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Case Reports

IDENTIFICATION OF SALMONELLA AND OTHER PATHOGENIC BACTERIA IN PET RED-EARED SLIDERS, *Trachemys scripta elegans*, FROM PET SHOPS IN THE KLANG VALLEY

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SUMMARY

Bacteriological isolation and identification were performed on 60 cloacal swabs and 15 aquarium water samples of pet red-eared sliders (*Trachemys scripta elegans*) obtained from aquarium shops in the Klang Valley, Central Peninsula Malaysia. The most common bacteria isolated was *Aeromonas* spp., which was present in both cloacal swabs (70%) and aquarium water (86.7%). *Klebsiella* spp. (50%), *Escherichia coli* (33.3%), *Yersinia* spp. (16.7%) and *Salmonella* spp. (15%) obtained form cloacal swabs were identified as pathogenic to both humans and animals. *Salmonella* spp. were isolated from both cloacal swabs and aquarium water. The *Salmonella* serotypes identified were *S. tennessee*, *S. typhimurium*, *S. brezany*, *S. pomona*, *S. corvallis* and *S. schwarzengrund*. Bacterial infections in humans associated with handling exotic pets directly or indirectly in contact with aquarium water have been described regularly, hence the zoonotic significance of owning a turtle infected with *Salmonella* spp. or any pathogenic bacteria therefore cannot be ignored.

Keywords: Salmonella spp., bacteria, red-eared slider, cloacal swab, aquarium water

INTRODUCTION

Exotic pets are becoming popular in households around the world, including Malaysia. Reptiles that are kept as pets include snakes, iguanas, lizards, tortoises and turtles. A survey of fifteen aquarium shops in the Klang Valley (this study), in the central peninsula Malaysia, revealed that red-eared sliders (*Trachemys scripta elegans*), was the most popular pet turtle sold in all the shops. This clearly shows that red-eared sliders are a popular exotic pet among Malaysians and frequently handled, especially by public and aquarium shop workers as well. The red-eared slider turtle, originating from the southeastern USA and Mexico, is now considered one of the invasive species that have successfully established its populations in many regions in the world.

Although various potentially pathogenic microbes have been isolated from pet turtles, most of the cases with confirmed zoonosis are related to salmonellosis (Nagano *et al.,* 2006; Ricard *et al*., 2015; Kuroki *et al*., 2015). Majority of the bacteria are known opportunistic pathogens which can cause diseases in both immunocompromised pet turtles and people who own them. In the USA for instance, turtle-associated salmonellosis is commonly reported among children despite numerous efforts including a long-standing ban against the sale and distribution of small reptiles like pet turtles, it is still readily acquired through multiple avenues (Bosch, 2016). Several other reports on confirmed zoonoses due to *Salmonella* from pet turtles were reported over the years in Europe, Japan and USA (Walters *et al*., 2016; Lafuente *et al.,* 2013; Nagano *et al.,* 2006). Immunocompromised individuals especially infants and children can succumb to salmonellosis with various degree of clinical symptoms such as gastroenteritis, myocarditis and even meningitis which can lead to sepsis and even fatality (Neuwirth *et al*., 1999, Ricard *et al*., 2015, Kuroki *et al*., 2015).

Hence, it is important to know the risks of zoonotic bacterial diseases that may be present in this species. The objectives of this study were to isolate and identify pathogenic bacteria from cloacal swabs of pet red-eared sliders and to investigate the potentiality of their aquarium water as a source of pathogenic bacteria.

MATERIALS AND METHODS

Consent and approval of study

This study was approved by the Faculty of Veterinary Medicine, Universiti Putra Malaysia final year project scientific committee and consents were obtained from the aquarium or pet shops that were approached for samples.

Cloacal swab and aquarium water sampling

Sixty turtles with carapace length of <8cm were sampled from 15 aquarium or pet shops in the Klang Valley, Central Peninsula of Malaysia. Four turtles were randomly selected from each aquarium shop. Cloacal swabs from individual turtles were placed in a sterile universal bottle containing 10 mL pre-enriched broth of buffered peptone water (BPW). A total volume of 10 mL water from the turtles' aquariums was collected in a sterile universal bottle aseptically and transported to the laboratory for further analysis.

