Carriage of antimicrobial resistant thermophilic *Campylobacter* in the intestines of household dogs in Morogoro Municipality, Tanzania

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SUMMARY

The genus *Campylobacter* includes many species, most of which are known to be human and animal pathogens causing gastrointestinal diseases. The drugs of choice for treatment of human infections caused by these organisms are known to be fluoroquinolones and macrolides. In the recent past, however, the organisms have been observed to display resistance to antimicrobial agents including the drugs of choice. The present study determined the occurrence of antimicrobial resistant thermophilic *Campylobacter* in the feces of purging and non-purging dogs of different age groups in Morogoro, Tanzania. Fecal samples were collected from 404 dogs and subjected to the Cape Town protocol for isolation of thermophilic *Campylobacter*. Obtained isolates were tested for resistance against ten antimicrobial agents. Out of 404 sampled dogs, 40 (9.90%) were infected with thermophilic *Campylobacter*. *C. jejuni* comprised 82.5% of the total number of isolates. There were no statistically significant differences in the proportions of positive samples for thermophilic *Campylobacter* between those collected from adults (9.39%, n=298) and young (11.32%, n=106), and also between those collected from male (11.32%, n=212) and female (8.33%, n=192) dogs. All the thermophilic *Campylobacter* isolates were resistant to nalidixic acid and cephalothin. The lowest frequency of resistance was observed for ciprofloxacin. Resistance to other antimicrobial agents ranged from 15.0% to 95.0%. Multi-drug resistance was observed in 32.5% of all isolates. The findings of this study indicate that dogs are potential sources of human infections with antimicrobial resistant thermophilic *Campylobacter*. Attempts to eliminate infections caused by *Campylobacter* should take dogs into consideration, also should consider use of drugs that are effective for treatment of humans against the disease in order to succeed in treatment of campylobacteriosis.

Keywords: Companion animals, Tanzania, Thermophilic *Campylobacter*

INTRODUCTION

In both developed and developing countries, *Campylobacter* forms the aetiology responsible for majority of the bacterial causes of gastro-intestinal illness (Rzewuska *et al.*, 2010; Gripp *et al.*, 2011; Newell *et al.*, 2011; Haruna *et al.*, 2013). More than 80.0% of those *Campylobacter* mediated illnesses in humans are caused by *C. jejuni* (Friedman *et al.*, 2000; Snelling *et al.*, 2005; Rajendran *et al.*, 2012); and the largest part of the remaining proportion by *C. coli* (Rajendran *et al.*, 2012). Raw or undercooked poultry meat is frequently incriminated as the source of the organisms for human infections (Ogden *et al.*, 2009; Lindmark *et al.*, 2009). Extra-intestinal infections with thermophilic *Campylobacter*, particularly *C. jejuni*, have been linked to post-infectious auto-immune neuromuscular disorder, Guillain-Barre´ syndrome, and reactive arthritis (Blaser, 1997; Rajendran *et al.*, 2012).

Most *Campylobacter* infections in animals

do not result into clinical disease and are therefore asymptomatic; whereas in humans the organisms cause acute and self-limiting intestinal infections (Takamiya *et al.,* 2011) which do not require therapy. However, antimicrobial therapy in human *Campylobacter* infections is indicated in cases of severe and prolonged gastrointestinal illnesses and extra intestinal infections, in which macrolides and fluoroquinolones are the drugs of choice (Guerrant *et al.,* 2001; Gupta *et al.,* 2004; McDermott *et al.,* 2004). Studies are increasingly reporting resistance of *Campylobacter* isolates to these (Gibreel and Taylor, 2006; Gallay *et al.,* 2007; Ghosh *et al.,* 2013; Komba et al., 2014; Komba *et al.,* 2015) and other antimicrobial agents.

