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Prevalence of *Campylobacter* spp and Antimicrobial Resistance of *Campylobacter jejuni* in Domestic Dogs in The Central Region of Algeria

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Abstract

Campylobacter spp rank among the primary bacterial agents of gastroenteritis worldwide, and dogs serve as a potential reservoir for these pathogens**.** This study aimed to investigate the prevalence of *Campylobacter* species in Algerian dogs, identify the species of the *Campylobacter* isolates and the antimicrobial susceptibility of *Campylobacter jejuni* isolates. Rectal swab samples were collected from 200 dogs whose ages ranged from two weeks to 24 months. They were subsequently analyzed following OIE recommendations. Antibiotic susceptibility was assessed using the disc diffusion method in accordance with CA-SFM guidelines. A questionnaire was completed for each animal. *Campylobacter* spp. were isolated from 26% (52/200), distributed among the diarrheic dogs as 16.25% (13/80) and non-diarrheic dogs as 32.5% (39/120). Statistically significant differences were recorded (P<0.05) within the age groups of all dogs examined: 36.7% of the young dogs (< 1 year) tested positive, compared to 19.7% of the old ones (> 2 years). A predominance of *Campylobacter jejuni* (22/52) followed by *Campylobacter upsaliensis* (20/52) was noted. *Campylobacter jejuni* strains exhibited high levels of resistance to quinolones tested; 63% (14/22) to ciprofloxacin and nalidixic acid and low levels of resistance to 27% (6/22) to tetracycline. All isolates of *Campylobacter jejuni* were susceptible to erythromycin and chloramphenicol. The result of this study, demonstrates that dogs in central Algeria might pose a zoonotic risk for humans. The high resistance levels to tetracycline and ciprofloxacin of strains of *Campylobacter jejuni* isolated may be considered a threat to public health.

Keywords: Algeria, Antibiotic- resistance, *Campylobacter*, dogs, diarrhea.

Introduction

Campylobacteriosis is one of the most widespread zoonotic diseases and the most common cause of bacterial enteritis in humans in many countries [1, 2]. *Campylobacter* spp. are gram-negative, spiral-shaped, and motile bacteria that grow best under microaerophilic conditions. *Campylobacter* causes more cases of diarrhea compared to other food-borne pathogens like Salmonella or Yersinia, in both developing and developed countries [3]. Several *Campylobacter* species are known to be pathogenic to humans [4]. More than 80% of the cases are caused by *C. Jejuni* and 10% are caused by *C. coli*. Other species of *Campylobacter*, such as *C. concisus*, *C. upsaliensi*, *C. lari* and *C. fetus* may also be

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associated with diarrhea in humans [5]. *Campylobacter*'s ubiquitous nature and extent of adaptation to wild farm animals and pets, contribute to the introduction and persistence of this bacterium in different biotopes [6**].** Many studies show that contact with pets, chiefly dogs were considered a possible source of *Campylobacter* infection in humans [7, 8, 9, and 10]. *Campylobacter* is generally not regarded as pathogenic in dogs [11, 12]. However, high isolation risks have been found in fecal samples from both healthy and diseased companion animals [13]. In addition to the high prevalence of *Campylobacter spp* in dogs which can reach amount to 87% [14] and which constitute a major problem for public health, *Campylobacter*, especially *C. jejuni* has shown a prolonged resistance to various antibiotics, including ciprofloxacin and erythromycin which are the major molecules for the treatment of *Campylobacter* infections.

In Algeria, *Campylobacter* in humans has been the subject of several studies [15, 16]. However, there are few works regarding *Campylobacter* species in animals, except for some studies in broilers, cattle and sheep [17, 18, 19]. But until now, no studies have been carried out on dogs.

The present study was performed on dogs in the central region of Algeria.

Therefore, this study was performed on dogs in the central region of Algeria to isolate and identify Campylobacter species from canine rectal swabs and to determine the antibiotic susceptibility of isolated C. jejuni strains.

