

A CASE OF ACUTE DIARRHEA DUE TO THE EMERGING PATHOGEN *CAMPYLOBACTER JEJUNI* SUBSP. *DOYLEI* IN SOUTHERN CHILE

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SHORT COMMUNICATION

ABSTRACT

The first documented case of acute diarrhea due to *C. jejuni* subsp. *doylei* in Chile is reported. The clinical findings, the absence of other enteropathogens, virus or parasites and the fact that *C. jejuni* subsp. *doylei* was the only bacteria isolated support the assumption that it was the etiological agent of this diarrheal case.

Key words: *Campylobacter jejuni* subsp. *doylei*, enteritis, emerging pathogen

Campylobacter enteritis is the most frequent form of acute bacterial diarrhea in developed countries (1) but, in developing countries it is considered as the second or third cause of infantile diarrhea (2,3). *C. jejuni* subsp. *jejuni* and *C. coli* are recognized as the most common agents of campylobacteriosis. In the last years, other *Campylobacter* species have emerged as enteropathogenic agents in humans (1). One of them is *C. jejuni* subsp. *doylei*, a Gram negative s-shaped rod, isolated for the first time in Australia from children with diarrhea (4). Although their characteristics are known since 1988 (5), little information is available with regard to ecological and geographical distributions, as well as prevalence in human diarrhea.

In order to provide additional information about the occurrence of *C. jejuni* subsp. *doylei* in different parts of the world, as well as about some of the clinical and bacteriological features, we report here the first case of acute diarrhea associated to this microorganism in Southern Chile.

A 1.5 year old child was admitted at the Pediatric Emergency Clinic of the Valdivia County Hospital (Valdivia City – Chile, 73° 11' Western, 39° 46' Southern latitude), with a two days history of watery diarrhea associated with vomiting. The onset started with liquid mucous stools, with no blood or pus, with a purging rate of 10 to 15 times daily. On physical examination, the child

presented signs of moderated dehydration and fever (38.2°C). The abdomen was normal but with hyperactive bowel sounds. The patient weighed 12.8 Kg and the height was 88.5 cm.

Three stool samples obtained every other day, were examined for ova and parasites. Other two stool samples were obtained, one tested for rotavirus and the other cultured for enteropathogenic *Enterobacteriaceae* and *Vibrio* but not for *Campylobacter* spp. The screening tests for enteric parasites, rotavirus and the standard cultures for conventional bacterial enteropathogens were negative. Urine analysis, uroculture, hemogramme, fecal pH and Benedict test results were negative or within normal parameters.

Parenteral fluid therapy and dietetic antidiarrheic regimen and no antimicrobial therapy were prescribed. The patient's conditions improved considerably being discharged home at the fourth day of hospitalization on the parent's demand.

Two days later, the patient relapsed with watery diarrhea. The stool samples were examined again for parasites, enteropathogenic *Enterobacteriaceae* and *Vibrio* as well as for *Campylobacter* spp. Stool culture for the classical thermophilic enteropathogenic *Campylobacter* species was done by direct inoculation onto a modified Skirrow medium plate (3). For the emerging *Campylobacter* species the membrane filter method (6) was used.

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In brief, a 47 mm 0.45 µm membrane filter (Millipore) was placed on a sheep blood agar plate and various drops (10-12) of 1/10 fecal suspension in physiological saline solution were placed onto the membrane filter. The membrane was removed after 30 min, by which time the fluid and *Campylobacter* cells will have passed through. The modified Skirrow medium plate was incubated at 42°C for 48h and the sheep blood agar plate at 37°C for up to five days, under microaerophilic atmosphere. A direct Gram smear was also performed, revealing thin, Gram negative curved and s-shaped bacteria, resembling *Campylobacter* morphology. No leukocytes were observed. No parasites, rotavirus nor classical enteropathogenic bacteria were found. No growth were found on modified Skirrow medium but, after three days of incubation, pinpoint, convex non hemolytic colonies were isolated on blood agar. Subcultures incubated at 26 and 42°C or aerobically failed to grow. Gram stain showed curved, s-shaped Gram negative rods. Oxidase and catalase tests gave positive reactions. Their growth and morphological characteristics as well as their biochemical properties allowed their identification as *C. jejuni* subsp. *doylei*.

After result of direct Gram stain was reported, erythromycin (50 mg/kg/day divided in four doses for 10 days) and dietetic regimen were prescribed. This was rapidly followed by a favorable outcome without further diarrhea relapses.

In the last years it has been reported that some *Campylobacter* species other than *C. jejuni* subsp. *jejuni* and *C. coli* can produce gastroenteritis in humans (1). One of them is *C. jejuni* subsp. *doylei* that has been isolated from patients with diarrhea in Australia (4,5), South Africa (6), Malasya (7), Spain (8), Sweden (9) and Brazil (10). This species grows slowly at 37°C and does not grow at 26°C nor at 42°C. It is hippurate hydrolysis positive, nitrate to nitrite reduction and gamma glutamyl transferase negative and it is more susceptible than *C. jejuni* subsp. *jejuni* to cephalotin and polymyxin (5,8). The strain described here was isolated at 37°C only in blood agar plate after a filtration procedure. It failed to grow at 26°C and 42°C and in media containing cephalotin and polymyxin and their biochemical behavior was compatible with that described for *C. jejuni* subsp. *doylei*.

According to the available data, *C. jejuni* subsp. *doylei* seems to produce diarrhea most likely in children under five years old (5). In this paper we report a 1.5 year old child with watery non mucous diarrhea and moderate dehydration, the latter due to vomiting and the high number of bowel movements per day. Initially, the illness presented a natural resolution but, two days after discharge, the patient relapsed with watery diarrhea, being *C. jejuni* subsp. *doylei* isolated from their stools, having a favorable outcome after erythromycin treatment.

The clinical features and the presence of liquid stools without blood and leucocytes suggest a secretory diarrhea. However, the virulence factors of *C. jejuni* subsp. *doylei* as well as reservoirs, ecological distribution and role in causing

human illness are not yet defined. Further studies are needed to demonstrate virulence factors and to identify the reservoirs and ecological distribution. Recently, we isolated *C. jejuni* subsp. *doylei* from stools of undernourished children without diarrhea (1.9%) and from stray dogs (6.7%) [unpublished data]. On the other hand, it is necessary that laboratories use suitable isolation methods, such as the filtration technique, that permit the isolation of this and other emerging *Campylobacter* species that could be misdiagnosed when solely the traditional selective media for *Campylobacter* are used.

The clinical findings of this case, the absence of other common enteropathogens, virus or parasites in stools and the fact that *C. jejuni* subsp. *doylei* was the only bacteria isolated, support the assumption that it was the etiological agent of this diarrheal case.

This is, to our knowledge, the first reported case of acute infantile diarrhea due to *C. jejuni* subsp. *doylei* in Chile and probably the second in South America.

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RESUMO

Um caso de diarréia aguda devido ao patógeno emergente *Campylobacter jejuni* subsp. *doylei* no sul do Chile

O primeiro caso documentado de diarréia aguda por *C. jejuni* subsp. *doylei* no sul do Chile é apresentado. As características clínicas, a ausência de outros enteropatógenos, vírus ou parasitas, e o fato de *C. jejuni* subsp. *doylei* ter sido a única bactéria isolada, permitem assumir que este microrganismo é o agente etiológico neste caso de diarréia.

Palavras-chave: *Campylobacter jejuni* subsp. *doylei*, enteritis, patógeno emergente.

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