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# Occurrence and Antimicrobial Susceptibility of *Campylobacter jejuni* and *C. coli* in Dog Feces from Public Parks in Southern Ecuador

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**ABSTRACT**

***Background*:** *Campylobacter jejuni* and *C. coli* are important agents of diarrhea in human beings. Several animals are reservoirs for these bacteria and they may contribute to environmental contamination through fecal shedding. The presence of dog feces in urban settings could be a risk of *Campylobacter* environmental contamination and a problem of public health concern. Besides environmental contamination, the increasing antimicrobial resistance among *Campylobacter* is another issue of public health significance. The aims of this study were to determine the prevalence of *C. jejuni* and *C. coli* in dog feces collected from public parks of Loja city, Ecuador, and establishing their susceptibility to six antibiotics.
***Materials, Methods & Results*:** A total of 70 fresh-looking non-diarrheal dog fecal samples were collected from public parks of Loja city. An amount (c. a. 1 g) of each fecal specimen was collected with a swab, placed in a tube containing Bolton enrichment broth and processed within the next six hours. *Campylobacter* spp. were isolated by direct plating onto Butzler selective agar and into Bolton enrichment broth, which were incubated at 42°C for 48 h in microaerophilic conditions. From Bolton enrichment broth subcultures to Butzler selective agar were made and also to blood agar, the latter using the passive filtration method in order to isolate *C. upsaliensis*. Identification was made firstly using the traditional phenotypic test (catalase, oxidase, oxygen tolerance, growth at 25°C, hippurate and indoxyl acetate hydrolysis, and cephalothin and nalidixic acid susceptibility) and corroborated by the genus-specific polymerase chain reaction (PCR) and by the multiplex PCR (m-PCR) method proposed for the characterization of the six most common clinical *Campylobacter* species. Susceptibility to ampicillin, amoxicillin/clavulanic acid, erythromycin, tetracycline, ciprofloxacin and gentamycin was performed by the disk diffusion method following the 2014 recommendations of The European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Committee for the Antibiogram of the French Society of Microbiology. *Campylobacter* was isolated in 7/70 samples (10%) corresponding 4 (5.7%) to *C. jejuni* and 3 (4.3%) to *C. coli. C. upsaliensis* was not found. All the strains were susceptible to ampicillin, amoxicillin/clavulanic acid, erythromycin and gentamycin, six were resistant to tetracycline but all the seven strains were ciprofloxacin-resistant.
***Discussion*:** The isolation frequency of *C. jejuni* and *C. coli* in dog feces collected in the public parks of Loja city was higher than that reported by Wright (4.6%) for similar type of samples. However, it was lower than that shown in the other reports when dogs were directly sampled. Besides the aesthetically undesirable aspect of the presence of feces in public settings, they may represent a hygiene and public health problem because dog feces may contain potentially pathogenic bacteria for humans, like *Salmonella* spp., *Escherichia coli*, *Yersinia* spp. and *Campylobacter* spp. All seven isolated strains were susceptible to ampicillin, amoxicillin/clavulanic acid, erythromycin and gentamycin, six were resistant to tetracycline and surprisingly all of them were resistant to ciprofloxacin. The latter fact reflects the overuse of fluoroquinolones in animals, representing an emerging epidemiological problem with implications for treatment in humans. Dog feces deposited in public parks of Loja city yielded *C. jejuni* and *C. coli* suggesting that dogs could be a common source of environmental contamination with the additional risk of widespread ciprofloxacin resistant strains.

**Keywords:** *Campylobacter*, dogs, environmental contamination, antimicrobial susceptibility, epidemiology.

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## INTRODUCTION

*Campylobacter jejuni* and *C. coli* have become very important agents of human infectious diarrhea, being considered the most common bacterial cause of gastroenteritis in the industrialized countries and the second or the third cause in developing countries [8,16,22]. Transmission to humans includes consumption of contaminated water and food of animal origin, handling of raw chicken and other poultry meats and direct contact with animals or their feces [2,5,9,16,21]. Being zoonotic bacteria, they used various wild and domestic animal species as natural reservoirs. Among the latter are poultry, cattle, swine and pets like cats and dogs [5,8,12,16,21,22].

