Two outbreaks of diarrhoea in nurseries in Norway after farm visits, April to May 2009

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During a 2009 nationwide outbreak of sorbitol-fermenting *Escherichia coli* O157 in Norway, the Norwegian Institute of Public Health was notified of diarrhoea outbreaks in two nurseries. A link to the nationwide outbreak was suspected and investigated, including retrospective cohort studies. Both nurseries had recently visited farms. Faecal specimens were obtained from symptomatic children as well as from the farm animals and tested for *Campylobacter, Salmonella, Yersinia, Shigella* and pathogenic *E. coli,* and isolates were further characterised. Nursery A had 12 symptomatic children, and we found the same strain of *C. jejuni* in faeces from children and lambs. Nursery B had nine symptomatic children, including one child with bloody diarrhoea carrying enterohaemorrhagic *E. coli* (EHEC) O26. EHEC O26 with a similar multiple-locus variable number tandem repeat analysis (MLVA)-profile was found in sheep. Five children had enteropathogenic *E. coli* (EPEC) O76. Animals were not tested for EPEC O76. We found no significant association between illness and risk factors for either nursery. The isolated pathogens differed from the one involved in the nationwide outbreak. In each nursery outbreak, the pathogens isolated from children matched those found in farm animals, implicating animal faeces as the source. Hygiene messages are important to prevent similar outbreaks.

Introduction

There are several reports from around the world of sporadic cases as well as outbreaks of zoonoses, especially among children, after farm visits [1,2]. The most commonly described pathogens in these incidents are different strains of *Escherichia coli* [3-12], but other pathogens including *Campylobacter* are reported as well [1,13].

Enterohaemorrhagic *E. coli* (EHEC) are known to cause infections that can lead to serious complications such as haemolytic-uraemic syndrome (HUS), especially in children, immuno-compromised persons and the elderly. The proportion of patients diagnosed with EHEC who develop HUS is around 10% [14,15], but varies by host factors and type of EHEC. In Europe, more than 50% of patients diagnosed with sorbitol-fermenting (SF) EHEC O157 (SF O157) develop HUS [14,16].

In the spring of 2009 there was a national outbreak of SF EHEC O157 in Norway, affecting 13 children, including nine HUS cases of whom one died [17,18]. This outbreak attracted a lot of media attention, reinforced by the public's memory of the first large EHEC outbreak in Norway in 2006, that affected 17 children including 10 HUS cases of whom one died [19].

In May 2009, as the Norwegian Institute of Public Health (NIPH) was investigating the national outbreak of EHEC SF O157, additionally the chief medical officers of two distinct Norwegian municipalities each notified an outbreak of diarrhoea in a nursery in their respective municipalities: On 12 May we received the notification from Nursery A in Rogaland County in south-western Norway, while on 14 May we received the notification from Nursery B in Akershus County in the eastern part of Norway. A stool specimen from a child with bloody diarrhoea from Nursery B was positive for *stx*2, a gene encoding one of the EHEC toxins. We also had information that children attending both nurseries had participated in farm visits. During the visits children had cuddled the farm animals. Nurseries in Norway function as pedagogical daycare facilities for children under the age of six years.

We initiated investigations of the two nursery outbreaks. Our aims were to decide whether they were associated with the concomitant national outbreak of EHEC SF O157, and to identify the source or sources of infection in order to stop the current outbreaks and

prevent similar outbreaks in the future. In order to reach our aims, we wanted to test the following hypotheses: (i) The pathogen causing the nursery outbreaks was EHEC SF O157. (ii) The nursery children who participated in the farm visit had a higher risk of becoming ill than those who did not.

Coincidentally with initiating the investigations we took preliminary measures to control the outbreaks by excluding ill children from attending the nurseries, as recommended in the NIPH guidelines for infection control in nurseries [20].

Materials and methods

Epidemiological investigation
The investigations were conducted by the NIPH in cooperation with the chief medical officers in the affected municipalities and the Norwegian Food Safety Authority (NFSA). We performed a retrospective cohort study in each nursery. We collected information on each child's nursery attendance, travel history and participation in gatherings preceding the outbreaks, symptoms of disease (if any), food consumption, participation in the farm visit, and animal contact at the farm. Questions about food consumption were based on menu lists of food and beverages served in the nurseries, collected by the local NFSA offices.

