Pulsed field gel electrophoresis identifies an outbreak of *Salmonella enterica* serotype Montevideo infection associated with a supermarket hot food outlet

EJ Threlfall, MD Hampton, LR Ward, IR Richardson, S Lanser, T Greener

**Summary:** In February 1996 *Salmonella enterica* serotype Montevideo infection in a patient in the North Tyneside area was attributed to consumption of cooked chicken bought from a supermarket hot food outlet. Isolates from the patient, leftover food, and environmental samples were indistinguishable by pulsed field gel electrophoresis (PFGE). PFGE also demonstrated that an outbreak of infection with *S.* Montevideo associated with the hot food outlet had occurred in late 1995 and early 1996.

This study shows the importance of microbial strain discrimination in outbreak investigations and illustrates the value of close liaison between microbiologists, epidemiologists, and environmental health officers in the control of salmonella outbreaks.

**Key words:** chickens, disease outbreaks, electrophoresis, gel, pulsed-field salmonella

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**Introduction**

In comparison with *Salmonella enterica* serotypes Enteritidis and Typhimurium, *S.* enterica serotype Montevideo is a relatively rare serotype in human infection in England and Wales, accounting for about 100 to 120 isolations a year\(^1\). The main reservoirs of infection are food animals, especially sheep, in which it can cause abortion\(^2\), but also chickens and cattle\(^3,4,\). Most human infections are acquired through eating contaminated food and 40 of the 65 isolations of *S.* Montevideo from foods for human consumption referred to the PHLS Laboratory of Enteric Pathogens (LEP) from 1993 to 1997 were from poultry or poultry products (table 1). From 1977 to 1983 one biogroup of *S.* Montevideo (2d) was responsible for almost all infections in humans, poultry, and cattle in England and Wales but for only a quarter of human infections in Scotland\(^5\). In contrast biogroup 10di predominated in all animals in Scotland but only in sheep in England and Wales. To our knowledge no method for the fine structure discrimination of *S.* Montevideo based on characterisation of the DNA of the organism has been published.

In February 1996 an elderly woman developed gastroenteritis some 20 hours after eating part of a whole roast chicken bought from a ‘hot roast’ department in a local supermarket in North Tyneside. She had stored the chicken at room temperature in her kitchen for 24 hours before consumption. Council officers retrieved remnants of the chicken carcass from her dustbin. A stool specimen and leftover chicken were submitted to Newcastle Public Health Laboratory (PHL) and *S.* Montevideo was isolated from both. Food samples and environmental swabs were obtained from the production and sales area eight days after the sale of the contaminated chicken and submitted to Newcastle PHL for examination.

**TABLE 1** *Salmonella enterica* serotype Montevideo isolations from humans and human foods: England and Wales, 1993 to 1997

<table>
<thead>
<tr>
<th>Year</th>
<th>Human faecal isolations(^a)</th>
<th>Isolations from human foods(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Poultry</td>
</tr>
<tr>
<td>1993</td>
<td>106</td>
<td>27</td>
</tr>
<tr>
<td>1994</td>
<td>130</td>
<td>21</td>
</tr>
<tr>
<td>1995</td>
<td>127</td>
<td>7</td>
</tr>
<tr>
<td>1996</td>
<td>107</td>
<td>6</td>
</tr>
<tr>
<td>1997</td>
<td>95</td>
<td>4</td>
</tr>
</tbody>
</table>

\(^a\) PHLS salmonella common data set
\(^b\) Isolations referred to LEP

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S. Montevideo was isolated from swabs from a cutting board and a serving fork used for portioning cooked chicken carcasses.

Epidemiological investigation of this incident required fine structure fingerprinting of DNA to confirm the association\(^5\). Pulsed field gel electrophoresis (PFGE) has been used for the fine structure fingerprinting of DNA of enterica subtypes Agona,\(^6\) Indiana,\(^7\) and Anatum\(^8\) in outbreak investigations). Plasmid profile typing and PFGE were used to define the strain of S. Montevideo responsible for the incident described and showed that an outbreak of S. Montevideo infection had been associated with the implicated supermarket hot food outlet.

**Methods**

**Epidemiological**

Data on the incidence of S. Montevideo infections in humans in England and Wales from 1993 to 1997 were obtained from the PHLS Salmonella Data Set. Specific information about human infections in the North Tyneside area were supplied by the North of Tyne Communicable Disease Control Unit, and data on isolations from human foods from 1993 to 1997 by LEP. Communicable Disease Control Unit, and data on information about human infections in the North Tyneside area were supplied by the North of Tyne Communicable Disease Control Unit, and data on isolations from human foods from 1993 to 1997 by LEP.

**Isolation and identification procedures**

Faecal isolates were recovered on deoxycholate citrate (DCA) and mannitol lysine crystal violet brilliant green (MCCLB) agar. Environmental strains were isolated on DCA and modified brilliant green (MBG) agars after initial resuscitation in buffered peptone water and enrichment in selenite cystine and soya Rappaport-Vassiliadis (RV) enrichment broths. Initial identification in the local PHL was by conventional serology and simple biochemistry, and in the reference laboratory isolates were confirmed as S. Montevideo by the methods of Kauffman\(^9\). All isolations were maintained on Dorset’s egg agar slopes at 18°C. The sources and years of isolation of the strains of S. Montevideo from patients in the North Tyneside area, including the index case described above. Two of these 17 isolations, one from a patient in December 1995 and one from a patient in February 1996, were unavailable for molecular typing.

