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Epidemiologic application of pulsed-field gel electrophoresis to an outbreak of *Campylobacter jejuni* in an Austrian youth centre

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SUMMARY

We report the first documented *Campylobacter jejuni* outbreak in an Austrian youth centre. Sixty-four children were involved of which 38 showed classical signs of campylobacter gastroenteritis. Since unpasteurized milk distributed by a local dairy was suspected to be the source of infection, stool samples were collected from 20 cows providing the milk. Five of the cows tested positive for *C. jejuni*. These isolates together with 37 clinical samples were compared by pulsed-field-gel electrophoresis (PFGE). The PFGE patterns, using the restriction endonucleases *Sma*I and *Sal*I, were identical for the human and bovine isolates.This finding confirmed that the outbreak was caused by the consumption of unpasteurized milk contaminated with *C. jejuni*.

INTRODUCTION

Campylobacter jejuni and *C. coli* are well-recognized causes of human gastroenteritis. Accurate identification of the sources of infections is especially difficult as the organism is very common in nature and most cases of campylobacter infections in humans occur sporadically. The National *Campylobacter* Surveillance Centre has determined the incidence of reported cases of campylobacter gastroenteritis in the USA as approximately 5–6 cases per 100000 population. However, if estimates of the number of milder and/or unreported infections are correct, and considering the difficulties in cultivation and identification of the bacteria from faeces, the true incidence may be 200 times or more the nationally reported rate, or 1 % of the population [1]. In Austria, the incidence of campylobacter infections, based on laboratory confirmed cases was 68·8/100000 inhabitants as determined in a national survey in the province Styria (1·2 million inhabitants) in 1998 [2], making it the second most common bacterial pathogen after *Salmonella* spp. causing diarrhoea. Contaminated drinking water, consumption of unpasteurized milk and poultry have been shown to be risk factors [3–5]. However, there have been no reports so far in Austria, where unpasteurized milk was involved in transmission of the pathogen.

During the last decade, traditional methods of strain differentiation, namely serological and phage typing [6–8], have been supplemented with molecular methods, such as plasmid fingerprinting, [9] ribotyping [10], PCR-based methods [11] and pulsed-field gel electrophoresis (PFGE) [12, 13]. PFGE has been shown to be a highly discriminative method for many species and in *Campylobacter* spp. several genotypes can be found within a serotype. The aim of the present study was to apply PFGE to trace-back the source of *C. jejuni* in an outbreak of gastroenteritis in an Austrian youth centre in September 1998.

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MATERIALS AND METHODS

Stool samples

The *C. jejuni* strains from human ($n = 37$) and bovine ($n = 5$) faeces were cultured on selective medium (mCCDA; Oxoid, UK) at 42 °C under microaerobic conditions for 48 h. Species identity was confirmed by Gram stain, cytochrome oxidase production, catalase activity and hydrolysis of hippurate.

Typing of isolates by PFGE

Isolates were cultured on 5% sheep blood agar plates (Oxoid, UK) in a Gas-Pak jar at 42 °C for 48 h under microaerobic conditions produced with a gas generator kit (GENbox microaer, bioMérieux, France). Bacterial cells were harvested from plates with a swab into 2 ml phosphate buffered saline, pH 7·3 (PBS; 130 mM NaCl, 10 mM NaH_2PO_4). The optical density of the suspension was adjusted to 1·2 at 600 nm [14] and 1·5 ml was centrifuged and the pellet resuspended in 300 μl PBS. The cells were incubated with 33 μl of 37% formaldehyde solution (Merck, BRD) for 1 h at room temperature to inhibit DNAse activity [15], and subsequently washed three times with PBS. 300 μl aliquots of each isolate were embedded in 1% agarose (pulsed-field certified, Bio-Rad Laboratories, USA) and the agarose plugs were incubated in 3 ml ESP lysis buffer (1% sodium dodecyl sulphate, 0·25 M EDTA. pH 8·0, 0·5 mg/ml proteinase K) at 55 °C for 48 h [16]. Digestion of the chromosomal DNA using restriction enzymes *Sma*I and *Sal*I (New England Biolabs, USA) was performed according to the manufacturer's instructions. The DNA fragments were separated in 1% agarose gels using a CHEF DR-III apparatus under the following conditions: *Sma*I: 5–40 s for 20 h at 6 V/cm, 14 °C; *Sal*I: 10–30 s for 20 h at 6 V/cm, 14 °C. The gels were stained with ethidium bromide and the profiles were photographed under UV-light.

RESULTS AND DISCUSSION

In autumn 1998, an outbreak of gastroenteritis occurred in a youth centre. Of the 64 persons attending the centre (aged 10–18 years), 38 showed classical signs of campylobacter infection such as stomach ache (100%), fever (72·2%), headache (63%), sickness (54·5%), nausea (22·7%) and rheumatism (18·2%). In most cases stools were highly liquid (54·5%) or soft liquid (36·4%). Blood and mucous was present in 19·1% and 23·8% respectively. The average duration of the diarrhoea as well as that of the illness was 7 days. Twenty-eight of 38 patients with diarrhoea, 8 children without clinical signs of infection and 1 healthy member of the staff were tested positive for *C. jejuni*.