Isolation and identification of bacteria

All samples were inoculated onto blood agar for bacterial isolation. Cloacal swabs were initially incubated at 37°C for 24 hours in the pre-enriched BPW,

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and later in enrichment Rappaport–Vassiliadis broth (RVB) for another 24 hours at the same temperature for *Salmonella* isolation. Aliquots from the RVB were inoculated onto XLT4 (xylose lysine tergitol 4) agar. XLT4 agar is a highly selective plating medium for detecting and isolating non-*typhi Salmonella* species. Bacterial isolates were identified using biochemical tests as described in the *Diagnostic Manual of Veterinary Clinical Bacteriology and Mycology* (Jang *et al*., 2004).

RESULTS

Cloacal swabs: A total of 227 bacterial isolates were obtained from the cultures of 60 cloacal swabs (Table 1). An average of three to four isolates were obtained from each turtle. The most common bacteria isolated was *Aeromonas* spp. (70%). Among the fewer common bacteria isolated were *Chromobacterium spp.*, *Proteus penneri*, *Providencia* spp., *Providencia stuartii*, *Staphylococcus* spp. and *Yersinia pseudotuberculosis*, with only one isolate each from the 60 samples obtained.

Of the 60 turtles sampled, nine were positive for *Salmonella* spp. Five *Salmonella* serotypes were identified, namely *S. typhimurium*, *S. brezany*, *S. corvallis*, *S. schwarzengrund* and *S. tennessee*. *Salmonella tennessee* was the most common serotype identified among the cloacal swabs positive for *Salmonella* spp. *Salmonella tennessee* was isolated from turtles within the same aquarium from one shop.

Aquarium water samples: There were 54 bacterial isolates from water samples (from 15 aquarium shops) (Table 1) with 14 types of bacteria identified. The most common bacteria isolated was *Aeromonas* spp. (86.7%). Only one sample was positive for *Salmonella* spp. (6.7%), and it contained the serotype *S. pomona*.

Table 1: Bacteria isolated and identified from cloacal sample and aquarium water of pet red eared sliders (*Trachemys scripta elegans*) from aquarium shops in Klang Valley, Malaysia.

No.	Bacteria	Cloacal swab		Aquarium water	
		Total isolates (n= 60)	Percentage (100%)	Frequency (n=15)	Percentage (100%)
1	*Acinetobacter spp.*[a]	5	8.3	0	0
2	*Acinetobacter iwoffii*	2	3.3	0	0
3	*Aeromonas spp.*	42	70.0	13	86.7
4	*Alcaligenes feacalis*	3	5.0	0	0
5	*Bacillus spp.*	5	8.3	0	0
6	*Bordetella spp.*	2	3.3	0	0
7	*Chromobacterium spp.*	1	1.7	1	6.7
8	*Citrobacter amalonaticus*	0	0	1	6.7
9	*Citrobacter freundii*	10	16.7	3	20.0
10	*Citrobacter spp.*	6	10.0	2	13.3
11	*Edwardsiella tarda*	1	1.7	0	0
12	*Enterobacter spp.*	20	33.3	11	73.3
13	*Escherichia coli*	20	33.3	9	60.0
14	*Klebsiella pneumoniae*	4	6.7	1	6.7
15	*Klebsiella spp.*[c]	26	43.3	3	20.0
16	*Morganella morganii*	2	3.3	0	0
17	*Plesiomonas spp.*	1	1.7	0	0
18	*Proteus mirabilis*	12	20.0	4	26.7
19	*Proteus penerri*	1	1.7	0	0
20	*Proteus vulgaris*	17	28.3	2	13.3
21	*Providencia spp.*[d]	1	1.7	1	6.7
22	*Providencia stuartii*	1	1.7	0	0
23	*Pseudomonas aeruginosa*	5	8.3	0	0
24	*Pseudomonas fluorescens*	11	18.3	0	0
25	*Salmonella brezany*	1	1.7	0	0
26	*Salmonella corvallis*	1	1.7	0	0
27	*Salmonella pomona*	0	0	1	6.7
28	*Salmonella schwarzengrund*	1	1.7	0	0
29	*Salmonella tennessee*	5	8.3	0	0
30	*Salmonella typhimurium*	1	1.7	0	0
31	*Serratia spp.*	9	15.0	0	0
32	*Staphylococcus spp.*	1	1.7	2	13.3
33	*Yersinia pseudotuberculosis*	1	1.7	0	0
35	*Yersinia ruckeri*	2	3.3	0	0
36	*Yersinia spp.*	7	11.7	0	0

