A Comparison of Self-reported Joint Symptoms Following Infection with Different Enteric Pathogens: Effect of HLA-B27

PETER SCHIELLERUP, KAREN A. KROGFELT, and HENNING LOCHT

ABSTRACT. ***Objective.*** We conducted a case-case comparison study to estimate the attack-rate of reactive joint pain (JP_{rea}) following intestinal infections, and evaluated whether the susceptibility and severity of joint symptoms was associated with the tissue-type HLA-B27.

Methods. Consecutive patients with positive fecal culture for *Salmonella*, *Campylobacter*, *Yersinia*, *Shigella*, and *E. coli* were addressed by questionnaires inquiring about gastrointestinal (GI) symptoms and the occurrence of joint pain in a previously healthy joint within 4 weeks after onset of infection. A blood sample was requested for HLA-B27 typing.

Results. Of 3146 patients invited, 2105 (67%) responded to the survey questionnaire. The triggering infections were *Campylobacter*, 1003; *Salmonella*, 619; *E. coli*, 290; *Shigella*, 102; and *Yersinia*, 91. JP_{rea} was reported by 294 subjects: *Campylobacter*, 131 (13.1%); *Salmonella*, 104 (16.8%); *Yersinia*, 21 (23.1%); *Shigella*, 10 (9.8%); and *E. coli*, 28 (9.7%). There was a significant association between severity of gastroenteritis and development of arthralgia ($p = 0.001$). The odds ratio (OR) for JP_{rea} in an HLA-B27-positive individual was 2.62 (95% CI 1.67–3.93) for the entire group. A significant association between JP_{rea} and HLA-B27 was found for *Salmonella*, *Shigella*, and *Yersinia*; not, however, for *Campylobacter* and *E. coli*. HLA-B27-positive patients had a significantly increased risk for severe joint symptoms.

Conclusion. Our study shows that JP_{rea} after GI infection is positively correlated to severity of GI symptoms. HLA-B27 is not associated with joint pain after *Campylobacter*. Intestinal *E. coli* seems to be an arthritogenic pathogen. A significant association between HLA-B27 and severity of joint pain was observed. (First Release Jan 15 2008; J Rheumatol 2008;35:480–7)

Key Indexing Terms:

JOINT PAIN | REACTIVE ARTHRITIS | HLA-B27 | *SALMONELLA*
YERSINIA | *SHIGELLA* | *CAMPYLOBACTER* | *E. COLI*

Reactive arthritis (ReA) is characterized by an asymmetrical inflammation in peripheral and axial joints as well as enthesopathy. Sometimes extraarticular manifestations occur, such as of conjunctivitis, urethritis, or mucocutaneous symptoms. ReA is triggered by infections elsewhere in the body, usually by microbes from the gastrointestinal (GI) or urogenital tract[1]. The main efforts of research in this field have been focused on the arthritides caused by *Salmonella*, *Yersinia*, *Shigella*, *Campylobacter*, or *Chlamydia*, although several other infections may produce a similar clinical picture. ReA after GI infections, particularly *Salmonella* and *Campylobacter*, have derived special attention due to the high incidence of these food-borne infections in industrialized countries. Knowledge about the numbers of individuals who develop ReA after episodes of infectious diarrhea varies considerably and originates mainly from reported community outbreaks or population-based studies. The incidence of ReA has been reported to occur in only a few percent in *Shigella*[2,3], 5%–20% in *Salmonella*[4-6] and *Campylobacter*[7,8] series, and exceeding 50% in one report describing a *Yersinia* outbreak[9]. Apart from the individual microbe, the ingested bacterial load, age, sex, and the presence of HLA-B27 are factors probably affecting the susceptibility for ReA. The close association between the class I major histocompatibility (MHC) antigen HLA-B27 and ReA has been known for more than 3 decades[10] and studies from rheumatology referral centers have shown that patients with severe arthritis and sometimes extraarticular complications often are HLA-B27-positive[11]. However, it is still uncertain to what extent HLA-B27, and presumably other risk factors, contributes to the susceptibility and clinical course of ReA when subjects are identified shortly after clinical symptoms from the triggering infection have appeared.

We conducted a case to case comparison study through collecting clinical data from individuals with *Campylo-*

From the Department of Gastrointestinal Infections and Department of Autoimmunology, Statens Serum Institut, Copenhagen, Denmark.

P. Schiellerup, MD; K.A. Krogfelt, PhD, Department of Gastrointestinal Infections; H. Locht, MD, Department of Autoimmunology.

Address reprint requests Dr. H. Locht, Department of Autoimmunology, Building 81/524, Statens Serum Institut, Artillerivej 5, DK-2300 Copenhagen S, Denmark. E-mail: hlo@ssi.dk

Accepted for publication October 24, 2007.

bacter, *Salmonella*, *Shigella*, *Escherichia coli*, or *Yersinia* infections verified by fecal cultures to identify possible risk factors for postinfectious joint pain (JP) and to estimate the role of HLA-B27 in this context. By comparing gastroenteritis caused by different bacterial species we were able to analyze differences in incidence, course, and outcome of postinfectious articular complications.

