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Review Article

SALMONELLA IN PET AND CAPTIVE REPTILES WITH REFERENCE TO SOME STUDIES IN MALAYSIA AND COUNTRIES WORLDWIDE

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SUMMARY

Reptiles, which included snakes, chelonians (turtles, tortoises), iguanas and lizards, have been reported as important reservoirs for *Salmonella*. In Malaysia, reports on *Salmonella* in reptiles are not many. A search for works in Malaysia on salmonellae in reptiles found 28.0% chelonians, 27.5 - 61.0% snakes, 26.5 – 36.0% lizards and 31.0% house geckos carried *Salmonella.* The prevalence in some countries worldwide which included the United States of America, Brazil, Japan, Nigeria and few others were shown. The public health risk of the presence of salmonellae particularly in pet reptiles are discussed.

Keywords: Salmonella, reptiles, antibiotic resistance, public health

INTRODUCTION

Reptiles, which include snakes, crocodiles, iguanas, lizards and chelonians (turtles and tortoises) carry a number of zoonotic bacteria in their intestines which they shed in their faeces, thus contaminating the environment. Among the pathogens the reptiles carry included *Aeromonas, Campylobacter, Yersinia, Mycobacterium* and by far *Salmonella* is the most frequently isolated. The reptiles are usually asymptomatic carriers and reported as natural reservoirs of *Salmonella* (Lukac *et al.*, 2015). The presence of salmonellae in the intestines of reptiles has been documented since 1939 (Mitchell and Shane, 2001). Exotic reptiles are found in zoos and many recreational parks. Many reports showed that popularity of exotic reptiles as pets has increased which lead to an increased in the occurrences of reptile-associated *Salmonella* infections. According to Saelinger *et al.* (2006), the Center for Disease Control (CDC) estimates the faecal carriage rate of *Salmonella* spp. in pet reptiles at >90.0%. Among reptiles, a high prevalence of *Salmonella* are in snakes and lizards and low in chelonians; however *Salmonella* were reported in over 70.0% of Europeans tortoises from captive centres (Hidalgo-Vila *et al.*, 2007). The studies in other countries worldwide found *Salmonella* infection in pet reptiles to be rather prevalent.

Salmonella nomenclature can be rather confusing. There are two species of *Salmonella*, namely *Salmonella enterica* and *S. bongori*. Under *S. enterica*, there are six subspecies (I to 1V or subsp. *enterica, salamae, arizonae, diarizonae, houtenae* and *indica)* and numerous serovars (serotypes). *Salmonella enterica* are commonly found in the intestines and the most frequently isolated in animals are *Salmonella enterica* subsp. *enterica* serovar *Typhimurium* (or for short, known as *Salmonella* Typhimurium) and *Salmonella enterica* subsp. *enterica* serovar *Enteritidis (Salmonella* Enteritidis*).*

Several methods are available for the isolation and identification of *Salmonella*. In animals, detection of salmonellae is commonly carried out on faecal and cloacal specimens and intestinal contents collected from the animals.

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The study by Eriksson and Aspan (2007) on 11 detection methods found that the culture method using Modified Semi-solid Rappaport Vassiliadis (MRSV) agar plates (Oxoid) performed best with accuracy of 99% and sensitivity of 98% when compared to other culture method (using XLD and BGA (Oxoid) agar plates) and ELISA techniques. The PCR-based methods used shown to have similar sensitivity and specificity as the MRSV method. However, using the MSRV culture method, the preliminary positive result can only be obtained after three days. The suspected colonies from cultures must then be confirmed using biochemical and serological agglutination tests or PCR assay (Eriksson and Aspan, 2007).

Prevalence studies on *Salmonella* in reptiles

Not many published studies on *Salmonella* in reptiles were found in Malaysia. The findings of these studies are described briefly in Table 1. Several studies on salmonellae in reptiles have been documented worldwide and some of these are included in Table 2.

The reptiles reported as negative in many studies may be truly free of salmonellae; however, at times it could be underdiagnosed if samples obtained lack appreciable faecal materials on the cloacal swabs or due to non-shedding of the organisms by the hosts at the time of sampling. Therefore, in some cases multiple samples may be required to detect the presence of salmonellae and to differentiate that the free-living or wild reptiles are not the natural carriers of *Salmonella* but acquired them when brought into captive and pet industry (Saelinger *et al.*, 2006).

Hidalgo-Vila *et al.* (2007) reported a higher occurrence of salmonellae in terrestrial turtles, 100.0% compared to aquatic turtles (in ponds, streams) which was low, at 12.0–15.4%. According to the authors, the *Salmonella* were washed from the skin and cloacae of the turtles which account for their lower persistence while in terrestrial habitats salmonellae tend to persist longer and are directly spread among individuals.

