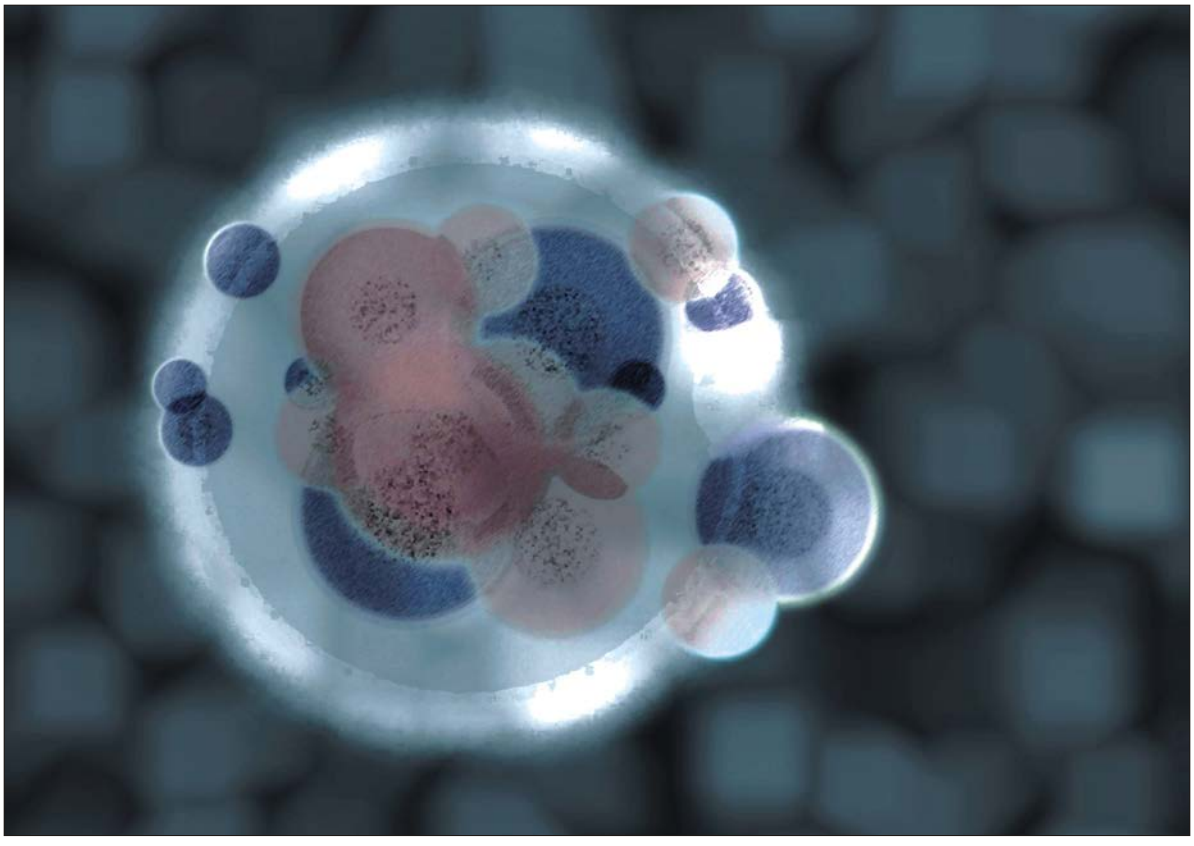

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Prevalence and Resistance to Antimicrobial Agents of *Campylobacter* sp. Isolated from Dogs in India

[1]Amit Kumar Verma, [2]Amit Kumar, [3]Shanker Kumar Singh, [4]Anu Rahal, [1]Iftekhar Ahmed,
[5]Deepti Singh, [3]Aashish Pratap Singh and [3]Lalit Singh
[1]Department of Veterinary Epidemiology and Preventive Medicine,
[2]Department of Veterinary Microbiology and Immunology,
[3]Department of Veterinary Clinical Medicine,
[4]Department of Veterinary Pharmacology and Toxicology,
College of Veterinary Sciences and Animal Husbondry
[5]College of Biotechnology, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu
Chikitsa Vigyan Vishwavidyalay Evum Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

