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Role of some wild birds in transmission of bacterial pathogens of zoonotic importance and poultry health and production

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Abstract

Cloacal swabs and drooping samples of 48 wild birds, including 16sparrows, 6 Brewer's blackbirds (Songbirds /starling),7 black crows (raven) ,10 Doves , 5 quail . 2 Parrots (parakeets), 1 Eagles and 1 hawk were collected and subjected for isolation of potentially pathogenic bacteria between Marchand June 2013 from different sites at 3 Districts located in Amran governorate (Yemen). Eleven bacterial species in 14 genera were isolated from wild birds. *E.coli* (15/48; 31.25%), *Salmonella Typhimurium*. (6/48; 12 %), *Proteus mirabilis* (6/48; 12 %), *Proteus vulgaris* (4/48; 8 %), *Citrobacter frundii* (6/48; 12 %), *Klebsiella pneumonia* (5/48; 10%) , *Pseudomonas aeruginosa* (5/48; 8%) , *Campylobacter jejuni* (4/48; 8%) , *Staphylococcus aureus* (4/48; 8%), *Enterococci* (7/48;14%) and *Clostridium perfringens* (6/48;12%) were the most prevalent bacterial groups recovered from examined wild birds. Bacterial species varied between bird species.

Our results indicated the importance of wild birds as potential vectors of bacterial pathogens this can threaten both human and domestic birds. Control measures should be considered to prevent transmission of such bacteria.

Key words: wild birds, bacterial pathogens, Gram negative bacteria, Gram positive bacteria.
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Introduction

The Republic of Yemen is very rich in bird's life. Yemen has a special responsibility and because of them, ranks as one of the most important countries in the Middle East for bird conservation. So why is Yemen so special for birds? There are in fact several reasons. Firstly, the high mountain block of the north Yemen highlands as an island surrounded by the Red and Arabian Seas on two sides and the sands of the Empty Quarter on the other. Second, Yemen is influenced by 3 major faunal regions of the world: the Palearctic, Orient and Afro-tropical; they overlap in Yemen and as a consequence the country has many species representative of each. Thirdly because Yemen is positioned at the foot - or funnel of Arabia, a number of migratory birds become concentrated on their long journey between their breeding grounds in Asia and their wintering areas in Africa. Finally, there is a spectacular range of habitats: from the mountain plateau and terraces down to the plains of the Tihama and desert, to the biologically very rich coast and its numerous off-shore islands. Each holds its own special bird communities. Around Yemen there are considerable numbers of wild birds living in urban and rural areas and coming into close contact with humans and other animals .Wild birds seem to be involved in the epidemiology of most zoonosis and serve as major reservoirs for transmission of zoonotic agents to domestic birds and humans. Many of the bacterial enteropathogens that affect poultry have also been isolated from wild birds, relatively little is known about their effect on wild populations, with the exception of outbreaks of lethal diseases ***Clare et al (2009).***

Migratory birds can disperse microorganisms across international borders and myriad pathogens harmful to poultry or other vertebrates have been associated with such birds ***(Hubalek,1994and 2004)*** **.**Moreover Wild birds are important to public health because they can be infected by a number of pathogenic microorganisms that are transmissible to humans including arboviruses such as *West Nile virus*, *Borrelia burgdorferi*, influenza A virus, enteric bacterial pathogens and drug-resistant bacteria. Wild birds are implicated in transmission in zoonoses as biological and mechanical carriers and as hosts and carriers of infected ectoparasites ***(Georgopoulou and Tsiouris 2008)*** **.** The presence of Wild birds droppings in urban environment may contribute to the spread of infectious agents as they could harbor various microorganisms ***(Tanaka et al2005).*** These might include human pathogens such as diarrheagenic *E.coli* strains, which are able to survive under adverse environmental conditions for extended periods of time if excreted in feces, thus creating potential for illness in humans and other animals ***(Pedersen et al 2006).*** Birds not only acquire pathogens from the environment, but also return them via excretion, potentially facilitating the dissemination of pathogenic organisms to both humans and other animals, especially through water. Livestock on many farms rely on rivers, streams and other untreated water sources for at least part of their drinking water ***(Reilly, 1981),*** and wild birds roosting in large numbers on or near water may contribute to its contamination and the spread of disease to other animals. Since many of the pathogens found in bird droppings originate from human sewage, it should follow that humans are highly susceptible to infection when the bacteria re-enter

the human food chain through drinking water supplies and bathing water.