Authors of several studies have determined risk factors for sporadic human *Campylobacter* infections through case-control studies. Identified risk factors for the disease have included handling and/or consumption of poultry, consumption of raw and unpasteurized milk, drinking untreated water, drinking bottled water, eating salad vegetables, handling and cooking food, contact with animals, swimming in natural water bodies and travel abroad (Kapperud *et al.,* 1992; Morgan *et al.,* 1994; Fahey *et al.,* 1995; Evans *et al.,* 1996; Eberhart-Phillips *et al.,* 1997; Studahl *et al.,* 2000; Kalman *et al.,* 2000; Rodrigues et al., 2001; Kapperud et al., 2003; Friedman et al., 2004; Carrique-Mas *et al.,* 2005;Wingstrand *et al.,* 2006; Uyttendaele *et al.,* 2006; Stafford *et al.,* 2007; Gallay *et al.,* 2008; Lindmark *et al.,* 2009; Komba *et al.,* 2015). Some other studies have specifically implicated contact with pets as a potential source of *Campylobacter* infections to humans (Wolfs *et al.,* 2001; Neimann *et al.,* 2003; Damborg *et al.,* 2004; Carrique-Mas *et al.,* 2005).

No study has been found in Tanzania reporting thermophilic *Campylobacter* carriage in dogs. Consequently the objective of this study was to determine the occurrence and antimicrobial resistance of thermophilic *Campylobacter* in domestic dogs. Results of the study shade some light on the possibility of dogs serving as sources of human infections with thermophilic *Campylobacter*.

MATERIALS AND METHODS

Study area

Dogs were reached out in different parts of Morogoro Municipality, Tanzania. Visited areas were Mazimbu, Kihonda, Mzumbe and staff houses of Main Campus of Sokoine University of Agriculture (SUA). Samples were also collected from the dogs brought to SUA Teaching Animal Hospital for different veterinary services. Analysis of the collected fecal samples from the dogs was conducted at microbiology laboratories of the Pest Management Centre of SUA.

Study design, sample size determination and sample collection

The carriage of antimicrobial resistant thermophilic *Campylobacter* in dogs in the study area was established in a cross-sectional study. The number of dogs sampled was determined using the formula developed by Thrusfield (1995) i.e. $n=Z^2p(1-p)/d^2$, where n is sample size; Z is the multiplier from the normal distribution, p is the expected prevalence and d is the desired absolute precision. The known prevalence of *Campylobacter* infections (p) in dogs was set at 50% as there is no previous report in the country to that effect. With Z value of 1.96 at 95% confidence interval (CI) and desired precision (d) of 0.05, the calculated sample size (n) was 385. Samples were collected from a total of

404 dogs. During collection, the samples were immediately placed in bijou bottles containing Bolton broth (Oxoid Ltd., Basingstoke, Hampshire, England) for enrichment. Location, age and sex of the dog were concurrently recorded in the sheet during sampling, and corresponding codes written on collection bottles. The samples were then conveyed on ice to the laboratory for analysis within 8 hrs of sampling. In the laboratory the faecal samples were incubated at 37°C for 24 hrs after which they were subjected to the Cape Town protocol for primary isolation of thermophilic *Campylobacter*.

Isolation and identification of thermophilic *Campylobacter* species

Following enrichment in Bolton broth, isolation of *Campylobacter* from collected faecal samples adopted the Cape Town protocol developed by Le Roux and Lastovica (1998) with some modifications as indicated by Jacob *et al* (2011). The organisms were isolated on an antibiotic-free blood agar following a filtration step as previously described by Komba *et al.* (2014; 2015). Following isolation, presumptive thermophilic *Campylobacter* were identified in two steps; 1) Preliminary identification using phenotypic tests 2) Confirmation of the isolates using a genotypic technique (polymerase chain reaction) using protocols explained in Komba *et al.* (2014) and Komba *et al. (* 2015)

