

# High Frequency of Reactive Joint Symptoms After an Outbreak of *Salmonella enteritidis*

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**ABSTRACT. Objective.** To study reactive symptoms following an outbreak of *Salmonella enteritidis* that occurred after a dinner party held January 15, 1999, in Copenhagen, Denmark. An epidemiological study pointed toward a dish of minced raw salmon, in which one of the constituents was unboiled eggs as the likely cause of the outbreak. Remnants of this dish were not available for bacteriological examination.

**Method.** All 94 guests and kitchen staff members were mailed a questionnaire about gastrointestinal, joint, and eye symptoms. Nonrespondents were contacted by telephone. Thirty-five individuals delivered blood samples for serological analysis mean 90 days (range 60–186) after the exposure.

**Results.** Answers were obtained from all participants and 91 were regarded as *Salmonella* exposed. Male/female ratio was 40/51, mean age 49 years. Fifty-two reported diarrhea (57%), 49 abdominal pain (54%), 33 fever (36%), and 12 vomiting (13%). Eight (9%) delivered stool samples, and all were positive for *S. enteritidis*. Seventeen fulfilled predefined criteria of reactive arthritis/arthralgia (ReA), and of these 13 had had enterocolitis. Joint pain from knees and ankles was most frequently reported. The mean duration of diarrhea among the patients reporting joint symptoms was 7.5 days, while in the group of patients with enterocolitis without joint symptoms it was 4.1 days ( $p = 0.00047$ ). Three participants, all from the ReA group, reported ocular redness and irritation compatible with conjunctivitis. Although there was a trend to higher IgG anti-*Salmonella* antibody levels among the patients with ReA the difference was not significant.

**Conclusion.** Reactive joint symptoms after food-borne *Salmonella* infection may be more frequent than previously thought. The duration of diarrhea is strongly correlated with the occurrence of joint symptoms. (J Rheumatol 2002;29:767–71)

**Key Indexing Terms:**  
REACTIVE ARTHRITIS  
INFECTION

SALMONELLA ENTERITIDIS  
EPIDEMIC

Reactive joint symptoms ranging from transient arthralgia to overt exudative synovitis, sometimes associated with extraarticular phenomena, may occur as a complication after a urogenital or gastrointestinal (GI) infection caused by *Chlamydia*, *Salmonella*, *Yersinia*, *Shigella*, or *Campylobacter*. Individuals possessing the HLA-B27 MHC class I antigen are considered to be at increased risk of developing reactive arthritis (ReA)<sup>1</sup>.

The incidence of zoonotic *Salmonella* infections has increased in recent years in a number of industrialized countries<sup>2</sup>. In Denmark, the number of registered cases peaked in 1997, with over 5000 cases within a population of 5 million<sup>3</sup>. Thus *Salmonella* is recognized as a leading cause of bacterial food-borne infectious gastroenteritis. However,

the extent to which *Salmonella* infections are followed by reactive symptoms such as ReA is not well established.

When calculating the risk of ReA following *Salmonella* food poisoning it is pivotal to know the exact size of the exposed population. In larger outbreaks this has often proved difficult to achieve and studies may be biased toward those with severe disease, leaving out patients with only mild transient ReA or enterocolitis or with no enteric symptoms at all. It is well known from studies and clinical practice that cases of ReA appear in which no triggering agent can be identified<sup>4</sup>. We were able to trace all exposed individuals who were involved in a localized outbreak of *S. enteritidis* and inquire about GI, eye, and joint symptoms. Blood samples from 35 participants were analyzed for antibodies against *Salmonella* with ELISA and compared to the Widal agglutination technique.

## MATERIALS AND METHODS

An outbreak of gastroenteritis due to *S. enteritidis* phage type 6 occurred January 15, 1999, in Copenhagen, Denmark. It took place at the annual New Year celebration event held by the Copenhagen Medical Association, and the guests were largely medical doctors and their spouses.

