



Investigating the Presence of *Salmonella* spp. in the Feces of Red Deer (*Cervus elaphus*) in Istanbul

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Abstract: Wildlife is an important source of many infectious agents for domestic animals and humans. Increasing interactions between humans, domestic animals, and wildlife may cause the infection of contagious agents between the species. Domestic animals can be easily infected with *Salmonella* serovars as a result of its asymptomatic presence in the intestinal flora of wild animals and its spreading around. The presence of *Salmonella* species in the feces of red deer (*Cervus elaphus*), which is an important member of wildlife in Turkey, was investigated in the present study. A total of 240 fecal samples were collected in sterile dry plastic containers from free-ranging deer at the Deer Breeding Farm in Istanbul Belgrad Forest, once a month for a year. The samples collected were examined through conventional culture and molecular methods (PCR). *Salmonella* spp. was not isolated from the samples examined. As a result, considering that wildlife may pose a potential health risk in terms of domestic animals and public health, it will be beneficial to conduct studies on both *Salmonella* agents and all bacterial agents in deer in terms of wildlife and public health.

Keywords: Deer, feces, isolation, PCR, *Salmonella*.

İstanbul'da Bulunan Kızıl Geyiklerin (*Cervus elaphus*) Dışkılarında *Salmonella* spp. Varlığının Araştırılması

Öz: Yaban hayatı, hem evcil hayvanlar hem de insanlar için birçok enfesiyöz etkenin önemli bir kaynağıdır. İnsanlar, evcil hayvanlar ve yaban hayatı arasındaki artan etkileşimler, enfesiyöz etkenlerin türler arası bulaşmasına neden olabilmektedir. *Salmonella* serovarlarının yaban hayvanlarının barsak florasında asemptomatik olarak bulunması ve çevreye yayılması sonucu kolaylıkla evcil hayvanlara bulaşabilmektedir. Bu çalışmada Türkiye'de yaban hayatının önemli bir üyesi olan kızıl geyik (*Cervus elaphus*) dışkılarında *Salmonella* türlerinin varlığı araştırıldı. İstanbul Belgrad Ormanı'ndaki Geyik Üretim Çiftliği'nde serbest halde yaşayan geyiklerden bir yıl boyunca her ay bir kez olmak üzere steril kuru plastik kaplara toplamda 240 dışkı örneği toplandı. Toplanan örnekler konvansiyonel kültür yöntemleri ve moleküler yöntemler (PCR) ile incelendi. İncelenen örneklerden *Salmonella* spp. izole edilmedi. Sonuç olarak yaban hayatının, evcil hayvan ve halk sağlığı açısından potansiyel bir sağlık riski oluşturabileceği göz önüne alındığında geyiklerde sadece *Salmonella* etkenlerinin değil tüm bakteriyel ajanlarla ilgili çalışmaların yapılması hem yaban hayatı hem de halk sağlığı açısından yararlı olacaktır.

Anahtar kelimeler: Dışkı, geyik, izolasyon, PCR, *Salmonella*.

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INTRODUCTION

Wildlife is an important source of infectious diseases with which humans can be infected. These diseases may include rabies, avian flu, cryptosporidiosis, Lyme disease, and salmonellosis, which are important for human life (Jijón et al., 2007). Salmonellosis is one of the important food-borne infections (Scallan et al., 2011). It is known that *Salmonella* species are enteric zoonotic pathogens that are widely found in the environment, wildlife, and domestic animals. *Salmonella* species are a member of the Enterobacteriaceae family and they are non-spore-forming and Gram-negative facultative anaerobic bacilli (Cakin et al., 2020). It has approximately 2600 serovars, of which most belong to the *Salmonella enterica* subspecies (Albufera et al., 2009; Botti et al., 2013; Rubini et al., 2016).

Salmonellosis is a globally significant disease caused by *Salmonella* species that emerges by vectors or by spreading between the species. Many serovars within the *Salmonella* species are pathogenic only in animals, only in humans, or in both animals and humans. *Salmonella* spp. can widely infect numerous living beings from domestic animals to reptiles, wild birds, and mammals. Moreover, it can be isolated from the intestinal contents of birds and mammalians. Infectious pathogens originating from wildlife are attracting attention all around the world, mostly due to their zoonotic potential in the breeding and health of farm animals (Hilbert et al., 2012).

Most of the domestic and wild animals with colonized *Salmonella* species carry this agent in their gastrointestinal system without showing any clinical symptoms and contaminate the environment (Sanchez et al., 2002). Wild animals have important ecological roles. As an example, their carrier and protective functions can be specified for the identification of infectious agents, which are important in terms of environmental health, showing that wild animals have global importance in the epidemiology of salmonellosis. Thus, wildlife is important in the One Health concept (Iovine et al., 2015).

