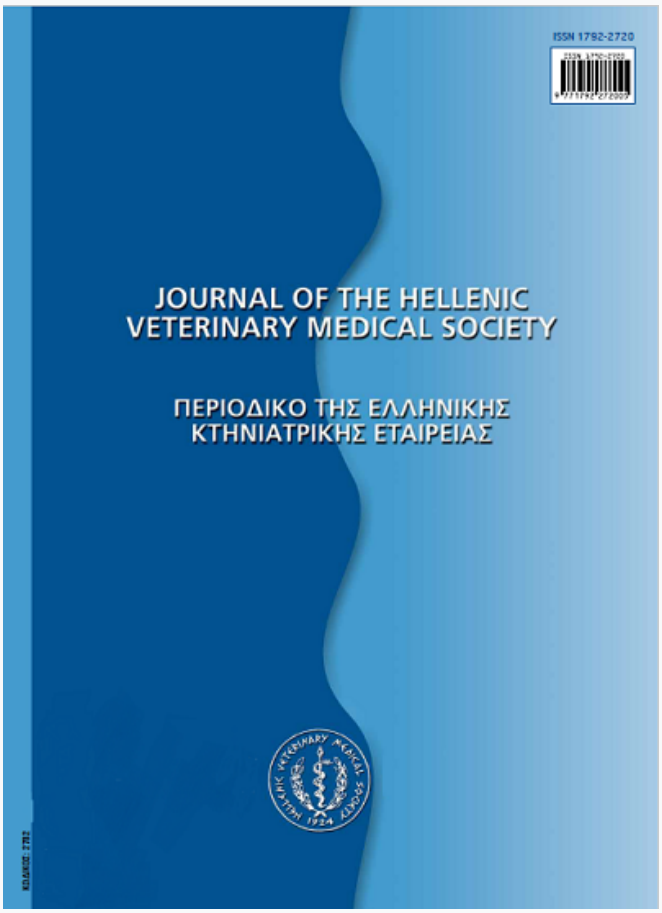
Journal of the Hellenic Veterinary Medical Society

Vol 68, No 3 (2017)



Detection of Salmonella spp. from zoo animals in Iran, determination of serovars, antibiotic susceptibility and genotyping by RAPD-PCR

B. N. FASAEI, I. A. TAMAI

doi: 10.12681/jhvms.15494

To cite this article:

FASAEI, B. N., & TAMAI, I. A. (2018). Detection of Salmonella spp. from zoo animals in Iran, determination of serovars, antibiotic susceptibility and genotyping by RAPD-PCR. *Journal of the Hellenic Veterinary Medical Society*, *68*(3), 377–384. https://doi.org/10.12681/jhvms.15494

J HELLENIC VET MED SOC 2017, 68(3): 377-384
ΠΕΚΕ 2017, 68(3): 377-384

Research article
Ερευνητικό άρθρο

Detection of *Salmonella* spp. from zoo animals in Iran, determination of serovars, antibiotic susceptibility and genotyping by RAPD-PCR

Bahar Nayeri Fasaei[1], Iradj Ashrafi Tamai[1, 2]

[1] *Department of Microbiology and Immunology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.*

[2] *Faculty of Veterinary Medicine, University of bu-ali sina, Hamedan, Iran.*

ABSTRACT. Salmonellosis is an important food-borne bacterial zoonotic disease that affects both people and animals. Contamination sources include direct or indirect animal contact. We determined and measured the risk of *Salmonella* infection from zoo animal to human contact by isolation of *Salmonella* serovars from zoo animals kept at Karaj zoo park, Iran. *Salmonella* was isolated from 21 (20%) of the 104 anal swap samples. From the 21 collected samples 4, 7 and 10 were originated from birds, mammals and reptiles, respectively. Serotyping detected nine different serotypes including *Enteritidis* (n=4), *Seftenberg* (n=1), *Typhimurium* (n=4), *Virchow* (3), *Berkeley* (n=1), *Kingabwa* (n=2), *Newport* (n=2), *Marina* (n=2) and *Havana* (n=1). All the isolates except one (serovar *Marina* subspecies *Hautenae* IV) were belonged to *Salmonella enterica* subspecies *enterica* I. *Salmonella enterica* serotypes *Typhimurium* and *Enteritidis* were the most commonly detected serotypes. All the isolates tested, were resistant to one or more antibiotics and five isolates including the monkeys' and long-eared owls' isolates were multiresistant. RAPD profiles of each isolate produced with two different primers were identical. These finding shows highly contamination of zoo animals from *Salmonella* especially from the multiresistant isolates. All of the animals in the zoo, pet owners, veterinarians, zookeepers and visitors are at high risk of salmonellosis with reptiles being the most important source of infection.

