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


Department of Veterinary Epidemiology and Preventive Medicine, Department of Veterinary Microbiology and Immunology, Department of Veterinary Clinical Medicine, Department of Veterinary Pharmacology and Toxicology, College of Veterinary Sciences and Animal Husbandry, College of Biotechnology, Uttar Pradesh Pandit Deen Dayal Upadhyay Pashu Chikitsa Vignan Vishwavidyalay Evum Go-Anusandhan Sansthan (DUVASU), Mathura, 281001, India

Abstract: Globally, Campylobacter 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 34.2%. 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, Amoxicillin, Amoxyclillin, Aztreonam, Lincomycin, Tetracyclin, Oxytetracyclin and Pericillin. A high rate of resistance was observed to Cefotaxim (97.35%), Peefloxacin (91.15%), Chloramphenicol (90.27%), Ofloxacin (84.07%), Ciprofloxacin (83.18%), Cefalosor (80.53%), Nitrofurazole (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.

Keywords: 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 Campylobacter 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; Hanna 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 Campylobacter 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 (Steinhauerova et al., 2000; Mawawa 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 Upadhyay Pashu Chikitsa Vignan 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 *Campylobacter* 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 occurrence of thermotolerant *Campylobacter* and their antibiotic 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™ broth base with addition of polymixin B sulphate, rifampicin, trimethoprim and cefoxitin were incubated at 42-43°C under microaerophilic conditions in the anaerobic 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

![Map of Uttar Pradesh showing Mathura as study area](image-url)

Fig. 1: Map of Uttar Pradesh showing Mathura as study area
cephalothin for primary isolation of 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), enrofloxacin (10 μg), gentamicin (10 μg), Lincomycin (10 μg), Nitrofurazone (100 μg), Norfloxacin (10 μg), Ofloxacin (5 μg), Oxytetracycline (30 μg), Peefloxacin (5 μg), Pencillin (10 units), streptomycin (10 μg) and tetracyclinc (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 34.2-4% (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).

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).

![Fig. 2: Microaerophilic jar with a lighting candle](image)

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

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Total animals</th>
<th>Total positive</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 1 year</td>
<td>197</td>
<td>92</td>
<td>46.70</td>
</tr>
<tr>
<td>&gt; 1 year</td>
<td>133</td>
<td>21</td>
<td>15.70</td>
</tr>
<tr>
<td>Total</td>
<td>330</td>
<td>113</td>
<td>34.24</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>257</td>
<td>87</td>
<td>33.85</td>
</tr>
<tr>
<td>Female</td>
<td>73</td>
<td>26</td>
<td>35.61</td>
</tr>
<tr>
<td><strong>Breed</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-descript</td>
<td>87</td>
<td>40</td>
<td>45.97</td>
</tr>
<tr>
<td>Doberman</td>
<td>18</td>
<td>06</td>
<td>33.33</td>
</tr>
<tr>
<td>Labrador</td>
<td>73</td>
<td>24</td>
<td>32.88</td>
</tr>
<tr>
<td>German shepherd</td>
<td>56</td>
<td>18</td>
<td>32.14</td>
</tr>
<tr>
<td>Pomeranian</td>
<td>50</td>
<td>15</td>
<td>30.00</td>
</tr>
<tr>
<td>Spitz</td>
<td>20</td>
<td>08</td>
<td>40.00</td>
</tr>
<tr>
<td>Boxer</td>
<td>06</td>
<td>02</td>
<td>25.00</td>
</tr>
<tr>
<td>Rottweiler</td>
<td>11</td>
<td>02</td>
<td>18.18</td>
</tr>
<tr>
<td>Bull mastiff</td>
<td>04</td>
<td>00</td>
<td>00.00</td>
</tr>
<tr>
<td>Cocker spaniel</td>
<td>01</td>
<td>00</td>
<td>00.00</td>
</tr>
<tr>
<td>Chihuahua</td>
<td>01</td>
<td>00</td>
<td>00.00</td>
</tr>
<tr>
<td>Pug</td>
<td>01</td>
<td>00</td>
<td>00.00</td>
</tr>
<tr>
<td><strong>Health status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G.I. disorders</td>
<td>197</td>
<td>93</td>
<td>47.21</td>
</tr>
<tr>
<td>Other problems</td>
<td>133</td>
<td>20</td>
<td>15.04</td>
</tr>
</tbody>
</table>
relation 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 apparently healthy or having some problem other than gastroenteritis (Table 1) showing that these animals can be carriers of *Campylobacter* species and may serve 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, Amoxicillin, Aztreonam, Lincomycin, Tetracycline, Oxytetracycline and Penicillin. A high rate of resistance was observed to Cefotaxim (97.35%), Peefloxacin (91.15%), Chloramphenicol (90.27%), Oxofloxacin (84.07%), Ciprofloxacin (83.18%), Cefadroxil (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-cloxacin, tetracycline, ofloxacin, ciprofloxacin etc were previous reported by various researchers like Little et al. (2008) and Maflin et al. (2007). Quinolones were considered as suitable drug for treatment of Campylobacteriosis (Uboi-Egbemei 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 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 therapeutic and/or prophylaxis for man as well as animals should be monitored because acquisition of antibiotic resistant strains of *Campylobacter* 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.

