Brazilian Journal of Microbiology (2009) 40: 952-959
ISSN 1517-8382

USE OF PCR-RFLP OF THE *FLA* A GENE FOR DETECTION AND SUBTYPING OF *CAMPYLOBACTER JEJUNI* STRAINS POTENTIALLY RELATED TO GUILLAIN–BARRÉ SYNDROME, ISOLATED FROM HUMANS AND ANIMALS

Scarcelli, E.[1] ; Piatti, R.M.[1] ; Harakava, R.[2] ; Miyashiro, S.[1] ; Campos, F.R.[1] ; Souza, M.C.A.[3] ; Cardoso, M.V.[1] ; Teixeira, S.R.[1] ; Genovez, M.E.[1]

[1]Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico, São Paulo, SP, Brasil; [2]Centro de Pesquisa e Desenvolvimento de Sanidade Vegetal, Instituto Biológico, São Paulo, SP, Brasil; [3]Agência Paulista de Tecnologia dos Agronegócios, Pólo Regional do Vale do Paraíba, Unidade de Pesquisa e Desenvolvimento de Pindamonhangaba, Pindamonhangaba, SP, Brasil.

Submitted: September 05, 2008; Returned to authors for corrections: November 12, 2008; Approved: May 15, 2009.

ABSTRACT

The objectives of the present study were the subtyping of *Campylobacter jejuni* subsp. *jejuni* strains obtained from humans and different animal species using PCR-RFLP, and the detection, by means of the same technique, of strains related to serotype PEN O19:LIO 7, the main *C. jejuni* serotype linked to Guillain-Barré Syndrome (GBS). Seventy *C. jejuni* strains isolated from human feces (n=33), primates (n=15), dogs (n=5), swine (n=2), bovines (n=1), abortion material from goats (n=2) and poultry carcasses (n=12), all collected in the state of São Paulo, were subtyped by means of PCR-RFLP of *fla* A gene, using restriction endonucleases *Hae* III, *Afa* I and *Mbo* I. Seven subtypes were observed when using the enzyme *Hae* III; eight when using *Mbo* I; and seven when using *Afa* I. The combination of the three endonucleases led to 16 *fla-RFLP* subtypes, from which ten subtypes shared strains of human and animal origin. From these, seven subtypes were observed in human and broiler strains. In eight subtypes, the other animal species shared patterns with human strains. It was inferred that, besides broilers, swine, goats, dogs and primates may be sources of infection for human in São Paulo. PCR-RFLP is a highly discriminatory technique that may be applied to molecular epidemiology studies of samples from different origins. Besides, the study also enabled the detection of two human strains and two primate strains related to serotype PEN O19: LIO 7.

Key words: *Campylobacter jejuni*, PCR-RFLP, subtyping, Guillain-Barré Syndrome

INTRODUCTION

Campylobacter jejuni subsp. *jejuni* is the most common cause of diarrhea in children in developing countries and the primary cause of enterites in industrialized regions (4, 27). Although recognized as an important pathogen for more than 20 years, its epidemiology is only partially understood (14), mainly in Brazil.

***Corresponding Author. Mailing address:** Centro de Pesquisa e Desenvolvimento de Sanidade Animal, Instituto Biológico, Av. Conselheiro Rodrigues Alves, 1252, CEP 04014-002, São Paulo, SP, Brasil.; E-mail: pinheiro@biologico.sp.gov.br

The internal variability observed in the flagelin gene sequence has been used as a base for *C. jejuni* subtyping methods, such as PCR-RFLP and sequencing (17, 24). The *fla* A gene has been frequently used in epidemiological studies in order to determine *C. jejuni* subtypes that may be involved in outbreaks, or even in sporadic cases of food borne disease (8, 11, 24).