Keywords: Zoo animal, *Salmonella*, αntimicrobial sensitivity, RAPD-PCR.

Correspondence:
Nayerib@ut.ac.ir
Address: P.O.Box: 141556453
Gharib str., Azadi str., Tehran- IRAN

Date of initial submission: 12.5.2016
Date of revised submission: 13.6.2016
Date of acceptance: 18.6.2016

INTRODUCTION

Salmonellosis is one of the most important food-borne bacterial zoonotic diseases that affect both people and animals, but little is known about the role of animals as carriers of this pathogen (Jang et al., 2008; Jardine et al., 2011). *Salmonella* spp. can cause disease in humans, livestock, zoo animals resulting in morbidity and mortality as well as economic losses (Okoh and Onazi, 1980; Forshell et al., 2006). Non-typhoidal *Salmonella* serotypes are transferred to humans through food products of animal origin. Other sources include direct or indirect animal contact at homes, veterinary clinics, pet products, zoological gardens and farm environments (Friedman et al., 1998; Neera et al., 2000; Campbell et al., 2001; Pitout et al., 2003). In addition, transmission of *Salmonella* between production animals and wildlife is probable (Pitout et al., 2003). Carrier animals are very important in the epidemiology of salmonellosis, because they can be the latent carriers and shed the bacteria without any obvious clinical signs (Jardine et al., 2011). The genus *Salmonella* consists of two species: *S. enterica* and *S. bongori*. Usually serovars that belong to the *enterica* species colonize the enteric tract of warm-blooded animals, while the others that belong to *bongori* species are found in cold blooded animals (Zahraeisalehi et al., 2013). Salmonellosis outbreaks have been associated with handling mammals, reptiles and birds (chicks and wild birds) (Abalem and Solari, 2001). Zoo animals (reptiles, birds and mammals) can be infected by food and fruits provided by zoo visitors, rodents and wild birds then can transfer infection to all other animals in the same and the other cages as well as to humans (Okoh and Onazi, 1980; Jang et al., 2008). *Salmonella*, especially *S. enterica* are found in the intestine of wild birds and these infected birds can transmit infection to humans (Stephen et al., 2007). Cold-blooded animals carry a wide variety of *Salmonella* serotypes in their intestine (Tizard, 2004). They are asymptomatic carriers and may shed *Salmonella* sporadically in their feces constituting a significant zoonotic risk factor to pet owners, zookeepers, zoo visitors and veterinarians (Corrente et al., 2004; Franciscus et al., 2011).

In regard to *Salmonella* spp zoonotic importance and the presence of few studies about the prevalence of *Salmonella* serovars in zoo animals of Iran; this study aimed at the detection of *Salmonella* spp from zoo animals, the identification of the serovars, the determination of the antibiotic susceptibility, and the genotyping of isolates by RAPD-PCR.

MATERIALS AND METHODS

Isolation and identification of *Salmonella*

Fecal samples from 104 animals (birds, mammals and reptiles) housed in Karaj zoo park, Iran, were collected by anal or cloaca swabs and cultured in 10 ml pepton water (Merck, USA) at 37°C for 24 h. 1ml of this culture was then transferred to 10 ml Selenit F and Rappaport broths (Merck, USA).

Samples were incubated at 37°C and 41.5°C for 24h and then each sample was inoculated on to Salmonella-Shigella agar, Brilliant Green agar (Merck, USA) and Chromogenic agar (Chromogenic agar, Paris France) plates. The plates were incubated at 37°C for 24 h. 3-5 colonies, suspected of *Salmonella* spp, were selected and subjected to preliminary biochemical identification using Triple Sugar Iron Agar (TSI), Urea agar and Lysine Iron agar (Merck, USA) (Quinn et al., 1994).

Serotyping of the isolated *Salmonella* strains was performed by commercial reliable antisera (Difco, Detroit, USA) and the results were interpreted according to the Kaufmann-White scheme (Popoff and Le Minor, 1992).

PCR assay

The biochemically identified isolates were analysed using Polymerase Chain Reaction (PCR) for *invA* gene, using S139: (5- GTG AAA TTA TCG CCA CTG TCG GGC AA-3) and S141: (5- TCA TCG CAC CGT CAA AGG AAC C-3) primers to confirm

