Enferm Infecc Microbiol Clin. 2013;31(1):32–35

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Brief report

Salmonella paratyphi B and *Salmonella litchfield* outbreaks associated with pet turtle exposure in Spain

Sarah Lafuente[a,*], Juan B. Bellido[b], Fernando A. Moraga[c], Silvia Herrera[d], Alberto Yagüe[e], Tomás Montalvo[a], Mercè de Simó[a], Pere Simón[a], Juan A. Caylà[a]

[a] *Agència de Salut Pública (ASPB), Barcelona, Spain*
[b] *Centro de Salud Pública de Castellón, Castellón, Spain*
[c] *Unidad de Patología Infecciosa e Inmunodeficiencias de Pediatría, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain*
[d] *Instituto de Salud Carlos III (ISCIII), Madrid, Spain*
[e] *Servicio de Microbiología, Hospital de La Plana, Villarreal, Spain*

ARTICLE INFO

Article history:
Received 21 February 2012
Accepted 27 May 2012
Available online 12 July 2012

Keywords:
Salmonella
Turtle
Transmission
Molecular epidemiology
Public health

ABSTRACT

Introduction: Salmonellosis is an important public health problem. Turtles are increasingly involved in the role of transmitters of this infection to humans.
Methods: Salmonella cases are reported to the local Surveillance Agency where interviews are carried out to address possible exposures. Molecular epidemiology techniques were used to identify species.
Results: In this article we report two examples of this type of infection in two places, 300 km apart in Spain. In Barcelona a turtle transmitted the disease to a small baby via her mother, and in Castellón 5 related cases of Salmonella infections were detected, and all were transmitted by imported turtles. Molecular epidemiology techniques confirmed the turtle-person transmissions and showed strong relationships between cases in Castellón and Barcelona.
Discussion: These examples represent the tip of the iceberg of what is happening with pet reptiles as regards transmission of this infection. We believe that it is important to assess the impact of this type of infection in each country, in order to subsequently promote prevention strategies such as: regulations for pet shops, and educating/informing families who buy reptiles as pets.

Brotes de *Salmonella paratyphi B* y *Salmonella litchfield* asociados a contacto con tortugas en España

Palabras clave:
Salmonella
Tortuga
Transmisión
Epidemiología molecular
Salud Pública

RESUMEN

Introducción: La salmonelosis es un problema con un impacto importante en salud pública. Cada día es más importante el papel de las tortugas como transmisoras de esta enfermedad.
Métodos: Los casos de salmonelosis son notificados a las Agencias de Vigilancia locales que realizan las encuestas pertinentes para valorar fuentes de exposición. Técnicas de epidemiologia molecular son utilizadas para identificar las especies.
Resultados: En este artículo exponemos ejemplos de este tipo de transmisión en dos puntos de España que distan 300 km. En Barcelona una tortuga transmitió la enfermedad a un bebé a través de su madre y en Castellón se registraron cinco casos de infecciones por Salmonella, todos ellos provocados por el contacto con tortugas. La epidemiologia molecular permitió confirmar la transmisión tortuga persona y así como una fuerte relación entre los casos de Barcelona y Castellón.
Discusión: Estos ejemplos representan la punta del Iceberg en cuanto al papel de los reptiles en la transmisión de Salmonella. Creemos que evaluar el impacto de esta transmisión en los países es básico para poder aplicar y promover medidas preventivas. Normativas específicas en los puntos de venta de reptiles así como información y educación de las familias que pretenden comprar un animal de este tipo podrían ser muy útiles para disminuir el impacto de esta transmisión.

* Corresponding author.
E-mail address: sarahlafuente@hotmail.com (S. Lafuente).

