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Salmonella Typhimurium and *Salmonella* Hessarek in wild birds

Vahşi kuşlarda *Salmonella* Typhimurium ve *Salmonella* Hessarek

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ABSTRACT

Objective: Sporadic *Salmonella* outbreaks were also documented in wild birds, although wild birds are the well-known carriers of salmonellosis. In this study, we investigated the causative agents of two death events of sparrows and black headed gulls occurring in the Çorum City Landfill and Bartın Port, respectively, between mid-autumn and winter of 2017-2018.

Methods: Septicemic salmonellosis was suspected based on necropsy findings of dead sparrows and black-headed gulls. In this context, isolation and identification was done according to conventional cultural method for the tissue samples (liver, spleen, heart) and ISO 6579:2002/Amd 1:2007 (Annex D) for small intestine samples, and serotyping were carried out according to Kauffman White Scheme.

Results: One of the two mortality events was seen in the Bartın Port. *S.* Typhimurium was found to be the causative agent of black-headed gulls' (*Larus ridibundus*) death. The other mortality event observed in sparrows (*Passer domesticus*) was determined in the Çorum City Landfill. *S.* Hessarek was determined to be responsible of the septisemic bacteremia of sparrows.

ÖZET

Amaç: Vahşi kuşlar, bilinen salmonelloz taşıyıcıları olmasına rağmen sporadik *Salmonella* salgınları da rapor edilmiştir. Bu çalışmada, 2017-2018 sonbahar ortası ile kış aylarında, sırasıyla Çorum İli Çöp Depolama Alanı ve Bartın Limanı'nda meydana gelen serçe ve kara başlı martıların iki ölüm olayının nedeni olan etken araştırılmıştır.

Yöntem: Ölü olarak bulunan serçe ve kara başlı martıların nekropsi bulgularına dayanarak septisemik salmonellosisten şüphelenilmiştir. Bu bağlamda konvansiyonel kültürel yöntemle dokulardan (karaciğer, dalak, kalp) ve ISO 6579:2002/Amd 1:2007 (Annex D) ile ince barsak örneklerinden izolasyonu, tanımlanması ve Kauffman White Şeması ile de serotiplendirmesi yapılmıştır.

Bulgular: İki ölüm vakasından biri Bartın Limanı'nda görülmüştür. *S.* Typhimurium kara başlı martıların (*Larus ridibundus*) ölümlerinin etkeni olarak tespit edilmiştir. Serçelerde (*Passer domesticus*) gözlenen diğer ölüm olayının Çorum İli Çöp Depolama Alanı'nda olduğu bildirilmiştir. Serçelerin septisemik bakteriyemisinden *S.* Hessarek'in sorumlu olduğu belirlenmiştir.

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Conclusion: In both cases, where the death cases observed were the places which had a close interaction with urban civilization. According to informations, while black-headed gulls were adapted to feeding on urban waste and showed tendency to scavenge for food at rubbish tips and sewage outfalls in the Bartın Port, sparrows fed from the Çorum City Landfill to obtain food during migration season. Circulation of S. Hessarek in Çorum where commercial layer flocks existed and S. Typhimurium, a zoonotic pathogen in the Bartın Port were thought not to be ignored for poultry and human health. The epidemiology of both agents should be examined in wild birds.

Key Words: S. Typhimurium, S. Hessarek, sparrow, black-headed gull

Sonuç: Her iki durumda da ölüm vakalarının görüldüğü yerler kent yaşamı ile yakın etkileşim içinde olan yerlerdir. Edinilen bilgilere göre Bartın Limanı'nda kara başlı martıların kentsel atıklarla beslenmeye adapte olup, çöplüklerde ve kanalizasyon çıkışlarında yiyecek arama eğilimi gösterdikleri, serçelerin göç mevsiminde yiyecek bulmak için Çorum İli Çöp Depolama Alanı'ndan beslendikleri bilgisine ulaşılmıştır. Ticari yumurtacı sürülerinin bulunduğu Çorum'da S. Hessarek ve Bartın Limanı'nda zoonotik bir patojen olan S. Typhimurium'un sirkülasyonunun kanatlı ve insan sağlığı açısından göz ardı edilmemesi gerektiğini düşündürmüştür. Her iki etkenin de vahşi kuşlarda epidemiyolojisi incelenmesi gerekmektedir.

