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PREVALENCE OF PATHOGENIC ENTERIC BACTERIA AND PARASITES IN WILD BIRDS NEAR POULTRY FARMS IN EGYPT

By

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ABSTRACT

In order to maintain the high production rates of poultry industry, it is necessary to prevent and control certain disease agents, which may occurred due to wild birds found near broiler houses. This study investigated the prevalence of bacteria from different organs (liver, kidney, heart, lung and content of intestine) of 76 apparently healthy resident and migratory free living birds.The gram-negative bacteria was identified as *E.coli*, *Proteus mirabilis*, *Klebsiella pneumonia, Klebsiella oxytoca , Enterobacter spp.,* and *Citrobacter freundii*, at the rate of 60.5%, 21%, 36.8%, 25%, 26.3%, and 35.5 respectively. On the other hand, a gram positive bacterium (*Staphylococcus aeures*) was isolated at the rate of 47.4%. Laughing dove and rock pigeon had the highest isolation percentage of these bacteria. An examination of gastrointestinal tract revealed the presence of nematode (*Ascardia galli),* three species of cestoda: *Hymenolepis demneota, Raillietina spp., Cotugnias spp.,* in resident wild birds. Ectoparasites recorded in this study were *Menopon gallinae*, *Argas persicus*.

Key words:

Wild birds, Migratory waterfowl, poultry, Enteropathogens, parasite, Egypt.

INTRODUCTION

Wild birds are these birds that live freely without the care of mankind. They may be terrestrial birds or marine birds. Birds are of different species, they may be resident birds that live in one area all their live or migratory birds that migrate from their home land to other places for given reasons. Since Egypt is one of the main routes for migratory birds on their migration from Europe and Asia during the spring and autumn to the warm areas in Africa. Wild birds can disperse microorganisms across international borders and many pathogens which are harmful to poultry or other vertebrates have been associated with such birds. For the efficient

dispersal of pathogens, wild birds serve as biological carriers of microbes or mechanical dispersers of vector that harbor pathogens **(Hubalek, 1995).** Today, many different species of wild birds are worldwide in distribution. Little is known about the incidence of these enteropathogens in wild birds near poultry facilities or their transmission to poultry **(Craven *et al.,* 2000 and Kaleta, 2001).** *Escherichia coli* are the most potential pathogen affecting birds. *E.coli* has been isolated from a range of bird species, including apparently healthy passerines and waterfowl **(Brittingham *et al.,* 1988; Foster *et al.,* 1998)**. Other bacterial species as *Pseudomonas spp.*, *Klebsiella spp., Staphylococcus aureus* and *Enterobacter spp.* were isolated from different species of wild birds **(Soad and Wafaa, 2003; El-Sheshtawy and Moursi 2005).** Wild resident birds and migratory anseriformes also harbor many gastrointestinal parasites **(Price, 1980; Foucher and Munster, 2009)**, which could pose threats to domestic poultry and human health **(Hatch, 1996).** The objectives of this study were to investigate prevalence of some enteric bacteria and parasites in wild birds in Egypt and the possible role of wild birds in transmitting these pathogens to poultry.

MATERIAL AND METHODS

Birds: 76 apparently healthy resident and migratory free living birds (10 Hooded Crow, 12 Cattle Egret, 13 Rock Pigeon 11 Laughing Dove 10 shovler duck, 10 Cotte duck and 10 Green Winged Teal duck) were collected by hunting from the following localities in Egypt, Giza, Cairo, Mansura and Port Said.

Sampling:

The specimens (heart blood, lung, liver, kidney and intestine) were taken under aseptic conditions for bacterial isolation.

Bacterial isolation and identification:

Loopful from internal organs were directly cultured into Nutrient broth, then incubated at 37°C for 18-24 hours, followed by subculturing on Eosin-methylene blue (EMB) agar, MacConkey agar, blood agar, and nutrient agar. Agar plates were incubated at 37◦C for 18-24 hours under aerobic conditions. The suspected colonies with characteristic growth of the bacteria were subcultured two times for pure culture and characterized by using Gram stain and standard biochemical testes according to **(Cruickshank *et al.,* 1975).** These testes included lactose, Indol, methyl red, citrate utilization, urease, and motility. The RAPID ONE ™ strips were used for confirming the bacteria species. The reactions were read according to the Reading Table provided and the identification obtained by using the Analytical Profile

Index. *Staphylococcus aureus* was cultured on Mannitol salt agar then further identification by coagulase test and catalase test.

