

## CAMPYLOBACTER JEJUNI OCCURRENCE IN CHICKEN FECAL SAMPLES FROM SMALL PROPERTIES IN PELOTAS, SOUTHERN OF BRAZIL

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### ABSTRACT

The aim of this study was to evaluate the occurrence of thermophilic *Campylobacter* species in broiler flocks from 26 small properties located near the campus of Federal University of Pelotas, RS, Brazil. A total of 404 chicken fecal samples were analyzed and after isolation, identification and biotyping was performed according to Lior's scheme. Twenty one strains (5.2%) of *Campylobacter jejuni* biotype II were isolated from animals from seven properties (26.9%), with a variation from 3% to 34.6% among the positive properties. We also analyzed twelve *C. jejuni* isolates by polymerase chain reaction (PCR), using specific primers that generate a fragment of 402 pb. Our study allowed us to observe that chickens raised in non-industrial establishments without sanitary attention can harbour *C. jejuni* in their intestinal tract, and by doing so, they could be a serious health risk to humans.

**Key words:** *Campylobacter jejuni*, thermotolerant species, diarrhea, chicken

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### INTRODUCTION

In the last two decades, thermophilic *Campylobacter* species have become recognized as one of the principal causes of human diarrhea in developed and developing countries (2). *C. jejuni* and *C. coli* are the most important agents that cause human enteritis and less frequently *C. lari* (28). This is a food-borne infection and the consumption of undercooked chicken meat has been identified as a significant risk factor (2,30). However, other factors, such as non-pasteurized milk (7,24) and contaminated water consumption (24,25), as well as contact with small and farm animals (2,24) have also been reported as sources of infection (26).

A great variety of mammals and wild birds can be reservoirs of thermophilic *Campylobacter* species, and thus potential sources of contamination for humans, animals, food and surface waters (10). The clinical manifestation of *Campylobacter* gastroenteritis differs between developed and undeveloped countries. In developed countries it appears as a sporadic infection while in developing countries it occurs as outbreaks

(26,27). The disease is not totally understood in developing countries, probably due to the lack of obligatory notification to the agencies of public health, and to difficulties with isolation techniques found in microbiology laboratories.

In Brazil, the presence of *Campylobacter* spp. in acute and chronic cases of diarrhea has been reported, as well as in asymptomatic individuals (3,13,15,23). Scarcelli *et al.* (21) reported the isolation of *C. jejuni* in 20% of fecal and carcass chicken samples from the state of São Paulo. In a different study Scarcelli *et al.* (22), using the PCR technique, analyzed 74 chicken carcasses and meat samples from state abattoirs, and found 5 positive *C. jejuni* samples.

Although *Campylobacter* species have been isolated from chicken meat (4-6), there is not sufficient information about cases of the disease occurrence and the animal carriage of thermophilic campylobacters, especially in the Southern region of Brazil. The aim of this study was to evaluate the occurrence of thermophilic *Campylobacter* species in broiler flocks in 26 small properties in Pelotas, Rio Grande do Sul, Brazil.

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## MATERIALS AND METHODS

### Material collection and culture conditions

The material for this study was collected on 26 small farms, dedicated family agriculture and kept broiler flocks with the purpose of meat production and eggs for the consumption of the family and commercialization of the excess. The birds were of different kinds (Plymouth Roch, New Hampshire, Rode Island Red, Embrapa 41, and cross breeds), were adult, created of half-intensive form, in poor conditions of hygiene, fed with maize and occasionally with commercial feed and available water *ad libitum*. A total of 404 fecal chicken swabs samples were collected and transported in enrichment broth (9), to the laboratory and incubated for 24 hours, at 42°C under microaerobic conditions (5% oxygen, 10% carbon dioxide), using the Microaerobac system (Probac do Brazil, São Paulo, SP, Brazil). After 24 hours of culture in enrichment media, the broth cultures were spread onto modified Skirrow plates (8) and the plates incubated for 48 hours in the same conditions as described above.