MATERIALS AND METHODS

Study design and population. This study was designed as a prospective case-case comparison study and was conducted between January 2002 and November 2003. Participants were recruited from the Danish Registry of Bacterial Enteropathogens (DRBE; results of positive fecal cultures are reported from microbiological laboratories in Denmark and stored in a central database) after a stool culture had confirmed bacterial gastroenteritis caused by *Campylobacter*, *Salmonella*, *E. coli*, *Shigella*, or *Yersinia*. The Statens Serum Institut in Copenhagen performs constant surveillance of infectious diseases in Denmark, and information on patient identity, referring doctor, and results of cultures is stored in the database. All patients older than age 18 years, who gave informed consent to participate, were included consecutively and sent a questionnaire. Subjects responding to the first questionnaire received a second one 6 months after onset of gastroenteritis. A reminder was mailed 3 weeks later to all nonrespondents. Only participants infected with one bacterial species were included. If the fecal culture contained more than one pathogenic bacterial strain, even if the genus was the same, the subject was not included. All patients were encouraged to visit their personal general practitioner (GP) to give a blood sample (serum and EDTA blood) for serological and genetic testing.

Letters of information were simultaneously sent to the patients and the referring GP; informed consent was obtained by the GP before taking the blood sample.

The Ethical Scientific Committee for Copenhagen approved the study (KF01-300/00).

The questionnaires. In questionnaire 1 (Q1) patients were asked to give information about their GI symptoms by marking boxes with "yes" or "no" for diarrhea, stomach pain, nausea, vomiting, fever, and blood in the stool, and whether they had been admitted to a hospital for enterocolitis. Duration of diarrhea in days (specified as ≥ 3 loose stools per day) and time of onset and termination of diarrhea were recorded. Based on the GI symptoms patients were assigned to 3 groups according to severity: "mild GI" was diarrhea and stomach pain or nausea (one or both); "moderate GI" was the above plus fever and vomiting (one or both). "Severe GI" was diarrhea and any one of the other symptoms plus blood in the stool and/or hospitalization.

Patients were asked whether joint pain had occurred in a previously healthy joint within 4 weeks after onset of gastroenteritis.

Severity of JP was estimated in 2 ways: on a visual analog scale (VAS) from 0 to 100 mm, people were asked to provide an overall evaluation about their JP ranging from "no pain" to "the worst imaginable pain." Further, patients should mark with "yes" or "no" to the following questions: "Were your joint symptoms mild, did the joint symptoms affect your daily work, did you take painkillers for joint pain, did you consult a doctor because of joint pain, and did you take sick-leave because of joint pain."

The joint symptoms were judged as "mild" when the subject had indicated the "mild" box, "moderate" if "not mild" and painkillers were needed and/or daily work was influenced, and "severe" if the individual had required sick-leave or seen a doctor or visited a hospital (inpatient or outpatient) for locomotor symptoms.

The Q1 included a graphic presentation of the body on which the person was asked to mark swollen or painful joints.

Participants were also queried about concurrent rheumatic disorders. Patients who claimed they suffered from chronic rheumatic conditions such as rheumatoid arthritis, psoriatic arthritis, or generalized osteoarthritis were excluded from the study. Worsening of symptoms in a previously painful joint (affected by surgery or from other causes) precluded participation in the study.

From questionnaire 2 (Q2), 6 months postinfection, we obtained information about persistence or resolution of joint symptoms.

Validation of questionnaires. Reproducibility of the data in Q1 was assessed by retesting the answers given in the questionnaire after 1 month in 10 randomly selected participants, who were mailed a second copy of Q1 and asked to complete it identically to the first. The reproducibility was 87% (range 55%–100%).

Similarly, validation of Q2 was done by telephone interviews of 10 participants randomly chosen among the first 100 responders. The reproducibility of Q2 was 96% (range 92%–99%).

The capacity of Q1 to detect infection-triggered joint symptoms was evaluated in a subcohort of patients from the Greater Copenhagen area (defined by postal codes). Eighty-three patients who reported joint pain were invited to a clinical examination performed by a rheumatologist (HL). Based on history and objective findings, patients were allocated into 3 groups: "definite ReA" if objective signs of synovitis, enthesopathy or axial joint involvement were diagnosed; "probable ReA" if a typical history and tenderness on palpation or motion were found; and "not ReA" if symptoms were unrelated to the gastroenteritis or suggested noninflammatory causes, e.g., neck tension, fibromyalgia, frozen shoulder, etc.

The rheumatologist, blinded to the HLA-B27 status and bacterial species causing the infection, assessed all the Q1 from patients with self-reported joint pain (JP_{sr}). If a patient satisfied the following criteria, thus suggesting a causal relationship between the triggering infection and onset of arthralgia, a diagnosis of reactive joint pain (JP_{rea}) was made. The criteria were onset of pain within 4 weeks after gastroenteritis, duration of arthralgia exceeding 7 days, the patient should have no concurrent rheumatic illness, and the questionnaire should be completed adequately (if important data were missing, precluding a reliable evaluation of the joint symptoms, the case was categorized as "not JP_{rea}").

The calculations were thus based on the entire group responding to the Q1 and complaining of JP_{sr}, the group of patients with JP_{rea}, and the number of individuals who delivered a blood sample for HLA-B27 typing.

