Pesq. Vet. Bras. 35(4):365-370, abril 2015
DOI: 10.1590/S0100-736X2015000400009

Occurrence and characterization of *Campylobacter* spp. isolates in dogs, cats and children[1]

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ABSTRACT.- Rodrigues C.G., Melo R.T., Fonseca B.B., Martins P.A., Ferreira F.A., Araújo M.B.J. & Rossi D.A. 2015. **Occurrence and characterization of *Campylobacter* spp. isolates in dogs, cats and children.** *Pesquisa Veterinária Brasileira 35(4):365-370.* Laboratório de Biotecnologia Animal Aplicada, Faculdade de Medicina Veterinária, Universidade Federal de Uberlândia, Rua Ceará s/n, Bloco 2D, Sala 43, Bairro Umuarama, Uberlândia, MG 38402-018, Brazil. E-mail: bialucas@yahoo.com.br

To improve the understanding of implications of *Campylobacter* spp. infections in pets and children of different environments were analysed 160 faecal samples from children and 120 from pets (103 dogs and 17 cats). *Campylobacter* spp. were detected in 6.87% of the children and in 18.3% of the dogs and cats. From 33 stool samples positive for *Campylobacter* spp., 57.6% were identified as *C. jejuni,* and 33.4% were identified as *C. coli*. More than 50% of the isolates in pets were resistant to ceftiofur, sulphazotrim, norfloxacin and tetracycline. In humans, most of the isolates were resistant to amoxicillin, cefazolin, ceftiofur, erythromycin and norfloxacin. From 19 isolates of *C. jejuni*, 11 isolates from children and 5 from dogs contained two to four of the virulence genes *flaA, pldA, cadF* or *ciaB.* We found an association between the presence of virulence genes and diarrhoea. Furthermore, an association was observed between the presence of *Campylobacter* spp. and diarrhoea in dewormed pets with blood picture suggestive of bacterial infection, and the therapeutic use of antibiotics was associated with more positive detection of *Campylobacter* spp. in the faeces of pets. Our data indicate that virulent strains of *Campylobacter* spp. can be risk factor to diarrhoea in animals, and that high resistance to antimicrobial agents is common in pets.

INDEX TERMS: *Campylobacter* spp., dogs, cats, children, diarrhoea, infection, epidemiology, virulence genes.

RESUMO.- [**Ocorrência e caracterização de isolados de *Campylobacter* spp. em cães, gatos e crianças.**] Com o objetivo de melhorar o entendimento das infecções por *Campylobacter* spp. em cães, gatos e crianças no Brasil, foram avaliadas 160 amostras fecais de crianças e 120 *swabs* retais de *pets* (103 cães e 17 gatos). Do total das amostras das crianças, 6,87% foram positivas para *Campylobacter* spp. e em cães e gatos a positividade foi de 18,3%. Das 33 amostras positivas para *Campylobacter* spp., 57,6% foram identificadas como *C. jejuni* e 33,4% foram identificadas como *C. coli*. Mais de 50% das amostras isoladas de pets foram resistentes a ceftiofur, sulphazotrim, norfloxacina e tetraciclina. Em crianças, a maioria das amostras foi resistente a amoxilina, cefazolina, ceftiofur, eritromicina e norfloxacina. De 19 isolados de *C. jejuni*, 11 isolados de crianças e cinco (5) de cães tinham dois (2) dos quatro (4) genes de virulência *flaA, pldA, cadF* or *ciaB*. Associação positiva entre a presença de *Campylobacter* spp. e diarreia em cães e gatos foi observada em animais desverminados e com hemograma sugestivo de infecção bacteriana. Também houve associação positiva entre a presença dos genes de virulência e a ocorrência de diarreia, e entre o uso de antibióticos e a positividade para *Campylobacter* spp. em suabes fecais de pets. Os dados desse trabalho indicam que cepas virulentas

[1] Received on February 15, 2014.
Accepted for publication on December 5, 2014.

