



## First Isolation of *Salmonella enterica* Serotype Newport (Sn) From Land Turtles (*Testudo Graeca Ibera*) Cultured in Turkey

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### ABSTRACT

Turtles are important carriers of *Salmonella* agents worldwide. In this study, fecal swab samples were taken from 43 adult turtles from a turtle farm in the west of Turkey. These examples were investigated for *Salmonella* spp. with bacteriological methods. *Salmonella enterica* serotype Newport (SN) were isolated from 6 out of 43 fecal swab samples (13.95%). The isolates were identified with Vitek 2 Compact fully automated identification device and with serological tests. SN causes significant food poisoning in humans through vegetables such as lettuce and cucumber. These data show that turtles can play a porter role in *Salmonella* infections in our country and can infect people through vegetables that grow in the soil. Although studies around the world show that SN infects people with vegetables, there is little data on the role of turtle porters in infection. This study also makes a contribution as it provides more data on a relatively overlooked subject. More detailed studies should be carried out to reveal the relationship between vegetables grown in the soil, turtles and Salmonellosis in Turkey. SN is also important with its rapidly developing antibiotic resistance.

**Keywords:** *Salmonella enterica* serotype Newport (SN), *Testudo graeca iberica*, Turtle

### ÖZ

## Türkiye’de Kültüre Edilen Kara Kaplumbağalarından (*Testudo Graeca Ibera*) İlk *Salmonella enterica* Serotype Newport (Sn) İzolasyonu

Kaplumbağalar dünyada *Salmonella* etkenlerinin önemli taşıyıcılarıdır. Bu çalışmada, Türkiye’nin batısındaki bir kaplumbağa çiftliğindeki 43 yetişkin kaplumbağadan fekal sıvab örneği alındı. Bu örnekler *Salmonella* spp. yönünden bakteriyolojik yöntemlerle muayene edildi. Kırk üç fekal sıvab örneğinin 6’sından (%13.95) *Salmonella enterica* serotype Newport (SN) izole edildi. İzolatlar Vitek 2 Compact tam otomatik identifikasyon cihazı ve serolojik testlerle tanımlandı. SN, marul ve salatalık gibi sebzeler vasıtasıyla insanlarda önemli gıda zehirlenmelerine neden olmaktadır. Bu veriler ülkemizde *Salmonella* enfeksiyonlarında kaplumbağaların portör rolü oynayabileceğini ve toprakta yetişen sebzeler aracılığıyla insanları enfekte edebileceğini göstermektedir. Ayrıca dünyadaki çalışmalar SN’nin sebzelerle insanlara bulaştığını gösterse de bulaşmada kaplumbağa portörlüğü hakkında az sayıda veri vardır. Bununla birlikte bu ülkemizde toprakta yetişen bitkiler, kaplumbağalar ve salmonellosis arasındaki ilişkiyi açığa çıkarabilmek için daha detaylı çalışmaların yapılması gerekmektedir. SN aynı zamanda çok hızlı gelişen antibiyotik dirençliliği yönüyle de önem arz etmektedir.

**Anahtar Kelimeler:** *Salmonella enterica* serotype Newport (SN), *Testudo graeca iberica*, Kaplumbağa

### INTRODUCTION

*Salmonella* is a Gram-negative, facultative intra-cellular bacterium which may penetrate ocular, nasal, oral or intestinal mucous membranes. Salmonellosis is an important problem in global public health. In the United States, Salmonellosis causes 1.4 million gastroenteritis cases (Mead et al. 1999) annually and several billion dollars of economic loss (Voetsch et al. 2004). 35% of these *Salmonella* cases result with hospitalization and 25% with death while 40% respond quickly to treatment (Scallan et al. 2011). According to CDC (2006), *S. newport* ranked in

the top three *Salmonella* serotypes associated with foodborne outbreaks in the United States. In U.S.A, childhood *Salmonella* cases were reported to be caused by turtles and turtle rearing was banned till the establishment of *Salmonella*-free turtle farms. Turtles are also kept as pets in gardens and homes because they are believed to bring good luck in Europe. Although there are no such beliefs in our country, turtles live widely in nature and contaminate food products grown on the ground with their fecal matter. Kristina (2014) and Angelo et al. (2014) have previously explained that SN with PFGE pattern JJPX01.0061 (outbreak strain) is the most pathogenic

strain of SN. This agent is also reported to be a source of contamination for vegetables grown in soil such as cucumbers. A total of 275 SN intoxications were reported in 29 districts in Columbia. Bayer et al. (2011) reported a *S. newport* outbreak originating from Brussels sprouts in Germany. In addition there is evidence of the spread of SN through turtles (Hemmes 1958).