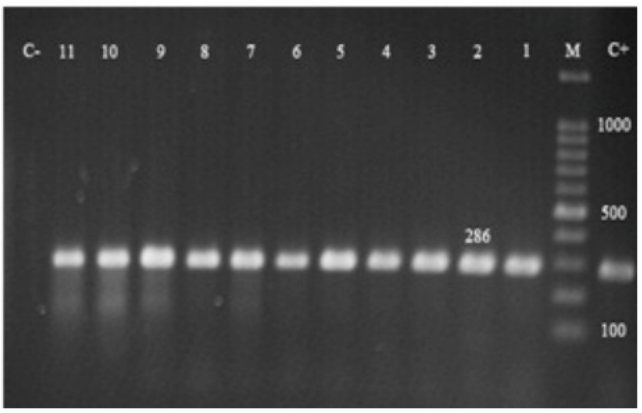


Fig 1. Results of *invA gene* fragment PCR; The expected 284 bp band was amplified from all *Salmonella* isolates. C+: positive control, C- :negative **control,** Lane 1 positive control (*S. typhimurium* (ATCC 14028), Lane 2 marker 100bp, lane 3-11 number of under study isolates, Lane 12 negative control.

Table 1: Species of the sampled animals, number of samples and number of positive results from each species of this study.

species	number	positive	species	number	positive
Long-eared owl (*Asio otus*)	5	2	Deer	4	0
Montagus barrier (*Circus pygargus*)	3	1	Elephant	1	0
Fischers lovebird (*Agapornis fischeri)*	7	1	Zebra	3	0
Golden hamster (*Mesocricetus auratus*)	7	1	Falcon	2	0
Iranian spiny-tailed lizard (*Uromastyx* asmussi)	3	2	Rabbit	10	0
Brandts hedgehog (*Paraechinus hypomelas)*	5	3	Fox	3	0
Red-eared slider (*Trachemys scripta*)	9	6	Pheasant& Pavo	20	0
Indian python (*Python molarus*)	5	2	Bear	3	0
Rhesus monkey (*Macaca mulatta*)	4	3	Chicken	10	0

the isolates at the genus level (Galan et al., 1992). A single colony of each isolate from an agar plate was picked and suspended in 200µl of distilled water. After vortexing, the suspension was boiled for 5min, centrifuged for 10 min at 15,000 g and 50 µl of the supernatant was collected. Reaction mixture consisted of 2.5 µl of 10X PCR buffer (500mM KCl, 200mM tris-HCl), 0.5 µl of dNTP mix (10mM), 2mM MgCl2, 0.5 µM of each primers, 1U of *Taq* DNA Polymerase and 1.5 µl of DNA templates. Amplification was conducted in thermocycler (Techne- TC 512, England) under the following conditions: initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 60 sec, 64°C for 30 sec, 72°C for 30 sec and final extension at 72°C for 7 min. PCR products were electrophoresed on 1.25% agarose gel stained with ethidium bromide (2µg/ml) for 15 min. PCR bands were visualized with a UV transilluminator (BIORAD, UK).

Randomly Amplified Polymorphic DNA analysis (RAPD- PCR)

To further analyse the isolates, RAPD- PCR was performed. Amplification reaction mixture contained 40 ng template DNA, 2mM MgCl2, 0.5 mM of primer, 1U of *Taq* DNA polymerase and 200µM of each dNTPs in 1X PCR buffer. The selected primers for this study were: OPA-4 (5- AATCGGGCTG-3) and 23L (5- CCGAAGCTGC-3).Thermal program and electrophoresis condition was performed as previously described by Lin et al. (1996).

Antibiotic resistance test

Antibiotics susceptibility of the *Salmonella* isolates to 14 antibiotics was determined using the disk diffusion method, as described by the Clinical and Laboratory Standards Institute (Bauer et al., 1966). The antibiotics were selected based on their application in human and veterinary medicine to control the *Salmonella* spp. The *Salmonella* isolates were cultured on tryptose soy broth (TSB) (Merck, USA) and incubated at 37°c for 1-2 hours, and then was calibrated based on the 0.5 McFarland BaSo4 turbidity standards. The suspension obtained was uniformly spread on to the surface of dry Mueller-Hinton (Merck) agar plates by an impregnate swap. Antimicrobial disks were aseptically disposed on the surface of inoculated agar media and the plates were incubated at 37°c for 24h. Diameters of the inhibition zones were measured in millimeters. The isolates were classified as susceptible (S), intermediate (I) and resistant (R) according to guideline published by CLSI.

