Salmonella associated with captive and wild lizards in Malaysia

Boey Yee Cheng, Siew Por Wong and Gary A. Dykes*

Abstract. Reptiles are well known reservoirs of *Salmonella* and are capable of carrying the pathogen without showing any clinical signs. Previous studies have found a high prevalence of *Salmonella* and predominantly *Salmonella enterica* subspecies *enterica* (I) in lizards. This subspecies is also commonly associated with mammals and is responsible for most cases of human salmonellosis. Our study investigated the prevalence of *Salmonella* species and subspecies in captive and wild reptiles in and around Kuala Lumpur, Malaysia. A total of 60 lizard (12 captive and 48 wild) faecal samples were tested for the presence *Salmonella*. Approximately 36% of the lizards sampled carried *Salmonella* in their faeces with a significantly ($p<0.05$) higher prevalence in captive (83.3%) as compared to wild lizards (25%). Four *Salmonella enterica* subspecies were isolated, namely *enterica* (I), *arizonae* (IIIa), *diarizonae* (IIIb) and *indica* (VI). The higher prevalence of *Salmonella* in captive lizards highlights a risk of acquiring reptile-associated salmonellosis from handling lizards while the level of *Salmonella* in wild lizards suggests some risk associated with their presence in and around dwellings.

Keywords. Lizards, *Salmonella enterica*, captive, wild, reptile-associated salmonellosis, Malaysia.

Introduction

The genus *Salmonella* is responsible for considerable human and animal disease worldwide. It consists of two species, namely *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into six subspecies, namely *S. enterica* subsp. *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV) and *indica* (VI) (Le Minor and Popoff, 1987). *Salmonella enterica* subsp. *enterica* is commonly found in mammals, including humans, where it is responsible for most *Salmonella* infections. The other subspecies are more commonly found in poikilotherms or in the environment (Geue and Löschner, 2002).

Reptiles are known to be reservoirs of *Salmonella*, carrying the pathogen without showing clinical signs (Geue and Löschner, 2002). Furthermore, a relatively high prevalence of *S. enterica* subsp. *enterica* (I) has been found in captive reptiles (Geue and Löschner, 2002; Kikillus, Gartrell and Motion, 2011). Several studies have reported a high rate of *Salmonella* in wild reptiles (Briones et al., 2004; Filippi et al., 2010). It has

been suggested that *S. enterica* subsp. *enterica* (I) from reptiles are more capable of invading human cells as compared to other subspecies (Pasmans et al., 2005). Over the last decade the number of households keeping exotic animals, especially reptiles, has increased. The increase in pet reptile ownership is believed to be the cause of increased incident of reptile-associated salmonellosis (RAS) which is now being recognized as a major public health concern. There are estimated 70000 cases of RAS in the USA annually (CDC, 2013).

In some countries, such as Malaysia, wild lizards roam freely through urban and peri-urban areas and could easily contaminate surfaces in dwellings with *Salmonella* if they carry it. This study examined the prevalence of *Salmonella* in captive and wild lizards in and around Kuala Lumpur, Malaysia.

Materials and Methods

A total of 60 faecal samples (Table 1) were collected from individual lizards in zoological parks and pet shops, and from wild lizards in and around residential areas and a recreational forest in Kuala Lumpur, Malaysia. Droppings were stored at 4°C and processed within 48 hours. Isolation of *Salmonella* was performed according to Geue and Lösschner (2002) with minor modifications. To confirm isolates as *Salmonella* and assign them to a particular subspecies, presumptively positive colonies

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School of Science, Monash University, Jalan Lagoon Selatan, 46150 Bandar Sunway, Selangor, Malaysia
*Corresponding author; e-mail:gary.dykes@monash.edu.

Table 1. Prevalence and subspecies of *Salmonella* from lizard faeces in Malaysia.

Lizard family	Origin of samples	No. of samples* (wild, captive)	Percentage *Salmonella* positive [wild (%), captive (%)]**	*Salmonella* subspecies
Iguanidae	Zoological park	2 (0, 2)	2 (100%) [0 (n/a), 2 (100%)]	*S. arizonae* (IIIa) *S. enterica* (I)
Agamidae	Pet shops, zoological park, wild lizards	36 (34, 2)	12 (33.3%) [10(29.4%), 2 (100%)]	*S. indica* (VI) *S. enterica* (I) *S. diarizonae* (IIIb)
Scincidae	Zoological park, wild lizards	3 (2, 1)	1 (33.3%) [0 (0%), 1 (100%)]	*S. enterica* (I)
Gekkonidae	Pet shops, Zoological park, wild lizards	17 (12, 5)	5 (29.4%) [2 (16.7%), 3 (60.0%)]	*S. enterica* (I) *S. diarizonae* (IIIb)
Varanidae	Zoological park	2 (0, 2)	2 (100%) [0 (0%), 2 (100%)]	*S. enterica* (I)
Total		**60 (48, 12)**	**22 (36.7%) [12 (25%), 10 (83.3%)]**	

*each sample was taken from an individual lizard.