Intestinal carriage of *Campylobacter* in healthy dogs varies from 16.9% to 51.4% in South American (SA) countries and from 7.9% to 58% in some industrialized countries [13,21]. *C. jejuni* and *C. coli* have also been isolated from different environment settings, where contamination occurs through animal feces [8,13,15,21,22] thereby the presence of dog feces in urban settings is considered to be a problem of public health concern [3,12].

Besides environmental contamination, the increasing antimicrobial resistance among *Campylobacter* is another issue of public health concern [20,21]. In SA countries, the emergence of *C. jejuni* and *C. coli* strains of human and animal origin resistant to erythromycin, tetracycline, ampicillin and quinolones has been widely reported, where quinolone resistance reached rates of over 60% [8].

The aims of this study were to determine i) the prevalence of *C. jejuni* and *C. coli* in dog feces collected from public parks of Loja city and ii) the susceptibility of the isolated strains to six antimicrobials.

## MATERIALS AND METHODS

### *Collection of samples*

A total of 70 fresh-looking non-diarrheal dog fecal samples were collected from public parks of Loja city, Southern Ecuador (3°59' Lat S; 79°12' Long W). An amount (c. a. 1 g) of each fecal specimen was collected with a swab, placed in a tube containing Bolton enrichment broth and transported to the microbiological laboratory at the Department of Health Sciences in the Universidad Técnica Particular de Loja, and subsequently processed within the next six hours.

### *Isolation and identification*

*Campylobacter* spp. were isolated by direct plating onto Butzler selective agar[1] and into Bolton enrichment broth[1], which were incubated at 42°C for 48 h in microaerophilic conditions. From Bolton enrichment broth subcultures to Butzler selective agar were made and also to blood agar[1], the latter using the passive filtration method in order to isolate *C. upsaliensis* [7].

*Campylobacter* spp. were firstly identified through Gram stain, catalase, oxidase, oxygen tolerance, growth at 25°C, hippurate and indoxyl acetate hydrolysis tests and cephalothin and nalidixic acid susceptibility tests as recommended by Debruyne *et al*. [4] and later, confirmed by the genus-specific polymerase chain reaction (PCR) of Linton *et al*. [10] and by the multiplex PCR (m-PCR) methods proposed for the characterization of the six most common clinical *Campylobacter* species of Yamazaki-Matsune *et al*. [24].

### *Antimicrobial susceptibility tests*

Susceptibility to ampicillin, amoxicillin/clavulanic acid, erythromycin, tetracycline, ciprofloxacin and gentamycin was determined by the disk (Antimicrobial disks)[1] diffusion method following the 2014 recommendations of The European Committee on Antimicrobial Susceptibility Testing- EUCAST and the Committee for the Antibiogram of the French Society of Microbiology [18].

## RESULTS

Of the 70 dog feces examined, 7 (10.0%) yielded *Campylobacter*, corresponding 4 (5.7%) to *C. jejuni* and 3 (4.3%) to *C. coli*. None of the analyzed samples were positive for *C. upsaliensis*. All the positive cultures were direct plate positive/enrichment positive.

With regard to antimicrobial susceptibility, all seven strains tested were susceptible to ampicillin, amoxicillin/clavulanic acid, erythromycin and gentamycin, six were resistant to tetracycline and surprisingly all of them were resistant to ciprofloxacin (Table 1).