### Characterization of the microorganisms

The identification of presumptive *Campylobacter* isolates was based on colony morphology and Gram stain. Identification to the species and biotypes level was based on sensitivity to nalidixic acid and to cephalothine, growth at 25°C, 37°C and 42°C, catalase and oxidase positive reactions and the biochemical tests proposed by Lior (H<sub>2</sub>S production, hippurate hydrolysis, DNase production) to establish the biotypes (17).

### Polymerase chain reaction

#### DNA Extraction

Colonies of *Campylobacter* were suspended in 100 µL of sterile Milli-Q water and washed twice at 14000 rpm for two minutes. The pellet was re-suspended in 100 µL of Milli-Q water and boiled for 7 minutes. After centrifugation (14000 rpm for 7 minutes), the supernatant was used as template for PCR.

#### DNA amplification

DNA amplification was performed with a specific pair of primers for *C. jejuni*, based on the sequence Pdt 1720 (5' TGA CGC TAG TGT TGT AGG AG 3' e 5' CCA TCA TCG CTA AGT GCA AC 3') (Life Technologies do Brasil Ltda, São Paulo, SP), producing a PCR product of 402 pb. The reactions were performed according to the method described by Ng (18), with some modifications. The total volume of the reaction was 50 µL, containing 0.2 mM of each dATP, dCTP, dGTP and dTTP, 0.5 U Taq DNA polimerase, 2 µL from each primer, 2 mM MgCl<sub>2</sub>, 50 mM KCl, 10 mM Tris-HCl (pH 8.3) and 2 µL DNA. The amplification was performed in a thermocycler 480 (Perkin Elmer Cetus, USA) with an initial denaturation period of 3 minutes at 95°C, followed by 25 amplification cycles (denaturation at 95°C

for 15 seconds, annealing at 48°C for 15 seconds and extension at 72°C for 30 seconds), and extension for 10 minutes at 72°C. The resulting product was analyzed through electrophoresis and 20 µL of the PCR product was applied in agarose gel at 1.5%, containing ethidium bromide (0.01%), and running at 100V for 45 minutes. The visualization was done under ultraviolet light and the results were documented using the Kodak Digital Science ID image capture system. *C. coli* ATCC 49941 strain was used as negative control and a molecular weight marker 800pb ladder (Amersham Pharmacia) was included.

## RESULTS AND DISCUSSION

Small properties were selected for this study owing to their prevalence in the vicinity of the campus of Federal University of Pelotas. Chicken farming in this area provides subsistence foods and provision for local people.

It has been demonstrated by different authors (4,11,14,23,24) that these fowl are intestinal carriers of *C. jejuni* and *C. coli*. In this work, *C. jejuni* was the only isolated specie from 21 samples (5.2%), collected on 7 properties (26.9%). Other authors (4,16,29-31) have reported higher percentages, as well as different thermophilic *Campylobacter* species. In a similar work, Tresierra-Ayala *et al.* (30) isolated *C. jejuni* in 27% of the fecal samples of free range chickens, belonging to families of low social-economic level (Iquitos city, Peru). In another study, the same authors reported the presence of *C. coli* (17%) and *C. lari* (10%) in fecal samples of domestic chicken (29). Here in Brazil, Levi and Ricciardi (16) reported that *C. jejuni* was isolated in 64% of 168 fecal chicken samples, and Carvalho *et al.* (4) found that 42% of the samples collected in poultry meat factories were contaminated with *C. jejuni*. This bacterial species has been also found in viscera (5,20) and chicken carcasses (1,6,16,20), which may be contaminated with the animals feces during processing, as well as by the equipment and handling (19).

Uyttendaele *et al.* (31) studying the occurrence of *C. jejuni* and *C. coli* in poultry products (carcass, skin, and meat) from a Belgian supermarket network, observed that 28.5 % of 733 samples collected were contaminated with these two agents. Also in the same report, Uyttendaele *et al.* (31) observed that products from Belgian (21.9%), France (30.2%), UK (54.5%), and Italy (15.4%) were contaminated with *C. jejuni* and *C. coli*, suggesting that even developed countries can have high *Campylobacter* contamination in poultry products.

