Neonatal Meningitis due to *Salmonella enterica* serotype Agona, and Review of
Breast milk associated Neonatal *Salmonella* Infections.

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Suggested Running Title: *Salmonella* Agona Neonatal Meningitis
No financial support sought and no conflicts of interest declared.
Abstract (49 words)

We present the first documented case of *Salmonella enterica* serotype Agona meningitis in a 6-day old baby. *Salmonella enterica* serotype Agona was isolated concurrently from infant CSF and parental faecal samples, and *Salmonella* spp. from breast milk. The role of breast milk in transmission of *Salmonella enterica* is discussed.
A six day old baby girl presented to the Paediatric Emergency Department with symptoms of poor feeding, grunting respiration, weight loss and fevers. She was born at term by spontaneous vaginal delivery with a birth weight of 3.52kg. After an uneventful observation period, mother and baby were discharged home. Two days later her parents noticed that she was feeding poorly and had a raised temperature. She had no diarrhea or vomiting. Her General Practitioner reviewed her, and reassured the parents. However she deteriorated over the next few days and required hospital admission having lost 600g of her birth weight. On examination she was febrile (38.3°C), her respiratory rate was 48 breaths/min and her heart rate was 190 beats/min. She appeared pale and dehydrated but she was responsive. She had no focal neurological signs. Her anterior fontanelle was full but not bulging. The rest of the systemic examination was unremarkable.

A diagnosis of neonatal sepsis was made and she was started empirically on intravenous benzylpenicillin and gentamicin. The C-reactive protein was greater than 250mg/L (normal value <2 mg/L), the initial peripheral white cell count was 5.7 x 10⁹/L (Normal at 7 days = 5 - 21 x 10⁹/L) and hemoglobin was 15.7g/dl (Normal at 7 days = 15.2 - 19.8g/dL). Lumbar puncture revealed cerebrospinal fluid (CSF) which was cloudy and blood-stained with a polymorph count of 3800 x 10⁶/l (Normal in neonates = 0); no lymphocytes (Normal in neonates < 20 x 10⁶/L) and a red blood cell count of 155,700 x 10⁶/L. CSF protein concentration was greater than 2g/L (Normal in neonates < 1g/L) and Gram-negative bacilli were seen on Gram’s stain.

On the basis of these CSF findings, intravenous cefotaxime (150mg/kg daily in three
divided doses) was started. The benzylpenicillin was stopped and the gentamicin was continued.

The baby had a complex in-patient stay, requiring intensive care admission for sepsis with respiratory distress. She received one dose of intravenous immunoglobulin for severe sepsis and required platelet support. Five days into her admission intravenous ciprofloxacin and metronidazole were added to her cefotaxime treatment for suspected necrotising enterocolitis. She made good progress thereafter. Repeat CSF and blood cultures were negative. After eighteen days in hospital she was discharged home on oral ciprofloxacin to complete four weeks treatment. Initial hearing assessments carried out soon after discharge were normal. Unfortunately, 12 weeks later, the baby developed post-meningitic hydrocephalus which required insertion of a neurosurgical shunt.

Salmonella species was cultured from CSF and blood cultures taken from the baby on admission. Stool samples from both parents were tested and Salmonella species isolated again. The baby’s stool samples were negative. Concurrent with the baby’s hospital admission, her mother noted bilateral breast induration and tenderness with a decline in milk production, both of which resolved after initiation of antibiotic treatment (see below). Thus expressed breast milk samples from the mother were also tested and three out of four separate samples taken over a two week period grew Salmonella species, which in two instances were mixed with coagulase-negative staphylococci (Table 1). All Salmonella isolates were susceptible to amoxicillin, cefotaxime, ciprofloxacin, gentamicin and co-trimoxazole. Four isolates (neonatal blood culture, neonatal CSF and both parents’ stool samples) were sent to the
Laboratory of Gastrointestinal Pathogens (LGP) at the Health Protection Agency Centre for Infections for further analysis (breast milk isolates were not available). Serotyping identified all four isolates as *Salmonella enterica* serotype *Agona* (serotype I 4,12: f,g,s:- ). The Resistance types (R-types) were identical, with all strains being fully sensitive, and phage typing identified them all as phage type 3 (PT3). All four isolates were indistinguishable by Pulsed Field Gel Electrophoresis, performed according to a standard protocol (11) (Figure 1). Thus molecular typing provided strong evidence that the baby had acquired the infection from one of her parents.

