the fluid. This is then could stand for 15 minutes, then was removed and placed with the wet side down on a clean slide for microscopical examination.

2.5. Sporulation of Eimeria oocyst

In clean glass Petri dishes, the positive fecal samples for *Eimeria* species were mixed with 2.5% potassium dichromate solution at the depth of 3-5 mm. Petri dishes were covered and stand at room temperature. They were daily aerated and were examined to follow up the process of sporulation according to (Mohammed and Hussein 1992). *Eimeria* spp. was identified according to Levine (1985).

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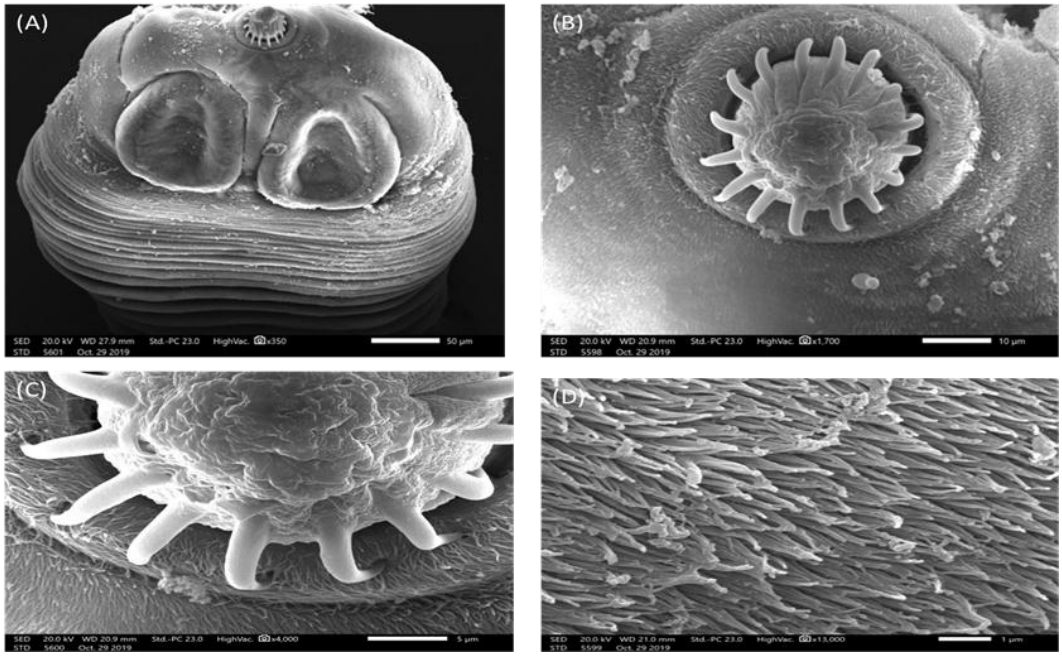

Figure 1: Scaning electron microscope of *Raillitina tetragona*. (A) The scolex of *R. tetragona* with 4 armed oval suckers. (B) Retractile rostellum with 1 row of hooks. (C) The shape of rostellar hooks. (D) Scale-like spines around the rostellar opening.

