Veterinary and Animal Science 6 (2018) 6–11

Contents lists available at ScienceDirect

Veterinary and Animal Science

journal homepage: www.elsevier.com/locate/vas

Prevalence, associated risk factors and antimicrobial susceptibility pattern of *Campylobacter* species among dogs attending veterinary practices at Veterinary University, Mathura, India

Iftekhar Ahmed[a], Amit Kumar Verma[a,*], Amit Kumar[b]

[a] Department of Veterinary Epidemiology and Preventive Medicine., College of Veterinary Sciences, U. P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh 281001, India

[b] Department of Veterinary Microbiology, U. P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, Uttar Pradesh 281001, India

ARTICLE INFO

Keywords:
Antimicrobial susceptibility
Campylobacter species
Dog
PCR
Risk factors

ABSTRACT

Campylobacteriosis is among the leading bacterial causes of human gastroenteritis all over the world and most of the isolates are resistant to different antibacterials. Pet rearing has been identified as a risk factor for *Campylobacter* infection in humans. The study was conducted to determine the prevalence of faecal *Campylobacter* shedding among dogs, to estimate the specific prevalence of *Campylobacter jejuni* shedding, to identify the associated risk factors and antimicrobial susceptibility pattern of *Campylobacter* spp. in dogs attending veterinary practice at Veterinary University, Mathura, India. Rectal swabs were aseptically collected and incubated using selective media and species isolation was further processed following standard protocols. In addition, genus and species specific polymerase chain reaction (PCR) was performed for species differentiation. A total of 134 dogs were included in this study. Among 134 faecal samples cultured, 38 samples (28.36%) were positive for *Campylobacter* species. *C. jejuni* was the most prevalent isolate in dogs. Breed, age, clinical signs of diarrhea and habitat sharing had statistically significant association with *Campylobacter* shedding. On drug sensitivity assay with 19 commonly used antibacterials 100% resistance was shown against amoxycillin, ampicillin, aztreonam, cefotaxim, lincomycin, oxytetracycline, penicillin, streptomycin and tetracycline. It was followed by pefloxacin (92.11%), chloramphenicol (86.84%), ciprofloxacin (84.21%), nitrofurazone (78.94%), ofloxacin (76.32%), norfloxacin (73.68%) and cefaclor (73.68%). The results of the present study revealed high prevalence of *Campylobacter* spp. among dogs. The prevalence was higher in dogs of nondescript breed, pups and dogs sharing their habitat. The antimicrobial resistance patterns showed a high rate of multi drug resistant isolates in the dog population. Therefore, awareness in handling of dogs is important to prevent the zoonotic transmission of bacteria from pets to human beings especially in children and immunocompromised patients.

Introduction

Campylobacter infections are among the leading zoonotic agents causing acute gastroenteritis in the developed countries (Campagnolo, Philipp, Long, & Hanshaw, 2018; Parsons et al., 2010; Verma et al., 2014). Among *Campylobacter* spp., *C. jejuni* is mostly associated with human and other domesticated animal's enteritis, followed by *C. coli, C. upsaliensis*, and other species (Campagnolo et al., 2018; Leahy, Cummings, Rodriguez-Rivera, Hamer, & Lawhon, 2017; Moore et al., 2005; Parsons et al., 2010). Campylobacters, present in the gastrointestinal tract of different domestic and wild animals, are widely distributed in nature (Iannino et al., 2017). Dog ownership has been shown to significantly increase the risk for pet-associated human *C. jejuni / coli* infection (Mughini et al., 2013). There are a number of reports of isolation of identical strains in humans and their pets (Campagnolo et al., 2018; Gras et al., 2013). The prevalence of *Campylobacter* spp. in dogs is variable, ranging from 4.81% (Andrzejewska, Szczepańska, Klawe, Spica, & Chudzińska, 2013) to 87% (Acke, Jones, & Collins, 2006). The bacterium has been isolated from both diarrheic (Guest, Stephen, & Price, 2007) and asymptomatic carrier dogs (Acke et al., 2006; Sandberg, Bergsjo, Hofshagen, Skjerve, & Kruse, 2002; Workman, Mathison, & Lavoie, 2005). Moreover, *Campylobacter* spp. are reported to be isolated in up to 56.0% of healthy dogs (Acke et al., 2009; Engvall et al., 2003; Kumar, Verma, Kumar,

