Prevalence of some zoonotic bacteria in wild birds in Kirkuk city

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Abstract

The object of this study was to isolate some of zoonotic bacteria from different organs of native wild birds included 21 individuals of House Sparrow, 15 individuals of White-Cheeked Bulbul, 20 individuals of Collared Dove and 20 individuals of Rock Dove. Samples of liver, kidney, blood and content of middle intestine of individual birds in Kirkuk city. Results revealed that many of zoonotic bacteria included *Listeria monocytogenes*, *Salmonella* spp., *Shigella* Spp., *Brucella abortus* and *Campylobacter* Spp. were isolated from all studied birds, House Sparrow had the highest isolation percentage of these bacteria and the content of middle intestine had the highest isolation percentage among other studied organs, *Salmonella* spp. was the highest isolation percentage among zoonotic bacterial isolates, that indicates an important and hidden role of native wild birds in causing and spreading of zoonotic diseases.

دراسة انتشار بعض الجراثيم المرضية ذات الطبيعة الانتقالية في الطيور البرية في مدينة كركوك

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الخلاصة

استهدف البحث الحالي عزل بعض الجراثيم المرضية ذات الطبيعة الانتقالية من أعضاء بعض الطيور البرية المحلية والتي شملت 21 من العصفور الدوري (House Sparrow) و15 من البلبل (White-Cheeked Bulbul) و20 من الحمامة الفاختة (Collared Dove) و20 من الحمامة المنزلية (Rock Dove). أخذت عينات من الكبد والكلى والدم ومن محتويات منتصف أمعاء أعداد من هذه الطيور في مدينة كركوك. بينت النتائج عزل عدد من الجراثيم المرضية المشتركة وشملت *Listeria monocytogenes* و *Salmonella* spp. و *Shigella* Spp. و *Brucella abortus* و *Campylobacter* Spp. من كافة أنواع الطيور البرية المدروسة، وإن أكثر نسبة عزل للجراثيم المرضية كانت من العصفور الدوري وكانت عينات محتويات منتصف الأمعاء الأكثر احتواء للجراثيم مقارنة ببقية الأعضاء المدروسة وان جرثومة *Salmonella* هي الأكثر نسبة عزل من بين الجراثيم المشتركة المعزولة، مما يدل على وجود دور كبير خفي للطيور البرية في نشر الإصابة بالأمراض المشتركة.

Introduction

Like other urban wastes and sewage, birds and animals feces may contain different kinds of pathogens that are infectious for different species of animals and plants as well as for humans. The origin and nature of organic wastes such as different types of sludge always causes a hygienic risk in storage, collection, processing, handling and utilization (1).

The main types of risks related to human and animal pathogens should be considered under public health aspects in processing and recycling sludge (2). These risks are occupational health considerations in collecting and processing of organic wastes and feces, and hygienic risks due to sludge and related products. The basic hygienic risk is the occurrence of pathogens in sewage sludge. This is the starting point for epidemiological reflections and necessary precautions (3).

From the variety of bacterial pathogens *Salmonella* spp. are the most relevant in animal feces since they can infect or contaminate nearly all living vectors from insects to mammals (4). The spectrum of pathogens found and in which concentrations depends on the origin of feces. Feces of animal origin will generally contain mostly animal pathogens or zoonotic agents.

Birds feces contained many genera of zoonotic bacteria, from a sample of 387 cloacal swabs from 364 passerines and woodpeckers. The prevalence of bacteria were as follows: *Escherichia coli* (1%), *Pseudomonas* spp. (22%), *Staphylococcus* spp. (15%), *Streptococcus* spp. (18%), and Y*ersinia* spp. (1%). The prevalence of *Streptococcus* spp. was higher in omnivorous species than in granivorous species (20% versus 8%) (5).

This study aimed to cover the hygienic aspects of some wild birds in Kirkuk city. However, undesired feces contamination of zoonotic bacteria can also cause risks and must be kept in mind.

Materials and Methods

Twenty one (12 males and 9 females) individuals of House sparrow, fifteen (8 males and 7 females) of White-Cheeked Bulbul, Twenty (10 males and 10 females) of Collared dove and Twenty (12 males and 8 females) of Rock dove were collected from different regions of Kirkuk city during summer season.

Blood samples(2 ml) were collected in sodium citrate anticoagulant tubes. Ten gm samples of liver, kidney, intestine content were collected separately in sterile screw capped bottles.