Anahtar Kelimeler: S. Typhimurium, S. Hessarek, serçe, kara başlı martı

INTRODUCTION

In last decades, wildbirds were well documented as a source of different zoonotic pathogens (1, 2). Particularly, enteric bacterial pathogens such as *Salmonella*, *Shigella*, *Listeria*, *Campylobacter*, *Enterococcus*, pathogenic *Escherichia coli* and viral pathogens such as Influenza virus, West Nile virus carriage of wild birds were reported worldwide as well in Turkey (3-7).

As enteric pathogen, *Salmonella* are found in the intestine. *Salmonella* present in wild birds for two reasons. In the first situation, *Salmonella* is adapted to the host and constitute it self as a part of the intestinal flora due to feeding habits of raptors, and sheeding in feces. In this case wild birds shed the microorganism permanently however they are not infected but the candidate of carriers. In the second situation, as a result of the environmental contamination, *Salmonella* exist in the feces for a short time (8, 9).

Although all birds are susceptible to *Salmonella* infections and wild birds are known as to be the *Salmonella* carriers (10), particularly, *Salmonella* Typhimurium. Also, S. Typhimurium was documented to be the causative agent of salmonellosis outbreaks in wild birds (10-12). Surprisingly, S. Hessarek was also reported in septisemic salmonellosis outbreaks of starlings and song trushes (13, 14). In sporadic *Salmonella* death events associated with wild birds, large numbers of susceptible birds are affected at bird feeders and feeding stations (12). Wild birds tend of being infected with enteropathogens due to their behaviours and feeding habits (15). Additionally, wild birds get them via scavenging on waste dumps and sewage sludge or from surroundings that have been contaminated by humans (15). Their flight ability to cover long distances during annual movements, they disperse the disease via feces and contaminated surface water and environment and also disperse the infections to both other animals and humans (10).

In this study, it was aimed to determine the causative agent of the dead events of wild birds with the suspicion of septisemic salmonelosis according to necropsy.

MATERIAL and METHOD

Case definition of wild birds

Between 2017-2018, a total of 45 wild birds including seven black-headed gulls (*Larus ribibundus*), 38 house sparrows (*Passer domesticus*) was transferred to the laboratory of the history of acute deaths by cold chain (Table 1).

Sparrows

The area involved in mortality events called the Çorum City Landfill (40° 45' 37.908"N, 34° 57' 18.2268") where 38 sparrows (*Passer domesticus*) were found. The period where the death events observed was coincided with the migration session mid-autumn and winter. Many migratory birds was observed to have a break during migration inorder to feed themselves from Çorum City Landfilled (Table 1).

Black-headed gulls

The area involved in mortality events called the Bartın Port (41°41'3.951"N, 32°13'32.7648") where seven black-headed gulls (*Larus ridibundus*) were found. We were informed that the black-head gulls fed from trash and sewage outfalls in the port when the mortalities were detected (Table 1).

Microbiological examination

Tissue samples (hearth, spleen, liver) were inoculated on 5% Blood Agar (Oxoid, CM0055), MacConkey Agar (Oxoid, CM0007), Brilliant Green Agar (Oxoid, CM0263) and aerobically incubated at 37°C (16). Each intestine samples of sparrows and black-headed gulls were examined according to ISO 6579:2002/Amd -1:2007 (Annex D)(17). Each minced intestine samples were added to 225 ml of buffered peptone water (BPW, Oxoid CM509) after preenrichment at 37 (±1) °C for 16-18 hours, 1 ml and 0.1 ml of each preenrichment culture were inoculated into each Mueller Kaufmann Tetrathionate-Novobiocin Broth (Oxoid; CM1048) and Modified Semi Solid Rappaport Vassiliadis Medium (HiMedia, M1428), respectively. Mueller Kaufmann Tetrathionate-Novobiocin broth (Oxoid; CM1048) and Modified Semi Solid Rappaport Vassiliadis Medium (HiMedia, M1428) enrichment cultures were incubated at 37°C and 41.5°C, respectively. After enrichment, a loopful of inocula were streaked on each Brilliant Green Agar (Oxoid, CM0263) and XLD agar (Oxoid, CM0469). Pure cultures were prepared from the suspected *Salmonella* colonies in Brain Hearth Infusion broth (Oxoid; CM1135). In order to identify the pure cultures of suspected colonies, biochemical assays were used: Triple Sugar Iron (Oxoid, CM0277), urea hydrolysis (Oxoid, CM0053B), H_2S, indole production, ONPG (ß-galactosidase; Oxoid, DD0013), lysine decarboxylase (Oxoid, CM038) and Voges Proskauer (Oxoid, CM0043) tests (17). Polyvalent and monovalent specific somatic and flagellar antisera (Statens Serum Instut, Denmark) were used to confirm and serotype all identified *Salmonella* spp. isolates according to Kauffmann-White scheme (18).

