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Histopathological and Molecular Study of *Raillietina echinobothrida* of Domestic and Wild Pigeons in Al-Muthanna Province

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ABSTRACT

This study was conducted to detect and distinguish *Raillietina echinobothrida* of domestic and wild infecting pigeons has been based on morphology, histopathological and molecular analysis. The present study was conducted on 115 pigeons (73 of S. Decaocto and 42 of C. livia) of different age groups and of both sexes. The results of the present study were revealed that only 86 out of 115 examined pigeons were infected by Raillietina spp at infection rate of 74.78%. Besides, the percentage of infection with Raillietina spp were recorded in S. decaocto is higher than C. livia in 44.34 % and 30.43% respectively. The most isolated parasites of examined birds were showed the cestoda R. echinobothrida with total number (168) adult worms from total number of examined pigeons infected (115). The histopathological examination of small intestine of pigeons infected with R. echinobothrida were showed different changes like, an infiltration of inflammatory cells in lamina properia and atrophy of villa of small intestine with vaculation of muscularis externain and loss of mucous membrane. The result of PCR were showed that specific primers (3S-F, BD2-R) were successfully for amplifying 944 bp with flanking region of ITS2 to R. echinobothrida. Also, the PCR result were revealed that 61(70.93%) out of 86 birds were positive to R. echinobothrida.

Keywords: *R.echinobothrida, domestic and wild pigeons, Pathological, Molecular, Iraq*

Introduction

Iraq has a wide range of geological diversity from the peaks of Kurdistan Mountains in the north to the deserts and semi-deserts in the west and the great marshes in the south, these contribute to the wide biological as well as faunal diversity[1].

In recent years, poultry farming has tremendously developed and become one of the most intensive forms of animal husbandry activities[2]. The Domestic pigeon (Columbia livia) is one of the commonest birds and tend to be closely contact with humans and birds as a source of protein, hobby, and recently as laboratory animals tend to be closely contact with humans and birds as a source of protein, hobby, and recently as laboratory animals[3,4].

Parasitic infections cause great economic losses to poultry in our country, the birds are under constant stress and are prone to parasitic infections[5]. Endoparasite of poultry are common in the tropical and sub-tropical where the standard of husbandry is poor and climatic conditions are favorable for their existence[6,7].

Raillietina echinobothrida is consider the most pathogenic and prevalent species infecting birds throughout the world[8,9]. This parasite inhabits in the jejunum and ileum of the definitive host, from where it obtains nutrition from the digested food of the host, whereas their larval stage (cyticercoid) resides in various invertebrate intermediate host, such as ants, beetles, small mini-wasps, or termites for completion of its life cycle. R. echinobothrida is a hermaphrodite worm having both the male and female reproductive organs in its body[10,11].

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R. echinobothrida is responsible for 'nodular tapeworm disease' in poultry and retarded growth of young birds, emaciation of adult and low egg production of hen, disrupt gastrointestinal tract digestion decrease in consumption of fodder and intestinal obstruction can occur in heavy infection[12]. In addition, this tapeworm does not cause gross pathological damages on well-nourished bird in the light infection, but do compete for food when they large numbers of parasites. If the heavy infection becomes, marked severe lesions on the intestinal walls due to the scolexs are penetrate the mucosal layer and causes characteristic hyperplastic enteritis associated with the formation of granuloma (nodules contain cheesy and calassified material) on the intestinal wall of the infected birds and diarrhea could arise, which ostensibly resulted in ill health[11,13].

The taxonomy of Cyclophyllidea traditionally depends upon the accurate descriptions of small differences in the size and shape of the scolex, rostellum (unarmed or armed with rows of hooks) suckers and gravid proglottids[9]. Nevertheless, with a rich resource of bourgeoning data the molecular approach is proving to be more rapid, sensible and reliable than that based on morphological data alone for phylogenetic analysis[14]. The objective of the present study was to study and recognize the histopathological changes result of the invasive R. echinobothrida in the small intestine, supplementing the morphological observation, also confirmed the diagnosis of R. echinobothrida in the birds by the molecular diagnostic PCR technique.

Materials and Method

Study Area and Sampling: A total number of 115 pigeon samples (73 of *Streptopelia Decaocto* and 42 of *Columba livia*) of different age groups and of both sexes were collected randomly selected from Samawah markets in Muthanna province, south of Iraq. The process of collecting pigeon samples were extended during the period from February to September 2017.

Samples Collection and Parasitological Examinations: Postmortem examinations were performed according to[15]. Occasionally, cestoda were very adherent to the intestines that have been cut off with the intestine wall then preserved them in hot water till passive separation[16]. The worms were preserved in 4% formalin according to[17].