Abstract: Globally, *Campylobacters* have been reported as leading cause of gastroenteritis in man as well as animals and considered as emerging zoonotic problem particularly in developing countries including India. A cross-sectional study was conducted to know the prevalence and epidemiological determinants for *Campylobacter* spp. in dogs in and around Mathura city, Uttar Pradesh, India. Based on isolation, cultural and biochemical characterization of bacteria, the prevalence of *Campylobacter* spp. was 34.24%. Younger dogs (less than 1 year of age) were more likely to carry *Campylobacter* spp. High prevalence of *Campylobacter* spp. supports the hypothesis that dogs, particularly younger animals, may be an important source of *Campylobacter* infection for humans. Breed-wise prevalence showed that non-descript dogs (45.97%) were more likely to carry *Campylobacter* infections. Dogs showing clinical signs of gastroenteritis were showing higher prevalence (47.21%) in comparison to that of animals without gastro-intestinal disorders (15.04%). Out of 113 *Campylobacter* isolates of canine origin, two isolates were resistant to all the nineteen antibiotics used in the study, while all the isolates were resistant to Streptomycin, Ampicillin, Amoxycillin, Aztreonam, Lincomycin, Tetracyclin, Oxytetracyclin and Penicillin. A high rate of resistance was observed to Cefotaxim (97.35%), Peefloxacin (91.15%), Chloramphenicol (90.27%), Ofloxacin (84.07%), Ciprofloxacin (83.18%), Cefaclor (80.53%), Nitrofurazone (76.11%), Norfloxacin (74.33%), Gentamicin (42.48%), Amikacin (40.71%) and Enrofloxacin (36.28%). Our results indicate Amikacin and Gentamicin as drugs suitable for the treatment of campylobacteriosis in dogs.

Key words: *Campylobacter*, dogs, animals, antibiotic sensitivity, diagnosis, isolation, prevalence, resistance

INTRODUCTION

Enteropathogenic bacteria are distributed worldwide and well to live as commensal in the gastrointestinal tract of man as well as animals. The zoonotic potential of these bacteria viz., *Salmonella* (Verma *et al*., 2007, 2008, 2011a, b; Sachan *et al*., 2013), *E. coli* (Malik *et al*., 2013; Kumar *et al*., 2013) and *Campylobacter* (Kumar *et al*., 2012a, b) was very well established. Among these *Campylobacters* are emerging food borne pathogens with increasing incidences and leads to severe gastroenteritis and other complications like Guillain-Barre Syndrome, reactive arthritis, haemolytic uraemic syndrome, meningitis, carditis, pancreatitis, septic arthritis, etc., (Stern and Line, 2000; Tenkate and Stafford, 2001; Hannu *et al*., 2002; Skirrow, 2006; Levin, 2007; Lindmark *et al*., 2009; Kirkpatrick and Tribble, 2011; Man, 2011; Rajendran *et al*., 2012; Bouwman *et al*., 2013; Hauri *et al*., 2013). In animals, these *Campylobacters* may cause diarrhoea and other reproductive disorders like abortion, early embryonic mortality and many other complications (Humphrey *et al*., 2007; Kumar *et al*., 2012a, b).

Various studies have reported the association of *Campylobacter* species with diarrhea in dogs (Steinhauserova *et al*., 2000; Misawa *et al*., 2002; Kumar *et al*., 2012a) however, their real role in canine enteritis is not clear (Koene *et al*., 2004). Generally,

Corresponding Author: Amit Kumar Verma, Department of Veterinary Epidemiology and Preventive Medicine, Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishwavidyalay, Evum Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

children are more susceptible to this infection (Wolfs *et al*., 2001; Chattopadhyay *et al*., 2001). Although, there is various reports from developed countries regarding prevalence of *Campylobacters* in dogs but there is little published information about *Campylobacter* infections and their drug resistance pattern in dogs of developing countries including India. Therefore, the present study was undertaken to determine the occurence of thermotolerant *Campylobacter* and their antibiogram in dogs of Mathura city and nearby areas, Uttar Pradesh, India.

MATERIALS AND METHODS

Study design, area and sample collection: This cross-sectional study was conducted during the period of almost one year in 2012-2013 in Mathura city and nearby areas, Uttar Pradesh India (Fig. 1). Geographically, the city is situated at the latitude 27°30'N and longitude 77°40'E with an elevation of 174 metre above sea level. A total of 330 rectal swabs for bacteriological culture were collected from dogs presented to Teaching Veterinary Clinical Complex, Mathura with the epidemiological information viz., breed, age, sex and health status.