**Bacterial strains**

The sources and years of isolation of the strains of S. Montevideo from the North Tyneside area are summarised in table 2. Strains from humans were isolated between May 1993 and February 1996; the food and environmental isolations in relation to this investigation were made in February 1996. For plasmid profile typing and PFGE analyses, strains isolated before the present incident were retrieved from the LEP culture collection, where they had been maintained on Dorset’s egg agar slopes at 18°C.

**Plasmid profile and pulsed field gel electrophoresis**

Plasmid DNA was extracted and sized as described elsewhere\(^9\). DNA for PFGE was prepared by a modified version of a published method\(^9\). Following digestion with Xba I linearised fragments were resolved using the CHEF DR II system (Biorad UK Ltd) at 4.8 v/cm for 60h, with pulse times of 5 to 60s. Fragments were sized in relation to a lambda 48.5 kb ladder (Sigma). Pulsed field profiles were assigned on a temporal basis and types were designated on the basis of at least four band differences between strains.

**Results**

**Epidemiological**

In the three years before this incident there had been ten isolations of S. Montevideo from patients in the North Tyneside area (table 2) – two in 1993, three in 1994, and five in 1995 (one in April, two in November, and two in December). Between 1 January and 14 February 1996 a further seven isolations were made from humans in the North Tyneside area, including the index case described above. Two of these 17 isolations, one from a patient in December 1995 and one from a patient in February 1996, were unavailable for molecular typing.

**Antibiogram analysis and molecular fingerprinting**

Eighteen strains of S. Montevideo - 15 from humans, one from leftover cooked chicken, and two from environmental swabs from the supermarket hot food area - were subjected to antimicrobial sensitivity testing, plasmid profile typing, and molecular fingerprinting (table 2). All strains were sensitive to the antimicrobial drugs used for test (see Methods). Two strains, both isolated in 1993, possessed plasmids of 65 MDa; the remaining 16 strains were plasmid-free. When analysed by PFGE four Xba I-generated pulsed field profiles (PFPs) were identified, with at least four band differences between the respective profile types (figure 1). In relation to the dates of isolation these profile types were designated Smont PFP X1 (1993) to Smont PFP X4 (December 1995/January 1996) (table 2).

All nine isolations made in 1996 and available for PFGE (including those from the index case, the cooked chicken, and the environmental swabs) and one from December 1995 had identical PFPs (Smont X4). This PFP differed from the PFPs identified in the eight other isolations made from 1993 to December 1995 (Smont PFPs X1 - X3).

**TABLE 2 Isolations of S. Montevideo from the North Tyneside area, 1993 to 1996: plasmids and pulsed field profiles**

<table>
<thead>
<tr>
<th>Year</th>
<th>Human</th>
<th>Food</th>
<th>Environment</th>
<th>Plasmids (MDa)</th>
<th>PFP</th>
<th>Smont</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>2 (2)</td>
<td>–</td>
<td>–</td>
<td>65</td>
<td>2</td>
<td>X1</td>
</tr>
<tr>
<td>1994</td>
<td>3 (3)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>X2</td>
</tr>
<tr>
<td>1995</td>
<td>5 (4)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3</td>
<td>X3</td>
</tr>
<tr>
<td>1996*</td>
<td>7 (6)</td>
<td>1</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>X4</td>
</tr>
</tbody>
</table>

MDa: megadaltons
PFP: pulse field profile
* 1 January to 20 February
Subsequent epidemiological investigations revealed that, in addition to the index case, another three of the six patients infected with S. Montevideo with the Smont X4 PFP between December 1995 and February 1996 named the supermarket in their food histories. These included the patient from whom S. Montevideo was isolated in late 1995. A fourth patient had a possible connection. The food history of the remaining two patients was not determined.

Discussion

An outbreak of S. Montevideo infection in the North Tyneside area in 1996 came to light as the result of an infection in an elderly patient, which was traced to cooked chicken bought from a supermarket’s hot food outlet. Isolations from the patient, the food, and the outlet (eight days later) were indistinguishable by PFGE. A retrospective examination of strains from patients in the North Tyneside area revealed that this was not an isolated incident, as six other patients were infected with the Smont X4 profile type, four of whom named the supermarket in their food histories.

Discriminatory typing by plasmid profile typing has proved useful for subdivision both within serotype and phage type in many outbreaks\(^5\). In this investigation the method was of limited value as only two isolations possessed plasmids. In contrast analysis of chromosomal DNA based on PFGE confirmed the association of an isolation of S. Montevideo from a patient with isolations from the supermarket hot food outlet and also demonstrated the existence of an outbreak associated with the supermarket, which lasted for six weeks in late 1995 and early 1996.

Investigations carried out by environmental health officers (EHOs) highlighted deficiencies in the handling, preparation, and cooking of the chicken products at the supermarket, including inadequate separation of ‘clean and dirty’ operations with an attendant risk of cross contamination between raw and cooked foods. Raw poultry is often contaminated with salmonella and the most likely cause of the outbreak described above was cross contamination of a cooked food product. The retailer promptly rectified deficiencies identified by EHOs, improving systems and practices, so that food safety was restored and further intervention was considered unnecessary.

This study shows the importance of strain discrimination coupled with food and environmental microbiology and targeted epidemiological investigations, and highlights the value of liaison between microbiologists and public and environmental health departments in the control of salmonella outbreaks associated with contaminated food products.

References