* Corresponding author.
E-mail address: drakverma79@gmail.com (A.K. Verma).

https://doi.org/10.1016/j.vas.2018.07.001
Received 30 June 2017; Received in revised form 30 June 2018; Accepted 10 July 2018
Available online 18 July 2018

Srivastava, & Lal, 2012a; Tsai, Huang, Lin, Lien, & Chou, 2007; Verma et al., 2014). In the last few years, members of *Campylobacter* spp. have shown an increasing level of resistant to antibacterials, especially to erythromycin, fluoroquinolones and betalactams (EFSA, 2015). Occurrence of resistance is mainly associated with the use of macrolides and quinolones in veterinary medicine (Ge et al., 2002; Kumar, Verma, Kumar, Srivastava, & Lal, 2012a; Verma et al., 2014).

Dogs are among the most popular companion animals in many parts of the world including India, and their ownership is beneficial to the physical and psychological health, emotional protection and social interaction of humans (McNicholas et al., 2005). Despite the benefits derived from the dogs, the transmission of zoonotic agents, including *Campylobacter* spp, is a risk and approximately 6% of human campylobacteriosis cases are reported due to contact with pets (Iannino et al., 2017; Tenkate & Stafford, 2001). Hence, studies on the epidemiology of *Campylobacter* in dogs are important to know their role as a possible source of zoonosis. Despite evidence of increasing human campylobacteriosis over the last decade, there is paucity of research based knowledge on epidemiology of faecal *Campylobacter* shedding among dogs in India. Therefore, the study aimed to determine the prevalence of faecal *Campylobacter* among dogs, to estimate the specific prevalence of *C. jejuni*, to identify the associated risk factors and antimicrobial susceptibility pattern of *Campylobacter* spp. in dogs.

Materials and methods

Study design and period

A cross-sectional study was conducted at the department of Veterinary Epidemiology and Preventive Medicine, U. P. Pt. Deen Dayal Upadhyaya Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan (DUVASU), Mathura, India between 2013 and 2015. This university has referral Teaching Veterinary Clinical Complex (TVCC) that provides services to Uttar Pradesh and nearby states in India. Dogs belonging to breed such as Dobermann, Pomeranian, German shepherd, Spitz and non-descript breed visiting the TVCC for treatment during the study period were the source population. Dogs less than one year of age were considered as pups and one or more than one year were grouped as adults.

Sampling technique and data collection

A total of 134 samples were collected from dogs that visited Teaching Veterinary Clinical Complex of Veterinary University, Mathura, India. Convenient sampling technique was used. After obtaining consent from the owner, faecal sample along with epidemiological data about the associated risk factors viz., breed, sex age, health status, co-habitation with other dogs and relevant clinical information were taken using pre-structured questionnaire. Rectal swab were collected from 134 dogs (male $n = 106$; female $n = 28$). Of the 134 dogs, 40 were healthy and 94 had diarrhoea. Dogs were divided into two age groups, namely, 1 year old or pups ($n = 80$), and > 1 year ($n = 52$).

Sample collection and processing

Fresh rectal swabs were collected aseptically from each dog using sterile swabs and transported immediately to the laboratory on ice. Rectal swabs were inoculated in *Campylobacter* Enrichment HiVeg^{TM} Broth Base (HiMedia, Mumbai) supplemented with polymixin B sulphate, rifampicin, trimethoprim and cycloheximide and incubated at 42–43 °C for 24 h in 5% CO_2 atmosphere using CO_2 incubator. After incubation, the inoculums were streaked onto selective media (*Campylobacter* selective agar, HiMedia, Mumbai) supplemented with 10% lysed horse blood and reconstituted contents of *Campylobacter* selective-I (HiMedia, Mumbai) supplemented with polymixin B, vancomycin, trimethoprim and cephalothin. These were incubated again at 42–43 °C for 48 h under microaerophilic conditions with 5% CO_2 concentration for the isolation of selective single colonies.

