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Antibiotic-Resistant *Salmonella*, isolated from cloacal swab samples from turtles in Guatemala

Salmonella *resistente a antibióticos, aislada de muestras de hisopados cloacales en tortugas de Guatemala*

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Resumen

Salmonellosis is a relevant public health threat worldwide. Reptiles are commonly involved in human cases. A microbiological survey was conducted from August to October 2018 to isolate *Salmonella* bacteria and determine if they were resistant to regularly used antibiotics in eight species of pet turtles (*Kinosternon acutum* sp., *K. leucostomum, K. scorpioides, Rhinoclemmys areolata* sp., *R. pulcherrima, Staurotypus salvinii* sp., *Trachemys scripta* and *T. venusta*) in Guatemala city, San Lucas Sacatepéquez and Antigua Guatemala. Cloacal swabs were taken from 63 turtles and cultivated in the Microbiology Laboratory at the Veterinary Medicine and Animal Husbandry Faculty, University of San Carlos of Guatemala, in Guatemala City. Three samples were positive to the presence of *Salmonella* sp. One of these isolates (from *Trachemys scripta*) was resistant to gentamicin, penicillin and amikacin, other isolate (from *T. scripta*) was partially resistant to amoxicilin + clavulanic acid and penicillin, and other (from *T. venusta*) to penicillin. These findings highlight the need for better biosecurity practices and show the capacity of bacteria to develop survival strategies that involve resistance to harmful substances like antibiotics.

Palabras claves: Salmonellosis, public health, epidemiology, zoonoses, multiresistant Gram negative bacteria

Abstract

La salmonelosis es una importante enfermedad zoonótica considerada una amenaza a la salud pública a nivel mundial. Los reptiles están comúnmente involucrados en la transmisión animal-humano. Con el objetivo de determinar la presencia de *Salmonella* y determinar su resistencia a antibióticos de uso común, se realizó un estudio exploratorio en ocho especies de tortugas (*Kinosternon acutum* sp., *K. leucostomum, K. scorpioides, Rhinoclemmys areolata* sp., *R. pulcherrima, Staurotypus salvinii* sp., *Trachemys scripta* y *T. venusta*) en Guatemala y en San Lucas Sacatepéquez. Se tomaron hisopados cloacales de 63 especímenes y se cultivaron en el Laboratorio de Microbiología de la Facultad de Medicina Veterinaria y Zootecnia de la Universidad de San Carlos de Guatemala. Tres muestras fueron positivas a la presencia de *Salmonella* sp. Uno de los aislados (de *Trachemys scripta*) fue resistente a gentamicina, penicilina y amikacina, otro aislado (de *T. scripta*) fue parcialmente resistente a amoxicilina + ácido clavulánico y a penicilina y un tercer aislado (de *T. venusta*) a penicilina. Estos hallazgos resaltan la necesidad de mejores prácticas de bioseguridad y muestran la capacidad de las bacterias para desarrollar estrategias de sobrevivencia que involucran la resistencia a sustancias que les son nocivas, como los antibióticos.

Keywords: SSalmonelosis, salud pública, epidemiología, zoonosis, bacterias Gram negativas multirresistentes

Introductión

Nontyphoidal *Salmonella* is an important public health threat, causing an estimated of 93.8 million cases of gastroenteritis globally, including 155,000 deaths (Majowicz et al., 2010). Over 2,500 *Salmonella* serotypes have been identified and more than half of them belong to *Salmonella enterica* subspecies *enterica* which causes most *Salmonella* infections in humans (Eng et al., 2015). Not only is salmonellosis one of the diseases affecting socio-economic development worldwide (World Health Organization, 2015), but is also a major contemporary concern due to its dynamic epidemic behavior (Ao et al., 2015; Besser, 2018) and to the growing ability of the causing bacteria serotypes to develop multiple resistance to antibiotics (Blairet et al., 2015; Ventola, 2015; Wong et al., 2015).