As soon as the epidemic became apparent an investigation was undertaken to identify its extent and source. This investigation was conducted at the end of January by the Danish Zoonosis Center in

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Submitted January 23, 2001; revision accepted October 18, 2001.

conjunction with Statens Serum Institut, Copenhagen, and consisted of a questionnaire followed by telephone contact to all members of the kitchen staff and to all guests according to the list of participants.

There was a significant association between disease (defined as onset of diarrhea between January 15 and 22) and intake of a dish of minced raw salmon in which one of the constituents was unboiled eggs<sup>5</sup>. However, there were no remnants of this dish, and the association could therefore not be confirmed bacteriologically. Support for this association was that one of the staff members brought a portion of that particular dish, and only that, home to her husband, who subsequently fell ill with enterocolitis. In Denmark *S. enteritidis* phage type 6 is almost exclusively found among egg-laying hens.

**Study population.** After 8 weeks another questionnaire was mailed to all 94 participants (83 guests and 11 staff) inquiring about symptoms of enterocolitis, duration of diarrhea, results of stool culture, and joint and eye symptoms. Patients were asked to mark in a schema if they had experienced tender joints within a period of 4 weeks after the exposure, and if the joints were also swollen. They were further asked to answer yes/no to questions about onset of pain during the same period in the lumbar or cervical spine that had previously been free of symptoms. For an estimate of the effect of the rheumatological symptoms on their daily lives, questions about duration of joint symptoms, need for analgesics, and absence from work because of joint pain were included.

Joint complaints were graded mild, moderate (if analgesics were required), or severe (if staying home from work was required). All were asked as well to deliver blood samples for serological analysis.

A case of enterocolitis was defined as having at least 2 of diarrhea, abdominal pain, nausea, vomiting, or fever following the exposure.

The development of pain in a previously asymptomatic joint or onset of cervical or lumbar back pain within 4 weeks of the exposure was regarded as ReA (which in this regard covers the spectrum from reactive arthralgia to overt arthritis) when other obvious causes, for example trauma or wrong movements, could be ruled out. Individuals with current rheumatological problems at the time of the outbreak were excluded.

**Widal agglutination test.** The Widal agglutination test was as described by Widal<sup>6</sup> and Faarup<sup>7</sup>. Two-fold dilutions of patient sera were incubated with suspensions of O- and H- antigens from *Salmonella* strains expressing specific antigens for either lipopolysaccharide (O-antigen) or flagella (H-antigen) and checked for agglutination after 24 hours. *Salmonella* strains used for preparing antigens for determinations of antibodies against *Salmonella* serotypes were: *S. enteritidis* (9,12:g,m), *S. typhimurium* (1,4,5,12:i:-), *S. typhi* (9,12:- and 9,12:d), *S. paratyphi* A (1,2,12:a:-), *S. paratyphi* B (4,5,12:-:- and 4,5,12:b:1,2). Serotyping of these *Salmonella* strains was performed by the National *Salmonella* Reference Center in Denmark with antisera produced by Statens Serum Institut. The Widal reaction was read as positive when the agglutination titer was  $\geq 50$ .

**ELISA for anti-*Salmonella* antibodies.** The ELISA procedure was performed essentially as described<sup>8</sup>. Briefly, microtiter plates (Nunc, Roskilde, Denmark) were coated with 1  $\mu\text{g}/\text{ml}$  of pooled phenol/water extracted lipopolysaccharide (LPS) from *S. typhimurium* and *S. enteritidis* (Sigma, St. Louis, MO, USA) in phosphate buffered saline (PBS) (0.1 mol/l, pH 7.5) overnight at 4°C. Plates were blocked overnight with 1% bovine serum albumin in PBS (pH 7.5) at 4°C. Serum samples collected a mean 90 days (range 60–186 days) after the exposure were diluted 1:1000 in PBS and incubated 2 h at room temperature. The plates were washed 3 times in PBS containing 0.05% Tween 20. Then alkaline phosphatase conjugated goat F(ab)<sub>2</sub> anti-human IgG, IgA, and IgM (Sigma) diluted 1:300, 1:100, 1:50, respectively, in PBS were separately incubated overnight at 4°C. Plates were washed 3 times in PBS-Tween and freshly prepared p-nitrophenylphosphate (Sigma) in diethanolamine buffer was added and incubated 30 min at room temperature. The optical densities were read at 405 nm and the results expressed in arbitrary units calculated from a standard curve. Fifty-six sera from blood donors served as controls in the IgG, IgA, and IgM anti-*Salmonella* ELISA. The cutoff level was mean + 3 SD.

