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# Salmonella Enteritidis in Captive Siberian Tiger: A Case Report from Iran

[1]Amirhossein Jangjou, [1]Alireza Mokhtari, [1]Pourdad Panahi, [2]Mahmoud Marashi and [3]Saeed Hosseini

[1]Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Environment, Iranian Environmental Protection Organization, Tehran, Iran
[3]Department of Physiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

**Abstract:** A female tiger of Siberian (or Amur) breed holding in captivity at zoo shown diarrhea. After stool culture and isolation causative bacteria, salmonelosis with *Salmonella enteritidis* has been diagnosed by culture and serologic tests and confirmed with PCR asay. Antibiogram performed on isolated bacteria by Kirby Bauer method. Treatment started with removing chicken meat from diet and using antibiotics for ten days (Furazolidone 4mg/Kg and Ciprofloxacin 10mg/Kg ). The tiger cured successfully.

**Key words:** Salmonellosis · Tiger · Siberian tigers · *Salmonella enteritidis*

## INTRODUCTION

Performing the therapeutic actions on animals such as tigers, because of the ferocity nature, mostly living in wildlife and approaching trouble is not simply possible and considered as the unique experience of a veterinarian so could guide other veterinarians in the treatment of similar infections. This article described the incidence of salmonellosis in two Siberian tigers (in captivity) and its successful treatment. Salmonellosis is an infection with Salmonella bacteria. Salmonella spp. are typically transmitted among humans and animals via a fecal-oral route, usually through the consumption of contaminated food or water [1]. This significant disease can lead to infection and even death is a wide range of animals such as Mammals, reptiles, birds and amphibians [2, 3, 4]. Due to the economical and genetically importance of Siberian tigers, severity and complication may made by salmonellosis, Fast and effective treatment of the disease is very important [5]. So far cases of infections with salmonella different species have been reported in tigers. In December 2010 AFP reported three Bengal tigers in south Indian zoo died of salmonellosis.

**Case History:** The Tiger that this article is referred to be among a pair of male and female tiger, aged 3.5 and 4 years, respectively and both breed were Siberian tigers imported from Russia. These tigers hold in captivity at Eram zoo in Tehran after transported from Russia to Iran.

**Clinical Observation:** Salmonellosis symptoms as severe diarrhea after the first acquaintance between male and female tiger were observed. The female tiger showed mild symptoms of the disease as diarrhea and anorexia.

**Bacteriology:** Sampling and cultivating of fecal samples took place two days after symptoms observed.

**Identification of Isolated Bacteria:** One g of fecal sample placed in 10ml Rappaport in screw cap tube after 24 and 48 h incubation, subcultures were made on plates of SS and Rambach agar. The plates were incubated at 37°C for 24 h. From the plates showing suspicious growth, two colonies were cultured on SIM, MRVP, Simone citrate, Urea and TSI and were further examined by appropriate biochemical and serological tests. These colonies were then picked onto triple sugar iron agar slants and incubated. Those that gave typical reactions of acid butt, alkaline slope were tested by the usual slide agglutination method with commercially obtained Salmonella polyvalent O and H and monovalent antisera purchased from Baharafshan.

Identification of *S. enteritidis* by multiplex Polymerase chain reaction.

**DNA Preparation:** DNA extracted by boiling method. Colonies on Fresh culture of isolated bacteria (24 hours cultures) on Luria-Berthani agar picked and suspended in 200 µl of distilled Water the suspension was boiled at temperature 95°C for 10 min, Then suspensions

**Corresponding Author:** Pourdad Panahi, Department of Microbiology, Science and Research Branch, Islamic Azad University, Tehran, Iran. E-mail: pourdadp@yahoo.com.