RESULTS

21 isolates of the 104 anal swap samples (20%) identified biochemically as *Salmonella* spp. PCR test for target gene, *invA*, was performed and in all cases

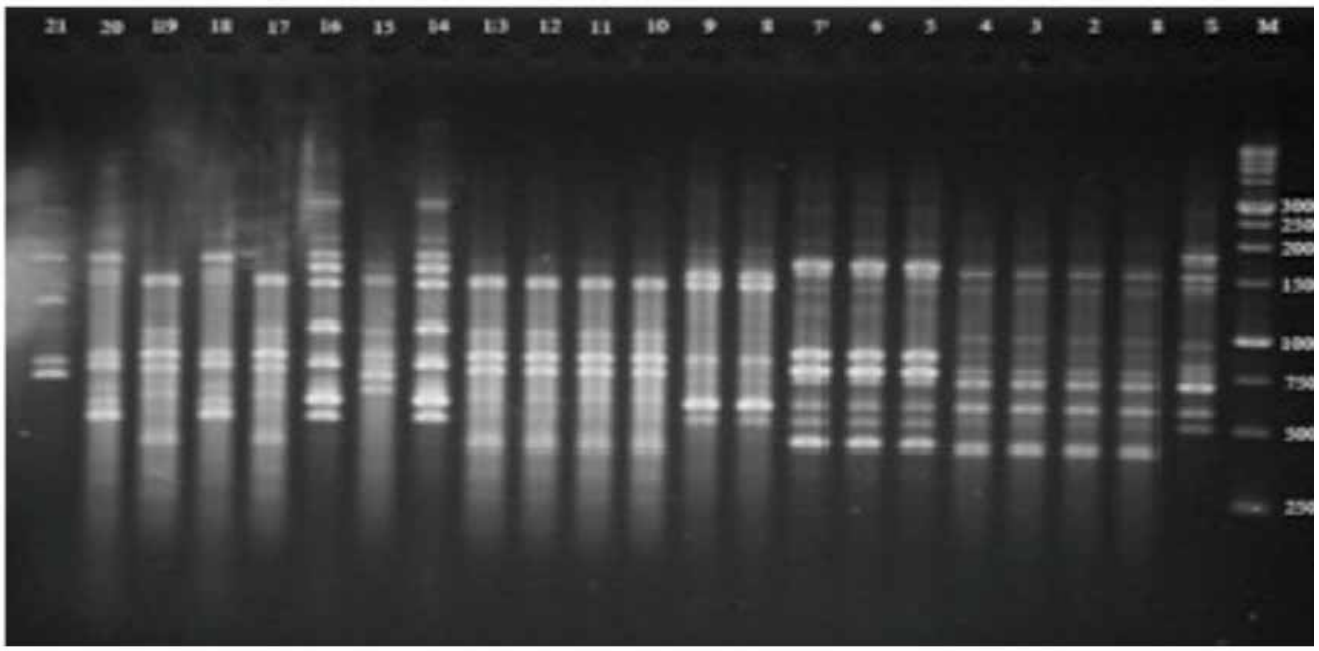


Fig 2. RAPD-PCR results of 21 *Salmonella* isolates from zoo animals with the 23L primer. Lane M Marker 1kb, Lane S *S. typhimurium* (ATCC 14028), Lane 1 Long-eared owl1, Lane 2 Long-eared owl2, Lane 3 Montagus barrier, Lane 4 Golden hamster, Lane 5 Rhesus monkey1, Lane 6 Rhesus monkey2, Lane 7 Rhesus monkey3, Lane 8 Indian python1, Lane 9 Indian python2, Lane 10 Brandts hedgehog1, lane 11 Brandts hedgehog2, Lane 12 Brandts hedgehog3, Lane 13 Iranian spiny-tailed lizard1, Lane 14 Red-eared slider1, Lane 15 Red-eared slider2, Lane 16 Red-eared slider3, Lane 17 Red-eared slider4, lane 18 Red-eared slider5, lane 19 Red-eared slider6, Lane 20 Iranian spiny-tailed lizard2, Lane 21 Fischers lovebird.