**Statistics.** Wilcoxon's 2 sample test and Fisher's exact test were used to compare data between ReA patients and patients with uncomplicated enterocolitis.

## RESULTS

Sixty-eight individuals returned the questionnaire within the following 2 mo. The 26 nonrespondents were all contacted by telephone and encouraged to return the questionnaire or to give their answers directly by phone. We thus obtained an overall response rate of 100%.

Three staff members denied having eaten from the minced salmon dish and were regarded as nonexposed (they had not presented any symptoms of either GI infection or ReA) and were excluded from further calculations.

The study population thus consisted of 91 (83 guests, 8 staff). Forty were men (44%), mean age 51 yrs (range 26–67), and 51 were women (56%), mean age 48 yrs (range 20–66). Thirty-five delivered blood samples for serological analysis.

**Enterocolitis.** Fifty-two participants (57%) reported diarrhea, of which 47 provided information about the length of diarrhea illness; 49 (54%) had abdominal pain, 36 (40%) nausea, 33 (36%) fever, and 12 (13%) had vomiting. The time relations between onset of diarrhea and exposure are shown in Figure 1.

Eight participants delivered stool cultures (9%); all had had diarrhea. They were found to be positive for *Salmonella* and 4 were further typed as *S. enteritidis* phage type 6. The remaining 4 isolates were not available for phage typing.

**Joint symptoms.** Twenty-one individuals reported joint symptoms or pain from the upper or lower spine. Four were ruled out as having ReA because of late onset, i.e., more than 4 weeks after the exposure or because they reported worsening of previous joint pain.

The ReA group thus consisted of 17 (19%), with a male to female ratio of 8 (47%) vs 9 (53%). Mean age for men was 48 yrs (range 26–57) and for women 53 yrs (range 42–66). Two participants commented that they previously had had ReA, in one as a complication of *Campylobacter jejuni* infection.

The most frequently reported pain was in knee and ankle joints, and the mean number of affected joints per person was 2.4. Two individuals reported onset of low back pain as the only manifestation (Figure 2).

Of the 52 patients with GI symptoms, 13 (25%) developed ReA, compared with 4 (10%) of 39 without GI symptoms (risk ratio 2.4,  $p = 0.13$ ).

All 4 patients without GI symptoms claimed to have had both tender and swollen joints, but their symptoms lasted less than one month. Five of the 13 with GI symptoms had tender and swollen peripheral joints and 2 had only low back or cervical neck pain. In 4 the symptoms lasted up to one month, in 3 symptoms lasted 1–3 months, and 6 had symptoms longer than 3 months.

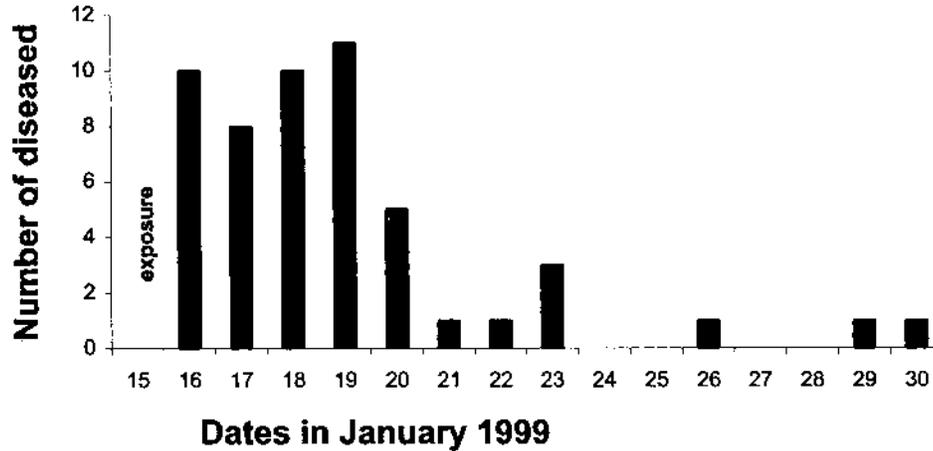


Figure 1. Time interval from exposure to *S. enteritidis* (January 15, 1999) to onset of diarrhea among 52 individuals who developed enterocolitis.