Parasitological examination:

Gastrointestinal tract contents were collected from each bird and examined microscopically for detection of any enteric parasites. Fecal samples were collected in clean sterile container; a portion of the sample was fixed in 10 % formalin followed by direct concentration and centrifugation in saturated salt solution. Gross helminthes passed in faces were identified after staining with borax carmine in case of cestodes. Nematodes were studied after clearing them in lacto phenol according to standard procedures. For cryptosporidium examination, ten *ul* of the concentrated sample was smeared on albumin coated slides, dried and stained using the modified- Ziehl-Neelsen stains. For ectoparasites, a commercially prepared insecticide was applied for 2 sec in areas where parasite could be seen. The bird feathers were then gentle unruffled so that the parasite could drop off onto a white sheet of paper, then fixed and stained for identification.

RESULTS

Table (1): Prevalence of enteric bacteria isolated from the wild birds captured from areas aroundpoultry farms from February 2015 through May 2016, with the significance difference between resident and migratory birds.

Bacteria	Birds				Total = 76 bird	
	Resident Birds no=46		Migratory birds no=30			
	Number positive	Prevalence (%)	Number positive	Prevalence (%)	Number positive	Prevalence (%)
Escherichia coli	**30**	**65.2**	**16**	**53.3**	**46**	**60.5%**
Proteus mirabilis	**10**	**21.7**	**6**	**20**	**16**	**21**
Proteus vulgaris	**4**	**8.7**	**5**	**16.7**	**9**	**11.8**
Citrobacterfreundii	**19**	**41.3**	**8**	**26.6**	**27**	**35.5**
Citrobacterdiversus	**7**	**15.2**	**0**	**0**	**7**	**9.2**
Klebsiella pneumoniae	**19**	**41.3**	**9**	**30**	**28**	**36.8**
Klebsiellaoxytoca	**13**	**28.3**	**6**	**20**	**19**	**25**
Enterobacter spp.	**14**	**30.4**	**6**	**20**	**20**	**26.3**
Shigella	**16**	**34.8**	**9**	**30**	**46**	**32.9**
Staphylococcus aeures	**16**	**34.8**	**20**	**66.6**	**36**	**47.4**

Table 2: Prevalence of selected enteric bacteria isolated from the 7 wild bird species (resident and migratory) captured from areas around poultry farms from February 2015 through May 2016, and the significance of the difference in prevalence between bird species.

Bacteria	Hooded Crow No=10		Cattle Egret No= 12		Rock Pigeon No: 13		Laughing Dove No=11		shovler duck. No= 10		Cotte duck No=10		Green Winged Teal duck No= 10		P-value
	Number positive	Prevalence (%)	Number positive	Prevalence (%)	Number positive	Prevalence (%)	Number positive	Prevalence (%)	Number positive	Prevalence (%)	Number positive	Prev (%)	Number positive	Prev (%)	
escherichia coli	5	50	4	33.3	10	76.9	11	100	3	30	5	50%	8	80	0.005 *
roteus mirabilis	2	20	1	8.3	3	23.1	4	36.4	3	30	2	20%	1	10	0.679
roteus vulgaris	1	10	0	0	2	10	1	9.1	2	20%	1	10%	2	20	0.785
robacterfreundii	4	40	3	25	5	40	7	63.6	4	40%	2	20%	2	20	0.349
robacterdiversus	2	20	2	16.7	1	20	2	18.2	0	0%	0	0%	0	0	0.387
bsiella pneumoniae	4	40	1	8.3	3	23.1	11	100	4	40%	3	30%	2	20	0.000 *
lebsiellaoxytoca	1	10	0	0	8	40	4	36.4	1	10%	2	20%	3	30	0.011 *
terobacter spp.	3	30	3	25	5	30	3	27.3	2	20%	2	20%	2	20	0.946
phylococcus acures	3	30	2	16.7	5	30	6	54.5	8	80-	7	70	5	50	0.047*
Shigella	3	30	5	41.6	2	30	6	54.5	3	30	3	30	3	30	0.581
Total	31		22		51		60		45		31	-	37		

*Prevalence significantly different between different wild bird species for the particular bacterium, P value <0.05.

(*Escherichia coli, Klebsiella pneumonia, Klebsiellaoxytoca, Staphylococcus aeures*).