Material and Methods

Sample collection

Rectal swab samples were collected from 200 domestic dogs, 80 diarrheal and 120 non diarrheal, of different ages randomly chosen in 10 private veterinary clinics in the Algiers region. There were 100 males and 100 female dogs which were classified into three age groups: under 12 months (68 samples), 12-23 months (68 samples) and over 24 months (64 samples).

A questionnaire was filled in upon sample collection that included questions on age, gender, health status, diarrhea, and the presence of antibiotic and/or corticosteroids therapy. Rectal swabs samples in Stuart's transport medium were transported to the central laboratory of clinical biology (CHU Nafissa hamoud-Ex Parnet, Algiers) and analyzed within 4 hours of collection.

Isolation and identification

Samples were cultured in a microaerophilic environment generated with CampyGen sachets (Oxoid, France) using an anaerobic jar. Within 4 hours of collection, the rectal swabs were directly streaked onto Karmali selective agar (Oxoid, France) supplemented with selective additives, then incubated at 37°C for 48 hours. Reference strains of *Campylobacter jejuni* (CIP 70.2) and *Campylobacter coli* (CIP 70.80) from the Pasteur Institute (France) served as controls. Colonies suspected of being *Campylobacter* were identified by their curved shape, Gram-negative staining, motility (rapid, darting), and positive oxidase and catalase reactions, along with lack of growth at 25°C.

These colonies were then subcultured onto Columbia blood agar (Bio-Rad, France) enriched with 5% horse blood (Institut Pasteur, Algeria) and identified to the species level using the API Campy identification system (bioMérieux, France).

Antibiotic sensitivity analysis

The antimicrobial resistance of the isolates was assessed using the disk diffusion method on Mueller-Hinton agar (Bio-Rad, France) supplemented with 5% defibrinated horse blood. Antibiotics tested included: ampicillin (10 µg), amoxicillin-clavulanic acid (10 µg), cephalothin (30 µg), cefotaxime (15 µg), gentamicin (15 µg), tobramycin (10 µg), erythromycin (15 IU), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 IU), and chloramphenicol (30 µg). Antibiotic selection was based on CA-SFM/2012 recommendations and WHO guidelines for national antimicrobial susceptibility testing.

A 0.5 McFarland turbidity standard suspension was prepared from 18-hour pure cultures. Swabs were used to inoculate the agar, and disks were placed on the surface before incubation at 42°C for 24–48 hours in a microaerophilic atmosphere. Inhibition zones were measured with calipers and interpreted according to the French Society for Microbiology guidelines. *Escherichia coli* (ATCC 25922) and *C. jejuni* (ATCC 33560) served as quality control strains.

Statistical analysis

Data were analyzed using SPSS software. Chi-square (χ^2) tests were employed to determine associations between variables, with statistical significance set at $p < 0.05$.

Results

Campylobacter spp. Prevalence and Species Distribution

Of the 200 dogs rectal swab samples examined, 52 (26%) yielded *Campylobacter* spp, of which 22 (42.3%) corresponded to *Campylobacter jejuni*, 20 (38.46%) to *Campylobacter upsaliensis*, 9 (17.3%) to *Campylobacter helveticus* and one strain (1.92%) to *Campylobacter coli* (Table, 1). Prevalence in the different groups of the dog population examined showed the following results:

Age influence

The presence of *Campylobacter spp*. was higher in older dogs compared to the younger ones. Isolation rates of 36.76% (n = 25), 20.58% (n = 14) and 18.57% (n = 13) were recorded in dogs less than 12 months, between 12 and 23 months and dogs over 24 months respectively. This difference is statistically significant (P =0.00).

Diarrhea influence

In spite of the variation of the isolation frequency of *Campylobacter* spp in non- diarrheic dogs (32.5%: 39/120) and diarrheic dogs (16.25%: 13/80), no significant association was found between the clinical signs and *Campylobacter* spp isolation in the fecal samples.

Of the 39 strains isolated in non-diarrheal dogs, 20 belong to *C. upsaliensis* (51.2 %); 9 *C. jejuni* (23.07%), the rest is distributed among the species *C. helveticus* (23.07 %) and *C. coli* (2.56 %). In diarrheal dogs, all isolates in this case correspond to *C. jejuni*. All the results are displayed in Table2.