Neither parent had a recent history of diarrheal illness or feeling unwell. Both recalled having an episode of severe gastroenteritis five years previously, after eating food from a UK take-away restaurant. Both parents received eradication treatment with ciprofloxacin 750mg twice daily after discussions with infectious diseases specialists, microbiologists and Public Health doctors. Subsequent stool samples from both parents and breast milk samples from the mother were negative (Table 1). The mother completed four weeks treatment while the father stopped treatment after two weeks because of concerns about side effects. In total, each parent had five negative stool samples in the month after treatment was commenced. The mother had three further negative stool samples at the end of her course of ciprofloxacin. Although the baby was initially breastfed, this was discontinued soon after admission and for several weeks after discharge. As the mother was keen to breastfeed, breastfeeding was restarted without complication following negative maternal stool and breast milk samples and completion of eradication treatment.
The advantages of breast feeding include protection against infections, particularly gastroenteritis, otitis media and upper respiratory tract infections. This is achieved through specific immunological factors (antibodies, T cells, B cells) and more general non-specific factors (complement, lactoferrin, properdin and glycoconjugates) (8). However, it is well recognised that occasionally breast milk can act as the vehicle of transmission of some bacteria and viruses to the infant. Neonatal bacterial infections documented as being linked to breast milk include Staphylococcus aureus, E coli, Serratia marcescens, Listeria monocytogenes, Klebsiella spp., Salmonella enterica, Mycobacterium tuberculosis, Brucella melitensis, Coxiella burnetii and Group B Streptococcus (12) (8). Thus screening programmes have been developed for breast milk banks, and breastfeeding is contra-indicated in specific maternal infections such as active tuberculosis (8).

Expressed breast milk frequently contains coagulase negative staphylococcus (CNS), diphtheroids and alpha-haemolytic streptococci. These represent normal skin flora and colonise the mammary ducts. They do not cause problems to the infants, as long as the breast milk is correctly stored so the mean bacterial count remains low. Outbreaks of infections such as Staphylococcus aureus, Streptococcus spp., E. coli and Salmonella enterica have chiefly occurred when expressed milk has not been correctly handled or stored.

In the case described above, four samples of breast milk were analysed over a two-week period. The breast milk was stored in sterile, screw-topped containers at 4°C for...
use within 24 hrs, or frozen at -20 ºC for use at a later date. *Salmonella enterica* spp. was isolated alone from one sample, in conjunction with CNS from two samples and the fourth sample yielded only CNS. Unfortunately there were no further samples available for culture, thus we were not able to perform sequential quantification of bacterial concentrations, which may have confirmed a temporal relationship with acquisition of infection. The mother states that she collected the samples under aseptic conditions, and the breast milk pumps were disinfected with hypochlorite before use. It is unclear how long the parents had been asymptptomatically excreting *Salmonella*, as they only recalled an episode of gastroenteritis 5 years previously. There was no relevant travel history, no occupational risks and their general health was normal. Five years would be an unusually long period of asymptomatic carriage. A review of 32 studies describing 2814 patients, all with Non Typhoidal Salmonella infections, found the median duration of excretion to be approximately five weeks, and that persistent carriage for over one year occurred in <1% of cases (2).