Identification of Campylobacter spp

The identification of *Campylobacter* spp. was performed by characteristic appearance on culture medium (moist, creamy-grey and flat-spreading), Gram stain, oxidase (Oxidase disc, HiMedia, India) and catalase testing (Gracia, Lior, Stewart, & Ruterbauer, 1985; Skirrow & Benjamin, 1980). DNA extraction from bacterial cultures was performed by phenol chloroform method (Sambrook & Russell, 2001). For the molecular identification, primers were custom synthesized to amplify members of the *Campylobacter* genus (16S rRNA; Linton, Owen, & Stanley, 1996) and isolates of *C. jejuni* (*cj0414* gene; Wang et al., 2002) were used. The in vitro amplification of DNA was performed in thermocycler (Eppendorf, Germany) using an initial denaturation step at 95°C for 15 min; 25 cycles of denaturation (95°C for 30 s), annealing (58°C for 90 s) and extension (72°C for 1 min); and a final extension step at 72°C for 7 min. About 10 µl of the PCR product was analysed by 1.0% agarose gel electrophoresis.

Antimicrobial susceptibility test

All the confirmed *Campylobacter* isolates obtained and confirmed during the present study were examined for their drug resistance pattern by disc diffusion method (Bauer, Kirby, Sherris, & Turck, 1966) using 19 antibacterial discs (Hi-Media, Mumbai) viz., amikacin (30 µg), ampicillin (10 µg), amoxycillin (20 µg), aztreonam (30 µg), cefaclor (30 µg), cefotaxim (30 µg), ciprofloxacin (30 µg), chloramphenicol (30 µg), enrofloxacin (10 µg), gentamicin (10 µg), lincomycin (10 µg), nitrofurazone (100 µg), norfloxacin (10 µg), ofloxacin (5 µg), oxytetracycline (30 µg), pefloxacin (5 µg), penicillin (10units), streptomycin (10 µg) and tetracycline (30 µg) as described in Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2006).

Data processing and statistical analysis

Campylobacter prevalence in dogs was stratified by the variables breed, sex, age (< 1 year, ≥ 1 year), occurrence of diarrhoea and co-habitation with other dogs. The possible role of these variables as risk factors in *Campylobacter* colonization was evaluated by chi-square test (Snedecor & Cochran, 1994). In all analyses, *P*-values less than 0.05 were taken as statistically significant.

Results

Prevalence of Campylobacter species

Among 134 faecal specimens cultured, *Campylobacter* species were isolated and subsequently confirmed by PCR in 38 samples representing the prevalence of 28.36%. The most frequent species identified in the present study was *Campylobacter jejuni* (68.42%), while 12/38 (31.57%) were other *Campylobaceter* spp. (Fig. 1).

Possible risk factors and their association with Campylobacter infections

Among the risk factors, sex and age showed no statistically significant association with *Campylobacter* culture positivity; whereas breed, health status and co-habitation with another dog had statistically significant association (Table 1). The positive rate of *Campylobacter* infection was the highest in nondescript dogs (36.84%), followed by Doberman (36.36%), Pomeranian (33.33%), German shepherd (30.00%) and, Spitz (21.43%). Significantly higher prevalence of *Campylobacter* spp. (> 3 times) had been observed in diarrhoeic dogs (34/94; 36.17%) compared to non-diarrheic dogs (04/40; 10.00%). The prevalence of *Campylobacter* spp. in dogs sharing their habitat (for