Evaluation of antimicrobial resistance of *Campylobacter* isolates

The disc diffusion method (Luangtongkum *et al.,* 2007) was adopted in testing of *Campylobacter* isolates for resistance against eight different antimicrobial agents. In summary, *Campylobacter* colonies were dissolved in normal saline to make suspensions of a turbidity equivalent to a 0.5 McFarland standard. The suspensions were then inoculated onto well dried Mueller-Hinton agar plates followed by distribution of antimicrobial discs using a BBL Sensi-disc dispenser. The plates were then incubated at 42°C for 48 hours under microaerobic conditions after which the diameters of zones of inhibition of microbial growth were measured. Results were interpreted based on guidelines developed by the National Committee on Clinical Laboratory Standards (currently known as Clinical and Laboratory Standards Institute) (NCCLS, 2002). Isolates were tested for resistance against the following antimicrobial agents; nalidixic acid (NA, 30 μg), ciprofloxacin (CIP, 5 μg), gentamycin (CN, 10 μg), cephalethin (CL, 30 μg), amoxycillin (AML, 25 μg), norfloxacin (NOR, 10 μg), erythromycin (E, 15 μg) and azithromycin (AZM, 15 μg) (Oxoid, Hampshire, UK). An isolate that was resistant to two or more classes of antimicrobial agents was referred to as multi-drug resistant.

Data analysis

Data were analyzed in MedCalc™ software following cleaning in Microsoft Excel™. Descriptive statistics were computed to determine prevalence of thermophilic *Campylobacters* in dogs; and proportions of isolates resistant to different antimicrobial agents. Proportions were compared for significant differences using a Chi square test at critical probability of $P < 0.05$.

RESULTS

Four hundred and four dogs were sampled, comprising 212 (52.47%) males and 192 (47.5%) females. Of the sampled dogs 298 (73.8%) were adult dogs (≥6 months) and 106 (26.2%) were puppies (<6 months). The overall prevalence of *Campylobacter* in dogs was 9.9%

(40/404). *C. jejuni* comprised 72.5% of and the remaining (12.5%) being *C. jejuni – C. coli* co-existing. The prevalence in adult dogs was 9.4% and that of puppies was 11.3%. The prevalence in males was 11.3% (n=212) while in females was 8.3% (n=192). There was no statistically significant difference of prevalence between adults and young, between health (9.6%; n=311) dogs and sick (10.8%; n=93) dogs, and also between males and females (p >0.05). Proportions of positive samples by sampling locations are as presented in table 1.

Antimicrobial resistance profiles of thermophilic *Campylobacter* isolates obtained from dogs in Morogoro Municipality

All the dog derived thermophilic *Campylobacter* isolates in this study were resistant to nalidixic acid and cephalothin. The lowest frequency of resistance was observed for ciprofloxacin. Results on resistance frequencies are shown in Table 2. Resistance to more than two classes of antimicrobial was observed in 32.5% of isolates.

Table 1. No significant differences were observed basing on sampling location

Name of the location	Proportions of positive samples (%)
SUA Main Campus	15.3
Mazimbu	7.8
Kihonda	9.3
Mzumbe	0.0
Falkland	25.0
SUA staff quarters	7.4

the total number of isolates, *C. coli* 15%

DISCUSSION

The findings from the study reveal intestinal carriage of thermophilic *Campylobacter* in 9.9% of the sampled dogs. This frequency is comparable to that of 13.0% obtained in a repeated cross-sectional study conducted in New-zealand (Mohan *et al.,* 2015). The proportion is however significantly low compared to those reported in other African countries (Turkson *et al.,* 1998; Salihu *et al.,* 2010) and elsewhere in Europe (Burnens and Nicolet, 1992; Torre and Tello, 1993; Hald *et al.,* 2004; Acke *et al.,* 2006; Acke *et al.,* 2009; Selwet *et al.,* 2014; Holmberg *et al.,* 2015). Various frequencies of *Campylobacter* infections in dogs have been reported in different countries including 47.2% in Kenya (Turkson *et al.,* 1998), 27.7% in Nigeria (Salihu *et al.,* 2010), 76.2% in Denmark (Hald *et al.,* 2004), 56.0% (Engvall *et al.,* 2003) and 37.0% (Holmberg *et al.,* 2015) in Sweden, 50.0% in Poland (Selwet *et al.,* 2014), 26.24% in Spain (Torre and Tello, 1993), 30.4% in Slovakia (Badlík *et al.,* 2014), 18.14% in Switzerland (Burnens and Nicolet, 1992); and 51.1% (Acke *et al.,* 2006) and 87.0% (Acke *et al.,* 2009) in two different studies conducted in Ireland. Differences in extent and frequencies of exposure could be determinants for the different proportions of infected dogs. Koziel *et al*. (2014) attribute the varied detection rates of *Campylobacter* species from dog faeces to geographical location and methodology used.

