Molecular Pathogens (online), 2011, Vol. 2
ISSN 1925-1998 http://mp.sophiapublisher.com

Research Article Open Access

An Invasive Species Red-eared Slider (*Trachemys scripta elegans*) Carrying *Salmonella* Pathogens in Hainan Island

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Molecular Pathogens 2011, Vol 2 No 4 DOI: 10.5376/mp.2011.02.0004
Received: 19 May, 2011
Accepted: 02 Jul., 2011
Published: 01 Aug., 2011

Preferred citation for this article as:
Shen et al., 2011, An Invasive Species Red-eared Slider (*Trachemys scripta elegans*) Carrying *Salmonella* Pathogens in Hainan Island, Molecular Pathogens, Vol.2 No.4 (doi: 10.5376/mp.2011.02.0004)

Abstract The red-eared slider (*Trachemys scripta elegans*) is a semiaquatic turtles belonging to the family Emydidae. It is native to the southern United States, but has become established in other places because of pet releases. It is the most popular pet turtle in the United States and also popular in the rest of the world. In China the red-eared slider listed as an invasive and quarantinable species due to carring the *Salmonella* pathogens. In this research we collected 68 individuls of the wild red-eared sliders from Nandujiang (Nandu river), Wanquanhe (Wanquan river) and Haikoudonghu (East Lake of Haikou City) in Hainan island of the most southern of China. We sampled the tissue and mucus from cloacae of the red-eared sliders used for *Salmonella* bacterial culture to observe its morphology, biochemical characteristics and serotypes. The result showed that the total carrier rate of *Salmonella* carring in the tested red-eared sliders reached 54.41% (n=68), of which the carrier rate in Nandujiang were 53.85% (n=26), in Wanquanhe 58.82%(n=34),and Haikoudonghu 50% (n=8). The serotypes of *Salmonella* in the tested red-eared sliders were indentified to be *S .litchfiled*, *S. chailey*, *S. senftenberg*, and *S. Stanley*. In this research we strong suggested that the wild red-eared slider carrying *Salmonella* pathogens in Hainan island be potential dangerous impact on the ecological system and island residents.
Keywords Red-eared slider (*Trachemys scripta elegans*); Invasive species; *Salmonella*; Hainan island

Background

Salmonella is an important zoonotic pathogen of *Enterobacteriaceae*, which is gram-negative, facultative anaerobic bacterium. Currently *Salmonella* has found 2449 serotypes in the world (Brenner et al., 2000), there are about 292 serotypes reported in China (Zhang and Zhu, 2002). As early as 1975, turtles were ascertained to carry and transmit *Salmonella*, red-eared sliders, as the same as many other reptiles can carry and transmit *Salmonella*, humans may *Salmonella* through direct or indirect contacting with red-eared sliders and other reptiles, thereby causing typhoid, paratyphoid, gastroenteritis and other diseases and even death (Ackman et al., 1995). Therefore, FDA (the Food and Drug Administration) in 1975 banned the pet turtle trade except for education purpose use in the USA.

In the United States, red-eared sliders is popular pet turtles, particularly loved by children. Each year about 1.4 million Americans infected with *Salmonella*. Reptile and amphibians exposure is associated with ~74,000 *Salmonella* infections annually in the United States (Mermin et al., 2004). Clearly, pet turtle has a serious public health impact because of carrying *Salmonella*. Previous studies of *Salmonella* focused on captive turtles (McCoy and Seidler, 1973) and commercial turtles (Siebeling et al., 1984). Under the conditions of captive wildlife, the detection of *Salmonella* in reptiles was higher than that in mammals and birds (Gopee et al., 2000). There are few studies of *Salmonella* on the turtles on the wild environment reported.

Red-eared sliders in China is a foreign invasive species, Guangdong and other southern regions of China have the habit of eating red-eared sliders, it was also the red-eared sliders as pets. So far, There is no information on wild red-eared sliders carrying *salmonella* reported. In recent years, red-eared sliders

have intruded on Hainan Island, populations have been found in red-eared sliders across the island, in order to know the impacts of alien species, red-eared sliders, on island ecosystems, especially on potential harms of environment and human in the cayying and transmiting of *Salmonella*. We collected samples of red-eared sliders in Nandu river, Wanquan river on Hainan Island, Donghu Lake in downtown of Haikou city of Hainan to isolate *Salmonella* bacteria from cloaca of red-eared sliders, in order to make clear the status of the wild populations of red-eared sliders carrying *Salmonella*.

