

## Sources of sporadic *Yersinia enterocolitica* infections in Norway: a prospective case-control study

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### SUMMARY

*Yersinia enterocolitica* is a recognized cause of gastroenteritis in northern Europe. During October 1988–January 1990, a prospective case-control study was performed to address risk factors associated with sporadic *Y. enterocolitica* infections in southeastern Norway. Sixty-seven case-patients (mean age 23·4 years, range 8 months–88 years) and 132 age-, sex- and geographically-matched controls were enrolled in the study. Multivariate analysis of the data showed that persons with *Y. enterocolitica* infection reported having eaten significantly more pork items (3·79 *v.* 2·30 meals,  $P = 0\cdot02$ ) and sausage (2·84 *v.* 2·20 meals,  $P = 0\cdot03$ ) in the 2 weeks before illness onset than their matched controls; only one patient had eaten raw pork. Patients were also more likely than controls to report a preference for eating meat prepared raw or rare (47 *v.* 27%,  $P = 0\cdot01$ ), and to report drinking untreated water (39 *v.* 25%,  $P = 0\cdot01$ ) in the 2 weeks before illness onset. Each of these factors was independently associated with disease, suggesting a link between yersiniosis and consumption of undercooked pork and sausage products and untreated water. Efforts should be directed towards developing techniques to reduce *Y. enterocolitica* contamination of pork and educating consumers about (1) proper handling and preparation of pork items and (2) the hazards of drinking untreated water.

### INTRODUCTION

*Yersinia enterocolitica* infections are an important cause of gastroenteritis in the developed world, particularly in northern Europe [1, 2]. *Y. enterocolitica* is a foodborne pathogen [1–4] which has caused foodborne outbreaks of disease [5–11]. In the United States, chocolate milk [5], pasteurized milk [6], soybean curd (tofu) [8], and bean sprouts [9] have been implicated as sources for outbreaks of *Y. enterocolitica* infection. These outbreaks, all of which occurred before 1983, were caused by *Y. enterocolitica* serotypes which have been infrequently associated with human disease (serotype O 13, O 18) or which no longer predominate in the United States (serotype O 8). More recently, preparation of raw pork intestines

(chitterlings) was associated with an outbreak of *Y. enterocolitica* O 3 infections among black US infants [11]; the organism was isolated from samples of the pork intestines.

*Y. enterocolitica* O 3 is the serotype most commonly associated with illness in northern Europe [12–14], and is being reported more frequently in the United States [15, 16]. Although yersiniosis appears to be more common in Europe than in the United States, only one foodborne outbreak has been reported in Europe [7]. In addition, few epidemiologic studies have been performed in either Europe or North America to investigate the sources of sporadic human infections. A 1985 study of *Y. enterocolitica* in Belgium identified consumption of raw pork as a risk factor for disease [17]. In a follow-up study to the chitterling-related outbreak mentioned above, sporadic *Y. enterocolitica* O 3 infections among US blacks were also associated with this product [18].

During the 1980s, Norwegian national surveillance data demonstrated a rising incidence of yersiniosis. Between 1986 and 1987, the number of reported cases doubled, and virtually all were due to *Y. enterocolitica* serotype O 3 [19]. The increase was seen in both children and adults and was not explained by changes in surveillance methods, culture techniques, or the occurrence of an outbreak. Raw pork is infrequently consumed in Norway, implying that risk factors for infection in Norway are different from those in Belgium. Therefore, in 1988 a prospective case-control study of sporadic yersiniosis was begun to identify sources for infection and guide control and prevention efforts.

#### METHODS

The case-control study was performed between October 1988 and January 1990 in Oslo city and the surrounding counties of Akershus, Buskerud, Østfold, and Vestfold, an area with a population of 1 533 000 persons (1990 census). Enrollees were residents of the study area from whom *Y. enterocolitica* had been isolated from a clinical specimen submitted to one of the county reference microbiology laboratories during the study period. If an outbreak or more than one case of yersiniosis in a household occurred, only the first identified case was eligible for enrollment. Case-patients were entered in the study after both the treating physician and patient consented to their participation.