Many animal species, both domestic and wild, are reservoirs of *Salmonella* and harbor these bacteria in their gastrointestinal tracts with no apparent signs of illness (MacDonald et al., 2019; Oludairo et al., 2013; Sanchez et al., 2002). Salmonellosis outbreaks are often associated with exotic pet ownership (Woodward et al., 1997). Turtles, specially inland species, were common sources of *Salmonella* in many of these outbreaks in United States (Bosch et al., 2016; Centers for Disease Control and Prevention [CDC], 2007; Harris et al., 2009; Harris et al., 2010; Warwick et al., 2001), Spain (Hernández et al., 2012; Hidalgo-Vila et al., 2007) and Japan (Nagano et al., 2006). By 1971, 280,000 turtle-associated cases were estimated to occur only in the United States (Lamm et al., 1972) and although some public health measures have been taken since then, turtles are still common sources of *Salmonella* infections (Bosch et al., 2016; Cummings et al., 2012; Hale et al., 2012). This, because turtles are the second most popular pets after birds (Bush et al., 2014) and sometimes live in frequent physical contact with their owners (Siegmund & Biermann, 1988).

Although salmonellosis is an important zoonosis in Latin America (Gil & Samartino, 2001), very few studies have addressed the role of domestic turtles in the cases or outbreaks in this region (Braun et al., 2015; Sacristán-Rodríguez et al., 2014; Tauxe et al., 1985). Considering the role of pet turtles in the epidemiology of salmonellosis in other parts of the world (Hoelzer et al., 2011; Sodgari et al., 2020), the objectives in this exploratory survey were to look for *Salmonella* organisms in turtle cloacal swab samples and to test the isolates for antibiotic resistance. The findings provide the first report on the circulation of *Salmonella*, including antibiotic-resistant isolates, in pet turtles in Guatemala.

Materials and methods

Study site

Samples were taken from households in Guatemala City and San Lucas Sacatepéquez, and from a rescue center in Antigua Guatemala. Turtle owners were contacted through a call placed in social networks and in the Veterinary Faculty of University of San Carlos of Guatemala website. A maximum of three specimens were collected from each sampling site except in the rescue center where nine samples were taken from turtles that had recently been brought from a domestic environment. Figure 1 shows the sampling sites distribution.

Sample collection

In a period of three months (August to October 2018) 63 cloacal swabs were taken from turtles of eight species (1 *Kinosternon acutum,* 3 *K. leucostomum,* 4 *K. scorpioides*, 14 *Rinoclemmys pulcherrima,* 3 *R. areolata,* 3 *Staurotypus salvinii,* 22 *Trachemys scripta* and 13 *Trachemys venusta*). The sampled turtles were kept either in backyards or in aquaria and all were being fed commercial food for turtles and, occasionally, vegetables.

Samples were collected from the cloaca, using a sterile peptone water-soaked swab, and transported in test tubes with peptone water to the Microbiology Laboratory of the Veterinary Faculty of University of San Carlos of Guatemala. Samples were received for processing when they had less than 4 hours of being taken.

Laboratory procedures for *Salmonella* isolation

Salmonella isolation was performed according to the ISO 6579 International Standard for *Salmonella* spp. detection (International Organization for Standardization, 2002). The samples were processed in duplicate to improve the chances of *Salmonella* isolation. Since this was an exploratory investigation, *Salmonella* serovars were not identified.