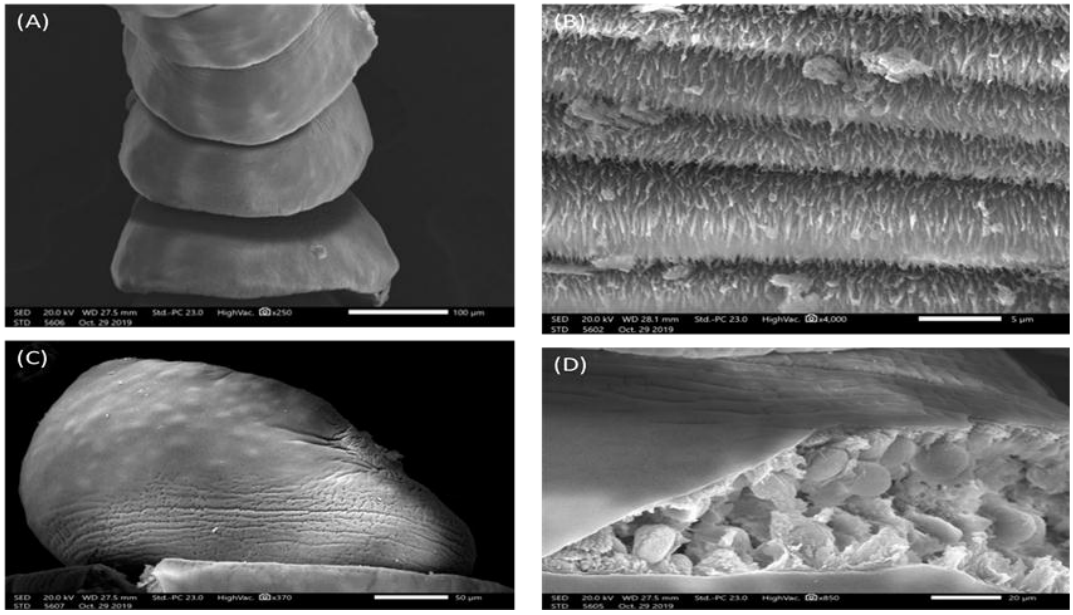

Figure 2: Scaning electron microscope of *Raillitina tetragona* strobila. (A) Mature segment of *R. tetragona*. (B) The surface of the strobila (C) shape of the last gravid segment. (D) Showing egg capsules each one contains several eggs.

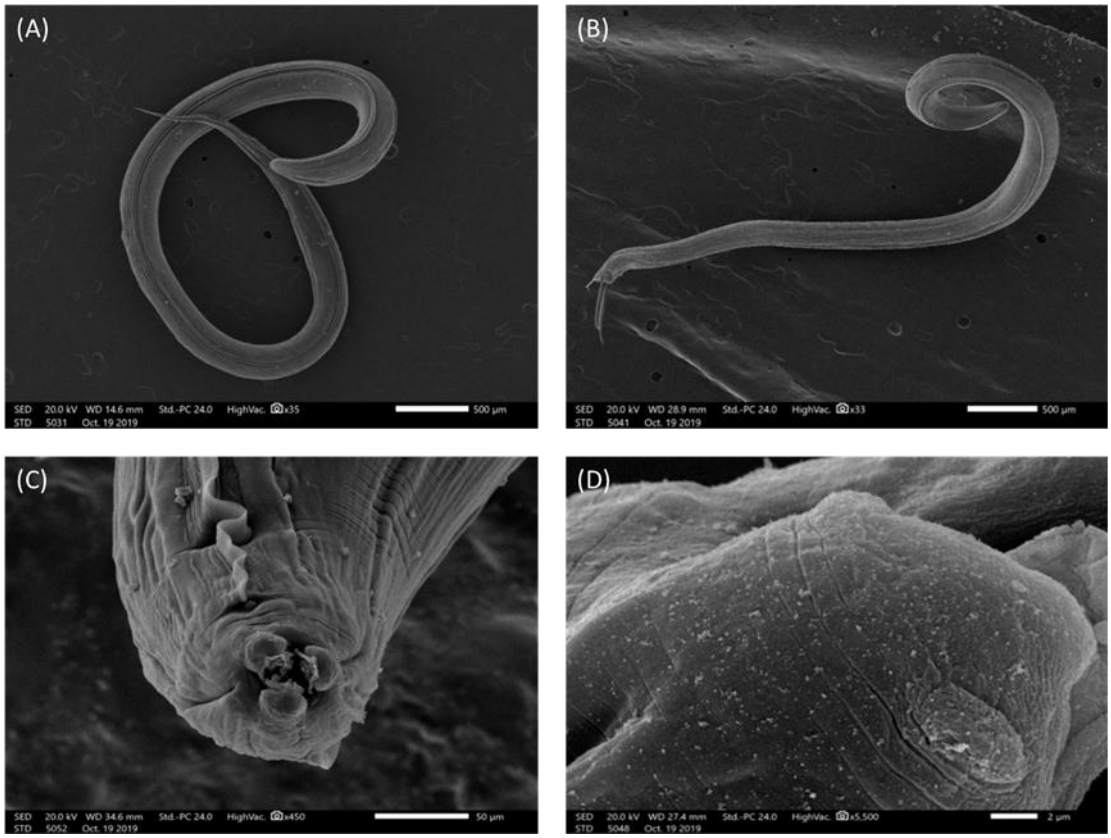

Figure 3: Electron microscope of *Heterakis gallinarum*. (A) Female with a tapered posterior end. (B) Male with two unequal spicules. (C) Anterior end with 3 lips and lateral cervical alae. (D) Sensory papillae in the anterior end.