Fecal cultures. Data on individual bacterial isolates were retrieved from the DRBE or from fecal cultures performed at the Laboratory for Intestinal Pathogens at the Statens Serum Institut. Bacteria were identified at species level and serotyped as *Salmonella enteritidis*, *Salmonella typhimurium*, or other zoonotic *Salmonella* species. *Yersina enterocolitica* was serotyped as O:3 or O:9. *Shigella* was serotyped as *S. sonnei*, *S. dysenteriae*, *S. boydii*, or *S. flexneri.* The group of *E. coli* consisted of enterotoxinogenic *E. coli* (ETEC), attaching and effacing *E. coli* (A/EEC), enteropathogenic *E. coli* (EPEC), and verocytotoxin (shigatoxin)-producing *E. coli* (VTEC).

Blood samples and HLA-B27 screening. DNA was extracted from EDTA blood samples with Nucleospin Blood Quick Pure® according to the manufacturer's protocol.

Prevalences of the tissue-type HLA-B27 gene were measured by a polymerase chain reaction (PCR), with primers and controls as described by Olerup[12]. The following primer pairs were used: B27-3 (5'-CAG TCT GTG CCT TGG CGT TGC-3') matching nucleotides 199 to 219, and B27-5 (5'-GCT ACG TGG ACG ACA CGC T-3') matching nucleotides 76 to 94 of the HLA-B27 gene. To ensure there was DNA present in the reaction, the primer pair SSPhgh-5 (5'-GCC TTC CCA ACC ATT CCC TTA-3') and SSPhgh-3 (5'-TCA CGG ATT TCT GTT GTG TTT C-3'), which amplifies the human growth hormone (hgh), was used together with the HLA-B27 primers. PCR was performed using the DNA polymerase AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). For the PCR solution we used Dynal PCR solution (Dynal Biotech Ltd., Bromborough, UK). The PCR program comprised denaturation at 95°C for 10 min, 10 cycles of denaturation at 95°C for 10 s and annealing at 61°C for 1 min, 20 cycles of denaturation at 95°C, annealing at 57°C for 50 s, and extension at 72°C for 30 s. The PCR product for HLA-B27 was 144 bp and the PCR product for hgh was 429 bp.

RESULTS

Study population. A total of 3146 subjects were invited to participate and 2105 (67%) responded to Q1. Fifty percent (n = 1558) of the total delivered a blood sample for HLA-B27 typing. Followup data on duration of JP_{rea} after 6 months were retrieved from the participants who responded to both questionnaires (n = 1670; 53%). Of these, 1359 (43%) persons were typed for HLA-B27.

The male/female ratio was 42.9%/57.1% and the median age in the study population was 42 years for men (range 18–79) and 38 years for women (range 18–79). There was an overrepresentation of women in the group with JP_{rea} (M/F 36.4%/63.6%) and their median age was slightly higher compared to the overall population.

The infections were as follows: *Campylobacter* (n = 1003), *Salmonella* (n = 619) [*S. typhimurium* 193 (31%), *S. enteritidis* 270 (44%), other zoonotic *Salmonellae* 156 (25%)], *E. coli* (n = 290) [EPEC 17 (6%), ETEC 112 (39%), A/EEC 138 (47%), VTEC 23 (8%)], *Shigella* (n = 102) [*S. sonnei* 70 (69%), *S. flexneri* 23 (22%), other *Shigellae* 9 (9%)], and *Yersinia* (n = 91) [*Y. enterocolitica* O:3 83 (91%), other 8 (9%)]. The median response time from onset of diarrhea until returning the Q1 was 42 days; 66% responded between 1 and 3 months after onset of gastroenteritis.

Rheumatological evaluation of Q1. Of the 2105 subjects returning Q1, 420 (20%) had JP_{sr}. One hundred twenty-six were excluded according to the predefined criteria for JP_{rea} (45 returned the questionnaire inadequately completed or presented no or inaccurate joint locations, 27 had preexisting rheumatic condition at the time of diarrhea, 49 had joint pain < 7 days, 5 had joint complaints beginning more than 4 weeks after onset of enterocolitis). Thus the group with JP_{rea} consisted of 294 (14%) individuals.

Clinical examination of patients with joint symptoms. Eighty-three persons with joint symptoms were invited to a clinical examination and 71 responded. Fifteen (21%) had objective signs of synovitis and were designated "definitive ReA," 30 (42%) had joint tenderness on palpation or motion and a history compatible with ReA and were judged as "probable ReA," and 26 (37%) had joint symptoms from other causes unrelated to the preceding infection and were termed "not ReA." The results of HLA-B27 typing in this group were as follows: 29% of the definitive ReA were B27-positive (4 of 14 tested), 24% of probable ReA were positive (5 of 21), and 5% of the not ReA subjects had the B27 antigen (1 of 22). In the group with definitive ReA, 6 were caused by *Salmonella*, 7 by *Campylobacter*, and 2 by *Shigella*. Among the probable ReA patients, 11 had *Salmonella*, 13 *Campylobacter*, 3 *Shigella*, and 3 had *E. coli* infections.