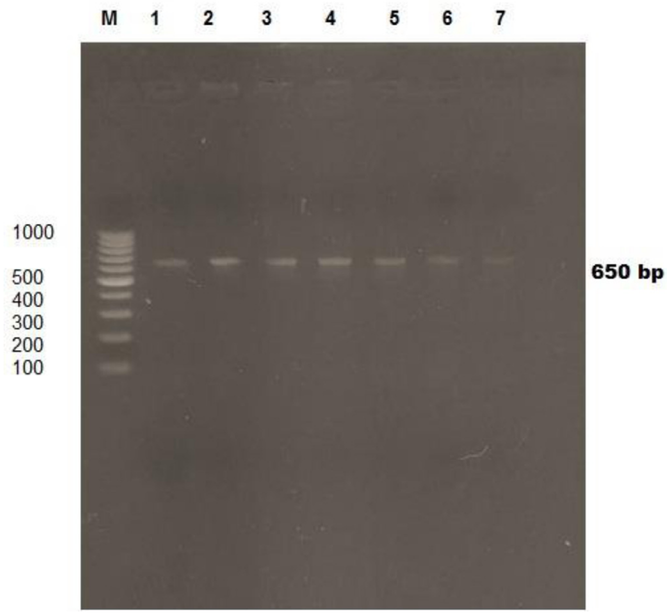

Fig. 1. Species-specific PCR amplicons were resolved after electrophoresis through a 1% agarose gel.
Lane M: 100 bp DNA ladder (in base pairs) is shown on the left-hand edge of the gel.
Lane N: Negative control
Lane 1-7: *Campylobacter jejuni* (650 bp) isolates.

Table 1
Campylobacter spp. detection in dogs.

Risk factors /Variables	Categories	Number of animals tested	Number of Positive animals	Percentage Positivity
Breed*	Boxer	7	1	14.29
	Bull mastiff	2	0	0.00
	Doberman	11	4	36.36
	German Shepherd	20	6	30.00
	Labrador	24	4	16.67
	Pomeranian	15	5	33.33
	Rottweiller	3	1	33.33
	Spitz	14	3	21.43
	Nondescript	38	14	36.84
	Total	134	38	28.36
Sex	Male	106	30	28.30
	Female	28	8	28.57
	Total	134	38	28.36
Age	< 1 year (Pups)	82	27	32.93
	≥ 1 year (Adult)	52	11	21.15
	Total	134	38	28.36
Diarrheic*	Yes	94	34	36.17
	No	40	4	10.00
	Total	134	38	28.36
Cohabitation with other dog*	Yes	64	24	37.50
	No	70	14	20.00
	Total	134	38	28.36

* Significant at ($P < 0.05$)

example in kennels or shelter) (24/64; 37.50%) was significantly higher than individually reared dogs (14/70; 20.00%).

Antimicrobial susceptibility patterns of the isolates

The results of antimicrobial susceptibility testing for *Campylobacter species* isolated from dogs against 19 selected antimicrobial agents are presented in Table 2. Of the 19 antimicrobial drugs used to determine antibiogram of *Campylobacter* isolates, amoxycillin, ampicillin, aztreonam, cefotaxim, lincomycin, oxytetracycline, penicillin, streptomycin and tetracycline revealed no zone of inhibition suggestive of resistance in all the isolates against these nine drugs. It was followed by pefloxacin (92.11%), chloramphenicol (86.84%), ciprofloxacin (84.21%), nitrofurazone (78.94%), ofloxacin (76.32%), norfloxacin (73.68%) and cefaclor (73.68%) Only three out of 19 antibacterials, enrofloxacin, gentamicin and amikacin revealed zone of inhibition suggestive of sensitivity against 31.58%, 23.68% and 18.42% isolates, respectively.

Table 2
Antibiogram of *Campylobacter* spp. isolated from dogs.