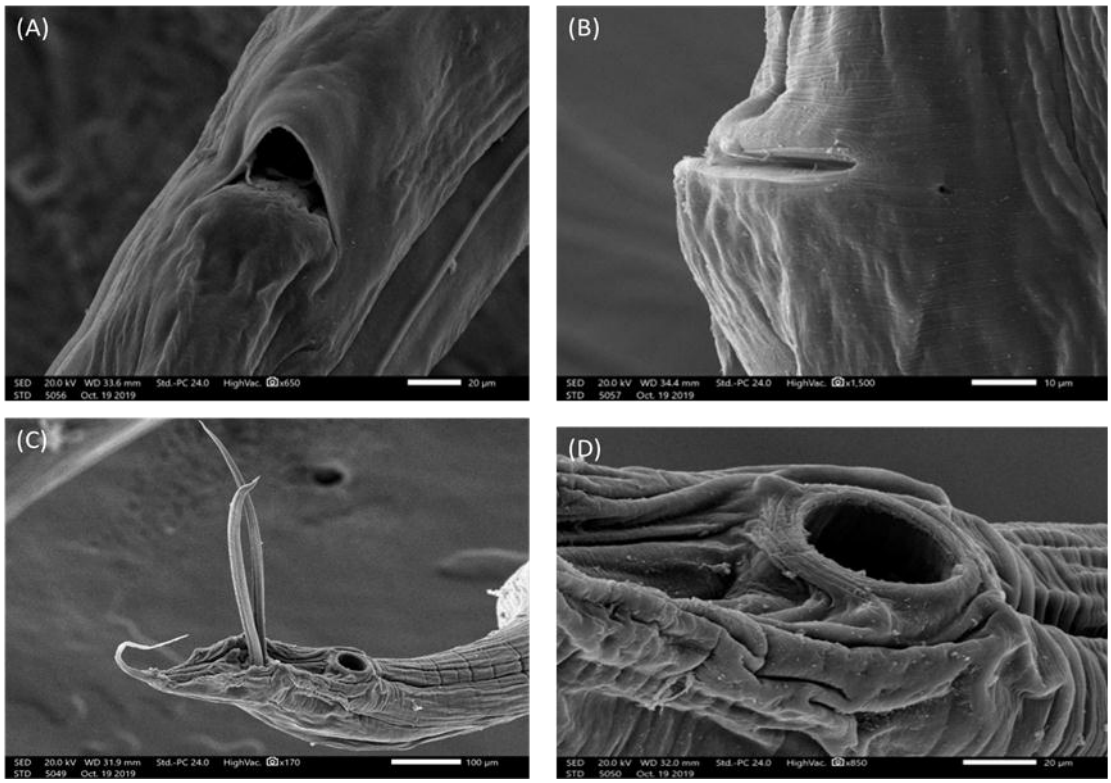

Figure 4: Electron microscope of *Heterakis gallinarum*. (A) Vulva opening of the female. (B) Anal opening of the female. (C) The 2 unequal spicules. (D) Circular pre-cloacal sucker.