Severity of GI symptoms. The patients were divided into those with mild, moderate, and severe GI symptoms according to the individual bacterial species. In the *Campylobacter* group, 285 had mild disease, 376 moderate, and 342 patients had severe enterocolitis. For *Salmonella* the figures were 174, 226, and 219; for *Yersinia* 49, 25, and 17; for *Shigella* 36, 28, and 38; and for *E. coli* 186, 62, and 42 patients, respectively. Among patients with *Campylobacter*, *Salmonella*, and *Shigella* the distribution was similar, in that about one-third of subjects had mild, moderate, or severe GI complaints. However, in the *Yersinia* and *E. coli* groups the majority of individuals, 54% and 64%, respectively, had only mild GI symptoms (*Yersinia* and *E. coli* vs *Campylobacter*, *Salmonella*, and *Shigella*, $p < 0.0001$).

Incidence of JP_{rea} according to bacterial species. Two hundred ninety-four patients had probable infection-related JP_{rea}. In the *Campylobacter* group there were 131 (13.1%) subjects, in *Salmonella* 104 (16.8%), in *Yersinia* 21 (23.1%), in *Shigella* 10 (9.8%), and in the *E. coli* group 28 (9.7%). The incidence of JP_{rea} in the *Yersinia* and *Salmonella* groups was significantly higher compared to the *Campylobacter* patients ($p = 0.01$ and $p = 0.04$, respectively; Table 1).

When incidence of JP_{rea} was correlated to GI symptoms, there was a significant tendency to an increased risk of postinfectious joint symptoms when people had severe intestinal disease. Of 730 subjects with mild GI symptoms, 76 (10.4%) developed ReA, 107 of 717 (15.2%) with moderate GI symptoms had JP_{rea}, and 109 of 658 (16.6%) with severe GI symptoms had JP_{rea} (mild vs severe GI symptoms, $p = 0.001$).

The OR for developing JP_{rea} in a person with severe enterocolitis (i.e., diarrhea plus blood in the stool or admission to hospital) for *Salmonella* was 1.94 (95% CI 1.08–3.47;

Table 1. The result of HLA-B27 typing in patients with reactive joint pain (JP_{rea}) according to bacterial species.

Species	Total, n	JP_{rea}, n (%)	HLA-B27 Typed, n	HLA-B27-positive, n (%)
Campylobacter	1003	131 (13.1)	91	12 (13)
Salmonella	619	104 (16.8)	86	19 (22)
S. typhimurium	193	29	23	7 (30)
S. enteritidis	270	49	44	10 (23)
Other	156	26	19	2 (11)
Shigella	102	10 (9.8)	6	2 (33)
S. sonnei	70	6	3	1 (33)
S. flexneri	23	4	3	1 (33)
Other	9	0	0	0
Yersinia	91	21 (23.1)	18	4 (22)
Y. enterocolitica 0:3	83	20	17	4 (24)
Other	8	1	1	0
E. coli	290	28 (9.7)	20	2 (10)
A/EEC	138	16	11	2 (18)
ETEC	112	8	6	0
EPEC	17	1	0	0
VTEC	23	3	3	0
All bacteria	2105	294 (14.1)	221	39 (17.6)

A/EEC: attaching and effacing *E. coli*, ETEC: enterotoxinogenic *E. coli*, EPEC: enteropathogenic *E. coli*, VTEC: verocytotoxin-producing *E. coli* (USA: STEC: Shigatoxin-producing *E. coli*).

p = 0.03) and was even more pronounced for *E. coli* with an OR of 3.56 (95% CI 1.47–8.63; p = 0.007; Table 2).

The median duration of diarrhea was 7 days [interquartile range (IQR) 5-12 days] in the patient group without joint symptoms and 8 days in subjects with JP_{rea} (IQR 5–14 days; p = 0.003). At species levels, patients *with Campylobacter* and *Salmonella* JP_{rea} had slightly longer periods of diarrhea than people with uncomplicated enteritis; however, the difference was statistically significant only for *Campylobacter* (data not shown).

HLA-B27. Of the 1558 participants tested for HLA-B27, 140 (9.0%) were positive, which is not significantly different from the overall prevalence of 9.4% in the Danish population (Svejgaard A, personal communication). There were no statistically significant differences in HLA-B27 prevalence among *Campylobacter*, 59/694 (8.5%), *Salmonella*, 51/503 (10.1%), *E. coli*, 20/212 (9.4%), *Yersinia*, 7/76 (9.3%), and *Shigella*, 3/73 (4%) (Table 3).

JP_{rea} and association with HLA-B27. Three hundred thirteen (20.1%) of 1558 patients had JP_{sr} and 45 (14.4%) were HLA-B27-positive, giving an overall OR of 2.03 (95% CI 1.39–2.97). When the "joint population" was narrowed to those with JP_{rea}, the fraction of HLA-B27-positive increased to 17.6% (39 of 221 subjects). The OR for JP_{rea} in a B27-positive patient was 2.62 (95% CI 1.76–3.92) for the entire group, indicating a considerable influence of HLA-B27 on susceptibility to postinfectious joint disease. At the microbial level there was a significant association between JP_{rea} and HLA-B27 in the *Salmonella* and *Shigella* subgroups. A borderline relationship was found for *Yersinia*, whereas no significant relation to HLA-B27 was established for *Campylobacter* and *E. coli* (Table 3).

