Surveillance and outbreak reports

THE FIRST REPORT ON *CAMPYLOBACTER COLI* FAMILY OUTBREAK DETECTED IN POLAND IN 2006

S Wardak (swardak@pzh.gov.pl)¹, J Szych¹, M Sadkowska-Todys²

1. Laboratory for Pathology and Medical Microbiology, Veldhoven, the Netherlands

2. Department of Hospital Infection Control, St Anna Hospital, Geldrop, the Netherlands

3. Department of Surgery, St Anna Hospital, Geldrop, the Netherlands

4. Department of Medical Microbiology and Infectious Diseases, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands

A family outbreak of gastroenteritis caused by *Campylobacter coli* occurred in May 2006 in Bielsko-Biala, in the south of Poland. Four members of a family had non-bloody diarrhea and abdominal cramps. *C. coli* were isolated in three of the four patients. PFGE and PCR-RFLP-flaA patterns confirmed the link between cases, showing the usefulness of these methods in outbreak investigation. At the same time, the epidemiological and environmental investigations of this outbreak were very limited and did not provide enough evidence to identify the source of infection, and thus to support the hypothesis formulated by the local epidemiologist. It is necessary to improve surveillance of campylobacteriosis mainly by multidisciplinary training of epidemiologists, microbiologists and general practitioners.

Introduction

Thermotolerant species of *Campylobacter* (mainly *C. jejuni* and *C. coli*) are among the most frequently isolated bacterial agents of human gastroenteritis in many developed countries [1]. Globally, more than 90% of *Campylobacter* spp. infections are caused by *C. jejuni*, followed by *C. coli* with 5-10% [1]. In an earlier study we showed that in Poland *C. jejuni* was the species most frequently isolated from humans with diarrhea (94.5%) followed by *C. coli* (5.5%) [2].

C. jejuni are found mostly in poultry, whereas *C. coli* are usually isolated from pigs but may also be found in poultry and cattle [3]. Most *Campylobacter* infections occur as sporadic cases; outbreaks are uncommon [1,4] and are mostly caused by *C. jejuni*, whereas *C. coli* outbreaks are extremely rare [3].

Since 2003, the reporting of campylobacteriosis has been mandatory in Poland; the European Union case definition was introduced in 2005. The surveillance system relies on general practitioners (GPs) and hospitals sending notifications to the local sanitary-epidemiological stations (SES). After the notification of a case/outbreak, the local epidemiologists conduct an investigation and report it through the regional sanitary-epidemiological station to the national level. The information is gathered at the national level, and verified and analysed by the Department of Epidemiology of the National Institute of Public Health – National Institute of Hygiene (NIZP-PZH).

Despite the national surveillance system, data about *Campylobacter* infections are restricted to some regions of the

country. This is mostly due to the limited number of laboratories performing the diagnosis of *Campylobacter*, which is based on isolation of the organisms from stool samples using selective media. In Poland, there are no more than 10 laboratories routinely performing culture of *Campylobacter*.

In this report, we describe an outbreak caused by *C. coli* and discuss the epidemiological situation regarding campylobacteriosis in Poland.

Outbreak description

In May 2006, four cases of gastroenteritis in a single family were notified to the SES in Bielsko-Biala, a city with around 180,000 inhabitants in southern Poland. The patients included a woman and a man in their forties, a nine-year old boy and a teenage girl. All of them presented with non-bloody diarrhea and abdominal cramps; in addition, the father and the son had also emesis. No fever or other clinical symptoms were observed. Upon notification to SES, the local epidemiologists launched an investigation, which included laboratory testing of stool samples taken from the affected patients.

Methods

The epidemiological investigation was limited to routine interviews of the patients by the local epidemiologists. The questionnaire used included demographic data, clinical symptoms and the date of onset of symptoms, treatment and diagnostic tests, and epidemiological data on housing conditions, travel history, animal exposure and food consumption in the past 72 hours.

The investigation was limited only to the affected family. No active case finding was conducted and no case control study was performed.

