Brief report

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

Sarah Lafuente, Juan B. Bellido, Fernando A. Moraga, Silvia Herrera, Alberto Yagüe, Tomás Montalvo, Mercè de Simó, Pere Simón, Juan A. Caylà

* 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 (ISCIIII), 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.

© 2012 Elsevier España, S.L. All rights reserved.

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

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 transmisores 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 epidemiología 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 epidemiología 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 de lo que está sucediendo con los reptiles como transmisores de esta infección. Creemos que es importante evaluar el impacto de este tipo de infección en cada país, con el objetivo de posteriormente promover estrategias de prevención como: regulaciones en puntos de venta de reptiles, y educar a las familias que quieran comprar un animal de este tipo para que sean más cuidadosos y minimizar el impacto de esta transmisión.

© 2012 Elsevier España, S.L. Todos los derechos reservados.
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, \(^2,14\) 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 \(^1,5\) 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 symptomatic treatment in out-patients. The two patients did not know each other and did not present any common antecedents, except that both had freshwater 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 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 Kaufman–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
Las *S. litchfield* (casos L1 y L2) fueron también de manera indistingüible. La similaridad entre humanos de *S. litchfield* (de casos J1–J3) y aquellos aislados de agua del acuario de la tortuga (estrainas A1 y A2) fue >90%.

En Barcelona, las dos estrainas estudiadas (B1 y A4) fueron identificadas como *S. paratyphi B*, var. Java, mutuamente indistinguibles utilizando PFGE-Xba. La similaridad entre las dos Barcelona *S. paratyphi B* estrainas y las del Castellón fue >90%. Esto sugiere una fuerte relación entre casos para este serotipo particular.

**Intervención**

Los intervinieron implementados en Barcelona y Castellón, con el fin de prevenir más casos, fueron similares. Aparte de la formación y el seguimiento de casos y la investigación ambiental, las familias se informaron sobre el CDC para estas recomendaciones, y las oficinas veterinarias oficiales realizaron inspecciones con el fin de reexaminar los permisos de los establecimientos donde se añadieron estos animales.

**Discusión**

En el presente estudio, se describieron 6 casos de salmonelosis en niños menores de 11 meses a 4 años con exposición a tortugas como mascotas en 2009.

Las tortugas son reservorios de *Salmonella* y su papel como transmisores de este problema ha sido estudiado.\(^{14-17}\) Dos diferentes serotipos estuvieron involucrados en estos 6 casos: *S. paratyphi B* var. Java y *S. litchfield*.\(^{5}\) Estudios de laboratorio se han realizado en investigaciones de serovars de *Salmonella* en reptiles y se encontró un caso de cada uno de ellos en nuestro país.\(^{18,19}\) El serotipo de la infección de tortugas se ha encontrado en 1972 en un estudio americano que mostró que hasta el 85% de las tortugas domésticas estaban contaminadas con esta bacteria y que una recientemente colombiano\(^{19}\) reportó que 35% de los muestras estuvieron positivas para *Salmonella* y la mayoría de los eran correspondientes a *Salmonella enteritidis*. Ninguno de los serotipos detectados en nuestro estudio ha sido encontrado en aquellos prevalencia en estudios de reptiles.\(^{16,19-21}\)

Sin embargo, es plausible que estas variedades de *Salmonella* pueden infectar reptiles y humanos, y mientras no son relevantes para estudios europeos de reptiles, este estudio ha sido precedido de identificación en el país. Un estudio controlado publicado en 2009 evaluó la fuerza de la asociación entre infecciones por *S. paratyphi B* y las exposiciones a tortugas, y un OR de 2.8 fue encontrado.\(^{22}\) Para *S. litchfield* esta relación ha sido previamente descrita solo una vez, en 2006, en los Estados Unidos.\(^{23}\) La relación entre tortugas y *Salmonella* (sin distinguir serotipos) ha sido evaluada en varios estudios, finding OR valores de 2.46\(^{24}\) y de 16.5.\(^{25}\) En los Estados Unidos, el 1.5% de los casos anuales de *Salmonella*, 74,000 están expuestos a reptiles.\(^{6,16}\) Ha sido estimado que con una prevalencia del 5% de todos los casos de salmonelosis existen asociaciones con reptiles.\(^{7}\)