HLA-B27 and severity of JP_{rea}. Patients with JP_{rea} were divided into those with mild, moderate, and severe joint disease according to the individual bacterial species. There were no significant differences between the microbe groups although we observed a tendency to more severe JP_{rea} among *Yersinia* patients (44%) compared to the *Campylobacter* patients (25%). In the entire group there were 93 with mild JP_{rea} of which 12 (12.9%) were HLA-B27-positive, 62 with moderate JP_{rea} of which 9 (14.5%) were HLA-B27-positive, and 66 with severe JP_{rea} of which 18 (27.3%) were HLA-B27-positive (Figure 1). There was a statistically significant difference between the mild and severe groups with respect to the proportion of HLA-B27-positive patients (p = 0.037; Figure 1).

VAS pain score and severity of JP_{rea}. Of the 294 patients with JP_{rea}, 133 (45%) were categorized as mild, 82 (28%) as moderate, and 79 (27%) as severe JP_{rea} according to the predefined criteria. The median VAS was 25.5 mm (IQR 15–38 mm) in the mild group, 45.0 mm (IQR 37–58 mm) in the moderate, and 66.5 mm (IQR 49–78 mm) in the severe JP_{rea} group. The difference between groups was significant, p < 0.0001.

The median VAS joint pain score for *Campylobacter* was 37.5 mm, for *Salmonella* 42.5 mm, *Yersinia* 62.0 mm, *Shigella* 39.5 mm, and for *E. coli* 35.5 mm. A significant difference was observed between *Yersinia* and *Campylobacter*

Table 2. Numbers of patients with JP_{rea} according to bacterial species and severity of gastrointestinal (GI) symptoms; mild: diarrhea and nausea, or stomach pain; moderate: diarrhea and fever and/or vomitting; severe: diarrhea and blood in stool and/or hospitalized.

	GI Symptoms							
	Mild,		Moderate,		Severe,		Mild vs Severe,	
Species	n	JP_{rea} (%)	n	JP_{rea} (%)	n	JP_{rea} (%)	OR (95% CI)	p
Campylobacter	285	28 (9.8)	376	56 (14.9)	342	47 (13.7)	1.46 (0.89–2.40)	0.16
Salmonella	174	19 (10.9)	226	43 (19.0)	219	42 (19.2)	1.94 (1.08–3.47)	0.03
Yersinia	49	12 (24.5)	25	5 (20.0)	17	4 (23.5)	0.95 (0.26–3.47)	0.80
E. coli	186	15 (8.1)	62	3 (4.8)	42	10 (23.8)	3.56 (1.47–8.63)	0.007
Shigella	36	2 (5.6)	28	2 (7.1)	38	6 (15.8)	3.19 (0.60–16.96)	0.30

Table 3. Distribution of patients with self-reported joint pain (JP_{sr}) and JP_{rea} among 1558 patients who were typed for HLA-B27. OR for joint pain in a subject who is HLA-B27-positive is given.

Species	No. Patients	HLA-B27+	JP_{sr}	HLA-B27+	OR (95% CI)	JP_{rea}	HLA-B27+	OR (95% CI)	p
Campylobacter	694	59	138	15	1.4 (0.76–2.63)	91	12	1.8 (0.91–3.53)	0.12
Salmonella	503	51	114	21	2.7 (1.48–4.99)	86	19	3.4 (1.83–6.37)	0.0001
Yersinia	76	7	20	5	8.8 (1.55–50.18)	18	4	5.2 (1.05–26.15)	0.05
Shigella	73	3	9	2	18.0 (1.47–228.21)	6	2	33.0 (2.44–446.1)	0.02
E. coli	212	20	32	2	0.6 (0.13–2.70)	20	2	1.1 (0.23–5.01)	1.0
All	1558	140	313	45	2.03 (1.39–2.97)	221	39	2.62 (1.76–3.92)	< 0.0001

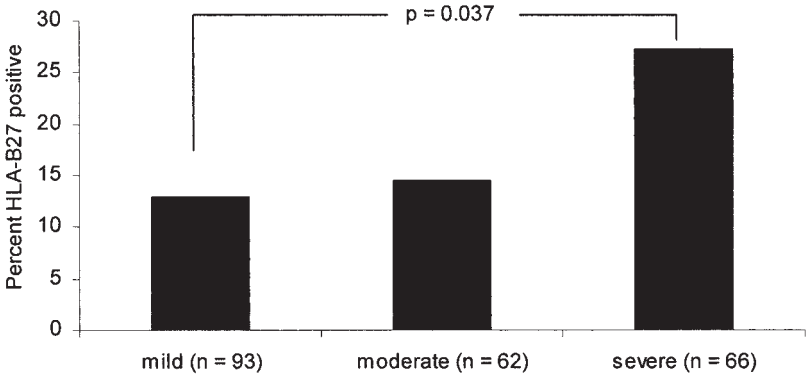

Figure 1. The percentage of HLA-B27 in the cohort of 221 patients with JP_{rea} after GI infection. Subjects are grouped according to severity of joint symptoms. Significantly more patients with severe joint disease were HLA-B27-positive.

(p = 0.006) and between *Yersinia* and *E. coli* (p = 0.04), indicating an increased overall burden of pain in the *Yersinia* JP_{rea} group. This is in accord with the finding of relatively more cases of severe JP_{rea} in this group.

When patients with VAS scores below and above 50 mm were compared with respect to proportion of HLA-B27, we found an OR of 2.18 (95% CI 1.04–4.59), indicating that people that were HLA-B27-positive predominated among individuals with the higher pain scores. This correlation is illustrated in Figure 2, showing the increasing number of HLA-B27-positives in the groups with the highest VAS scores (p = 0.0024, Wilcoxon test for trend).

