Symptomatic Acute Reactive Arthritis After An Outbreak of *Salmonella*

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ABSTRACT. ***Objective.*** In 2005, 592 individuals in Ontario developed acute gastroenteritis, predominantly after consuming bean sprouts contaminated with *Salmonella enteritidis*. *Salmonella* is a known trigger of reactive arthritis (ReA). We describe the population affected by the *Salmonella* outbreak in terms of clinical presentation of self-reported arthritic symptoms and HLA-B27 genotyping.

Methods. Subjects were mailed a questionnaire, which assessed symptoms consistent with ReA. Subsequently, subjects were asked to submit saliva samples, which were analyzed for HLA-B27. Simple descriptive statistics were performed for analysis of survey responses, and the genetic component was analyzed by chi-square or Fisher's exact tests.

Results. Most respondents were female (71.3%), with a mean age of 46.0 years. The mean duration of diarrhea symptoms was 16.5 days. 62.5% of respondents reported extraintestinal symptoms that were consistent with ReA. The most commonly reported features were joint pain, swelling or stiffness (46.2%), stiffness > 30 min (35.6%), ocular symptoms (24.0%), and visibly swollen joints (19.2%). Subjects with *Salmonella* infection had a similar incidence of HLA-B27, regardless of whether they developed symptoms consistent with ReA or not. Notably, HLA-B27 was present more frequently in those who developed *Salmonella* infection than in healthy controls (OR 3.0).

Conclusion. The study, one of the largest for a dysenteric outbreak, revealed a high event rate of self-reported symptoms consistent with ReA in those infected with *Salmonella*. Our results showed that HLA-B27 may have rendered individuals more susceptible to *Salmonella* infection, but did not contribute to the development of symptoms consistent with ReA after infection. We note that the methods used in this study, including self-report, are not ideal for diagnosis of inflammatory arthritis. However, given the rarity of large outbreaks of *Salmonella*, the study adds valuable knowledge about the course of ReA. (First Release June 1 2008; J Rheumatol 2008;35:1599–602)

Key Indexing Terms:
REACTIVE ARTHRITIS OUTBREAK *SALMONELLA*

Reactive arthritis (ReA) refers to a sterile synovitis that occurs after an extraarticular infection, usually of the gastrointestinal or genitourinary tract[1]. Its clinical manifestations can be diverse, involving both the musculoskeletal system and extraarticular sites[2]. Affected individuals frequently develop peripheral and axial arthritis, enthesitis, mucocutaneous lesions, and ocular inflammation[2]. ReA shares several features with ankylosing spondylitis (AS), in terms of both clinical presentation and in its association with HLA-B27[1-3]. The association of ReA with non-HLA genetic factors is less clear.

The natural history of ReA has been largely elucidated through analysis of sporadic cases occurring in clinic populations. There are few studies that prospectively follow the rheumatic complications of dysenteric outbreaks. The occurrence of a point-source outbreak affords a unique opportunity to obtain information regarding the clinical features of ReA. Epidemiologic and genetic information may be analyzed in a comprehensive manner not possible through investigation of isolated cases. Additionally, prospective analysis of an outbreak population reduces ascertainment bias, which may occur when patients try to associate previous noninfectious gastrointestinal or genitourinary symptoms with their arthritis. Although questionnaires are not an ideal method for elucidating arthritic symptoms, they are a good option in outbreaks with broad geographical spread that are difficult to assess clinically.

Between October and December 2005, approximately

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600 people developed acute gastroenteritis after consuming contaminated bean sprouts. In most cases, the offending organism was determined to be *Salmonella enteritidis*, phage type 13, through stool culture. *Salmonella* is a known trigger of ReA, and has been shown to produce both acute and chronic arthritic symptoms in previous outbreaks[4,5]. We aimed to describe the population affected by the *Salmonella* outbreak in terms of clinical presentation, as determined through self-report, and through HLA-B27 genotyping.

MATERIALS AND METHODS

Patients. In Ontario, the Health Protection and Promotion Act (Regulation 558/91) requires that physicians submit a report to the local health unit if a patient develops an infection that is communicable, including *Salmonella*[6,7]. Because of this mandatory reporting, virtually all patients affected by an outbreak are documented by Ontario public health units. Subjects with dysenteric symptoms that were stool-culture-positive for *Salmonella* between October and December 2005 were identified through the assistance of local health units in Ontario. Affected individuals were sent a package containing study information, consent forms, and a short questionnaire.