0213-005X/$ – see front matter
http://dx.doi.org/10.1016/j.eimc.2012.05.013

Introduction

Salmonellosis is still an important public health problem worldwide. In the USA alone it has been estimated that there are approximately 1.5 million cases per year.[1] In Spain it is the second cause of bacterial gastroenteritis, after that caused by *Campylobacter*.[2,3]

Salmonellosis is a zoonosis, the main reservoir being the intestinal tract of domestic and wild animals. There are over 2500 serotypes of *Salmonella*, 2000 of which can affect humans. Infection by *Salmonella* may involve diarrhoea, vomiting and fever. The clinical course of the infection is usually self-limiting; however it may also present a more severe ailment, and even end fatally in high risk patients such as babies, the elderly or patients with immunodeficiencies.[4]

In recent years an increase has been observed of *Salmonella* cases in humans directly or indirectly related with reptiles, mainly due to the increasing numbers of families who have such an animal at home.[1,5–9] Usually the infection in these animals is asymptomatic, although it can become serious or even fatal.[5]

In Europe the number of families with small children who acquire a reptile as a pet is continually increasing and although it seems that the number of cases of salmonellosis associated with this form of exposure is increasing, we do not as yet have any data on this phenomenon.[10] Before any programme for preventing this form of exposure can be set up, it is important to quantify the impact of the problem. The aim of the present article is to describe two outbreaks of infection by *Salmonella* in humans, associated with the same turtle type, which were detected in 2009 in Spain, in two towns 300 km apart.

Description of the investigation

Investigation in Castellón

Between March and July 2009 two cases of infection by *S. paratyphi B* were reported to the Epidemiology Unit of the Castellon Public Health Centre (CSPCS), which covers an area with 450,000 inhabitants, and 71 towns. No case of such an infection [*S. paratyphi B*] had been reported during the previous 5 years. Both patients, aged 11 months (case J1) and 4 years (case J2), resided in the same town. The antibiograms of the two strains were identical. A CSPCS epidemiologist contacted the patients and their paediatrician in order to initiate an epidemiological investigation.

The symptoms consisted of gastroenteritis with fever lasting 3–5 days which was resolved with symtomatic treatment in outpatients. The two patients did not know each other and did not present any common antecedents, except that both had fresh-water turtles acquired prior to the onset of symptoms at the same pet-shop in their home town. During the investigation a third case (J3) was identified, the 2-year old brother of case J1 who had suffered similar symptoms and whose copro-culture revealed the same infection.

Samples of aquarium water were taken in the homes of case J2 and of cases J1 and J3. Moreover, the investigation made it possible for one of the turtles to be taken to and kept alive at the CSPCS, where 3 more samples of water were taken, on 9 July, and 14 August, this is important since excretion is not continuous.[11] The five water samples were all positive to *Salmonella*. Three of the strains isolated were serotyped and were found to be: two *Salmonella litchfield* (strains A1 and A2, taken July 8) and one *Salmonella paratyphi B* var. Java (strain A3, taken July 9). The turtle was identified as being a *Trachemys scripta troosti* [Fig. 1].

Given these findings, all cases of salmonellosis diagnosed during the first half of 2009 in the CSPCS jurisdiction area were checked and

Fig. 1. *Trachemys scripta troosti.*

two cases of gastroenteritis due to *S. litchfield* were identified. These corresponded to two children (cases L1 and L2) both 2 years old and living in the same town as the above cases, whose symptoms had appeared in January and May 2009, the only common antecedent being exposure at home to fresh-water turtles bought in the same pet shop as the other cases.

Investigation in Barcelona

On 5 November 2009 the Barcelona Public Health Agency (ASPB), which covers the Barcelona city area with 1.6 million inhabitants, was notified of the case of an infant aged 11 months who had been admitted to the Hospital Universitario Vall d'Hebrón, after 9 days with vomiting, fever, bloody diarrhoea and dehydration (case B1). Microbiological tests isolated *Salmonella*. The infant's situation normalised following treatment with antibiotics and intravenous fluid therapy and was discharged on the seventh day. An ASPB epidemiologist contacted the infant's mother in order to initiate the field study. It was found that the family did not have a history of trips or of eating out (the infant was still being fed formula milk and purees of fruit or vegetables, all of which were always prepared by the mother). Moreover, the grandparents, brother and mother also had gastroenteritis during the same period. However, *Salmonella* was not isolated in the copro-cultures taken from any of the family members. The only exposure factor which could be associated with the infection was the family's possession of a pet turtle. This animal was identified as *T. scripta troosti*, and was mainly looked after by the mother, who reported that the baby neither had any direct contact with the turtle nor its aquarium. On November 24 a sample of aquarium water was taken, which was positive to *Salmonella* (strain A4). Serotyping was carried out on the strains isolated from the baby's copro-culture, and from the turtle's aquarium water, both being identified as *S. paratyphi B* var. Java.