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de *Campylobacter* spp. são fatores de risco para diarreia em cães e a resistência antimicrobiana é comum em isolados de cães.

TERMOS DE INDEXAÇÃO: *Campylobacter* spp., cães, gatos, crianças, diarreia, infecção, epidemiologia, genes de virulência.

INTRODUCTION

In humans, *Campylobacter* sp. is the most common bacterial cause of foodborne enteritis (EFSA 2009, EFSA 2010). In addition to ingestion of contaminated food and water, direct contact with animals may be a possible source of *Campylobacter jejuni* infection (Damborg et al. 2004, Houf et al. 2008). In dogs, *Campylobacter* spp. are frequently isolated from animals with symptoms of enteritis and asymptomatic animals (Baker et al. 1999, Moser et al. 2001, Chaban et al. 2010). Although symptoms of enteritis may be associated with campylobacteriosis, few veterinarians understand the importance of this bacterium in clinical practice. Antimicrobial resistance is an important factor for *Campylobacter* spp. in different regions of the world in humans and pets.

Dogs and cats are increasingly present in family life, especially with children. In many cases, close contact with animals occurs regularly, and *Campylobacter* spp. can be transmitted to humans by contact (Lengerh et al. 2013).

Knowledge of the incidence, association of clinical symptoms of virulent strains of *Campylobacter* spp., antimicrobial resistance, and epidemiology of *Campylobacter* spp. infection in pets and children enables measures to prevent human infections and guide the treatment and care provided for animals.

This study aimed to identify dogs, cats and children positive for *Campylobacter* spp. through faecal samples, to establish associations between the presence of the microorganism and gastrointestinal disorders, and to evaluate the presence of virulence genes and antimicrobial resistance in isolated strains.

MATERIALS AND METHODS

We analysed 103 samples from dogs, 17 samples from cats of different ages, and 160 samples from up to 5-year-old children. After authorization, the samples were collected from patients in the Department of Internal Medicine of Small Animal Veterinary Hospital (HVet-UFU) and sectors of the Emergency and Clinic Department of the Clinics Hospital (HC-UFU), both at the Federal University of Uberlândia. Samples were collected from July 2010 to March 2011. Dogs and cats with clinical symptoms of diarrhea or other diseases (dermatitis, ear infections, ocular infections and accidents) and children with symptoms of diarrhea or anal itching were chosen randomly. Type of diarrhea (as colour, appearance of faeces, time course) and antibiotic usage in the animals and children were recorded. Blood picture of dogs and cats with diarrhoea showed changes suggestive of bacterial infection. The experiment was approved by the Ethics Committee on Animal Use and the Ethics Committee on Humans from the Federal University of Uberlândia, number 089/2010.

The collection of animal faeces was performed directly from the rectum of animals with sterile swabs. Faeces samples were collected from children and stored in sterile jars by health professionals. The samples were immediately transported to the laboratory and analysed. We collected information regarding the presence of pets in the residences of children positive for *Campylobacter* spp. We also verified whether the animals treated at HV-UFU that were positive for *Campylobacter* spp. lived in the residences of children.

In the laboratory, Bolton broth (Oxoid®) with an antibiotic supplement (Selective Supplement Oxoid®) was added, and the stool samples were homogenised. The samples were supplemented with 5% horse blood hemolysate and subjected to pre-enrichment in a microaerophilic atmosphere (Probac of Brazil®) with anaerobic jars at 37°C for 24 hours. After pre-enrichment, the material was seeded in selective Campylobacter Blood-Free Selective Agar Base modified medium (mCCDA) (Oxoid®), added to the supplement antibiotic (Oxoid®) and 5% horse blood hemolysate and incubated at 37ºC for 48 hours in anaerobic jars under microaerobic conditions (Probac of Brazil®). Suspected colonies were confirmed by the modified Gram stain by observing the morphology of spiral and curved rods or "gull wings".