We collected this information from the nursery staff, using detailed questionnaires based on the NIPH's standardised outbreak questionnaire [21], adjusted for the respective nurseries. For Nursery A the farm visit took place on 5 May, the questionnaires were filled out on 15 May, and the questions covered the period from 4 May. The time frame for travel history was 1–7 May. The question about gatherings was not included in the questionnaire for each child, but the nursery staff were asked if they were aware of anyone in the nursery group participating in any gatherings during the week before the outbreak. For Nursery B the farm visit took place on 29 April, the questionnaires were filled out on 19 May, and the questions covered the period from 27 April. The time frame for travel history and gatherings was 27–30 April.

We collected information on gastrointestinal illness for each child in the query period. For those who were ill, we asked about specific symptoms including diarrhoea, vomiting, nausea, abdominal pain, fever, bloody stools and joint pain.

Case definitions
We defined a case for Outbreak A as a child that attended Nursery A in April and May 2009 and a case for Outbreak B as a child that attended Nursery B in the period from April 29 to May 19, with the following additional criteria: Suspected cases were those who showed symptoms of gastroenteritis in the query period (general gastroenteritis, vomiting and/or diarrhoea). Because the microbiological results later indicated that the outbreak in Nursery A was caused by *Campylobacter jejuni* and the outbreak in Nursery B by *E. coli*, the definition for confirmed cases was chosen accordingly as: those who tested positive for *C. jejuni* in Nursery A and those who tested positive for pathogenic *E. coli* in Nursery B.

Statistical analyses
We conducted descriptive statistics and univariate analyses using Stata (version 11). In the univariate analyses we calculated the relative risk (RR) with 95% confidence intervals (CI) for association between illness and different risk factor exposures such as participation at farm visit, sex and age of the child as well as consumption of diverse food items and beverages. We performed the analyses both for confirmed cases only, and for suspected and confirmed cases combined.

Microbiological investigations

Human specimens
We aimed to collect faecal specimens from all children with symptoms. The initial analyses were performed at the regional medical microbiological laboratories, and included testing for *Campylobacter, Salmonella, Yersinia, Shigella* and pathogenic *E. coli* according to the standard protocols of the respective laboratories. The specimens from the children in Nursery A were also tested for rotavirus and adenovirus by immunochromatography [22]. The specimens from the children in Nursery B were not tested for viruses after we had identified pathogenic *E. coli* as the pathogen of the outbreak in this nursery. For the bacterial isolates that we suspected as possible causative infecting agents, we conducted further verification and typing (described below) at the reference laboratory at the NIPH.

From children who tested positive for pathogenic *E. coli*, we collected specimens repeatedly, until we considered them not to be contagious anymore and hence allowed them to attend nursery again. According to NIPH guidelines [20], for EHEC this requires five consecutive negative tests of faecal specimens collected a minimum of 24 hours apart.

Animal specimens
The district offices of the NFSA collected faecal specimens from animals and transported them at ambient temperature to the laboratory at the Norwegian Veterinary Institute (NVI) for examination within 24 hours.

From the farm visited by Nursery A, specimens from six lambs were collected and tested for *Campylobacter* according to an ISO-method [23]. In addition we evaluated the bacterial flora by plating out on non-selective media, and performed standard bacteriological testing. From the farm visited by Nursery B, we collected 36 specimens from sheep and 17 from cattle (one test per animal), and tested them individually for *E. coli*

O26 by automated immunogenic separation (AIMS) as described previously [24]. We did not perform standard bacteriology on these animal specimens, and as adequate methods were unavailable we did not analyse them for *E. coli* O76.

Typing and comparisons of human and animal isolates

At the DNA-analysis laboratory of the Department of Foodborne Infections at the NIPH, we typed and compared all animal isolates with the human isolates from the corresponding outbreak. We ascertained the DNA profiles of *C. jejuni* isolates by combining three different methods: clustered regularly interspaced short palindromic repeat (CRISPR) polymorphism, single nucleotide polymorphism (SNP) typing and binary gene typing (BGT) [25-28]. We assessed the DNA profiles of *E. coli* by multi-locus variable number of tandem repeats analysis (MLVA). We implemented a generic *E. coli* MLVA assay for all non-O157 isolates as detailed previously [29,30]. For the *E. coli* O26 isolates, we examined virulence, including detection of *eae* and the *stx* genes, as described elsewhere [31,32].

Environmental investigations

The district offices of the NFSA inspected the farms and the nurseries and collected specimens of food and drinks from Nursery A on 13 May. We also asked for water specimens from both nurseries. On 15 May the district office of the NFSA inspected and collected specimens of food and garbage from the kitchen in the home of the child with bloody diarrhoea in Nursery B.

