Work-related symptoms and *Salmonella* antibodies among wastewater treatment plant workers

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SUMMARY

Wastewater treatment plant workers are exposed to microbes, including *Salmonella*, but the prevalence of antibodies against S*almonella* species or serovars in their serum samples has not been studied. Antibodies against *Salmonella* Infantis and lipopolysaccharide antigen common to *S*. Enteritidis and *S*. Typhimurium in immunoglobulin classes IgA, IgM and IgG were measured from 79 serum samples of wastewater treatment plant workers and from 79 blood donor samples. Faecal samples for S*almonella* and C*ampylobacter* were studied. Gastrointestinal, dermal and other symptoms were compared between 81 wastewater treatment plant workers and 89 food-processing workers. The blood donors had more antibodies against all of the tested antigens expect for *S*. Infantis in IgM and IgA classes, even though the wastewater treatment plant workers had more gastrointestinal symptoms than the controls. No S*almonella* or C*ampylobacter* were found in any faecal samples. *Salmonella* is not a probable cause of symptoms among wastewater treatment plant workers.

INTRODUCTION

Wastewater treatment plant workers suffer from more gastrointestinal tract symptoms than controls [1]. An additional risk of respiratory symptoms has been reported in most but not all studies [2]. The cause of these symptoms may be related to the exposure to endotoxin from Gram-negative bacteria [1].

Workers in wastewater treatment plants are exposed to bioaerosols containing pathogenic microbes such as hepatitis A [3, 4] and hepatitis C [5], intestinal parasites [6], and also to bacterial structures such as endotoxins [7]. Many *Salmonella* serovars have been isolated in wastewater samples [8] and enteric micro-organisms have been found in aerosol samples in wastewater treatment plants [9]. However, although the workers are exposed to certain specific microbes, they do not seem to experience an increased risk of these diseases, as emphasized in a review of the risk for hepatitis A among sewage treatment workers [3].

Since we have found different *Salmonella* serovars in samples taken from Finnish wastewater treatment plants [8], we compared the content of antibodies against some *Salmonella* between wastewater treatment plant workers and controls. Respiratory, gastrointestinal and dermal symptoms were surveyed by a questionnaire.

SUBJECTS AND METHODS

Subjects

We asked 84 wastewater treatment plant workers from seven different treatment plants to participate in the

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study. The questionnaire was returned by 81 (96%) of them and blood samples for antibody testing were received from 79 (94%) subjects. Faecal samples were supplied twice in 2001 (May–June and November–December) by 81 (96%) of the wastewater treatment workers in both samplings. The target group for the questionnaire survey and faecal sampling was the same.

Employees in the food-processing industry were used as controls for the symptoms (questionnaire study). All the reference subjects worked in the clean areas of the plants and none were exposed to livestock or their excreta. They were recruited from six factories situated in the same geographic areas as the wastewater treatment plants. The questionnaire was returned by 90 (89%) and a faecal sample supplied by 91 (90%) in first sampling and 89 (88%) in second sampling of the control group.

The mean age of wastewater treatment plant workers was 46 years and the mean age of controls was 45 years. The smoking status was rather similar: the proportion of never smokers in both groups was 38%, 53% of the wastewater treatment plant workers were ex-smokers compared to 54% of the controls. The prevalence of current smoking was 9% and 8% respectively. The groups differed in their gender status; 90% of the wastewater treatment plant workers and 66% of the controls were male.

For the antibody analysis we used the serum samples of 79 blood donors as controls. No background information was available on the donors. All blood donations in Finland are voluntary and unpaid.

The questionnaire survey

The questionnaire contained 14 pages with the respondents identified by name. The main body of the questionnaire included questions on asthma, chronic bronchitis, respiratory infections, organic dust toxic syndrome, dermal symptoms and smoking and were taken from the Tuohilampi questionnaire, which is a Finnish questionnaire for population studies of respiratory and dermal hypersensitivity diseases [10]. Questions about work history, time spent in different tasks in the current job, previous diseases such as salmonellosis and hepatitis were added to the survey. In addition to stomach pain, nine other gastrointestinal symptoms described by Neal et al. [11] were inquired about for two different time periods (for the last month and for the last 12 months).