During the last few years, an association between *C. jejuni* infection and two neurological emergent diseases has been demonstrated: Guillain-Barré Syndrome (GBS) and Muller–Fisher Syndrome (MFS), a rare GBS variant (9,10). GBS is mostly characterized by acute inflammatory demyelinization of the peripheral nervous system (12). In serological studies, it has been observed that 40% of the GBS patients showed recent infection by *C. jejuni,* mainly associated with serotype PENO19:LIO 7 (1, 2, 20). Dourado *et al.*(7) demonstrated in Rio Grande do Norte, Brazil, an association between GBS and the detection of antibodies anti-*Campylobacter jejuni* and anti-gangliosides (GM1).

The objectives of the present study were the subtyping of different *Campylobacter jejuni* subsp. *jejuni* strains isolated from humans and different animal species using PCR-RFLP, and to detect, by means of this technique, strains related to serotype PEN O19: LIO 7, in the state of São Paulo.

MATERIALS AND METHODS

Thirty seven *Campylobacter jejuni* strains, previously isolated from bacteriological analysis (23) of 1,094 biological samples from different animal species and several locations in the state of São Paulo, were received in the *Laboratório de Doenças Bacterianas da Reprodução* at *Instituto Biológico*. Other 33 *C. jejuni* strains of human origin, isolated from adults and children affected by diarrhea, were also studied. These feces samples were analyzed in clinical laboratories in the city of São Paulo (Table 1). The reference strain used was *Campylobacter jejuni* subsp. *jejuni* Lior 7, from the *National Enteric Reference Centre, Laboratory Center for Disesases Control,* Ottawa, Ontario, Canada, serotype LIO 7, according to the classification by Lior *et al.* (16).

Table 1. List of the 70 *C. jejuni* field strains used in PCR-RFLP of *fla* A gene.

Sample source (type of sample)	Total of samples	Strain denominations
Bovines (feces)	1	B1
Swine (feces)	2	S1- S2
Dogs (feces)	5	D1-D5
Poultry (carcass)	12	P1-P12
Monkeys (feces)	15	M1-M15
Caprines (aborted fetuses)	2	CAPR1-CAPR2
Humans (feces)	33	H1-H33

DNA preparation and PCR of *fla* A gene

C. jejuni strains were grown at 37°C for 48 h under microerophilic atmosphere (5% CO_2) on blood Brucella agar (Difco-BBL). DNA was extracted by boiling, according to Nishimura et al. (18) and On et al. (19). Colonies obtained from 2 to 3 day-old cultures were used to prepare *C. jejuni* suspensions in 1.0 mL of ultrapure water (Milli-Q, Millipore Inc.), corresponding to 8 on the McFarland turbidity scale (2.3×10^9 bact/mL). These suspensions were heated at 100°C for 10 min. Five microliters of the suspension supernatant were used as templates. The oligonucleotides used as PCR primers were forward *fla* A primer 5'-TA CTA CAG GAG

TTC AAG CTT-3' and reverse *fla* A primer 5'-GT TGA TGT AAC TTG ATT TTG-3' that represented the variable (V1) region, according to Nishimura *et al.* (18). PCR was performed with 1 x PCR buffer (Invitrogen), 200 µM dNTPs, 2.5 mM $MgCl_2$, 40 pmol of each primer, 2.5 u Taq DNA polymerase (Invitrogen), and 5 µl DNA template. A 30-cycle reaction was run in a PTC 200 thermocycler (MJ Research) with 48 sec denaturing at 94°C, 36 sec annealing at 55°C, 2 min extension at 72°C and 10 min final extension at 72°C. The resulting product was approximately 702 bp. The amplified product from gene *fla* A was analyzed by means of 2.0% agarose gel electrophoresis in 0.5 X TBE buffer (0.045M TRIS-Borate and 1mM EDTA, pH 8.0). The gel was submitted to constant voltage equal to 5-6 V/cm, using a 100bp ladder as the standard marker (Invitrogen). Gel was stained with ethidium bromide 0.5 µg/mL, for 15 min and photographed under UV light (300-320nm) using a Kodak Digital Camera DC/120 Zoom. Images were analyzed by the 1D Image Analysis software (Kodak Digital Science).

