

# First Isolation of *Salmonella* Hessarek from *Sturnus vulgaris* in Turkey: A Case Report

Asiye DAKMAN <sup>1</sup>  Özlem ŞAHAN YAPICIER <sup>2</sup> Aylin YAŞARER <sup>1</sup> Metin GÜLEÇ <sup>1</sup>

<sup>1</sup> Etlik Veteriner Kontrol Merkez Araştırma Enstitüsü, TR-06020 Etlik, Ankara - TURKEY

<sup>2</sup> Mehmet Akif Ersoy Üniversitesi, Veteriner Fakültesi, Mikrobiyoloji Anabilim Dalı, TR-15030 Burdur - TURKEY

Article Code: KVFD-2016-16242 Received: 06.06.2016 Accepted: 25.10.2016 Published Online: 10.11.2016

## Citation of This Article

**Dakman A, Şahan Yapıcıer Ö, Yaşarer A, Güleç M:** First isolation of *Salmonella* Hessarek from *Sturnus vulgaris* in Turkey: A case report. *Kafkas Univ Vet Fak Derg*, 23 (2): 343-346, 2017. DOI: 10.9775/kvfd.2016.16242

## Abstract

In this paper, the first isolation of *Salmonella enterica subsp. enterica* serovar Hessarek (*S. Hessarek*) was reported from *Sturnus vulgaris* in Çorum, Turkey in 2010 and 2015. Eleven dead *Sturnus vulgaris* were necropsied, and *S. Hessarek* was isolated from seven of dead bird's tissue samples. The automated ribotyping was performed for epidemiologic typing of the all seven *S. Hessarek* isolates. The number and relationship of bands obtained by ribotyping were analyzed with a similarity index by using the Dice coefficient and unweighted pair group method with mathematical averaging (UPGMA) in GelCompar TM software. The six isolates from 2015 were evaluated as closely related. But, the other isolate apart from them was unrelated. In conclusion, we thought that there were two unrelated *S. Hessarek* outbreaks in a studied region.

**Keywords:** *Salmonella* Hessarek, *Sturnus vulgaris* (Common Starling, European Starlings), Turkey, Wild birds

## Türkiye'de İlk Kez *Sturnus vulgaris*'ten *Salmonella* Hessarek İzolasyonu: Olgu Sunumu

### Özet

Bu yazıda Çorum ilinde *Sturnus vulgaris*'ten 2010 ve 2015 yıllarında *Salmonella enterica subsp. enterica* serovar Hessarek (*S. Hessarek*)'in ilk defa izolasyonları bildirilmiştir. Onbir adet ölü *Sturnus vulgaris*'in nekropsisi yapıldı ve yedi ölü kuşun organ örneklerinden *S. Hessarek* izole edildi. *Salmonella* Hessarek izolatlarına epidemiyolojik tiplendirme için otomatize ribotiplendirme uygulandı. Ribotiplendirme ile elde edilen bantların sayısı ve ilişkileri GelCompar TM jel analiz programı kullanılarak; küme analizi Dice benzerlik katsayısı ve UPGMA ilişki kuralı parametreleriyle değerlendirildi. 2015 yılında izole edilen altı izolat birbiriyle yakından ilişkili bulundu. Fakat 2010 yılındaki izolat diğerleri ile ilişkili bulunmadı. Sonuç olarak, çalışmanın yapıldığı bölgede iki farklı salgın olduğu düşünüldü.

**Anahtar sözcükler:** *Salmonella* Hessarek, *Sturnus vulgaris* (Sığırcık, Avrupa sığırcığı), Türkiye, Vahşi kuşlar

## INTRODUCTION

*Salmonella* agents have a broad range of animal hosts, especially wild birds that are known as natural hosts for *Salmonella* throughout the world. The transmission of *Salmonella* agents from infected wild birds to the environment has a common risk for animals and humans <sup>[1,2]</sup>.