**Table 1.** Antimicrobial susceptibility patterns of seven *Campylobacter jejuni/coli* strains isolated from dog feces collected prom public parks in Loja city, Southern Ecuador.

| Strain | AMP | AMOX-CLAV | TETRA | CIPRO | ERYT | GENTA | SPECIES |
|---|---|---|---|---|---|---|---|
| 13 | S | S | R | R | S | S | *C. coli* |
| 19 | S | S | R | R | S | S | *C. jejuni* |
| 25 | S | S | R | R | S | S | *C. coli* |
| 35 | S | S | R | R | S | S | *C. coli* |
| 37 | S | S | R | R | S | S | *C. jejuni* |
| 39 | S | S | R | R | S | S | *C. jejuni* |
| 48 | S | S | S | R | S | S | *C. jejuni* |

AMP: ampicillin; AMOX-CLAV: amocicillin-clavulanic acid; TETRA: tetracycline; CIPRO: ciprofloxacin; ERYT: erythromycin; GENTA: gentamycin. S: susceptible; R: resistant.

## DISCUSSION

The results showed that the isolation frequency of *C. jejuni* (5.7%) and *C. coli* (4.3%) in dog feces collected in the public parks of Loja city was higher than that reported by Wright (4.6%) for similar type of samples [23]. However, it was lower than that shown in the other reports when dogs were directly sampled. In fact, Procter *et al*. [12], studying *Campylobacter* carriage in pet dogs visiting dog parks in Canada, found that *Campylobacter* spp. were present in 43.0% of fecal samples. Of the isolated *Campylobacter* strains, 86.1% were *C. upsaliensis*, 13.0% were *C. jejuni* and 0.9% was *C. coli*. However, we did not isolate *C. upsaliensis*, and the prevalence of *C. jejuni* found in our study (10.0%) was similar to the 13.0 % reported by Procter *et al*. [12] while *C. coli* prevalence in our study was considerably higher (4.3% versus 0.9%). These and other data suggest that in developing countries, *C. coli* can be isolated in higher rates from different sources, including human, animals, environmental or food samples, than in developed countries [8].

In some studies, which include dog fecal samples collected from the streets, a higher isolation rates have been reported, reaching frequencies over 40% [8,12,21]. The difference could be explained by the consideration that most of the samples analyzed in our study were from animals belonging to known owners that walked their dogs in the parks, which may indicate a good standard of care. The fecal samples of the streets could also be from stray dogs, whose loitering habits reflect bad standards of care. There were remarkable differences shown between stray dogs and pet dogs in the incidence of intestinal carriage of *Campylobacter* [1,6,17].

The antimicrobial susceptibility showed that all seven strains tested were susceptible to ampicillin, amoxicillin/clavulanic acid, erythromycin and gentamycin, six were resistant to tetracycline and surprisingly all of them were resistant to ciprofloxacin. The latter is a fact that reflects the overuse of fluoroquinolones in animals and represents an emerging worldwide epidemiological problem with implications for treatment in humans [11,14,19, 22].

Besides the aesthetically undesirable aspect of the presence of feces in public settings, they may represent a hygiene and public health problem because dog feces may contain potentially pathogenic bacteria for humans, like *Salmonella* spp., *Escherichia coli*, *Yersinia* spp. and *Campylobacter* [3]. In our study, we found that dog feces deposited in public parks yielded *C. jejuni* and *C. coli* suggesting that dogs could be a common source of environmental contamination with the additional risk of widespread ciprofloxacin resistant strains.

To our knowledge, this is the first attempt to demonstrate the presence of *Campylobacter* species in the environment in Southern Ecuador, as well as their antimicrobial susceptibility patterns. Further research is needed to contribute to a better understanding of the epidemiology of *Campylobacter* spp. and their antimicrobial behavior in developing countries. This could help to develop management and control programs, which can be used to reduce the risk of transmission from animals, food and environmental sources to humans.

## CONCLUSIONS

Dog feces deposited in public parks of Loja city yielded *C. jejuni* and *C. coli* highly resistant to ciprofloxacin, suggesting that dogs could be a common source of environmental contamination that represents a hygiene and public health problem because dog feces may contain potentially pathogenic bacteria for humans, with the additional risk of widespread ciprofloxacin resistant strains.

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