Outbreak of Recurrent Abdominal Cramps Associated with *Arcobacter butzleri* in an Italian School

P. VANDAMME,1 P. PUGINA,2 G. BENZI,2 R. VAN ETTERICK,3 L. VLAES,4 K. KERSTERS,1 J.-P. BUTZLER,5 H. LIOR,5 AND S. LAUWERS3*

Department of Microbiology, Ospedale Civile, Rovigo, Italy; Department of Microbiology, Universiteit Gent, B-9000 Ghent,1 Department of Microbiology, Academisch Ziekenhuis, Vrije Universiteit Brussel, B-1090 Brussels,3 and World Health Collaborating Center for Enteric Campylobacter, Universitair Ziekenhuis St. Pieter, B-1000 Brussels,4 Belgium; and National Laboratory Center for Enteric Pathogens, Laboratory Center for Disease Control, Tunney’s Pasture, Ottawa K1A 0L2, Canada*

Received 10 March 1992/Accepted 22 June 1992

In the autumn of 1983, an outbreak of recurrent abdominal cramps occurred in a nursery and primary school in the Rovigo area in Italy. None of the 10 affected children had diarrhea. An atypical *Campylobacter*-like organism was isolated from feces in all cases. Conventional enteropathogens were searched for but not detected. The *Campylobacter*-like organism was identified as *Arcobacter butzleri* by using sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins and cellular fatty acid analysis. Its identity was corroborated by DNA-DNA hybridizations versus *Arcobacter* reference strains. All of the preserved outbreak strains have identical protein profiles and phenotypic characteristics and belong to serogroup 1 of the Lior serotyping scheme on the basis of slide agglutination of crude and absorbed antisera of *A. butzleri* reference strains versus heat-labile antigens of live bacteria. These data point to an epidemiological relationship. The successive timing of the cases suggests person-to-person transmission.

Organisms previously known as aerotolerant campylobacters are now included in the genus *Arcobacter* (11). Two *Arcobacter* species have been associated with human disease (2, 4, 10, 13). These organisms are isolated mainly from stool specimens of patients with diarrhea (2). Only a minority of the human isolates belongs to *Arcobacter cryaerophilus*, whereas the majority belongs to a new species known as *A. butzleri* (2, 13). Our knowledge of the clinical significance of these organisms is very restricted. More than 50% of 22 patients with *A. butzleri*-associated diarrhea suffered from abdominal pain and nausea; fever, chills, vomiting, and malaise were other frequently reported features (3). The only risk factor noted was exposure to potentially contaminated water (3). These findings were corroborated by the results of Lior and Woodward (6), who found water and sewage as the most frequent nonhuman sources for 131 strains of *A. butzleri*.

In this report, we describe an outbreak of *A. butzleri* in a nursery school in Fratta (Rovigo area), Italy. The outbreak took place in autumn 1983. In that period, all clinical and epidemiological and some serological and phenotypic data were recorded. Recently, the preserved outbreak strains were identified by using gel electrophoresis of whole-cell proteins and cellular fatty acid analysis and confirmed by DNA-DNA hybridizations versus *Arcobacter* reference strains.

(Preliminary data of this study were presented earlier [7].)

**MATERIALS AND METHODS**

Setting. The outbreak occurred in a nursery and primary school in Fratta, Italy, attended by 65 children. Twenty-five children, 2 to 5 years old, were in the nursery school, and 40 children, 6 to 8 years old, were in the primary school consisting of three grades.

**Case definition.** Any child attending the nursery school and suffering from a clinical syndrome characterized mainly by recurrent abdominal cramps during September to October 1983 was included in the study.

**Isolation procedure.** Stool specimens were cultured for conventional enteric pathogens and for campylobacters. For isolation of campylobacters and related organisms, a selective medium (8) and an enrichment procedure (9) were used. Briefly, the selective medium consisted of Columbia agar base (Oxoid) supplemented with 7% lysed horse blood, FBP (Oxoid SR 84), and a selective supplement (Oxoid SR 98). Plates were incubated for 48 h in a microaerobic atmosphere at both 37 and 42°C. Cold enrichment (at 4°C) was performed in thioglycolate broth (Oxoid) with supplements identical to those used for the agar medium.

Three months after the outbreak, stool cultures were repeated for all case patients.