Characteristics of the two outbreaks are summarised in Table 1.

Microbiological investigation

On arrival at the National Centre of Microbiology (CNM) strains of *Salmonella* were classified as S. *paratyphi B* and *S. litchfield* using the Kauffman–White technique.[12] All strains of serotype *paratyphi B* belonged to the Java variety.

All human strains and 4 of those isolated from aquariums were of molecular-type using the pulsed field gel electrophoresis technique (PFGE-Xbal) following the protocol and interpretation criteria of PulseNet[13] [Fig. 2].

In Castellón, all strains of *S. paratyphi B* var. Java, both from humans (cases J1–J3) and from the aquarium of one of the turtles (strain A3) were mutually indistinguishable. The human strains of

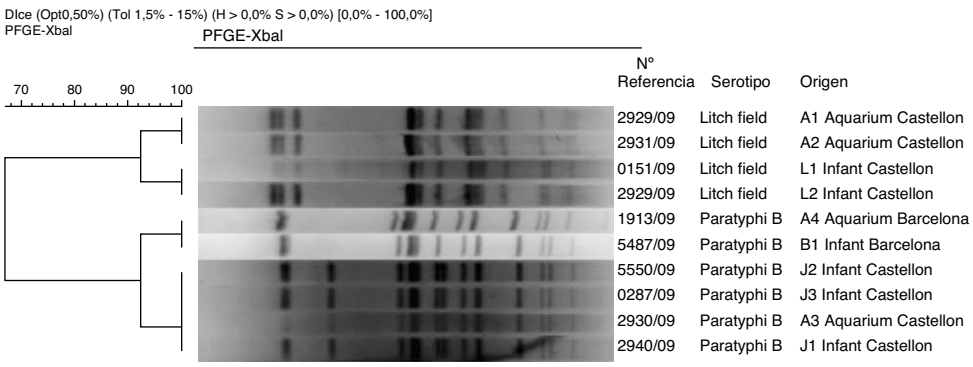

Fig. 2. Profile of the genomic DNAs of *Salmonella enterica* serotypes *Paratyphi B* and *Litchfield* after digestion with Xbal (from outbreaks in Barcelona and Castellon).

Las *S. litchfield* (cases L1 and L2) were also mutually indistinguishable. The similarity between human strains of *S. litchfield* (from cases J1–J3) and those isolated from the turtle's aquarium water (strains A1 and A2) was >90%.

In Barcelona, the two strains studied (B1 and A4) were identified as *S. paratyphi B*, var. Java, mutually indistinguishable using PFGE-Xba. The similarity between the two Barcelona *S. paratyphi B* strains and those of Castellón was >90%. This suggests a strong relationship between cases for this particular serotype.

Intervention

The interventions employed in Barcelona and Castellón, with the aim of preventing more cases, were similar. Apart from follow-up of cases and environmental investigation, the families were given health education based on CDC recommendations for these cases, and official veterinary services carried out inspections with the aim of revising licenses in the pet-shops where these animals were sold.

Discussion

In the present study we have described 6 cases of salmonellosis in children ranging in age from 11 months to 4 years related with exposure at home to pet turtles in 2009.