For the above mentioned , this work was initiated to investigate the incidence of various bacterial pathogens among some common wild birds in Amran governorate(located at the north Yemen highlands plateau land and rocky slopes- about 45 km from Sana'a city).

Material and Methods

Collection of samples:

Cloacal and fresh fecal swab samples from 48 wild birds, including: 16 sparrows , 6 Brewer's blackbirds (Songbirds /starling) ,7 black crows (raven), 10 Doves, 5 quail,2 Parrots (parakeets) , 1 Eagle and 1 hawk were collected. These samples were collected between March and June 2013from different sites at 3 Districts located in Amran governorate (Yemen).

Sample preparation:

Coloacal swabs were placed in buffered glycerol saline solution and the entire contents of the container were thoroughly mixed while fresh dropping was collected in sterile caped tubes. The collected samples were sent to the laboratory in a clean cooled labeled container.

The Culture media:

Fluid media including nutrient broth and selenite-F-broth media as well as solid agar media including MacConkey agar media for Enterobacteriaceae, nutrient and blood agar media for Gram- positive bacteria as well as Skirrow's, Butzler and Skirrow's media for Campylobacter

and Nutrient agar medium for *Pseudomonas aeruginosa* were prepared and used according to ***Collee et al (1996).***

Bacterial isolation:

The cotton swabs immediately were streaked for isolated colonies onto used culture media plates. All inoculated culture media were incubated for the recommended temperature, time and precaution then examined for bacterial growth. All bacterial colonies were isolated in pure culture for further identification.

Bacterial identification:

Identification of the obtained bacterial isolates was done on the basis of cultural, morphological and biochemical characteristics according to ***Quinn et al. (1994) and Collee et al. (1996)*** as shown in table (1).

Results and Disscusion

Although free-living birds are an important part of a healthy environment and may have considerable beneficial aspects, on occasion they may contribute to the spread of pathogens from animals to man and vice versa. In recent decades, migratory birds and wild life have been incriminated in and associated with emergent and resurgent diseases,***(Hubalek, 2004 and Jourdain et al., 2007).*** They are implicated in the transmission of Zoonoses and other microbial pathogens by three main mechanisms , namely biological carriers , mechanical carriers and carriers of infected ectoparasites ***(Hubalek, 2004)***.The importance of wild birds as potential vectors of disease has received recent empirical interest, especially regarding human health.

Understanding the spread of bacterial. Pathogens in wild birds may serve as a useful model for examining the spread of other disease causing organisms.

Types and frequency of the microorganisms found in the different wild birds in some Districts of Amran governorate is summarized in tables (2).

Results of this study pointed and supporting the fact that wild birds play crucial roles in the dissemination of zoonotic and entero-pathogenic bacteria like *Campylobacter* and salmonellae (table 2). These bacteria may also harmful to many domestic birds species. The incidence of human infection with *Campylobacter jejuni* is increasing and even though poultry is considered to be a major source, it is evident that other reservoirs may exist ***(Skirrow, 1977 and Tomar et al., 2006***).

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Table (1): Characteristics of the isolated bacteria according to ***Quinn et al. (1994)*** ***and Collee et al. (1996)***.