Histopathological Examinations and DNA Extraction: A pieces of intestines from the infected pigeons were collected. A histological study was conducted according to[18]. The Mini Kit DNA QIAamp/QIAGEN/Germany were using to isolate genomic DNA from adult wormaccording to [19]. The PCR program were performed of 94C° at 2 min followed by 35 cycle of 94C°for 1min, 57C° for 1min and 72C° for 1min, this was followed by a final extension step at 72 °C for7 min[19].

Results

The results of the present study were revealed that only 86 out of 115 examined pigeons were infested by Raillietina spp during the study period from February to May 2017 at infestation rate of 74.78% (86/115).Also, the result were showed that both S. adecaocto and C. livia (males and females) were infested with different the species of Raillietina. The number of Cestoda which were isolated from each bird may be reach to 5-7 worms. Furthermore, the percentage of infection with Raillietina spp were recorded in S. decaocto is higher than C. livia in 44.34 % (51/115) and 30.43% (35/115) respectively.

The histopathological examination of small intestine of pigeons infected with *Raillietina echinobothrida* were characterized by varying degrees of degenerative changes to destruction and sloughing of epithelial layer of intestinal mucosa in heavy infections as showed in figure 3. Also, the results of this examination were showed different pathological changes like, desquamation, destruction and loss of mucous membrane of villi as showed in figure 4.

Fig. 1: *Raillietina echinobothrida* worm with alum carmine stain, Scolex

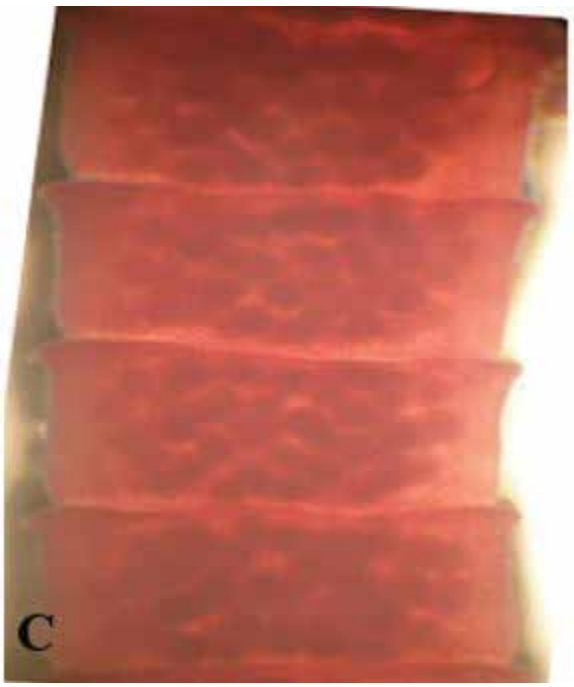

Fig. 2: *Raillietina echinobothrida* worm with alum carmine stain, Graved segment with egg capsules

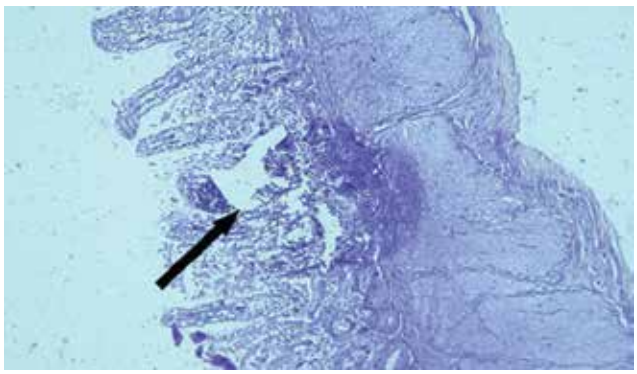

Fig. 3: Small intestine of pigeons infected with *Raillietina echinobothrida* showed destruction and sloughing of epithelial layer of intestinal mucosa in heavy infections

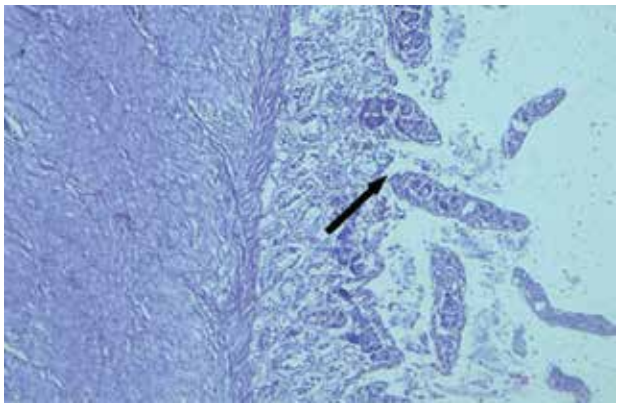

Fig. 4: Small intestine of pigeons infected with *Raillietina echinobothrida* showed destruction and desquamation of villi