The stool samples from patients were examined for the presence of *Campylobacter* as well as *Salmonella*, *Shigella*, *Yersinia*, enteropathogenic (EPEC) and verotoxic (VTEC) *Escherichia coli* in the SES laboratory in Bielsko-Biala. Isolates of *C. coli* were sent to the Department of Bacteriology in NIZP – PZH for confirmation and further investigation. Species-level identification of *Campylobacter* isolates was based upon hippurate and indoxyl acetate hydrolysis tests and polymerase chain reaction (PCR) [5]. The *C. coli* ATCC 33559 and *C. jejuni* ATCC 33560 strains were used as controls. To determine differences or similarities between *C. coli* isolates,

PFGE using Smal, PCR-RFLP-flaA and antimicrobial susceptibility studies were performed. To test the value of the genotypic methods, the molecular fingerprints of the outbreak-related isolates were compared with the patterns of three additional epidemiologically unrelated control isolates of *C. coli* obtained from patients with diarrhea from the Bielsko-Biala region. These *C. coli* control isolates were obtained in the same laboratory between March and September 2006. PFGE and flaA-RFLP analysis was carried out as described on the Campynet website (<http://campynet.vetinst.dk/PFGE.html>). The minimum inhibitory levels (MIC) of tetracycline, ciprofloxacin, nalidixic acid, ampicillin, erythromycin and gentamycin for *C. coli* isolates were determined by the E-test method (AB Biodisc, Solna, Sweden) with Mueller-Hinton agar with 5% sheep blood (bio Mérieux, France) according to the CLSI standard (formerly NCLLS) [2,6] and technical guidelines provided by the manufacturer.

As a result of the outbreak investigation, a standard inspection of food items and kitchen area of the restaurant that was implicated in the course of the investigation was also performed by inspectors from food safety unit of SES. This routine inspection, however, did not include testing food and environmental samples for *Campylobacter*.

Results

The investigation revealed that the only meal common to all family members was chicken shoarma with vegetable salad they had consumed a day before the onset of symptoms in a restaurant in town. This prompted the local epidemiologist to suspect the identified food items as a possible source of infection and undertake routine inspection of the restaurant and investigation of incriminated food. The quantitative results of swabs taken from the restaurant's kitchen utensils and chopping boards showed that they were microbiologically contaminated, but particular pathogens were not identified. This indicated poor hygienic practices in the restaurant, which could have contributed to this outbreak.

Laboratory analysis revealed the presence of *C. coli* in stool samples taken from three out of four members of the family (the mother and two children). No other causative agent has been identified in stool samples taken from the patients.

The outbreak-related isolates had the same pattern according to PFGE (pulsed-field gel electrophoresis) and PCR-RFLP (polymerase chain reaction - restriction fragment length polymorphism) of flaA methods. This pattern, however, was different from those observed in epidemiologically unrelated control isolates obtained from patients with diarrhea from the same region.

The outbreak-related isolates had identical patterns of antimicrobial susceptibility. They were resistant to ciprofloxacin (MIC >32 µg/ml), nalidixic acid (MIC > 256 µg/ml), ampicillin (MIC > 256 µg/ml), and were susceptible to erythromycin (MIC 0.5 µg/ml) and gentamicin (MIC 1 µg/ml). These findings support the assumption of a common source of the outbreak.

Discussion

In 2006, a total of 175,561 cases of campylobacteriosis were reported from 21 European Union Member States. Specifically, countries neighbouring with Poland, Germany, Czech Republic, Slovakia and Lithuania reported 52,035; 22,571; 2,718 and 624 confirmed cases, respectively [7]. In 2006, in Poland, only 156 *Campylobacter* infections were notified, with incidence of 0.4 per

100 000 population. For comparison, in the same period, 12,502 confirmed cases of *Salmonella* infection were reported in Poland, with incidence of 32.8 per 100,000. Despite the fact that we observed an increase in the number of reported *Campylobacter* cases between 2005 and 2006 – from 47 to 156 – we are very far from estimating the true number of cases and incidence of campylobacteriosis in our country.

