

Short Communication / Kısa Bilimsel Çalışma

First isolation of enteropathogenic *Escherichia coli* from land turtles (*Testudo graeca ibera*) cultured in Turkey

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Summary: Enteropathogenic *Escherichia coli* was isolated from the internal organs and intestinal contents of 3 land turtles submitted to the laboratory for diagnostic purposes from a turtle farm located in western Turkey. As a result of the antibiotic susceptibility testing, isolates showed intermediated susceptibility to ceftiofur and gentamycine. This is the first report of the disease in turtles in Turkey.

Keywords: *Escherichia coli*, *Testudo graeca ibera*.

Türkiye’de kültürü yapılan kara kaplumbağalarından (*Testudo graeca ibera*) ilk enteropatojenik *Escherichia coli* izolasyonu

Özet: Türkiye’nin batısındaki bir kaplumbağa çiftliğinden hastalık teşhisi amacıyla gönderilen üç yavru kara kaplumbağasının iç organ örnekleri ve bağırsak içeriklerinden enteropathogenic *Escherichia coli* izole edildi. Yapılan antibiyogram testinde izolatların ceftiofur ve gentamisine orta derecede duyarlı olduğu tespit edildi. Bu veriler ülkemizde kaplumbağalardan sağlanan ilk hastalık verileridir.

Anahtar sözcükler: *Escherichia coli*, *Testudo graeca ibera*.

Turtles are reared as pets as well as being wild animals. Turtle farms exist in many countries (China, Japan, Southeast Asia, United States, Caribbean, Europe) including Turkey. Rearing these animals with a high population density may cause an increase in disease prevalence. *Aeromonas* spp., *Pseudomonas* spp., *Citrobacter* spp., *Mycoplasma* spp., are among the most important bacterial agents of turtles (6, 8, 10). Enteropathogenic *E. coli* is the other microorganism closely associated with mortalities in turtles (12, 13).

Turtles can be carriers for many zoonotic bacteria. Most important ones are *Edwardsiella* spp., *Mycobacterium* spp., and *Salmonella* spp. In the United States of America, in 1960’s 14% of *Salmonella* cases in children were related to turtles; as a result the turtle production lines were prohibited till the establishment of *Salmonella* free farms in 1971 (2, 5, 10). However, enteropathogenic *E. coli* found incidentally in turtles, especially can cause infections in children who play with them.

Economic impact of turtle farming is also important. The farm where this study was carried out had a population of 50.000 land turtles and the annual export

revenues to European countries were stated to be around 100.000 Euros. The choice of turtles as pets in Europe may be due to their easy maintenance and lack of any harmful impact on the environment as well as a superstition about turtles bringing luck to the household (6).

Still, turtle farming is relatively new in Turkey and the diseases of the land tortoise is one of the least studied areas. The aim of this study is to present a case caused by enteropathogenic *E. coli*, to create awareness about turtle farming and to draw attention to the zoonotic diseases of turtles.

In this work, after mortalities occurred in turtles reared in a farm in western Turkey, 3 newly dead turtles (1 adult, no 3; 2 juveniles, no 1 and 2) were submitted to the laboratory. Case history revealed that mortalities occurred in adults within few days following diarrhea and without any symptoms in the young. During the necropsy, after an incision in the midsection of the bodies, internal organs were exposed. Macroscopically; exudate formation in the lungs and enteritis were the findings in the adult, while in the young, enteritis was observed (Figure 1). Liver, spleen and lung samples were

streaked onto Columbia blood agar (Oxoid) and MacConkey agar (Merck) plates. Also, enrichment of intestinal samples was carried out in Rappaport Vassiliadis (RVS) Broth (Merck) and Muller Kauffmann Tetrathionate Broth (Oxoid), afterwards inoculations from these media were made on MacConkey Agar. Inoculated media were incubated under aerobic conditions at 37°C for 24 hours (12).