Nursery A brought its own water from a water processing plant approved by the NFSA to the farm visit. Nonetheless, on 26 May we collected a water specimen from the farm, which was served by groundwater from a well. Eurofins Environment Testing Norway AS, Stavanger, analysed this specimen for total bacterial count at 22°C, coliform bacteria, generic *E. coli*, *Campylobacter*, and *Clostridium perfringens*.

We also collected faecal specimens from the floor of two lamb pens at the farm visited by Nursery A. We investigated these specimens in the same way as the faecal specimens taken from the animals.
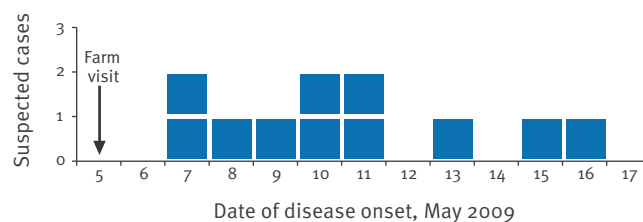
Results

Descriptive epidemiology

Nursery A

Of the 24 children attending Nursery A, 12 met the definition of a suspected case (attack rate (AR): 50%). The suspected cases were all between three and six years of age with median age four years, as for the nursery group in general. Ten of the suspected cases were girls. The first child became ill on 7 May. The 12 suspected cases included one child that became ill on 16 May, the day after the end of the query period. We included the information on this child's disease upon later notification from the chief medical officer of the

municipality. Of the 24 children in the nursery, three did not participate in the farm visit. One of these had symptoms defining her as one of the 12 suspected cases (date of onset 11 May).

We aimed to exclude all children with symptoms of gastroenteritis from nursery attendance until 48 h after cease of symptoms, as recommended by the NIPH guidelines for infection control in nurseries [20].

Nursery B

Of the 16 children attending Nursery B, seven met the definition of a suspected case (AR: 44%). The suspected cases were all between one and five years of age, as for the nursery in general. The median age for the suspected cases was two years, compared with three years for the nursery in general. Three of the suspected cases were girls. The first child became ill on 2 May, while the latest reported illness onset was on 8 May. One of the 16 children did not participate in the farm visit. This child did not become ill.

FIGURE 2

Number of suspected cases in Nursery B by date of onset of disease, Norway, April–May 2009 (n=7)
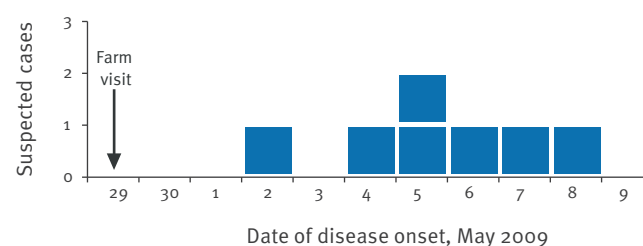
Microbiological results

Nursery A
We sampled and analysed four of the 12 suspected cases. These four specimens all yielded *C. jejuni* with identical DNA-profiles, and no other pathogens.

Specimens from four of the lambs on the visited farm were positive for *C. jejuni* with the same DNA-profile as the human isolates. We detected no other pathogens in the specimens from the lambs.

Nursery B
We analysed specimens from the seven suspected cases in Nursery B. In addition, two further children were sampled, who are not considered in the epidemiological analysis above because they had more general symptoms not included in the final suspected case definition. From specimens of the suspected case with bloody diarrhoea, we isolated EHEC O26, *stx1* negative, *stx2a* positive and *eae* positive. In addition we identified atypical enteropathogenic *E. coli* (aEPEC) O76, all with an identical MLVA-profile, from the specimens of a further five children, including one who did not fulfill the suspected case definition.

We aimed to exclude all children with faecal specimens positive of EHEC or EPEC from nursery attendance until they had repeated negative faecal specimens, as recommended for EHEC cases by the NIPH guidelines for infection control in nurseries [20].

We identified EHEC O26, with the same virulence genes as the human isolate, from two specimens of cattle and four specimens of sheep on the farm visited by Nursery B. The MLVA-profiles of the human and animal isolates were almost identical, differing in one locus only.

Environmental results
Nursery A had visited a farm with about 290 sheep and 430 lambs. The children were allowed to enter lamb pens. There was a sink in the barn, but the children did not use it to wash their hands. The staff from Nursery A brought hand disinfection that the children used prior to their meal. They ate outside in the yard sitting on the ground on seating pads. The analyses of the water samples from this farm did not yield positive results. Faecal specimens from the floor of the lamb pens tested positive for *C. jejuni*.