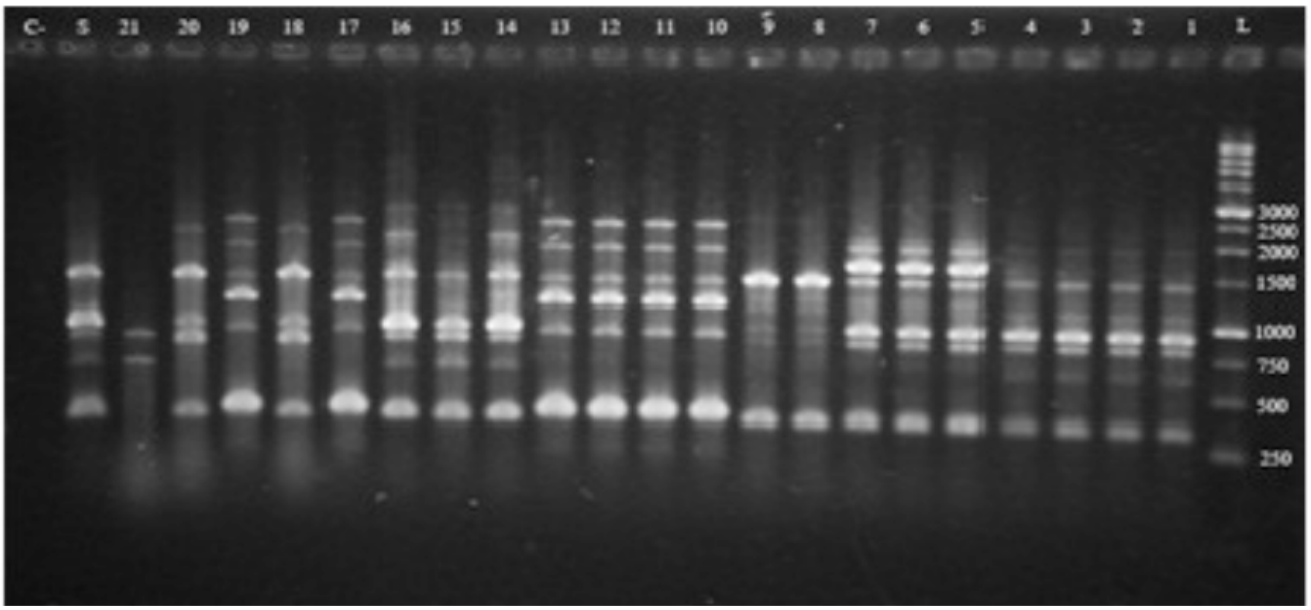


Fig 3. RAPD-PCR results of 21 *Salmonella* isolates from zoo animals with the OPA-4 primer. Lane L Marker 1kb, Lane 1 Long-eared owl1, Lane 2 Long-eared owl2, Lane 3 Montagus barrier, Lane 4 Golden hamster, Lane 5 Rhesus monkey1, Lane 6 Rhesus monkey2, Lane 7 Rhesus monkey3, Lane 8 Indian python1, Lane 9 Indian python2, Lane 10 Brandts hedgehog1, lane 11 Brandts hedgehog2, Lane 12 Brandts hedgehog3, Lane 13 Iranian spiny-tailed lizard1, Lane 14 Red-eared slider1, Lane 15 Red-eared slider2, Lane 16 Red-eared slider3, Lane 17 Red-eared slider4, lane 18 Red-eared slider5, lane 19 Red-eared slider6, Lane 20 Iranian spiny-tailed lizard2, Lane 21 Fischers lovebird, Lane S *S. typhimurium* (ATCC 14028).

the characteristic band of 284 bp was observed in the agarose gel (Fig 1). From the seven mammalian positive samples three originated from monkeys, one from hamster and three from Brandt's hedgehogs. From the four birds' samples, two were isolated from long-eared owl, 1 from Montagu's harriers and 1 from Fischer's lovebirds. Finally from the ten samples collected from reptiles two came from Iranian spiny-tailed lizards, six from turtles and 2 from snakes (Indian python).

Serotyping of the isolates was performed by using commercially reliable antisera. Of 21 positive isolates of *Salmonella* spp. 9 different serotypes were detected: *Enteritidis*, *Seftenberg*, *Typhimurium*, *Virchow*, *Berkeley*, *Kingabwa*, *Newport*, *Marina* and *Havana*. *Salmonella enterica* serotype *Typhimurium* and *Enteritidis* were the most commonly detected serotypes from these animals. The number of samples, the species of the sampled animals and the number of positive results from each species were listed in Table 1. The serotypes of *Salmonella* spp. isolated from Karaj zoo park was listed in Table 2.

The results of the antibiotic resistance patterns are shown in Table 3. All the isolates tested, were resistant to one or more antibiotics and 5 isolates including the ones from monkeys and long-eared owls were multiresistant. Nine (42.8%) isolates were resistant to amoxicillin and nine (42.8%) isolates were resistant to tetracycline. All the isolates showed susceptibility pattern to cephalothin, gentamicin, norfloxacin, ciprofloxacin, cefotaxime, enrofloxacin and chloramphenicol.

RAPD-PCR profiles were identical in isolates of every nine serotypes with each primer. RAPD profiles of isolates are shown in Figure 2 and 3.

DISCUSION

Zoonotic disease outbreaks from zoos are rare but zookeepers, zoo visitors and zoo veterinarian are at risk of infection from animal carriers. This study showed the prevalence of *Salmonella* spp. in zoo animals. Since some of them are kept as pets, *Salmonella* spp. can be transmitted to their owners.