Evaluation of the questionnaires regarding the possible source of infection revealed two possible risk factors: poultry meat, and unpasteurized milk together with the breakfast cereal. Sixty-three (98%) of the children had consumed poultry meat, but only 32 (50%) had unpasteurized milk. Twenty-three (72%) of the milk consumers were ill, whereas 9 (2%) stayed healthy. In the group of children that had not consumed raw milk 15 (47%) were ill and 17 (53%) were healthy. The poultry meat served 2 days before onset of the outbreak was not available for testing but milk samples from the local dairy, that distributed the milk directly to the youth centre, were collected by the Austrian Food Surveillance authority. *C. jejuni* was not detected in any of them. This was not surprising as cross contamination from faeces to raw milk, as a result of inadequate hygiene measures was suspected as the route of infection. Therefore, rectal swabs of all cows on the dairy farm ($n = 20$) were taken and *C. jejuni* was recovered from five of these.

By PFGE, 37 human and 5 bovine isolates showed identical macrorestriction patterns using enzymes *Sma*I (Fig. 1) and *Sal*I. Additionally, ten isolates sent to the Institute of Hygiene in Hamburg (Professor Aleksic) were typed as serotype 062. It was therefore concluded that the consumption of contaminated milk provided by the local dairy, caused the outbreak in the centre. However, there were clearly other routes of infection as some of the children developed illness without having consumed the milk in the centre. In these cases human-to-human transmission probably occurred, suggesting that the infective dose of organisms was low as the hygiene standards of the centre were sufficient. Moreover, a member of staff who cleaned the toilets contracted the infection but had no direct contact with the affected children or contaminated milk. This suggests possible person-to-person spread of campylobacter infection resulting from contact with contaminated human faeces.

The value of PFGE as a discriminant method of DNA fingerprinting isolates for epidemiological studies has been commented on by other writers [13, 16]. It has also been shown previously, that *Sma*I defined macrorestriction types of *C. jejuni* can be further differentiated by the use of a second enzyme such

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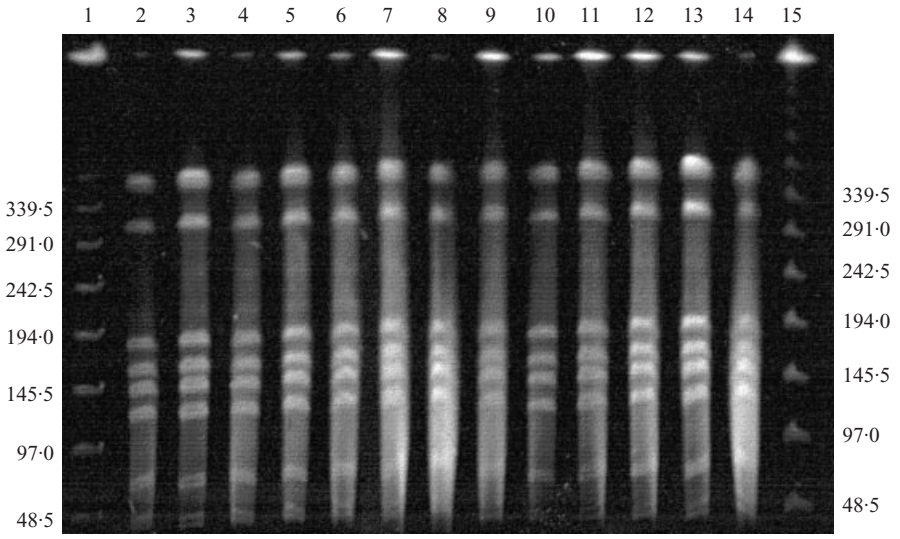

Fig. 1. PFGE patterns of DNA from 13 human (lanes 2–14) and 5 bovine (15–19) *C. jejuni* isolates digested with *Sma*I. Lanes 1 and 20: molecular weight standard in kb (lambda concatemers).

as *Sal*I [17]. It is noteworthy that all isolates showed the same susceptibility pattern to seven antimicrobial compounds (data not shown) and this corroborates the finding of a single DNA pattern for all isolates examined.

During the evaluation of a parallel study comparing the PFGE profiles of poultry and human campylobacter isolates, four additional clinical isolates, drawn from the same region in Austria, from persons originally not connected to the youth centre could be linked to this outbreak strain. Further questioning of these individuals revealed that all of them had had contact with the farm, either as consumers or visitors. This finding underlines the importance of establishing national DNA fingerprint databases based on PFGE.

We have documented the first outbreak of *C. jejuni* attributed to unpasteurized milk in Austria. Apart from other sources and routes of contamination the handling and sale of unpasteurized milk should be considered a potential risk for gastroenteritis and clear guidelines on hygienic measures should be given to minimize the risk of infection. Because many of the reported campylobacter infection cases are among children, individuals involved in youth activities must be alert to the danger of consumption of raw milk.

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