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Tiny turtles purchased at pet stores are a potential high-risk for *Salmonella* human infection in the Valencian region, Eastern Spain

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Abstract

Although humans usually become infected with *Salmonella* through ingestion of *Salmonella*-contaminated food, 6% of *Salmonella* infections overall are acquired from direct or indirect contact with reptiles. Under a public health point of view, turtles are considered unsafe pets, particularly in households with children. The objective of the present study was to assess *Salmonella* carriage by turtles in pet stores and private owners in order to inform the public of the potential health risk and allow them to make informed choices around pet selection. During the period between September and October 2013, 24 pet stores and 96 private owners were sampled in the Valencian Region (Eastern Spain). *Salmonella* identification procedure was based on ISO 6579: 2002 recommendations (Annex D). *Salmonella* strains were serotyped in accordance with Kauffman-White-Le-Minor technique. The rate of isolation of *Salmonella* was very high from pet store samples (75.0±8.8%) and moderate for private owners (29.0±4.6%), Serotyping revealed 18 different serotypes among two *Salmonella enterica* subspecies: *S. enterica* subsp. *enterica* and *S. enterica* subsp. *diarizonae*. Most frequently isolated serotypes were *S.* Typhimurium (39.5%, 17/43) and *S.* Pomona (9.3%, 4/43). Serotypes identified have previously been reported in turtles and child *Salmonella* infections associate with pet turtle exposure. The present study clearly demonstrates that turtles in pet stores, as well as in private owners could be a direct or indirect source of a high risk of human *Salmonella* infections. In addition, pet stores should advise their customers of the potential risks associated with reptile ownership.

Keywords: Salmonellosis; Pet Turtles; Pet shop; Owners; Children.

Introduction

Salmonellosis is one of the most prevalent zoonosis worldwide (European Food Safety Authority, 2012). 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). In Europe, salmonellosis is responsible for 99,020 cases of illnesses (European Food Safety Authority, 2012). This zoonose represents an important public health problem and controlling the disease has become a challenge in most countries (European Food Safety Authority, 2012).

Although humans usually become infected with *Salmonella* through ingestion of *Salmonella*-contaminated food, 6% of *Salmonella* infections overall are acquired from direct or indirect contact with chelonians (Mermin et al. 2004). The most popular reptiles are chelonians, though there are also a large number of lizards and snakes kept as pets (Lafuente et al. 2013). Wild and pet reptiles are generally known to be asymptomatic carriers and natural reservoirs of several *Salmonella* serotypes (Mermin et al. 2004, Nakadai et al. 2005, Hidalgo-Vila et al. 2007, Marin et al. 2013). From a public health point of view, turtles commonly shed *Salmonella* and are therefore unsafe pets, particularly in households with children (Harris et al. 2010). Nevertheless, as the proportion of worldwide salmonellosis infections in humans transmitted by reptiles is low (Editorial team et al. 2008, Cooke et al. 2009, Lafuente et al. 2013, Pees et al. 2013), it is considered sporadic. Moreover, the popularity and number of exotic reptiles kept as pets has risen, leading to an increase in the number of reptile-associated zoonotic pathogen infections, especially in vulnerable patients such as infants, young children, the elderly or immunocompromised adults (Schröter et al. 2004; Hernández et

al. 2011, Centers for Disease Control and Prevention, 2012). Based on this information, many countries have recognized the risk of the increasing number and popularity of pet reptiles (Lukac et al. 2015) and it has been recognized as a significant public health issue in Europe and the United States (Editorial team et al. 2008, Centers for Disease Control and Prevention, 2012). Multiple reports since 2004 suggest exposure to small pet turtles is a persistent source of salmonellosis in young children (Voetsch et al. 2004).

The objective of the present study is to assess *Salmonella* carriage by turtles in pet stores and private owners in order to inform the public of the potential health risk and allow them to make informed choices around pet selection.