Table 1. Main informative parameters and definitions of the studied areas and wildbird species

Area	Common name	Wild bird species	Number of examined wild birds
Bartın Port 41°41'3.951"N,32°13'32.7648"	Black-headed gull	*Larus ridibundus*	7
Çorum City Landfilled 40° 45' 37.908"N, 34° 57'18.2268"	Sparrow	*Passer domesticus*	38
TOTAL			**45**

RESULTS

Gross macroscobic findings

Except three sparrows, the remaining was suitable for diagnostic examination. The main necropsy findings in 35 sparrows were poor body condition, splenomegaly, absence of fat deposits, pectoral muscle atrophy. In addition to these findings, severe hemorrhage in the proventriculus and in the intestine was observed in 11 sparrows. Moderate hepatomegally, multifocal pinpoint foci of necrosis in the internal organs (hearth, spleen, liver) was observed in 17 sparrows. Multifocal encephalitis was seen in five sparrows. Multifocal pinpoint foci of necrosis in the internal organs (hearth, spleen, liver), and severe hemorrhage in the proventriculus and in the intestine were observed in seven black-headed gulls. Four out of seven expressed splenomegally (at least twice normal size). These findings in both two death events in Çorum and Bartın guided us to suspect *Salmonella* originated bacterial septicemia as the cause of deaths.

Microbiological examination results

According to macroscobic lesions in the internal organs, tissue samples (spleen, liver, hearth) of sparrows and black-headed gulls were examined for the presence of *Salmonella*. As emphasized before, thirty-five out of 38 sparrows was suitable for the macroscobic examination. Of the examined tissue samples of 35 sparrows, *Salmonella* spp. was isolated from all tissue samples. Eighteen out of 35 minced intestine samples of sparrows *Salmonella* spp. was isolated. All *Salmonella* spp. isolated sparrows were identified as *S.* Hessarek after serotyping. All black-headed gulls' intestine samples were found to harbour *Salmonella* spp. In addition to this, *Salmonella* spp. was isolated from seven spleen, four hearth, five hearth samples of black-headed gulls. All of the *Salmonella* spp. from black-head gulls were identified as *S.* Typhimurium. As a result, *S.* Hessarek and *S.* Typhimurium were decided to be the responsible agents of the death events for sparrows and black-headed gulls, respectively.

DISCUSSION

Salmonella carriage of wild birds was well documented (2, 6). Malekian et al. (7) discovered 36%, 30%, 26%, and 23% *Salmonella* positive in black headed gulls, slender billed gulls, starlings, and rooks, respectively, in landfills near Isfahan City, Iran (7). In general, these bacteria are not dangerous to the wildbirds, although certain salmonellosis outbreaks that caused the death of bird colonies in the United States have been reported (10). In a study conducted between 1985-2004 on wild bird mortality events in the United States, of the 3,472 total events, 5.4% were determined as avian wildlife *Salmonella*-related mortality events. *Salmonella* serovars including *S.* Typhimurium (n=96), subsequently 4,5,12:i-monophasic (n=7), *S.* Litchfield (n=1), *S.* Rubislaw (n=1), *S.* Uganda (n=1) were reported to be determined in the study (10). *Salmonella* serovars especially *S.* Typhimurium are commonly found in the intestine of wild birds. They appear to be relatively resistant to salmonellosis however may serve as effective carriers of *Salmonella*. Therefore they are the source of infection for other animals and humans (1, 2, 12).