**Bacterial strains and growth conditions.** Fourteen outbreak strains (LMG 10897 [=SL 2188], LMG 10898 [=SL 2189], LMG 10899 [=SL 2190], LMG 10900 [=SL 2191], LMG 10901 [=SL 2192], LMG 11118 [=SL 4091], LMG 11119 [=SL 4092], LMG 11120 [=SL 4093], LMG 11121 [=SL 4094], LMG 11122 [=SL 4095], LMG 11123 [=SL 4096], LMG 11124 [=SL 4097], LMG 11125 [=SL 4098], and LMG 11126 [=SL 4101]) were studied. For identification, all strains were grown on a medium containing (per liter) 10 g of Special Peptone (Oxoid L72), 5 g of Lab Lemco powder (Oxoid L29), 5 g of yeast extract (Oxoid L21), 5 g of sodium chloride (Merck 6404; Merck, Darmstadt, Germany); 2 g of sodium succinate hexahydrate (RPL 1785; RPL, Leuven, Belgium); 2 g of sodium L-glutamate monohydrate (Merck 6445), 1 g of magnesium chloride hexahydrate (Merck 5833), 16 g of Agar no. 3 (Oxoid L13); and 5% (vol/vol) horse blood, unless specified otherwise. The plates were incubated at 37°C in a microaerobic atmosphere containing 5% O2, 10% CO2, and 85% N2, unless indicated otherwise.

**Chemotaxonomic studies.** Polymyxin B sulfate gel electroph-
resis of whole-cell proteins, subsequent numerical analysis of the protein profiles, and fatty acid methyl ester analysis were performed as described earlier (13). For the polycrylamide gel electrophoresis study, all strains were grown on Mueller-Hinton agar (Oxoid CM337) supplemented with 5% horse blood and incubated in a microaerobic atmosphere containing approximately 5% O₂, 3.5% CO₂, 7.5% H₂, and 84% N₂, in accordance with our standard procedure for identification of campylobacters and campylobacterlike organisms (12).

Genotypic studies. Preparation of high-molecular-weight DNA, DNA-DNA hybridization experiments, and determination of DNA base composition was performed as described previously (13).

Phenotypic tests. All classical phenotypic tests were performed as described previously (13).

Antibody determination. Antibodies against Campylobacter jejuni and C. fetus were determined by complement fixation with a commercially available antigen (Institut Virion AG, Zürich, Switzerland) in all affected children and many members of their families. Acute- and convalescent-phase specimens were available in 8 of 10 cases.

Serotyping. In 1984, all strains were typed by using the LAU heat-stable C. jejuni-C. coli serotyping system (5). In the current study, all strains were serotyped by the Lior scheme, i.e., by bacterial agglutination with absorbed and unabsorbed A. butzleri antisera (6).

RESULTS AND DISCUSSION

Epidemiological data. From 29 September until 17 October 1983, 10 children (four boys and six girls) were affected by a clinical syndrome characterized mainly by abdominal cramps (Table 1). The affected children attended the same school in Fratta. Five children were in the nursery school, two in the first grade and three were in the second grade of the primary school. They all lived in Fratta or Lusia (villages on the outskirts and near Rovigo) and made use, as did many others, of the school dining room. It was not practical to perform cultures on either staff members or the food. It was ascertained that farm and breeding animals were kept on the school grounds. Apparently, no other inhabitant of the area suffered from the same symptoms in the autumn and winter of 1983. Nevertheless, in January 1984, it was discovered that a school teacher had also shown the same symptoms at the time of the outbreak.

Clinical data. The syndrome was characterized by symptoms that varied only slightly in intensity and duration from case to case. An overview of the demographic and clinical data is given in Table 1. Constant features were (i) recurrent abdominal pain, two or three times a day initially, lasting about 2 h and unrelated to meals; (ii) sudden, acute attacks in otherwise healthy subjects, with good health in the intervals between attacks; (iii) no response to common antispasmodic or antimicrobial drugs or cortisone; (iv) absence of diarrhea and fever; (v) gradual spontaneous disappearance of the syndrome after day 3 of illness; and (vi) a recovery time (5 to 10 days) correlated with both the intensity and the duration of the attacks. Other, occasional characteristics were (i) inconstant episodes of biliary vomiting accompanying the pain attacks, limited to day 1 of illness in two cases (cases 4 and 8) and the first 2 days in one case (case 1), and (ii) low-grade fever for 1 day in one patient (case 4).

The symptoms caused serious anxiety in both parents and pediatricians and were severe enough to require hospitalization of 3 of the 10 children.

Laboratory investigations. Routine hematological and biochemical parameters were normal in all patients. From the feces of all 10 children, an atypical catalase-producing campylobacterlike organism was isolated on the selective medium incubated at 37°C. This organism also grew at 25°C, was resistant to cephalothin and nalidixic acid, and did not hydrolyze hippurate or produce hydrogen sulfide. The bacteria were still recovered from feces 3 to 10 days after onset of the symptoms by direct plating (except for cases 6 and 9) and up to days 9 to 25 after enrichment. Clearly, enrichment improved detection of the organism. No other enteric pathogens were found, and all control cultures after 3 months were negative.