PCR-RFLP

After amplification, DNA products were digested with restriction enzymes *Hae* III, *Mbo* I and *Afa* I (BioAgency BR). The reaction was carried out in a final volume equal to 20 µL for each enzyme containing; enzyme buffer, 6 µL amplified product and 10 U of enzyme, and was incubated for 3 h at 37°C (18). The digested product was analyzed by means of 3.0% agarose gel electrophoresis, using the same procedure described above.

RESULTS

PCR-RFLP using restriction endonuclease *Hae* III, applied to the 70 strains isolated and to the Lior 7 reference strain, produced 7 different restriction patterns, as shown in Table 2 and in Figure 1a.

PCR-RFLP applied to 70 strains and to the reference strain, using restriction endonuclease *Mbo* I, produced 8 different restriction patterns, as presented in Table 3 and Figure 1b.

PCR-RFLP applied to 70 strains and to the reference strain Lior 7, using restriction endonuclease *Afa I*, produced 7 different restriction patterns, as presented in Table 4 and Figure 1c.

The combination of the three restriction endonucleases - *Hae* III, *Mbo* I and *Afa* I – applied to the 70 *C. jejuni* strains and to the reference strain Lior 7, showed 16 different *C. jejuni* subtypes differentiated by PCR-RFLP, as shown in Table 5. The procedure showed high heterogeneity between the subtypes.

Table 2. Restriction patterns of 70 *Campylobacter jejuni* strains, and the reference strain, obtained using restriction endonuclease *Hae* III applied to PCR-amplified 702 pb fragment of *fla* A gene.

PCR-RFLP *Hae* III Pattern	**Strain denominations**
Pattern 1	P3, P5, P6, P7, P10, H4, H6, H8, H12, H13, H14, H15, H22, H23, H26, H27, H28, H30, H32, H33, M6, M11, Capr1, Capr2
Pattern 2	P1, P2, P4, P11, P12, H1, H2, H3, H9, H10, H11, H18, H19, H20, H24, H25, H29, M1, M2, M3, M7, M8, D1, D2, D3, D4, D5, B1
Pattern 3	H16
Pattern 4	H5, H7, P5, P15, Lior 7
Pattern 5	P8, P9, H17, H21, H31, S1, S2
Pattern 6	M4, M13, M14
Pattern 7	M9, M10, M12

B= Bovine, D= Dog, P= Poultry, H= Human, M= Monkey, S= Swine, Capr=Caprine, Lior 7= reference strain

Table 3. Restriction patterns of 70 *Campylobacter jejuni* strains, and the reference strain, obtained using restriction endonuclease *Mbo* I applied to PCR-amplified 702 pb fragment of *fla* A gene.

PCR-RFLP *Mbo* I Pattern	**Strain denominations**
Pattern 1	P1, P2, P7, P8, P9, P11, H4, H9, H10, H11, H13, H15, H16, H17, H19, H20, H22, H23, H24, H25, H26, H28, H29, H30, H33, M7, M9, B1, D1, D5, S1, S2, Capr1, Capr2
Pattern 2	H18, M1, M2, M3, D2, D4
Pattern 3	H1, H2, D3, H5, H7, H8, H33, M5, M15, Lior 7
Pattern 4	P4, P5, P6, P12, H3, H6, H12, H14, H32, M8
Pattern 5	M4, M13, M14
Pattern 6	P3, P10, H27, M6, M11
Pattern 7	H21
Pattern 8	M10, M12

B= Bovine, D= Dog, P= Poultry, H= Human, M= Monkey, S= Swine, Capr=Caprine, Lior 7= reference strain

Table 4. Restriction patterns of 70 *Campylobacter jejuni* strains, and the reference strain, obtained using restriction endonuclease *Afa* I applied to PCR-amplified 702 pb fragment of *fla* A gene.