Analysis of *Salmonella* antibodies

Antibodies to *Salmonella* were measured by enzyme immunoassay (EIA) principally as described earlier [12]. Two *Salmonella* antigen preparates were used: (1) phenol/water-extracted lipopolysaccharides (LPS) of *S.* Typhimurium and *S.* Enteritidis (Sigma, St. Louis, MO, USA) together and (2) sodium dodecyl sulphate (SDS) extract (made in our laboratory) of whole *S.* Infantis bacteria. Every serum sample was studied for IgM, IgA and IgG antibodies to both of these antigens. In laboratory diagnostics for *Salmonella* infections, antibodies are usually determined by using combined LPS of two *Salmonella* (antigen 1). This antigen detects most effectively antibodies against these two *Salmonella* bacteria and against other *Salmonellae* belonging to groups B and D, because of the identical or closely similar O-polysaccharide chains among these *Salmonellae*. This antigen also detects antibodies developed against all other *Salmonellae* because of the lipid A part of LPS that is quite similar among all *Salmonellae* [12]. In Finland, $\sim 70\%$ of *Salmonellae* isolated from patient samples belong to groups B or D. However, as the frequency of antibodies to *S.* Infantis (belonging to group C) is high in the Finnish population [13] they were studied separately.

In EIA, polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated with *Salmonella* antigens. Diluted serum samples were incubated on the plates for 2 h at 37 °C. After washing the plates, alkaline phosphatase-conjugated rabbit anti-human-IgM, IgA or IgG (Dako, Glostrup, Denmark) were incubated on the plates overnight at room temperature. After the plates had been washed, p-nitrophenylphosphate in diethanolamine-magnesium chloride buffer (Oy Reagena Ltd, Kuopio, Finland) was added and incubated for 30 min at 37 °C; the reaction was stopped with 1 mmol sodium hydroxide. The optical density (OD) was measured with a VICTOR™ 1420 Multilabel counter (Wallac, Turku, Finland).

Faecal samples

Faecal sample tubes with an address envelope were given to subjects so that they could send the fresh sample directly to Kuopio University Hospital laboratory where *Salmonella* and *Campylobacter* were studied with routine cultivation methods.

Table 1. *The number of male and female wastewater treatment plant workers and controls who reported weekly gastrointestinal symptoms during the previous 12 months and during the previous month. Statistical significance was tested by Fisher's exact test for males and females separately*

Symptom	Males		Females	
	Sewage workers ($n=73$)	Controls ($n=59$)	Sewage workers ($n=8$)	Controls ($n=30$)
Habitual stomach pain				
During the last 12 months	7	0**	2	1
During the last month	8	1**	2	1
Loose or watery stools				
During the last 12 months	18	7**	0	3
During the last month	11	5	0	3
Varying bowel habits				
During the last 12 months	14	12	3	7
During the last month	11	3*	1	5
Need to strain to open bowels				
During the last 12 months	4	3	0	6
During the last month	4	1	0	5
Need to rush to toilet				
During the last 12 months	14	6	2	4
During the last month	9	3	1	5
Need to go back to toilet				
During the last 12 months	5	1	1	2
During the last month	4	1	0	3
Passed slime or mucus				
During the last 12 months	0	0	0	1
During the last month	0	0	0	1
Swollen abdomen after a meal				
During the last 12 months	9	4	1	9
During the last month	5	3	1	8
Need to loosen clothing after a meal				
During the last 12 months	6	2	0	4
During the last month	4	2	0	3

* $P<0.10$, ** $P<0.05$.

Statistical methods

The reported frequency of symptoms was compared between the two groups. Since the groups differed in their gender status, males and females were analysed separately. Smoking and age were not controlled in the analysis since the groups did not differ in these two respects. The differences in the prevalence of symptoms were compared by Fisher's test.

The antibody contents of the two groups were compared by a method we have described earlier [14]. Briefly, both the exposed workers and their controls are sorted according to the absolute value of the serological test from the highest value to the lowest value. In this ordered series, the risk ratio and its 95% confidence interval (CI) are calculated and plotted for each subject. By this method the difference in antibody content between the exposed and control workers regardless of the pre-set cut-off point between 'high' and 'low' antibody content can be observed. In clinical settings the difference between 'high' and 'low' values is set by its clinical significance, i.e. by the ability of the test to find those with the particular clinical condition. In epidemiology, we are more interested about the overall difference over the whole distribution. By rolling the cut-off point over the

Table 2. *The number of male and female wastewater treatment plant workers and controls who reported ever having had work-related symptoms. Statistical significance was tested by Fisher's exact test for males and females separately*

Symptom	Males		Females	
	Sewage workers ($n=73$)	Controls ($n=59$)	Sewage workers ($n=8$)	Controls ($n=30$)
Dry cough	12	7	2	3
Dyspnoea	3	0	1	1
Dyspnoea in strain	10	4	0	0
Passing mucus while coughing	11	7	2	2
Wheezing	3	2	1	3
Rhinitis	14	8	1	8
Sore throat	12	6	1	6
Red eyes	8	11	3	7
Itching or redness of the skin	20	4***	0	5
Stomach pain	13	5*	1	2
Nausea	8	1**	0	2
Vomiting	3	1	1	0
Headache	24	22	0	12**
Muscle pains	24	21	2	13
Joint pains	19	13	2	14
Fever and chills	6	6	1	4
Dizziness	2	3	0	1
Tiredness	18	17	1	13

* $P<0.10$, ** $P<0.05$, *** $P<0.01$.

whole ordered series possible differences at any level of antibody content can be found. From this ordered series the points with the highest significant difference can also be found. This difference is at the point where the risk ratio is highest and the lower 95% CI exceeds 1.