Two age-, sex-, and geographically-matched controls were obtained for each enrolled case-patient. Potential controls were selected using the Norsk Folke-register, a population-based registry of all Norway residents that is updated quarterly. Ten sex-matched persons closest in age to the patient who lived in the patient's same or adjacent postal code area were identified in the registry, contacted by mail, and sequentially telephoned until two agreed to act as controls. Exclusion criteria for controls were (1) a history of yersiniosis, or (2) diarrhoea or abdominal pain with fever in the preceding month.

Face-to-face interviews were conducted with all patients and controls (or their parents for persons under age 15 years) by trained interviewers from the National Institute of Public Health using a standardized questionnaire. Patients were queried about the 2-week period before the onset of illness; if the date of onset could not be specified, they were asked about the 2 weeks before the first specimen

yielding *Y. enterocolitica* was collected. To minimize recall problems, controls were asked about the 2-week period before the interview date. Enrollees were asked about travel activities, water and food sources, and foods eaten during the period of interest. The food history concentrated on dairy, meat, fish, and vegetable products. Enrollees were also questioned about cooking preferences, food preparation, and food handling practices. Because many questions concerned foods common to the Norwegian diet, enrollees were asked to estimate how frequently they had eaten each item during the 2-week period.

#### *Laboratory methods*

During the study period, all specimens submitted to the county reference laboratories were routinely cultured for *Yersinia* spp. within 2 days of collection. Culture methods varied by laboratory, but each used cefsulodin-irgansan-novobiocin (CIN) agar and most employed desoxycholate-citrate agar and enrichment in selenite broth at 22 °C. Verification, biochemical characterization, and serogrouping of strains identified as *Y. enterocolitica* were performed at the National Institute of Public Health in Oslo. Strains were identified by standard criteria [20], biotyped by the methods of Wauters and colleagues [21], and serogrouped by slide agglutination against absorbed rabbit antisera for *Y. enterocolitica* O-antigen factors 1–34 [22].

#### *Statistical analysis*

Univariate analysis of dichotomous risk factor variables was performed using procedures for matched datasets in the computer program EpiInfo (Centers for Disease Control and Prevention, Atlanta, GA). For continuous variables, conditional logistic regression analysis was performed using SAS (SAS Institute Inc., Cary, NC). Conditional logistic regression analysis was also used to evaluate which risk factors were independently associated with illness as well as to evaluate interactions among these variables. All reported odds ratios (OR) are for matched datasets and all *p*-values are 2-tailed.

The analysis of food information was first performed by comparing the frequency of consumption by patients and controls of each of the items listed on the questionnaire. The individual food items were then broken down into their constituent component(s), and items with the same constituent were aggregated into broader categories. For example, ham steak and homemade pork cakes were first analysed individually; both were then combined, along with other appropriate items, into an aggregate variable termed 'pork'. Variables were also created for beef, poultry, sausage products, cold cuts, spekemat (dried, cured meat products), and fish by grouping the appropriate food items. Each aggregate food group was mutually exclusive from the others, and each represents the sum of the number of meals eaten by the patient or control.

## RESULTS

Sixty-seven persons with *Y. enterocolitica* infection, representing 61 % of eligible patients, were enrolled in the case-control study. Non-enrolled patients were similar to enrollees with respect to age, sex, and county of residence. Two age-, sex-, and

Table 1. *Univariate analysis of selected dichotomous risk factors for sporadic Y. enterocolitica infection, southeastern Norway, 1988–90*