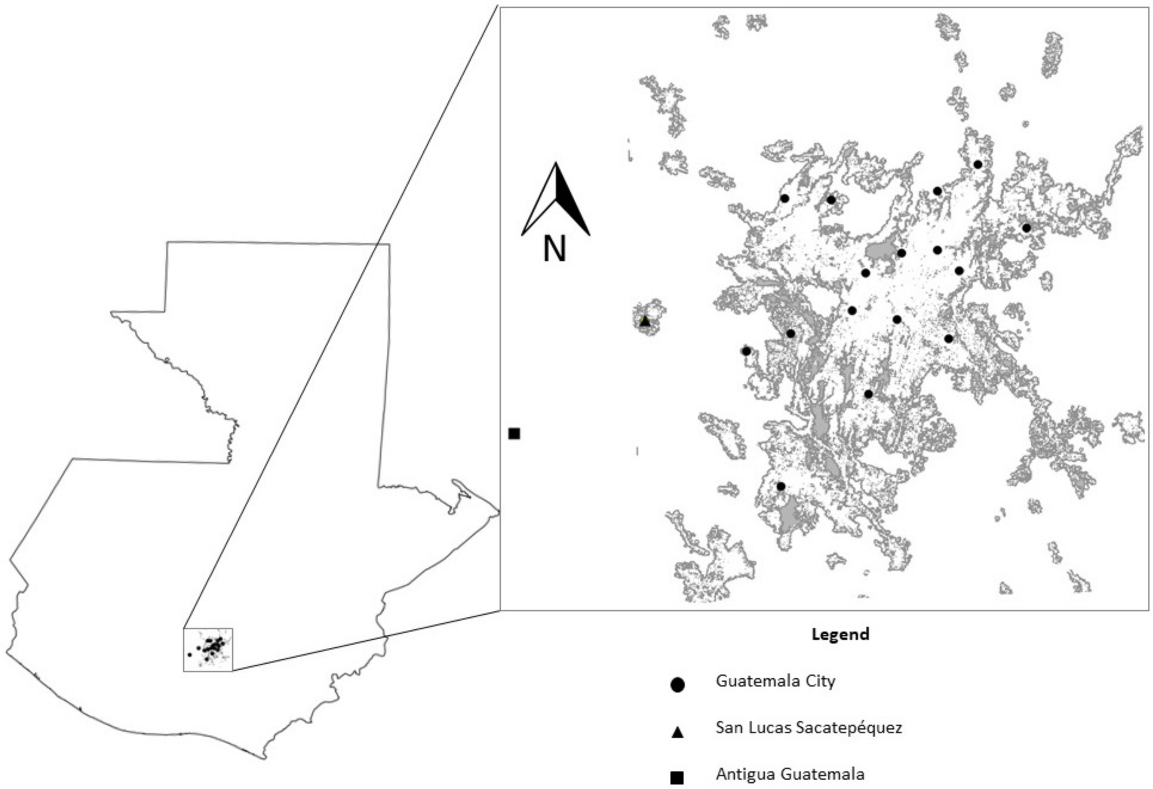

Figure 1. Turtle sampling sites in Guatemala City, San Lucas Sacatepéquez and Antigua Guatemala.

Antibiotic resistance test

The *Salmonella* isolates were tested for resistance against amoxiciline + clavulanic acid, cefotetan, oxytetracycline, gentamicine, penicillin, amikacin, nalidixic acid, doxycycline, and ceftriaxone, following the disk sensitivity test procedures presented by Sacsaquispe and Velásquez (2002).

Results

Salmonella bacteria was isolated from three of the 63 cloacal swabs. Even though the samples were taken from eight turtle species reported for Guatemala (Acevedo et al., 2010) the three isolates came only from turtles of the genus *Trachemys* (Table 1). Although not an objective of this study, *Escherichia coli*, *Proteus* sp., and *Enterobacter* sp. were common findings in all cloacal swabs.

The *Salmonella* isolates were resistant or partially resistant to at least one antibiotic. The first isolate (from *Trachemys scripta*) was resistant to amikacin, gentamicin and penicillin. The second isolate (from *T. venusta*) was partially resistant to amoxicillin/clavulanic acid and penicillin. The third isolate (from *T. scripta*) was partially resistant to penicillin.

Discussion

The prevalence of *Salmonella* isolates in our sample was rather low (4.7%). This level of detection is consistent with other investigations in turtle populations where the prevalence was close to zero (Brenner et al., 2002; Mitchell & McAvoy, 1990; Richards et al., 2004; Saelinger et al., , 2006; Way & Allender, 2011). However, other studies have detected prevalences ranging from 8 to 18.75% (Charles-Smith et al., 2009; Readel et al., 2010; Sharma et al., 2020; Tomastikova et al., ;

Tabla 1

Distribution of Salmonella isolates in the sampled turtle specimens

Species	*n*	Number of isolates
Kinosternon acutum	1	0
Kinosternon leucostomum	3	0
Kinosternon scorpioides	4	0
Rhinoclemmys areolata	3	0
Rhinoclemmys pulcherrima	14	0
Staurotypus salvinii	3	0
Trachemys scripta	22	2
Trachemys venusta	13	1
Total	63	3