Salmonella-infected subjects who agreed to participate were clinically stratified in accordance with their self-reported symptoms as follows; Group 1: patients with arthritis and extraarticular features (EAF); Group 2: patients with arthritis alone; Group 3: patients with EAF alone; Group 4: patients with neither. DNA samples from a healthy cohort were also genotyped, for comparison. Patients were excluded if they were under the age of 18 years at the time they received the survey package.

Survey tool. The questionnaire was based on a tool previously developed for ReA, the Questionnaire Utilizing Epidemic Spondyloarthropathy Traits 2 (QUEST2)[8]. QUEST2 has been shown to have high sensitivity, specificity, and retest reliability[8]. A modification of QUEST2 was used in this study; the modified survey was not revalidated for the purposes of the study. The modified instrument was called the Acute Reactive Arthritis (AReA) questionnaire. The AReA questionnaire assessed the duration of diarrhea, the presence of swelling or stiffness in joints, the presence of morning stiffness, heel pain, ocular symptoms, mucosal ulceration, urethritis, and rash in *Salmonella*-positive subjects. Patients were sent the AReA questionnaire once; limited access to case information due to privacy guidelines prevented repeat mailings. Privacy legislation also prevented us from contacting affected cases by other means, such as telephone, until they had responded to the initial mail-based questionnaire.

HLA-B27 genotyping. Genetic samples were gathered using the Oragene DNA Self-Collection Kit (DNA Genotek Inc., Ottawa, ON, Canada). With this method, patients were asked to submit salivary samples to be analyzed for DNA in buccal epithelial cells and white blood cells. The samples may be stored at room temperature for long periods of time without DNA degradation. Patients were asked to collect their own saliva samples and return them for analysis.

A duplex polymerase chain reaction (PCR) was performed to amplify a 135-bp HLA-B27 segment (exon3) and a 268-bp ß-globin fragment[9]. The ß-globin was used as an internal control for PCR. The HLA-B27 primers recognized all HLA-B27 subtypes except HLA-B*2707, and the sequences are: forward primer, 5'-GGG TCT CAC ACC CTC CAG AAT-3'; and reverse primer, 5'CGG CGG TCC AGG AGC T-3'. The ß-globin reaction used the forward primer 5'-CAA CTT CAT CCA CGT TCA CC-3' and the reverse primer 5'-GAA GAG CCA AGG ACA GGT AC-3'. The reaction mix contained 100 ng genomic DNA template, 1× Taq buffer (NEB), 0.2 mM dNTPs (Roche), 0.9 µM HLA-B27 primers, 0.45 µM ß-globin primers, and 0.5 units of Taq DNA polymerase (NEB) in a 20 µl reaction. The PCR conditions were 94°C for 2 min; 30 cycles of 94°C for 1 min, 57°C for 1 min, 72°C for 2 min; and after the 30th cycle, 72°C for 10 min. The PCR products were run on a 1.5% agarose gel to resolve the 2 components, stained with ethidium bromide, and visualized on an Bio-Rad imager.

Statistical analysis. Simple descriptive statistics were performed for analysis of survey responses. For the genetic component, statistical analyses were carried out using chi-square and/or Fisher's exact tests[10]. Statistical significance was set as a 2-sided α of 0.05.

RESULTS

Clinical features. In total, 592 affected individuals were identified though the assistance of local public health units. All individuals under the jurisdiction of participating health units were mailed the survey questionnaire. Of these affected individuals, 123 responded to the survey; 19 responses were incomplete, leaving 104 for final analysis.

The majority of respondents were female (71.3%), and the mean age of respondents was 46.0 years (range 19.0–92.0 yrs). The mean duration of diarrhea was 16.5 days (range 1–120 days). About 62.5% of respondents reported extraintestinal symptoms that were consistent with ReA. The features reported most commonly were joint pain, swelling or stiffness (46.2%), stiffness > 30 min (35.6%), ocular symptoms (24.0%), and visibly swollen joints (19.2%). The least frequently reported symptoms were genital rash (4.8%) or palmar/plantar rash (3.8%); 37.5% of respondents reported that they did not have any symptoms aside from diarrhea. The reported symptoms are summarized in Figure 1.

Many subjects reported a combination of joint pain plus one other symptom (Figure 2). The most common symptom that accompanied joint pain was joint stiffness.