Name of antibacterial	Symbol	Number of isolates (38)				
		R	I	S	Sensitivity %	Resistant %
Amikacin	Ak	16	15	07	18.42	42.11
Amoxycillin	Am	38	–	–	0	100
Ampicillin	A	38	–	–	0	100
Aztreonam	At	38	–	–	0	100
Cefaclor	Cf	28	06	04	10.53	73.68
Cefotaxim	Ce	38	–	–	0	100
Chloramphenicol	C	33	03	02	5.26	86.84
Ciprofloxacin	Cip	32	04	02	5.26	84.21
Enrofloxacin	Ex	16	10	12	31.58	42.11
Gentamicin	G	23	15	09	23.68	60.53
Lincomycin	L	38	–	–	0	100
Nitrofurazone	Nr	30	08	–	0	78.94
Norfloxacin	Nx	28	10	–	0	73.68
Ofloxacin	Of	29	08	01	2.63	76.32
Oxytetracycline	O	38	–	–	0	100
Peefloxacin	Pf	35	03	–	0	92.11
Penicillin	P	38	–	–	0	100
Streptomycin	S	38	–	–	0	100
Tetracycline	T	38	–	–	0	100

S- Sensitivity, I – Intermediate, R- Resistant (based on chart provided by manufacturer

Discussion

This study showed the prevalence of *Campylobacter* species in dogs presented to veterinary practices, and Mathura was 28.36%, that is within the range (4.81%–75%) of prevalence data previously reported (Bojanic et al., 2017; Engvall et al., 2003; Giacomelli, Follador, Coppola, Martini, & Piccirillo, 2015; Holmberg et al., 2015; Kumar et al., 2012a, Kumar, Verma, Kumar, Srivastava, & Lal, 2012b; Leahy et al., 2017; Procter et al., 2014; Sandberg et al., 2002; Verma et al., 2014). The variation in prevalence could be due to differences in geographical location, differences in the populations investigated, or in the detection methods used (Acke et al., 2009; Guest et al., 2007; Parsons et al., 2010; Verma et al., 2014). The species distribution of *Campylobacter* isolates from dogs differs considerably among publications and years. The *C. jejuni* (Andrzejewska et al., 2013; Badlik, Holoda, Pistl, Koscova, & Sihelska, 2014; Giacomelli et al., 2015) and *C. upsaliensis* (Holmberg, Rosendal, Engvall, Ohlson, & Lindberg, 2015; Mughini et al., 2013; Procter et al., 2014; Sandberg et al., 2002) have been found to be the most common species in dogs. However, the season, geographical area, breeds etc may have different prevalence for different species. Most *Campylobacter* positive dogs in the present study were colonized by *C. jejuni.* This is an important finding from a public health standpoint, since *C. jejuni* is the species most frequently associated with human gastroenteritis (EFSA and ECDC, 2015; Moore et al., 2005) and associated also with asymptomatic human disease (Szczepanska, Andrzejewska, Spica, & Klawe, 2017) in middle income countries.

The distribution of *Campylobacter* species between male and females dogs was not statistically significant, that agrees with the previous studies (Badlik et al., 2014; Kumar, Verma, Kumar, Srivastava, & Lal, 2012b; Leahy et al., 2017; Parsons et al., 2010; Verma et al., 2014), those also suggested that campylobacter colonization in dogs was not sex dependent.

The high prevalence of *Campylobacters* in non-descript dogs in the present study was in agreement with the previous study (Verma et al., 2014). The possible reason of higher prevalence might be the way of living of non-descript dogs as these dogs used to roam outside the home freely leading to more exposure and chances of getting infection from stray dogs or animals in and around areas (Kumar et al., 2012b; Verma et al., 2014). The high prevalence (39.3%) of *Campylobacter* spp. in wild birds (Workman et al., 2005) is of importance because nondescript dogs can easily meet the faeces of these infected birds during roaming in streets and parks.