Turtles are reservoirs of *Salmonella* and their role as transmitters of this disease has been studied.[14–17] Two different serotypes were involved in these 6 cases: *S. paratyphi B* var. Java and *S. litchfield*.[5] Seldom studies have been done on investigations of serovars of *Salmonella* on reptiles and just one of them was done in our country.[16] Highest prevalence of turtles infection has been found in 1972 in an American study that showed that up to 85% of domestic turtles were contaminated with this bacteria[18] and a recently published Colombian study[19] reported as 35% of the samples were positive to *Salmonella* and most of them were corresponding to *Salmonella enteritidis*. None of the serotypes detected in our work have been detected in those prevalence studies in reptiles.[16,19–21] However, it is plausible that these varieties of *Salmonella* may infect both reptiles and humans, and while there are no relevant European studies showing this association, this relationship has been previously identified in the USA. A case-control study conducted in 2009 evaluated the strength of association between infections by *S. paratyphi B* and previous exposure to turtles, and an OR of 2.8 was found.[22] For *S. litchfield* such relation has been previously described only once, in 2006, in the United States.[23] The relationship between turtles and *Salmonella* infection (without distinguishing serotypes), has also been evaluated in various studies, finding OR values of 2.46[24] and of 16.5.[25] In USA, of the approximately 1.5 million cases annually of *Salmonella*, 74,000 are attributed to exposure to reptiles.[6,10] It has been estimated that in between 3 and 5% of all cases of salmonellosis there is some association with reptiles.[7]

In Sweden, between 1990 and 2000, a total of 339 cases were reported of *Salmonella* infections associated with reptiles.[26]

In the present study we describe 5 cases of minor infection, and one serious one; this is a reminder that while in most cases *Salmonella* infection is banal and self-limiting, it can still lead to complications requiring hospitalisation and invasive treatment. There are many cases of serious infections by *Salmonella* associated with contact with turtles,[27] serious cases in babies[28] or in immunodepressed patients,[29] and even fatal cases.[23]

It is becoming ever more common for families with small children to acquire a turtle as a pet, and therefore the number of *Salmonella* infections due to this exposure is increasing. We have no data on the incidence or impact of these infections in Spain, and although there are various reports of such infections in Europe, there are no global indicators that measure their magnitude. Given the high prevalence of infections associated with turtles, since 1975 legislation in the USA prohibits sale and distribution of small turtles (under 10.2 cm in length).[26] This legislation has been associated with an important decline in the annual number of infections of this nature. However, the fact that the law is not rigorously obeyed and that it has certain exceptions (sale for educational purposes), means that cases continue to appear.[23,30] Prior to 1996 in Sweden there was a law that required imported turtles to be certified free of *Salmonella* infection. This requirement ceased to exist after Sweden joined the European Union (1996). There is some evidence that this legislative change provoked a rise in the incidence of this pathology, from 0.15/100,000 to 0.79/100,000 cases.[26] When this became known, an educational television campaign was mounted, which reduced the incidence to 0.46/100,000, showing that both restrictions on importation and the information campaigns are effective

Table 1
Outbreak characteristics.

	Barcelona	Castellón
Year	2009	2009
Report method	Familiar outbreak	Cluster of cases
Confirmed cases	1	5
Hospital admission	1	0
First case	November 2009	January 2009
Last case	November 2009	May 2009
Cases' age	11 months	11 months to 4 years
Families affected	1	4
Stools cultures to cases	*S. paratyphi* B. Java	*S. paratyphi* B. Java (3 cases) *S. litchfield* (2 cases)
Aquaria water samples (all resulted positive)	1	4
Result of aquaria samples	*S. paratyphi* B. Java	*S. paratyphi* B. Java S. *litchfield*
Turtle type	*Trachemis scripta troosti*	*Trachemis scripta troosti* [all of them bought in the same pet shop]

measures for dealing with human salmonellosis associated with reptiles.

After important restrictions in turtle importation and commercialisation in USA, this business was redirected to other places such as Europe, where until now there are no importation or selling regulations regarding this matter.

The present article shows that in Spain there is a clear relationship between human salmonellosis and pet turtles exposure. Even though limited to the description of a few cases identified in Barcelona and Castellón as a result of a professional collaboration, we are certain that a considerable number of turtle-related cases *Salmonella* must occur which could neither be investigated nor be counted. Moreover, the number of families acquiring exotic turtles, normally from the USA, is continually increasing.