Nursery B had visited a farm with around 60 cattle and 90 sheep. The children had close contact with cows and lambs in the barn and did not wash hands before their meal, which they ate outside in the yard. The water supply both in the nursery and at the farm was a water processing plant approved by the NFSA, with no reports from other recipients indicating contamination of the water. Therefore the local NFSA office regarded the water supply to be of good quality and did not collect any water specimens.

As the microbiological results incriminated farm animals as the source of infection in both outbreaks, we did not analyse the food specimens taken from Nursery A, or the food and garbage specimens taken from the kitchen in the home of the child with bloody diarrhoea and EHEC in Nursery B.

Analytical epidemiology

Nursery A
In total we examined 69 risk factors. By univariate analysis we found that children who ate carrots during the farm visit were more likely to become ill (RR: 2.1; 95% CI: 1.4–3.2), but it has to be noted that this result is based on a single child who ate carrots. We found no other exposure significantly associated with disease. Table 1 shows examples of the risk factors examined for nursery A and their association with being a case (suspected or confirmed).

Nursery B
In total we examined 55 risk factors. By univariate analysis we found no exposure among the children increasing the risk of becoming ill. Table 2 shows examples of the risk factors examined for nursery B and their association with being a case (suspected or confirmed).

The exposures shown in Table 1 and Table 2 are chosen to illustrate the different categories of risk factors

Table 1

Selected results from univariate analysis for Nursery A, Norway, May 2009 (n=24)

Exposure		Exposed		Unexposed		Risk ratio	95% Confidence interval
		Cases/total	%	Cases/total	%		
Farm visit 5 May	Participation	11/21	52	1/3	33	1.6	0.30–8.2
	Close contact with lambs	11/21	52	1/3	33	1.6	0.30–8.2
	Eating carrots	1/1	100	11/23	48	2.1	1.4–3.2
	Eating fish cakes	11/21	52	1/3	33	1.6	0.30–8.2
Food and beverages consumed in the nursery 4–7 May	Mutton sausage	12/23	52	0/1	0	-	-
	Cucumber	12/23	52	0/1	0	-	-
	Tap water	12/23	52	0/1	0	-	-

Selected results from univariate analysis for Nursery B, Norway, April–May 2009 (n=16)

Exposure		Exposed		Unexposed		Risk ratio	95% Confidence interval
		Cases/total	%	Cases/total	%		
Farm visit 29 April	Participation	8/15	53	0/1	0	-	-
	Eating grilled minced steaks	8/15	53	0/1	0	-	-
Food and beverages consumed in the nursery 27–30 April	Honey dew melon	8/16	50	0/0	0	-	-
	Saveloy	0/0	0	8/16	50	-	-
	Liver paste	6/14	43	2/2	100	0.43	0.23−0.78
	Mayonnaise	8/16	50	0/0	0	-	-
	Margarine	4/12	33	4/4	100	0.33	0.15−0.74
	Gherkin	6/11	55	2/5	40	1.4	0.41−4.5
	Tap water	8/16	50	0/0	0	-	-

examined. Some were included because they had been pointed out as sources of earlier outbreaks in Norway (for instance mutton sausage [19]) or abroad.

Overall, the staff in both nurseries had given very similar answers on the questionnaires for all children. As univariate analysis did not yield any positive associations between exposures and illness, and as the number of subjects was low, we considered multivariate analysis not appropriate.

Discussion

We found that the causative pathogens of the outbreaks were *C. jejuni* in Nursery A, and *E. coli* O26 and O76 in Nursery B. Thus, we excluded an association with the concomitant national outbreak of *E. coli* SF O157.

In each outbreak, we found the same pathogens in faecal specimens from farm animals and from the sick children, implicating the animals as source of the outbreaks, directly or indirectly. The association between illness and eating carrots at the farm visited by Nursery A could only explain one of the 12 cases, and is therefore not plausible as the source of the outbreak.

Earlier publications of outbreaks in Norway due to transmission of zoonoses by animal contact are scarce. However, in 2005 a small outbreak of cryptosporidiosis among students and workers at a farm used for training by the Norwegian School of Veterinary Science was traced to contact with calves [33]. Two other outbreaks of cryptosporidiosis, which occurred in March 2009 and March 2012 among schoolchildren staying in a wildlife reserve, have also been attributed to animal contact [34,35]. In addition, animals were discussed as the cause of an outbreak of *E. coli* O145 in a third nursery [36] in September and October 2009. Generally, animal health in Norway has been regarded as good for many years. For example, the national surveillance programme did not detect any *Salmonella* among domestic animals in 2009 [37]. In contrast, a recent study identified Norwegian sheep flocks as an important

reservoir for potentially human-pathogenic *E. coli* O26 [31,38]. Our findings are especially relevant in light of the popularity of visiting farms with children; similar outbreaks might occur again.