Bacterial Species	**Isolation**		**Identification**
	Culture	**Colonial morphology**	
E.coli	MacConkey agar	Large 2-4 mm Lactose fermenting colonies	Gram negative usually motile rods. Most strains are indole positive, citrate negative.
	Blood agar	1-4mm colonies appear mucoid and some strains are hemolytic.	
Salmonella typhimurium	MacConkey agar	Colorless (pale non lactose colonies). KIA: pink-red slope and yellow butt.	Gram negative bacilli .Motile, indole and urease negative, H2S positive.
Proteus mirabilis	MacConkey agar	Non-lactose fermenting colonies, Cultures have a distinctive smell.	Gram - ve pleomorphic rods, Actively motile, indole negative, Urea and phenylalanine deaminase positive.
	Blood agar	Produce swarming growth	
Proteus vulgaris	MacConkey agar	Non-lactose fermenting colony, Cultures have a distinctive smell.	Gram negative, pleomorphic rods, Actively motile, indole ,Urea and phenylalanine deaminase are positive e.
	Blood agar	produce swarming growth	
Citrobacter frundii	MacConkey agar	Positive and negative lactose fermenting colonies	Gram negative usually motile rods. Indole negative, citrate positive.
Klebsiella pneumonia	MacConkey agar	Large lactose fermenting mucoidcolnies .	Gram negative usually non-motile rods. Indole negative but citrate positive.
Pseudomonas aeruginosa	MacConkey agar	Non-lactose fermenting colonies with yellow- green in medium.	Gram negative motile rods. Rapidly oxidase positive.
Campylobacter jejuni	Campy-Cefex agar	Effuse moist droplet-like colonies, microaerophilic (5% oxygen and 10% CO2).	small Gram negative rods comma or S- shaped; oxidase ,catalase positive, Urea negative
Staphylococcus aureus	MacConkey agar	Small-non lactose colonies	Gram positive cocci singly and in group Positive coagulase, DNAse and catalase .
	Blood agar	Smooth ,1-2mm cream colored	
Streptococcus faecalis (Enterococci)	MacConkey agar	Minute pink or red colonies	Gram positive cocci arranged in short chains. Ferment lactose, positive litmus milk test.
	Blood agar	Non haemolytic.	
Clostridium perfringens	Blood agar	Large beta-haemolytic colonies Facultative anaerobe.	Large gram positive capsulated rods with straight ends, lactose and lecithinase positive.

Table (2): Results of Bacterial isolation from samples collected from different wild birds in Amran governorate.

Bird Species	No of birds/species	Type and Number of Bacterial Isolates											Total/species
		E. coli	*S.typhimurium*	*P. mirabilis*	*P. vulgaris*	*C. frundii*	*K. pneumonia*	*Ps. aeruginosa*	*C. jejuni*	*S. aureus*	*Enterococci*	*Cl.perfringens*	
Sparrows	**16**	**4**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**1**	**14**
Partridges Songbirds	**6**	**2**		**1**		**1**	**1**		**1**		**1**		**7**
Black crows	**7**	**2**	**1**	**1**	**1**	**1**		**1**	**1**	**1**	**1**	**1**	**11**
Doves	**10**	**2**	**1**	**1**	**1**		**1**					**1**	**7**
Quail	**5**	**2**	**1**		**1**	**1**	**1**	**1**		**1**	**1**	**1**	**10**
Parrots	**2**	**1**	**1**	**1**		**1**		**1**			**1**	**1**	**7**
Eagles	**1**	**1**	**1**					**1**	**1**	**1**	**1**		**6**
Hawks	**1**	**1**		**1**		**1**	**1**				**1**	**1**	**6**
Total	**48**	**15**	**6**	**6**	**4**	**6**	**5**	**5**	**4**	**4**	**7**	**6**	**68**

Various apparently healthy wild birds have been found to contain *Cl. jejuni*, suggesting that this organism may be a normal component of the intestinal flora of at least some bird species (like migrating ducks, common starlings, sparrows and other wild birds***(Waldenstrom et al., 2007).*** The reasons for Birds are being ideal hosts for *Campylobacter* is due to their relatively high body temperature (42°C). Similarly, A variety of Salmonella species specially *(S.Typhimurium and S.Enteritidis)* have been found in both apparently healthy and obviously diseased wild birds like many species of gulls, pigeons and sparrows ***(Pennycott, et al., 2006 and Kobayashi, et al., 2007).*** A high proportion of the dropping samples in our study were found to have *E.coli* spp. (31.3%)

(Table 2) considering it the most common gram-negative bacteria were found. Although *E.coli* is part of the normal flora of the intestinal tract of vertebrates, virulent and sometimes lethal toxin-producing pathogenic strains do exist ***(Hunter, 2003).*** Studies that have addressed the incidence of such strains in the intestinal tract of wild birds suggest that there is an avian reservoir ***(Foster et al., 2006).***

In this work *Proteus* spp, *Citrobacter frundii, K. pneumonia, Ps. aeruginosa, Enterococcus, Staph. aureus,* and *Cl. Perfringens*are also isolated from different wild birds with different proportion**.** Proteus occasionally causes embryonic death, yolk sac infections and mortality in young chickens, turkeys, and ducks ***(Baruah et al., 2001 and Salmon and Watts, 2000).***Septicemia due to *Proteus* has occurred in quail ***(Sah et al., 1983).*** *Klebsiella* species appear to be relatively common avian pathogens ***(Fudge, 2001 and Hernandez et al., 2003).*** *Ps. aeruginosa* is considered to be an opportunist common avian pathogen ***(Walker et al., 2002)***.It affects the upper respiratory tract, causing rhinitis, sinusitis and laryngitis ***(Bailey et al., 2000).*** Infections are also associated with septicemia and hemorrhagic enteritis in Psittacines ***(Rich, 2003*****) and** corneal ulcers in captive cranes ***(Miller et al., 1995)*****.**