For these reasons we believe that it would be important to evaluate the extent of this problem in Spain and in Europe, based on the individualised notification and investigation of cases.

The vast majority of these turtles are exotic, and although importers ensure their *Salmonella* free culture proceedings, bad conditions of their transportation and their maintenance in pet shops facilitates bacterial growth and increases the risk of associated infections.[31]

Based on the available evidence, it is not easy to affirm that turtles are free of *Salmonella*[32] due to the irregular secretion of the bacteria and hence it would not be appropriate to require them to have a certificate to this effect. We believe that flat prohibition of turtle sales is also too drastic as a measure.

However, controlled import quotas, promotion of educational campaigns for both the general public and pet shops, and getting the shops to comply strictly with regulations would all be basic steps to help prevent this type of infections.

Conflicts of interest

The authors have no conflicts of interest to declare.

Acknowledgments

The authors would like to thank the patients and families for their collaboration during the interviews. They are also sincerely grateful to the paediatricians who treated the children: Inmaculada Rius and Jaume Izquierdo, and to all the people related to microbiology work: Pilar Pons, Julia Quirós and Ana Gimeno in Castellón and Virginia Rodriguez and M.ª Dolors Ferrer in Barcelona. Other important collaborators of this study for which the authors would like to acknowledge are: Antonio L. García-Sanz, Judith Hidalgo, Milagros Sanz and Carmen Díaz-Paniagua.