Table 1. *Salmonella* in reptiles in Malaysia

Authors	Findings	*Salmonella* subspecies / seovars	Antibiotic resistance of isolates
Boey *et al.* (2014)	Faecal samples were collected from 60 (12 captive and 48 wild lizards; 36.0% were positive for salmonellae – higher prevalence in captive (83.0%) lizards (zoological parks and pet shops) as compared to wild (25.0%) ones (in and around residential areas).	*S. enterica* subsp. *enterica* most frequently isolated, followed by subsp. *arizonae, diarizonae, indica*	NA
Abatcha *et al.* (2013)	Cloacal swabs from 42 snakes (22 wild and 20 captive) were collected; 38.0% were found positive for *Salmonella*.	S. *enterica* and serovars/serotypes Typhimurium, Corvallis, Mbandaka, Poona were reported.	The isolates showed resistance to cephalexin, cephalothin and amoxicillin-clavulanic acid.
Norazrena *et al.* (2008)	Clocal swabs were taken from 90 chelonians (45 turtles and 45 tortoises) in recreational parks, aquarium shops and homes (as pets). 28.0% were positive for *Salmonella*.	*Salmonella* serovars isolated were Newport, Pamona, Typhimurium, Tenessee, Arizona, Brezany and Corvallis	NA
Fazhana *et al*. (2007)	Gastrointestinal contents of 32 house geckos (*Hemidactylus frenatus*) were collected. Ten or 31.0% were *Salmonella* positive – 43.0% from residential homes and 28.0% were from shoplots including eateries	*Salmonella* spp.	All isolates were resistant to erythromycin. 50.0% of the isolates were resistant to ampicillin and streptomycin.
Nur Mahiza *et al.* (2007)	Cloacal swab from 40 pet snakes were collected. *Salmonella* was detected in 27.5% .	*S*. Albany and *S*. Typhimurium	Isolates were resistant to polymixin B (50.0%), followed by neomycin and streptomycin
Noor Asyikin *et al.* (2006)	Cloacal swab were taken from 34 green iguanas in zoos (27), pet shops (4) and homes (3); 26.5% were *Salmonella* – positive.	*Salmonella* spp.	NA
Saravanan (2004)	*Salmonella* was detected in 61% of the cloacal swabs taken from 41 snakes in a snake farm.	*Salmonella* spp.	NA

NA – not available / not done

Table 2. *Salmonella* in reptiles in other countries worldwide

Authors	Countries	Findings
Lukac *et al.* (2015)	Croatia	Apparently healthy reptiles, privately owned or at a zoo. 13.0% positive for *Salmonella* – 48.0% in lizards, mainly iguanas and bearded dragons, 9.0% in snakes and 4.0% in turtles. *Salmonella enterica* subsp. *enterica* was frequently isolated (35.0%) and 5 serovars identified.
Hidalgo-Vila *et al.* (2008)	Spain	Samples from 172 free-living and 39 pet turtles found 13 animals or 6.2% positive for *Salmonella*. *S. enterica* were frequently isolated and 9 serovars were identified.
Nakadai *et al.* (2004)	Japan	*Salmonella* were detected in 74.0% of the 112 faecal samples collected from turtles, lizards and snakes in a pet shop. *Salmonella enterica* subsp. *enterica* was frequently isolated and 28 serovars were identified. *S.* Bardo, *S.* Newport and *S.* Panama were predominant.
Saelinger *et al.* (2006)	USA	Cloacal swab samples from 94 wild North American turtles were collected and none (0%) were positive.
Chambers and Hulse (2006)	USA	61 of 64 turtles (10) and snakes (54) or 95.3% were *Salmonella* positive. *S.* Typhimurium and *S.* Entertidis were two most common serovars isolated.
Schroter *et al*. (2004)	Germany	Fresh stool samples from 16 pet snakes found 81.0% were positive for *Salmonella*.
Abalem de Sa and Solari (2001)	Brazil	Of the 97 faecal samples from pet reptiles which included snakes, lizards and chelonians, 39.0% were *Salmonella*-positive, mainly in lizards. Subspecies I predominated (45.0%) with serovars Albany, Entertidis and Typhimurium were most common.
Pasmans *et al.* (2005)	Belgium	*Salmonella* was isolated from 76.0% (25 of 33) of cloacal samples and 59.5% (47 of 79) of faecal samples from captive lizards. Four subspecies with mainly subspecies I and 44 serovars were identified.
Otokunefor *et al.* (2003)	Nigeria	Of the 176 intestinal contents from 3 species of pest lizards, 69 or 39.0% were positive for *Salmonella*
Callaway *et al.* (2011)	Australia	100 Asian house gecko were collected and prevalence of *Salmonella* in the intestinal contents was 7.0%.

Public health risk

According to CDC Reports (MMWR 1999, 2007, 2010), approximately 93,000 or 6.0-7.0% cases per year of *Salmonella* spp. infections are attributable to contact with pet reptiles or amphibians. In the United States of America (USA), the number of commercialised reptiles, especially iguanas, imported per year has increased considerably to ≈ 1 million. The number of human cases of salmonellosis, especially in very young children, increased dramatically in parallel with pet reptiles ownership. Most reptiles have a stable mixture of *Salmonella* serovars in their intestinal tracts and intermittently or continuously shed *Salmonella* organisms in their faeces (Chomel *et al.*, 2007).