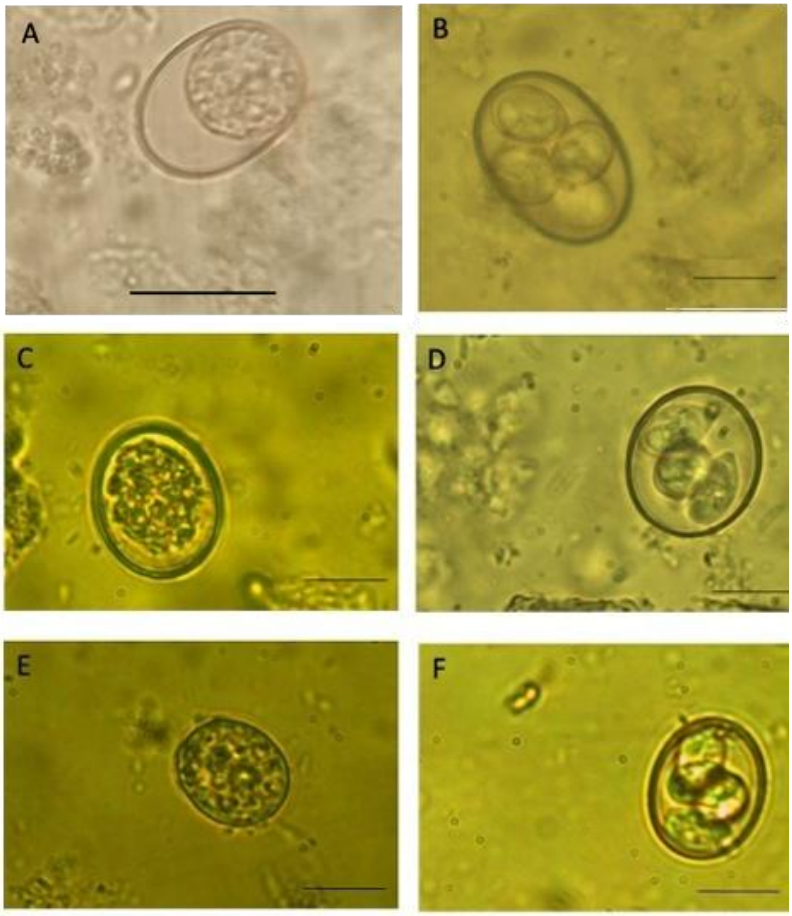

Figure 5: Photomicrograph of oocyst of *Eimeria* species reported from quail. *E. bateri* unsporulated (A) and sporulated (B) oocysts, *E. tsunodai* unsporulated (C) and sporulated (D) oocysts, and *E. uzura* unsporulated (E)and sporulated (F) oocysts.Scale bar= 10 µm

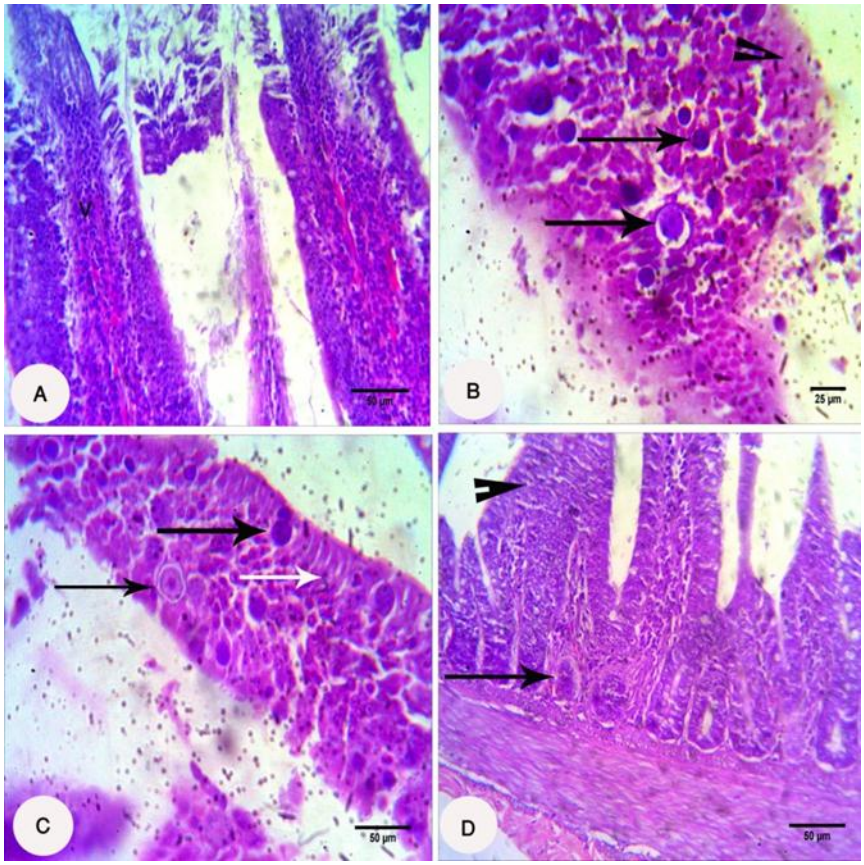

Figure 6: Photomicrograph of quail intestine stained with hematoxylin and Eosin. (A) Control non-infected birds with the normal histological structure of intestinal villi (V) and with no stages of *Eimeria*. (B) Intestinal villi with different stages of *Eimeria* (arrows), sloughing of epithelial cells and necrotic enteritis (arrow head). (C) Intestinal villi with different stages of *Eimeria* (black arrows) and degenerative changes in epithelial cells with congestion (white arrow). (D) The propria-submucosa Showing *Eimeria* stage (arrow) with slight changes and necrosis in epithelium (arrowhead).