**the percentages were calculated by comparing the number of positive faecal samples from wild and captive lizards to the respective total number of faecal samples collected.

were subject to a multiplex PCR protocol as described by Lee et al. (2009). Isolates which could not be identified at the subspecies level using the multiplex PCR were identified biochemically using the Microbact GNB 24E system (Oxoid, Australia) according to the manufacturer's instructions.

All isolates were tested for antimicrobial sensitivity to five antibiotics by standard methods. The antimicrobial agents and concentrations used were ampicillin (AMP) 10 µg, gentamycin (CN) 10 µg, tetracycline (TE) 30 µg, chloramphenicol (C) 30 µg and trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 µg).

A Fisher's exact test was used for comparisons of samples with an expected number of less than five (SPSS Ver. 16.0 for Windows; SPSS Inc. Chicago). A Chi-Squared test with Yate's correction was performed for other comparisons.

Results

Salmonella were found in 22 of the total number of 60 lizard faecal samples (36.7%), with captive lizard faecal samples (10 of a total of 12, 83.3%) having a significantly higher prevalence of *Salmonella* compared to wild lizard faecal samples (12 of a total of 48, 25%) (Fisher's exact test, p<0.05) (Table 1). Most of the faecal samples were obtained from members of the Agamidae (n=36) and Gekkonidae families (n=17) with no significant difference in *Salmonella* prevalence being found between the two lizard families ($\chi^2_{(1)} = 9.9 \times 10^{-4}$, P>0.05). Four different *S. enterica* subspecies (I, IIIa, IIIb and VI) were isolated. *S. enterica* subspecies *enterica* (I) was most abundant among the *Salmonella*

positive samples (68.2%) followed by subspecies *arizonae* (IIIa) (18.2%), *diarizonae* (IIIb) (9.1%) and *indica* (VI) (4.6%). There was no significant difference in the prevalence of *S. enterica* subsp. *enterica* (I) or any of the other subspecies between the Agamidae and the Gekkonidae (Fisher's exact test, p>0.05). Furthermore there was no significant difference in the prevalence of *S. enterica* subsp. *enterica* (I) or any of the other subspecies between captive and wild lizards (Fisher's exact test, p>0.05).

Only two of the isolates were found to be resistant to multiple antibiotics while others were sensitive to all antibiotics tested. One of the multiple-drug resistant strains showed resistant to 4 antibiotics (TE, SXT, C and AMP) while another one was resistant to 3 different antibiotics (SXT, C and AMP).

Discussion

The higher rate of *Salmonella* prevalence in captive lizards as compared to wild lizards in our study was similar to findings reported by Geue and Löschner (2002) who suggested that captivity may increase the acquisition of *Salmonella* in lizards by horizontal transfer from humans and other animals. In our study the prevalence of *Salmonella* in lizards was not species dependent as no significant difference was found between the two lizard families (Agamidae and Gekkonidae) sampled. However, other factors such as diet, season and lizard age might play a role and a larger sample size may reveal species specific difference in the prevalence of *Salmonella* (Geue and Löschner, 2002).

Salmonella enterica subsp. *enterica* (I) dominated the

subspecies isolated in our study although several other *S. enterica* subspecies were isolated. Although a similar scenario has been noted (Kikillus, Gartrell and Motion, 2011), some authors have suggested that reptiles are more likely to carry *S. enterica* subsp. II, IIIa, IIIb, IV and VI (Geue and Löschner, 2002, Briones et al., 2004). It is thought that captive lizards are more likely to have a diet more contaminated with mammal-associated pathogens, such as *S. enterica* subsp. *enterica*, and consequently are more likely to be colonized by these pathogens. However, the absence of a difference in prevalence of *S. enterica* subsp. *enterica* (I) between captive and wild lizards in our study suggests that exposure to human activities might not be a contributing factor to the *Salmonella* subspecies distribution in and around cities where all lizards are exposed to human activity of some form. This finding is consistent with that of Geue and Löschner (2002).

Our study provided no evidence that different species of lizard are more or less likely to carry *S. enterica* subsp. *enterica* (I). The sample size in our study was relatively small (60) compared to other studies which involved more than 100 reptiles but still provides indicative information on occurrence of *Salmonella* in lizards in Kuala Lumpur. In addition, the majority of the lizards sampled in our study are insectivores and there are therefore an insufficient number of samples from carnivorous and herbivorous lizards to draw conclusions about these species. For example, Franco et al. (2011) found that 98.4% of the land iguanas (which are herbivorous) in Galapagos Islands are carriers of *Salmonella.*

Two multidrug-resistant *Salmonella* strains were isolated from two monitor lizards (*Varanus salvator*) housed in the same confinement area of a zoological garden. Antimicrobial agents may have been applied to these animals for therapeutic use but we do not have information confirming this.

Our results demonstrated a high prevalence of *Salmonella*, especially *S. enterica* subsp. *enterica* (I), in Malaysian lizards in and around Kuala Lumpur. This suggest a risk for the acquisition of RAS since *S. enterica* subsp. *enterica* (I) is known to be the main cause of salmonellosis in humans (Park et al., 2009). The higher prevalence of *Salmonella* in captive lizards highlights a risk of acquiring reptile-associated salmonellosis from handling lizards while the level of *Salmonella* in wild lizards suggests some risk associated with their presence in and around dwellings.

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