Yersinia enterocolitica O:9 infections associated with bagged salad mix in Norway, February to April 2011

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In March 2011, the Norwegian Institute of Public Health identified a possible outbreak involving 21 cases of *Yersinia enterocolitica* O:9 infection with similar MLVA-profiles. Preliminary results of epidemiological and microbiological investigations indicate bagged salad mix containing radicchio rosso (also known as Italian chicory) as a possible source. As a result of the investigation, bagged salad mixes of a specific brand were voluntarily withdrawn from the market by the producer.

Introduction

In March 2011, the Department of Infectious Disease Epidemiology at the Norwegian Institute of Public Health (NIPH) was informed by the National Reference Laboratory (NRL) for enteropathogenic bacteria of an unusually high number of *Yersinia enterocolitica* serotype O:9 isolates from geographically disparate areas in Norway. After being notified of five cases of *Y. enterocolitica* O:9, which is rare in Norway, a multidisciplinary investigation team was established on 18 March 2011 to find the source and prevent further illness.

Yersiniosis is a mandatorily notifiable disease and the fourth most commonly reported cause of bacterial diarrhoeal disease in Norway [1]. In the past 10 years, between 80 and 150 cases of yersiniosis were reported annually. More than 98% of yersiniosis cases in Norway are due to serotype O:3, which is also the dominant cause of yersiniosis in Europe, Japan, Canada and parts of the United States [2].

Descriptive epidemiology

A confirmed case was defined as an individual with laboratory-confirmed *Y. enterocolitica* O:9 infection with the outbreak MLVA-profile identified between 1 January and 5 May 2011. By 5 May, the reference laboratory had registered 21 cases with the outbreak strain of *Y. enterocolitica*. Of the 21 confirmed cases, 15 were female and six were male. The age range of patients was from 10 to 63 years with a median age range of 30–39 years (Figure 1).

Cases occurred in geographically disparate areas of the country, across ten different municipalities (Figure 2).

For four patients, the date of symptom onset was unavailable and the date of positive microbiological sample was used for the epidemic curve (Figure 3). Between week 6 (7–13 February) and week 11 (14–20 March), 17 patients with positive microbiological samples became ill.

Epidemiological investigation

When there are outbreaks in Norway where the cases are geographically widespread, the NIPH is responsible for coordinating the outbreak investigation. As is often done in foodborne outbreaks in Norway, after being notified of a microbiologically confirmed outbreak case, the NIPH contacted the respective municipal doctor and asked them to contact the patient in order to get consent for the district Food Safety Authority office to visit the home, collect food samples and conduct an interview. The first seven cases were interviewed using a trawling questionnaire, designed to collect information on food consumption in the seven days prior to onset of symptoms, animal contact and environmental exposures, as well as clinical and demographic

FIGURE 1

Age and sex distribution of cases of *Yersinia* O:9 infection, Norway, February–April 2011 (n=21)

information. Following these interviews, the questionnaire was shortened to focus on categories of foods of most interest, and used to conduct a case-control study. In particular, from the trawling interviews, bagged salad mix was suspected as the source of infection. The case-control study was conducted in week 13 (28 March–1 April 2011). At that time, nine patients had been interviewed using the shortened questionnaire. In order to ensure enough statistical power in the case-control study given the small number of cases, three controls for each case were selected from the national population register. Controls and cases were matched by age, sex and municipality of residence. Potential controls were excluded if they reported having had diarrhoea during the last 14 days.

The results from the trawling interviews revealed that limited number of cases had consumed pork products. Salad mix and arugula were consumed by a notable number of cases, with at least four specifically stating they had consumed a specific brand of salad mix containing arugula. Preliminary results of the case-control study corroborate the hypothesis of bagged salad mix as the suspected source. Among the nine cases, six had eaten bagged salad mix in the week prior to onset of illness compared with three of 25 controls (matched odds ratio (mOR):13.7; 95% confidence interval (CI): 1.6–116.3). We included eight significant food items in a conditional multivariate logistic regression model. A forward selection procedure was used by starting with the most significant item and including the other items one by one. The only food item which remained significant in the model was the bagged salad mix.