En Suecia, desde 1990 y 2000, un total de 339 casos fueron reportados de *Salmonella* asociados con reptiles.\(^{26}\)

En el presente estudio, se describen 5 casos de infección minor, y una seria uno; esto es un recordatorio de que en muchos casos *Salmonella* es banal y autolimitante, pero puede dar lugar a complicaciones requiriendo hospitализación e invasiva tratamiento. Hay muchos casos de infecciones serias de *Salmonella* asociadas con contacto con reptiles,\(^{27}\) serios casos de seres humanos de inmunodepresión,\(^{29}\) y casos fatales.\(^{23,26}\)

Esto está cada vez más común para familias con niños pequeños para adquirir un tortuga como mascota, y la cantidad de *Salmonella* infecciones debido a esta exposición está aumentando. No tenemos datos sobre la incidencia o impacto de estas infecciones en España, aunque en España hay varias publicaciones de estas infecciones en Europa, en España hay siete nacionales que miden su magnitud. Dado que el número de casos de infección asociados con reptiles, desde la legislación de la USA prohibe el comercio y la distribución de pequeños tortugas (menores de 10.2 cm en longitud).\(^{26}\) Esta legislación ha sido acompañada con un importante declive en el número de infecciones de este problema. Sin embargo, el hecho de que el Código de Comercio no se cumple y que esto puede ser particularmente grave en el caso de estrofes educacionales, mecanismos que continúan para aparecer.\(^{23,30}\) 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

<table>
<thead>
<tr>
<th></th>
<th>Barcelona</th>
<th>Castellón</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td>2009</td>
<td>2009</td>
</tr>
<tr>
<td>Report method</td>
<td>Familiar outbreak</td>
<td>Cluster of cases</td>
</tr>
<tr>
<td>Confirmed cases</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Hospital admission</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>First case</td>
<td>November 2009</td>
<td>January 2009</td>
</tr>
<tr>
<td>Last case</td>
<td>November 2009</td>
<td>May 2009</td>
</tr>
<tr>
<td>Cases’ age</td>
<td>11 months</td>
<td>11 months to 4 years</td>
</tr>
<tr>
<td>Families affected</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Stools cultures to cases</td>
<td><em>S. paratyphi B</em>, Java</td>
<td><em>S. paratyphi B</em>, Java</td>
</tr>
<tr>
<td>(3 cases)</td>
<td><em>S. litchfield</em>, (2 cases)</td>
<td></td>
</tr>
<tr>
<td>Aquaria water samples (all resulted positive)</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Result of aquaria samples</td>
<td><em>S. paratyphi B</em>, Java</td>
<td><em>S. paratyphi B</em>, Java</td>
</tr>
<tr>
<td><em>S. litchfield</em></td>
<td><em>S. litchfield</em></td>
<td></td>
</tr>
<tr>
<td>Turtle type</td>
<td>Trachemis scripta troosti</td>
<td>Trachemis scripta troosti</td>
</tr>
<tr>
<td>[all of them bought in the same pet shop]</td>
<td>[all of them bought in the same pet shop]</td>
<td></td>
</tr>
</tbody>
</table>
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 individual notified investigation 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. Based on the available evidence, it is not easy to affirm that turtles are free of Salmonella 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 Jaime Izquierdo, and to all the people related to micro-specialisation 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 individual notified investigation 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. Based on the available evidence, it is not easy to affirm that turtles are free of Salmonella 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 Jaime Izquierdo, and to all the people related to microbiology work: Pilar Pons, Julia Quiroés and Ana Gimeno in Castellón and Virginia Rodriguez and M. 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