In comparing subjects who reported arthritic symptoms and those who did not, the groups appeared to be very similar. Specifically, the mean duration of diarrheal illness did not differ significantly between groups (15.2 vs 20.4 days,

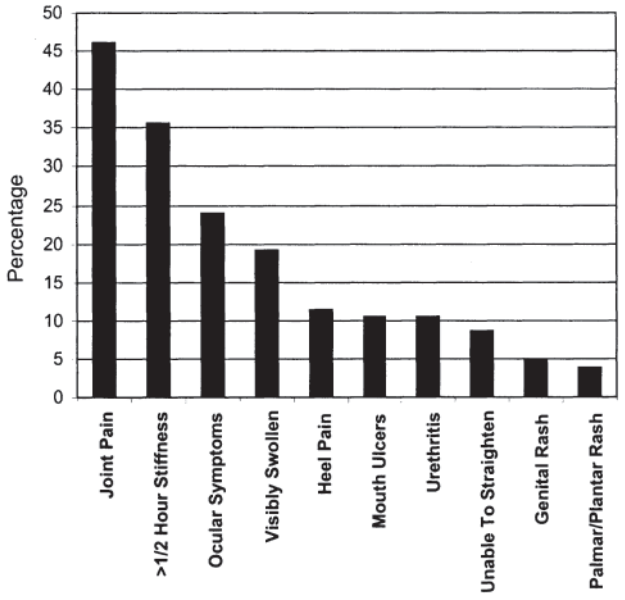

Figure 1. Symptoms reported by respondents to the AReA survey (n = 104); note that 37.5% of respondents reported no symptoms.

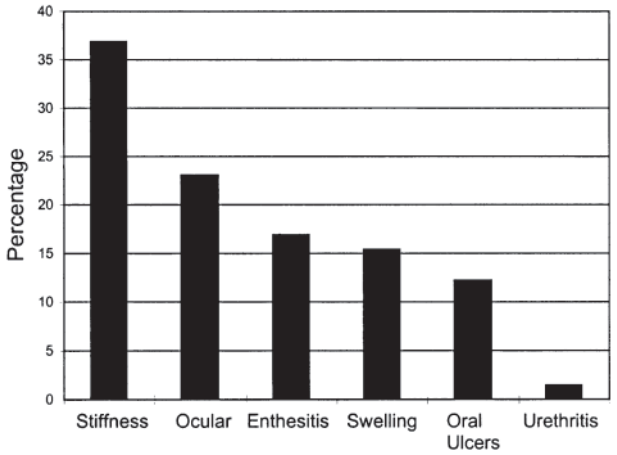

Figure 2. Symptoms in addition to joint pain reported by respondents to the AReA survey (n = 104).

respectively). The mean age of those who developed arthritis (48.4 yrs) also did not differ significantly from the mean age of those without arthritis (42.3 yrs).

HLA-B27 genotyping. Results of HLA-B27 genotyping are presented in Table 1. Subjects with *Salmonella* infection had a similar incidence of HLA-B27, whether they developed symptoms consistent with ReA or not. Notably, HLA-B27 was present more frequently in those who developed *Salmonella* infection than in controls (OR 3.0).

DISCUSSION

After an outbreak of *Salmonella*, 62.5% of survey respondents described symptoms consistent with ReA. The most frequently reported symptoms were joint pain, joint stiffness, and ocular symptoms. Many respondents had both joint pain and another symptom, such as duration of stiffness greater than a half hour, ocular symptoms, and heel pain suggestive of enthesitis.

The study revealed a high event rate of symptoms consistent with ReA in those infected with *Salmonella*. Of the 592 cases documented by public health units, 11.5% (65/592) reported symptoms consistent with ReA. This number is likely an underestimate, as the overall complete response rate to the AReA survey was only 17.6%. This is likely true even if a selection bias would cause those with symptoms to be more likely to respond to the AReA survey.

Table 1. Results of HLA-B27 genotyping in study subjects and controls.

Study Group	HLA-B27 Positivity (%)	p, Fisher Exact Test	Odds Ratio
Group 1 (arthritis + EAF)*	5/25 (20)		
Group 2 (arthritis alone)	0/12 (0)		
Group 3 (EAF alone)	0/9 (0)		
Group 4 (no symptoms)	6/28 (21.4)		
Groups 1–3	5/46 (10.9)		
Groups 1–3 vs Group 4		0.12	
Groups 1–4	11/74 (14.9)		
Controls	5/91 (5.5)		
Groups 1–4 vs controls		0.029*	3.0

* EAF: extraarticular features.