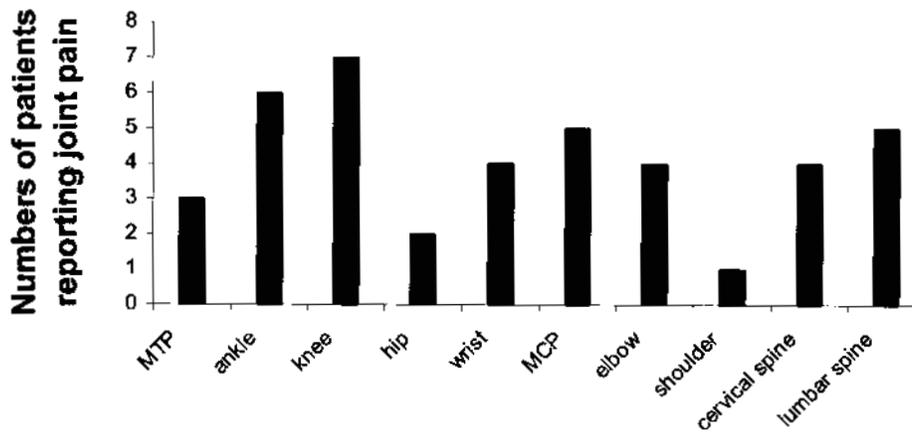


Figure 2. Joint involvement within the first 4 weeks after exposure to *S. enteritidis* among 17 individuals with ReA. MCP: metacarpophalangeal, MTP: metatarsophalangeal.

The frequencies of individual GI symptoms for ReA compared to enterocolitis patients were nausea 77% vs 58%, vomiting 38% vs 16%, abdominal pain 85% vs 87%, diarrhea 100% vs 87%, and fever 69% vs 53%. It follows that the 13 ReA patients with GI symptoms, although not statistically significant, generally had more severe GI disease than those with enterocolitis only.

When duration of diarrhea was compared between the ReA patients and those who had only enterocolitis, it showed that the ReA population had a mean duration of diarrhea of 7.5 days, while the duration for enterocolitis patients was 4.1 days. Eleven individuals had prolonged diarrhea of at least 7 days' duration, and 8 (73%) of these experienced ReA compared with 5 (14%) of 36 patients with diarrhea of less than 7 days (risk ratio 5.2,  $p < 0.001$ ) (Figure 3).

Seven of the cases were classified as mild, 5 moderate, and 5 as severe joint complaints.

**Eye symptoms.** Three participants, all from the ReA group, reported irritation and redness of the eyes.

**Serology.** Among the 35 persons who delivered blood samples a mean 90 days (range 60–186) after the exposure, 10 came from the ReA group and 20 from the enterocolitis group. Five sera were from participants who did not experience any symptoms.

Nineteen sera were positive for IgG anti-*Salmonella* antibodies, 5 for IgA, and one for IgM. When measured by Widal agglutination, 8 sera were positive, all of low titers between 1:50 and 1:400. Six of the Widal positive sera were also positive by ELISA (data not shown).

There was a trend toward higher levels of IgG anti-*Salmonella* antibodies in the group with ReA symptoms; however, this was not significantly different from the non-ReA group (Figure 4).