When considering the effect of wildlife on carrying and contaminating the pathogenic agents such as *Salmonella* spp. to humans and domestic animals, the number of studies examining *Salmonella* species from wild animals in Turkey is limited. In the present study, the presence of *Salmonella* spp. was investigated in the fecal samples taken from the free-ranging deer at the Deer Breeding Farm at Istanbul Belgrad Forest.

MATERIAL AND METHOD

In this study, a total of 240 fecal samples, including 20 samples per month, were collected from free-ranging wild red deer (*Cervus elaphus*) in an area of 100

hectares in Bahçeköy Deer Breeding Farm, under the body of the General Directorate of Nature Conservation and National Parks of the Ministry of Agriculture and Forestry in Istanbul Belgrad Forests between April 2019 and March 2020 for the isolation and identification of *Salmonella*. Fresh fecal samples collected every month regularly from different regions of a 100-hectare land were taken into sterile dry vessels containing feces and delivered to the laboratory as soon as possible within cold chain (Carroll et al., 2015).

Among the samples, isolation of *Salmonella* species were performed according to the Protocol of the World Health Organization on *Salmonella* spp. isolation from Food and Animal feces (WHO 2010). The samples delivered to the laboratory were incubated for 18-20 hours at 37°C in Buffered Peptone Water for pre-enrichment. After the pre-enrichment process, cultures were passaged into Tetrathionate Broth (Oxoid) and Rappaport-Vassiliadis Soya Broth (Hi-Media) for selective enrichment and incubated for 18-24 hours at 37°C and 41.5°C, respectively. Hektoen Enteric Agar (Condalab) including novobiocin (15 µg/ml final concentration) and MacConkey Agar (Hi-Media) were used for the selective agar for isolation (Cakin et al., 2020). Black colonies on Hektoen Enteric Agar and lactose-negative semi-transparent yellowish colonies on MacConkey Agar were passaged to obtain pure cultures. As a result of the biochemical tests performed from the pure cultures, the isolates which were urease negative and methyl red, citrate positive, and formed H₂S and gas in Triple Sugar Iron Agar, were evaluated as presumptive *Salmonella* spp. These isolates were examined by PCR by using *Salmonella* spp.-specific *invA* gene region primer-139 (5'GTG AAA TTA TCG CCA CGT TCG GGC AA3') and primer-141 (5'TCA TCG CAC CGT CAA AGG ACC C3') (Rahn et al., 1992).

As a positive control, *Salmonella* Typhimurium ATCC 14028, from the culture collection of Department of Microbiology, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, was used. *Salmonella* spp.-specific gene region was sought in the electrophoresis that was conducted after the DNA extraction and DNA amplification procedures.

RESULTS

According to the colony morphology, 67 presumptive- *Salmonella* isolates were detected in 240 fecal samples examined. As a result of the biochemical tests conducted on these isolates, 14 *Salmonella* spp.-suspicious isolates were found. No *invA* gene region was found in the isolates as a result of the PCR applied for the *invA* gene region. *Salmonella* spp. was not isolated in the 240 fecal samples examined.

DISCUSSION AND CONCLUSION

Wildlife is an ecosystem formation that naturally hosts plants and animals. It is possible to classify the animals in this formation as mammalians, birds, reptiles, and invertebrates (Oğurlu, 1988). Deer, one of the most well-known members of wildlife, are animals that identify the *Cervus* genus in the family Cervidae in the class mammals (Linnaeus, 1758) (ITIS, 2012).

Among the important bacterial diseases affecting deer, various infections such as bovine tuberculosis, paratuberculosis, yersiniosis, leptospirosis, brucellosis, pasteurellosis, anthrax, colibacillosis, malignant edema, tetanus, and soft kidney disease can be regarded. Tuberculosis, brucellosis, salmonellosis, and anthrax are also important infections in terms of public health (Mackintosh et al., 2002; Ayanegui-Alcerreca., 2007; French et al., 2010; Sieber et al., 2010). *Salmonella* species, members of Enterobacteriaceae family, are primarily colonized in small intestine of different animal types and they are non-spore-forming and Gram-negative bacilli (Popoff & Le Minor, 2015). Rapid spreading of *Salmonella* serovars among the animals and their zoonotic characters are important risk factors in terms of human and animal health. *Salmonella* species can affect many mammalians, including deer (Sato et al., 1999).