Antibiotic Susceptibility of the Isolates

Except for the resistance to quinolone and tetracycline antibiotics, low levels of resistance were noted for the 52 strains of *Campylobacter* spp. and *C. jejuni* isolated in dogs to the various antibiotics tested. All strains showed susceptibility to aminoside antibiotics, in particular to erythromycin.

The resistance rates of *Campylobacter* spp. and *C. jejuni* to different antibiotics are shown in Table 3.

A 5.7% of *Campylobacter* spp. and 13.68 % of *C. jejuni* strains from dogs were concurrently resistant to tetracycline and quinolones (ciprofloxacin and nalidixic acid). The antimicrobial resistance profile of the examined *Campylobacter* strains is presented in Table4.

Discussion

A variation in the prevalence of *Campylobacter* has been found between the various studies in different parts of the world in dogs and in different animal species.

In the present study, the prevalence (26%) of *Campylobacter* spp. is in agreement with the rare studies carried out in Africa, such as that of Salihu et al. [22] in Nigeria that recorded a prevalence of 27.7% of *Campylobacter spp*.

However, it remains (considerably) fairly lower than the estimates reported in the following European countries such as: Sweden (56%), Denmark (76.2%), and Ireland (41.5%) [23, 24].

In South America, Lopez et al. [13] and Aquino et al. [25], in Argentina and Brazil respectively noted the same prevalence of 17% which is low compared to our results.

Many factors may have influenced the variation in the prevalence of *Campylobacter spp*. between the studies. Many researchers have suggested that the structure of the dog population studied may be responsible for the variation in *Campylobacter* prevalence in dogs [8, 24, 27, 28].

Dogs living in a community with other dogs show a higher infection rate of *Campylobacter* spp. [26, 29, 30, 31]. The higher prevalence of *Campylobacter* spp. in dogs living in groups in shelters is due to cross-infection [14].

Definitely, stray animals were found to be more infected with *Campylobacter* than domestic animals that are mostly kept indoors [29]. Wandering animals that are not sheltered can easily come into direct or indirect contact with wild birds that are considered carriers for *Campylobacter* species [32].

The design of the study also plays an important role in the variation of the isolation rate of *Campylobacter* spp. It should be noted that in our research, each dog was sampled only once which may have given a lower isolation rate compared to the longitudinal studies i.e. dogs sampled several times and / or taken into account for all isolates per sample [2, 23].

In addition, the age of the dogs has a significant impact on the probability of isolating *Campylobacter* species [31, 33]. A variation in the frequency of isolation according to the age of the dogs was recorded during our study. The younger dogs had higher levels of *Campylobacter* isolation compared to older dogs. This corresponds to the studies carried out by Aquino et al. [25] and Workman et al. [8].

In our study, non-diarrheic dogs showed a significantly higher positivity rate (32.5%) compared to diarrheal dogs (16.5%), which corresponds to the results of Chaban et al. [12]. In contrast, other researchers found no difference in the prevalence of *Campylobacter* species in healthy dogs and diarrheal dogs [8, 11, 13].

The results obtained in this study concerning the distribution of different *Campylobacter* species are similar to the other studies carried out in the world, which reported that *C. jejuni* and *C. upsaliensis* are the most commonly isolated species in dogs [24, 26, 27, 28].

The isolation frequency of *C. jejuni* in the present study is slightly higher than that of other species, which is comparable to the studies performed by Lopez et al [13] in Brazil and Aquino et al [25] in Argentina in 2002. Whereas several other researchers in different countries of the world have found a predominance of *C. upsaliensis* [8, 12, 34].

In non-diarrheic dogs, we obtained a predominance of *C. upsaliensis* followed by *C.*

jejuni, which corresponds to the results of the other studies [8, 13, 25].