PCR-RFLP *Afa I* Pattern	**Strain denominations**
Pattern 1	P1, P2, P3, P4, P5, P8, P9, P10, P11, P12, H3, H6, H9, H10, H11, H14, H16, H17, H19, H20, H21, H24, H25, H27, H22, H23, H31, M6, M7, M8, M9, M14, D1, D5, S1, S2
Pattern 2	M4, M13, B1
Pattern 3	P6, P7, H1, H2, H4, H12, H13, H15, H26, H28, H29, H30, H32, D3, Capr1, Capr2
Pattern 4	M11
Pattern 5	H18, M1, M2, M3, D2, D4
Pattern 6	M10, M12,
Pattern 7	H5, H7, H8, M5, M15, H33, Lior 7

B= Bovine, D= Dog, P= Poultry, H= Human, M= Monkey, S= Swine, Capr=Caprine, Lior 7= reference strain

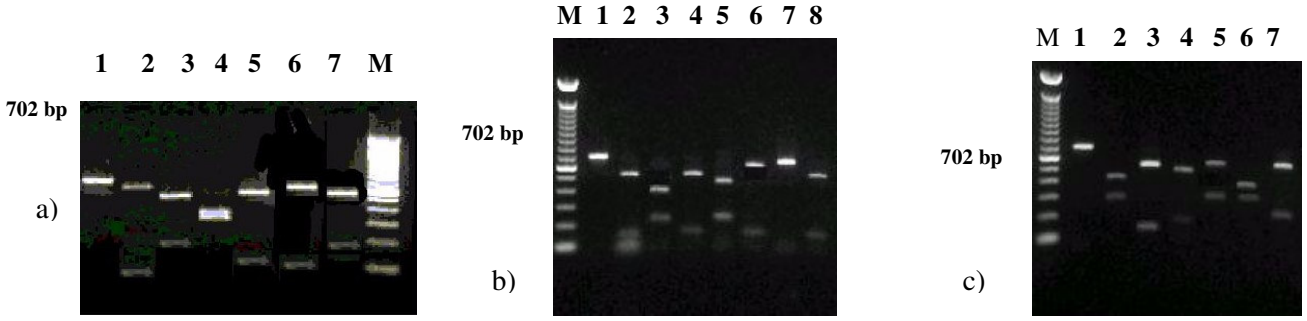

Figure 1. Representative restriction endonuclease digests of PCR-amplified 702 bp fragment of *Campylobacter jejuni fla* A gene. RFLP patterns were as follows: seven *Hae* III patterns, lanes 1-7, respectively (a); eight *Mbo* I patterns, lanes 1-8, respectively (b); seven *Afa* I patterns, lanes 1-7 respectively (c). Lane M: 100 bp DNA Ladder (Invitrogen).

Table 5. Subtypes based on the combination of the restriction profiles obtained with the three restriction endonucleases used, *Hae* III, *Mbo* I and *Afa* I, applied to the 70 *Campylobacter jejuni* strains and to the reference strain Lior 7.

RFLP Subtypes (*Hae* III, *Mbo* I and *Afa* I)	**Strain denominations**
fla-RFLP 1	D1 D5 P1 P2 P11 H9 H10 H11 H19 H20 H24 H25 M7
fla -RFLP 2	D2 D4 M1 M2 M3 M10 M12 M13 H18
fla-RFLP 3	P7 H4 H13 H15 H26 H28 H30 M11 Capr1 Capr2
fla-RFLP 4	P8 P9 H17 H21 H31 S1 S2
fla-RFLP 5	P4 P12 H3 M4 M8 M14
fla-RFLP 6	P5 H6 H14 M6
fla-RFLP 7	H5 H7 M5 M15 Lior 7
fla-RFLP 8	H1 H2 D3
fla-RFLP 9	P3 H27 P10
fla-RFLP 10	P6 H12 H32
fla-RFLP 11	H22 H23
fla-RFLP 12	H16
fla-RFLP 13	M9
fla-RFLP 14	H8 H33
fla-RFLP 15	B1
fla-RFLP 16	H29

B= Bovine, D= Dog, P= Poultry, H= Human, M= Monkey, S= Swine, Capr=Caprine, Lior 7= reference strain

Table 5 shows that the combination of the three restriction endonucleases produced 16 *fla-RFLP* subtypes. From these, four subtypes comprised only strains of human origin (*fla-RFLP* 11, 12, 14 and 16*);* two subtypes (*fla-RFLP* 13 and 15*)* involved only strains of animal origin and the other subtypes shared human and animal strains. The subtypes *fla-RFLP* 1, 3, 4, 5, 6, 9 and 10 comprised human and poultry strains and the subtypes *fla-RFLP* 1, 2, 3, 4, 5, 6, 7 and 8 the other animal species shared patterns with human strains.

