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Campylobacter species isolated from poultry and humans, and their analysis using PFGE in southern Brazil

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ABSTRACT

Campylobacteriosis is a bacterial disease transmitted to humans through ingestion of contaminated food. Six hundred samples were collected, 200 from human stool samples, 200 from poultry products and 200 from poultry feces in Southern Brazil, and then inoculated on blood agar plates. A total of 58% of the poultry feces, 17% of the poultry meat, and 2% the of human stools tested positive for *Campylobacter*. Positive *Campylobacter* colonies were identified as *Campylobacter jejuni* or *Campylobacter coli* by multiplex PCR. *Campylobacter* isolates were analyzed using PFGE to compare different profiles according to the source. This study demonstrated that there are different *Campylobacter* clones distributed in different aviaries in Southern Brazil. In addition, PFGE molecular profiles suggested that broilers can be a source of contamination of poultry products. However, the human isolate studied did not show any relationship with other strains examined.

1. Introduction

Campylobacteriosis is a foodborne disease. Common symptoms are gastroenteritis, diarrhea, abdominal pain, cramps, and fever (Blaser, 1997). The disease can also lead to complications such as Guillain–Barré syndrome (Nachamkin et al., 1998; NINDS, 2014), and hemolytic–uremic syndrome (FDA, 2013). Campylobacteriosis occurs differently in developed and developing countries. In developing countries, it affects children under 5 years of age more frequently than other age ranges (Padungton and Kaneene, 2003). Conversely, the disease can affect all ages in developed countries (Padungton and Kaneene, 2003). However, more recently, the number of cases in the UK has been increasing among elderly people (PHE, 2012).

Campylobacter is the most common diarrheic disease in the United States (CDC, 2014). It is estimated that more than 1.3 million people are affected every year, of which approximately 76 die (CDC, 2014). In Brazil, the number of cases of people infected is not well known. Lima et al. (1993) showed that 12% of children under 5 years of age with diarrhea had *Campylobacter*. However, children without symptoms can also be infected by *Campylobacter* (Quetz et al., 2010).

Avian species, including poultry, are a natural reservoir of *Campylobacter*. Chickens can be infected without displaying any symptoms (Van Deun et al., 2008). This bacterium does not trigger the inflammatory response of chickens' intestinal cells, acting as a commensal microorganism (Shaughnessy et al., 2009). Thus, its control in the aviary can be difficult to manage (Germano and Germano, 2003). In Brazil, there are no regulations to control *Campylobacter* in flocks or in food, hence, the percentage of contamination of poultry feces can reach 96.6% (Chaves et al., 2010).

Poultry meat contamination is one of the primary sources of human campylobacteriosis (Powell et al., 2012). Infected poultry are responsible for the contamination of carcases during processing (Allen et al., 2007; Elvers et al., 2011). Some points are especially important for cross contamination, such as the evisceration process (Powell et al., 2012), and the immersion chilling (Smith et al., 2005). A study in Southern Brazil found that 71.3% of broiler carcases contained *Campylobacter* (Franchin et al., 2007). Hygiene and control methods in the poultry industry are essential for decreasing the initial carcass contamination (Bashor et al., 2004; Kuana et al., 2008). However, these methods are not enough to eliminate contamination from the final product (Bashor et al., 2004; Kuana et al., 2008). *Campylobacter* can be found at different points in the slaughter line process (Franchin et al., 2005). Therefore, flocks free of initial contamination could become contaminated during the slaughter process even if the flocks are slaughtered on subsequent days (Perko-Makela et al., 2011). Different studies showed the prevalence of *Campylobacter* in poultry products sold in the market. Northern Brazil, for example, had 20.7% prevalence compared to 93.7% in Southern Italy (Freitas and Noronha, 2007; Nobile et al., 2013). The main

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species identified in flocks are *Campylobacter jejuni*, followed by *Campylobacter coli* (Hald et al., 2000; Heuer et al., 2001; Powell et al., 2012).

The prevention of *Campylobacter* human infection through contaminated food relies on a better understanding of the epidemiology of *Campylobacter* (Meinersmann et al., 2005). To establish the importance of *Campylobacter* in public health, it is necessary to study the occurrence of this bacterium in intestinal infections, as well as the link between the food contamination during the processing and the final product. The presence of clones of *Campylobacter* has been reported in animals, food, and humans in different countries (Levesque et al., 2008; Lyhs et al., 2010; Ragimbeau et al., 2008). However, there is a lack of research in Brazil to allow us to understand the epidemiology of campylobacteriosis in the food chain, and such knowledge is essential to develop effective plans to control the disease. Assessing the possible occurrence of *Campylobacter* clones in different points within the epidemiological chain of infection is the base for further studies. The aims of this research were to assess the presence of *Campylobacter* in poultry feces, poultry products, and in human stool isolated in Southern Brazil, as well as to discuss the similarity among the different DNA profiles isolated in the area studied.

2. Material and methods

2.1. Sampling

Two hundred samples were collected from poultry feces aged between 40 to 45 days old from 40 different aviaries (5 samples from each aviary). Two hundred *in natura* poultry products (40 thighs/legs, 40 wings, 40 chicken backs, 40 livers, and 40 ground chicken) were collected at the retail level. Two hundred samples were collected from human stool samples submitted to clinical analysis. All samples were collected in Southern Brazil.