Thermophilic *Campylobacter* isolation and identification: The samples in the *Campylobacter* enrichment HiVeg TM broth base with addition of polymixin B sulphate, rifampicin, trimethoprim and cycloheximide were incubated at 42-43°C under microaerophilic conditions in the microaerophilic jars with a lighting candle (Fig. 2). After incubation, the enriched samples were properly shaken and sub-cultured onto *Campylobacter* selective agar (HiMedia, Mumbai) supplemented with 10% defibrinated sheep blood and addition of Polymixin B, vancomycin, trimethoprim and

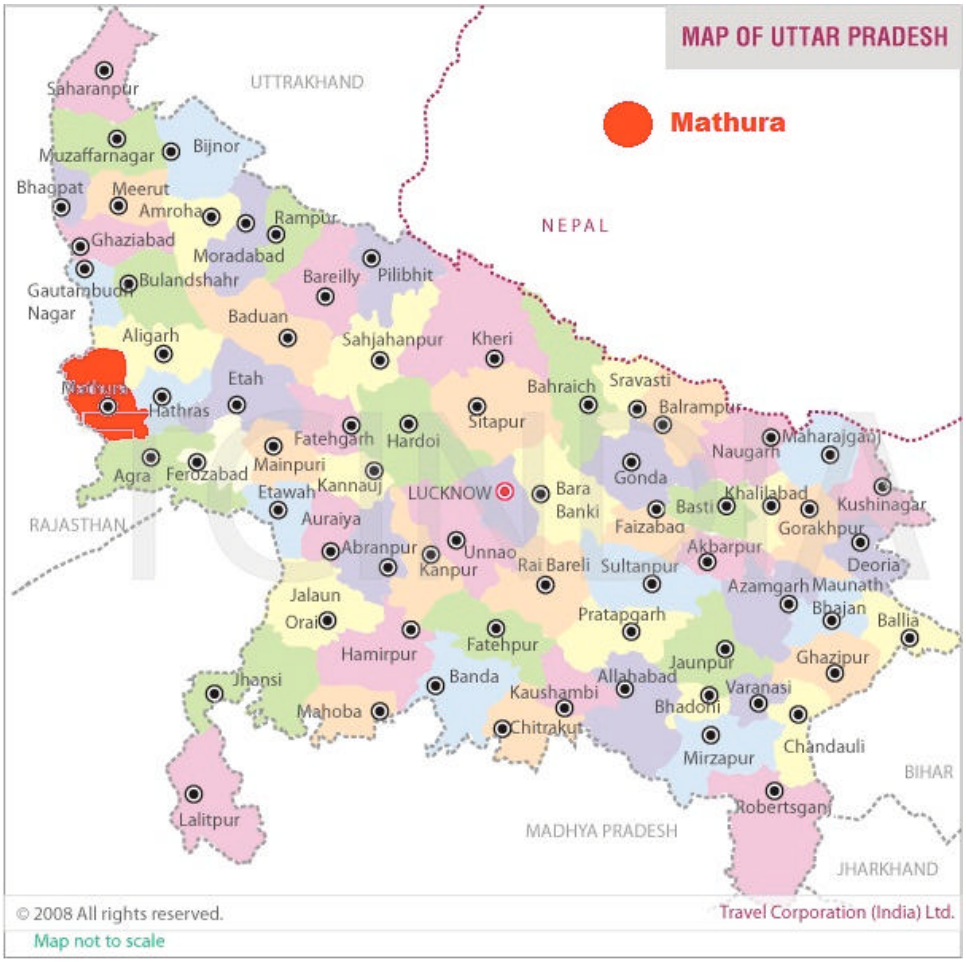

Fig. 1: Map of Uttar Pradesh showing Mathura as study area

Fig. 2: Microaerophilic jar with a lighting candle

cephalothin for primary isolation ofc thermophilic *Campylobacter*. The inoculated petridishes were incubated at 42°C for 48 h under microaerophilic conditions. Suspected thermophilic *Campylobacter* colonies that were Gram negative, curved, or spiral rods and showed corkscrew-like motion, were confirmed biochemically (Skirrow and Benzamin, 1980; Garcia *et al*., 1985).