Subtype fla-RFLP 1 was the most frequent one, involving 13 (18,6%) from the 70 field strains analyzed, followed by subtype *fla*-RFLP 2 with 9 (12,8%) and *fla*-RFLP 3, with 10 (14.3%) strains, and by subtype *fla*- RFLP 4, with seven strains (10%). The other strains were grouped in the 12 remaining subtypes (*fla*-RFLP 5 and 16), ranging from one to six strains per subtype (Table 5).

Detection of serotype PENO19:LIO:7 using PCR-RFLP

Serotype PENO19:LIO:7 was detected by means of PCR-RFLP in the reference strain Lior 7 and in four *C. jejuni* strains: two human strains (H5 and H7) and two strains from primates (M5 and M15). Restriction pattern of *fla*-RFLP 7 (Table 5) obtained by the combination of the enzymes *Hae* III (Pattern 4), *Mbo* I (Pattern 3) and *Afa* I (Pattern 7) corresponded to subtype Cj-1, described by Nishimura *et al.* (18) as the molecular standard significantly associated with serotype PENO19:LIO 7 (18, 26).

DISCUSSION

Bacteria in the Campylobacteraceae family are not frequently studied in microbiological routine. They are microaerophylic, adding a limiting factor for their isolation and to the systematic study of the epidemiology of different species and subtypes in the genus *Campylobacter*, mainly

clinical strains (13, 14, 22). Besides, the genus has great public health importance because it involves several diarrhea-causing species (5).

In the comparison of *C. jejuni* strains obtained from different animal species and human strains previously isolated in clinical laboratories in the city of São Paulo, PCR-RFLP showed that several human strains could be differentiated in distinct patterns or subtypes and grouped with strains from different animal species (Tables 2, 3 and 4).

In the present study, the 12 *C. jejuni* strains isolated from poultry were grouped with human strains in seven of the 16 subtypes generated by PCR-RFLP (Table 5).

The five *C. jejuni* strains isolated from dogs were grouped with human strains by PCR-RFLP in three subtypes. These animals were considered to be sources of *C. jejuni* infection for the group of humans studied.

The only bovine strain analyzed showed an exclusive PCR-RFLP pattern (*fla* –RFLP 15) and was not grouped with any human strain. In Europe and the United States, *Campylobacter jejuni* is responsible for sporadic outbreaks of intestinal campylobacteriosis (3, 21), with bovines playing a significant role as sources of human campylobacteriosis (Saito *et al*., 2005). According to Saito *et al.*(21), in Japan, the importance of bovines as sources of *C. jejuni* infection have not been totally elucidated, but studies indicate that a large proportion of bovines bred in Japan are intestinal carriers of *C. jejuni*. This fact would facilitate the occurrence of cross contamination in this country, once bovine liver and intestines are popular items in certain restaurants.

PCR-RFLP grouped samples common to poultry, humans and primates in four subtypes each. It should be considered that poultry meat was part of the diet of these animals, and may have been the source of infection for them.

The subtyping study showed that two strains isolated from two aborted goat fetuses from the same farm presented identical restriction patterns (*fla*-RFLP 3), indicating that the contamination of the two animals came from a common source. It should also be emphasized that goat strains were grouped with human strains, showing that this species may also be a source of human infection.

Due to the diversity of patterns grouping human strains with those of different animal origins, it may be observed that the source of *Campylobacter* spp. for humans was not restricted to poultry, in samples collected in the state of São Paulo. Swine, goats, dogs and primates may also be sources of human infection in our state.

