Serological Markers of Recent *Campylobacter jejuni* Infection in Patients with Guillain–Barré Syndrome in the State of Piauí, Brazil, 2014–2016

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Abstract. In countries where poliomyelitis has been eradicated, Guillain–Barré syndrome (GBS) is the leading cause of acute flaccid paralysis. The range of infections that precede GBS in Brazil is unknown. *Campylobacter jejuni* infection is the most frequent trigger of GBS worldwide. Given the lack of systematic surveillance of diarrheal diseases, particularly in adults, the incidence of enteritis caused by *C. jejuni* in developing countries is unknown. From 2014 to 2016, pretreatment serum samples from 63 GBS patients were tested by immunoglobulin M (IgM) enzyme-linked immunosorbent assay for *C. jejuni*. *Campylobacter jejuni* IgM antibodies were detected in 17% (11/63) of the samples. There was no association between serological positivity (IgM) for *C. jejuni* and the occurrence of diarrhea among the investigated cases ($P = 0.36$). Hygiene measures, basic sanitation, and precautions during handling and preparation of food of animal origin may help prevent acute flaccid paralysis.

Campylobacter jejuni is the leading cause of bacterial diarrhea in developed countries, where the rate of this infection is higher than that with *Shigella* sp. and *Salmonella* sp.¹ *Campylobacter jejuni* is a Gram-negative microaerophilic bacillus that is transmitted to humans by the ingestion of contaminated and undercooked meat, untreated water, and unpasteurized milk. Contact with animals with diarrhea and handling of raw meat (particularly poultry meat) are also implicated in its transmission.² Most cases of *C. jejuni* enteritis (CJE) have a benign and self-limited course and occur in small sporadic outbreaks. Acute infection is characterized by fever, myalgia, vomiting, abdominal pain, and inflammatory diarrhea with different combinations of symptoms.³ Acute complications such as dehydration, peritonitis, and sepsis may occur in infants, elderly individuals, and immunocompromised individuals. In addition, rare late complications have been described, including reactive arthritis, hemolytic uremic syndrome, and Guillain–Barré syndrome (GBS).^{2,3}

The incidence of CJE in developing countries is unknown because usually there is no systematic surveillance of diarrheal diseases. The available estimates in Brazil are based on surveys of samples from a few laboratory or hospital services, where the reported positivity rates for *C. jejuni* in diarrheal stool are 5–20%.⁴

Campylobacter jejuni infection is the most common precipitating factor for GBS. A study estimated that in CJE patients, the risk of developing GBS within 2 months after developing symptomatic CJE was 100 times higher than that in the general population, and approximately 0.03% of CJE patients developed GBS.⁵ GBS is the leading cause of acute flaccid paralysis in children and adults in countries where

poliomyelitis has been eradicated.⁶ As a rule, two-thirds of individuals affected by GBS report symptoms of previous and recent infection of the respiratory or gastrointestinal tract. Cytomegalovirus, Epstein–Barr virus, and *Mycoplasma pneumoniae* are also involved in GBS trigger, as reported in prevalence and case–control studies conducted in several countries.^{7–9} In January 2017, the World Health Organization concluded that there are causal relationships between the Zika epidemic and the increase in the number of GBS cases in several countries.¹⁰ To date, the range of infections associated with the onset of GBS in Brazil is unknown.

The Health Department of Piauí State, Brazil, initiated a hospital-based program for the active surveillance of infectious, parainfectious, and postinfectious cases of neurological syndromes. In 2014, the surveillance of neurological syndromes was intensified because of the detection of the first case of a severe neuroinvasive disease caused by the West Nile virus (WNV) in Brazil, in Aroeiras do Itaim municipality (Piauí, Brazil).¹¹ Since then, cases of aseptic meningitis, encephalitis, and acute flaccid paralysis (including those involving adult patients) have been reported and investigated.

From 2014 to 2016, 73 patients were diagnosed with GBS in Piauí (0.83 cases per 100,000 patients per year). The annual incidence of GBS in this period was lower than the global estimate of one to two cases per 100,000 inhabitants per year,¹² but the surveillance program evaluated only hospitalized patients. Sixty-three samples were collected from 63 GBS patients in the acute phase of the disease, before the administration of hyperimmune human immunoglobulin. The samples were analyzed in the Bacteriology Section of the Evandro Chagas Institute. Serum immunoglobulin M (IgM) antibodies specific for *C. jejuni* were determined by using commercial enzyme-linked immunosorbent assay (ELISA) kits (Serion ELISA Classic, Institut Virion/Serion, Würzburg, Germany), using a preparation of the outer membrane of *C. jejuni*, according to the manufacturer's recommendations. None of the evaluated samples were positive for WNV by in-house

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TABLE 1

Association between serological positivity (IgM ELISA) for *Campylobacter jejuni* and the report of diarrhea in patients with Guillain-Barré syndrome

IgM ELISA for <i>C. jejuni</i>	Report of diarrhea		Total
	Present (n)	Absent (n)	
Positive	3	8	11 (17%)
Negative	7	45	52 (83%)
Total	10 (16%)	53 (84%)	63 (100%)

ELISA = enzyme-linked immunosorbent assay; IgM = immunoglobulin M. $P = 0.36$, by Fisher's exact test.

antibody capture IgM-ELISA or reverse transcription-polymerase chain reaction in real time.

