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Short Communication

Highly pathogenic *Salmonella* Pomona was first isolated from the exotic red-eared slider (*Trachemys scripta elegans*) in the wild in China: Implications for public health

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HIGHLIGHTS

- Highly pathogenic *Salmonella* Pomona was first isolated from the exotic species red-eared sliders in the wild in China.
- The total carrying rate of *S.* Pomona in collected red-eared sliders reached 39%.
- This research implied that the widespread red-eared sliders may impact on public health and the ecological system of China.

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ABSTRACT

Salmonella Pomona, a highly pathogenic serotype, can cause severe human salmonellosis, especially in children. Turtles and other reptiles are reservoirs for *S.* Pomona, and these cold-blooded animals remain a source of human *Salmonella* infections. Since the 1980s, this serotype has become a significant public health concern because of the increasing number of cases of *S.* Pomona infection in humans. To date, outbreaks of *Salmonella* Pomona infection in humans have mainly occurred in the United States, with some in other countries (e.g. Belgium, Germany, Canada), and most of the infections in humans were associated with turtles and other reptiles. In China, *S.* Pomona was first isolated from the feces of an infant in Shanghai in 2000, and two further cases of *S.* Pomona infection in humans were later found in Guangzhou. No one knew the source of *S.* Pomona in China. In this study, for the first time we isolated *S.* Pomona from free-living exotic red-eared sliders in the wild in China. *Salmonella* serotype (*S.* Pomona) was isolated from 16 turtle samples. The total carrying rate of *S.* Pomona in the collected red-eared sliders was 39% ($n = 41$) overall: 40% ($n = 25$) in juveniles and 38% ($n = 16$) in adult turtles. This study suggests that the widespread exotic red-eared sliders may impact on public health and ecosystems of China by transmitting *S.* Pomona. Additional steps should be considered by the governments and public health agencies to prevent the risk of turtle-associated *Salmonella* infections in humans in China.

1. Introduction

Salmonellosis, one of the important anthropozoonosis, is caused by *Salmonella*. There are about 1.3 hundred million people infected by *Salmonella* and suffer from enterogastritis in the world each year (Pang et al., 1995). In the United States, Salmonellosis is a significant public health concern, and causes about 1.4 million illnesses and 400 deaths each year (Voetsch et al., 2004). The genus *Salmonella* is comprised of two species, *Salmonella enterica* and *Salmonella bongori*. As of 2007, 2,557 serovars of *S. enterica* and 22 serovars of *S. bongori* have been recognized. The majority of human clinical isolates, including *Salmonella* serovars Enteritidis, Typhimurium, and Typhi (etiologic agent of typhoid fever) are found within *S. enterica* subspecies *enteric* (Mikoleit, 2010a).

Salmonella Pomona (or *S. enterica* serovar Pomona), a highly pathogenic serotype, was first isolated from turkeys (Hinshaw et al., 1944). Later, this serotype was also isolated from turtles, other reptiles, and humans (Tauxe et al., 1985; Woodward et al., 1997; Franco et al., 2011). *S.* Pomona is an uncommon *Salmonella* serotype associated with humans (Woodward et al., 1997). Since the 1980s, however, this serotype has become a significant public health concern because of the increasing number of cases of *S.* Pomona infection in humans (Tauxe et al., 1985; Bertrand et al., 2008; Centers for Disease Control Prevention, 2007, 2012). In children, *S.* Pomona infections can be severe

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and can result in bloody diarrhea, cramps, fever, and occasionally death (Tauxe et al., 1985; Centers for Disease Control Prevention, 2007). Currently, the outbreaks of *S.* Pomona infection in humans mainly occurred in the United States, as well as some cases in other countries (e.g. Belgium, Germany, Canada), and most of the infection in humans were associated with turtles and other reptiles (Woodward et al., 1997; Bertrand et al., 2008; Centers for Disease Control Prevention, 2007, 2012).

In China, *S.* Pomona was isolated from the feces of an infant in Shanghai in 2000 (Xu et al., 2002), and two further cases of *S.* Pomona infection in humans were found in Guangzhou later (Li et al., 2009; Sun et al., 2007). However the source of *S.* Pomona in China was unknown. Prior to this report, there were no reports of animals carrying *S.* Pomona in China. In this study, for the first time, we isolated *S.* Pomona from free-living exotic red-eared sliders in the wild in China.

2. Materials and methods

2.1. Sample collection

All the red-eared sliders were collected from Guangdong Gutian Nature Reserve, Guangdong, China (114° 46′–114° 49′ E, 23° 05′–23° 09′ N, 200–1,071 m a.s.l.), about 29 km north of South China Sea, and 95 km northeast of Hong Kong (Fig. 1). This nature reserve is in the subtropical humid monsoon climate zone, and is rainy and humid. The mean annual temperature is 21.7 °C, and mean annual precipitation is 1,904 mm. The vegetation consists primarily of subtropical evergreen broad-leaf forest. Within the last few decades much of the forest has been destroyed by human activities and has been replaced by secondary forest.