The season is also an important factor that may have influenced the distribution of the *Campylobacter* species. Carbonero et al. [35] observed that the prevalence of *C. jejuni* in dogs was significantly higher in spring than in winter. Additionally, they noted an increased prevalence of *C. upsaliensis* during the summer months.

The prevalence of *C. coli* was very low (1.9%), which is consistent with the results of other studies [23, 24, 36].

Campylobacter spp. are recognized as emerging pathogens in humans, and concern over the prevalence of campylobacteriosis has grown in recent years, largely due to the frequent detection of antimicrobial-resistant strains in both humans and animals [37, 38]. Given the frequent isolation of resistant antimicrobial strains in humans and animals, there was a growing concern about the prevalence of Campylobacteriosis.

The effects of antimicrobial use in small animal veterinary practice are thought to be no different from those observed in human medicine and animal production [39].

Several studies suggest a potential link between antimicrobial use and the development of antimicrobial resistance in companion animals. [40].

The resistance rates obtained in our study in dogs are low compared to the resistance rates observed in other animal species in Algeria [18, 19]. This may be due to the use of a different range of antibiotics in domestic animals compared to slaughter animals, especially in broiler chickens.

Few studies have studied the resistance profile of *Campylobacter* strains in dogs around the world. In India, resistance rates of 80.23% (41/51), 88.23% (45/51) and 45 (88.3%) were registered against ciprofloxacin, tetracycline and erythromycin respectively [41].

Erythromycin administered for Campylobacteriosis in humans is one of the antibiotics of choice. In our study, all of the tested *Campylobacter* isolates from dogs showed sensitivity to erythromycin. This result is reassuring from a public health point of view. The same result has been reported in other studies [11, 33].

However, resistance to quinolones was exhibited by the majority of canine *C. jejuni* isolates in this study, while cross-resistance to nalidixic acid and ciprofloxacin was always observed.

Indeed, it has been recognized that the use of fluoroquinolones in animals has led to the selection and dissemination of *Campylobacter* species which are resistant to enrofloxacin (in veterinary use) as well as cross-resistance to fluoroquinolones, such as ciprofloxacin, used in humans [42; 43].

Tetracycline is an alternative drug for Campylobacteriosis treatment [44]. 7% (6/22) of the *C. jejuni* strains showed resistance to tetracycline. The use of this antibiotic for therapeutic purposes may be related to the increase in the level of resistance.

Changes in resistance levels in different antimicrobial agents reflect various veterinary practices in the use of antimicrobials for treatment and prevention from one country to another [45].

Conclusion

To our knowledge, this is the first study investigating the prevalence of *Campylobacter* in dogs in Algeria. Dogs are certainly potential sources of *Campylobacter* in humans, especially because these animals cohabitate with human beings. The high resistance levels to tetracycline and ciprofloxacin of strains of *C. jejuni* isolated in dogs may be considered a threat to public health. On the other hand, the sensitivity of *Campylobacter* strains to erythromycin is reassuring from a public health perspective

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Declaration of Conflict of Interest

The authors state that they have no conflicts of interest to declare

TABLE1. Species identification of *Campylobacter spp.* isolated in dogs

Campylobacter species	Number of strains	Isolation frequency
C. jejuni	22	42.3%
C. upsaliensis	20	38.46%
C. helveticus	09	17.3%
C. coli	01	1.92%
Total	52	26%

Value P_1> 0.05: Value of the difference between the isolation rates of the different *Campylobacter* species

TABLE 2.Distribution of *Campylobacter* species

Variable	Category			N° of infected animals			
		N	n(%)	*C.jejuni*	*C.upsaliensis*	*C.helviticus*	*C.coli*
Sex	Male	100	30	22 (73.33)	06(20)	02(6.66)	-
	Female	100	22	-	14(63.63)	07(31.81)	01(4.54)
Age	< 12 Months	68	25 (36.76)	16(64)	09(36)	-	-
	12-23 Months	68	14 (20.58)	02(14.28)	08(54.14)	04(28.57)	-
	> 24 Months	64	13 (20. 31)	04(30.76)	03(23.07)	05(38.46)	01(7.69)
Diarrhea	Yes	80	13(16.25)	13(100)	-	-	-
	No	10	39 (32.5)	09 (23.07)	20(51.2)	09(23.07)	01(2.56)