During the present investigation *Salmonella* serotypes were isolated from 21 of the 104 Samples (20%) from zoo animals include reptiles, mammals and birds with the biggest number of isolates obtained from reptiles. Among the serovars found during this survey, the serotypes at the highest frequency were *S. Enteritidis* and *S. Typhimurium* the most common serovars causing salmonellosis in humans, too. Zahraei et al. in 2010 determined the prevalence of *Salmonella* serovar infection in 64 animals comprising small mammals (rodents, rabbits, squirrels, hamsters, and guinea pigs) and reptiles (turtles and lizards) in Tehran. 13.6% of reptiles were positive for *Salmonella* which belonged to serogroup B and C. Tamimi et al. (2014) isolated *Salmonella* spp. specially serogroups B and C from reptiles kept as pets. All the isolates in the present study were identified as subspecies I except one (serovar *Marina*) which was subspecies IV. *Salmonella enterica* subspecies I serovars are associated with infection of warm blooded animals, but in some studies they have been isolated from reptiles, too as in this study (Nakadai et al., 2005; Jang et al., 2008).

The sources of *Salmonella* isolates from captive animals in the zoo may have been food, especially raw meat and mice. Other sources may include native rodents and birds that approach zoo animals via contact with human handlers or food which may be contaminated with human strains (Okoh and Onazi, 1980; Neera et al., 2000).

In this study 4,7,10 of 21 isolates were related to birds, mammals and reptiles respectively. During the last few years, a high percentages of human salmonellosis have been associated with pets especially reptiles (Olsen et al., 2001).

Reptiles especially terrestrial and turtles as pets may be a serious source of infection for humans. All *Salmonella* serovars in this study should be considered potential human pathogens, despite reptiles usually don't show clinical signs. Human salmonellosis associated with pets results in diarrhea, abdominal cramp, vomiting and fever. The infection can also spread to the blood-stream, bone marrow and nervous system. Children, the elderly, pregnant women and immunosuppressive patients are at high risks (Ebani et al., 2004). Nowadays, Salmonellosis and other oral-fecal transmitted pathogens are very important in societies where immunosuppressive diseases are prevalent such as Iran. Results of this study shows that these susceptible patients, zoo visitors and also pet owners are at high risk of salmonellosis. Zahraei et al. (2010) and Tamimi et al. (2014) showed that rodents and reptiles

Table 2: Results from the tested animals

Animal source	O	H1	H2	Salmonella serotype
Long-eared owl (*Asio otus*)1	D(1,9,12)	g.m	--	Entritidis
Long-eared owl (*Asio otus*) 2	D(1,9,12)	g.m	--	Entritidis
Montagus barrier (*Circus pygargus*)	D(1,9,12)	g.m	--	Entritidis
Golden hamster (*Mesocricetus auratus*)	D(1,9,12)	g.m	--	Entritidis
Rhesus monkey (*Macaca mulatta*)1	C1(6,7)	r	1,2	Virchow
Rhesus monkey (*Macaca mulatta*)2	C1(6,7)	r	1,2	Virchow
Rhesus monkey (*Macaca mulatta*)3	C1(6,7)	r	1,2	Virchow
Indian python (*Python molarus*)1	U(43)	y	1,5	Kingabwa
Indian python (*Python molarus*)2	U(43)	y	1,5	Kingabwa
Brandts hedgehog (*Paraechinus hypomelas)*1	B(1,4,5,12)	i	1,2	Typhimurium
Brandts hedgehog (*Paraechinus hypomelas)*2	B(1,4,5,12)	i	1,2	Typhimurium
Brandts hedgehog (*Paraechinus hypomelas)*3	B(1,4,5,12)	i	1,2	Typhimurium
Iranian spiny-tailed lizard (*Uromast*yx asmussi)	B(1,4,5,12)	i	1,2	Typhimurium
Red-eared slider (*Trachemys scripta*)1	C2(6,8)	e,h	1,2	Newport
Red-eared slider (*Trachemys scripta*)2	U(43)	a	1,5	Berkeley
Red-eared slider (*Trachemys scripta*)3	C2(6,8)	e,h	1,2	Newport
Red-eared slider (*Trachemys scripta*)4	Y(48)	g,z_{51}	--	Marina
Red-eared slider (*Trachemys scripta*)5	G2(1,13,23)	f.g	--	Havana
Red-eared slider (*Trachemys scripta*)6	Y(48)	g,z_{51}	--	Marina
Iranian spiny-tailed lizard (*Uromastyx asmussi*)2	G2(1,13,23)	f.g	--	Havana
Fischers lovebird (*Agapornis fischeri)*	E2(1,3,19)	g.s.t	--	senftenberg