Near the border of the reserve, there is a temple, and some followers of Buddhism often release turtles and other animals around the temple, and the exotic red-eared slider is the most common species for release. Based on 2 years of field survey, we found that red-eared sliders are able to survive and reproduce in the reserve. In this study, a total of 41 red-eared sliders (16 adults and 25 juveniles) were collected by cage-trapping from the stream in the reserve during April–November 2012. Following the method of Gibbons and Greene (1990), we designated individuals <100 mm plastron length and without male secondary sexual characteristics (or, females <160 mm plastron length) as juveniles. The rectal sample of each turtle was collected with bioclean swab. All the rectal swab samples were placed immediately in Cary-Blair transport medium and transported to the laboratory for *Salmonella* detection within 24 hours of collection. All the identification works were done in the laboratory of the Guangdong Province Center for Disease Control and Prevention, Guangzhou, China.

2.2. Morphological identification of Salmonella

Following the technique of Mikoleit (2010b), we inserted a sterile swab into the rectal sample and then dropped the swab into the tube of selenite broth. Following 18–24 hours of incubation at 37 °C in a non-CO_2 incubator, the selenite broth was plated to the Xylose Lysine Desoxycholate Agar (XLD) plate. We collected a small amount of selenite broth sample, and rolled the swab over the first quadrant of XLD plate, and incubated at 37 °C for 18–24 hours in a non-CO_2 incubator. *Salmonella* typically produce clear to light pink colonies with distinct black centers on XLD. In addition, for further identification, a sterile plastic bacterial loop was used to transfer an aliquot of enriched broth to the surface of a Chromogenic *Salmonella* Agar (CSA). Streaked plates were incubated at 37 °C for 18–24 hours in a non-CO_2 incubator. *Salmonella* typically produce purple colonies, diameter 2–3 mm on CSA.

2.3. Biochemical identification of Salmonella

Based on the identification of XLD and CSA, suspect colonies of *Salmonella* were selected for further biochemical test. All tests were performed using pure cultures. Following the technique of Mikoleit (2010a), three well isolated suspect colonies of *Salmonella* from each plate were picked using an inoculating needle for biochemical test. All suspect colonies were screened with five kinds of biochemical test mediums, consists of: triple sugar iron agar (TSI), lysine iron agar (LIA), motility–indol–ornithine agar (MIO), citrate agar, and urea agar, at 37 °C for 18-24 hours. The result profiles were compared to standard biochemical tables to differentiate the genus *Salmonella* from other

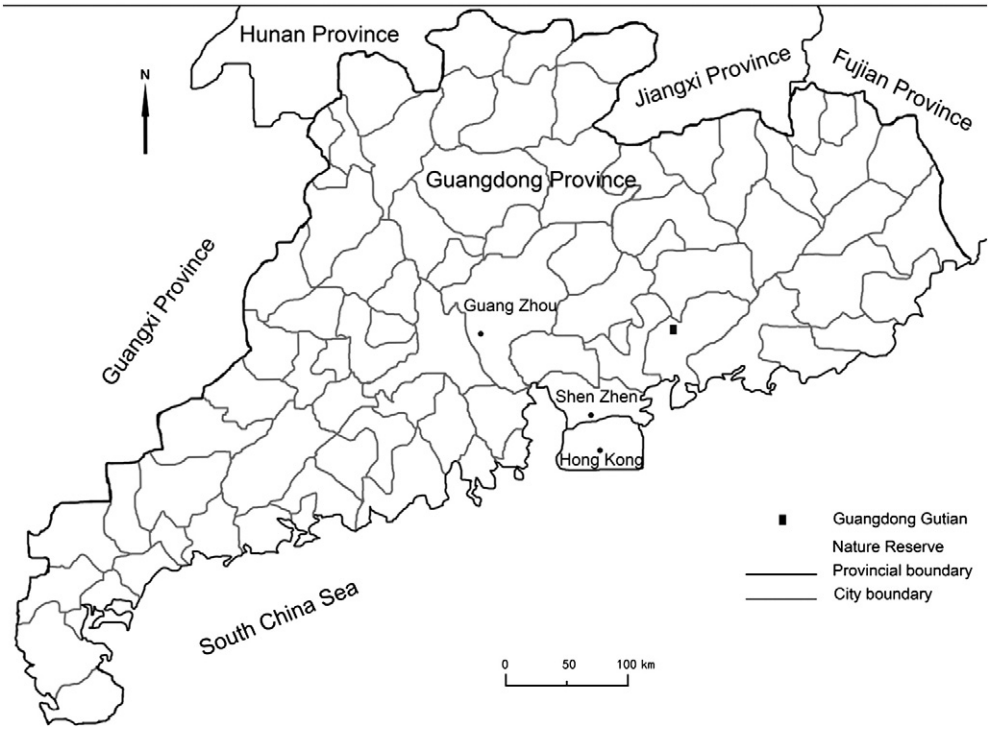

Fig. 1. The location of the Guangdong Gutian Nature Reserve, Guangdong, China (solid quadrate).