Identification of the isolates. The protein profiles of 14 strains isolated from the 10 affected children were compared with the protein profiles of more than 600 campylobacters and related organisms, including about 80 Arcobacter strains that were characterized previously (13). All 14 isolates had the same protein profile and were identified as A. butzleri (data not shown). Strain LMG 11118 was chosen as the reference for the outbreak strains and was used in further studies.

The fatty acid composition of strain LMG 11118 is as follows: 12:0, 5.4%; 14:1 cis 7, 3.1%; 14:0, 2.9%; 14:0 3OH, 10.6%; 16:1 cis 9; 20.5%; 16:1 trans 9, 13.4%; 16:0, 24.0%; 18:1 cis 11, 16.1% (fatty acids that occur only in trace amounts are not given; therefore, the percentages do not total 100%). This fatty acid composition corresponds to the average fatty acid profile of A. butzleri strains as determined previously (13). One of the two phenotypic subgroups within

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex*</th>
<th>Age (yr)</th>
<th>Date of onset</th>
<th>Duration of symptoms (days)</th>
<th>Abdominal pain</th>
<th>Vomiting</th>
<th>Fever</th>
<th>Diarrhea</th>
<th>Hospitalization (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>4</td>
<td>29/9</td>
<td>7</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>7</td>
<td>1/10</td>
<td>10</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>7</td>
<td>1/10</td>
<td>8</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>3</td>
<td>3/10</td>
<td>10</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>7</td>
<td>3/10</td>
<td>8</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>7</td>
<td>5/10</td>
<td>6</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>7</td>
<td>8/10</td>
<td>5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>F</td>
<td>4</td>
<td>14/10</td>
<td>8</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>4</td>
<td>15/10</td>
<td>7</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>3</td>
<td>17/10</td>
<td>7</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

* M, male; F, female.
**A. cryaerophilus** (the so-called **butzleri** phenotype [2]) has a similar average fatty acid profile (13).

The DNA base composition of strain LMG 11118 is 28 mol%, which corresponds to the overall percent G+C content of the genus **Arcobacter** (13). We measured a DNA-binding value of 70% between strain LMG 11118 and the type strain of **A. butzleri** (LMG 10828). No significant DNA-binding values (<30%) were found with reference strains of **A. nitrofigilis**, **A. cryaerophilus**, or **A. skirrowii** (data not shown). These data indicate that the outbreak strains belong to **A. butzleri**.

**Phenotypic traits.** The following phenotypic traits were determined for all epidemic strains. Gram-negative, motile curved rod. Microaerobic growth at 15, 25, 30, and 37°C, not at 42°C. Aerobic growth at 30°C. Presence of oxidase activity and weak catalase activity. Hydrolysis of indoxyl acetate. No hydrolysis of hippurate. No hydrogen sulfide production in triple sugar iron agar. Nitrate is reduced. Growth in the presence of 1% glycine, no growth in the presence of 3.5% NaCl. In contrast with the antibiotic susceptibility tests performed in 1984, we found that all strains were susceptible to nalidixic acid (30-μg disc) and resistant to cephalotin (30-μg disc). The results of additional phenotypic tests performed on strain LMG 11118 only are given in reference 13.

**Typing results.** Thirteen strains from the 10 children were not typeable in the heat-stable **C. jejuni-C. coli** serotyping scheme, but all of the strains reacted at high titers with an antiserum prepared against one of the epidemic strains (LMG 10899). In the Lior serotyping scheme versus the heat-labile antigens of **A. butzleri** (6), all of the strains belong to serogroup 1.

**Antibody response.** All of the sick children had complement fixation antibodies against **C. fetus** (low titers of 10 to 40), and nine also had complement fixation antibodies against **C. jejuni** (titers, 10 to 20) in convalescent serum. Seroconversion was observed in eight children. No members of the children’s families had complement fixation antibodies. This antibody response against **C. fetus** and **C. jejuni** antigens may be explained by cross-reaction between **C. fetus**-**C. jejuni**, and **A. butzleri** antigens.

In conclusion, an outbreak of an unusual illness consisting of recurrent abdominal cramps without diarrhea, involving 10 children attending the same school, occurred in the autumn of 1983 in Fratta, Rovigo, Italy. This outbreak was caused by **A. butzleri** serogroup 1 strains, which were recovered from the feces of all cases. One month after the onset of the illness, **A. butzleri** strains were no longer recovered. This was probably due to self-limitation, as no response to therapy was noted. The fact that all available outbreak strains belong to the same serogroup and have identical protein profiles and phenotypic characteristics suggests an epidemiological relationship. The timing of the cases suggests person-to-person transmission. Our results support the hypothesis of a pathogenic role of **A. butzleri** in gastrointestinal disease.

**ACKNOWLEDGMENTS**

P.V. is indebted to the National Fund for Scientific Research (Belgium) for a position as a senior research assistant. K.K. is grateful to the Fund for Medical Scientific Research (Belgium) for research and personnel grants.

**REFERENCES**