This high incidence rate of symptoms consistent with ReA is greater than rates previously reported in outbreak situations. A large study of a *Salmonella* outbreak in police officers noted an event rate of acute ReA of 7.3%[4]. However, the reported incidence of ReA after an outbreak of *Salmonella* varies greatly, from 1.6% to 19%[11-13].

Interestingly, we found no association between the duration of diarrheal illness and the development of self-reported symptoms of ReA. Prior studies of *Salmonella* outbreaks demonstrated that the development of both acute and chronic arthritic sequelae is related to the duration of dysentery[5]. However, we do note that recall of the duration of diarrheal illness is fraught with numerous sources of bias.

We did not find a higher frequency of HLA-B27 positivity in those subjects who developed symptoms compatible with a clinical diagnosis of ReA. Studies have described higher frequencies of HLA-B27 in subjects with ReA[1,4,5], although the frequency in outbreaks is typically lower than those in hospital series[14]. This discrepancy in HLA-B27 rates suggests that subjects who are HLA-B27-positive may have more severe clinical manifestations, necessitating referral to a hospital. Our results showed that HLA-B27 may have rendered individuals to be more susceptible to *Salmonella* infection, but it did not contribute to development of ReA after infection. Interestingly, *Salmonella* has been noted to increase intracellular replication in HLA-B27-expressing monocytes[15].

Our study has some shortcomings imposed by the design of the followup study, which relied on questionnaire response. Determination of symptoms compatible with ReA was based on patient self-assessment. Some of the respondents may certainly have exaggerated the severity of their symptoms. We acknowledge that the use of a questionnaire in this outbreak setting may misidentify cases of arthritis. Indeed, we can only report on the symptoms patients observed themselves rather than true physician-identified arthritis. In a previous Ontario outbreak (May 2005), we made an attempt to send a more comprehensive questionnaire that included checklists of specific joints and asked respondents to indicate if each joint was swollen or tender, and the duration of these symptoms. Unfortunately, this previous survey had a very poor response rate, and it was felt that the length of the questionnaire was a deterrent factor for potential participants. Another shortcoming of the study was the lack of physician examination to confirm the diagnosis of true inflammatory arthritis. Based on the information that we gathered from the AReA questionnaire, we can only report on the patients having symptoms consistent with ReA, but we lacked the physical examination confirmation

for an unequivocal diagnosis of ReA. An attempt was made to interview and examine those who reported symptoms; unfortunately, very few study subjects were willing to undergo this process, in part because of the geographical spread of the subjects. In an ideal examination of an epidemic, each affected individual should be examined by an objective professional. The AReA questionnaire was distributed in English only, and may have excluded some groups for whom bean sprouts may be a regular part of the diet. The use of an English questionnaire may have selected an ethnic population that was homogeneous. This homogeneity would make genetic analysis easier, but increased diversity might have allowed us to discover some differences in the predisposing genetic factors between different populations. The AReA questionnaire was modified from a previously validated survey instrument, but was not independently tested for reproducibility or validity. This decision was made in light of the short timeframe we had to gather responses in an outbreak situation. Another limitation of the study is that our overall response rate was low, although consistent with that from other mail-based questionnaires.

The major strength of our study is that it is one of the largest ever completed in a dysenteric outbreak. Given the sparse information available in these settings, we felt that any investigation would be worthwhile. Indeed, the execution of the study itself offers some valuable lessons for conducting outbreak research. The obstacles to studying outbreak cohorts include obtaining timely ethics approval, addressing privacy legislation, coordinating multiple sites, and working with numerous stakeholders. An outbreak investigation necessitates rapid decision-making in regard to study design, which can lead to later difficulties in analysis. A positive consequence of our outbreak experience was recognition that a larger network for studying epidemics is needed in Ontario.

The setting of a dysenteric outbreak provides researchers with a unique opportunity to learn more about the rheumatologic complications of such events. For disease processes such as ReA that have a definite infectious trigger, the study of outbreaks plays a critical role in addressing pathogenesis and clinical features of the disease. We hope that our experience can be applied to future outbreaks of gastroenteritis, so that we may understand ReA more fully.

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