2.6. Collection of helminths

Each bird was dissected and the alimentary tract was opened in a petri dish containing physiological saline, then examine under a dissecting microscope. The worms were collected with the aid of a plastic pipette.

2.7. Preparation of collected helminths for SEM

Freshly collected worms were kept in 2.5% buffered glutaraldehyde (for Fixation) in 0.1 M PBS pH 7.4 at 4°C for 2h. Then submitted to Electron Microscope Unit, Faculty of Science, Alexandria University.

2.8. Histopathological examination

Specimens were obtained from the gizzard, liver, lung, and intestine and were preserved in 10% formalin. The paraffin embedding block was sectioned at 5µm thickness and was stained with Hematoxylin and Eosin (H&E) according to the method described by Bancroft et al. (1996).

2.9. Statistical analysis

The effect of locality, sex, type of quail on infection rate was analyzed by Chi-square test using the SPSS program Version 16. The results were considered significant at $P < 0.05$.

Table 1: Prevalence of parasites in domesticated and migratory quails from Edko and Rashid

Quail	Number examined	Number infected	Infection %
Domesticated	50	35	70%
Migratory	50	20	40%
Total	100	55	55%
X^2	9.09		
P	0.003		
Sig.	**		

Significant at *P*<0.05, * sig, ** highly sig., and *** very high sign.

Table 2: Prevalence of parasitic infection according to the sex of examined quails

Bird sex	Number examined	Number infected	Infection %
Male	51	29	56.86%
Female	49	26	53.06%
Total	100	55	55%
X^2	0.146		
P	0.702		
Sig.	Non		

Significant at *P*<0.05, * sig, ** highly sig., and *** very high sign.

Table 3: Helminths and protozoa infection in examined quails

Quail	Number of infected	Number infected with helminths	Number infected with protozoa
Domesticated	35	2	33
Migratory	20	16	7
Total	55	18	40
X^2	26.43		
P	0.000		
Sig.	***		

Significant at *P*<0.05, * sig, ** highly sig., and *** very high sign.

Table 4: Infection rate according to locality

Locality	Number of examined	Number of infected	Infection %
Edko	44	35	79.54%
Rashid	56	20	35.71%
Total	100	55	55%
X^2	19.12		
P	0.000		
Sig.	***		

Significant at *P*<0.05, * sig, ** highly sig., and *** very high sign.

3. Results

3.1. Parasitic infection rate in examined quails

The total prevalence in examined quail was 55% (55/100). The prevalence was 70% (35/50) in domesticated quail, while prevalence in migratory quail was 40% (20/50) (Table 1). The prevalence was significantly affected by type of examined quails (X^2 = 0.09, P=0.003). According to sex, the prevalence in the examined males was 56.86% (29/51) but in females was 53.06% (26/49) (Table 2). The prevalence was not significantly affected by sex (X^2=0.146, P=0.702) (Table 2). Prevalence of helminths was 32.72% (18/55) but of protozoa was 72.72% (40/55) in the form of *Eimeria* spp. (Table3). The prevalence of protozoa or helminths was significantly affected by type of examined quail (X^2=26.43, P=0.000). The parasitic infection rate in examined quails from Edko was 79.54% (35/44) while from Rashid was 35.1% (20/56) (Table 4). The prevalence of parasites significantly affected by the location of the collected quails (X^2 = 19.12, P=0.000).