There are several possible routes of transmission of *Salmonella enterica* serotype Agona from the parents to the baby, and the precise role of breast milk remains unproven. *Salmonella enterica* spp. was cultured repeatedly from the maternal breast milk, but it is unclear if the baby acquired the infection primarily from the breast milk (which had already been infected through haematogenous or lymphatic spread within the mother), or the baby acquired the organism through an alternative route and then infected the breasts through suckling. As the mother was systemically well and afebrile, but recalled bilateral breast tenderness and a fall in milk production when the baby was admitted to hospital, it is proposed that infection of maternal breast milk most likely occurred during breast feeding, due to local contamination. Alternative
routes of acquisition by the baby include from perineal contamination at the time of delivery, or via the faeco-oral route from either parent, presumably via transient hand carriage. It is unusual for both parents to be asymptomatic carriers, and unclear whether this was related to their severe episodes of gastroenteritis five years previously.

There are five published cases of neonatal salmonella infection, probably due to acquisition from breast milk, and three reported outbreaks (Table 2). In one published case, the mother reported a concurrent systemic illness (4) and there was an additional report of neonatal Salmonella infection associated with mastitis (6). One of the most compelling reports of Salmonella transmission through breast milk involved quantification of S. Typhimurium DT104 in sequential breast milk samples by real-time PCR (12). The rise and fall in concentration of the organism was consistent with an infectious course (i.e. colonisation, pathogen growth and immune response), rather than sporadic external contamination of milk or breast-pump machines. There was a plausible temporal relationship between breast milk concentration and neonatal infections, in that the Salmonella breast milk concentration peaked day 13-15 post-delivery, and the twin infants became unwell on days 16 and 19. This coincides with the characteristic 2-3 day incubation period for Salmonella infections. In this published case (12), the mother was asymptomatic throughout the entire period. One proposed explanation was that the initial route of entry into the breast was via the skin, which then lead to colonisation of the mammary ducts. An alternative hypothesis proposed by Qutaishat was the involvement of macrophages in the priming of Salmonella organisms into the mammary gland, after gastrointestinal ingestion (12).
Lactogenic hormones are involved in regulating selective homing procedures, which result in high abundance of immune cells in breast milk during lactation.

There are three published reports of outbreaks of *Salmonella* infections on neonatal units (Table 2). An epidemic of *Salmonella* Typhimurium in Czechoslovakia in 1989 affected 11 neonates who all received mixed breast milk, pooled from many mothers (4). One mother was positive for *S.* Typhimurium in breast milk (taken under strict aseptic conditions) and stool, and gave a history of a short febrile illness (‘one loose stool and temperature lasting a few hours’). She was also reported to have a positive serology test. One environmental sample (details not provided) was also positive (4).

The second and third outbreaks involved *Salmonella* Kottbus. One of these affected 7 of 22 infants on a neonatal intensive care unit in the USA in the 1970s. A case-control investigation identified the only risk factor as consuming milk from a single donor, whose milk was subsequently found to be contaminated with *Salmonella* Kottbus. The donor had no evidence of gastrointestinal infection or mastitis and it was hypothesised that improper handling and storage of the milk enabled the *Salmonellae* to multiply to a number sufficient to cause disease (14).

There are several reports of individual cases of neonatal *Salmonella* infections possibly acquired through breast milk (Table 2). A 3-month old baby in India, who had been exclusively breast fed since birth, had *Salmonella* Senftenberg isolated from blood, stool, throat and gastric aspirate cultures. *Salmonella* senftenberg with an identical antibiogram was cultured from the mother’s breast milk, while all other individuals in the nursery were negative. The mother had no evidence of fever, diarrhea, or breast lesions (13). A 4-month old baby girl from Scotland, also
exclusively breast fed since birth, had *Salmonella* Virchow cultured from her stool. Her mother reported mastitis for 48 hours, and breast milk and her stool samples grew *Salmonella* Virchow. It was thought that the baby acquired the infection from infected milk or, directly or indirectly, from contamination of the mother’s skin. The maternal mastitis could have arisen by haematogenous spread, as in typhoid fever, but, in the absence of a febrile illness, ascending infection seemed more likely. The authors recommended that whereas breast feeding should continue in cases of sporadic puerperal mastitis, if coincidental gastroenteritis occurs in the baby or mother, breast feeding should be temporarily stopped until results of bacteriological investigations are available (6).