Non-significant relationship was found between age and *Campylobacter* positive status. Similarly, the previous studies (Leahy et al., 2017; Tsai et al., 2007; Wieland et al., 2005) also reported age as non predisposing factor for *Campylobacter* infection. However, contrary to the present findings, higher positivity of *Campylobacter* isolation in pups in comparison to adult dogs was reported in earlier studies (Acke et al., 2006, 2009; Engvall et al., 2003; Guest et al., 2007; Holmberg et al., 2015; Sandberg et al., 2002; Verma et al., 2014 Badlik *et al.,* 2014). These reports suggested younger dogs more likely to be carriers of *Campylobacter spp.* and to shed the bacteria more commonly than older dogs probably as consequence of age-related immunity (Iannino et al., 2017). In Denmark, the incidence of *Campylobacter* spp. in particular *C. upsaliensis*, peaked at 13–15 months aged pet dogs (Hald, Pedersen, Wain¸, Jargensen, & Madsen, 2004). Another report suggested that age of dog had a quadratic effect, with young dogs and senior dogs having an increased probability of shedding *Campylobacter* spp. compared with adult dogs (Procter et al. (2014).

In this study, diarrhoea had statistically significant association with isolation of *Campylobacter* species among dogs. This is consistent with the previous studies (Engvall et al., 2003; Sandberg et al., 2002; Workman et al., 2005). However, in contrasts to these findings, several authors reported no association between *Campylobacter* infection and digestive disorders in dogs (Acke et al., 2006; Giacomelli et al., 2015; Parsons et al., 2010). Thus, such reports may call into question the presumed association of *Campylobacter* with gastrointestinal disease in dogs. In 2010, Parson and coworkers further suggested the presence of *Campylobacter* spp. in the faeces as an important risk indicator for diarrhoea in dogs. High infection rate was seen in dogs that shared their habitat with other dogs, indicating the direct association between *Campylobacter* spp. infection and pets. The present findings are in agreement with the findings of previous studies (Badlik et al., 2014; Guest et al., 2007; Parsons et al., 2010). The overcrowding, cross-infection, stress, frequent dietary changes might be the factors for increased incidence of gastrointestinal disease suffered by animals in pounds or kennels (Acke et al., 2006, 2009; Burnens, Wick, & Nicolet, 1992; Torre & Tello, 1993). Contrary to this, several studies found no association between a dog's *Campylobacter* carrier status, and whether or not they lived with other animals (Acke et al., 2006; Engvall et al., 2003; Hald et al., 2004; Parsons et al., 2010; Sandberg et al., 2002), suggesting *Campylobacter* as a commensal (Engvall et al., 2003).

Although gastroenteritis due to *Campylobacter* species is mostly mild and self-limiting in nature but occasionally severe dehydration become life-threatening and requires antibacterial treatment. In the present study, ninteen commonly used antibacterials were tested against 38 isolates of *Campylobacter* species. Majority of *Campylobacter* spp. isolates showed resistance to at least 3 of the antibacterials tested, indicating multi-drug resistance. In the present study, isolates were sensitive to amikacin (18.42%), gentamicin (23.68%) and enrofloxacin (31.58%). Among these, enrofloxacin are not prescribed for dogs due to their contradictions. The antimicrobial susceptibility test indicated that gentamicin and amikacin are the most efficient antibacterials against *Campylobacter* spp. isolated from dogs in vitro. A 100% of resistance of *Campylobacter* isolates against amoxicillin, amoxycillin, ampicillin, aztreonam, cefotaxim, lincomycin, oxytetracycline, penicillin, streptomycin and tetracycline was also observed. A high rate of resistance was observed against pefloxacin (92.11%), chloramphenicol (86.84%), ciprofloxacin (84.21%), nitrofurazone (78.94%), ofloxacin (76.32%), norfloxacin (73.68%) and cefaclor (73.68%). These results are consistent with other studies in dogs, which found low resistance to gentamicin and medium-high resistances to quinolones (ciprofloxacin and nalidixic acid), cephalosporins, and tetracyclines (Di Giannatale et al., 2014; Lengerth et al., 2013). This high rate may be due to indiscriminate use of these drugs in the studied area that leads to increased resistance.