Starlings, small wild birds, are among the most widespread birds in Europe and in the urban environment, and have been nominated to the list of the "100 World's Worst" invaders by the Invasive Species Specialist Group <sup>[3,4]</sup>. Starlings damage agriculture by consuming crops destined for human and livestock consumption <sup>[5-7]</sup>. In addition to the

influence of animal and human health, these birds cause economic loss to agriculture; for example the economic loss in the U.S. was estimated at \$800 million annually <sup>[8,9]</sup>.

Salmonellosis can occur in starlings most commonly their omnivorous diet due to feed on the ground, food contaminated with fecal matter and live or feed in contaminated water <sup>[10,11]</sup>. Thus starlings can catch the disease and die or further excrete the disease through their feces to livestock, especially poultry and egg producers, and to humans <sup>[12,13]</sup>.

*Salmonella enterica subsp. enterica* serovar Hessarek (*S. Hessarek*) is not a common serotype in Turkey. It was



### İletişim (Correspondence)



+90 312 3260090



asiyedadman@hotmail.com

originally isolated from a Common Raven (*Corvus corax*) in Iran<sup>[14]</sup> and numerous occasions in outbreaks of septicemic salmonellosis in starlings<sup>[15]</sup>. It has also been reported that *S. Hessarek* caused an epidemic of salmonellosis amongst Blackbirds *Turdus merula*<sup>[16]</sup>.

This paper describes two mortality events involving *Salmonella* Hessarek in *Sturnus vulgaris* in Turkey and epidemiological typing of the isolates.

## CASE HISTORY

### Case Definition

The area involved in both mortality events is a park called Hıfzı Veldet Velidedeoğlu Park (formerly called Yunus Emre Park) in the town of Çorum (40°33'18.8"N, 34°58'5.17"E, Turkey), where eleven *Sturnus vulgaris* were found. Five of dead birds in 2010 and six dead birds in 2015 were found. Starlings fed from trash in the park and mortalities were detected in this place.

### Necropsy

Necropsies were performed on all dead birds. Among all of the examined birds, the most consistent lesion was necrosis loci on the internal organs (spleen, liver, and heart).

### Microbiological Examination

Tissue samples were inoculated on 5% blood agar and incubated aerobically at 37°C. The presumptive colonies of *Salmonella* were identified by biochemical tests: Triple Sugar Iron (Oxoid, CM0277), urea hydrolysis (Oxoid, CM0053B), H<sub>2</sub>S, indole production, ONPG ( $\beta$ -galactosidase; Oxoid, DD0013), lysine decarboxylase (Oxoid, CM038) and Voges-

Proskauer (Oxoid, CM0043) tests<sup>[17]</sup>. Then all presumptive *Salmonella* positive isolates were confirmed serologically with polyvalent and monovalent specific somatic and flagellar antisera (Statens Serum Institut, Denmark) and serotyping was performed based on the Kauffmann-White scheme<sup>[18]</sup>. A total of seven *Salmonella* spp. were isolated from only liver samples and these isolates were identified as *S. Hessarek*, named A, B, C, D, E, F (strains were isolated in 2015), and G (strain was isolated in 2010). But, there was no isolation from spleen and heart samples.