Zhang et al., 2016). It is known that *Salmonella* shedding could be intermittent (Kaufmann et al., 1967) and stress-related in reptiles (Burnham et al., 1998; Chiodini & Sundberg, 1981; DuPonte et al., 1978), so, this apparent prevalence should be taken with caution. Cloacal swab sampling could as well not be the best technique for *Salmonella* detection in comparison to better –but more invasive– methods like mucosa scrapings. On the other hand, *Salmonella* has been a common finding in imported turtles from captive breeding facilities, mostly from the USA (D'Aoust et al., 1990; Lukac et al., 2015; Shane et al., 1990).

It should be noted that *Trachemys* turtles were the only source of the *Salmonella* isolates in this study. *Trachemys scripta* is a common pet turtle worldwide. In Guatemala, it is sold at a very affordable price (around 2.5 US $ each) in most pet stores around the country. Over 36,000 *T. scripta* turtles were imported to Guatemala in 2018 to supply the pet market (R. De León, personal communication, April 20, 2019). This turtle species has been considered one of the most common sources of *Salmonella* to humans (Hidalgo-Vila et al., 2008; Lamm et al., 1972; Nagano et al., 2006; Pasmans et al., 2002; Readel et al., 2010; Shen et al., 2011) and also an alien invasive species (Kuzmanova et al., 2018; Ma & Shi, 2017; Zhang et al., 2020).

Based on the premises that some of the sampled turtles in the present investigation were shedding Salmonella bacteria with some degree of antibiotic resistance and that thousands of turtles are being imported annually to Guatemala for the pet market, we can assume that there is some risk of acquiring salmonellosis from the contact with these animals or their environment.It has been stressed out that the risk of turtles as a source of human salmonellosis, especially for young children, should not be underestimated (Sodagari et al., 2020). Furthermore, not only could *Salmonella* cause highly pathogenic cases in humans (Nagano et al., 2006), but also hard to treat cases due to antibiotic resistance (Eng et al., 2015; Liang et al., 2015).

Resistance to commonly use antibiotics –such as erythromycin, sulfisoxazole, gentamycin, amoxycillin and ampicillin– by *Salmonella* isolates have been previously reported for various species of reptiles (Ebani et al., 2005). *Salmonella* strains from turtles imported from the USA have shown to be resistant to gentamicin and other antibiotics commonly used for treating turtles after the 1974 small turtle trade ban (Shane et al., 1990). Antibiotic use as supplement in livestock and other captive bred animals like turtles has been identified as one of the main causes of the antibiotic resistance crisis (Angulo et al., 2014; Eng et al., 2015; Ventola, 2015). As the household size become smaller in modern societies, people look for more suitable pets like turtles to fit small apartments. Children could then be the more exposed age group to become infected with *Salmonella* from turtles because they use to play with their pets (Kaibu et al., 2006; Pees et al., 2013; Siegmund & Biermann, 1988) and because turtles are one of the most commercialized pets worldwide (Bush et al., 2014).

Our preliminary data suggests that only *Trachemys* turtles harbor *Salmonella* bacteria amongst inland turtle species in Guatemala. Interesting also, is the fact that we found the bacteria in imported (*T. scripta*) and native (*T. venusta*) specimens of this genus of turtle. This could be the result of inter-species transmission between imported *T. scripta* and native *T. venusta* housed together or coexisting in free ranging conditions. *Salmonella* was already found in wild populations of naturalized *T. scripta* in China (Gong et al., 2013). *Salmonella* shedding and transmission to naturalized turtles could even threaten native turtle populations. More research needs to be done to understand the ecology of *Salmonella* bacteria across the inland and marine species of turtles in Guatemala in domestic or wild environments.

The results of this investigation reinforce the idea that captive raising of farm or wild animals could provide the necessary conditions for the establishment and dissemination of *Salmonella*. Furthermore, the practice of administering preventive or therapeutic antibiotics to captive populations favors the evolution of multi-resistant bacteria strains which represent a contemporary public health issue (Kariuki et al, 2015; Medalla et al., 2017; Ventola, 2015).

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