The main reason of the underreporting of campylobacteriosis in Poland is the limited laboratory capacity for *Campylobacter* detection, available only in some regions of the country. For example, in 2005-2006 in the region of Bielsko-Biala, *Campylobacter* was the second after *Salmonella* most frequently isolated pathogen, and accounted for 41% (n=70) of all cases of bacterial gastroenteritis [8].

In 2006, 22 countries of the European Union reported 5,710 food-borne outbreaks, involving 53,568 people; 2,709 were considered family outbreaks. *Salmonella* was the most common cause of food-borne outbreaks (53.9% of all reported outbreaks). *Campylobacter* was the third most common cause associated with 6.9% of all food-borne outbreaks. In Poland, in the same year, a total of 561 food-borne outbreaks were reported (378 family outbreaks), affecting 6,974 people. The predominant causative agent was *Salmonella* spp. accounting for 292 (52%) of these outbreaks. In about 28% of reported outbreaks, the etiological agent was not identified.

In 2005, no outbreak of campylobacteriosis was reported in Poland, in 2006 only three outbreaks were notified, all were considered family outbreaks. Two of these were caused by *C. jejuni* (involving eight people) and one by *C. coli*. According to our knowledge this is the first report of a *C. coli* outbreak in Poland.

Our study shows that genotyping methods such as PFGE and PCR-RFLP flaA may be useful in investigating outbreaks due to *Campylobacter*. The results of these tests allow to link cases and thus identify outbreaks and look for their sources. The outbreak-related *C. coli* isolates were highly resistant to ciprofloxacin (MIC >32 µg/ml). The high prevalence of *Campylobacter* isolates resistant to fluoroquinolones is an emerging problem in Poland. In our previous study conducted between 2003 and 2005 we showed that 55.9% of *C. jejuni* and four out of six *C. coli* isolates were resistant to ciprofloxacin. This situation may reflect the broad use of this group of antibiotics in veterinary medicine in our country.

The outbreak described here provides more evidence of the importance of *C. coli* as a food-borne pathogen and underlines the need to strengthen surveillance of campylobacteriosis in Poland. It also reveals limitations of the epidemiological investigation conducted in relation to this outbreak. Not enough information was collected on food consumed by the affected patients to formulate the hypothesis that the source of infection was a meal containing chicken consumed at a particular restaurant in town. As the incubation period of campylobacteriosis is 3-4 days (range of 1 to 7), other common meals within the family before the visit to the restaurant should have been considered as a possible source of infection at the beginning of the investigation. In particular, information on the consumption of pork should have been gathered as, apart from poultry, *C. coli* are most frequently found in this kind of meat. In addition, once the hypothesis was formulated, there was no attempt at active case-finding, which could have confirmed the restaurant meal as a source of infection.

In Poland a lot of effort is still needed to improve the surveillance of campylobacteriosis. The NIZP-PZH provides training and education programmes that include both practical and theoretical courses on the diagnosis, treatment and epidemiology of campylobacteriosis. In recent years, on average about 80% of recorded *Campylobacter* isolates in Poland have been sent voluntarily by microbiology laboratories to NIZP-PZH where the diagnosis is confirmed by biochemical and PCR tests and isolates undergo antimicrobial resistance testing. This allows us to evaluate the quality of particular laboratories performing the diagnosis of campylobacteriosis. However, these actions would be enhanced if *Campylobacter* was included in the set of enteric pathogens (*Salmonella*, *Shigella*, *Yersinia*, VTEC, EPEC) routinely tested for in cases of diarrhea.

Acknowledgments

This study was supported by a grant from the Polish Ministry of Science and Higher Education N404 2532 33. We thank U. Duda and J. Klimczak for kindly providing *C. coli* isolates. We also wish to thank R. Gierczynski for useful advice.

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This article was published on 28 February 2008.

Citation style for this article: Wardak S, Szych J, Sadkowska-Todys M. The first report on *Campylobacter coli* family outbreak detected in Poland in 2006. *Euro Surveill*. 2008;13(9):pii=8052. Available online: <http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=8052>