## DISCUSSION

Spontaneous outbreaks of bacterial enterocolitis are experiments of nature that allow us to study clinical and laboratory

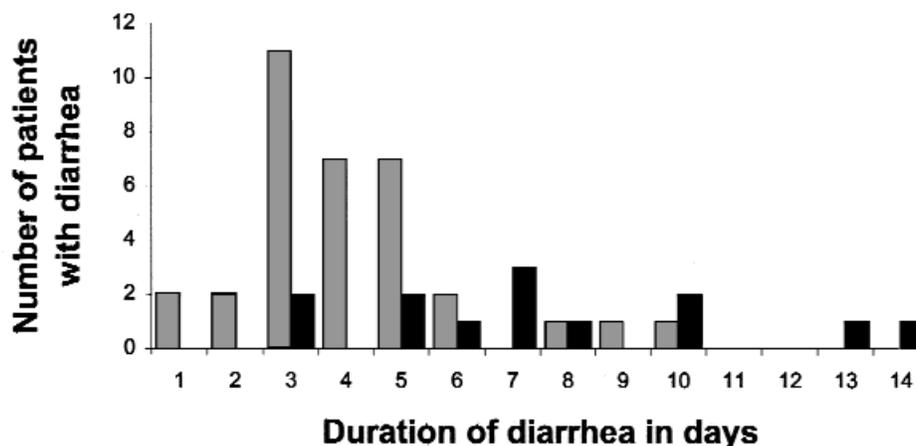


Figure 3. Duration of diarrhea among 13 patients with ReA (black bars) and 34 with uncomplicated enterocolitis (shaded bars).

variables among a group of individuals exposed simultaneously to the same bacterial pathogen. The common epidemiological method in these situations is to distribute survey questionnaires to all affected persons, and a prerequisite for obtaining reliable data is of course a high response rate. One problem in this type of research is a bias toward including only those individuals with overt GI or articular symptoms. Individuals with minor symptoms may be less motivated to respond to the questionnaires or may simply pass unnoticed by the investigators.

In this study over 70% returned the questionnaire, but we reached 100% by contacting nonrespondents by telephone. As the majority were medical doctors familiar with the clinical issues under investigation, most gave detailed answers with a precise description of their symptoms.

The frequency of ReA in this study was 19%, which is a higher percentage than estimated in other reports of *Salmonella* outbreaks published recently<sup>9-15</sup>. This is most likely because the ReA incidence here is based upon self-reported symptoms and not examination by medical professionals, who may exclude more discrete articular manifestations like arthralgia and cervical and lumbar pain in the absence of objective clinical findings. As well, most rheumatological examinations were often carried out several weeks or months after the outbreaks, which may rule out cases with only minor transient joint symptoms.

In this study one-third of the ReA group (6 out of 17) had symptoms lasting longer than 3 months and 5 of these patients scored their arthritic problems as severe (i.e., causing the patient to stay home from work because of arthritic pain).

The distribution of joint pain was not different from that seen in other studies<sup>9,11,14,15</sup>. Knee and ankle joints were most frequently affected, but pain from finger joints, wrist, elbow, and axial skeleton almost reached the same numbers.

We previously described an outbreak of *Salmonella* enteritidis<sup>11</sup> that in many aspects was identical to this one,

with one major exception: the fraction of participants who fell ill with enterocolitis was nearly 100% in the former, whereas in this outbreak it was 57%. The severity of diarrhea was probably also different, in that 14% were hospitalized (16 out of 108 with enterocolitis) in the previous study, but only one person stayed one night in hospital in the present outbreak. Whether this was due to the virulence of the strain, to the bacterial load ingested, or to differences in host defense in the 2 populations is unclear.

In spite of this the attack rate of ReA was practically the same (15% vs 19%) in the 2 outbreaks. This raises the question, to what extent do people who escape overt GI symptoms develop joint symptoms? Of the 17 patients with ReA, 4 denied having had any signs of enterocolitis. Among those, 2 were classified as mild, one as moderate, and one as severe; however, all 4 claimed that their joint symptoms had resolved within one month. A similar figure of 24% of ReA patients without diarrhea was found by Mattila, *et al*<sup>13</sup>, and it is known that some patients apparently experience no symptoms preceding arthritis<sup>16-19</sup>. The duration of diarrhea was strongly correlated to the development of articular symptoms, an association confirmed by others<sup>9,10,14,16,20</sup>, but this was not found in a recent large outbreak in Finland<sup>15</sup>.