Gulls are the omnivorous wild birds termed as opportunistic scavengers which feed at sites where the raw sewage released (19). They are recognized to be *Salmonella* carriers. Serovar diversity among the gulls was variable, however *S.* Typhimurium was seen the most commenest serovar among the studies (20). In our investigation, *S.* Typhimurium was isolated from each of the seven black-headed gulls that were transferred to the laboratory with a recent history of acute mortality between midautumn and winter from the Bartın Harbour. In our study, death events observed in black-headed gulls were coincided with the annual migratory season (3). Their ability to fly freely and cover long distances proned them to disperse *Salmonella* via feces. Although they are known to be the carriers

of *Salmonella* and not affected, in our study seven *Larus ribibundus* were found to be death. In the United States, proportional mortality among the wild birds between 1985-2004 were taken into account, *Salmonella*-related death events were determined in *Larus delawerensis* was declared to be 10.8% (19). *Salmonella* is quite frequently carried by gulls, but this is rarely linked to any manifestation of disease. Nevertheless, clinical salmonellosis in larids is occasionally recorded as a substantial cause of gull morbidity and mortality (as lethargy, fluffed-up plumage, difficult swallowing, enteritis, and neurological signs including incoordination) (19).

During migration, they roost for the purpose to obtain feed. We assumed that decreased fish population in the Black Sea at last five years force the gulls to feed from sewage outfalls, urban waste and garbage (3). Gulls eat small terrestrial animals such as rodents (particularly voles), kitchen scraps (remains of meat, fish, baked goods, rice, etc.), although they are less likely to eat plants (grains, cherries) (15). Seagulls and people have a tight interaction as a result of sharing same coasts and people feeding them, which also increases the spread of bacteria between two species (9, 11, 20). Dolejská et al. (21) showed significant similarities with clinical isolates from Australia, suggesting their human origin. We can not interprate our *S.* Typhimurium isolates as a result of a close interraction with the human population in our study because we did not compare the human origin and wildbirds origin *S.* Typhimurium isolates. However, one of the reason of the death events might be interraction with human population. Also, *Salmonella* Typhimurium phage type DT40, DT56 variant and DT 160 accounted for an important cause of mortality events in wild birds such as garden birds greenfinches and house sparrows between 1993 to 2003 in England and Wales (22). In further studies we decided to determine the phage types. The circulation of similiar phage types of *S.* Typhimurium during migration might be one of the other reason of the death events.

Between midautumn and winter 2017-2018, we were informed about 38 sparrows that was found dead in the Çorum City Landfill. And then, conventional cultural examination was performed from spleen, liver, heart and intestine of the dead sparrows for the presence of *Salmonella* spp. A hundred percentage of the *Passer domesticus* were found to harbour *S.* Hessarek. This was the second report of *S.* Hessarek from wild birds in Çorum, Turkey. Dakman et al. (23) reported *S.* Hessarek A,B,C,D,E,F from the liver samples of dead starlings that was transferrred to the laboratory in 2015 and the same researchers declared *S.* Hessarek G strain from the liver samples of dead starlings that was transferrred to the laboratory, in 2010. Starlings (*Sturnidae*) and Song Thrushes (*Turdus philomelos*) has apparently affected from *S.* Hessarek due to high specifity and and pathogenicity of the agent for these bird species, however other susceptible species of birds are known, including the house sparrows (*Passer domesticus*), the Eurasian blackbird (*Turdus merula*) and White Wagtail (*Motacilla alba*) was also reported to be affected (13, 24). In sparrows death event, *S.* Hessarek circulation in the same city should not be ignored due to being the localisation of large commercial layer flocks and pullets. The city landfill of Çorum was the attraction point for sparrows due to abundant food supplies. The feeding habits related to garbage have largely been pose the risk of microbiological infection on sparrows. And also, when they rest during migration to find feed, sparrows might have gained this pathogen from city landfill. City lanfills are one of the essential elements of urban civilisation. The possibility of transmitting infectious agents to both humans (garbageman) and other animals is a very serious public health issue, especially during the migration season when the uncontrollable flights of the migrant wards and their usage of urban garbage dumps as a place to find food (25-28). They take pathogens from the contaminated food there or contaminating these areas if they are the carriers

of *Salmonella*, are a problem (7, 8, 12, 25, 26)

Consequently, in our study, *Salmonella* might have been acquired after exposure to human altered environments, particularly to those related to garbage and sewage (9). In two different acute dead events occured in the Bartın Port and Çorum City Landfill, *S.* Typhimurium and *S.* Hasserek were isolated, respectively. *Salmonella* can survive in the environment for long periods (29). Hence, as zoonotic pathogen *S.* Typhimurium in the Bartın harbour and possible potential epidemic of *S.* Hessarek in Çorum which is a localisation of commercial layer flocks should not be ignored.

ETHICS COMITTEE APPROVAL

* This study does not require Ethics Comittee Approval.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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