Salmonella can be transmitted to humans by direct or indirect contact with a pet reptile or its faeces. Pet turtles, iguanas and snakes which are often allowed to roam freely in the owners' (keepers') homes which have been identified as the sources for human infection (Schroter *et al.*, 2004). Also, it should be noted that water in turtle bowls or aquariums can amplify the salmonellae shed by the turtles.

Salmonellae in humans usually cause gastroenteritis with symptoms of headache, malaise, nausea, fever, vomiting, abdominal pain and diarrhoea (with or without blood). However, according to reports, children aged less than 5 years, pregnant women, elderly people and immunocompromised persons seems to be particularly prone to reptile-associated salmonellosis and often experience severe clinical infections, including fatalities due to septicaemia and meningitis (Schroter *et al.*, 2004; Woodward *et al.*, 1997; CDC 1999,2007, 2010).

Since 2006, USA experienced a number of multistate turtle-associated *Salmonella* outbreaks due to increased pet turtle ownership. Majority of the cases reported were found exposed to turtles with shell lengths < 4 inches purchased either at pet stores, flea markets or other outlets. As a matter of fact, USA has banned commercial distribution of pet turtles < 4 inches long in 1975 and that move had prevented an estimated 100,000 cases of turtle-associated salmonellosis in children aged between 1-9 years in 1976 (CDC, 2007). However, an exception was made for small turtles to be sold legally for scientific, educational or exhibition purposes, which may have increased the popularity of small turtles as pets. In Malaysia, these small turtles are being sold openly in pet shops, aquarium shops and other outlets. The CDC (2008) reported an interesting case of salmonellosis in two girls who had swum in an unchlorinated, in-ground swimming pool together with two pet turtles with carapace length <4 inches purchased from a pet shop. *Salmonella* Paratyphi B var. Java was isolated from the girls and turtle's habitat water. The *Xba*I pattern of the isolates from the water was indistinguishable by pulsed-field gel electrophoresis or PFGE from the isolates of one of the girls.

A recent report which examined 175 cases of *Salmonella* infections in children under 5 years old in South West of United Kingdom (2010 – 2013) found 48 patients had been exposed to reptiles and about half were hospitalised (Kennedy, 2015). Chomel *et al.* (2007) reported that more than 25 outbreaks of human infectious diseases were associated with visitors that have visited the animal exhibits. An interesting report by Friedman *et al*. (1998) described an outbreak of salmonellosis at Colorado zoo which detected 65 cases (most of them in children) which were associated with touching a wooden barrier around the Komodo dragon exhibit. *Salmonella* organisms were isolated from 39 case-patients, a Komodo dragon, and the wooden barrier. Children who did not become infected were more likely to have washed their hands after visiting the exhibit. Also, secondary transmission of salmonellae can occur by contact with handlers who had not washed their hands thoroughly. Saravanan (2004) swabbed the palms of snakes' handlers after handling *Salmonella*-infected snakes found their hands contaminated with the bacteria. Washing of hands removed 83.0% while good hygiene practice using antiseptic soap could remove almost all the salmonellae from the hands.

Petting zoos, where children are allowed to approach and pet or handle and sometimes feed certain captive wildlife and domestic animals, have been linked to several zoonotic outbreaks, including infections caused by salmonellae and *Escherichia coli* O157:H7 (Chomel, 2007). Thus, these places should provide hand-washing facilities or hand sanitisers.

In Asia, house geckos or house lizards are commonly found crawling on ceilings and walls of homes and premises, to catch insects for food. The salmonellae-infected lizards are most likely to shed salmonellae in the faeces which they would excrete indiscriminately; as such this would contaminate the home environment with salmonellae-laden faeces (usually unnoticeable, as on average it is the size of a large rice grain). This could also be a source of salmonellosis in toddlers and children upon crawling and playing on such contaminated floors. Otokunefor *et al.* (2003) reported that salmonellae survive longer in lizard dropping exposed to dry environments, up to 8 weeks, than wet ones. Thus, the house lizards may play a significant role in occurrence of sporadic salmonellosis (Callway *et al.*, 2011). Pet owners with children should be advised regarding *Salmonella* in pet reptiles. Besides that, they must teach their children to wash hands thoroughly after handling the animals, to restrict the pets' from access to the children areas and if possible, to send three faecal samples collected from their pets one week apart for bacterial culture.

The occurrence of antibiotic-resistant pathogenic bacteria is of public health utmost concern worldwide especially in relation to treatment failure. Several reports showed an increased in the prevalence of antibiotic resistant *Salmonella* and the emergence of multidrug resistant strains in animal and human populations with more than 80.0% isolates resistant to tetracycline, sulphonamides, streptomycin and chloramphenicol. Hence, infections in humans by such resistant salmonellae from animals could be a serious problem and thus require close monitoring.

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