Of the 63 screened patients, 38 (60%) were men and 25 (40%) were women, and their mean age was 43 years. Only 11 (17%) patients lived in rural areas. The GBS report forms registered the occurrence of diarrheal symptoms in 10 (16%) patients (Table 1). The GBS cases were classified in accordance with the diagnostic certainty of the Brighton Collaboration Criteria case definitions for GBS, as follows: 11 (17%) patients met level 1, 43 (69%) met level 2, and 9 (14%) met level 3 of diagnostic certainty. *Campylobacter jejuni* IgM antibodies were detected in 17% (11/63) of the serum samples. In other countries, serological surveys of patients with GBS using a variety of methods and control groups have shown that a significant proportion of GBS patients have antibodies against *C. jejuni* antigens^{8,9,13-15} (Table 2). The wide range of levels of positivity could reflect local population characteristics, fine specificities of the assay, different criteria for positivity, and timing of the serum sample collection. The present study used the presence of IgM against *C. jejuni* as evidence of a recent infection. IgG was not used as a criterion because high IgG reactivity against *C. jejuni* may persist for months or years after infection. However, the detection of short-lived *C. jejuni*-specific IgM in GBS patients strongly indicates that the infection occurred within the previous weeks.¹⁶

There was no association between serological positivity (IgM) for *C. jejuni* and the reporting of diarrhea among the investigated cases ($P = 0.36$, by Fisher's exact test) (Table 1).

Two studies performed in Brazil used bacterial genome assays and coproculture tests and reported that the prevalence of *C. jejuni* in the stool was similar between symptomatic and asymptomatic individuals (5.8–9.6% in patients with diarrhea and 4.9–7.2% in patients without diarrhea).^{17,18} In developing countries, symptomatic presentations are more common in children, whereas subclinical infections are more usual in adults.¹⁹ Subclinical *C. jejuni* infections may be associated with GBS, and IgM antibodies may remain detectable for up to 90 days in the serum of infected individuals. Some IgM-negative samples may have been collected from patients who had diarrhea caused by microorganisms of high incidence in the country, such as norovirus and rotavirus.¹⁷ The GBS report form did not indicate whether the onset of diarrheal symptoms occurred before or concomitantly with neurological symptoms or it occurred as a result of complications during hospitalization. In addition, the available sample size provided inadequate statistical power to demonstrate a difference in the prevalence of diarrhea between the IgM-positive and IgM-negative subgroups.

Campylobacter jejuni infections are associated with more severe types of GBS, with axonal involvement rather than demyelinating peripheral nervous system involvement.²⁰ Meanwhile, no differences were found between the electrophysiological pattern and the outcome among the *C. jejuni* IgM-positive and IgM-negative subgroups of the present study. However, there was no homogeneity between the proportion of patients submitted to electromyography in each subgroup, and the sample included only hospitalized patients. Therefore, patients who showed mild GBS and did not require hospitalization were not included in this study.

The gold-standard method for the detection of *C. jejuni* infection is coproculture using specific media.³ However, most GBS patients present neurological symptoms after the resolution of diarrheal symptoms, when *C. jejuni* is no longer detectable or isolated in their stool samples. This study was based on the epidemiological surveillance investigation of GBS cases in Piauí. Therefore, the control group was not available for comparing the frequency of serological positivity in the evaluated cases.

TABLE 2

Prevalence of serological markers of recent infection with *Campylobacter jejuni* in serum samples of patients in the acute phase of Guillain-Barré syndrome (GBS), reported in the five largest studies conducted in other countries

Authors and year of publication	Study period	Countries	Study design	Sample size (GBS cases)	Positivity criteria	Prevalence of <i>C. jejuni</i> antibodies indicating recent infection
Mishu et al., 1993	1983–1990	USA	Case control	118	Detection of IgG, IgA, or IgM	36%
Vriesendorp et al., 1993	1985–1991	USA	Case control	58	Detection of two or more immunoglobulin classes (IgG, IgA, and/or IgM)	17%
Saida et al., 1997	1990–1996	Japan	Case control	205	Detection of two or more immunoglobulin classes (IgG, IgA, and/or IgM) or high titer of IgA or IgM	45%
Jacobs et al., 1998	1986–1996	The Netherlands	Case control	154	Detection of IgM, IgA, or high titer IgG	32%
Hadden et al., 2001	1993–1995	Australia, Europe, and North America	Prevalence	229	Detection of two or more immunoglobulin classes (IgG, IgA, and/or IgM), or one class and recent diarrhea	23%

IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M.

This study is the first to investigate the presence of serological markers (IgM antibodies) of recent *C. jejuni* infection in a case series of hospitalized GBS patients in Brazil. Additional studies are necessary to assess the role of CJE in diarrheal diseases in Brazil and in GBS onset in other Brazilian states, with longer duration, larger sample size, and control groups. The search for other infections associated with GBS, particularly of those with epidemic potential, gains relevance in the current epidemiological scenario in Brazil, wherein several arboviruses are present. The results of the present study demonstrate the possible epidemiological importance of *C. jejuni* as a pathogen associated with GBS. Therefore, the use of strategies to prevent the transmission of CJE in Brazil, including the adoption of hygiene measures, basic sanitation, and precautions during handling and preparation of food of animal origin, may help prevent acute flaccid paralysis.

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