Isolation of *Salmonella* Spp.: A 10 ml of pre warmed (37 C) selenite-cystine broth was added to the 3g sample. Samples were mixed thoroughly before incubation. The covers of the screw-cap bottles were placed on loosely. Incubation was at 37 C for 24 hr. The incubated samples were mixed and streaked onto Brilliant Green Bile Agar plates. The sample was streaked onto four different plates. Straw-colored colonies preliminary confirmation (6). To perform a statistical evaluation, 4 presumptive colonies were picked from each sample and inoculated the lysine-iron agar slants by stabbing method and the tubes incubated for 24 hr at 37 C. Production of a straw-colored butt was considered a negative Salmonella reaction, and these tubes were discarded. A neutral or alkaline butt, with or without H2S production, was indicative of a presumptive positive Salmonella culture (7).

Isolation of the *Listeria monocytogenes*: After preparation of sterile media, a 2.5 gm each of a 25 ml Trypton Soya Yeast Extract Broth (HiMedia, India) was inoculated according to standard protocols, in which a portion of analytic sample was added to 9 portions of

Listeria enrichment broth (8,9). For a homogenous distribution of the sample in the medium it was shaken for 2-3 minutes inside stomacher. Media so inoculated were then incubated at 30 C° for 24-48 hours. After 24 and 48-hour enrichment processing, the culture was made on the Enrichment Listeria Broth, Modified (ELBM). The planted plates were incubated for 24-48 hours at (35-37) C° under aerobic conditions. At the end of the incubation, they were evaluated as typical colonies with a blue-green colored, smooth, convex, circular or creanated with entire margins and opaque halo of 1-3 mm in diameter while the others *Listeria* spp. appear as blue-green colonies. The typical *Listeria monocytogenes* colonies observed on the culture medium were selected and cultured separately in Trypton Soya Yeast Extract Agar (HiMedia, India). After that, the colonies were checked morphologically and their purity controlled by gram staining. Later, the separated colonies were then subjected to carbohydrate fermentation tests (mannitol, D-xylose, rhamnose), catalase activity, oxidase activity, modified motility test with triphenyltetrazolium chloride salts (Umbrella formation), esculin hydrolysis (8). The colonies isolated as *Listeria* were then subjected to β-hemolysis in a 7% sheep blood agar for species identification.

Isolation and identification of *Campylobacter* spp.: Ten grams of tissue and blood material was weighed and put into 90 ml of Bolton Both (Campylobacter Enrichment Broth, Bury, England) and incubated at 41°C for 24 h in a incubator with N_2. One loopfull (10 µl) of enrichment culture was spread onto modified *Campylobacter* Charcoal Differential Agar (mCCDA) plates, which were incubated in the same conditions. In addition, one loopful (10 µl) samples was directly cultured on mCCDA(10).

Isolation of *Shigella* spp.: samples was inoculated in *Shigella* Broth (SB) with 0.5 and 3.0 µg/ml novobiocin, were used all incubated at 37 °C (SB with 3.0 µg/ml novobiocin also at 42 °C) and Buffered Brilliant Green Bile Glucose Broth with 1.0 µg/ml novobiocin incubated at 37 and 42 °C growth of *Shigella* spp.(11).

Isolation of *Brucella abortus*: samples was inoculated in Trypticase Soy Broth and incubated at 37ºC for 3 days. Loopfull growth were cultured on Trypticase Soy Agar plates and incubated at aerobic atmosphere at 37ºC for 3 days for bacterial identification (12). *Brucella abortus* like colony were performed using gram staining and identification of the biochemical profile: catalase, oxidase, citrate, indole, nitrate, motility, fermentation in TSI medium and urease (7,13).

Results

Table (1) appeared that high % of isolation of *Salmonella* was in the intestine of House Sparrow and Rock Dove species that recorded 21 from 21(100%), and 20 0f 20 (100%) respectively, while in collared dove 18 from 20 (90%) and in White Cheeked Bulbul species10 from 15(66.6%). Bacterial isolation of *Shigella* Spp from House Sparrow and Rock Dove species recorded 19 case (90.4%) and 18 (90%) respectively, while in White Cheeked Bulbul species13 (86.6%) and Collared dove 15(75%). *Listeria monocytogenes* isolated from House Sparrow 20 (95.2%) and Collared Dove species recorded 8 (40%) while in White Cheeked Bulbul species 4 (26.6%) and Rock Dove 5(25%). *Brucella abortus* was isolated only from House Sparrow species recorded 19(90.4%), but *Campylobacter* Spp. isolated in House Sparrow and Collared Dove was 2 and 1 (9.5% , 5%**)** respectively.