There is no report on SN isolation from turtles in Turkey. The aim of this study is to report the first isolation of SN in cultured turtles in Turkey and point out the fact that turtles can contaminate vegetables with SN.

## MATERIALS and METHODS

### Materials

43 adult turtles randomly selected from a turtle farm in the west of Turkey were used in this study. Fecal swab specimens taken aseptically were transported to the laboratory under cold chain. Main stocks of turtles in this farm were brought from all over Turkey. Farm environment was observed to be identical to natural conditions and the turtles lived in the soil under the trees (Figure 1). The population on the farm varied between 10-50.000 individuals for export periods.



**Figure 1.** An image of the turtle farm in which the study was carried out (Turtles living in a natural habitat).

### Methods

#### Isolation of *Salmonella* spp.

Swab samples were transferred to 9 ml Buffered Peptone Water (BPW) and incubated aerobically for 16–18 h. at 37°C. Then, 0.1 ml of the incubated BPW was inoculated into Rappaport–Vassiliadis Medium (Rappaport–Vassiliadis Broth (RVS), Lab M, Lancashire, UK), and incubated at 41.5°C for 24 h. 1.0 ml of the incubated RVS was inoculated in Muller-Kauffmann Tetra-Thionate-Novobiocin Broth (MKTT-Lab M, Lancashire, UK), and incubated at 37°C for 24 h. Then plating on Xylose Lysine Deoxycholate Agar (XLD) and Mac Conkey Agar was carried out and they were aerobically incubated at 37°C for 24 h (ISO 2002).

#### Identification of *Salmonella* isolates

We used Microscopy, Vitek 2 Compact device and serological methods for identification. Serology was performed using slide agglutination tests with polyvalent somatic and flagellar antisera (SIFIN, Berlin, Germany) according to Kauffmann-White serotyping scheme (Popoff 2001). Isolates' antibiotic susceptibility patterns were investigated with the disc diffusion method (Koneman

1997) and zones were evaluated according to reference values (CLSI 2000).

## RESULTS

In this study, 6 SN were isolated from fecal swab samples of 43 adult turtles. *Salmonella* isolates were found to be susceptible to ceftiofur (30 µg) and gentamycin (10 µg), and resistant to florfenicol (30 µg), penicillin G (10 µg), streptomycin (10 µg), oxytetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), amoxicillin (25 µg) and enrofloxacin (5 µg) (Oxoid).

## DISCUSSION

Kristina (2014) and Angelo et al. (2014) stated in their study that people diagnosed with SN were infected with vegetables which caused the food poisoning epidemic. However, researchers have not touched on the topic of contamination of vegetables with SN, which could be caused by turtles. On the other hand, our findings direct our attention to turtle portership. Turtles are important carriers of *Salmonella*. They have a fondness for raw vegetables such as lettuce and cucumbers. They move with their cloacae in close contact with the ground. Therefore, they are prone to be carriers of pathogens leading to food poisoning. Although turtles' natural habitat includes Turkey's agricultural land, similar food poisonings are too sporadic to constitute a significant threat to public health. However, a proper ecological risk/benefit assessment should be taken into account if they are to be included in disease monitoring/combat programs. SN isolates resistant to at least nine antimicrobials (including extended-spectrum cephalosporins), especially, known as serotype Newport MDR-AmpC isolates, have been rapidly emerging as pathogens in both animals and humans throughout the United States (Zhao et al. 2003).

Antibiotic resistant foodborne pathogens can be associated with an increased risk of hospitalization for infected people. Furthermore, a large proportion of resistance mechanisms are encoded on genetic mobile elements, which can be transmitted horizontally to other bacteria potentially present in the intestinal tract (CDC 2006). The fact that *Salmonella* bacteria isolated in the study is resistant to ceftiofur and other antibiotics but susceptible to gentamycin supports this data. On the other hand, turtle diseases are among the least studied diseases in our country. Therefore, this study includes original data. This study may also help new business ideas flourish in our country because there is only one turtle farm in Turkey and the export aspect is strong.

## CONCLUSION

SN were isolated from 6 out of 43 fecal swab samples (13.95%) of adult turtles in a turtle farm in Turkey for the first time. It is also evident that the turtles in our country can infect humans with SN through raw vegetables. In addition, further studies are necessary to reveal the relationship between vegetables grown in the soil, turtles and Salmonellosis in Turkey.

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