Materials and Methods

This project is included in the LIFE + Biodiversity section, which aims to develop innovative projects or demonstrations that contribute to the implementation of the objectives of the Commission communication (COM (2006) 216 final) "Halting the loss of Biodiversity for 2010- and beyond."

Sample collection

During the period between September and October 2013, 24 pet stores and 96 private owners were sampled in the Valencian Region (Eastern Spain). The Conselleria de Infraestructuras, Territorio y Medio ambiente (regional administration) gave permission to take samples in the pet stores. Private owners were contacted by advertising the project through the University community (CEU Cardenal Herrera University,

University of Valencia and Polytechnic University of Valencia). As bacteria excretion is not continuous, water samples were taken after two days without filtration or water changes according with Marin et al. (2013). Water samples were collected in a Sterile 0.5-L polypropylene bottles were filled by hand in the deepest part of the aquariums. Samples from the pet stores were collected by veterinary inspectors. Private owners personally brought samples to the laboratory. In both cases, all samples were processed within 6 h of collection. Each of the participants were provided with a questionnaire that was completed and submitted together with the samples to the laboratory. The questionnaire containing specie, number of animals in the aquarium, age of the animal, aquarium volume, housing (indoor vs. outdoor) was completed.

Detection of *Salmonella spp*.

The procedure was based on ISO 6579: 2002 recommendations (Annex D). Samples were pre-enriched in 1: 10 vol/vol Buffered Peptone Water 2.5% (BPW, Scharlau®, Barcelona, Spain) and then incubated at 37±1°C for 18±2 hours. The pre-enriched samples were transferred onto Semi-Solid Modification Rappaport Vassiliadis agar plate (MSRV, Difco®, Valencia, Spain) and incubated at 41.5±1°C for 24-48 hours. The culture obtained in MSRV was inoculated onto Xylose-Lysine-Desoxycholate (XLD, Liofilchem®, Valencia, Spain) and Xylose-Lysine-Tergitol-4 (XLT4, Biokar Diagnostics®, Pantin Cedex, France) and incubated at 37±1°C for 24-48 hours. After incubation, 5 typical colonies were streaked onto the surface of pre-dried nutrient agar plates (Scharlab®, Barcelona, Spain) 37±1°C for 24±3 hours. Then, a biochemical test using API (API-20®, bioMérieux, Madrid, Spain) was performed to confirm *Salmonella* spp. *Salmonella* strains isolated were serotyped by the Ministry of

Agriculture, Fisheries and Food Reference Laboratory (Algete, Madrid, Spain) in accordance with Kauffman-White-Le-Minor technique.

Statistical analysis

Data relative to number of animals in the aquarium, age of the animals and aquarium volume was analysed using a General Linear Model (GLM) with sample origin (pet store and private owner [indoor and outdoor]) as a fixed factor. Additionally, a GLM, which assumed a binomial distribution for *Salmonella* shedding, was fitted to the data to determine whether there was an association with sample origin (pet store and private owner). Analyses were performed with SPSS 21.0 software package (SPSS Inc., Chicago, Illinois, USA, 2002). Values were considered statistically different at $P<0.05$. Results are reported as least square means with standard error of the mean (SEM).

Results

During the study, 120 samples (Table 1) were evaluated. They were distributed as follows: 20.0% (24/120) from pet stores and 80.0% (96/120) from private owners. The private owners housing distribution was as follow: 62.4% (77/120) from indoor turtles and 15.8% (19/120) from outdoor turtles. Details of sample origin, number of animals in the aquarium, age of the animals, aquarium volume and housing are provided in Table 1. A total of 35 species were identified (Table 2). The 10% of the samples were obtained from aquariums with turtles of different species. In the pet stores, the most common species identified were, in decreasing order: *Graptemys pseudographica* (45.8%), *Pseudemys rubriventris* (12.5%) and *Pseudemys nelsoni* (8.3%). In the private