3.2. Scanning electron microscope of some collected helminths

3.2.1. Raillitina tetragona

R. tetragona has a small round rostellum and ovoid suckers. *R. tetragona's* rostellar hooks are placed in a single row. The genital pore opens unilaterally in a mature proglottid. Several eggs per egg capsule in the gravid proglottid. The entire body covering, referred to as the tegument, is thickly covered with microtriches, which give the surface a velvety texture. The microtriches showed variations in their distribution on different segments or proglottids along the body proper or strobila. Those in the neck region are thin, slender with pointed ends, and uniformly distributed, while those in the strobilar surface (from immature to gravid proglottids) are thick, elongated, conical, and wider at the base and slightly tapering towards the tip (Fig. 1, 2).

3.2.2. Heterakis gallinarum

The nematode *Heterakis gallinarum* is collected from the caeca of the domestic and migratory quails. Scanning electron microscopy is used to describe the worms' surface topography. The worm was small and white with a length of 13mm and has a curved tip. The mouth opening sensory papillae, vulva copulatory spicules, and copulatory papillae were all described (Fig. 3, 4). Three equal-sized lips encircled the mouth entrance (Fig. 3 C, D). On both sides, narrow lateral alae extended almost to the end of the body (Fig. 3C), and the oesophagus ended in a well-developed bulb containing a valvular apparatus. The female's back end was long, narrow, and pointed. The female's vulva was not prominent and was positioned slightly posteriorly (Fig. 4 A, B). The male has a straight and thinned caudal extremity that is laterally winged by two well-marked allae and sustained by 12 pairs of papillae that include two pedunculated, ribbon-like, shorted precloacal pairs and another six postcloacal pairs, two sessile and four pedunculated pairs (Fig. 4 C). The spicules are extremely uneven and dissimilar (Fig. 4 C). The precloacal sucker is rounded, with a diameter of about 90 µm and a chitinous inner ring (Fig. 4D).

3.3. Morphology of Eimeria species

Eimeria bateri, *Eimeria tsunodai*, and *Eimeria uzura* were among the protozoa identified.

3.3.1. E. bateri

E. bateri sporulated oocysts were ovoidal to ellipsoidal, measuring 20µm by 13 µm. The oocyst wall was bilayered and smooth (colorless outer layer and brownish inner one). There were one or two refractive polar granules present. The oocyst's micropyle and residual body were missing. The sporocysts were ovoid in shape and measured 10-13.2 µm by 6.3-8 µm with a more usual range of 12.5×7.5 µm. They possessed a spherical sub-stieda body and a nipple-like stieda body. The sporocyst residual body was apparent at the expanded extremities as tiny granules. dispersed among the sporozoites which were in pairs with refractive globules visible in the enlarged extremity (Fig. 5 A, B).

3.3.2. E. tsunodai

E. tsunodai sporulated oocysts were spherical to ellipsoidal in form and were 15 µm by 13 µm. Some oocysts had a smooth bi-layered wall (colourless outer layer and brownish inner layer) with one flattened end. There were one to four refractive polar granules. The oocyst's micropyle and residual body were missing. The sporocysts were oval in shape and measured 10-12 µm by 5-6 µm, with a more usual range of 10×5 µm. A little triangular or nipple-like stieda body and a rectangular, scarcely detectable sub-stieda body protruded from the finer end. The sporocysts' residual body was apparent as tiny granules interspersed among sporozoites in pairs with refractive globules seen in the expanded extremities (Fig. 5 C, D).

3.3.3. *E. uzura*

E. uzura sporulated oocysts were ovoidal to ellipsoidal in form, measuring 20µm by 15µm. The oocyst wall was bilayered and smooth (colorless outer layer and brownish inner one). There were one to four refractive polar granules (sometimes with a massive aspect not refractive). The oocyst's micropyle and residual body were missing. The sporocysts ranged in size from ovoid to elongate, measuring 11-13.9 µm by 5.2-7 µm, with a more typical range of 12.5×6.25 µm. They have a conspicuous spherical sub-stieda body and a piriform or knob-like or half-moon shape stieda body. The sporocysts' residual body was apparent as tiny granules interspersed among sporozoites in pairs with refractive globules seen in the expanded extremities (Fig. 5 E, F).

3.4. Histopathological examination of infected tissue

3.4.1. Macroscopical lesions

The protozoa-infected intestine appeared abnormal and filled with bloody faecal material, as well as thickening of the intestinal mucosa with heamorage.