A logistic regression model identified the following risk factors for development of JP_{rea}: carriage of HLA-B27 allele, severe gastroenteritis, age 30 to 59 years, and female sex. OR were, respectively, 2.62 (95% CI 1.74–3.95), 1.77 (95% CI 1.28–2.45), 2.30 (95% CI 1.66–3.18), and 1.43 (95% CI 1.06–1.94) (Table 4).

Followup of JP_{rea} and correlation to HLA-B27. Of the 294 participants with JP_{rea}, 125 (42.5%) had persistent joint pain when answering Q2 after 6 months. The response rate to Q2 was 78% for the group with JP_{rea} and 79% for the whole study population, thus probably alleviating the potential bias that subjects with joint symptoms might be more inclined to respond to the survey questionnaires. HLA-B27 was positive in 17.6% (39 of 221 JP_{rea} patients) of those responding to Q1 and in 21.3% (23 of 108) of those with persistent joint symptoms according to Q2. This difference was, however, not statistically significant.

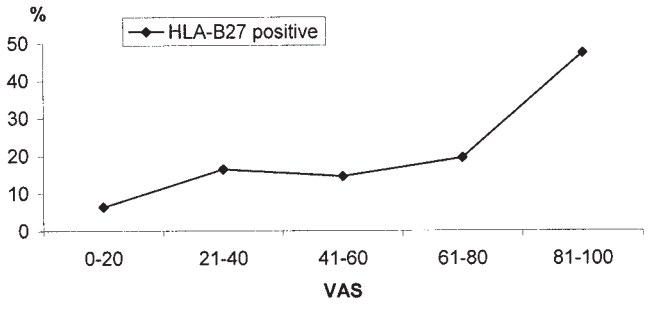

Figure 2. Percentage of HLA-B27-positive subjects according to severity of JP_{rea}, measured by VAS pain score (mm). p = 0.0024; Wilcoxon test for trend.

Table 4. A logistic regression model showing contribution of individual risk factors for development of ReA.

Risk Factor	OR	95% CI
HLA-B27-positive	2.62	1.74–3.95
Age group 30–59 yrs	2.30	1.66–3.18
Severe gastroenteritis	1.77	1.28–2.45
Female sex	1.43	1.06–1.94

DISCUSSION

Due to the prospective design of this study, where cases were collected during a period of nearly 2 years, the spectrum of bacterial microorganisms causing infectious enterocolitis reflects the overall pattern in the Danish population. It is notable that the incidence rates obtained for different enteric pathogens do not reflect the true exposure *per se* but merely represent the numbers of individuals (inevitably with some degree of case selection) who consult their doctor because of diarrhea. However, as patients are unaware of the microbial strain causing their problems, it is reasonable to assume that the threshold for seeking medical advice is the same, regardless of the triggering bacterium. We obtained an acceptable response rate of about 70%. There were slightly more female responders, probably illustrating that women were keener to respond to questionnaires and participate in epidemiological studies. The female preponderance in the group who had a blood sample drawn by their GP (60% women, 40% men) strengthened this impression. There was, however, no indication that women in general were overrepresented among individuals with a registered positive fecal culture because the female/male ratio in the DRBE data was 1:1.

Data relying on the patient's own observations should be regarded with caution, but due to the comparative design of the study it is possible to compare the incidence and course of arthralgia following enteric infection between various arthritogenic microbes. As all patients and their physicians were informed about the purpose of the study it cannot be excluded that recall bias may have interfered. People were informed that joint pain could follow as a complication to infectious gastroenteritis thus eventually leading to an overestimate of JP_{rea}. However, to evaluate the test instrument (Q1) we conducted a clinical examination of 71 individuals with joint pain recruited from a local area. Based on a typical history and objective findings, 63% of subjects had definitive or probable ReA. The subsequent evaluation of all Q1 data from people with joint pain led to exclusion of 30%, resulting in only 70% where infection-triggered joint disease was found to be likely. It cannot be excluded that bias may have occurred as the questionnaires were evaluated subjectively, and in several instances a JP_{rea} diagnosis had to be rejected based on inadequately completed survey forms.

As no generally accepted standards exist for classification of gastroenteritis into mild, moderate, and severe disease we developed an arbitrary set of criteria and applied these on the study population.

When subjects were grouped according to severity of GI symptoms, there was a significant overrepresentation of mild cases among the patients with *Yersinia* and *E. coli*. A large British study described to what extent people with infectious enterocolitis in the population actually sought medical advice and had a stool culture performed. The ratio between cases in the community (who were actively looked for) and the numbers who visited their GP varied from 1 to 2.1 for *Salmonella*, *Shigella*, and *Campylobacter* species, whereas it reached 11.7 for *Yersinia* and between 4 and 13 for the various *E. coli* subtypes[13]. This indicated that the majority of people with gastroenteritis caused by *Yersinia* and *E. coli* had only minor or transient GI symptoms and thus did not visit their physician.