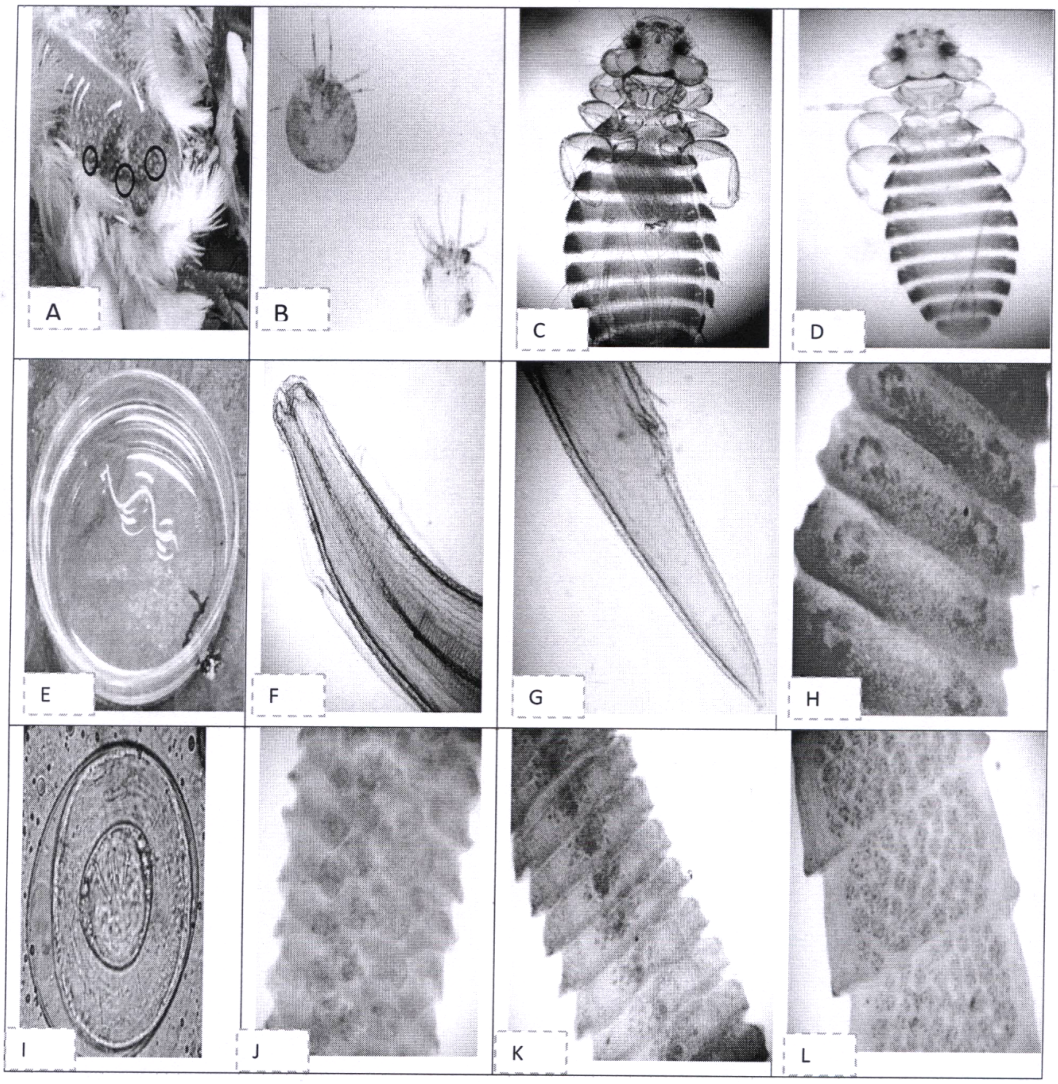

Fig (1): parasitological finding; A) Soft tick attached pigeon skin,B) Argas spp. Larva 10x C) male Menopon ;Biting louse D) female Menopon; Biting louse E) Ascaridia galli adult F) Ascaridia galli anterior.end G) Ascaridia galli, female posterior end H) *Cotugnia ,* mature segment I) *Hymenolepis carioca* egg 40x J) *Hymenolepis* mature segment K) *Raillietina* spp., mature segment *L)Raillietina* spp., Gravid segment.

Table (3): Prevalence of parasites identified from free-living birds captured from areas around poultry farms between February 2015 and May 2016, and the significance of the difference in prevalence between bird species.

Species	External parasite	Helminths	Species	Number examined	Positive number	Positive %
Hooded Crow	**-**	**Cestodes**	***Hymenolepis carioca***	**10**	**1**	**10**
Cattle Egret	**Lice**	**Nematodes**	***Ascaridiagalli***	**12**	**1**	**8.3**
Rock Pigeon	**Soft tick**	**Cestodes** **Nematodes**	**Cotognea** ***Raillietina*sp.** ***Ascaridia*sp,**	**13** **13**	**4** **2**	**30.7** **15.4**
Laughing Dove	**-**	**Cestode** **Nematodes**	***Raillietina*sp.** ***Ascaridia*sp,**	**11** **11**	**5** **3**	**45.4** **27.2**
Migratory waterfowl *	**-**	**-**	**-**	**30**	**0**	**0**

***The prevalence of parasites in migratory waterfowl reveled that, there is no external or internal parasites were found in the 30 examined birds include 3 duck species (shovler duck, Cotte duck, and Green Winged Teal duck).**

