Rev. Inst. Med. trop. S. Paulo
48(6):307-310, November-December, 2006

SURVEY OF CHICKEN ABATTOIR FOR THE PRESENCE OF *Campylobacter jejuni* AND *Campylobacter coli*

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SUMMARY

The genus *Campylobacter* is of great importance to public health because it includes several species that may cause diarrhea. These species may be found in water, food and in the intestinal tract of chickens. This study investigated the presence of *Campylobacter jejuni* and *Campylobacter coli* in chicken abattoirs in São Paulo State, Brazil. A total of 288 samples of feces, feathers, scald water, evisceration water, chiller water, and the rinse water of eviscerated, not eviscerated and chilled carcasses were collected in six chicken abattoirs. Polymerase Chain Reaction (PCR) was performed in *Campylobacter* spp.-positive isolates using the gene *HIP*, specific for hippuricase enzyme from *Campylobacter jejuni* and aspartokinase gene, specific to detect *Campylobacter coli*. The percentage of positive isolates of *Campylobacter jejuni* was 4.9% (14/288). Isolation was greater in feces samples (22%, 8/36). One sample was positive for the species *C. coli*. In conclusion, the results indicate that it is necessary to improve quality control for *Campylobacter* spp. in chicken abattoirs.

KEYWORDS: Abattoir; *Campylobacter coli; Campylobacter jejuni;* Chicken; PCR.

INTRODUCTION

The genus *Campylobacter* is of great importance to public health. Several species of this genus may cause diarrhea and have been isolated with increasing frequency in humans, animals, food and water[26]. A great variety of species can harbor this microorganism in the intestinal tract: pigs, sheep, bovines, dogs, cats, and domestic and wild birds. During the slaughter this pathogen can be uploaded from the poultry intestine to the meat surface[17].

In Brazil, *Campylobacter* spp. have been reported in cases of acute and chronic diarrhea, and even in individuals without disease symptoms[21]. In the United States, it is estimated that more than 2.5 million cases of enteritis caused by *C. jejuni* occur on a year, surpassing the number of cases of salmonellosis and shigellosis[10].

The transmission of *Campylobacter* spp. is usually associated with the consumption of contaminated food[22]. Nevertheless, a great number of outbreaks have been related to water, which has been considered an important transmission route[12]. Campylobacteriosis outbreaks have been associated mainly to chicken meat and their subproducts[9,19]. The consumption of food contaminated by *C. jejuni*, especially inadequately processed or partially cooked poultry has been considered the main transmission route of enteritis in human beings[20].

Two major species of the genus *Campylobacter* which occur in the poultry industry are *Campylobacter jejuni* and *Campylobacter coli*. Growth temperature ranges from 30 to 45 °C, with optimum temperature of multiplication at 42 °C[11]. The species *C. coli* is closely related to *C. jejuni*. It is associated with poultry and swine and has been related to diseases in human beings and animals[11]. The species *C. jejuni* is widely present in the gastrointestinal tract of mammals and also domestic and wild birds[17,24].

Campylobacter jejuni is a frequent cause of human bacterial gastroenteritis cases in industrialized and developing countries and represents a considerable drain on economic and public health resources[1]. The infections caused by *C. jejuni* represent the most common antecedent infection to a neuromuscular paralysis in human beings, known as Guillain-Barré syndrome[7].

CARVALHO *et al.*[3] have performed microbiological analysis of 291 samples collected in chicken abattoirs and *C. jejuni* has been isolated from 42% of the feces samples, 38% of the feather samples, 26% of the scald water samples, 61% of the rinse water of eviscerated carcasses, and 36% of the samples of carcasses after evisceration. The authors concluded that evisceration and plucking are critical points of contamination during slaughter and processing in chicken abattoirs.

The specific mechanism for the spread of *Campylobacter* spp. from animals to humans has not been elucidated yet, although it has been extensively studied. It is indispensable to establish reliable methods to

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study the mechanism of *Campylobacter* spp. spread[23]. Currently, *C. jejuni* isolates have been typed using the Polymerase Chain Reaction (PCR) and hippuricase gene (*HIP*)[10].

The objectives of this study were to evaluate the presence of *C. jejuni* and *C. coli* and identify these bacteria in the processing line of chicken abattoirs.