The incidence of JP_{rea} was highest among patients with *Yersinia* and *Salmonella*, and significantly higher relative to the *Campylobacter* group. Although no comparative studies have been performed, some investigators have found a particularly high frequency of infection-triggered joint symptoms following *Yersinia* infections, and it even appeared that these patients experienced a more severe course of arthritis[9,14,15]. Patients with serious GI symptoms, such as hospitalization or blood in the stools, were more prone to develop JP_{rea}. A significant association was found for *Salmonella* and *E. coli* as well as for the entire group. It cannot be ruled out that people who have experienced severe abdominal discomfort will also be more observant on extraintestinal symptoms, thus causing overreporting of joint pain in that group. However, we found an analogous association in previous studies investigating JP_{rea} after *Salmonella* and *Campylobacter*[6,16] where GI severity, measured as duration of diarrhea in days, seemed to be a risk factor for joint problems. An explanation could be that the increased bacterial load or impaired host defense system leading to serious gastroenteritis might also promote the development of joint inflammation.

The prevalence of HLA-B27 among patients with ankylosing spondylitis is over 95% in many hospital series[17,18], whereas the occurrence in patients with ReA varies from more than 80% in hospital-based followup studies[11,19] to around 40% or less in population-based series or localized outbreaks[4,20-22].

We found a highly significant OR for JP_{rea} when the individual was HLA-B27-positive, most pronounced for the *Salmonella* and *Shigella* species, indicating a considerable influence of this allele on susceptibility to contracting JP_{rea}. The borderline association with *Yersinia* probably depended on the relatively few patients in this group. In *Campylobacter* enteritis, representing the largest subgroup in the study, we found no statistically significant relation between JP_{rea} and HLA-B27. It is generally believed that *Campylobacter* belongs to the HLA-B27-related arthritides. However, this concept is mainly based on case reports or studies containing only a few patients. In a review of reported cases in 1994, 56% of 29 cases were HLA-B27-positive, and a more severe course of arthritis was associated with this tissue type[23,24]. In a recent outbreak caused by *Campylobacter* the investigators found a frequency of HLA-B27 of 33% among the relatively small number of patients with ReA[25]. However, a population-based survey conducted by the same authors showed the same rate of HLA-B27 among ReA subjects (14%) as in the control group[8].

The allocation of subjects with JP_{rea} into mild, moderate, and severe disease was based on arbitrarily predefined criteria. But there seemed to be a reliable discrimination between groups as the median VAS pain scores were significantly different (25.5, 45.0, and 66.5 mm, respectively). This strengthens the impression that JP_{rea} clinically runs a continuum from very light to serious joint disease. The proportions of patients with mild, moderate, and severe joint symptoms were comparable between groups, but patients with *Yersinia* developed more severe disease manifestations, reflected by higher median VAS scores, which is in agreement with previous observations[9,11]. When individuals positive and negative for HLA-B27 were compared for the entire population, there was a clear statistically significant trend that B27-positive subjects were overrepresented in the group with severe joint disease. This accounted for the subgroups defined by the mild–severe arthritis criteria as well as for the VAS estimates. These findings corroborate the assumption that patients who carry the HLA-B27 gene often get more advanced joint disease, which likely explains the observation of a high frequency of patients with this genotype presenting to rheumatology referral centers.

We performed a logistic regression analysis of the putative risk factors for JP_{rea} and found that HLA-B27 had the highest OR, followed by age 30 to 59 years, severe gastroenteritis, and female sex. In followup reports describing patients from hospital series there was a pronounced male preponderance[11,19,26,27]. It is therefore interesting that women accounted for an OR of 1.43 in our study. Obviously, it cannot be ruled out that men are more susceptible to severe arthritis when followed over the years.

At the 6-month followup fewer than half of the patients had persisting joint complaints (42.5%). Slightly more patients were B27-positive among the JP_{rea} subjects at 6 months than at onset of symptoms (17.6% vs 21.3%). This difference was not statistically significant, however.

We included *E. coli* in this study although no confirmed cases of postenteric joint complication have been established following infection with this microorganism. Yet we found 9.7% (the lowest incidence among all the bacterial species) with JP_{rea} in this group, mostly due to the A/EEC subtype. Interestingly, *E. coli* patients tended to have less severe GI symptoms (mild GI accounted for 64.1%). This fraction was much higher compared to patients with *Campylobacter* and *Salmonella*. Nevertheless, the *E. coli* patients with the most severe GI disease also had the highest incidence of joint symptoms (OR 3.56). *E. coli* JP_{rea} was not associated with HLA-B27. In the group that was clinically examined we found 3 *E. coli* patients classified as having "probable ReA." Thus, it is possible that *E. coli* may be an arthritogenic microorganism. That this has evaded detection may be because the search for pathogenic *E. coli* strains is not performed routinely at most microbiological laboratories and due to our findings of predominately mild diarrhea caused by this bacterium.

In this large population-based study we compared the occurrence of infection-triggered joint pain between patients with different enteric pathogens. We established close association between HLA-B27 and the development of JP_{rea} for *Salmonella*, *Shigella*, and probably *Yersinia*, but not for *Campylobacter* or *E. coli.* We showed that the incidence of JP_{rea} is dependent on the severity of diarrhea, where more pronounced GI symptoms increased the risk for joint complications. Additional risk factors were middle age and female sex. JP_{rea} is likely to occur after GI infection with *E. coli*, thus this microorganism should be included in the panel of arthritogenic bacteria.