Antibiogram: All the *Campylobacter* isolates were assessed for their antimicrobial susceptibility testing using disc-diffusion method (Bauer *et al*., 1966) following the NCCLS guidelines (NCCLS 2002). A total of 19 antimicrobial discs (Hi-Media, Mumbai) of commonly used antibacterial drugs 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), enrofolxacin (10 μg), gentamicin (10 μg), Lincomycin (10 μg), Nitrofurazone (100 μg), Norfloxacin (10 μg), Ofloxacin (5 μg), Oxytetracyclin (30 μg), Peefloxacin (5 μg), Penicillin (10 units), streptomycin (10 μg) and tetracyclin (30 μg) were used to assess the drug resistance pattern of *Campylobacter* isolates.

RESULTS AND DISCUSSION

A total of 330 faecal samples were collected from dogs with and without clinical signs of gastroenteritis and isolation of bacteria was attempted in all the samples. Based on isolation, cultural and biochemical characterization of bacteria, the prevalence of *Campylobacter* spp. was 34.24% (Table 1). The prevalence of *Campylobacter* spp. in faecal samples of dogs is within the range (17-59%) as reported in various studies (Sandberg *et al*., 2002; Engvall *et al*., 2003; Koene *et al*., 2004; Rossi *et al*., 2008; Parsons *et al*., 2010; Kumar *et al*., 2012a, b). The variation between these studies might be either due to different study population or methods used for detecting the bacteria (Guest *et al*., 2007; Rossi *et al*., 2008; Acke *et al*., 2009; Parsons *et al*., 2010; Kumar *et al*., 2012a, b).

Table 1: Occurrence of *Campylobacter* infection in dogs influenced by age, sex, breed and health status

Risk factor	Total animals	Total positive	Percent positive
Age			
Less than 1 year	197	92	46.70
≥1 year	133	21	15.70
Total	330	113	34.24
Sex			
Male	257	87	33.85
Female	73	26	35.61
Breed			
Non descript	87	40	45.97
Doberman	18	06	33.33
Labrador	73	24	32.88
German shepherd	56	18	32.14
Pomeranian	50	15	30.00
Spitz	20	06	30.00
Boxer	08	02	25.00
Rottweiler	11	02	18.18
Bull mastiff	04	00	00.00
Cocker spaniel	01	00	00.00
Chihuahua	01	00	00.00
Pug	01	00	00.00
Health status			
G.I. disorders	197	93	47.21
Other problems	133	20	15.04

Younger dogs (less than 1 year of age) were more likely to carry *Campylobacter* spp. (Table 1). High prevalence of *Campylobacter* spp. supports the hypothesis that dogs especially the puppies (less than 1 year of age) may be an important source of *Campylobacter* infection for man. Our findings were similar to previous studies (Engvall *et al*., 2003; Acke *et al*., 2006, 2009; Guest *et al*., 2007). But contrary to our findings, a small number of studies conducted by Wieland *et al*. (2005) and Tsai *et al*. (2007) have suggested that age is not a predisposing factor for *Campylobacter* infection. Breed-wise prevalence showed that non-descript dogs (45.97%) were more likely to carry *Campylobacter* infections (Table 1). This might be due to the way of living of non-descript dogs. As they roam outside the home freely in comparison to other breeds of dog leading to more exposure and chances of getting infection from stray dogs or animals in and around areas (Kumar *et al*. 2012b).

Dogs showing clinical signs of gastroenteritis were showing higher prevalence (47.21%) in comparison to that of animals without gastro-intestinal disorders (15.04%) (Table 1). Similar to our study, various researchers (Guest *et al*., 2007) reported the association between *Campylobacter* infection and clinical signs, especially in younger dogs (Fox *et al*., 1983; Nair *et al*., 1985; Burnens *et al*., 1992). However, contrary to our findings, there are various reports suggesting no

Table 2: Drug susceptibility pattern of all the *Campylobacter* isolates (n = 113) from dogs