Recurrent *Salmonella* Panama meningitis has been reported in a 13 day old, exclusively breast fed baby, which was thought to have been acquired through contaminated breast milk. The mother reported no symptoms of gastroenteritis or mastitis, and maternal stool and blood cultures were negative. The mother continued to excrete the organism asymptomatically for at least 2 weeks (3).

*Salmonella enterica* serotype Agona is uncommonly isolated from humans in England and Wales. Between 1981 and 2004 its average incidence was 154 human cases per year, while from 2005 to 2007 it's average incidence dropped to 85 per year. In 2008 there were 228 human cases, and *Salmonella enterica* serotype Agona ranked as the fourth most common non-typhoidal *Salmonella* isolated from humans in England and Wales.
Salmonella enterica serotype Agona has been responsible for several significant food-borne outbreaks (9, 10, 15). Of interest, an outbreak of gastroenteritis due to Salmonella enterica serotype Agona started in February 2008, affecting residents in the UK, Ireland and Finland. By August 2008 there were 119 cases internationally (77 in England and Wales) and by mid-September there were 105 cases in England and Wales alone (Anonymous, 2008). Molecular typing of strains from human cases and from contaminated meat products confirmed that the cases were linked to an Irish food production company and a retail outlet chain supplied by this company (10). The strain responsible was characterised as a new phage type, PT39, and by PFGE as SAGOXB.0066. This strain was distinct from the strain in this case study, which was defined by the Laboratory of Gastrointestinal Pathogens (LGP) as PT3, SAGOXB.0076. Four unrelated isolates of PT3 isolated earlier in 2008 were also distinct from each other and from the strain in this study.

In conclusion, Salmonella Kottbus was initially thought to have a specific predilection to colonise the mammary glands (14). This current case and the literature review extends the list of serotypes associated with possible breast-milk acquired infection to Typhimurium (5, 12), Senftenberg (13), Panama (3), Virchow (6) and Agona (this case) (Table 2). Salmonellae may colonise the breast directly via the mother’s bloodstream (possibly contained within macrophages), or indirectly via infection of the suckling neonate.

Acknowledgements: We would like to thank Dr Kathy Bamford, Imperial College for helpful discussions and Elizabeth DePinna, Unit Head, Salmonella Reference Laboratory, HPA, Colindale.
Figure 1: PFGE of *Salmonella enterica* serotype Agona isolates. The infant CSF isolate (Lane 2), the parents' faecal isolates (lane 3 and 4) and the infant blood isolate (lane 5) show identical band patterns following electrophoresis of genomic DNA digested with XbaI. Lanes 6 to 9 are examples of other strains of *Salmonella enterica* serotype Agona PT3. The molecular weight standard *S. enterica* serotype Braenderup H9812 is in lanes 1 and 10 (7). Electrophoretic conditions were as standardised by Salm-gene/PulseNet Europe (200 V for 22 hrs in 2.0 L TBE½ running buffer at 14°C with a ramp of 2 - 64 sec) (11).
Table 1: Characteristics of Isolates from infant and parents