ACKNOWLEDGMENT

Severin Olesen-Larsen, former head of the Department of Biostatistics, Statens Serum Institut, Copenhagen, is cordially thanked for help with the statistical evaluation.

REFERENCES

1. Granfors K, Marker-Hermann E, de Keyser F, Khan MA, Veys EM, Yu DT. The cutting edge of spondylarthropathy research in the millennium. Arthritis Rheum 2002;46:606-13.
2. Noer HR. An "experimental" epidemic of Reiter's syndrome. JAMA 1966;198:693-8.
3. Simon DG, Kaslow RA, Rosenbaum J, Kaye RL, Calin A. Reiter's syndrome following epidemic shigellosis. J Rheumatol 1981;8:969-73.
4. Mattila L, Leirisalo-Repo M, Pelkonen P, Koskimies S, Granfors K, Siitonen A. Reactive arthritis following an outbreak of Salmonella Bovismorbificans infection. J Infect 1998;36:289-95.
5. Locht H, Kihlstrom E, Lindstrom FD. Reactive arthritis after Salmonella among medical doctors — study of an outbreak. J Rheumatol 1993;20:845-8.
6. Locht H, Molbak K, Krogfelt KA. High frequency of reactive joint symptoms after an outbreak of Salmonella enteritidis. J Rheumatol 2002;29:767-71.
7. Vogt RL, Sours HE, Barrett T, Feldman RA, Dickinson RJ, Witherell L. Campylobacter enteritis associated with contaminated water. Ann Intern Med 1982;96:292-6.
8. Hannu T, Mattila L, Rautelin H, et al. Campylobacter-triggered reactive arthritis: a population-based study. Rheumatology Oxford 2002;41:312-8.
9. Tertti R, Granfors K, Lehtonen OP, et al. An outbreak of Yersinia pseudotuberculosis infection. J Infect Dis 1984;149:245-50.
10. Brewerton DA, Caffrey M, Nicholls A, Walters D, Oates JK, James DC. Reiter's disease and HL-A27. Lancet 1973;2:996-8.
11. Leirisalo M, Skylv G, Kousa M, et al. Followup study on patients with Reiter's disease and reactive arthritis, with special reference to HLA-B27. Arthritis Rheum 1982;25:249-59.
12. Olerup O. HLA-B27 typing by a group-specific PCR amplification. Tissue Antigens 1994;43:253-6.
13. Wheeler JG, Sethi D, Cowden JM, et al. Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. The Infectious Intestinal Disease Study Executive. BMJ 1999;318:1046-50.
14. Hannu T, Mattila L, Nuorti JP, et al. Reactive arthritis after an outbreak of Yersinia pseudotuberculosis serotype O:3 infection. Ann Rheum Dis 2003;62:866-9.
15. Lindholm H, Visakorpi R. Late complications after a Yersinia enterocolitica epidemic: a follow up study. Ann Rheum Dis 1991;50:694-6.
16. Locht H, Krogfelt KA. Comparison of rheumatological and gastrointestinal symptoms after infection with Campylobacter jejuni/coli and enterotoxigenic Escherichia coli. Ann Rheum Dis 2002;61:448-52.
17. Locht H, Skogh T, Kihlstrom E. Anti-lactoferrin antibodies and other types of anti-neutrophil cytoplasmic antibodies (ANCA) in reactive arthritis and ankylosing spondylitis. Clin Exp Immunol 1999;117:568-73.
18. Leirisalo-Repo M, Turunen U, Stenman S, Helenius P, Seppala K. High frequency of silent inflammatory bowel disease in spondylarthropathy. Arthritis Rheum 1994;37:23-31.
19. Leirisalo-Repo M, Helenius P, Hannu T, et al. Long-term prognosis of reactive salmonella arthritis. Ann Rheum Dis 1997;56:516-20.
20. Ekman P, Kirveskari J, Granfors K. Modification of disease outcome in Salmonella-infected patients by HLA-B27. Arthritis Rheum 2000;43:1527-34.
21. McColl GJ, Diviney MB, Holdsworth RF, et al. HLA-B27 expression and reactive arthritis susceptibility in two patient cohorts infected with Salmonella typhimurium. Aust NZ J Med 2000;30:28-32.
22. Mattila L, Leirisalo-Repo M, Koskimies S, Granfors K, Siitonen A. Reactive arthritis following an outbreak of Salmonella infection in Finland. Br J Rheumatol 1994;33:1136-41.
23. Toivanen P, Toivanen A. Two forms of reactive arthritis? Ann Rheum Dis 1999;58:737-41.

24. Peterson MC. Rheumatic manifestations of Campylobacter jejuni and C. fetus infections in adults. Scand J Rheumatol 1994;23:167-70.
25. Hannu T, Kauppi M, Tuomala M, Laaksonen I, Klemets P, Kuusi M. Reactive arthritis following an outbreak of Campylobacter jejuni infection. J Rheumatol 2004;31:528-30.
26. Fox R, Calin A, Gerber RC, Gibson D. The chronicity of symptoms and disability in Reiter's syndrome. An analysis of 131 consecutive patients. Ann Intern Med 1979;91:190-3.
27. Leirisalo-Repo M, Skylv G, Kousa M. Follow-up study of Reiter's disease and reactive arthritis. Factors influencing the natural course and the prognosis. Clin Rheumatol 1987;6 Suppl 2:73-82.