DISCUSSION

Fee living birds including migratory species could move for many reasons as food supplies, breeding habitat. Birds travel across national and international borders, many avian species can sometimes move as far as 50-100 km. They carry and also transmit viable pathogens to distant sites during erratic movement **(Kurt *et a*l., 2002).** The infected bird often shed the agent, sometimes for a prolonged period while in some bird species the shedding of a pathogen is more intense and clinical signs more obvious in younger birds than in adult as in Salmonellosis **(Chuma *et al.,* 2000)**. Isolation of enteropathogenic bacteria from free living birds found near poultry farms agree with many authors who have recorded isolation of bacteria with variation in prevalence rates attributed to the species of wild bird examined, type of sample, localities and bird feeding habits **(Soad and Wafaa, 2003**; **El-Sheshtawy and Moursi 2005 and Hedawy and El- Shorbagy, 2006).** Similar results were recorded by

(Brittingham *et al.*, 1988; Glunder, 1989; Aguirre *et al.*, 1992; Pennycott, 1998; Teresa *et al.*, 1999; Prange *et al.*, 2000 and Hideki *et al.*, 2002) who isolated *E.coli, Pseudomonas, Enterobacter* and *Staphylococcus aureus* from wild birds. The most isolated bacteria were *E.coli* (60.5%). These results were similar to **(El-Sheshtawy and Moursi 2005)**. The highest prevalence among free living birds was 100% for Laughing Dove followed by 76.9 % for Rock Pigeon (Table 2), these higher prevalence of *E.Coli* in these species may result from the urban habits of those birds which were usually found feeding on grains in feed storage facilities as previously supported by **(Vilela *et al.*, 2012)** on the other hand **(Adesiyun, 1999)** could not isolate *E.coli* from wild birds. The isolation of *E.coli, Proteus mirabilis, Citrobacter freundii*, *Klebsiella pneumonia, Klebsiella oxytoca, Enterobacter spp* were higher in resident birds than migratory waterfowl (Table 1) these agree with **(Sato and Asagi 1979)**; who reported low occurrence of Enterobacteriaceae in wild ducks. In contrast, we had a high prevalence of *E. coli* in our study and this may be a reflection of the contamination level of the habitat **(Fallacara *et al.*, 2001)**. The possible role of migratory wild duck for transmitting of infectious agent to poultry farms was low to some extent and that agree with **(Schelling *et al.*, 1999)** who reported that, wild waterfowl were only observed in close contact to poultry in 3% of visited poultry holdings. The duck holders observed wild ducks nearby domestic flocks more often than chicken holders. There were significant differences in prevalence of *E.coli, Klebsiella pneumonia,* and *Staphylococcus aeures.* Among studied wild birds ($P<0.05$). The variation in *isolated bacteria prevalence* rates may be attributed to the species of wild bird examined, localities and bird feeding habits as shown in (Table 2). Parasitological examination of intestinal content revealed presence of one type of cestode only (*Hymenolipis spp*.) In crow (Table 3) Similar results were reported by **(Naderman and Pence, 2006)** who detected 2 *Acanth-ocephalan spp* and *hymenolipis spp.* in the Crow and with **(Maha *et al.*, 2009*)*** who found two types of cestodes (*Raillietina spp*. and *Hymenolipis spp*.) while Parasitological examination of cattle egret revealed presence of external parasite female and male *Menopon*, Biting louse Fig. (1) and one type of nematode only (*Ascaris galli)* Similar results were reported by **(Kamdi *et al.*, 2015)** who examined about one and half-year-old Indian cattle egrets and observed that intestine had the small thread like round worms, confirmed to be *Ascardia galli* depending on their morphological characteristics. Of the 24 Rock Pigeons and Laughing Doves examined, the birds were found to be infested by helminthes parasites (Table 3) nematode as *Ascaridia galli*, cestodes; *Raillietina* spp. and

Cotugnia. This result is similar to some extent to **(Natala *et al.* 2009)** who reported *Raillietina tetragona*(4.9%),*R.cesticillus* (3.0%),*R.echinobothrida*(7.6%), *Ascaridia columbae* (1.2%), *A. galli* (1.2%) and *Cappillaria anatis* (0.8%) when examined pigeons for helminths. Higher incidence was recorded by **(Begum and Sehrin 2012)** who examined 60 pigeons (25 males and 35 females) they observe helminth parasite infection and found six species of cestoda: *Hymenolepis columbae* (63.33%), *Raillietina echinobothrida* (100%), *R. bonini* (43.33%), *R. cesticillus* (100%), *Cotugnia celebesensis* (68.33%), *C. cuneata* (100%); and one species of Nematoda: *Ascaridia columbae* (28.33%).

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