1 Results and Analysis

1.1 Red-eared sliders collection and *Salmonella* identification

We collected 68 of wild red-eared sliders in Nandujiang (Nandu river), Wanquanhe (Wanquan river) and Haikoudonghu (East Lake of Haikou City) during the period between Feb. to Sep. 2010 (Figure 1), of which Nandujiang was 26, Wanquanhe was 34 and Haikoudonghu was 8. Total tentative 98 *Salmonella* isolates were obtained from 68 samples of red-eared sliders. Further biochemical identification showed that 79 *Salmonella* bacteria were detected out of Total tentative 98 *Salmonella* isolates, of which 37 red-eared sliders carried *Salmonella*, Total carrying rate of *Salmonella* in collected red-eared sliders was 54.41%, of which Nandujiang was 53.85%, Wanquanhe was 58.82%, Haikoudonghu was 50%.

1.2 Biochemical identification of *Salmonella*

In this study we used *Salmonella* biochemical identification kit produced by Huankai Biotechnology Co., Ltd. Guangdong to determine serological reactions of *Salmonell*a. The employed Kit contained three response categories (A1, A2 and A3) with six standard identifications of *Salmonella* (Table 1). The results showed that 24 individuals of red-eared sliders response to A1−1 reaction.

Figure 1 Selected photos of Red-eared sliders (*Trachemys scripta elegans*) collected in Hainan island

According to biochemical test positive data, further statistics showed that the tested sample of 68 red-eared sliders had a total carrying rate of 54.41%, of which the red-eared sliders from Nandujiang was 53.85%, Wanquanhe was 58.82% and Haikoudonghu was 50.00%. The sample of group A from Nandujiang carries 75.00% of *Salmonella*, and group B 50.00% of *Salmonella* (Table 2). 34 of red-eared sliders from Wanquanhe in Group A carried 66.67% of *Salmonella*, group B carried 56.00% of *Salmonella*, Whereas 8 of red-eared sliders in group A carried 42.85% of *Salmonella*, group B was not for statistical analysis due to small sample size.

Table 1 Biochemical test response to *Salmonella* of red-eared sliders

Types of biochemical reactions	H_2S	Indole	Urea	KCN	Lysine decarboxlase	No of positive individuls detected
A1	A1−1	+	−	−	+	24
	A1−2	+	−	−	−	2
	A1−3	+	−	+	+	32
	A1−4	+	+	−	+	0
A2	A2−1	+	−	−	+	2
A3	A3−1		−	−	+/−	19

Note：“+” stands positive; “−” stands negative

Table 2 Positive carrying rate of *Salmonella* by biochemic test

Sampling locations	Carrying rate A group (%)	Carrying rate B group (%)	Total Carring rate (%)
Nandujiang	75.00	50.00	53.85
Wanquanhe	66.67	56.00	58.82
Haikoudonghu	42.85	–	50.00
Carrying rate of group	60.00	53.19	54.41

Note: A group: Carapace length of a red-eared slider less than 4–inch; B group: Carapace length of a red-eared slider equal or more than 4–inch; "–"stands no statistical analysis with small sample size; Carrying rate = individuals of red-eared slider carrying *Salmonella* divided by total tested red-eared slider

1.3 Identification of *Salmonella* serotypes

The employed *Salmonella* diagnostic serum kit (Model 60) produced by Lanzhou Institute of Biological Products can theoretically detect *Salmonella* OA-F serotypes, while *Salmonella* diagnostic kits produced by Thailand S & A company can detect OA-I serotypes. The results showed that the two sampling points of Nandujiang and Wanquanhe detected out Salmonella common serotypes as *S. litchfiled, S. chailey, S. senftenberg*, and *S. Stanley.* We were unable to detect out any Salmonella common serotypes in samples of *Salmonella* isolated from Haikoudonghu, which indicated that the *Salmonella* serotypes of isolates from red-eared sliders in Haikoudonghu should be outside of the A-F group (Table 3).