The phenotypic identification of differentiated species was performed as recommended by Silva et al. (2007) using the oxidase test, catalase production, hippurate hydrolysis, nitrate reduction, H_2S production, growth at 25°C and 42°C and the DNAse test. All the tests were performed in duplicate, and *C. jejuni* (ATCC 33291) was used as the positive control.

In parallel with the phenotypic identification, typical colonies were subjected to molecular identification using a multiplex PCR technique. The DNA was extracted from colonies by thermal extraction and amplified using the primer described by Harmon et al. (1997). The reaction solution (Invitrogen®) was prepared and the amplification cycle (Eppendorf®) was performed according to Harmon et al. (1997). As a positive control, we used the standard strains of *Campylobacter jejuni* ATCC 33291 and *Campylobacter coli* ATCC 43478. The amplified products were separated by electrophoresis, stained with SYBR® solution Safe DNA gel stain (Invitrogen®), supplemented with a 100 bp molecular weight marker (Invitrogen®) and visualised in UV light in a transluminator (L PIX EX Loccus Biotechnology).

The primer pairs and PCR assay described for Hänel et al. (2004) and Zheng et al. (2006) were used for the analysis of virulence factors associated with adhesion and cell invasion using multiplex PCR analysis. The final volume of the amplification reaction (50µL) comprised 20ng of bacterial DNA and the following reagents: 10mM Tris-HCL, 50mM KCl, 200 mM of each deoxynucleotide triphosphate (dNTP), 2.6mM MgCl2, 10 picomoles of primer for flaA and pldA, 40 picomoles for gene cadF and ciaB and 1.25 U Taq DNA polymerase. The determination of each gene was performed individually. The positive control *C. jejuni* NCTC 11351 and a negative control were used in all of the amplification reactions. Amplification was performed in a thermocycler (Eppendorf ®) according to the following cycles: 1 cycle starting at 95°C for 10 min, 35 cycles consisting of 3 (three) steps including denaturation at 95°C for 1 min, annealing at 45°C for 1 min and extension at 72°C for 2 min and a final cycle with extension at 72°C for 10 min. Separation and visualisation of the amplified products

were performed using the same technique as described for multiplex PCR to identify the species.

The selected antimicrobials were based on indicated by the CLSI, the participants of the main classes in public/ health (third-generation cephalosporins, fluoroquinolones and macrolides) and used in the animal clinic. The profile of antimicrobial susceptibility testing was performed by disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI 2010) for the following antimicrobials: amoxicillin (10mg), erythromycin (15µg), gentamicin (10mg), neomycin (30µg), norfloxacin (10mg), sulphazotrim (25µg) and tetracycline (30µg) (Laborclin ®). Standard samples of *C. jejuni* (ATCC 33291) were used for the control test.

To determine the epidemiological association measures, we used chi-square tests and odds ratios (OR) (p≤0.05) using the statistical program Graphpad Prism 5.

RESULTS AND DISCUSSION

In the children, 6.87% (11/160) of the faecal samples tested were positive for *Campylobacter* spp. The 120 stool samples analysed from pets included 103 samples from dogs and 17 from cats. Overall, 18.3% (22/120) of the pet samples tested were positive for *Campylobacter* spp. including 19.41% (20/103) of dog samples and 11.7% (2/17) of cat samples.

The rate of *Campylobacter* spp. isolated from children was slightly lower compared with the results of other authors. In Rio de Janeiro, Mangia et al. (1993) found that 9.9% (15/153) of stool samples from children under 5 years of age with and without diarrhoea were positive for *Campylobacter* spp.