Isolation of some zoonotic bacteria in liver of some birds species appeared in (table - 2). *Salmonella* Spp. in House Sparrow observed 19 case (90.4%), in both Collared Dove

and Rock Dove 17 (85%) and finally in White-Cheeked Bulbul was 5(33.3%) . *Brucella abortus* have been isolated from House Sparrow species only in 14.2%, in spite of *Campylobacter* Spp. was not isolated from liver of any species. The prevalence of some zoonotic bacteria in kidney is shown in table (3) it appears that *Salmonella* spp. was only isolated than other pathogens and House Sparrow recorded 8 case (38%), in Collared Dove and Rock Dove was 5 (25%) then White-Cheeked Bulbul (13.3%).

Bacteria which were detected in blood circulation are shown in table (4) in which *Salmonella* Spp. in Rock Dove was (35%) Collared Dove (30%) and House sparrow (28.5%) and White-Cheeked Bulbul (26.6%). *Shigella* Spp. and *Campylobacter* Spp. Have not been isolated from blood in our study.

Table (1) Bacterial prevalence in the intestine

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove	Rock Dove
***Salmonella* Spp.**	21/21 % 100	10/15 % 66.6	18/20 % 90.0	20/20 % 100
***Shigella* Spp.**	19/21 % 90.4	13/15 % 86.6	15/20 % 75	18/20 % 90.0
Listeria monocytogenes	20/21 % 95.2	4/15 % 26.6	8/20 % 40.0	5/20 % 25.0
Brucella abortus	19/21 % 90.4	–	–	–
***Campylobacter* Spp.**	2/21 % 9.5	-	1/20 % 5.0	–

Table (2) Bacterial prevalence in the liver

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove	Rock Dove
***Salmonella* Spp.**	19/21 % 90.4	5/15 % 33.3	17/20 % 85.0	17/20 % 85.0
***Shigella* Spp.**	6/21 % 28.5	3/15 % 20.0	7/20 % 35.0	6/20 % 30.0
Listeria monocytogenes	21/21 % 100.0	10/15 % 66.6	13/20 % 65.0	15/20 % 75.0
Brucella abortus	3/21 % 14.2	–	–	–
***Campylobacter* Spp.**	–	–	–	–

Table (3) Bacterial prevalence in the kidney

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove	Rock Dove
***Salmonella* Spp.**	8/21 % 38.0	2/15 % 13.3	5/20 % 25.0	5/20 % 25.0
***Shigella* Spp.**	–	–	–	–
Listeria monocytogenes	–	–	–	–
Brucella abortus	–	–	–	–
***Campylobacter* Spp.**	–	–	–	–

Table (4) Bacterial prevalence in the blood

Bacteria species	House Sparrow	White-Cheeked Bulbul	Collared Dove	Rock Dove
***Salmonella* Spp.**	6/21 % 28.5	4/15 % 26.6	6/20 % 30.0	7/20 % 35.0
***Shigella* Spp.**	–	–	–	–
Listeria monocytogenes	5/21 % 23.8	–	1/20 % 5.0	2/20 % 10.0
Brucella abortus	3/21 % 14.2	1/15 % 6.6	3/20 % 15.0	2/20 % 10.0
***Campylobacter* Spp.**	–	–	–	–

Listeria monocytogenes was not isolated in blood of White-Cheeked Bulbul, but isolated in House Sparrow, Rock Dove and Rock Dove (23.8%,10% and 5%) respectively. *Brucella abortus* isolated from Collared Dove was (15%), in House sparrow was (14.2%), then in Rock Dove was (10%) and (6.6%) from White-Cheeked Bulbul.

Discussion

Fee living birds including migratory species have to exploit seasonal opportunities for breeding habitat, food supplies and birds travel across national and international borders (17). Avian mobility and migration are remarkable biological phenomena also they posses crucial epizootiologic factors in which these birds play a significant role in the ecology and circulation of pathogenic organism (15,16). They carry pathogens, even sedentary avian species can sometimes move as far as 50-100 km and nomadic bird species can transport viable pathogens to distant sites during erratic movement (17), that can be transmitted to domestic animals and human (18).

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 (19,20).

Programmers for zoonosis control and prevention are passed on many steps that have been adopted by international health organization such as FAO, WHO and OIE as prevention, control, eradication, neutralization of reservoir, reducing potential contact increasing host resistant, implementing consumer protection strategies, identifying animals appropriately, maintaining health, communication and education (21).

Salmonella spp. are the most relevant in animal feces since they can infect or contaminate nearly all living vectors from insects to mammals (4). Birds feces contained many genera of zoonotic bacteria including *Escherichia coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Streptococcus* spp., and Y*ersinia* spp. (5).

We concluded that wild birds harbor many pathogenic organism which are capable to produce disease conditions in human beings (zoonotic).