Table 3: The results of antibiotic resistance test

Antimicrobial agent	S(Sensitive)	I (Intermediate)	R(Resistant)
AM	(19) 90.47%	(2) 9.53%	0
TE	(1) 4.76 %	(11) 52.39%	(9) 42.85%
GM	(21)100%	0	0
KF	(21)100%	0	0
NOR	(21)100%	0	0
K	(15) 71.4%	(3)14.3%	(3) 14.3%
SXT	(18) 85.7%	0	(3) 14.3%
FM	(18) 85.7%	(1) 4.76%	(2) 9.52%
AMC	(1) 4.76%	(11) 52.4%	(9) 42.84%
CIP	(21)100%	0	0
CTX	(21)100%	0	0
FD	(15)71.4%	(3)14.3%	(3)14.3%
ENF	(21)100%	0	0
C	(21)100%	0	0

AM: ampicillin , TE: tetracycline, GM: gentamaycin, KF: cephalotin, NOR: norfloxacin, K: kanamycin, SXT: Sultrim, FM: flumequine, AMC: amoxiclave, CIP: ciprofloxacin, CTX: cefotaxime, ENF: enrofloxacin, C: chloramphenicol

were found to be potential carriers of pathogenic *Salmonella* in humans and animals.

To confirm isolates molecular techniques like PCR and RAPD-PCR as well as serotyping were used. The 284 bp fragment of the *invA* gene was amplified from all the isolates verifying the use of this protocol for the identification of *Salmonella*. Malorny et al. (2003) concluded that the PCR method for detecting the *invA* gene using S141, S139 in the identification of *Salmonella* is very beneficial (Malorny et al., 2003; Ebani et al., 2004). The results of RAPD-PCR showed identity between the same serovars. Madadgar et al. (2009) reported that the use of this method revealed identical profiles of isolates in the outbreak investigation. Transmission rate between animals seemed relatively high because the isolates belonged to a serotype, presented the same profile.

In the present study, high percentage of *Salmonella* spp. isolated from zoo animals showed multiple antimicrobial resistance which indicated virulent identity of the isolates. Fortunately, all the strains showed a common pattern of sensitivity to cephalothin, gentamicin, norfloxacin, ciprofloxacin, cefotaxime, enrofloxacin and chloramphenicol. Enrofloxacin is one of the choice′ antibiotics against *Salmonellae* (Neera et al., 2000; Mitchel et al., 2001). These resistant isolates of *Salmonella* spp. may have been transmitted to zoo animals via raw meat or milk fed to them (Neera et al., 2000). Sporadic shedding of serotypes of *Salmonella* spp. from apparently healthy zoo animals, presenting resistance to many antimicrobial agents is very important to public health.

Zookeepers, pet owners, veterinarians and zoo visitors must follow some simple rules to prevent salmonellosis and other bacterial zoonoses. They should wash the hands after handling animals and cages, disinfect the places and things that they use, especially their eating places.

The results of this study indicated that the determination of zoonotic pathogens presence in zoo animals and the prevention of human infection are very important. Furthermore, keeping the reptiles along with humans especially immunosuppressive infected ones, could be very dangerous. Finally, it seems salmonellosis and the other oral-fecal bacterial infections should be controlled by adding the antibiotics in food of zoo animals.

ACKNOWLEDGMENT

This study was supported by the Research Council of University of Tehran, Tehran, Iran.

REFERENCES

Abalem IV and Solari CA (2001) *Salmonella* in Brazilian and imported pet reptiles. Brazil J Microbiol 32: 293-297.

Bauer AW, Kirby WM, Sherris JC, Turck M (1966) Antibiotic susceptibility testing by a standardized single disk metod. Amer J Clin Patho 45: 493-496.

Campbell JV, Mohle-Boetani J, Reporter R, Abbott S, Farrar J, Brandl M, Mandrell R, Werner SB (2001) An outbreak of *Salmonella* serotype Thompson associated with fresh cilantro. J Infec Dis 183: 984-987.

Corrente M, Madio A, Friedrich KG, Greco G, Desario C, Tagliabue S, Dincau M, Campolo M, Buonavoglia C (2004) Isolation of *Salmonella* strains from reptile faeces and comparison of different culture media. J Appl Microbiol 96: 709-715.

Ebani VV, Cerri D, Fratini F, Meille N, Valentini P, Andreani E (2004) *Salmonella enterica* isolates from faeces of domestic reptiles and a study of their antimicrobial in vitro sensitivity. Res Vet Sci 78: 117-121.

Forshell LP and Wierup M (2006) *Salmonella* contamination: A significant challenge to the global marketing of animal food products. Revue Scientific et Technique de L,Office International Des Epizooties. 25: 541-554.

Franciscus ST, Lightfoot D, Holz P (2011) Prevalence of *Salmonella* in Australian reptiles. J WildL Dis 47: 1-11.

Friedman CR, Torigian C, Shillam PJ, Hoffman RE, Heltze LD, Beebe JL, Malcolm G, Dewitt WE, Hutwagner L, Griffin PM (1998) An outbreak of salmonellosis among children attending a reptile exhibit at a zoo. J Pediatr 132: 802-807.