TABLE 3. Antimicrobial resistance rates of *Campylobacter ssp. and Campylobacter jejuni* isolated strains

	Profil	AM	AMC	CF	CTX	GM	TM	K	E	AN	Cip	TE	C
Campylobacter ssp.	N°	12	12	8	10	4	0	0	0	14	22	7	0
N=52	%	23,0	23,08	15,3	19,2	7.6	0	0	0	26.9	42.3	13.46	0
C. jejuni	N°	2	3	3	2	2	0	0	0	14	14	6	0
N=22	%	9.1	13.6	13.6	9.1	9.1	0	0	0	63.6	63.6	27.27	0

TABLE 4.Antimicrobial resistance profile of *Campylobacter spp*. and *C.jejuni* isolates from dog

Antimicrobial resistance profile	*Campylobacter spp. N(%)*	*Campylobacter jejuni N(%)*
TE+ NA	5(9.61)	4(18.18)
TE+ NA+ Cip	3 (5.76)	3 (13.63)
Total	8 (15.37)	7(31.81)

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مدى انتشار أنواع *Campylobacter* ومقاومة *Campylobacter jejuni* للمضادات الحيوية لدى الكلاب المنزلية في المنطقة الوسطى من الجزائر

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الملخص

تهدف هذه الدراسة إلى التحقق من مدى انتشار أنواع العطيفة لدى الكلاب في الجزائر، وتحديد أنواع العزلات، بالإضافة إلى تقييم الحساسية المضادة للميكروبات لعزلات العطفية الصائمية (*C jejuni*.). تم أخذ مسحات مستقيمية من 200 كلب وفحصها. وقد تم الحصول على العينات من حيوانات يتراوح عمرها بين أسبوعين و24 شهرًا، وتم تحليلها لاحقًا وفقًا لتوصيات المنظمة العالمية لصحة الحيوان. تم تحديد مدى الحساسية للمضادات الحيوية باستخدام طريقة انتشار الأقراص، وذلك وفقًا لإرشادات لجنة المعايير الفرنسية لمضادات الميكروبات. كما تم ملء استبيان لكل حيوان على حدة. تم عزل *العطفية* من 26% من الكلاب (200/52)، حيث وُجدت في الكلاب المصابة بالإسهال بنسبة 16.25% (80/13)، وفي الكلاب غيرالمصابة بالإسهال بنسبة 32.5% (120/39). وقد سُجِّلت فروق ذات دلالة إحصائية بين الفئات العمرية للكلاب التي خضعت للفحص؛ إذ أظهرت النتائج أن 36.7% من الكلاب الصغيرة (أقل من سنة واحدة) كانت إيجابية، مقارنةً بـ 19.7% من الكلاب الأكبر سنًا (أكثر من سنتين). لوحظ تفوّق في عزلات *C. jejuni* (22من 52)، تليها *C. upsaliensis* (20 من 52). وأظهرت سلالات *C. jejuni*مستويات مرتفعة من المقاومة للمضادات الحيوية من فئة الكينولونات؛ حيث بلغت نسبة المقاومة 63% (14 من 22) لكل من السيبروفلوكساسين وحمض النالیديكسيك، في حين سُجِّلت مستويات منخفضة من المقاومة للتتراسيكلين بنسبة 27% (6 من 22). وقد كانت جميع عزلات *C. jejuni*حساسة للإريثروميسين والكلورامفينيكول. تشير نتائج هذه الدراسة إلى أن الكلاب في وسط الجزائر قد تُشكّل خطرًا بانتقال العدوى من الحيوان إلى الإنسان. وتُعدّ المستويات المرتفعة من المقاومة للتتراسيكلين والسيبروفلوكساسين في عزلات *C. jejuni* تهديدًا محتملاً للصحة العامة.

الكلمات الدالة: الجزائر، العطيفة، مقاومة المضادات الحيوية،الكلاب، الاسهال.