<table>
<thead>
<tr>
<th>Sample</th>
<th>Individual</th>
<th>Date of sample</th>
<th>Culture result</th>
<th>Characterisation of <em>Salmonella enterica</em> serotype Agona</th>
<th>PFGE *</th>
</tr>
</thead>
</table>
| CSF          | Baby       | 18/10/08       | *salmonella enterica* serotype Agona | Biotype I  
Antigenic Structure  
4,12: f.g.s-  
Phage Type 3  
R-Type: sensitive | Fig 1, lane 2  
SAGOXB.0076 |
|              |            | 28/10/08       | Negative                |                                                          |        |
| Blood Cultures | Baby       | 23/10/08; 25/10/08 | *salmonella enterica* serotype Agona | Biotype I  
Antigenic Structure  
4,12: f.g.s-  
Phage Type 3  
R-Type: sensitive | Fig 1, lane 5  
SAGOXB.0076 |
<p>|              |            |                | Negative                |                                                          |        |</p>
<table>
<thead>
<tr>
<th>Sample</th>
<th>Subject</th>
<th>Date(s)</th>
<th>Result</th>
<th>Organism</th>
<th>Biotyping Information</th>
<th>Phage Type</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stool</td>
<td>Baby</td>
<td>20/10/08; 20/12/08; 13/12/08</td>
<td>Negative</td>
<td><em>Salmonella enterica</em> serotype <em>Agona</em> Biotype I</td>
<td>Antigenic Structure 4,12:f,g,s:- Phage Type 3 R-Type: Sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22/10/08</td>
<td></td>
<td><em>Salmonella enterica</em> serotype <em>Agona</em> Biotype I</td>
<td>Antigenic Structure 4,12:f,g,s:- Phage Type 3 R-Type: Sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mother</td>
<td>22/10/08</td>
<td></td>
<td><em>Salmonella enterica</em> serotype <em>Agona</em> Biotype I</td>
<td>Antigenic Structure 4,12:f,g,s:- Phage Type 3 R-Type: Drug sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Father</td>
<td>22/10/08</td>
<td></td>
<td><em>Salmonella enterica</em> serotype <em>Agona</em> Biotype I</td>
<td>Antigenic Structure 4,12:f,g,s:- Phage Type 3 R-Type: Drug sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Date</td>
<td>Sample</td>
<td>Results</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>----------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11/12/08</td>
<td>Urine, Baby</td>
<td>Non lactose-fermenter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18/10/08</td>
<td>Breast milk</td>
<td>Non lactose-fermenter, Salmonella species,</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>plus CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23/10/08;</td>
<td></td>
<td>29/10/08 Salmonella species, plus CNS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30/10/08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>06/11/08</td>
<td></td>
<td>Positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CNS = coagulase negative staphylococcus

CSF = cerebrospinal fluid

- The nomenclature of *Salmonella* pulsed-field patterns used by the Laboratory of Gastrointestinal Pathogens is consistent with Salmgene/Pulse-Net. Upper case letters are based on the serotype examined and the macrorestriction enzyme used, followed by a dot, then an arbitrary number (usually based on the order in which the patterns are identified).
Table 2: Published cases of neonatal infection with *Salmonella enterica*, associated with breast milk

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Outbreak / number of babies infected</th>
<th>Outbreak – details of transmission</th>
<th>Individual case (age, sex, feeding method, clinical details)</th>
<th>Individual case / Details of mother</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typhimurium</td>
<td>Single case</td>
<td>Donor was asymptomatic. Transmission may have been due to improper handling or storage of milk</td>
<td></td>
<td></td>
<td>(5)</td>
</tr>
<tr>
<td>Kottbus</td>
<td>7 of 22 infants infected</td>
<td>Donor was asymptomatic. Transmission may have been due to improper handling or storage of milk</td>
<td></td>
<td></td>
<td>(14)</td>
</tr>
<tr>
<td>Kottbus</td>
<td>XX</td>
<td>XX</td>
<td></td>
<td></td>
<td>(1) (Outbreak 1)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>11 neonates all neonates</td>
<td></td>
<td>Gastroenteritis</td>
<td></td>
<td>(4)</td>
</tr>
<tr>
<td>Nonstandardizable</td>
<td>Infected &amp; Received</td>
<td>Pooled Breast Milk</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Virchow</td>
<td>4 month old girl, exclusively breast fed</td>
<td>Maternal mastitis</td>
<td>6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senftenberg</td>
<td>3 month old, exclusively breast fed</td>
<td>Asymptomatic</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Typhimurium DT104</td>
<td>Yes - twins</td>
<td></td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Panama</td>
<td>13 day old, exclusively breast fed</td>
<td>Asymptomatic</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agona</td>
<td>6 days old, meningitis</td>
<td>Mastitis</td>
<td>this case</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References