It is possible that some of the sick children were secondary cases who acquired the infection from nursery mates. Such secondary transmission of zoonotic agents has also been described after visits to a petting zoo in Canada [9] and is likely in a nursery environment due to the difficulty of ensuring good hand hygiene among young children. The incubation period of campylobacteriosis ranges between one and 10 days [39], indicating secondary transmission for the child in Nursery A that became ill on 16 May. The incubation period of EHEC ranges between two and 10 days, but is probably shorter for EPEC [40], not excluding the possibility of secondary transmission for the children in Nursery B with later disease onset. Both nurseries aimed to exclude children from the nursery while they were symptomatic, but possible failure to achieve this completely could explain secondary transmission.

In two earlier campylobacteriosis outbreaks related to farm visits, the reported ARs for *Campylobacter* ranged from 0.5% [1] to 53% [13], whereas the AR in the outbreak in Nursery A was 50%. In previously described outbreaks of pathogenic *E. coli* infection after farm visits, the ARs ranged from 0.06% to 18% [8,10,12], whereas the AR in the outbreak in Nursery B was 44%. The AR depends on the dose of ingested organisms, but for both pathogens the infectious dose is low [39,40]. The fact that the children in Nursery B did not wash their hands after close animal contact and before their meal, suggests that many of the children could have ingested an infective dose of the bacteria. This can explain the high AR seen in this outbreak compared with previously described farm-related outbreaks of pathogenic *E. coli*. However, ARs are subject to substantial variation in small cohorts and should be interpreted with caution.

Limitations

The small number of affected children hampered the epidemiological investigations. For example, we could not assess the risk ratio of participating in the farm visit, since almost all children participated. It is possible that we could have obtained more conclusive indications of appropriate prevention measures if we had included more detailed questions about the children's behavioural pattern on the farm, as has been described in a Swiss study [41].

Recall problems probably influenced the nursery personnel's answers to the questionnaires, reflected by the similarity between their answers for the different children in each nursery. It is conceivable that the nursery personnel had problems remembering details about food consumption and behaviour of each child. A possible differential recall of exposures by case status is also understandable.

For Nursery A we applied a combined method for DNA typing of *C. jejuni* that is as yet unpublished. However, the basic work has been described in several publications [25-28]. As we received faecal specimens from only four of the 12 suspected cases in Nursery A, we had to use the suspected case status together with the confirmed case status for the epidemiological analyses, rather than the confirmed case status alone.

For Nursery B, we did not examine the animal faeces for EPEC O76. The DNA profile of human and animal EHEC O26 isolates differed in one locus. When employing methods with large discriminatory power like MLVA, it is not unexpected for such small variations in DNA-profiles to occur within the short time frame of an outbreak. They reflect recent evolutionary divergence from a common ancestor, and do not preclude our conclusion regarding the source of infection. However, genotyping results must always be seen in context with the other results from the outbreak investigation.

Conclusion

The outbreaks affecting Nursery A and B were not part of the concomitant national outbreak of *E. coli* SF O157. This was an important finding, since the EHEC SF O157 outbreak caused nine HUS cases of whom one died, and identification of the source was a major priority at the time. Furthermore, we concluded that the nursery outbreaks were caused by contact with animal faeces during the farm visits. This is only the third time an outbreak in Norway has been traced to animal contact.

Recommendations

Increased popularity of petting farms may lead to the occurrence of similar outbreaks in the future. Consequently, authorities in Norway as well as in other countries need to enforce hygienic measures when visiting farms with children. We did not advise the farms and nurseries described here to stop arranging farms visits with children, but we recommended letting only the oldest children enter the animal pens, and keeping them away from animals with diarrhoea, in addition to focusing on hand hygiene. Studies have reported that there is room for improvement concerning farm visitors' information on hygiene and hand washing in general [41-43]. To reduce human exposure to livestock faeces, several studies recommend a strict separation between picnic areas and animals, and to reinforce the importance of providing hand-washing facilities [2,42]. Previous findings suggest that active rather than passive interventions are more effective for increasing compliance [43].

The NIPH has published guidelines for farm visits with children [44]. In light of our findings, we recommend further efforts to spread and implement these guidelines among farmers and nursery staff.

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