MATERIAL AND METHODS

Sampling: A total of 288 samples of feces, feathers, scald water, evisceration water, chiller water and rinse water of eviscerated, not eviscerated and chilled carcasses were collected, by the authors, during processing in three chicken abattoirs with Federal Inspection Service - SIF (B, C and F) and three chicken abattoirs with State Inspection Service - SISP (A, D and E) in São Paulo State, Brazil. After the samples were collected they were kept in refrigerated boxes and transported to the laboratory and directly processed.

Feces samples were collected at the Receiving Department and placed into sterile polyethylene bags. Feather samples were collected near the feather-plucking machine and placed into sterile polyethylene bags. The samples of scald water, evisceration water and chiller water were collected into sterilized glass flasks (500 mL) in a volume of 400 mL. The samples of rinse water of eviscerated, not eviscerated and chilled carcasses were collected during processing by placing the carcasses into sterile polyethylene bags containing 300 mL of 0.1% peptone water. The resulting solutions were then poured into sterilized glass flasks (500 mL) in a volume of 400 mL.

Isolation of *Campylobacter* spp. and DNA extraction: Feces samples: one g of feces was diluted with 10 mL of 0.9% sterile saline solution, mixed, and decanted for five minutes[5]. Feathers samples: 25 g of feathers were added to 45 mL of fluid thioglycollate medium (Merck). Water and carcass samples: the scald water, evisceration water, chiller water and the rinse water of eviscerated, not eviscerated and chilled carcasses were incubated for 24 hours at 42 °C. Afterwards, 50 mL of the total volume were centrifuged at 8,000 g for 30 minutes at 4 °C[2].

Two loops of each sample were inoculated in Brucella Agar (Merck) added with *Campylobacter* growth supplement (FBP, 0.025% ferrous sulphate, 0.025% sodium pyruvate (Merck), 0.025% sodium metabissulphite), 2% of antibiotic mixture (10 $mg.mL^{-1}$ vancomycin, 5 $mg.mL^{-1}$ trimethoprim, 2.5 $UI.mL^{-1}$ polymixin, 5 $mg.mL^{-1}$ amphotericin B, 15 $mg.mL^{-1}$ cephalothin) and 7% of sheep blood, and incubated for 48 hours at 42 °C in a microaerophilic atmosphere[2]. Suspicious colonies of *Campylobacter* spp. were Gram-stained and identified according to the morphology by optical microscopy.

The DNA of positive isolates of *Campylobacter* spp. was extracted according to NISHIMURA *et al.*[16]. Briefly, *Campylobacter* spp. colonies were grown for two or three days, and three to five colonies were diluted in 1 mL of ultra-pure water (Milli Q®) and boiled for 10 minutes.

Identification of *Campylobacter jejuni*-positive isolates: The oligonucleotides *HIP*400F (5'-GAA GAG GGT TTG GGT GGT G-3') and *HIP*1134R (5'-AGC TAG CTT CGC ATA ATA ACT TG-3')[13] were used to amplify the DNA of *C. jejuni*, targeting the hippuricase gene (*HIP*). PCR was performed with 1 x PCR buffer (Gibco- BRL), 200 µM dNTPs, 2.5 mM $MgCl_2$, 40 pmol of each primer, and 2.5 u Taq DNA polymerase (Gibco- BRL), and 10 µL of template DNA. The cycling was as follows: initial denaturation at 94 °C for five minutes, 30 amplification cycles of denaturation (one minute at 94 °C), annealing (one minute at 58 °C) and extension (one minute at 72 °C), followed by a final extension step for 10 minutes at 72 °C[13]. Finally, the samples were loaded to 1.4% agarose gels in 0.5 X TBE buffer (0.045M TRIS-Borate and 1 mM EDTA, pH 8.0) to be analyzed. Positive control strains of *Campylobacter jejuni* used were from Canada Center for Disease Control and Prevention (CDC): CDC 913/PC-264. A negative control without template DNA was included in each PCR run. Amplified products of *C. jejuni* (gene *HIP*) were analyzed in 1.4% agarose gel at a constant voltage of 5-6 $V.cm^{-1}$, using a 100-bp DNA ladder (Gibco-BRL) as molecular size standard. The gel was photographed under UV light (300-320 nm) with a Kodak Digital Camera DC/120 Zoom. The image was analyzed using the 1D Image Analysis software (Kodak Digital Science) electrophoresis.