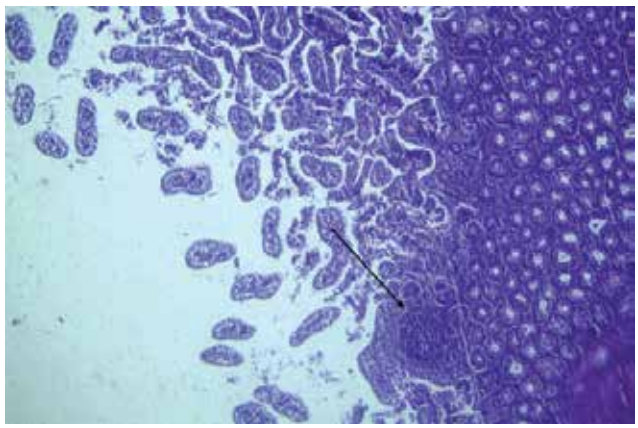

Fig. 5: small intestine of pigeons infected with *Raillietina echinobothrida* showed infiltration with heterophils throughout the mucosa, especially in the lamina propria

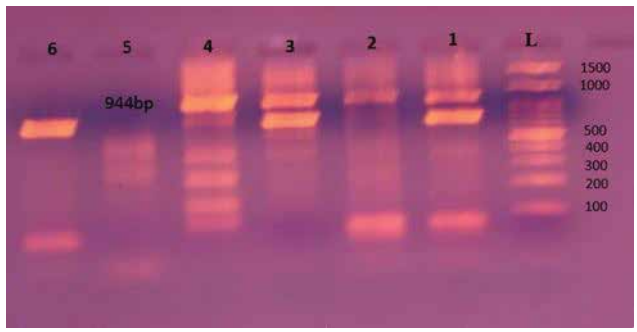

Fig. 6: Agarose gel electrophoresis of amplified DNA of *R. echinobothrida* by using primers (3S-F, BD2-R) region of region of 28S, Lane L DNA ladder, Lanes 1,2,3,4, positive to 944 bp.

Discussion

The result in the present study were revealed that only 86 out of 115 examined pigeons were infested by Raillietina spp during the study period from February to May 2017 at infestation rate of 74.78% (86/115). Also, the results in this study were recorded and confirm that Raillietina spp spread throughout the months of the year with high prevalence. This result agreed with most studies as [21,22, 23, 24]. But, this result was disagreed with [25, 26] who reported rate of infection 25.3%, and 17.4% respectively.

In addition, the spread of tapeworms and presence in all seasons of the year is the result of a natural of life cycle of the tapeworm that transmitted from birds to another through contamination feed and water with mature segment led to easily spread between birds[24,27,28]. Finally, there are many studies confirm that changes in the climate factors like temperature or humidity had no directly effect

in incidence infection and severity, therefore Raillietina spp is found in all months the year[24,29].

The results were agreement with many previous studies[31,32,33] and disagreement with[34].This agreement between the studies were recorded that the male's bird were more infected than females may be because consuming greater quantities of food as result to fly away from the nest and for long distance, While, the females remains near the nest near her eggs. Also, the females are concentrated in the food on the limestone material that need in building egg shells which reduce the handling of food[31,32,35].

The duodenum and jejunum cavities of Raillietina spp infested birds was congested and filled with greenish or yellowish feces with a very soft to liquid consistency and containing much mucous exudate. In cases with a heavy infection of parasites the mucosa was thickened and also revealed hemorrhagic spots. These finding are agreement with other studies[24,26].The explanation of results the gross examination in the present study is attributed to the adhesion site of adult worms in the small intestine by scolex and change his location in wall of intestine continuously and the worms migrated up and down the intestinal lumen away from the congestion to get nutrition when present in large numbers and also aggregate in the lower half of the intestine. Moreover, probably this lesion is results from anther secondary bacterial infection in the intestine due to destruction of intestinal epithelium by parasites, hemorrhage have been noticed in others may be due to irritation of heavy infection[28].

On the other side, the histopathological examination of the small intestine of pigeons infected with *Raillietina echinobothrida* were showed necrosis of villous epithelium, massive hemorrhages and infiltration of inflammatory cells in the lamina propria and sub mucosa. These results are agreement with previously studies[24,28]. Each parasite have a degree of adverse impact on host and may be a change in texture of tissue, or take change in overall impact. The parasites occurs a damage to their hosts like; injurer and metabolic outcomes affecting enzymes and hormones of the host or consume food the host[31,36].

Also In the present study the reason to chosen these sets of primers may be attributed to the constancy and stability of the second internal transcribed spacer (ITS2) region of ribosomal DNA gene makes them a prominent target for species variation. In addition, the gene sequences of these locations have been developed for molecular phylogenetic studies[38].

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Conflict of Interest: This research is a personal non-profit work and there is no conflict of interest.

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Ethical Clearance: Ethical clearance was obtained from the Faculty Scientific Committee (Department of Anatomy College of Veterinary Medicine/AL Muthanna University/Iraq,) to study the histopathological and molecular of *Raillietina echinobothrida* of domestic and wild pigeons in Al-Muthanna Province

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