Only two of the children positive for *Campylobacter* spp. were reported to have had contact with animals, and the corresponding animals negative for *Campylobacter* spp. Among all the positive pets that lived with children, only 5 cases provided corresponding stool samples from children, and all were negative for *Campylobacter* spp. faeces. Wolf et al. (2001), Damborg et al. (2004) and Houf et al. (2008) studied possible transmission of Campylobacter from household pets to humans via constant direct physical contact. In our study it was not possible to evaluate the importance of dogs as reservoirs for the infection of children, because few samples were obtained from children living with pets positive for *Campylobacter.*

Our study included only a small number of cats, but the number of dogs allowed to verify the high occurrence of bacteria in the dogs (19.41%). In Nigeria, Salihu et al. (2010) noted the importance of healthy dogs and cats as reservoirs of *Campylobacter* (27.7% of dogs and 18.3% of cats were positive for *Campylobacter* spp.). In an impoverished community in Buenos Aires, Lopez et al. (2003) reported a prevalence of *Campylobacter* sp. of 16.96% and 20% in the faeces of dogs and cats, respectively. Chaban et al. (2010) found that 58% of the healthy dogs and 97% of the diarrhoeic dogs shed detectable levels of *Campylobacter* spp. using qPCR (quantitative PCR) in a region of Canada. These findings show the importance of pets as reservoirs of *Campylobacter* spp.

The presence of *Campylobacter* spp. was higher in dogs compared with children (Fig.1). As studies comparing the occurrence of *Campylobacter* spp. in children and animals are not common, we had difficulty to interpret this result. Despite the importance of our study to show the incidence and virulence genes of the strains of *Campylobacter* spp. in dogs, cats and children, the majority of children and dogs of this study did not live with each other and samples were from different locations, different hygiene habits and social classes.

The results of the occurrence of *Campylobacter* spp. in faeces of children, and dogs are shown in Table 1. The genotypic and phenotypic identification for *Campylobacter jejuni* and *Campylobacter coli* using multiplex PCR demonstrated homology of 100%. There was no significant difference between the percentage of isolation of *C. jejuni* (57.6% (19/33)) and *C. coli* (33.3% (11/33)). Among the 20 isolated from dogs, 15 were identified as *C. jejuni*, 4 were identified as *C. coli* and 1 (one) was identified as *Campylobacter* sp. (Table 1). Although *C. upsaliensis* has been reported to be the most prevalent species in dogs in different parts of the world (Steinhauserova et al. 2000, Tsai et al. 2007, Salihu et al. 2010, Parsons et al. 2011), this was not verified in our study. The prevalence of the strain may vary from region to region.

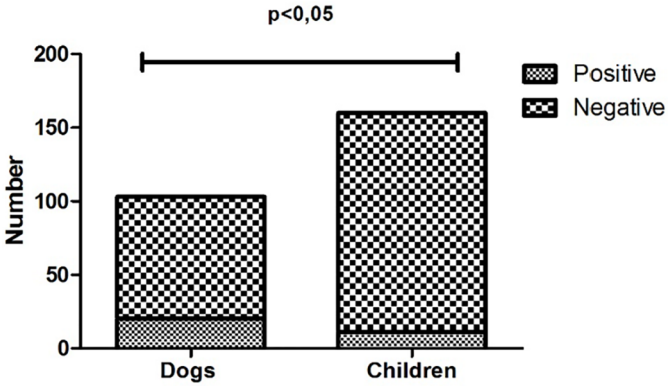

Fig.1. Occurrence of *Campylobacter* spp. in faeces of children and dogs seen at the Veterinary Hospital and Clinics Hospital of the Federal University of Uberlândia.

Table 1. Species identification of *Campylobacter* spp. isolated from feces of dogs, cats and children by multiplex PCR

	C. jejuni		*C. coli*		*Campylobacter* sp.	
	N	%	N	%	N	%
Dogs	15	79.0	4	36.4	1	33.4
Cats	0	0.0	2	18.1	0	0.0
Children	4	21.0	5	45.4	2	66.6

N = Number.