Table 1: Nucleotide sequence and primers used for identification of *S. enteritidis* by multiplex PCR

| Primer Name | Target Gen | Sequence | Lengh | Ref |
|---|---|---|---|---|
| ST11 | Random[a] | 5'-GCCAACCATTGCTAAATTGGCGCA | 429 | [17] |
| ST14 | Sequence | 5'GGTAGAAATTCCCAGCGGGTACTGG | | |
| S1 | Spv[b] | 5'-GCCGTACACGAGCTTATAGA | 250 | [1] |
| S4 | | 5-ACCTACAGGGGCAATAAC | | |
| SEFA2 | SefA[c] | 5'-GCAGCGGTTACTATTGCAGC | 310 | [1] |
| SEFA4 | | 5'-TGTGACAGGGACATTTAGCG | | |

a. Randomly cloned sequence specific for the genus Salmonella, b. *S. enteritidis* fimbrial antigen gene (specific for the *S. enteritidis*) and c. Salmonella plasmid virulent gene

centrifuged for ten minute at 7000 RCF. 50 µl of the supernate containing extracted DNA removed to the fresh tube and stored in 4°C.

**DNA Primers:** For identification *S. enteritidis* by multiplex PCR assay three set of primers were selected: ST11-ST14 (429bp), SEFA2-SEFA4 (310 bp) and S1-S4 (250 bp) [6]. The primers sequences and their corresponding genes are shown in Tables 1.

**DNA Amplification:** Multiplex PCR was performed in a reaction of 25 µl containing reaction buffer (50 mM KCl, 1.5 mM MgCl2, 10 mM Tris-HCl pH = 8.3) (CinaGen, Iran), 2 µl of DNA sample, 200 µM dNTPs, 1 U Taq Polymerase (CinaGen, Iran) and 1 µm of each primer (CinaGen, Iran). The multiplex PCR amplification program for *S. enteritidis* confirmation was similar to the protocol by Pan *et al.* [7]. On the other hand, the multiplex PCR program for mentioned genes were 3 min at 94°C followed by 35 cycles of 30 s at 94°C, 90 s at 56°C, 30 s min at 72°C and final extension 10 min at 72°C.

The PCR product was electrophoresed in 1.2% agarosis gel (Fermentas) with 1X TBE and then stained with ethidium bromide. Stained gel washed with DW and visualized by UV light illumination (Bio- rad, Molecular Imager, Gel DocTM, XR Imaging system, USA).

**Antibiogram Assay:** Pure culture of bacteria is isolated from stool culture standardized using a McFarland turbidity standard 0.5 and Antibiotic susceptibility tests for isolates were performed according to the Kirby Bauer method [8, 9]. The Mueller Hinton agar was used as growth medium for standard disc diffusion test and growth was spread on plates with the help of a sterilized cotton swab to form a smooth bacterial lawn. Commercially prepared standard susceptibility test discs purchased from Padtan teb impregnated with known agent and strength were dispensed on the agar surface. Within 15 minutes of application of the discs, plates were incubated overnight at 37°C. Characterization of strains as sensitive or resistant was based on the size of inhibition zone around the disc compared with the interpretation standards provided by the manufacturers. antimicrobial agents (concentration in µg) used were: Neomycin10, Ampicilin 10, Erythromycin 15, Flumequine 30, Enrofolxacin 5, Lincomycine 2, Lincospectine 15/200, Gentamycin 10, Floramphenicol 30, Sultrim 25, Choloramphenicol 30, Tetracycline 30, Oxytetracycline 30.

## RESULTS

Present of Salmonella in stool cultures in male tiger were negative and female tiger was positive. Serotype of Isolated salmonella was $DO_9$. Bacteria species also confirmed by polymerase chain reaction. Multiplex PCR assay was applied to confirm *S. enteritidis* in fecal sample which were confirmed to was Salmonella positive by culture and serotyping method. Results showed isolates bacteria was *S. enteritidis* with three bands (ST11- ST14, SEFA2-SEFA4 and S1-S4) amplifying the expected 429, 310 and 250 bp fragments respectively.