Poultry products samples were kept in the original package in isothermal containers, and taken to the laboratory. Samples were collected from nine different brands. One hundred retail samples were obtained from the brand A (main brand commercialized in the retail market). The other 100 samples were collected from eight different brands (B to I) commercialized in the same region.

Human and poultry feces samples were collected with sterile swabs, and transported to the laboratory in Cary Blair media (Himedia, Mumbai, India) in isothermal containers with ice. Poultry feces were collected after the slaughter in the slaughterhouse belonging to brand A. The large intestine was longitudinally sectioned immediately after the cecum, and feces were collected with a swab. Human stools were collected from samples that had been sent to a clinical laboratory for parasitological analysis. No information about the patients' symptomatology were given. Human feces analysis was performed to investigate the number of people with gastrointestinal disorder who were also infected with *Campylobacter*.

2.2. Isolates

Each poultry product was placed in a sterile plastic bag containing 50 ml of Brucella broth (Acumedia, Lansing, Michigan, USA), and rubbed for 2 min. One aliquot (20 μL) of the broth was plated onto Columbia agar (Merck, Darmstadt, Germany) supplemented with 0.4% (*w*/*v*) activated charcoal, 5% (*w*/*v*) oxygen reduction solution FBP (George et al., 1978), and *Campylobacter* I supplement (Blaser–Wang, Himedia) which contains antibiotics to inhibit growth of other microorganisms. Swabs containing feces were plated onto Columbia agar added with the aforementioned supplements. Plates were incubated at 42 °C for 48 h under microaerobic conditions.

All spreading colonies which presented with a shiny and moist appearance were re-streaked to a fresh Columbia agar plate. Colonies were analyzed by Gram staining for identification of *Campylobacter* by morphology (Gram negative colonies with typically curved or "S" shaped rods ("gull wings")), and then were tested for catalase and oxidase. When it was not possible to obtain a pure culture, colonies were scraped and resuspended in 0.85% saline solution, filtered with 0.45 μm filter to remove contaminants, and plated on Columbia agar plate.

Pure cultures identified phenotypically as *Campylobacter* were cryopreserved in media containing 25 ml glycerol, 1 g neopeptone, 0.5 g NaCl, and 75 ml distilled water. When necessary, strains were recovered on Columbia agar at 42 °C for 48 h under microaerobic conditions.

The *Campylobacter* isolates were identified for species *C. jejuni* or *C.* coli by multiplex polymerase chain reaction (PCR), according to Harmon et al. (1997). The DNA was isolated with Illustra Bacterial GenomicPREP Mini Spin Kit (GE Healthcare, Buckinghamshire, UK) following the manufacturer instructions. Two pairs of primers were used: the first primer pair, Pg 3/Pg 50, amplifies a highly conserved region within flagellin genes in *C. jejuni* and in *C. coli*; the second primer pair, C-1/C-4, amplifies a specific region only present in *C. jejuni*. *C. jejuni* ATCC 33291 and *C. coli* CCAMP1003 were used as positive controls. These strains were kindly sent from *Campylobacter* sector from Fundação Oswaldo Cruz (Oswaldo Cruz Foundation) in Rio de Janeiro.

2.3. Molecular profiles

Campylobacter isolates were analyzed using Pulsed Field Gel Electrophoresis technique (PFGE) to compare DNA profiles following the suggested protocol from Centers for Disease Control and Preventions — CDC, available at http://www.cdc.gov/pulsenet/protocos.htm (Ribot et al., 2001). Briefly, DNA isolated from each sample was digested with *Sma*I, and analyzed on a 1% agarose gel using Pulsed Field Gel Electrophoresis (Bio-Rad Laboratories) apparatus. The gel was then stained with ethidium bromide, and visualized with ultraviolet light. Band profiles obtained were analyzed through Bionumerics 6.1 software (Applied Maths NV, Sint-Martens-Latem, Belgium) to generate dendrograms with a band position tolerance of 3%, and an optimisation coefficient of 0%. The PFGE cluster was based on a similarity cut off of 95%.

3. Results

Campylobacter was isolated from 116 (58%) samples out of 200 poultry feces analyzed. This microorganism was present in 33 of 40 poultry farms sampled. Nine aviaries had all samples tested positive for *Campylobacter*. Eighty isolates from poultry feces were analyzed by PFGE (remaining isolates could not be maintained in vitro), 63 (78.8%) were identified as *C. jejuni*, and 17 (21.2%) as *C. coli*. The distribution of species within the aviaries was analyzed considering the 5 samples obtained from each one. Twenty-three aviaries had only *C. jejuni*, five aviaries had only *C. coli*, and only one had both species present.

Of the 200 samples of poultry products analyzed, *Campylobacter* was isolated from 34 (17%), of which 20 were *C. jejuni* and 6 were *C. coli* (Table 1). Eight isolates did not grow when re-streaked. *C. jejuni* was the main species isolated from all types of poultry products analyzed.

Table 1
Poultry products samples positive for *Campylobacter*.

Poultry products	No of samples	*Campylobacter* Isolated	Species		
			C. jejuni	*C. coli*	Non identified
Livers	40	15	11	2	2
Backs	40	9	4	2	3
Wings	40	6	3	1	2
Thighs/legs	40	4	2	1	1
Ground	40	0	0	0	0
Total	200	34	20	6	8