Risk factor	Cases* Number (%)	Controls* Number (%)	Matched odds ratio	95 % confidence interval	P
Foreign travel	4 (6.0)	3 (2.3)	2.67	0.60–11.92	N.S.
Travel in Norway	26 (39.4)	49 (37.1)	1.18	0.63–2.21	N.S.
Ate at					
Restaurant	39 (59.1)	72 (55.0)	1.34	0.68–2.65	N.S.
Street kitchen	15 (22.7)	29 (22.0)	1.05	0.48–2.28	N.S.
Sausage stand	14 (21.9)	17 (12.9)	1.79	0.82–3.87	N.S.
Bought meat at					
Butcher	13 (20.6)	24 (19.0)	1.05	0.50–2.22	N.S.
Market	58 (90.6)	116 (92.1)	0.73	0.24–2.17	N.S.
Ate meat purchased abroad	22 (36.1)	50 (39.7)	0.83	0.44–1.56	N.S.
Prefers meat raw/rare	28 (46.7)	35 (26.7)	3.58	1.52–8.44	< 0.005
Ate raw minced meat	7 (10.6)	2 (1.5)	7.59	1.44–55.95	< 0.01
Cleaned counter with soap and water	10 (16.4)	39 (33.1)	0.41	0.18–0.90	< 0.05
Drank untreated water	24 (38.7)	31 (25.4)	2.76	1.19–6.43	< 0.05

\* Number of cases = 67 and number of controls = 132. However, within each risk category the denominators vary because of 'unknown' responses.

geographically matched controls were enrolled for each of the case-patients except for an 88-year-old man with *Y. enterocolitica* O 3 bacteraemia for whom no acceptable controls could be identified. Only 1 of the 133 persons contacted as potential controls refused to participate in the study, and none was excluded because of recent illness or a history of yersiniosis.

Except for the 88-year-old man with bacteraemia and a 56-year-old woman with enteric *Y. enterocolitica* O 9 infection, all the enrolled patients had *Y. enterocolitica* O 3 isolated from stool. The mean age of the patients was 23.4 years (range, 8 months–88 years), and 34% were under 5 years of age. There were 34 (51%) females and 33 (48%) males. Clinical and demographic features of *Y. enterocolitica* illness among the 67 case-patients have been described elsewhere [19]. All but five case-patients provided an illness onset date which defined the 2-week period used for questioning; the median interval between this date (or the specimen submission date for those not reporting an onset date) and the interview was 32 days.

All the enrollees had been in Norway during all or most of the interview period (Table 1). The few enrollees who had left the country went to either Sweden or Denmark. A similar proportion of patients (39%) and controls (37%) had made an overnight trip within Norway. Thus, neither domestic nor international travel was a risk factor for yersiniosis. Illness was also not associated with visiting a farm, contact with farm animals (including pigs), frequency and duration of contact with cats or dogs, soil consumption, or visiting a kindergarten.

Patients were no more likely than controls to report having eaten at a

Table 2. *Univariate continuous analysis of food consumption histories of case-patients and matched controls for individual food items, southeastern Norway, 1988–90*

Food item	Mean no. of meals		Univariate matched <i>P</i> value
	Cases*	Controls*	
Homemade beef cakes	1.11	0.50	< 0.01
Homemade pork cakes	0.52	0.28	< 0.05
Pork spare ribs	0.43	0.16	< 0.05
Pork items, not otherwise listed	0.68	0.37	< 0.05
Yogurt	3.27	1.88	< 0.05
Scooped ice cream	0.76	1.28	< 0.05

\* Does not include persons who gave an 'unknown' response for a listed item.

Table 3. *Univariate continuous analysis of food consumption histories of case-patients and matched controls for aggregated food groups, southeastern Norway, 1988–90*

Food category	Mean no. of meals		Univariate matched <i>P</i> value
	Cases*	Controls*	
Fish items	4.58	3.83	N.S.
Coldcuts	4.78	3.52	0.05
Poultry items	1.05	0.72	< 0.05
Sausage	2.84	2.20	< 0.05
Spekemat	5.42	3.72	< 0.05
Beef items†	4.62	3.50	< 0.01
Pork items†	3.79	2.30	< 0.001

\* Does not include persons who gave an 'unknown' response for any of the items included in the food group.

† Beef and pork variables exclude sausage and coldcut items, which are analysed as separate categories.

restaurant, street kitchen, or sausage stand. Among those who ate outside the home, the number of times eating out was similar for cases and controls.