owners, the most common species identified were, in decreasing order: *Chelydra serpentina* (34.4%), Trachemys scripta elegans (19.8%), *Macrochemys temmindxii* (5.2%) and *Pseudemys nelsoni* (4.2%). All turtles in the pet stores were kept indoor. Pet store showed a significant higher number of animals in the aquarium (30±3.1) when compared to outdoor private owner aquariums (6±3.4, $p<0.001$) and indoor private owner aquariums (2±1.7, $p<0.001$). Pet stores also had much younger turtles (2±10.6 months, $p<0.001$) when compared to private owners (131±11.7 and 39±5.9 months for outdoor and indoor private owner, respectively). In addition, pet store showed significant differences in the aquarium volume (43±109.0, $p<0.001$) when compared to outdoor private owner aquariums (705±134.6, $p<0.001$) and indoor private owner aquariums (8±80.24, $p<0.001$).

The location of sample collection was significantly associated with *Salmonella* carriage, with frequency of carriage higher for samples from pet stores (75.0±8.8 % vs 29.0%±4.6%, for pet stores *vs* private owners' samples, respectively, $p < 0.001$). When we compared private owners' indoor and outdoor enclosure, no significant differences were observed (37.0±11.1% *vs* 27.0%±5.1%, for outdoor *vs* indoor samples, respectively, $p < 0.001$).

Of the 46 *Salmonella* isolates, 43 could be identified as 18 serotypes. *S. enterica* subspecies represented 95.3% (41/43) of all isolates (Table 3). Most frequently isolated serotypes were *S.* Typhimurium (39.5%, 17/43) and *S.* Pomona (9.3%, 4/43). Among the 12 serotypes isolated from pet stores, 3 were also isolated from private owners: *S.* Pomona, *S.* Urbana and *S.* Thompson.

Discussion

The present study clearly demonstrate that turtles in pet stores, as well as in private owners could be a direct or indirect source of human *Salmonella* infections. The percentage of isolation of *Salmonella* was high from pet store samples (75%) and lower for private owners (29%), consistent with those of other countries such as Japan (Nakadai et al. 2005). This finding was not unexpected if we take in to account that reptiles and amphibians have long been known to harbor *Salmonella* (Caldwell and Ryerson 1939) and to cause human infection (Door 1958). Effectively, Mermim et al. (2004) estimated that 74,000 *Salmonella* infections, during the 1996–1997, in the United States were associated with reptile or amphibian contact. Consistent with this, recent studies clearly demonstrates the association of reptile associated salmonellosis with hospitalisation and invasive disease at young age (Meyer Sauteur et al. 2013, Murphy and Oshin 2015, Walter et al. 2016), in particular infants <6 months of age (Meyer Sauteur et al. 2013). Because turtles are slow moving and are perceived to be gentle pets, they are more likely than other reptiles to be given to infants or young children (de Jong et al. 2015), who in turn handle small turtles more closely than they might handle other reptiles, including kissing the turtles or putting them in their mouths (de Jong et al. 2015). In addition, turtle terrariums frequently contain a reservoir of water that serves as a site of amplification of *Salmonella* bacteria (D'Aoust et al. 1990); children might play with a turtle in the terrarium and splash the contaminated water, increasing their risk of infection. The difficulty in enforcing children's good hygiene practices, including adequate handwashing immediately after contact with a reptile, compounds this problem (Harris et al. 2010).