Antimicrobial	Resistance	Intermediate	Sensitive
Amikacin	46 (40.71)	46	21
Amoxycillin	113 (100.00)	-	-
Ampicillin	113 (100.00)	-	-
Aztreonam	113 (100.00)	-	-
Cefaclor	91 (80.53)	14	08
Cefotaxim	110 (97.35)	03	-
Chloramphenicol	102 (90.27)	07	04
Ciprofloxacin	94 (83.18)	12	07
Enrofloxacin	41 (36.28)	36	36
Gentamicin	48 (42.48)	43	22
Lincomycin	113 (100.00)	-	-
Nitrofurazone	86 (76.11)	27	-
Norfloxacin	84 (74.33)	29	-
Ofloxacin	95 (84.07)	16	02
Oxytetracyclin	113 (100.00)	-	-
Peefloxacin	103 (91.15)	10	-
Penicillin	113 (100.00)	-	-
Streptomycin	113 (100.00)	-	-
Tetracycline	113 (100.00)	-	-

relationship between diarrhoea and *Campylobacter* spp. infection status (Engvall *et al*., 2003; Acke *et al*., 2006; Rossi *et al*., 2008; Parsons *et al*., 2010; Kumar *et al*., 2012b), suggesting that the organism is a commensal (Engvall *et al*., 2003). *Campylobacter* infection were detected in 15.04% of the dogs either apprarantly healthy or having some problem other than gastroenteritis (Table 1) showing that these animals can be carriers of *Campylobacter* species and may a source of infection for other pets and human beings (Acke *et al*., 2006; 2009; Kumar *et al*., 2012b).

All the *Campylobacter* isolates were tested for drug sensitivity using 19 antibacterial drugs and the results were shown in Table 2.

Out of 113 *Campylobacter* isolates of canine origin, two isolates were resistant to all the nineteen antibiotics used in the study, while all the isolates were resistant to Streptomycin, Ampicillin, Amoxycillin, Aztreonam, Lincomycin, Tetracycline, Oxytetracycline and Penicillin. A high rate of resistance was observed to Cefotaxim (97.35%), Peefloxacin (91.15%), Chloramphenicol (90.27%), Ofloxacin (84.07%), Ciprofloxacin (83.18%), Cefaclor (80.53%), Nitrofurazone (76.11%), Norfloxacin (74.33%), Gentamicin (42.48%), Amikacin (40.71%) and Enrofloxacin (36.28%). Antibiotic sensitivity studies on *Campylobacter* isolates were conducted by various researchers in different countries and reported varying degree of resistance to same drug (Little *et al*., 2008; Moran *et al*., 2009; Kumar *et al*., 2012a). High resistance to ampi-cloxacillin, tetracycline, ofloxacin, ciprofloxacin etc were previous reported by various researchers like Little *et al*. (2008) and Miflin *et al*. (2007). Quinolones were considered as suitable drug for treatment of Campylobacteriosis (Uaboi-Egbenni *et al*., 2011) but higher resistance to ciprofloxacin and ofloxacin was seen, which might be either due to genetic mutations interfering with bacterial DNA gyrase (Greene and Watson, 2003) or selection pressure due to injudicious use of antibiotics (Norma *et al*., 2007; Biasi *et al*., 2011). Present results indicated Amikacin and Gentamicin as drugs suitable for the treatment of canine campylobacteriosis. This also opens up therapeutic possibilities for these drugs in human medicine.

This cross-sectional study has the some limitations in its design; nevertheless, it is performed in only little veterinary practice involving small number of dogs and few related determinants. Therefore, there is a requirement of detailed study considering larger population in order to establish a better understanding of the epidemiology of *Campylobacter* infection in dogs of developing countries like India and ultimately help in making strategies to control or reduce the risk of this infection in man. The use of antibiotics as threauptic and/or prophylaxis for man as well as animals should be monitored because acquisition of antibiotic resistant strains of *Campylobacters* by man has serious public health implications.

CONCLUSION

From the present study, it can be concluded that *Campylobacter* infection was prevalent in dogs of study area suggesting their possible role in transmission to human beings. Younger dogs (less than 1 year of age) were more likely to carry *Campylobacter* spp. it is worth highlighting that dogs, particularly puppies may be an important source of *Campylobacter* infection for humans. High levels of resistance to antibiotics commonly used for prophylaxis and therapeutic is of public health significance. Present results indicated Amikacin and Gentamicin as drugs suitable for the treatment of canine campylobacteriosis. However, the results emphasize the use of antibiotic sensitivity test be conducted before prescribing the antibiotics.

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