References

1. Martens, W. & Böhm, R. (2002). Public health aspects connected to the use of sludge on land. AMIRAN 2002.10 th International Conference. Hygiene Safety Proceedings. Štrbské Pleso, High Tatras, (Slovak Republic) May 14-18, 2002.
2. Strauch, D. (1998). Pathogenic micro-organisms in sludge. Anaerobic digestion and disinfection methods to make sludge usable as fertilizer. European Water Management, 2(2): 12-26.
3. Hickey, J. K. S. & Reist, P. L. (1975). Health significance of airborne microorganisms from wastewater treatment processes. J. WPCF., 47(12): 2741-2773.
4. Strauch, D. (1991). Survival of pathogenic micro-organisms and parasites in excreta, manure and sewage sludge. Rev. Sci. Techn. Off. Int. Epiz., 10. 813-846.
5. Brittingham, M. C.; Temple, S. A. & Duncan, R. M. (1998). A survey of the prevalence of selected bacteria in wild birds. J. Wildlife Dis., 24(2): 299-307.
6. Bhatia, T. R.; McNabb C. D.; Wyman, H. & Nayar, G. P. (1979). *Salmonella* isolation from litter as an indicator of flock and carcass contamination. Avian Dis., 24(4):838-847.
7. Cruickshank, R.; Duguid, J. P.; Marmoin, B. P. & Swain, R. H. (1975). Medical Microbiology. 12th ed. Churchill Livingstone. Edinburg, London and New York. P. 403-419.
8. Hitchins, A. D. (2003). Detection and Enumeration of *Listeria monocytogenes* in Foods. Chapter 101. In: Jackson, G.J. (coordinator), Bacteriologial Analytical manual. 10th ed., Revision A., AOAC Int., Gaithersburg, M.D., USA.
9. Pagotto, F.; Daley, E.; Farber, J. & Warburton, D. (2001). Isolation of *Listeria monocytogenes* from all food and environmental samples. MFHPB-30, Health products and food branch, HPB Method, Ottawa, Canada.
10. Marjaana H.; Heiska, H. & Hänninen, M. (2007). Prevalence of *Campylobacter* spp. in cattle in Finland and antimicrobial susceptibilities of ovine *Campylobacter jejuni* strains. Appl. and Env. Microbiol., 73 (10) :3232-3238.
11. Uyttendaele, M.; Bagamboula, C. F.; De Smet, E.; Van Wilder, S. & Debevere, J. (2001). Evaluation of culture media for enrichment and isolation of *Shigella sonnei* and *S. flexneri*. Inte. J. of Food Microbiology, 70(3): 255-265.

12. Al-Obaidi, F. A.; Al-Ramahi, H. S. & Al-Shadeedi, S. M. (2009). Proceeding of the 9th scientific conference of the College of Veterinary Medicine, University of Baghdad, during the period of March 31st to April 1 st of 2009, Vol. 1: 221–227.
13. Keid, L. B.; Soares, R. M.; Morais, Z. M.; Richtzenhain, L. J. & Vasconcellos, S. A. (2004). *Brucella* spp. Isolation from dogs from commercial breeding kennels in Sao Paulo state, Brasil. Brazilian J. of Microbiol., 35:161-166.
14. Hubalek, Z. (2004). An annotated checklist of pathogenic microorgamisms associated with migratory birds. J. wildlife Dis.,40(4):639-659.
15. Gill F. B. (1995). Migration .In Orniyhology,2nd Ed W.H. Freeman and Co., New York, 287-309.
16. Ioanna, G. & Vasilios, T. (2008). The potential role of migratory birds in the transmission of zoonoses. Vet. Italiana, 44 (4):671-677.
17. Kurt, D.; Jennifer, K.; James, S. & Sanjay, K. (2002). Bird migration and emerging zoonoses. Cli. Med. and Res., 1 (1): 5-12.
18. Ehrilich, P. R.; Dobkin, D. S. & Wheye, D. (1988). In the birders handbook: a field guide to the natural history of North American birds. 1sted. Simon and Schuster Inc, New York, P. 720.
19. Chuma, T.; Hashimoto, S. & Okamoto, K. (2000). Detection of hermophilic, Cambylobacter from sparrows by multiplex PCR: the role of sparrows as a source of contamination of broilers with campylobacter. J. Vet. Med. Sci., 62: 1291-1295.
20. Roppole, J. H. & Hubalek, Z. (2003). Migratory birds and West Nile virus J. Appl. Microiol., 94: 475-585.
21. Hugh-Hones, M.; Hubbert, W. T. & Hagstad, H. V. (1995). Principles of zoonoses control and prevention . In Zoonsis, recognition, control and prevention .1St ed. Iowa State University Press. Ames. P. 79-120.