High antimicrobial resistance was observed in *Campylobacter* spp. isolates. The antimicrobial resistances observed in *C. jejuni* and *C. coli* from animals and humans are shown in Table 2. *Campylobacter coli* showed high resistance to the β-lactams amoxicillin and ceftiofur, and *C. jejuni* showed high resistance to ceftiofur in the isolates from

Table 2. Antimicrobial resistance of *Campylobacter jejuni* and *C. coli* isolated from pets and children

	Campylobacter jejuni		*Campylobacter coli*	
	Children	Pets[a]	Children	Pets
Nalidixic acid	25.0%	33.3%	40.0%	33.0%
Amoxicillin	75.0%	26.7%	60.0%	50.0%
Cefazolin	75.0%	40.0%	60.0%	67.0%
Ceftiofur	75.0%	53.3%	40.0%	50.0%
Enrofloxacin	0.0%	40.0%	40.0%	50.0%
Erythromycin	75.0%	46.7%	60.0%	67.0%
Gentamicin	0.0%	33.3%	20.0%	50.0%
Lincomycin	0.0%	60.0%	60.0%	67.0%
Neomucin	0.0%	40.0%	0.0%	17.0%
Norfloxacin	50.0%	46.7%	40.0%	67.0%
Sulfazotrim	25.0%	66.7%	40.0%	100.0%
Tetracycline	50.0%	60.0%	20.0%	50.0%

[a] Dogs and cats.

pets (Table 2). Modolo et al. (2003) also observed isolated highly resistant to β-lactams. More than 50% of the isolates of *C. coli* showed resistance to enrofloxacin and norfloxacin. Kuana et al. (2008) also found high rates of resistance to quinolones, particularly enrofloxacin, in *Campylobacter* spp. Rossi et al. (2008) reported that the extensive use of fluoroquinolones in dogs and cats in Italy resulted in a high rate of resistance to nalidixic acid and ciprofloxacin in *Campylobacter* spp. In our study, more than 50% of the isolatesof *C. coli* and *C. jejuni* were resistant to erythromycin and tetracycline. Our results showing resistance to these antibiotics are highly relevant because these antibiotics are the drugs of choice for the treatment of infections (Kuana et al. 2008).

The isolates in our study showed high levels of resistance. Resistance was observed mainly for drugs class commonly used in the clinical treatment of gastroenteritis in animals, such as sulphazotrim. Resistance to antibiotics is concerning and shows the necessity of proper antimicrobial use in the clinic to minimize the selection pressure for these microorganisms.

Of the isolates from children identified as *C. jejuni*, 75% were resistant to the antibiotics amoxicillin, cefazolin, erythromycin and ceftiofur. Higher resistance to amoxicillin, cefazolin, erythromycin and lincomycin (60%) was observed in isolates identified as *C. coli*. Isolated from pets and children showed more resistance to β-lactam, macrolide and quinolone antimicrobials. Macrolides and fluoroquinolones are the antibiotics of choice for treatment in children (Engberg et al. 2001, Moore et al. 2005).

Due to the self-limiting nature of campylobacteriosis, antimicrobials are generally not recommended for treatment in humans except in severe cases in which fluoroquinolones and macrolides are recommended (Yates 2005). However, resistance to norfloxacin (fluoroquinolone) was confirmed in this study. Studies conducted in the United States, Poland and other EU countries have also confirmed this trend (Gupta et al. 2004, Rozynek et al. 2008, NARMS 2007, EFSA 2010).