References

1. Mermin J, Hutwagner L, Vugia D, Shallow S, Daily P, Bender J, et al. Reptiles, amphibians, and human *Salmonella* infection: a population-based, case-control study. Clin Infect Dis. 2004;38 Suppl. 3:S253–61.
2. Infecciones por Salmonella no tifoidea de origen humano en españa. sistema de información microbiológica. años 2000–2008. Boletín Epidemiol Semanal. 2009;17:193–204.
3. Infecciones por salomonella typhi y Salmonella partatyphi en españa. sistema de información microbiológica. años 1998–2008. Boletín Epidemiol Semanal. 2009;17:205–16.
4. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis. 2001;32:263–9.
5. Warwick C, Lambiris AJ, Westwood D, Steedman C. Reptile-related salmonellosis. J R Soc Med. 2001;94:124–6.
6. Mermin J, Hoar B, Angulo FJ. Iguanas and *Salmonella* marina infection in children: a reflection of the increasing incidence of reptile-associated salmonellosis in the United States. Pediatrics. 1997;99:399–402.
7. Woodward DL, Khakhria R, Johnson WM. Human salmonellosis associated with exotic pets. J Clin Microbiol. 1997;35:2786–90.
8. Corrente M, Totaro M, Martella V, Campolo M, Lorusso A, Ricci M, et al. Reptile-associated salmonellosis in man, Italy. Emerg Infect Dis. 2006;12:358–9.
9. Dessi S, Sanna C, Paghi L. Human salmonellosis transmitted by a domestic turtle. Eur J Epidemiol. 1992;8:120–1.
10. Bertrand S, Rimhanen-Finne R, Weill FX, Rabsch W, Thornton L, Perevoscikovs J, et al. *Salmonella* infections associated with reptiles: the current situation in Europe. Euro Surveill. 2008;13:18902.
11. Kaufmann AF, Feeley JC, DeWitt WE. *Salmonella* excretion by turtles. Public Health Rep. 1967;82:840–2.
12. Grimont P, Weill FX. Antigenig formulas of the *Salmonella* serovars WHO collaborating centre for reference and research on Salmonella. 9th ed. Paris: Institut Pasteur; 2007.
13. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and shigella for PulseNet. Foodborne Pathog Dis. 2006;3:59–67.
14. Briones V, Tellez S, Goyache J, Ballesteros C, del Pilar Lanzarot M, Dominguez L, et al. *Salmonella* diversity associated with wild reptiles and amphibians in spain. Environ Microbiol. 2004;6:868–71.
15. Hidalgo-Vila J, Diaz-Paniagua C, de Frutos-Escobar C, Jimenez-Martinez C, Perez-Santigosa N. *Salmonella* in free living terrestrial and aquatic turtles. Vet Microbiol. 2007;119:311–5.
16. Hidalgo-Vila J, Diaz-Paniagua C, Perez-Santigosa N, de Frutos-Escobar C, Herrero-Herrero A. *Salmonella* in free-living exotic and native turtles and in pet exotic turtles from SW Spain. Res Vet Sci. 2008;85:449–52.
17. Lamm SH, Taylor Jr A, Gangarosa EJ, Anderson HW, Young W, Clark MH, et al. Turtle-associated salmonellosis, I. an estimation of the magnitude of the problem in the united states, 1970–1971. Am J Epidemiol. 1972;95:511–7.
18. Keymer IF. The unsuitability of non-domesticated animals as pets. Vet Rec. 1972 Oct 14;91:373–81.
19. Sanchez-Jimenez MM, Rincon-Ruiz PA, Duque S, Giraldo MA, Ramirez-Monroy DM, Jaramillo G, et al. *Salmonella enterica* in semi-aquatic turtles in colombia. J Infect Dev Ctries. 2011;5:361–4.
20. Kikillus KH, Gartrell BD, Motion E. Prevalence of *Salmonella* spp., and serovars isolated from captive exotic reptiles in new zealand. N Z Vet J. 2011;59:174–8.
21. Pedersen K, Lassen-Nielsen AM, Nordentoft S, Hammer AS. Serovars of *Salmonella* from captive reptiles. Zoonoses Public Health. 2009;56:238–42.
22. Harris JR, Bergmire-Sweat D, Schlegel JH, Winpisinger KA, Klos RF, Perry C, et al. Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007–2008. Pediatrics. 2009;124:1388–94.
23. Centers for Disease Control Prevention (CDC). Turtle-associated Salmonellosis in humans—United States, 2006–2007. MMWR. 2007;56:649–52.
24. Aiken AM, Lane C, Adak GK. Risk of *Salmonella* infection with exposure to reptiles in England, 2004–2007. Euro Surveill. 2010;15:19581.
25. Centers for Disease Control Prevention (CDC). Multistate outbreak of human *Salmonella typhimurium* infections associated with pet turtle exposure—United States, 2008. MMWR Morb Mortal Wkly Rep. 2010;59:191–6.
26. de Jong B, Andersson Y, Ekdahl K. Effect of regulation and education on reptile-associated salmonellosis. Emerg Infect Dis. 2005;11:398–403.
27. Nagano N, Oana S, Nagano Y, Arakawa Y. A severe *Salmonella enterica* serotype paratyphi B infection in a child related to a pet turtle, *Trachemys scripta elegans*. Jpn J Infect Dis. 2006;59:132–4.
28. Van Meervenne E, Botteldoorn N, Lokietek S, Vatlet M, Cupa A, Naranjo M, et al. Turtle-associated *Salmonella* septicaemia and meningitis in a 2-month-old baby. J Med Microbiol. 2009;58 Pt 10:1379–81.
29. Stam F, Romkens TE, Hekker TA, Smulders YM. Turtle-associated human salmonellosis. Clin Infect Dis. 2003;37:e167–9.
30. Centers for Disease Control Prevention (CDC). Multistate outbreak of human Salmonella typhimurium infections associated with aquatic frogs—United States, 2009. MMWR Morb Mortal Wkly Rep. 2010;58:1433–6.
31. Kaplan M. Reptile rehabilitation. In: Ackerman L, editor. The biology husbandry and health care of reptiles. New Jersey: T.F.H. Publishing; 1998. p. 898–941.
32. Mitchell MA, Adamson TW, Singleton CB, Roundtree MK, Bauer RW, Acierno MJ. Evaluation of a combination of sodium hypochlorite and polyhexamethylene biguanide as an egg wash for red-eared slider turtles (*Trachemys scripta elegans*) to suppress or eliminate *Salmonella* organisms on egg surfaces and in hatchlings. Am J Vet Res. 2007;68:158–64.