**Therapeutic Actions:** Regarding the origin of *Salmonella enteritidis* contamination in most cases is poultry, the alteration was made to the diet and chicken meat were removed from food [10, 11]. According to the antibiogram result Following drug administrated and used for ten day long:

Furazolidone 4mg/Kg body weight
Ciprofloxacin 10mg/Kg bodyweight
Sign of recovery observed on 2 weeks after treatment started.

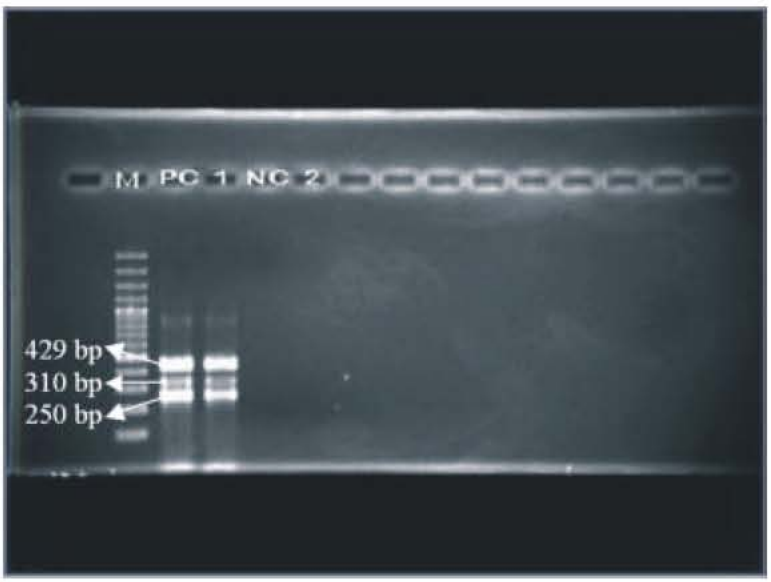


Fig. 1: Multiplex PCR with three pairs of primers for detected *S. enteritidis* isolated; M:marker 100 bp; PC: positive control; NC: negative control (*E. coli*]; lane 2: Product without the DNA template; lane 1 lane for positive *S. enteritidis*

## DISCUSSION

Salmonellosis is a disease of animals and humans caused by a group of Salmonella [1]. These can live in the intestine of a wide range of mammals, including man, as well as birds and reptiles [2, 12, 13, 14]. They may cause food poisoning when foodstuffs are contaminated with faces. Infection may also follow contact with infected animals or contaminated items or environment [15, 16, 17]. Infection is usually fairly short-lived and may not cause any obvious disease; however, moderate, severe or fatal disease may also occur with diarrhea, abortion, blood poisoning, etc. In a few cases infected animals or people may carry certain strains of the bacteria for prolonged periods [18, 1]. Over 2,500 Salmonella serovars are known [6]. Some of these affect or mainly certain species of animal; others occur in a wide range of species among the most important are *S. enteritidis* [15, 19, 1]. Salmonella is capable of prolonged survival outside the intestine [20, 21, 22, 23]. There is concern over the increasing resistance of some Salmonella serovars to antimicrobials drug [24, 25]. Due to wide range of host and easily Transfer through contaminated food and water, salmonella occurrence is fairly possible in captive animal.

As a far as we study there is no documented study of salmonella infection in tiger although there are many news reported death of tigers in zoo inconsequence of salmonelosis. According to this matter this merit case report can be useful to help veterinarian to encounter with salmonellosis in tigers. We decided to use oral antibiotic because the danger of anesthesia for this priceless animal. It seems feeding with chickens meat rise the risk of infection with salmonella in carnivorous.

**Recommendation:** Salmonella have wide range of host and tigers also can be a victim of salmonelosis if the diseases not properly managed. Finding the source of contamination and effective antibiotic for treatments are among the first action should be done. It seems for better understanding of pathogenesis and virulence factor of the isolated bacteria should be done.

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