2 Discussions

According to the literature, 14% of human *Salmonella* infections in is caused by the spread of turtles (Cohen et al., 1980), Richard et al (2004) detected 0% of wild turtles carrying *Salmonella*. Saelinger et al (2006) detected less than 5% of wild turtles carrying *Salmonella*, Vila et al (2007) detected 12% of overall detection rate of *Salmonella* in one kind of upland tortoises and two species of freshwater turtles, Gaertner (2008) detected *Salmonella* in a variety of wild freshwater turtles that the total carrier rate was 51%, of which the wild red-eared sliders carried *Salmonella* rate of 38%. The results of this study for the total rate of wild red-eared sliders carrying *Salmonella* was 54.41% (n = 68).

There is a little difference of *Salmonella* carrying rate among three different sampling locations, it indicated that habitation should have no effect on the carrying rate of *Salmonella*, which was consistent with Gaertner's report (Gaertner, 2007). Nandujiang is a kind of brackish water river, where *Salmonella* in the sample was detected out, indicating that *Salmonella* as less salt tolerant baterium, can be carried by amphibians reptiles living in these aquatic environment, *Salmonella* detection rate (50.00%) of red-eared sliders from semi-artificial Lake of Haikoudonghu was lower than that of the natural rivers, which does not match the *Salmonella* carring rate of turtles in the artificial environment reported in the previous studies (Keymer, 1972). The reason might be due to regularly release bleaching powder,

Table 3 Serotypes detection of *Salmonella* isolates in collected red-eared sliders

Locations	Serotypes	Name of *Salmonella* isolates	No of strains	Serotypic groups
Nandujiang	O6,8:Hz4,z23:e,n,z15	*S. chailey*	2	C2
	O6,8:He,h:1,2	*S. newport*	1	C2
	O6,8:Hl,v:1,2	*S. litchfield*	1	C2
	O4,5:He,h:−	To be comfirmed	1	B
	OA-F	To be serotyped	2	–
Wanquanhe	O4,12:Hd:1,2	*S. stanley*	1	B
	O1,3,19:Hg,t:-	*S. senftenberg*	1	E4
	O4,5:He,h:-	To be comfirmed	1	B
	OA-F	To be serotyped	3	–
Haikoudonghu	No detected out			

Note: *Salmonella* serotypes of isolates from red-eared sliders in Haikoudonghu should be outside of the A-F group

potassium permanganate and other disinfection reagents to sanitize the lake of Haikoudonghu, which may be causing the *Salmonella* carrying rate decrease, but the specific cause needs further study.

Comparison of two groups by carapace length, *Salmonella* carrying rate of red-eared sliders with small carapace length was higher than that of red-eared sliders with big carapace length, in which red-eared sliders with small carapace length from Nandujiang carried highest rate of *Salmonella* that was consistent with the findings of Keymer (1972).

Amphibians and reptiles carrying *Salmonella* usually without any symptoms (Anonymous, 1999). Mitchell (1980) and other studies have shown that *S. Urbana*, *S. Lichfield*, and *S. Java* were the most human infection serotypes caused by turtles in the *Salmonella*, especially affecting infants and young children. Other serotypes can also be detected from humans (Geue and Loschner, 2002). This study detected the *S. Lichfield*, which indicated the invasive red-eared sliders in china might cause potential harm to humans. Detected *S. Stanley* also is a common pathogens causing human food poison. In this study, we might have a preliminary suggestion that the invasive alien red-eared sliders carrying some *Salmonella* serotypes closely to human health, which may have a direct impact on human health (OIsen et al., 2001).

In China, red-eared sliders, albeit an exotic invasive species, but is still widely used as food turtle and pet turtle. Obviously, the red-eared sliders, as one of the main disseminators of *Salmonella* in reptiles, have entered the human food chains and ecosystem, human health and the environment posed by the potential risks and dangers.