**ACKNOWLEDGMENTS**

This study was supported by Uttar Pradesh Pandit Deen Dayal Upadhayay Pashu Chikitsa Vigyan Vishvavidyalaya Evam Go-Anusandhan Sansthan (DUVASU), Mathura, India under university funding scheme. The authors are highly thankful to Head, Department of Veterinary Microbiology and Immunology; Department of Veterinary Epidemiology and Preventive Medicine, Director, Teaching Veterinary Clinical Complex, Dean, College of Veterinary Sciences and Animal Husbandry, Director Research and Hon’ble Vice Chancellor, Uttar Pradesh Pandit Deen Dayal
REFERENCES


Campylobacter and Salmonella in raw red meats in the United Kingdom: Prevalence, 

Incidence and drug resistance pattern of 

Man, S.M., 2011. The clinical importance of emerging 

Antibiotic resistance in Campylobacter jejuni and 

Isolation and characterization of 
Campylobacter Helicobacter and 
Anaerobiospirillum strains from a puppy with 

Campylobacter spp. in raw retail poultry in 


Prevalence of resistance to 11 antimicrobials 
among Campylobacter coli isolated from pigs on 

Parsons, B.N., C.J. Porter, R. Ryvar, J. Stavisky and N.J. Williams et al., 2010. Prevalence of 
Campylobacter spp. in a cross-sectional study of 

and identification of Campylobacter in stool samples 
of childrens and animals from Vellore, South India. 

Occurrence and species level 
diagnostics of Campylobacter spp., enteric 
Helicobacter spp. and Anaerobiospirillum spp. in 


Sandberg, M., B. Bergsjo, M. Hofshagen, E. Skjerve and 

Skirrow, M.B. and J. Benzamin, 1980. Differentiation of 

Skirrow, M.B., 2006. John McFadyean and the centenary of the first isolation of 


Campylobacter. In: The Microbiological Safety and Quality of Food, 
Lund, B.M., T.C. Baird-Parker and G.M. Gould (Eds.). 
Aspen Publishers Inc., Gaithersburg, Maryland, 
pp: 1040-1056.

Salmonella and Campylobacter in 
household and stray dogs in Northern Taiwan. Vet. 

Uabo-Egbemi, P.O., P.O. Bessong, A. Samie and C.L. Obi, 2011. 
Prevalence, haemolysis and 
anti-biograms of Campylobacters isolated from pigs from 
three farm settlements in Venda region, 

Salmonellosis in apparently healthy dogs. J. Vet. 

Micro-Agglutination Test (MAT) based 
sero-epidemiological study of salmonellosis 

Detection of Salmonella from clinical 
samples of dogs by PCR. Indian J. Anim. 