RESULTS

Five controls but only one wastewater treatment plant worker reported asthma diagnosed by a physician. The prevalence of allergic rhinitis was also more common among the reference subjects than among the exposed workers, 19 and 13 cases respectively. Four wastewater treatment plant workers and one control subject met the criteria of suffering from chronic bronchitis. The food-processing plant workers reported more previous *Salmonella* infections (eight cases, 9%) than the wastewater treatment plant employees (three cases, 4%), but the difference failed to reach statistical significance.

The reported gastrointestinal symptoms are described in Table 1. It can be seen that the male wastewater treatment plant workers more frequently had habitual stomach pain, loose or watery stools and varying bowel habits than the controls. Similar differences were also found for females, but the number of females was too small for the differences to become statistically significant. Three subjects out of the 15 wastewater treatment plant workers who had worked for < 14 years in the treatment plants had experienced weekly stomach pain during the last 12 months. For those who had worked for ≥ 14 years, the proportion was only half of that. However, the difference was not statistically significant. Similarly loose or watery stools were reported more often by those who had worked for < 14 years (27% for the last year and 20% for the last month) compared to more experienced workers (19 and 13% respectively).

Table 2 describes the differences in the prevalence of work-related symptoms between the groups. It can be seen that the male wastewater treatment plant workers more frequently reported dermal symptoms, nausea and stomach pain than the controls. None of the female wastewater treatment plant workers
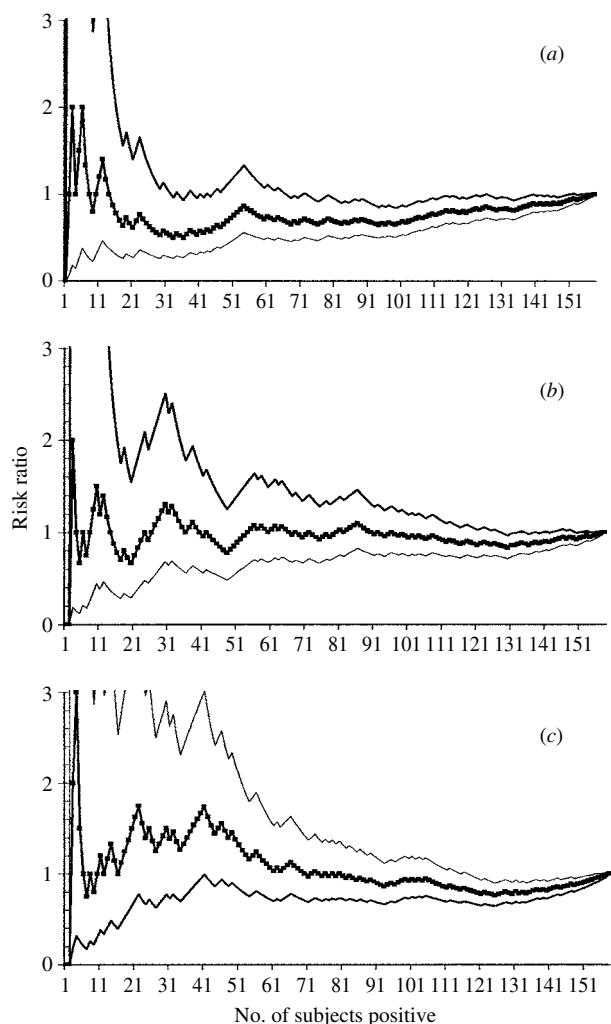
Fig. 1. The risk ratio plots comparing the *Salmonella* Infantis antibody contents in (*a*) IgA, (*b*) IgM and (*c*) IgG classes between 79 wastewater treatment plant workers and 79 blood donors. The number of subjects considered positive are on the *x*-axis and the corresponding risk ratios and their 95% confidence intervals (upper and lower curves respectively) are on the *y*-axis.

Fig. 2. The risk ratio plots comparing *Salmonella* Enteritidis and *S.* Typhimurium antibody contents in (*a*) IgA, (*b*) IgM and (*c*) IgG classes between 79 wastewater treatment plant workers and 79 blood donors. The number of subjects considered positive are on the *x*-axis and the corresponding risk ratios and their 95% confidence intervals (upper and lower curves respectively) are on the *y*-axis.

reported headache compared to 12 out of 30 control subjects.

All faecal samples given in two seasons by wastewater treatment and food industry workers were negative for *Salmonella* and *Campylobacter*.

