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Azhar A Al-Moussawi
Iraq Natural History Research Center and Museum, University of Baghdad, Iraq

Wasan Addai Al-Marsomy
College of Science for Females, University of Baghdad, Iraq

Mustafa M Saeed
College of Science for Females, University of Baghdad, Iraq

Correspondence
Azhar A Al-Moussawi
Iraq Natural History Research Center and Museum, University of Baghdad, Iraq

Infection of local chicken *Gallus gallus domesticus* Linnaeus, 1758 (Galliformes, Phasianidae) with the cestode *Raillietina echinobothrida* (Megnin, 1881) (Cestoda: Cyclophyllidea) and intestinal microorganisms

Azhar A Al-Moussawi, Wasan Addai Al-Marsomy and Mustafa M Saeed

Abstract
Twenty five of local chicken *G. g. domesticus* Linnaeus, 1758 in Baghdad area were dissected and searched for cestodes and intestinal microorganisms. Intestines of five individuals (20%) were found infected with the cestode *Raillietina echinobothrida* (Megnin, 1881) and nine bacterial species (*Bacillus cereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Escherichia coli*, *Lactobacillus* sp., *Proteus vulgaris* and *Staphylococcus epidermidis*). Relationship between the cestode and bacteria was discussed. Morphology of bacterial colonies and biochemical tests of bacterial growth in intestines were provided.

Keywords: *Gallus gallus*, *Raillietina echinobothrida*, intestinal microorganisms

1. Introduction
Chicken is a rich source of protein, minerals and vitamins [1]. It is susceptible to some parasites that affect poultry causing significant losses [2]. Previous studies that dealt with helminthic parasites of *Gallus domesticus* have been carried out, from those which conducted in Iraq that revealed recording many protozoan species, *Eimeria* spp. by Shamsuddin and Jasim [3], Al-Khalidi *et al*. [4], Al-Alousi [5] and Al-jaumeili and Aljoburi [6]; *Toxoplasma gondii* by Al-Kennany *et al*. [7], Alkhaled *et al*. [8], Al-Sanjary and Hussein [9], Al-Mayali *et al*. [10] and Al-Nasrawi *et al*. [11]; *Haemoproteus* spp., *Leucocytozoon* spp. and *Plasmodium* spp. by Abd Allah [12] and Hasson [13]; the ectoparasites found were *Argas persicus*, *Campanulotes bidentatus comopare*, *Columbicola columbae*, *Cotugnia* sp., *Cuclotogaster heterographus*, *Dermanyssus gallinae*, *Goniocotes gallinae*, *Gonoides gigas*, *Menacanthus stramineus*, *Menopon gallinae* and *Pseudolynchia canariensis* [6; 14; 15]; the nematodes, *Amidostumum anseris*, *Ascaridia galli*, *Capillaria obsignata*, *Capillaria* sp., *Cheliospirura hamulosa*, *Dispharynx nasuta*, *Epomidiostumum* sp., *Heterakis gallinarum*, *Subulura brumpti* and *S. suctoria*, *Tetrameres Americana* [4; 15; 16; 17 ;18] and the cestodes, *Amoebotaenia sphenoides*, *Choanotaenia infundibulum*, *Davainea proglottina*, *Hymenolepis carioca*, *H. cantainana*, *Raillietina cesticellus*, *R. echinobothrida* and *R. tetragona* [4, 15, 17, 18]. *Raillietina echinobothrida* is a widely-dispersed avian cestode which occurs in the small intestine of the fowl in most parts of the world [19]. It is the most pathogenic and prevalent species infecting *Gallus* [20]. It was isolated from *G. domesticus* by Al-Khalidi *et al*. [4] in Iraq. The gut of poultry is populated with microorganisms [21] which are essential components of the intestinal ecosystem [22], Bacteria are predominant in this ecosystem [23]. Most birds are hosts to some species of cestodes, *R. echinobothrida* is one of the most pathogenic tapeworms, because of its association with nodular disease of chickens in heavily infested [24].
The present study is an attempt to study the intestinal bacteria of the local chicken *Gallus gallus domesticus* and the interaction between it and the with the cestode *Raillietina echinobothrida* infects the chicken.

2. Materials and methods
Twenty five individuals of local chicken *G. g. domesticus* were purchased from the local market in Baghdad city during the period from 1-1-2017 to 1-9-2017,

they brought to the laboratory, dissected and the intestines were searched for cestodes and intestinal microorganisms. The isolated cestodes were killed, cleaned, stained with acetocarmine, dehydrated by passing through a series of alcohol concentrations, cleared by xylene and mounted in canada balsam. Recovered cestode was identified as in Sawada [25]. Contents of the guts were treated by adding 1gm of it into 9 ml of sterile distilled water. The mixture was thoroughly shaken well to precipitate the microorganisms. A serial dilution up to 10^{-5} was prepared. An aliquot (0.1ml) was inoculated to nutrient agar plates from each of 10^{-4} and 10^{-5} dilution. Plates were allowed to be dry then incubated at 37°C for 18-24 hr., bacterial colonies were transferred to separated agar plates and sub-cultured thrice to obtain pure cultures. Cultures were maintained on agar slants and examined for colony morphology and characteristics following Jin [26].