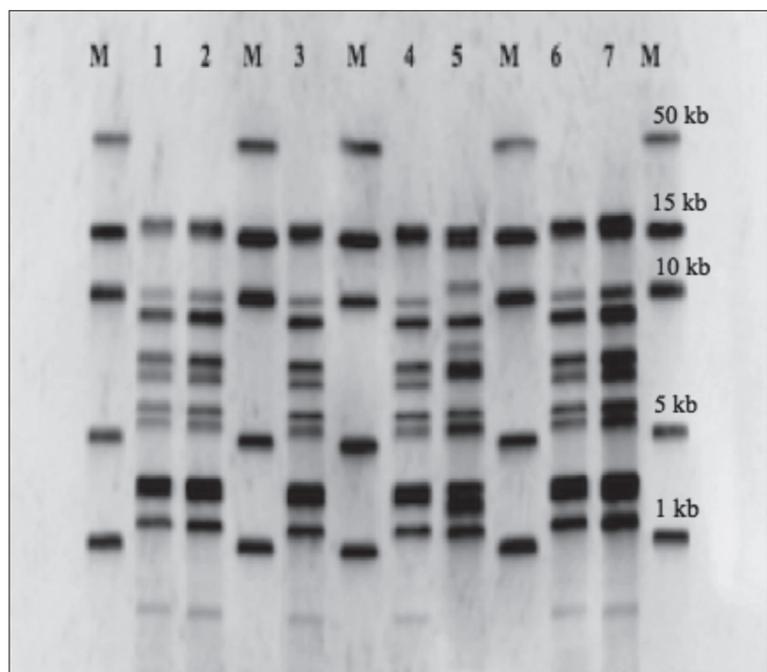
### Molecular Typing

Ribotyping was performed as described in the user manual of RiboPrinter (Dupont Qualicon, Wilmington, DE, USA)<sup>[19]</sup>. The number and relationship of bands obtained by ribotyping were analyzed with a similarity index by using Dice coefficient with optimization and position tolerance set at 1-1.5%, respectively, and unweighted pair group method with mathematical averaging (UPGMA) in using Gelcompar II (version 6.5; Applied Maths, Saint-Martens-Latem, Belgium) software. According to the automated ribotyping, seven isolates separated into one cluster and one unique ribotype with a coefficient of similarity of 70%. The cluster included six isolates (A-F) that had been isolated in 2015. The unique ribotype, included one isolate (G) that had been isolated in 2010 (Fig. 1, Fig 2). The isolate A and B were showed 100% similarity. Also, the isolates C to F were 100% similar. The similarity of these two groups was 95.2%.

## DISCUSSION

In this study, *Salmonella* Hessarek was isolated from starlings for the first time in Turkey. *Salmonella* Hessarek was isolated from dead starlings at an interval of five years.

**Fig 1.** Banding patterns of *S. Hessarek* generated by PvuII. M: molecular size marker (kilobases). Lanes 1- 6: *S. Hessarek* isolates from 2015; Lane 7: *S. Hessarek* isolate from 2010





**Fig 2.** The dendrogram of *S. Hessarek* isolates that were analyzed by ribotyping

Similarity is high between two *S. Hessarek* isolates in 2010-2015. It is showed that similar strains could lead to birds in two cases and these strains could be persistent in this area. Therefore, it was revealed that migrating birds were exposed continuously to these strains. A similar study was performed in wild birds, Song Thrushes, in Spain [20]. *S. Hessarek* was isolated from wild birds in two cases and had high similarity between isolates with pulsed field gel electrophoresis (PFGE).

*Salmonella* Hessarek was isolated for the first time in Iran from a raven (*Corvus corax*) in 1953 [4]. However the first isolation of *S. Hessarek* from European Starlings in Isreal was notified that starlings as a reservoir for human [15]. Birds can acquire these pathogens from contaminated environments and spread it directly to humans or indirectly by contaminating commercial livestock vehicles [21]. In particular, the transmission route of *Salmonella* to humans occurs both by direct contact to contaminated fecal materials and by consumption of their contaminated meat [22].

In this study, there was no data about course of the disease in both cases. It was detected only in necrotic foci on internal organs, especially the liver, spleen, and heart. Other studies have reported that *Salmonella* Hessarek also can cause poor body condition, splenomegaly, moderate to severe pectoral muscle atrophy, hepatomegaly, fibrinous serositis, and hemorrhage in the proventriculus or intestine [15,20].

It has been stated that PFGE and RAPD were performed for diversity of *Salmonella* serovars, especially clonally related *Salmonella* isolates from the environment and feces or wildlife reservoirs that affected public health [13,23]. Ribotyping was an essential method for epidemiological typing in this study [24,25]. *Salmonella* Hessarek isolates were separated into seven ribopatterns and two clusters although they had similar profiles. This shows that in 2010-2015, isolates could have come from a different sources.

In conclusion, this knowledge may help to further identify potential epidemic *Salmonella* Hessarek in the wild, and control measures against the migration routes of wild birds avoid transmission of this infection both in humans and animals.

## ACKNOWLEDGEMENTS

We thank to DVM, M.Sc. Selahattin Şen who is the head of Bacteriological Diagnostic Laboratory of Etlik Veterinary Control Central Research Institute, for his contribution.

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