Similar results were obtained by Nishimura *et al.* (18), using for the first time a combination of the same restriction endonucleases in PCR-RFLP of a 702 pb fragment of *fla* A gene. These authors observed 25 distinct patterns for 154 *C. jejuni* strains obtained from humans in China and Japan. Steinhauserova *et al* (25), in the Czech Republic, using the same fragment of *fla* A gene and the same enzymes, showed 22 patterns in 156 *C. jejuni* samples from humans and poultry. Tsai *et al.*(26), in Taiwan, obtained 45 different PCR-RFLP patterns using a combination of the three enzymes for the analysis of *Campylobacter jejuni* from 220 strains obtained from humans, poultry and dogs.

In the present study, in spite of the analysis of a greater diversity of animals, the number of patterns (n=16) was not substantially greater that observed by other authors (18, 25, 26).

Nishimura *et al.*(18) showed a correlation between PCR-RFPL and serotyping of strains from patients presenting Guillain-Barré Syndrome (GBS) and carrying serotype PENO19:LIO 7. These authors concluded that PCR-RFLP analysis for *C. jejun* may not only contribute for epidemiological subtyping studies, but also for the analysis of *C. jejuni* subtypes related to GBS.

In the present study, it was observed that subtype *fla*-RFLP 7 was grouped together with reference strain Lior 7, corresponding to serotype PENO19:LIO 7, two human strains (H5 and H7) and two primate strains (M5 and M15). Patterns obtained for these strains by means of the three restriction enzymes were similar to those observed by Nishimura *et al.* (18) in Japan and China, for human strains,

and by Tsai *et al.* (26) in Taiwan, for dog strains. Therefore, the presence of serotype PENO19:LIO 7, main serotype related to Guillain-Barré Syndrome (GBS), was demonstrated in the state of São Paulo.

Control and prevention strategies may not be developed and implemented without the adequate understanding of campylobacteriosis (14).

PCR-RFLP of *fla* A gene demands less equipment when compared with other subtyping techniques, such as sequencing and PFGE (6, 15). Besides, it is a simple, cheap and simple technique for the subtyping of *Campylobacter jejuni* strains and it is discriminatory enough to be applied to molecular epidemiology studies in samples isolated from different origins. Therefore, it is feasible to be used in human and veterinary laboratories (8, 14), enabling the detection of serotype PENO19:LIO 7 strains associated with GBS (Nishimura *et al.* 1996).

ACKNOWLEDGEMENTS

This study was financially supported by FAPESP – Fundação de Amparo a Pesquisa do Estado de São Paulo – research project # 2003/10776-4.

We thank Dra. Angela Cristina Rodrigues Ghilardi, from the Seção de Bacteriologia, Instituto Adolfo Lutz, São Paulo, SP, Brazil, for donation of the reference strain Lior 7.