The number of studies conducted for the presence of *Salmonella* species in deer is quite limited throughout the world. Renter et al. (2006) isolated *Salmonella* serovars in five of 500 fecal samples (*S. Lithfield*, *S. Dessau*, *S. Infantis* and *S. Enteritidis*) and Sato et al. (1999) isolated *S. Typhimurium* from the internal organs of 7 deer that died in a park with 30 deer in total. While Henderson and Hemmingsen (1983) did not isolate *Salmonella* spp. from 3810 deer feces collected from farms, Lillehaug et al. (2005) did not isolate *Salmonella* spp. from 618 deer feces. The limited number of studies have revealed that isolation of *Salmonella* agents from the deer feces is relatively low (Henderson & Hemmingsen 1983; Sato et al., 1999; Renter et al., 2006). Besides, Sato et al., (1999) highlighted that *S. Typhimurium* may be responsible for the deaths in deer depending on both agent isolation and histopathological findings. The presence of *Salmonella* agents in other members of wildlife is also striking. While Simpson et al. (2018) identified *S. Paratyphi B Java* and *S. Wangata* serovars from wild mammals, birds, and reptiles, Gorski et al. (2011) reported that they detected *S. Give*, *S. Typhimurium*, *S. Montevideo*, *S. Kumasi*, *S. Kentucky*, and *S. Infantis* serovars from wild birds, jackals, deer, wild boars, and skunks. Farias et al. (2014) examined 225 fecal samples of wild animals and isolated *Salmonella enterica* serovars in 56 of them (*S. Typhimurium* (64.3%), *S. Newport* (32.1%), and *S. Heidelberg* (5.3%)). Onbüyük et

al., (2020) examined 180 fecal samples of wild birds and detected *Salmonella* serovars in 16 (8.9%) feces.

In the present study, *Salmonella* spp. was not isolated from the deer feces that were collected from the free ranges of the deer living at Bahçeköy Deer Breeding Farm at Istanbul Belgrad Forest. Other studies, in which *Salmonella* agents were not isolated, reported that deer cannot carry these agents for a long time, which may be associated the absence of gallbladder in deer (Henderson & Hemmingsen 1983). A study conducted in 2010 reported that *Salmonella* agents can be carried and colonized through the gallbladder tissue and gallstones, which supports these hypotheses (Crawford et al., 2010). Another study was stated that direct PCR from feces, in addition to bacteriological culture study, may be effective in increasing the chance of identification (Renter et al., 2006).

The number of deer, which has decreased due to overhunting and the destruction of their habitats, has been increased by the studies carried out in deer breeding centers established in various regions of Turkey. Increasing the number of deer may pose a risk in terms of both human health and domestic animal health and also food hygiene. Factors increasing the contact of humans with wildlife, such as the expansion of urban borders and the destruction of forest areas, can affect the spread of infectious agents and the epidemiology of zoonotic agents (Dos Santos et al., 2020). Although there is no direct contact with infected animals, various insects, reptiles, and birds may infect humans, domestic animals, and animal food products with *Salmonella* agents (Skov et al., 2008). In addition, hunting activities can also pose a risk to human health (Paulsen et al., 2012). It is thought that the presence of *Salmonella* serovars in wildlife may be responsible for the infected domestic animal herds and human activities (Sato et al., 1999; Scallan et al., 2011). When the *Salmonella* cycle between wildlife, humans, and domestic animals is examined, it is thought that wildlife may have an important role as a transmission vector for salmonellosis seen in humans. These transmission pathways include direct contact of wildlife with humans, relation with farm animals as a vector of accumulation and contamination, and contamination of food and food production units. Free-range of farm animals and free land conditions, which are one of the requirements of organic agriculture, increase the contact of farm animals and wildlife as a part of this cycle. Bi-directional contamination seen as a result of this contact causes the spreading of *Salmonella* agents. In addition, it is thought that the wild animals that carry the agent to the water resources such as irrigation ponds, canals, and dams may have a direct or indirect role in the contamination cycle of *Salmonella* agents (Henderson & Hemmingsen 1983; Hilbert et al., 2012; Dos Santos et al., 2020).

The study conducted by Carillo-Del Valle et al at the wild animal breeding center in Mexico (2016) reported some pathogenic *Escherichia coli* serotypes in the feces of red deer. Interestingly these strains were similar to human strains detected in Mexican people, suggesting wildlife may cause a potential health risk for domestic animals and public health.

Considering the factors such as the limited number of studies on bacterial diseases of deer in Turkey, growing of animal population, increasing wildlife and human interaction, it will be beneficial to conduct studies on both *Salmonella* agents and all the bacterial agents in terms of wildlife and public health.

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