In the present study a higher proportion of positive samples were present in pet stores compared with private owners. A plausible explanation for the difference could be the fact that turtles from private owners are less or not even exposed to stress factors that increase shedding rates. Intermittent shedding of *Salmonella* in reptiles and the wide array of collection and sampling techniques have been proposed to be the main reasons for the variability in detection rates (Scheelings et al. 2011). We hereby used a protocol evaluated two year ago in our laboratory, with higher detection rates compared with cloacal swabs (Marin et al. 2013). In the cloaca of turtles, the presence of *Salmonella* is lower than in the intestinal content (Marin et al. 2013). *Salmonella* shedding from the gastrointestinal tract is facilitated by stress due to transport, overcrowding at pet stores, or incorrect, inadequate housing (Smith et al. 2012). It is possible that the high number of young pet turtles (>30 of about 2 months aged) housing at the same aquarium resulted in a stress situation, resulting in more *Salmonella* excretion. It is known that the risk of transmission is increased in young reptiles kept indoors, living, i.e., in breeding farms and transport from farms to stores, where a high diversity and number of animals are kept in close contact (Mitchell and Shane 2000). Moreover, the primary transmission route for pathogens is via faecal-oral ingestion (Lamm et al. 1972) and perharps turtle-to-turtle transmission within aquarium will be extremely rapid, and the majority of turtles in a positive aquarium will be colonized within only a few days. More research is requiered to corroborate such hypotheses.

In the present study, two subspecies of *S. enterica* belonging to 18 different serotypes were isolated (*S. enterica enterica* [I] and *S. enterica diarizonae* [III]). The proportion of subspecies I serotypes was the highest (95.3%), belonged to those most-frequently seen in humans in the European Union, where *S.* Enteritidis, *S.* Typhimurium

monophasic, *S.* Typhimurium, *S.*Infantis, and *S.* Stanley were the top five serotypes associated with human illness in 2012 (Hugas and Beloeil 2014). In addition to the *S. enterica enterica* subspecies, *S. enterica diarizonae* has also been described as causative agents in human infections associated with direct or indirect contact with pet reptiles (Editorial team et al. 2008). The serotypes most frequently identified were *S.* Typhimurium and *S.* Pomona, *S.* Thompson and *S.* Urbana, isolated in both pet stores and privately owned turtles. All of these serotypes have previously been reported in turtles and child *Salmonella* infections associate with pet turtle exposure (Dessì et al. 1992, Xu et al. 2000, Brédart et al. 2007, Bertrand et al. 2008, Editorial team et al. 2008, Li et al. 2009, Kocianova et al. 2010, Centers for Disease Control and Prevention, 2013, Walter et al. 2016), an exception of *S.* Thompson that has been identified in turtles but not directly related with infant infection (Marin et al. 2013). Centers for Disease Control and Prevention reported a multistate outbreak of human *S.* Typhimurium infections associated with pet turtle exposure (Centers for Disease Control and Prevention, 2010). Previously, Dessì et al. (1992) disclosed human salmonellosis transmitted by a domestic turtle. Additionally, 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 and Prevention, 2013). Specifically, a case of a four-month-old girl who suffered of septicaemia due to *S.* Pomona was described in 2007 (Editorial team et al. 2008). The source of infection was established to be the family's pet turtle (Brédart et al. 2007). In China, *S.* Pomona was isolated from the feces of an infant in Shanghai in 2000 (Xu et al. 2000), and two further cases of *S.* Pomona infection in humans were found in Guangzhou later (Li et al. 2009). Finally, *S.* Urbana infection in a two-year-old child was caused by pet water turtles (Kocianova et al. 2010).

In conclusion, we had found a high and moderate frequency of *Salmonella* positives in both pet store and private owners, respectively. In our study, serotypes most frequently identified (*S.* Typhimurium, *S.* Pomona and *S.* Urbana) have previously been reported in children *Salmonella* infections associate with pet turtle exposure. More than a half-century after the first reports of turtle-associated *Salmonella* in humans, and the continued occurrence of turtle-associated salmonellosis indicates that existing prevention efforts need to be enhanced to protect the public health. Families with young children should be aware of the health risks associated with having a turtle as a family pet. In addition, pet stores should advise their customers of the potential risks associated with reptile ownership. The effectiveness of any potential intervention requires monitoring of the *Salmonella* status at breeding farms and transport from farms to stores to determine the source of infection

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Author Disclosure Statement

No competing financial interests exist.

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