FIGURE 2
Geographical distribution of cases of *Yersinia* O:9 infection, Norway, February–April 2011 (n=21)

International notifications

On 26 April 2011 the NIPH sent a message via the European Centre for Disease Prevention and Control (ECDC) Epidemic Intelligence Information System asking whether other countries had also experienced an increase in cases of *Y. enterocolitica*. The Norwegian Food Safety Authority sent a notification through the Rapid Alert System for Food and Feed (RASFF) on 15 April 2011. International requests for information produced no reports of similar yersiniosis outbreaks in European countries. However, it is possible that few countries routinely perform serotyping of *Y. enterocolitica*.

Microbiological investigation

At the NIPH-located NRL all isolates of *Y. enterocolitica* from human patients are routinely characterised phenotypically, bityped and serogrouped against O:3 and O:9 as well as a range of other serogroups. The *Y. enterocolitica* isolates were MLVA-typed by the method described by Gierczyński et al. [3], locally adjusted to capillary electrophoresis.

Food samples were sent to the Norwegian Veterinary Institute for analysis. A total of 61 samples consisting of two chicken meat products, two pork products and 57 diverse salad products and bagged salad mix products were collected from patient homes, retail and the company producing the bagged salad mix products. All products were analysed according to NMKL 117B, an adaptation of ISO 10273. Additionally, samples were cold enriched for 21 days according to NMKL 117. All enriched broths and colonies isolated were further examined for the *ail* gene, an indicator for pathogenic *Y. enterocolitica*, using PCR (NMKL 163, Part A (1998)). PCR positive colonies were characterised by biochemical reactions and their serogroup was determined.

Diverse *Yersinia* spp. including *enterocolitica* were isolated from 11 of the salad products of which two were consistently positive by PCR. These strains were

FIGURE 3
Cases of *Yersinia* O:9 infection by week of symptom onset, Norway, 7 February–20 March 2011 (n=21^a)

^aIncluding the four cases for which information on date of symptom onset was not available.

isolated from one particular salad type, radicchio rosso, imported from Italy, and mixed salad products, which also contain radicchio rosso. However, these isolates were not serogroup O:9.

Discussion and conclusion

The geographically widespread occurrence of the yersiniosis cases and the illness onset dates indicate that the suspected source of infection is likely a product that was widely distributed but available only for a relatively short period of time. In addition, the number of female cases compared to male cases indicated that the source was a food product more commonly consumed by women. Radicchio rosso is the only variety of salad included in the suspected bagged salad mixes that keeps long enough to fit with the duration of this outbreak. Radicchio rosso is stored at -1°C before it is supplied to the market. The storage conditions may increase growth of *Y. enterocolitica* as this bacterium is able to grow down to -2°C .

Yersiniosis outbreaks are often associated with consumption of pork, as the pig is the only animal consumed by humans which regularly harbours the pathogenic serovars O:3 and O:9 [2]. Although most cases of yersiniosis in Norway are sporadic, there have been several previous outbreaks, including an outbreak of *Y. enterocolitica* O:9 in 2005–2006 due to a Norwegian ready-to-eat pork product ('sylte') [4]. Published literature on yersiniosis outbreaks linked to salad and/or fresh vegetables is limited. Although previous outbreaks of *Salmonella*, *Shigella* and *Escherichia coli* in Norway have been linked to the consumption of fresh vegetables [5–8], this is the first outbreak of yersiniosis in Norway to be linked to consumption of vegetables.

As of 5 May 2011, no new outbreak cases have been reported. The supplier voluntarily withdrew suspected bagged salad mixes containing radicchio rosso from the market based on the information collected through the interviews, case-control study preliminary results and positive PCR results, as well as their own risk assessments. Information obtained through RASFF indicates that while the exporter of radicchio rosso implicated in this outbreak also sends the product to the United Kingdom, the batch in question was only distributed in Norway. Although the epidemiological evidence incriminates bagged salad mix, the ongoing trace-back investigation of the product has been complicated. *Yersinia* is challenging to cultivate from food products [9] and microbiological testing is also still in progress.

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