The ReA group tended to show higher values of IgG anti-*Salmonella* antibodies, but the results were difficult to interpret mainly because the intervals from the time of exposure to the time samples were drawn varied considerably. It has been shown that increased antibody levels tend to persist longer among patients with ReA compared to those with uncomplicated enterocolitis<sup>21</sup>. In salmonellosis a response in all immunoglobulin classes is seen, in contrast to *Yersinia* triggered ReA, where IgA antibodies and particularly those of the secretory IgA class and the IgA2 subclass persist<sup>22</sup>. We compared the results obtained from analyzing sera with the Widal test and an ELISA technique and found, like others<sup>8,23</sup>, that ELISA is by far the most sensitive method to detect anti-*Salmonella* antibodies. Only 8 sera were weakly

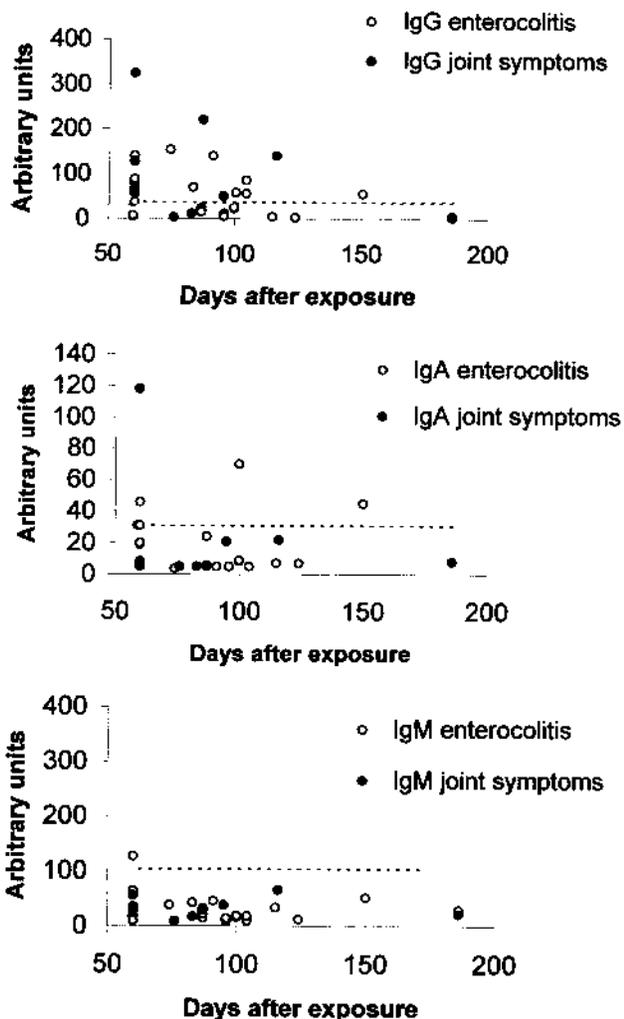


Figure 4. ELISA determination of anti-Salmonella antibodies of IgG, IgA, and IgM isotypes from 35 individuals exposed to *S. enteritidis*. Results are plotted according to timing of samples. Broken lines represent the mean + 3 SD from 56 blood donor controls.

positive in the Widal assay, whereas 19 were positive for IgG anti-Salmonella by ELISA.

Three participants, all from the ReA group, reported eye symptoms compatible with conjunctivitis, thus bringing the number with possible Reiter's syndrome (urethritis not included) down to 3%.

This study of a *Salmonella* outbreak illustrates that a significant proportion of exposed individuals can develop reactive joint symptoms regardless of the presence of GI complaints. The duration of diarrhea has a strong effect on the risk of ReA; but overall the joint symptoms were mostly mild and transient.

#### ACKNOWLEDGMENT

The authors thank B. Cederholm, MD, Department of Microbiology, Lund University, Lund, Sweden, for performing the anti-*Salmonella* ELISA.

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