Identification of *Campylobacter coli*-positive isolates: The DNA extracted from positive isolates of *Campylobacter* spp. was amplified using the oligonucleotides CC18F (5'-GGT ATG ATT TCT ACA AAG CGA G-3') and CC519R (5'-ATA AAA GAC TAT CGT CGC GTG-3'), which are specific for aspartokinase gene[13]. PCR was performed with 1 x PCR buffer (Gibco- BRL), 200 µM dNTPs, 2.5 mM MgCl2, 40 pmol of each primer, and 2.5 u Taq DNA polymerase (Gibco- BRL), and 10 µL of template DNA. The cycling was as follows: denaturation for five minutes at 94 °C, 30 amplification cycles of denaturation (one minute at 94 °C), annealing (one minute at 60 °C) and extension (one minute at 72 °C). Samples were submitted to an extension step for 10 minutes at 72 °C[13]. Positive control strains of *Campylobacter coli* used were from USA CDC: ATCC A3315. A negative control without template DNA was included in each PCR run. Finally, samples were analyzed and photographed as described previously.

Statistical analyses were performed using the software SAS 8.0 System (SAS Institute, 2002).

RESULTS

The Table 1 exhibits the percentage of *Campylobacter jejuni*-positive isolates in each collection points. The highest isolation percentages of *C. jejuni* were found in feces samples (22.2%, 8/36), following by feathers and rinse water of eviscerated carcasses (5.6%, 2/36). Positive isolates (Fig. 1) for *C. jejuni* were in number of 14 (4.9%) and distributed as follows: five in abattoir D, four isolates in abattoir E, three isolates in abattoir B, one isolate in abattoir C and F. No positive sample was found in abattoir A. From the 288 evaluated in the specific PCR 14 samples were positive for *C. jejuni*. One feather sample was positive for *C. coli* in the abattoir E, which has SISP inspection.

DISCUSSION

From a total of 288 samples, 4.9% (14/288) were positive for the species *C. jejuni*. SCARCELLI *et al.*[20] analyzed 55 chicken samples

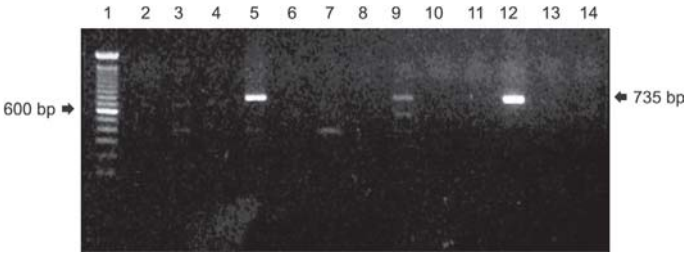

Fig. 1 - Electrophorese gel of *Campylobacter jejuni* samples, targeting the hippuricase gene *(HIP)*, from chicken abattoirs. *C. jejuni*-positive samples (lanes 5, 9); *C. jejuni*-negative samples (lanes 2, 3, 4, 6, 7, 8, 10, 11, 14); positive control, CDC 913/PC-264 (lane 12); negative control without DNA (lane 13); 100 bp DNA Ladder (lane 1).

Table 1
Positive isolates for *Campylobacter jejuni* collected in the processing lines of chicken abattoirs from March 2003 to August 2004, São Paulo State, Brazil

Samples	Number of samples	C. jejuni-positive samples	
		n	%
Feces	36	8	22.2
Feathers	36	2	5.6
Scald water	36	1	2.8
Evisceration water	36	1	2.8
Chiller water	36	0	0.0
Rinse water of not eviscerated carcasses	36	0	0.0
Rinse water of eviscerated carcasses	36	2	5.6
Rinse water of chiller carcasses	36	0	0.0
Total	288	14	4.9

and reported 20% *C. jejuni*-positive isolates, CASON *et al.*[4], in United States, have reported an occurrence of 20% of *Campylobacter* spp. in chicken carcasses. Both researches show values higher than the present findings.

In the present study *C. coli* was detected only in 0.35% of the samples. These results are lower than the values reported by ERTAS *et al.*[8], who analyzed liver and intestine samples of 200 chickens and reported 11% of positivity for *C. coli*. NIELSEN & NIELSEN[15] stated that the occurrence of positive samples for *C. coli* was 15% out of 156 samples in different poultry products. These results demonstrate high variation in the occurrence of *C. coli*. Studies on the prevalence of campylobacteriosis in poultry have indicated that *C. jejuni* is more commonly found than *C. coli*[8,11].