3. Results and discussion

Five of 25 specimens (20 %) of *G. g. domesticus* found infected with 9 specimens of the cestode *Raillietina echinobothrida* which found attached to the small intestines of the chicken during the present study. *R. echinobothrida* was isolated in Iraq from three species of the genus *Gallus*; *G. bankiva*, *G. ferrigineus* and *G. g. domesticus* [4, 25]. Other reports were in the rock dove *Columba livia* [27; 28], in the collared dove *Streptopelia decaocto* [28] and in the house sparrow *Passer domesticus biblicus* [29; 30].

Results obtained from the present study have demonstrated that the most common microorganisms of chicken intestines were (*Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, and *Lactobacillus* sp.) as shown in table (1 & 2). They seem as normal in the chicken intestine as it cleared by Saliu *et al*. [31] who indicated that *Bacillus cereus*, *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Lactobacillus* sp., *Proteus vulgaris* and *Staphylococcus epidermidis* occurred in the digestive tracts of the free ranger chickens. Pan and Yu [21] reported *Escherichia coli* and *Lactobacillus* as predominant bacteria in the chickens.

Table 1 and 2 have shown that there are varieties of bacterial growth in chicken intestine. *Enterobacter* sp., *Enterococcus* sp. and *Lactobacillus* sp. were considered as normal microorganisms found in the intestine. Although *E. coli* is also found as normal microflora of the intestine of chicken, but its presence is restricted by the growth of *Lactobacillus* sp. which suppresses *E. coli* by producing lactic acid, which lowers the pH to a level which is suitable for its growing, whilst the most other bacteria, such as *E. coli* cannot [32]. Meanwhile the parasite-infected intestines have shown lower ranges of *Lactobacillus* sp. growth than uninfected ones, this gives a chance for *E. coli* to grow as the pH of the intestine is tended to be 6.3 [33]. *Staphylococcus epidermidis* was isolated from some of infected intestines, it is considered as an opportunistic bacterium, which can be pathogenic under appropriate conditions [34]. *Bacillus* spp. have been associated with embryo mortality in chickens; *Lactobacilli* and specific strains of *Bacillus* spp. such as *Bacillus subtilis* can inhibit colonization with *E. coli* in the digestive tract [24].

In the present study, we noticed that the mucosa of the infected small intestines was found with thickened wall and there are many small scattered nodules (Fig. 1), we think this may due to the infection with *R. echinobothrida*, this agrees with findings of Mönnig [19] who mentioned that the penetration of *R. echinobothrida* into the mucosal and submucosal layers of the duodenum cause the formation of nodules, and the young worms of *R. echinobothrida* cause the destruction of these two layers, that may allow bacteria to enter the tissues, as we noticed in this study, the presence of lesions and necrosis may be caused by bacterial infections in the internal surface of infected intestines with the cestode (Fig. 2), this explains the infection of *G. g. domesticus* in the present study with bacteria.

Previous studies have discussed the relationship between bacteria and the parasitic worms that inhabit the same environment, Zaiss and Harris [35] indicated that infection with helminthes can affect the intestinal microorganisms by altering the intestinal physiology and production of antimicrobial peptides. While Johnson and Reid [36] and Biswal *et al*. [37] and Biswal *et al*. [23] suggested an intimate relationship between them by the role of the bacteria contributing the development of the parasite by fermenting the food products producing acid that use by the cestode as a source of nutrition and energy.

Fig 1: Shows scattered nodules on the surface of the small intestine of the chicken.

Fig 2: The internal surface of infected intestine of the chicken shows lesions.

Table 1: Bacteria biochemical test

Isolates	Lactose Fermenter	Oxidase Test	Indole Test	MR. VP. Test		Simmon's Citrate Test	Urease Test	Motility Test	Nitrate Reductase Test	Triple Sugar Iron Test				Bacteria
				MR.	VP.					Slant	Butt	H_2S	Gas	
1	+	-	+	+	-	-	-	+	+	A	A	-	+	*E. coli*
2	+	-	-	-	+	+	-	+	+	A	A	-	+	*Enterobacter aerogenes*
3	+	+	-	+	-	+	-	-	-	A	A	-	-	*Bacillus subtilis*
4	+	-	-	-	-	-	-	-	-	A	A	+	-	*Lactobacillus sp.*
5	-	-	+	+	-	-	+	+	+	A	A	+	+	*Proteus vulgaris*
6	-	-	-	+	-	+	-	-	-	A	A	-	-	*Bacillus megaterium*
7	+	-	-	+	-	-	-	-	-	A	A	-	-	*Enterococcus faecalis*
8	+	-	-	+	-	-	+	-	+	A	A	-	+	*Staphylococcus epidermidis*
9	-	+	-	-	+	+	-	+	-	K	A	-	+	*Bacillus cereus*

A= acid, K= alkaline

Table 2: Bacterial colony morphology

Isolates	Gram Reaction	Shape	Margin	Elevation	Size	Textures	Pigmentation
1	Negative	Circular	Entire	Raised	Small	Muciod	Colorless
2	Negative	Circular	Entire	Convex	Small	Shiny	Yellow
3	Positive	Irregular	Wavy	Umbonate	Large	Rough	White
4	Positive	Circular	Entire	Convex	Puncture	Smooth	White
5	Negative	Circular	Entire	Convex	Small	Muciod	Tan
6	Positive	Irregular	Wavy	Convex	Large	Rough	White
7	Positive	Circular	Entire	Convex	Large	Shiny	White
8	Positive	Circular	Entire	Convex	Pinhead	Muciod	White
9	Positive	Irregular	Wavy	Umbonate	Large	Rough	White

4. Conclusion

In the future an investigating works should be held on intestinal bacterial communities in hosts which are endemic for helminthic infections in Iraq.

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