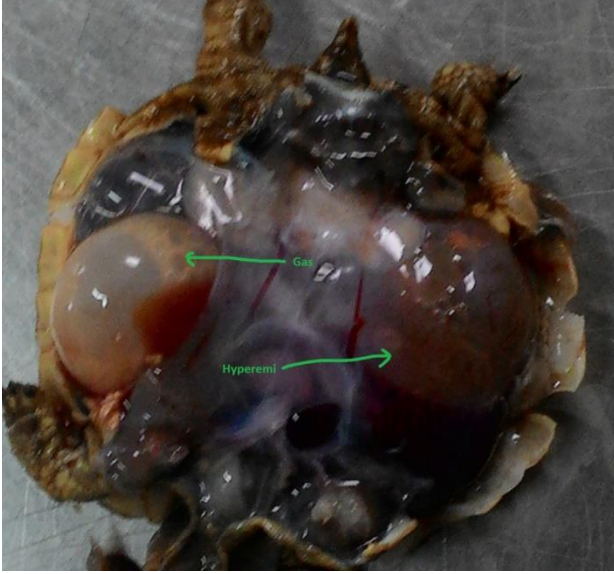


Figure 1. Enteritis in the young (number 1).

Şekil 1. Genç kaplumbağada enteritis (1 nolu kaplumbağa).

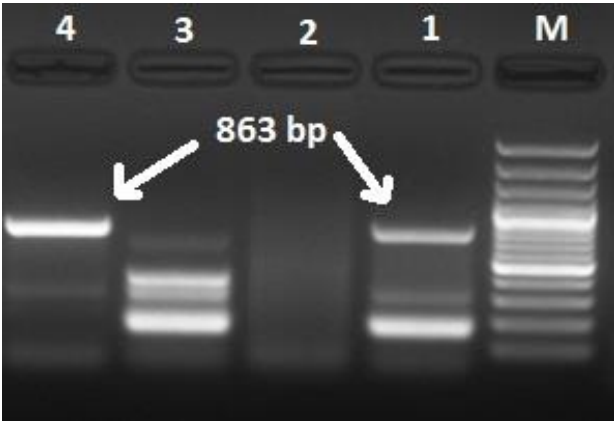


Figure 2. The gel electrophoresis image showing the presence of *eae* genes in *E. coli* isolates from turtles. M: Marker, 100 bp. Line 1: Positive control, *E. coli* field isolate (from no 1 turtle), 863 bp. Line 2: Negative control, distilled water. Line 3: Negative control, no enteropathogenic *E. coli* isolate (from a lamb), Line 4: *E. coli* field isolate (from no 1 turtle), 863 bp.

Şekil 2. Kaplumbağalardan izole edilen *E. coli* izolatlarındaki *eae* gen varlığını gösteren jel elektroforez resmi. M: Marker, 100 bp. Sütun 1: Pozitif kontrol, *E. coli* saha izolatu (1 no' lu kaplumbağadan), 863 bp. Sütun 2: Negatif kontrol, distile su. Sütun 3: Negatif kontrol, enteropatogenik olmayan *E. coli* suşu (kuzudan), Sütun 4: *E. coli* saha izolatu (2 no' lu kaplumbağadan), 863 bp.

Colonies were identified by conventional microbiological methods (3, 12) and Vitek 2 Compact identification system. According to these results *E. coli* was isolated from the internal organs and intestinal contents of turtles. After isolates were subcultured on Minca Agar (Sifin), serum agglutination test was performed with K99 antiserum (7). *E. coli* B41 reference strain (0101:K99/F41) was used as a positive control. The presence of *eae* gene encoding the intimin protein to verify enteropathogenic features of the *E. coli* isolates was investigated with PCR (14). As positive control, one of *E. coli* isolates forming the clear band in the PCR. As negative control, distilled water and no enteropathogenic *E. coli* isolate were used.