DISCUSSION

An estimated 90% of all reptiles, including turtles, carry various pathogenic bacteria, which are shed in the faeces. For this reason, exotic pets such as cold-blooded vertebrates, in particular turtles, represent important reservoirs of *Salmonella* as well as other pathogenic bacteria (Woodword *et al*., 1997, Abalem de Sa, 2000). In the present study, a total of 33 bacterial species were isolated from cloacal swabs while 14 from aquarium water samples. These bacteria can be a potential source of infection for humans. In below-optimum environmental conditions, captive reptiles may develop depressed immune systems (Evans, 1963), with possible invasion of opportunistic pathogens. The species of bacteria isolated and its composition can vary with turtle age, gut health and environmental conditions (Peng *et al.,* 2020). These bacteria are transmitted through direct contact between a turtle with another susceptible turtle or man.

The bacteria commonly classified as major pathogens are *Escherichia coli*, *Salmonella* spp. and *Yersinia* spp., while others are classified as opportunistic pathogens (Quinn *et al*., 2002). In the present study, *E. coli* was one of the common pathogenic bacteria isolated from both the cloacal swabs and aquarium water. *Escherichia coli* is a common inhabitant of the gastrointestinal tract of many cold- and warm-blooded animals, including humans (Wasteson, 2002). Hence, the isolation of *E. coli* from the aquarium water samples indicates the presence of faecal contamination by the turtles.

In the present study, two *Yersinia* spp. was isolated from cloacal swabs namely *Y. pseudotuberculosis* and *Y. ruckeri*. These two *Yersinia* species was also isolated from cloacal swabs of European pond turtles in Poland (Nowakiewicz *et al*., 2015). In an abstract by MacDonald in 1998 (personal communication), *Y. pseudotuberculosis* was isolated from the oral flora of chelonians (*Testudo* spp.) from the United Kingdom. *Yersinia pseudotuberculosis* causes subclinical enteric infections in a variety of wild and domestic animals. In humans, *Y. pseudotuberculosis* can cause enterocolitis and mesenteric lymphadenitis (Quinn, 2002).

Salmonellosis is one of the most frequently reported zoonosis transmitted by reptiles, especially turtles in captivity (Lamm *et al*., 1972, Stam *et al*., 2003, Harris *et al*., 2010). *Salmonella* has been isolated from water in turtle bowls or aquariums that harbour the chelonian (McCoy *et al*., 1973, Morse et al., 1976, Neuwirth *et al*., 1999). In a study done in Malaysia, microbiological assessment of 90 captive pet chelonians, (45 turtles and 45 tortoises), found that 28% of samples were positive for *Salmonella*. The aforementioned study detected several *Salmonella* species, including Typhimurium, Brezany, Corvallis, Pomona, Newport and Tennessee (Norazrena and Saleha, 2008). Pond water harbouring turtle hatchling from two commercial breeding farms in southern Louisiana, USA was found contaminated with either *Salmonella newport*, *S. arizonae* or *S. poona* (Shane *et al.,* 1990). Adding to that, *Salmonella* spp. detection was reported in more than 70% of European tortoises from captive centres (Hidalgo-Vila *et al*., 2007) however, report by Saelinger *et al.* (2006) revealed that 94 wild North American turtles were tested negative for *Salmonella* spp. from the cloacal and gastrointestinal mucosa samples. There are few possible explanations for these contradictory findings where wild chelonians may not be active shedders or carriers of *Salmonella* spp. In some cases, multiple samples may be required to detect *Salmonella* spp. from faecal samples via bacterial culture, perhaps because of intermittent shedding of the bacteria. Having said that, differences in bacteria population between wild and captive chelonians are attributed to water contamination and high population density where even apparently healthy turtles may shed the bacteria under stressful conditions.