Similarly resistance to tetracycline with *Campylobacter* isolates from humans, dogs and other animals were reported in the range of 15–94% (De Vega et al., 2005; Gaudreau & Gilbert, 1998; Saenz et al., 2000). The concurrence to previous reports of high resistance to ampi-cloxacillin for *Campylobacter* spp. i.e. 57.3% (Little, Richardson, Owen, Pinna, & Threlfall, 2008) and 65.7% (Saenz et al., 2000) in pigs and 43.1% (Han, Jang, Choo, Heu, & Ryu, 2007) and 40.8% (Miflin, Templeton, & Blackall, 2007) in chicken, the resistance to ampi-cloxacillin was recorded 88.23%.

All the *Campylobacter* isolates showed resistance to cefotaxim, and this is alarming because it is the 3rd generation cephalosporin and is the drug of choice for local vets in the treatment of diarrhoea and gastroenteritis in pets. An increased resistance to quinolones is probably due to genetic mutations interfering with bacterial DNA gyrase (Greene & Watson, 2003). Selective pressure caused by the indiscriminate use of these drugs in aviculture might be a contributory factor. Previous studies (Biasi, DeMacedo, Malaquias, & Franchin, 2011; Saenz et al., 2000) reported the highest resistance of *Campylobacter* isolates to quinolones among various antibacterials similar to results obtained in the current study with 80.39% of the isolates resistant to ciprofloxacin. Contrary to our findings, different reports showed 100% sensitivity to ciprofloxacin in *Campylobacter spp*. isolated from chicken in Australia (Miflin et al., 2007); domestic animals and poultry in India (Baserisalehi, Bahador, & Kapadnis, 2007); isolates from environmental samples (Baserisalehi & Bahador, 2008). A report from Canada showed only 0.3% resistance against ciprofloxacin in cattle isolates (Inglis et al., 2005). The resistance patterns displayed by *Campylobacter* isolates in dogs to fluoroquinolone (ciprofloxacin) and macrolides (erythromycin) classified as second line. The first line antimicrobials like aminoglycosides and cephalosporins are of particular importance, since patients suffering from campylobacteriosis are usually treated with these antimicrobials agents (Uaboi-Egbenni, Bessong, Samie, & Obi, 2011). The resistance to chloramphenicol was also observed previously in humans (Bardon, Kolar, Cekanova, Hejna, & Koukalova, 2009) and chickens (Miflin et al., 2007). The great variability in this antibacterial's efficacy is probably due to its worldwide use in cattle, both at therapeutic or low doses; this would increase selective pressure on bacteria.

Recent scientific studies have shown that campylobacter antimicrobial resistance are related to some specific genes, and the dissemination of these genes of microorganisms to their progeny and across to other unrelated co-habitat bacterial species through extra-chromosomal DNA fragment called the plasmid (Baserisalehi & Bahador, 2008). Antimicrobial resistance particularly multi drug resistance observed in the present work might be due to the indiscriminate and irrational use of antimicrobials (Tambekar, Dhanorkar, Gulhane, & Dudhane, 2007) in animals for preventive or therapeutic purposes irrespective of etiological agents.

Conclusion

The present study indicates a high prevalence of *Campylobacter* species particularly of *C. jejuni* among dogs. *C. jejuni,* the most frequently associated with the occurrence of the disease in humans are present in Indian dogs. The prevalence was higher in dogs of nondescript breed, pups and dogs sharing their habitat. The high rate of antibacterial resistance and higher percentage of multi durg resistant isolates may be due to frequent prescription of drugs without drug susceptibility testing and inappropriate usage of the commonly available drugs in the market. There is an urgent need to develop awareness strategies of the *Campylobacter* risk from dogs to reduce its transmission from dogs to children and immunocompromised human beings.

Acknowledgement

We express our gratitude to the Dean, College of Veterinary Science and Animal Husbandry; Hon'ble Vice Chancellor, DUVASU, Mathura for supporting our research activity. We are also very thankful to the staff of the Teaching Veterinary Clinical Complex, DUVASU, Mathura for its valuable help in the sampling and gathering information about the animals. Authors also wish to thank the technician Smt. Mamta for her excellent support.

Disclosure statement

No potential conflict of interest was reported by the authors.

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