The primer sequences were SK1-fo CCCGAATTCCGGCACAAGCATAAGC and SK2-re CCCGGATCCGTCTCGCCAGTATTCG. PCR amplification was performed in a 25 µl volume with 1 × PCR buffer (2 mM MgCl₂), 0.2 mM of each deoxynucleoside triphosphate (dNTP), 0.2 µM of each primer, 2.5 U Taq DNA polymerase (MBI, Fermentas) and 2 ng of template DNA. The *eae* PCR assay specific for *E. coli eae* gene was performed for 30 cycles at 94°C for 30 s, 52°C for 60 s, and 72°C for 60 s preceded by an initial incubation at 94°C for 2 min and followed by a final extension step at 72°C for 5 min (Technne, TC-412). After PCR amplification, 4 µl of each product was added into a 1.0% agarose gel, electrophoresed (Thermo, Primo™). DNA sizemarker 100 DNA Ladder (MBI Fermentas) was used. Bands (863 bp) were visualised with designated equipment (Vilber Lourmant, E-BOX VX5) (Figure 2). As a result, *E. coli* isolates from turtles were seen to be enteropathogenic. Isolates' antibiotic susceptibility patterns were investigated with the disc diffusion method (1) and zones were evaluated according to reference values (4, 9). *E. coli* isolates were seen to be susceptible to cefiofur (30 µg) and gentamycine (10 µg), and resistant to florfenicol (30 µg), penicillin G (10 µg), streptomycine (10 µg), oxytetracycline (30 µg), trimethoprim-sulfamethoxazole (25 µg), amoxycillin (25 µg) and enrofloxacin (5 µg) (Oxoid). A field visit was organized after reporting period to the farm so that test results could be relayed and discussed with the producers (Figure 3). During the inspection of the grounds, a poultry farm was seen to be located nearby the premises with its ventilation exhaust directed towards the facility. Also, a large number of stable flies were observed to swarm in the farm and producers were advised to take the necessary biosafety and hygiene measures.

Nowakiewicz et al. (11), as a result of their work on the cloacal swabs of 130 turtles raised in a controlled environment, reported the isolation of 17 different bacterial species in juvenile turtles and 36 different

species in adults. Most common isolates in juveniles were *Cellulomonas flavigena* (77/96), *Enterococcus faecalis* (96/96), *E. coli* (58/96) and *Proteus mirabilis* (41/96); while in adults, isolates showed more diversity and zoonotic quality. They were *Salmonella enterica* serovars *Newport*, *Daytona* and *Braenderup*; *Listeria monocytogenes*, *Yersinia enterocolitica*, *Yersinia ruckeri*, *Klebsiella pneumoniae*, *Vibrio fluvialis*, *Serratia marcescens*, *Aeromonas sobria*, *Aeromonas caviae*, *Hafnia alvei*, *Edwardsiella tarda* and *Citrobacter braakii*. The results obtained in this work are compatible with this report.

In this work, symptoms of the *E. coli* infection found in the turtles are generally compatible with the findings of Owuamanam et al. (12), the only exception being the exudate formation in the lungs. This presentation in the lungs can be interpreted as the existence of a secondary infection in which the agent is unable to grow due to suppression by *E. coli*. On the other hand, Owuamanam et al. (12) have stated that, enteropathogenic *E. coli* isolated from turtles could be of human or environmental origin. The enteropathogenic *E. coli* isolates from this work might also be contaminants from an external source. Animal production facilities around the tortoise farm seem to be the likeliest source. The owner's statement that the outbreak occurred after the establishment of these neighboring facilities also strengthens this possibility. Still, further phylogenetic analyses are needed for a conclusive result.



Figure 3. A view of the tortoise farm.
Şekil 3. Kaplumbağa çiftliğinden bir görüntü.

Although disinfection is of great importance in *E. coli* outbreaks, it is not considered to be a practical solution for the tortoise farm in question. As seen in Figure 3; the farming area was created by separating a part of the natural environment. The bedding made up of dirt and grass was impossible to disinfect. For this reason the farm has to be transformed into a facility where appropriate disinfection and hygiene measures can be taken. Natural environment can cause parasitic invasions followed by secondary bacterial infections. Also, the large fly population in the premises may be an important problem. For these reasons, disease control strategies should be applied in turtle farms.

As a result, enteropathogenic *E. coli* was isolated from 3 land turtles from a turtle farm in Turkey. This is the first report of a disease outbreak in farmed land turtles in Turkey.

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Geliş tarihi:16.11.2015 /Kabul tarihi:19.01.2016

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