3 Materials and Methods

3.1 Experimental samples collected

We collected 68 of wild red-eared sliders in the two major rivers of Hainan Island, Nandujiang and Wanquanhe, and in the downtown lake of Haikou city, Haikoudonghu during the period between February to September in 2010, of which Nandu were 26, Wanquan were 34, and Haikoudonghu were 8. In this study we have collected wild red-eared sliders divided into three groups, Nandu was as first group, Wanquan as second group, Haikoudonghu as third group. Meanwhile each group was sub-divided as subgroups, the carapace length of a red-eared sliders less than 4-inch divided into group A, greater than or equal to 4 inches into the B group. 26 samples from Nandu (First Group) were gone into Group A of 4 red-eared sliders and Group B of 22 red-eared sliders. 34 samples from Wanquan (Second Group) were gone into Group A of 9 red-eared sliders and Group B of 25 red-eared sliders. 8 red-eared sliders from Haikoudonghu were grouped into 7 of Group A and 1 of group B.

3.2 Standard strains of *Salmonella*

In this study, *Salmonella typhimurium* was used as the standard strains, the standard accession number of the *Salmonella typhimurium* is CMCC (B) 50071, come from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP).

3.3 Bacterium culture medium

Nutrient broth, *Salmonella* chromogenic Medium, vassiliadis enrichment broth, and *Salmonella* biochemical identification kit, provided by HuanKai Biotechnology Co., Ltd. Guangdong; *Salmonella* diagnostic serum (60 reactions) were purchased from Lanzhou Institute of Biological Products; *Salmonella* diagnostic serum for 0A-I, purchased from S & A company in Thailand.

3.4 Method for *Salmonella* Identification

Salmonella bacteria were identified by following the procedures of GB 4789.4-2010 established as the Chinese National Standard Food Safety Inspection.

3.4.1 Sampling and enrichment

Bacterium samples were collected with a steriled cotton swab in red-eared sliders cloaca, then add 1 mL nutrient broth, cultured 6~8 h at 37℃; then took 60 μL medium transferring into 1mL magnesium chloride malachite green, cultured 16~18 h at 37℃.

3.4.2 Bacterial isolation of *Salmonella*

Took one loop of *Salmonella* cultured medium with the inoculation loop to inoculate by crossing the plate of Salmonella chromogenic medium and incubated 16~18 h at 37℃. If there is purple colonies appeared, the isolate will be considered as tentative *Salmonella* bacterium. Pick three purple single colonies ready for

further purified culture. If no any colony appeared purple, then extend the incubation time to 48 hours to observe whether there is purple colonies appeared, The tentative colonies of *Salmonella* were conducted by the assay of Gram staining, Gram-negative bacteria were considered as *Salmonella*.

3.4.3 Biochemical identification of *Salmonella*

Salmonella biochemical identification was carried out by using biochemical identification kit. The colonies on nutrient agar slant was inoculated in 1 mL of nutrient broth to make as bacterial suspension for incubation 4~6 h, and then took 70 μL of the liquid of enriched bacteria to inoculate in 10 reaction bottles of biochemical identification kit (of which three are in control), after incubating 18~24 hours at 37℃ by following guideline, all observation items except for lysine decarboxylase reaction can be done, in addition to lysine decarboxylase, the result of the reaction can be observed. If there was no any reaction of lysine decarboxylase observed in incubation of 18~24 h, incubation will be extended 4 days and then re-observed.

3.4.4 Serological identification of *Salmonella*

Positive *Salmonella* isolates identified by the biochemical identification was inoculated to *Salmonella* chromogenic medium and then the purple colonies were picked to steriled petri dishes on a clean slide for agglutination test. The first agglutination test was for OA-I serum agglutination, positive strains then used for OA-F slide agglutination serum test, *Salmonella* strains were genotyped by comparing the common *Salmonella* antigen.

Author contributions

LS is the executor of experimental research in this study, LS, DL and XPP completed data analysis and manuscript preparation; HTS and RPW are the persons who conceived the project and took responsibility to make the experimental design, data analysis, paper writing and revising. All authors have read and agreed the final text.

Acknowledgments

The study was funded by the National Natural Science Foundation of International Cooperation Project (NO: 30910103916). The authors thank to the two anonymous peer reviewers for their critical comments and revising suggestion. Regarding the reagent suppliers mentioned in this article in our experiments, this is not to provide recommend or endorsement for their products and services.

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