Its possible to observe high *Campylobacter* spp. contamination level in properties that do not have Federal Veterinary inspection, however, the same it happens in places where it exists. Tresierra-Ayala *et al.* (30) reported similar contamination levels when studying samples from family producers (54%) as well as commercial poultry farms (35%) with Federal Veterinary inspection.

We were not able to isolate thermotolerant *Campylobacter* species from 19 properties (73.08%). However, these results do not surprise us, since Humphrey *et al.* (14) also reported groups of birds free of *Campylobacter*, suggesting that the absence of *Campylobacter* was due to inhibitory metabolic processes produced by the cecal microflora. Also, Scarcelli *et al.* (22) analyzed 74 carcass samples from abattoirs (São Paulo, Brazil) were unable to isolate any *Campylobacter* species, in spite of having identified 5 (6.75%) positive samples of *C. jejuni* by PCR.

According to the scheme proposed by Lior (17), all the isolated *C. jejuni* strains belonged to biotype II. Similar results were reported by Fernández and Torres (11) in fecal samples from chicken reared in domestic environments in three regions of Chile. In another study conducted in Peru (30), biotype II was also frequent, however, biotypes I and III were also found.

The utilization of specific primers in the reaction confirmed the strains as *C. jejuni*, previously identified by their phenotypic characteristics.

The *Campylobacter* isolation frequency was relatively low in this study when compared with other research. However, we observed in individual properties high isolation percentages, up to 34.6% (as shown in Table 1). These results indicated that birds raised in non-industrial establishments and without sanitary attention may harbour thermophilic *Campylobacter* species, especially *C. jejuni*. One might consider this an important risk factor, since there is a correlation between the presence of these bacteria in poultry products and its concentration in the intestinal tract (6,20).

In order to prevent the *Campylobacter* infection some good hygiene practices must be adopted: washing hands with soap before and after handling raw food, avoiding cross contamination with other foods, and washing the utensils immediately after use with hot water and detergent. Many studies have shown that one main source of *Campylobacter* contamination is water, so clean and chlorate water might reduce contamination, and by doing so reduce the amount of contaminated meat that reaches consumers (12).

**Table 1.** *Campylobacter jejuni* frequency in chicken fecal samples in Pelotas, Southern of Brazil

Local	Nº of Samples	Nº of Isolations	%
1	06	01	16.7
2	26	09	34.6
3	14	04	28.6
4	13	01	7.7
5	06	02	33.3
6	11	03	27.3
7	30	01	3.3
<b>Total</b>	106	21	19.8

## RESUMO

### Ocorrência de *Campylobacter jejuni* em amostras fecais de galinhas em pequenas propriedades de Pelotas, sul do Brasil

O objetivo deste estudo foi avaliar a ocorrência de espécies termofílicas de *Campylobacter* em galinhas de 26 pequenas propriedades localizadas próximo ao campus da Universidade Federal de Pelotas, RS, Brasil. Um total de 404 amostras fecais de galinhas foram analisadas e após o isolamento, a identificação e a biotipificação foram realizadas de acordo com o esquema de Lior. Vinte e uma amostras (5,2%) de *Campylobacter jejuni* biotipo II foram isoladas de animais de sete propriedades (26,9%), com uma variação de 3,3% a 34,6% entre as propriedades positivas. *Primers* específicos, que geram um fragmento de 402pb, foram usados em PCR para analisar 12 isolados de *C. jejuni*. Concluímos que galinhas criadas em estabelecimentos não industriais e sem atenção sanitária, são portadoras de *C. jejuni* e, desta forma, podem ser consideradas um fator de risco para infecção humana.

**Palavras-chave:** *Campylobacter jejuni*, espécies termotolerantes, diarreia, galinhas

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