Enrollees were questioned about the frequency of consumption of 62 individual food items. Homemade (as opposed to packaged or store bought) beef cakes, homemade pork cakes, pork spare ribs, pork items which were not specifically listed on the questionnaire (categorized as 'other pork'), and yogurt were all significantly associated with illness on matched univariate analysis (Table 2). Scooped (as opposed to packaged) ice cream was eaten significantly less often by patients than controls.

Matched univariate analysis of each of the aggregate food groups revealed that patients reported eating significantly more of each of the following: poultry, sausage products, spekemat, beef, and pork (Table 3). Sources for meat (butcher shop versus food market) and the frequency of eating meat procured outside of Norway was similar for patients and controls (Table 1).

Only one patient and one control reported eating raw pork, although 11% of patients and 2% of controls reported eating raw minced meat (OR 7.59),  $P < 0.01$ . Forty-seven percent of patients but only 27% of controls preferred their meat to be either raw or rare (OR 3.58,  $P < 0.005$ ). When questioned about kitchen hygiene practices, patients were less likely than controls to report that

their countertops were cleaned with soap and water (as opposed to only water or not cleaning) between preparing food items (16 versus 33%, OR 0.41,  $P < 0.05$ ).

Study enrollees reported drinking water from multiple sources during the 2-week period of interest. There was no difference between patients and controls in the type of water (treated versus untreated) used in the primary residence. However, patients were more likely than controls to report drinking untreated water during the 2-week period (39 versus 25%, OR 2.76,  $P < 0.05$ ).

Conditional logistic regression analysis was performed to determine which of the above factors were independently associated with disease. Variables included in the multivariate analysis included those found to be statistically associated with disease and those which represented potential confounding factors. No significant interactions were found among any of the risk factors included in the multivariate analysis. When simultaneously evaluated in the final model, the only items found to be independently and significantly ( $P < 0.05$ ) associated with *Y. enterocolitica* infection were the pork aggregate variable ( $P = 0.02$ ), sausage ( $P = 0.03$ ), drinking untreated water ( $P = 0.01$ ), and a preference for eating undercooked meats ( $P = 0.01$ ).

#### DISCUSSION

This study strengthens the evidence that sporadic yersiniosis in northern Europe results from consumption of domestic pork products that harbour pathogenic *Y. enterocolitica* [1–4, 17]. In Norway, persons with yersiniosis reported eating a number of food items more often in the 2 weeks before they became ill than did their matched controls. Some of these food items contained pork, others did not. However, on multivariate analysis, the only food categories that remained independently associated with illness contained pork.

This reinforces the findings of the Belgian study conducted in 1985 [17]. However, in contrast to the findings of that study, consumption of raw pork may play only a limited role in the development of yersiniosis in Norway, since this was reported by only one patient. Transmission could possibly have occurred via cooked pork and sausage products, because of the preference of patients for eating their meat undercooked or because improper handling of these items in the kitchen led to reintroduction of the organism into pork items after cooking. The Norwegian study also extends the association between pork and yersiniosis to older children and adults. In Belgium, 68% of patients were under 5 years of age, while in Norway 66% were over 5 years of age.

In the multivariate analysis, none of the items which made up the aggregate pork variable remained statistically associated with *Y. enterocolitica* infection. This suggests that the risk associated with pork resulted from frequent consumption of a broad variety of pork-containing foods, and that any pork product should be considered as potentially harbouring the organism. Norwegian consumers should therefore assure that all pork products are appropriately cooked. Because patients were more likely to report kitchen hygiene practices that might facilitate cross-contamination of *Y. enterocolitica* to other food items, increased attention to the handling of raw meat items, including the use of soap and water to clean food-preparation surfaces after preparing pork, is also a useful prevention measure.

*Y. enterocolitica* appears to be a domestically acquired infection in Norway, since only 4% of case-patients had travelled outside the country. In contrast, during the study period, 69% of salmonella infections, 85% of shigella infections, and 37% of campylobacter infections in Norway were reported to be associated with foreign travel [23]. Patients were no more likely than controls to have eaten meat obtained from outside the country, in contrast to a recent study of sporadic campylobacter infections in Norway, which demonstrated an association with poultry from foreign sources [24].