The index of association OR showed that children positive for *Campylobacter* spp. in faeces were 3.57 times more likely to have diarrhoea compared with the children that tested negative; however, the association was not statistically significant . In São Paulo, Brazil, Palma et al. (1997) found that the second most common agent isolated from cases of childhood diarrhoea was *Campylobacter* spp. (3/40, 7.5%). Our findings revealed asymptomatic children that were positive for *Campylobacter* spp., which is consistent with a previous study. Mendes et al. (1987) mentioned that people in developing countries did not always exhibit clinical symptoms when infected with *Campylobacter* spp. The recovery of *Campylobacter* organisms from children without diarrhoea is common in developing countries. Acquisition of the pathogen as a result of poor sanitation and contact with animals early in life may cause infections in healthy children (De Wit et al. 2001). Blaser (1997) found that healthy children and adults in developing countries were constantly exposed to *Campylobacter* spp. antigens in the environment. As a consequence, serum antibodies to *Campylobacter* spp. develop early in life in children in developing countries, and the levels of the antibodies tend to be higher compared with children in developed countries.

There was no association between antibiotic use and the occurrence of *Campylobacter* spp. in children. We were unable to effectively evaluate this finding, and little or no data concerning this topic exist in the literature.

There was an association between positive test results for *Campylobacter* spp. and clinical symptoms of diarrhoea in pets. The frequency of *Campylobacter* spp. infection was 7.38 times higher in animals with diarrhea (Table 3). An association between the presence of *Campylobacter* spp. in stools and diarrhoea in dogs was also observed by Burnens et al. (1992), who analysed the rates of *Campylobacter* spp. in animals with gastroenteritis and asymptomatic animals. Chaban et al. (2010) found that 58% of healthy dogs and 97% of diarrhoeic dogs shed detectable levels of *Campylobacter* spp. using qPCR (quantitative PCR) in a region of Canada. Rossi et al. (2008) studied different species of Campylobacter strains isolated from dogs and cats and found a possible association between the isolation of these microorganisms in animals with clinical disease and antimicrobial resistance of the strain.

Table 3. Association between clinical symptoms of diarrhea and positivity for *Campylobacter* spp. animal feces pets treated at Veterinary Hospital

Campylobacter spp.	Diarrhea	Asymptomatic	Total
Positive	17	5	22
Negative	31	67	98

p<0.05; OR=7.38.

Except for 1 (one) dog positive for *Campylobacter* spp. that was 11 years old and had chronic black diarrhoea, abdominal pain, fever, vomiting and anorexia, all the other animals positive to *Campylobacter* spp. had episodes of watery diarrhoea. These cases presented acute watery diarrhoea with blood and mucus lasting for approximately 4 (four) days along with the presence of vomiting and fever. These animals were vaccinated against major diseases that affect dogs (distemper, parvovirosis, infectious hepatitis, diseases caused by adenovirus type 2, canine parainfluenza virus, coronavirosis, leptospirosis), showed no parasites in the blood or faeces and received anti-parasite treatments. In addition, animals had blood picture suggestive of bacterial infection. Only 2 (two) of the 17 animals positive for *Campylobacter* spp. and diarrhoea were cats. Only the cats with diarrhoea tested positive for *Campylobacter* spp., and these cats were less than 3 months old. Of the 15 dogs with positive test results and diarrhoea, 10 were about 5 (five) months old, 4 (four) were 2 (two) years old and 1 (one) dog 11 years old. Other authors describe that age is an important factor for campylobacteriosis in animals and that dogs under 6 months of age have a greater risk of contracting the disease (Aquino et al. 2002, Ackey et al. 2006). Our findings are important because little is known about the importance of *Campylobacter* spp. as pathogens in pets in veterinary clinics in countries such as Brazil. Similar to humans, the importance of *Campylobacter* spp. in immunosuppressed

dogs and cats and the presence of autoimmune diseases (Yu et al. 2006) should be studied in future clinical pets.

A total of 17 of the 22 positive animals had diarrhoea, and 5 (five) were asymptomatic. Only 2 (two) of the 17 animals positive for *Campylobacter* spp. and diarrhoea were cats. Only the cats with diarrhoea tested positive for *Campylobacter* spp., and these cats were less than 3 months old. Of the 15 dogs with positive test results and diarrhoea, 10 dogs were approximately 5 (five) months old, 4 (four) dogs were 2 (two) years old and 1 (one) dog 11 years old.