Galan JE, Ginocchio C, Costeas P (1992) Molecular and Functional characterization of the *Salmonella* invasion gene *invA*: homology of *invA* to members of a new protein family. J Bacteriol 174: 4338-4349.

Jang YH, Lee SL, Lim JG, Lee HS, Kim TJ, Park JH, Chung BH, Choe NH (2008) The rate of *Salmonella* spp. Infection in zoo animals at seoul grand park, Korea. J Vet Sci 9: 177-181.

Jardine C, Reid-Smith RJ, Janecko N, Allan M, McEwen SA (2011) *Salmonella* in raccoons (procyonlotor) in southern Ontario, Canada. J wildL Dis 47: 344-351.

Lin AW, Usera MA, Barrett TJ, Godsby RA (1996) Application of random amplified polymorphic DNA analysis to differentiate strains of *Salmonella enteritidis*. J Clin Microbiol 34: 870-876.

Madadgar O, Zahraeisalehi T, Ghafari MM, AshrafiTamai I, Madani SA, Yahyareyat R (2009) Study of anusual paratyphoidepornitic in canaries (Serinuscanaria). Av Pathol 38: 437-441.

Malorny B, Hoofar J, Bunge C, Helmuth R (2003) Multicenter validation of the analytical accuracy of *Salmonella* PCR towards international standard. Appl Environ Microbiol 69: 590-596.

Mitchell MA, Shane SM, Nevarez J, Pesti D, Sanchez S, Wooley RE, Ritchie B (2001) Establishing a *Salmonella*-free iguana, Iguana iguana, model using enrofloxacin. In: Proceedings of the VIII annual conference, association of reptilian and amphibian reptiles. pp. 189-190.

Nakadai A, Kuroki T, Kato Y, Suzuki R, Yamai S, Yaginuma C, Shiotani R, Yamanouchi A, Hayashidani H (2005) Prevalence of *Salmonella* spp. in pet reptiles in Japan. J Vet Med Sci. 67: 97-101.

Neera VG, Abiodun AA, Kenneth C (2000) Retrospective and longitudinal study of salmonellosis in captive wilelife in Trinidad. J wildL Dis 36: 284-293.

Okoh AEJ and Onazi M (1980) Notes on *salmonella* isolated from wildlife in Kano zoological gardens. J wildL Dis 16: 7-10.

Olsen SJ, Bishop R, Bernner FW, Roels TH, Bean N, Tauxe RV, Slutsker L (2001) The changing epidemiology of *Salmonella*: trend in serotypes isolated from humans in the United states. J Infect Dis 183: 753-761.

Pedersen K, Lassen-Nielsen AM, Nordentoft S, Hammer AS (2008) Serovars of *Salmonella* from captive reptiles. Zoonoses Public Health 56: 238-242.

Pitout JD, Reisbig MD, Mulvey M, Chui L, Louie M, Crowe L, Church L, Elsayed S, Gregson D, Ahmed R, Tilley P, Hanson ND (2003) Association between handling of pet treats and infection with *Salmonella enterica* serotype Newport expressing the AmpC betalactamase, CMY-2. J Clin Microbiol 41: 4578-4582.

Popoff MY and Le Minor L (1992) Antigenic formulas of the *Salmonella* serovars.6th ed. WHO collaborating centre for Reference and Research on *Salmonella*. Institute Pasteur Paris, France.

Quinn PJ, Carter ME, Markey PK, Carter GR (1994) Enterobacteriaceae. In: clinical veterinary Microbiology. pp 209-236, 2nd ed. London: Wolfe publishing.

Stephen J, Swanson MD, Cynthia Snider MHS, Christopher R, Braden MD (2007) Multidrug-resistant *Salmonella enterica* serotype Typhimurium associated with pet rodents. New England J Med 356, 21-28.

Tamimi N, Rostami A, Majidzadeh K, Bahonar A, Esmaeili H, Niazi shahraki S. The survey of *Salmonella* infection in pet reptiles in Tehran and the associated risk factors in their owners Iranian J Epidemiol 10(2): 24-32.

Tizard I (2004). Salmonellosis in wild birds. Seminars in avian and exotic pet medicine. 13: 50-56.

Zahraei Salehi T, Mahzounieh M, Khaksar E (2010) Detection of Salmonella serovars in zoo and pet reptiles, rabbits, and rodents in Iran by culture and PCR methods. Comp Clin Pathol 19: 199–202

Zahraei salehi T, AskariBadouei M, Madadgar O, Ghiasi SR, AshrafiTamai I (2013) Shepherd dogs as a common source for *Salmonella enterica* serovar Reading in Garmsar, Iran. Turk J Vet Sci 37: 102-10