In the present study, all of the six *Salmonella* serotypes detected have been previously isolated from chelonians, except *S. schwarzengrund*, which is a new serotype reported in the present study. High-level fluoroquinolone resistance was reported in *S. schwarzengrund* in different regions of the world (Baucheron *et al*., 2005) and hence poses a risk for zoonosis, and more worryingly, anti-microbial resistance. The *Salmonella* serotypes detected from the turtles in the present study can be used as an epidemiological marker in this region to study the incidence of *Salmonella* infection in turtles and the associated transmission to humans. Woodword *et al*. (1997) reported that turtle–human salmonellosis by *S. pomona* was detected in 30 human patients. Hence, in the present study, the *S. pomona* isolated from aquarium water suggests a definite health hazard to humans. There was a report in a young child that developed severe gastroenteritis complicated with sepsis caused by *Salmonella* serotype Paratyphi B from pet turtle *Trachemys scripta elegans* (Nagano *et al.,* 2006), but this particular serotype was not isolated in this study.

Here, *Aeromonas* spp. was the most common bacteria isolated from both red-eared slider cloacal swabs and aquarium water samples. Due to increasing reports of acute diarrhoea in humans caused by these bacteria, *Aeromonas* spp. can be considered a relatively common enteropathogen (Janda and Duffey, 1988); nevertheless, other reviews have pointed out that several *Aeromonas* spp. have been considered emerging pathogens with public health significance (Isoken *et al.,* 2012). Studies have shown that several Aeromonads including *Aeromonas caviae, Aeromonas enteropelogenes, Aeromonas veronii and Aeromonas hydrophila,* and, have been isolated from healthy pet turtles which may have antimicrobial resistance (Wimalasena *et al*., 2017). *Aeromonas* spp. is a commonly isolated from turtles, therefore, it is important that pet owners practice good hygiene while caring for their pets due to potential for antimicrobial resistance.

Almost 50% of the turtles were positive for *Klebsiella* spp. which was the second most common bacteria isolated from the cloacal samples. *K. pneumoniae* and *K. oxytoca* were isolated from pet turtles from Korea where the isolates were found to have resistance to ampicillin and several other antimicrobials (Hossain *et al*., 2020) which suggests that the occurrence of virulence and antimicrobial resistance in *Klebsiella*

spp. may pose a potential public health concern. These bacteria are known pathogens for respiratory infections affecting turtles and other reptiles (Pees *et al*., 2007); while causing a wide range of diseases including respiratory tract infections such as pneumonia, urinary tract infections and septicaemia (Bengoechea *et al*., 2019). Adding to that, *Klebsiella* spp. are commonly found in aquarium water of turtles as well (McCoy *et al.,* 1973), hence contact with contaminated aquarium water can facilitate opportunistic infections to those who are handling and washing the aquarium tanks.

The present study presents several pathogenic bacteria of public health importance isolated from pet turtles (red eared slider) and their aquarium water as well other bacteria that can be opportunistic pathogens in immunocompromised individuals. The occurrence of other Gram-negative bacteria such as *Citrobacter freundii, Edwardsiella tarda, Pseudomonas aeruginosa* and *Proteus mirabilis* have been commonly reported as well from pet turtles (De Silva *et al.,* 2017). Majority of these bacteria are also etiologic agents of diseases causing shell rot, skin infections, conjunctivitis and ulcerative stomatitis in ectothermic animals including turtles (Vega-Manriquez *et al.,* 2018; Di lanni *et al*., 2015).

CONCLUSION

Faecal contamination of the environment accounts for widespread distribution of these pathogenic organisms which can contribute to the occurrence of opportunistic infections in humans and animals. Human–reptile bacterial infections have been regularly described, hence the zoonotic significance of owning a turtle infected with *Salmonella* spp. or any other pathogenic bacteria cannot be ignored. More surveillance should be carried out, particularly epidemiological studies related to turtle– human infections, especially in Malaysia. Molecular detection techniques could be further explored to facilitate rapid detection of these pathogens. Thus, reducing and preventing the potential risk of contracting pathogenic bacteria, especially *Salmonella* infections, from pet turtles is a measure of responsible ownership, public awareness and education.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

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