The epidemiologic findings of the present study complement veterinary studies documenting ubiquitous carriage of *Y. enterocolitica* O 3 in Norwegian pigs. In three separate surveys, *Y. enterocolitica* O 3 was isolated from the oral cavities of between 32 and 83% of pigs at the time of slaughter [25–27]. Significant intestinal carriage of the organism has also been found [4, 11, 28, 29], as has transfer of faecal organisms to the surface and cut sections of pig carcasses [27, 30]. The organism appears to be easily transferred to the surface and cut sections of the carcass during the slaughtering process; contamination may occur during evisceration of intestines, excision of the tongue and tonsils, incision of mandibular lymph nodes during inspection, and deboning of head meat [4]. Transfer of the organism during removal of the rectum appears to be especially critical [4, 30]. Improved hygiene and modified slaughtering procedures may be required to avoid contamination during these activities.

*Y. enterocolitica* O 3 has also been isolated from retail pork products in Norway. In a study conducted in 1983, the organism was found in only 1 (0.8%) of 127 retail pork samples [31]. However, a second survey in 1988–9 which used improved culture methods demonstrated *Y. enterocolitica* O 3 in 18% of pork items taken from meat-processing plants; colony hybridization using a DNA probe found that over 60% of these samples were contaminated with pathogenic strains of *Y. enterocolitica* [32]. These findings show that use of conventional isolation procedures for the organism may lead to considerable underestimation of *Y. enterocolitica* in port products. Additionally, since *Y. enterocolitica* can readily proliferate at refrigerator temperatures [4, 33, 34], the level of contamination in the home may be even higher than at the meat processing or retail level if products are stored in the refrigerator for any length of time. Studies to develop methods to reduce yersinia contamination at the farm, slaughterhouse, and meat processing stages are needed.

Drinking untreated water, reported by 39% of patients, was independently associated with yersiniosis. This practice may be related to the Norwegian custom of visiting a rural hut or cabin on weekends and holidays. During these visits, water is often taken directly from a stream or lake, and the huts are frequently served by shallow, untreated wells which are susceptible to contamination with surface runoff from rain or snow melt. Such runoff may become faecally contaminated by wild or domesticated animals, leakage from septic tanks, or open latrines in the surrounding areas. In 1981, an outbreak of *Y. enterocolitica* O 8 in Washington state occurred in association with consumption of tofu packed in untreated spring water [8]. The outbreak serotype was isolated from the spring water samples. Two other yersinia outbreaks have been associated with well water. One occurred among members of a Pennsylvania girl scout troop after they ate bean sprouts grown in contaminated well water [9]; the other was a familial

outbreak of yersiniosis in Canada [35]. The association between yersiniosis and untreated water suggests that disease incidence may be reduced by systematic monitoring of well water for contamination, or the use of bottled, boiled, or chemically disinfected drinking water when visiting rural cabins.

Although the relationships between sporadic yersiniosis in Norway and eating pork, undercooking meat, and drinking untreated water are plausible, caution is needed in interpreting these findings. In general, patients reported eating more of a number of food items. This may result from differences in recall or in responses to questions between patients and controls. The patients may have had knowledge or received information on the general foodborne nature of the infection from their physician. Future studies in Norway and other high-incidence areas may be helpful to validate our findings and would also serve to monitor the impact of potential interventions on the occurrence of disease.

The consequences of yersiniosis are severe and include prolonged acute infections, pseudoappendicitis, and long-term sequelae such as reactive arthritis and erythema nodosum, particularly in northern Europe where the prevalence of the HLA-B27 histocompatibility type is high [1, 2, 36]. These consequences make *Y. enterocolitica* infection a financial and public health problem of greater magnitude than the actual number of cases would suggest [19]. Our findings suggest that changes in slaughterhouse practices as well as modification of consumer food preparation and water use patterns offer the potential for substantial reductions in the burden of yersiniosis in Norway.

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