Different strains of Streptococcus and *Enterococcus* species have also been isolated from birds in association with septicemic disease ***(Droual et al., 1997).*** Infections with *Staph. aureus* are frequently secondary to impairment of the host defense mechanisms, or are due to a compromised immune system ***(Wobeser and Kost, 1992).*** In addition to

being a major disease-producing organism for poultry, approximately 50% of typical and atypical *Staph. aureus*s trains produce enterotoxins that can cause food poisoning in human beings ***(Evans et al., 1983).***

Clostridium perfringens is another ubiquitous bacterium often found in the intestinal tract of healthy birds, causing outbreaks of both acute clinical disease and subclinical disease in broiler and turkey flocks. It is also of concern to human health, as it can cause food borne disease by transmission through poultry products ***(Engstrom et al., 2003).*** The detection of *Cl. perfringens* in dropping from wild birds near broiler chicken houses suggests that wild birds that gain entry to poultry houses have the potential to transmit the pathogen to poultry ***(Craven et al., 2000).***

In conclusion on the basis of our findings, the prevalence of both pathogenic Gram negative bacteria (*Salmonella, Proteus, Klebsiella, P. aeruginosa* and *Campylobacter*) and Gram positive bacteria*(Staph. aureus and Cl. perfringens)* are relatively high in healthy wild birds. From literature, such birds are able to spread and transmit bacterial pathogens to man as well as animals. Therefore; control measures should be considered to prevent transmission of such bacteria, and to protect wild birds against our polluted environment.

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دور بعض الطيور البرية في نقل مسببات الأمراض البكتيرية ذات الأهمية على الصحة العامة وصحة الدواجن والإنتاج

الملخص

تم تجمع 48 عينة من مسحات مجمع وعينات زرق من الطيور البرية، والتى تضمنت 16من العصافير (sparrows)، 6 بلاك بيرد بيرة Brewer's blackbirds (Songbirds /starling) (الطيور المغردة / زرزور)، 7 غربان سوداء (black crows) (الغراب)، 10 الحمائم (Doves)، 5 السمان (quail). 2 الببغاوات Parrots (الببغاوات)(parakeets) ، 1 نسور (Eagle) و1 صقور (Hawk) كما تم فحص للبكتيريا المسببة للأمراض فى الفترة بين مارس و يونيو 2013 من اماكن مختلفة في 3 مناطق تقع في محافظة عمران (اليمن).

وقد تم عزل أحد عشر نواعا لبكتيريا من الاربعة عشرة جنسا من الطيور البرية المزكورة . كانت المجموعات البكتيرية الأكثر انتشارا فن الطيور البرية التى تم فحصها الميكوب القولوني (E.coli) (48/15، 31.25٪)، السالمونيلا التيفية (S.typhimurium) (48/6 و 12٪)، المتقلبة الرائعة (Pr.mirabilis) (48/6 و 12٪)، المتقلبة الاعتيادية (Pr.vulgaris) (48/4، 8٪)، السيترورباكتر فروندى (C.frundii) (48/6و 12٪)، الكلبسيلة الالتهاب الرئوي (Klebsiella pneumonia) (48/5 و 10 ٪)، الزائفة الزنجارية (Ps.aeruginosa) (48/5، 8٪)، العطيفة الصائمية (Campylobacter jejuni) (48/4، 8٪)، المكورات العنقودية الذهبية (Staph,aureus) (48/4، 8٪)، المكورات المعوية (Enterococci) (48/7، 14٪) والمطثية الحاطمة (Cl. perfringens) (6 / 48 و 12٪). حيث تفاوتت الأنواع البكتيرية بين انواع الطيور التى فحصت.

وتشير النتائج الى أهمية الطيور البرية كنواقل محتملة لمسببات الأمراض البكتيرية والتى يمكن أن تهدد كل من الإنسان والطيور المحلية. ولذا ينبغي النظر في تدابير الرقابة لمنع انتقال هذه البكتيريا.