ZHAO *et al.*[26] analyzed 184 samples of raw chicken meat in the United States, from which the majority (70.7%) was positive for *Campylobacter* spp. In Brazil, high percentages of *C. jejuni* and *C. coli* (from 25% to 50%) have been isolated from feces of healthy chickens[5,14]. It has been reported that the prevalence of *C. jejuni* in poultry products might vary from 0 to 100%[11]. The presence of *C. jejuni* and *C. coli* in poultry carcasses might be fundamental to transmission to human beings[8].

In this work, despite the high frequency of *Campylobacter* spp. in feces samples (22.2%), only one sample (2.8%) of the evisceration water presented contamination with *C. jejuni*, and the presence of *Campylobacter* spp. in chiller water samples and rinse water of chilled carcasses was not observed. Differently from the present findings, CASTRO *et al.*[5] have detected higher percentages of *C. jejuni* contamination in the evisceration water (35.7%) and have also detected *Campylobacter* spp. in scald and chiller water samples. The scalding at 58 °C decreased *Campylobacter* spp. contamination in the carcasses, but did not eliminate it completely[18].

In abattoir A, there were no positive samples for *C. jejuni* probably because there was no contamination of the flocks that were processed in the sampling days. The chicken flocks evaluated in the present study originated from different places and there was no isolation of *Campylobacter* spp. from the samples of some flocks. Therefore, it is possible that such flocks were not infected by *Campylobacter* spp., probably because antibiotics had been added to the diet in the farms.

Reproducible and discriminatory methods are necessary to identify the origin of infections and to outline the transmission routes[6]. Besides, new laboratory techniques are necessary to differentiate between non-epidemiologically related outbreaks[10]. Frequent food contamination by multiple strains of *Campylobacter* spp. has been hindering the delimitation of transmission routes and the recognition of outbreaks[6].

Data corroborate the hypothesis that chickens might play an important role as a transmission route of *Campylobacter* spp. to human beings. These results emphasize the importance of improving control measures and both hygiene and sanitary conditions in chicken abattoirs.

The statistical analysis (Fisher test, $\alpha = 0.05$)[25] indicated that there were no significant differences between the presence and absence of *Campylobacter* spp. in the six abattoirs ($p > 0.05$). There were also no significant differences between the presence and absence of *Campylobacter* spp. in the different abattoirs evaluated (SIF or SISP) ($p > 0.05$). On the other hand, there were significant differences between the presence and absence of *Campylobacter* spp. in the eight sampling points evaluated ($p \leq 0.05$).

RESUMO

Pesquisa de *Campylobacter jejuni* e *Campylobacter coli* em abatedouros de aves

O gênero *Campylobacter* tem grande destaque em saúde pública, principalmente por pertencerem a este gênero várias espécies que podem causar diarréia. Estas espécies podem ser encontradas em amostras de água, alimentos e no trato intestinal das aves. Este estudo investigou a presença de *Campylobacter jejuni* e *Campylobacter coli* em abatedouros de aves no Estado de São Paulo. As 288 amostras foram coletadas em seis estabelecimentos e incluíram: fezes; penas; água de escaldamento, de evisceração e de resfriamento; e água de enxaguadura de carcaça não eviscerada, eviscerada e resfriada. Após o isolamento microbiológico das amostras positivas de *Campylobacter* spp. foi realizada uma Reação em Cadeia da Polimerase (PCR) utilizando o gene *HIP*, da hipuricase, específico para *Campylobacter jejuni* e o gene da enzima aspartoquinase, específico para

Campylobacter coli. A porcentagem de amostras positivas para *Campylobacter* spp. foi de 4,9% (14/288), sendo que o isolamento foi maior em amostras de fezes (22%, 8/36). Foi isolada uma amostra positiva para *C. coli*. Em conclusão, os resultados indicam que há uma necessidade de melhorar a qualidade higiênico-sanitária do controle de *Campylobacter* em abatedouros de aves.

ACKNOWLEDGEMENTS

The authors gratefully thank Fabio Kiyohara for critically reading the manuscript. This research work was financially supported by Brazilian National Council for Scientific and Technological Development (CNPq) and by the State of São Paulo Research Support Foundation (FAPESP).

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Received: 19 October 2005
Accepted: 29 June 2006