REFERENCES

1. Allos, B.M. (1997). Association between *Campylobacter* infection and Guillain-Barré syndrome. *J. Infect. Dis.* 176 (2), 125-128.
2. Allos, B.M.; Lippy, F.T.; Carlsen, A.; Washburn, R.G.; Blaser, M.J. (1998). *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome. *Emerg. Infect. Dis.* 4 (2), 263-268.
3. Altekruse, S.F.; Swerdlow, D.L.; Stern, N.J. (1998). Microbial food borne pathogens. *Campylobacter jejuni*. *Vet. Clin. North Am. Food Anim. Pract.,* Review. 14 (1), 31-40.
4. Carvalho, A.C.T.; Ruiz-Palacios, G.M.; Ramos-Cervantes, P.; Cervantes, L.; Jing, X.; Picckering, L.K. (2001). Molecular characterization of invasive and noninvasive *Campylobacter jejuni* and *Campylobacter coli* isolates. *J. Clin. Microbiol.* 39 (4), 1353-1359.
5. Cortez, A.L.L.; Carvalho, A.C.F.B.; Scarcelli, E.; Miyashiro, S.; Vidal Martins, A.M.C.; Burger, K.P. (2006). Survey of chicken abattoir for the presence of *Campylobacter jejuni* and *Campylobacter coli. Rev. Inst. Med. Trop. S. Paulo,* 48 (6), 307-310.
6. Desai, M.; Logan, J.M.J.; Frost, J.A.; Stanley, J. (2001). Genome sequence-based fluorescent length polymorphism of *Campylobacter jejuni*, its relationship to serotyping, and its implications for epidemiological analysis. *J. Clin. Microbiol.* 39 (11), 3823-3829.
7. Djordjevic, S.P.; Unicomb, L.E.; Adamson, P.J.; Mickan, L.; Rios, R. (2007). The Australian *Campylobacter* Subtyping Study Group. Clonal Complexes of *Campylobacter jejuni* identified by Multilocus Sequence Typing are reliably predicted by Restriction Fragment Length Polymorphism analyses of the *fla A* gene. *J. Clin. Microbiol*. 45 (1), 102-108.
8. Dourado, M.E.; Duarte, R.C.; Ferreira, L.C.; Queiroz, J.W.; Illa, I.; Perez-Perez, G.; Guerrant, R.L.; Jerônimo, S.M.B. (2003). Anti-ganglioside antibodies and clinical outcome of patients with Guillain-Barré Syndrome in northeast Brazil. *Acta Neurol. Scand*. 108, (2), 102-108.
9. Duim, B.; Win Ang, C.; Van Bellkum, A.; Rigter, A.; Van Leeuwen, N.W.J.; Endtz, H.P.; Wagenaar, J.A. (2000). Amplified fragment length polymorphism analysis of *Campylobacter jejuni* strains isolated from chickens and patients with gastroenteritis or Guillain-Barré or Miller Fisher syndrome. *Appl. Environ. Microbiol.* 66 (9), 3917-3923.
10. Endtz, H.P.; Ang, C.W.; Van Den Braak, N.; Duim, B.; Rigter, A.; Price, L.J.; Woodward, D.L.; Rodgers, F.G.; Johnson, W.M.; Wagenaar, J.A.; Jacobs, B.C.; Verbrugh, H.A.; Van Belkum, A. (2000). Molecular characterization of *Campylobacter jejuni* from patients with Guillain-Barre and Miller Fisher syndromes. *J. Clin. Microbiol.* 38 (6), 2297-2301.
11. Fitzgerald, C.; Helsel, L.O.; Nicholson, M.A.; Olsen, S.J.; Swerdlow, D.L.; Flahart, R.; Sexton, J.; Fields, P.I. (2001). Evaluation of methods for subtyping *Campylobacter jejuni* during an outbreak involving a food handler. *J. Clin. Microbiol*. 39 (7), 2386-2390.
12. Fujimoto, S.; Yuki, N.; Itoh, T.; Amako, K. (1992). Specific serotype of *Campylobacter jejuni* associated with Guillain-Barre syndrome. *J. Infect. Dis.* 165 (1), 183.
13. Giacoboni, G.; Echeverría, M.G.; Perfumo, C. (2005). PCR-RFLP for *Campylobacter jejuni* subtyping. *Rev. Argent. Microbiol*. 37 (2), 81-83.
14. Gillespie, I.A.; O'Brien, S.J.; Frost, J.A.; Adak, G.K.; Horby, P.; Swan, A.V.; Painter, M.J.; Neal, K.R. (2002). The *Campylobacter* Sentinel Surveillance Scheme Collaborators. A Case-Case Comparison of *Campylobacter coli* and *Campylobacter jejuni* Infection: A Tool for Generating Hypotheses. *Emerg. Infect. Dis*. 8 (9), 937-942.
15. Kéller, J.; Wieland, B.; Wittwer, M.; Stepphan, R.; Perreten, V. (2007). Distribution and genetic variability among *Campylobacter* spp. isolates from different animal species and humans in Switzerland. *Zoon. Public Health.* 54, 2-7.