The comparison of the quantity of *Salmonella* antibodies is given in Figures 1 and 2. For IgM and IgG classes of *S.* Infantis antibodies, there were no statistically significant differences between the groups. The amount of *S.* Infantis IgA antibodies was smaller among the wastewater treatment plant workers. When the cut-off point was at the 33rd highest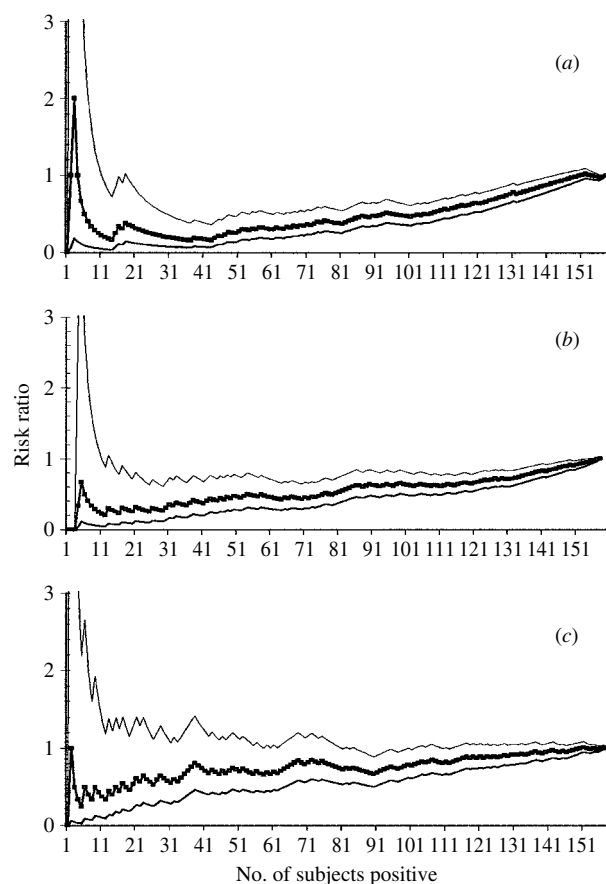 value, the risk was 0·5 (95% CI 0·26–0·96). For the common LPS antigen of *S.* Enteritidis and *S.* Typhimurium, the wastewater treatment plant workers had fewer antibodies in all three immunoglobulin classes than their controls with the differences being the greatest for the IgA and IgM classes.

DISCUSSION

Gastrointestinal symptoms were more common among wastewater treatment plant workers than among controls, which is in agreement with the literature survey performed by Thorn & Kerekes [1]. The differences found in this study were related to habitual stomach pain, loose or watery stools and varying bowel habits. The male wastewater treatment plant employees more frequently reported nausea than controls. Nausea is a common symptom related to gastrointestinal tract infections.

Dermal symptoms were also more common among wastewater treatment plant workers as reported earlier. However, this was only found for the male subjects. Among the females the dermal symptoms were more common among the controls working in food-processing plants. This is probably due to the fact that in both industries the tasks of male and female workers differ with respect to dermal exposure and irritation of hands.

Since the wastewater treatment plant workers are exposed to bioaerosols containing *Salmonella* serovars, we hypothesized that they would have more *Salmonella* infections than employees in the food-processing industry and have higher levels of *Salmonella* antibodies than the blood donors, but we found the opposite.

In the reporting of previous *Salmonella* infections there may be detection and reporting bias, since in the food-processing industry *Salmonella* infections are controlled and special measures are taken for their detection, i.e. there may be a higher detection rate. However, this higher detection rate does not explain the results of the antibody analysis.

Blood donation in Finland is voluntary and the non-paid donors are generally in good health. There is no reason to suspect that people with previous salmonellosis would be more likely to become blood donors. Thus, we can presume that the blood donors studied here represent the general Finnish population experience with respect to contact to *Salmonella*.

Thus, the wastewater worker group exposed to bioaerosols also containing *Salmonella* serovars had fewer antibodies against *Salmonella* than the blood donor group. The wastewater treatment plant workers are exposed to *Salmonella* mainly via the respiratory route. This exposure may evoke a different kind of antibody formation than that occurring via the intestinal route. The antibodies we measured are those found after salmonellosis, which is normally contracted orally. Respiratory priming by *Salmonella* may protect the intestinal tract, since microbial mucosal priming also defends other distant mucosal organs [15]. If this were the case, our findings of fewer cases of salmonellosis among wastewater treatment plant workers would not be a biased result.

Our study shows that wastewater treatment plant workers experience more gastrointestinal symptoms and male wastewater treatment plant workers more dermal symptoms than controls working in the food-processing industry, but they do not have more *Salmonella* antibodies than blood donors even though they are exposed to bioaerosols containing *Salmonella*. It is likely that the symptoms are not caused by *Salmonella*, but by some other microbial agents not measured in our study.

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