A positive association was observed between *Campylobacter* and concurrent use of antibiotics (Table 4). Pets treated with antimicrobials were 57.41 times more likely to test positive in faeces compared with untreated pets (Table 4). The previous use of antimicrobials may reduce the natural microflora of the gut and favours the colonisation and multiplication of *Campylobacter* after infection. Antimicrobial use in dogs and cats should be evaluated. In healthy humans the disease is self-limiting and in most cases it does not need treatment. In dogs and cats very little is known about the pathogenesis and disease progression. Thus, it would be interesting knowledge of isolated by antimicrobial resistance test.

Table 4. Association between antibiotic use and presence of *Campylobacter* spp. in animal feces pets treated at Veterinary Hospital

Campylobacter spp.	In use of antibiotics	No use of antibiotics	Total
Positive	22	0	22
Negative	43	55*	98

p<0.05; OR=57.41; * asymptomatic dogs.

Among the strains of *Campylobacter jejuni* isolates detected in the dogs, 4 (four) virulence genes were simultaneously detected in one sample, and 3 (three) genes were simultaneously detected in 2 (two) samples (*flaA, cadF* and *ciaB*, or *flaA, pldA* and *cadF*). Two isolates contained the *flaA* gene or *cadF* and *ciaB* genes, and 2 (two) isolates contained only 1 (one) virulence gene (*cadF* or *flaA*). All the strains isolated from the children's stool samples contained the *flaA* and *cadF* genes. Two of the isolates demonstrated the *ciaB* gene, and 1 (one) contained the *pdlA gene*. We found an association between the presence of 4 (four) virulence genes in *Campylobacter jejuni* and the presence of diarrhoea in pets and children (Table 5). Virulence genes were present in all the diarrhoeal specimens isolated from pet and human samples (Table 5). Our results are consistent with Hänel et al. (2004) and Zheng et al. (2006), who report that the genes *flaA*, *ciaB, cadF* and *pldA* are important and can be used as a reference in the study of the mechanisms of pathogenicity of *C. jejuni.*

Table 5. Association between the presence of virulence genes in *Campylobacter jejuni* and the occurrence of diarrhea in children under 5 (five) years treated at Clinics Hospital and pets treated at Veterinary Hospital

Virulence genes	Diarrhea	Asymptomatic	Total
Presence of genes	12	0	12
No presence of genes	5	2	7

p<0.05; OR=11.36.

The *flaA, cadF, pldA* and *CiaB* genes encode proteins involved in adhesion and invasiveness of *C. jejuni.* The *flaA* gene is required for the adhesion and invasion of epithelial cells (Wassenaar et al. 1991), and the *ciaB* gene encodes a protein involved in cell invasion (Rivera-Amill et al. 2001). The *cadF* gene encodes a protein that interacts with fibronectin in the extracellular matrix of the host cell participating in the colonisation (Monteville et al. 2003). The *Plda* gene is related to cellular invasion and encodes a protein involved in the synthesis of outer membrane phospholipase (Ziprin et al. 2001). These results show that the genes studied in this work help identify virulent strains of *C. jejuni* in animals and humans.

CONCLUSIONS

This study shows high antimicrobial resistance in strains of *Campylobacyter jejuni* and *C. coli* isolated from children and pets.

It also shows that the presence of *Campylobacter* spp. is associated with diarrhoea in animals, which may be associated with the presence of virulence genes. In addition, antimicrobial therapy is associated with increased likelihood of positive *Campylobacter* spp. in the faeces of pets.

Conflict of interest statement.- None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgements.- This study was financed by the "Fundação de Amparo a Pesquisa de Minas Gerais" (FAPEMIG) (Minas Gerais Research Support Foundation) and "Conselho Nacional de Desenvolvimento Científico e Tecnológico" (CNPq) (Federal Brazilian Research Support Foundation).

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