Enterobacteriaceae (Mikoleit, 2010a). After the detection of the five kinds of biochemical mediums, the VITEK 2 Compact, a fully automated microbiological identification system (produced by BioMerieux, France), was used to identify the *Salmonella*.

2.4. Identification of Salmonella serotypes

Pure cultures of *Salmonella* were used to determine somatic (O) and flagellar (H) antigenic types. The *Salmonella* O monovalent/multivalent antisera were used to detect *Salmonella* O antigens. After O antigen detection, relevant H antisera were used to detect *Salmonella* H antigens. On the basis of the serologic identification of O and H antigens, the genus is further sub-divided into serovars using the White–Kauffmann–Le Minor scheme, which summarizes antigenic formulae of all known *Salmonella* serovars (Grimont and Weill, 2007).

3. Results and discussion

Based on the identification of XLD and CSA, total 63 suspect colonies of *Salmonella* were obtained from 21 samples of red-eared sliders. Additional biochemical testing of the five kinds of mediums confirmed 48 suspect colonies of *Salmonella* from 16 samples of red-eared sliders. The VITEK 2 Compact fully automated microbiological identification system gave the consistent result with the testing of the five kinds of mediums. Finally, 16 red-eared sliders (6 adults, 10 juveniles) were confirmed to carry *Salmonella*. Serotyping revealed that only one rare serovar, *S.* Pomona [antigenic formula: 28:y:1,7] was identified in all the colonies of *Salmonella*. Total carrying rate of *S.* Pomona in the collected red-eared sliders was 39% ($n = 41$) overall: 40% ($n = 25$) in juveniles and 38% ($n = 16$) in adult turtles. This finding, for the first time, indicates *S.* Pomona is carried by turtles in the wild in China, and adds new knowledge to the current understanding of the epidemiological features of *Salmonella* spread in the region.

Turtles and other reptiles are the major reservoirs of *S.* Pomona, and most of human *S.* Pomona infections were associated with turtles and other reptiles (Bertrand et al., 2008; Centers for Disease Control Prevention, 2012). In the United States, *S.* Pomona infection in humans is common (Centers for Disease Control Prevention, 2007, 2012). Tauxe et al. (1985) thought that millions of turtles exported annually from the United States are an important potential route for global dissemination of human salmonellosis. For example, a significant outbreak of *S.* Pomona infection occurred in Puerto Rico in 1984 following the importation of turtles from Louisiana, USA, and 15% of all infant salmonellosis could be attributed to turtles in the year (Centers for Disease Control Prevention, 1984), about 89% pet turtles in Puerto Rico yielded *S.* Pomona (Tauxe et al., 1985). In order to prevent turtle-associated salmonellosis, the government of the United States made a 1975 law prohibiting the sale or distribution of small turtles, and that prohibition led to a substantial decline in human salmonellosis cases associated with turtles (Cohen et al., 1980).

In China, millions of exotic turtles (as pets or food) have been imported illegally/legally from the United States and other countries, and most of the turtles are red-eared sliders (Lee et al., 2004; Cheung and Dudgeon, 2006; Gong et al., 2009; Liu et al., 2011). Because of Buddhism releasing or other unreasonable releasing, red-eared sliders have occurred widely in the wild in China, especially in southern China (Liu et al., 2011). Without solid evidences and background survey data, we are not sure the main route of *S.* Pomona transmission and infection in China. However, obviously, lots of red-eared sliders and other turtles in pet/food markets and in the wild are potential huge reservoirs for *S.* Pomona, and pose a great potential threat to public health and ecosystems of China by transmitting *S.* Pomona. Currently there is no law prohibiting release of red-eared sliders into the wild, or prohibiting the sale or distribution trade of this exotic species in China. Additional steps should be considered by the governments and public health agencies to prevent the risk of turtle-associated *Salmonella* infections in humans in China.

Author contributions

S.G., F.W. and H.S. wrote the article including conception and design, acquisition, analysis of data. P.Z. and Y.G. collected the samples of red-eared sliders and rectal swab samples, carried out morphological identification of *Salmonella*. L.H. and W.L. conducted the biochemical identification of *Salmonella*, identification of *Salmonella* serotypes. All authors read and approved the final manuscript.

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