16. Lior, H.; Woodward, D.L.; Edgar, J.A.; Laroche, L.J.; Gill, P. (1982). Serotyping of *Campylobacter jejuni* by slide agglutination based on heat-labile antigenic factors. *J Clin Microbiol*, 15 (5), 761-768.
17. Meinersmann, R.J.; Helsel, L.O.; Fields, P.I.; Hiett, K.L. (1997). Discrimination of *Campylobacter jejuni* isolates by fla gene sequencing. *J. Clin. Microbiol,* 35 (11), 2810-2814.
18. Nishimura, M.; Nukina, M.; Yuan, J.M.; Shen, B.Q.; M.A.J.J.; Ohta, M.; Saida, T.; Uchiyama, T. (1996). PCR-based restriction fragment length polymorphism (RFLP) analysis and serotyping of *Campylobacter jejuni* isolates from diarrheic patients in China and Japan. *FEMS Microbiol. Lett.* 142 (2-3), 133-138.
19. On, S.; Harrington, C.S. (2001). Evaluation of numerical analysis of PFGE-DNA profiles for differentiating *Campylobacter fetus* subspecies by comparison with phenotypic, PCR and 16S rDNA sequencing methods. *J. Appl. Micrbiol*. 90 (2), 285-293.
20. Saida, T.; Kuroki, S.; Hao, Q.; Nishimura, M.; Nukina, M.; Obayashi, H. (1997). *Campylobacter jejuni* isolates from Japanese patients with Guillain-Barré syndrome. *J. Infect. Dis.* 176 (2), 129-134.
21. Saito, S.; Yatsuyanagi, J.; Harata, S.; Ito, Y.; Shinagawa, K.; Suzuki, N.; Amano, K.; Enomoto, K. (2005). *Campylobacter jejuni* isolated from retail poultry meat, bovine feces and bile, and human diarrheal samples in Japan: comparison of serotypes and genotypes. *FEMS Immunol. Med. Microbiol.* 45 (2), 311-319.
22. Sanz, J.C.; Rios, R.R.; Lopez-Portoles, J.A.; Taveira, J.A.; Simon, C.; Echeita, M.A. (2006). Descripción de um brote de gastroenteritis por *Campylobacter jejuni* y caracterización molecular de la cepa implicada. *Enferm. Infecc. Microbiol. Clin.* 24 (7), 437-439.
23. Scarcelli, E.; Genovez, M.E.; Cardoso, M.V.; Souza, M.C.A.M.; Grasso, L.M.P.S.; Souza, C.A.I.; Torres, A.P. (1998). Avaliação do potencial de disseminação de *Campylobacter* spp por diferentes espécies animais. *Arq Inst Biol.* 65 (1), 55-61,
24. Scarcelli, E.; Piatti, R.M.; Harakava, R.; Miyashiro, S.; Campos Fernandes, F.M.; Campos, F.R.; Francisco, W.; Genovez, M.E.; Richtzenhain, L.J. (2005). Molecular subtyping of *Campylobacter jejuni* subsp. *jejuni* strains isolated from different animal species in the state of São Paulo, Brazil. *Braz. J. Microbiol.* 36 (4), 378-382.
25. Steinhauserova, I.; Ceskova, J.; Nebola, M. (2002). PCR/Restriction fragment length polymorphism (RFLP) typing of human and poultry *Campylobacter jejuni* strains. *Lett. Appl. Microbiol,* 34 (5), 354-358.
26. Tsai, H.J.; Huang, H.C.; Tsai, H.L.; Chamg, C.C. (2006). PCR-based restriction fragment length polymorphism (RFLP) analysis of *Campylobacter jejuni* isolates from humans, chickens and dogs in northern Taiwan. *J. Vet. Med. Sci*. 68 (8), 815-819.
27. Zorman, T.; Heydrickx, M.; Uzunovic-Kamberovic, S.; Smole-Mozina, S. (2006). Genotyping of *Campylobacter coli* and *C. jejuni* from retail chicken meat and humans with campylobacteriosis in Slovenia and Bosnia and Herzegovina. *Int .J. Food Microbiol.* 110 (1), 24 -33.