3.4.2. Microscopical lesions

Hyperpalasia of epithelial cells with presence of different developmental stages of parasites (shizonts, macro, and microgametes). Descomation of intestinal villi and necrosis of intestinal epithelium were observed (Fig. 6).

4. Discussion

Poultry producers were looking for alternatives to chicken meat, which will be available in the future as pigeon and quail meat, to contribute to an increase in the livestock sector's gross domestic product (Urquhart, 1996). Several parasites are highly pathogenic to their host, causing great economic losses in quail breeding and limiting the development of this industry (Seok et al., 2003). The present study revealed a prevalence of parasitic infection in quails of 55%. A higher prevalence rate of 62.5% was reported in Sharkia, Egypt (Abedel-Aal and El-Sayed 2003). A lower prevalence of 21% was reported (Shakshouk et al., 1992). The prevalence of parasites was higher in domesticated quails (70%) than in migratory ones (40%). Opposite findings were recorded in Sharkia, Egypt of higher prevalence in migrant than domesticated ones of 90% and 76.6%, respectively (Abedel-Aal and El-Sayed 2003). This difference may be due to a different number of collected birds and the season of collection.

In the present study, the infection rate with parasites was higher in males (56.86%) than females (53.06%). This result is different from that recorded by Mohamed et al. (2011). Also in our study, the prevalence of protozoa was higher (72.72%) than the prevalence of helminths (32.72%) which domesticated quail showed a higher infection rate of *Eimeria* spp. (94.28%) than migratory ones (35%). On the contrary, migratory quails showed a higher prevalence of helminths (80%) than domesticated ones (5.71%). This occurs due to migratory quail is susceptible to taking intermediate hosts as snails during migration which contain the infective stage of helminths, so the infection rate of helminths was higher in migratory quails than in domesticated ones.

In the current study, the prevalence of helminths in domesticated quails was 5.71%. This result was higher than 1.5% in Egypt (Sheire 2008), 17.5% in Bangladesh (Islam et al., 2020), 44% in Turkey (Koroglu and Tasan 1996), and 51.6% in India (Rinesh et al., 2003). In this study, the prevalence of helminths in migratory quails was 80%. This result was higher than 75.26% in Egypt (ElShabrawy et al., 2016) and lower than 100% in Texas, USA (Dunham et al., 2017).

The prevalence of *Eimeria* spp. in domesticated quails was 94.28%. This result was higher than14.9% in the United States (Duszynski and Gutierrez 1981) and 31.5% (Abd-El-Maged 2005), 37.5% (Otify1988), and 89.8% (El-Madawy 2001) in Egypt. In the present study the prevalence of *Eimeria* in migratory quails was 35% this result was lower than 49.4% in Iraq (Mohammad 2012), 90% in Egypt (Otify 1988), and 100% in the USA (Ruff et al. 1985). The difference in the percentage of infection of *Eimeria* species in migratory quails may be due to the age of examined birds, which were collected randomly. Moreover, the variation in the prevalence of *Eimeria* infection in farm quails may be due to the different systems of rearing and management in quail farms.

The collected *Eimeria* species in this study were *E. tsunodai*, *E. uzura*, and *E. bateri*, which was agreed with Ruff (1984) in South Carolina and Mohamed (2012) in Egypt. Ahmed et al., 2017 discovered two species (*E. bateri* and *E. tsunodai*), while Otify (1988) discovered four species (*E. uzura*, *E. coturniria*, *E. bahli*, and *E. bateri*) in Egypt.

In this study, the morphology of collected helminths was described by Scanning electron microscope and the descriptions agreed with that described by Ilie et al. (2008) who mentioned that the scolex of *R. tetragona* has rostellum armed with hooks arranged in one or two rows and the suckers are oval-shaped armed with minute hooks. Also, the morphology of *H. gallinarum* in this study was similar to that recorded by the same author. The morphology of collected Eimeria spp. was studied using a light microscope, and the results were consistent with those previously published (Pellerdy 1974; Elmorsy et al., 2020; Hassan et al., 2020).

In conclusion, this study presented the prevalence of parasitic infection in domesticated and migratory quails in two cities of El-Behera governorate and recorded the morphology of collected helminths by Scanning electron microscope and also the pathological changes in infected tissue with *Eimeria* parasites.

Competing Interests

The authors have no conflict of interest.

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