Table 2. Antimicrobial resistance test results for 40 *Campylobacter* isolates obtained from dogs in Morogoro Municipality

Name of antibiotics	Antimicrobial class	Proportion of resistant isolates (%)
Gentamycin	Aminoglycoside	45
Erythromycin	Macrolide	15
Azithromycin	Macrolide	25
Norfloxacin	Quinolone	75
Ciprofloxacin	Quinolone	10
Cephalothin	Cephalosporin	100
Nalidixic Acid	Quinolone	100
Amoxycillin	Penicillin	95

Findings of this study corroborate those of previous studies in Kenya (Turkson *et al.,* 1998), Slovakia (Badlík *et al.,* 2014), Spain (Torre and Tello, 1993) and Italy (Giacomelli *et al.,* 2015) which reported higher frequencies of isolation of *C. jejuni* followed by *C. coli* in dogs. Different observations were made by a study in Nigeria and those in European countries (Engvall *et al.,* 2003; Hald *et al.,* 2004; Acke *et al.,* 2006; Acke *et al.,* 2009; Salihu *et al.,* 2010; Holmberg *et al.,* 2015). In these studies faecal samples from dogs yielded higher frequencies of *C. upsaliensis* followed by *C. jejuni*. In a study by Hald *et al.* (2004) *C. lari* followed after *C. jejuni* and then *C. coli,* while in a study conducted in Ireland by Acke *et al.* (2009) *C. coli* occurred at a higher frequency than *C. lari*. Holmberg and colleagues (2015) didn't report the occurrence of *C. coli* at all among thermophilic *Campylobacter* isolates from dogs in Sweden. The authors indicated *C. helveticus* to be the third species detected. Two different studies conducted in Switzerland reported similar frequencies of occurrence of *C. upsaliensis* and *C. jejuni* among dog faecal samples (Burnens and Nicolet, 1992; Amar *et al.,* 2014). The findings in these different studies indicate inconsistencies in the species distribution of thermophilic *Campylobacter* isolates in dogs. This could be an attribute of factors which require further investigation. Chaban *et al.* (2010) points out to a correlation between the number of thermophilic *Campylobacter* species obtained and the method used in detection. The authors reveal an increase in species richness in faeces of diarrheic animals in a study that employed quantitative PCR.

Worldwide, *C. jejuni* is the most commonly termophilic *Campylobacter* species involved in human infections (Newell and co-authors, 2000; Komba *et al.,* 2015). The frequent occurrence of *C. upsaliensis* among dogs in some locations suggests the role of pets as an important reservoir for *C. upsaliensis* infections in people (Bourke and colleagues, 1998). Human infections caused by this particular termophilic *Campylobacter* species have been associated with gastrointestinal illness, bacteraemia, haemolytic-uraemic syndrome and an autoimmune disorder, the Guillan-Barré Syndrome (Lastovica *et al.,* 1989; Goossens *et al.,* 1995; Carter and Cimolai, 1996; Jenkin and Tee, 1998; Bourke *et al.,* 1998)

The present study found no association of the occurrence of thermophilic *Campylobacter* in dogs with sex, age or healthy status of a dog. Studies in Ireland (Acke *et al.,* 2009) and Canada (Chaban *et al.,* 2010) attributed higher prevalence of thermophilic *Campylobacter* in dogs to diarrhea. The authors reported higher frequencies of thermophilic *Campylobacter* among diarrheic dogs as compared to healthy dogs. Chaban *et al.* (2010) further reported thermophilic *Campylobacter* species richness among isolates from diarrheic dogs as compared to those from healthy dogs. Other authors (Sandberg *et al.,* 2002; Acke *et al.,* 2006), however, reported comparable detection frequencies of thermophilic *Campylobacter* from dogs with and without diarrhoea. Several other studies have compared frequencies of

detection of thermophilic *Campylobacter* between young and adult dogs; and found young dogs to be more frequently infected (Torre and Tello, 1993; Engvall *et al.*, 2003; Acke *et al.*, 2009; Holmberg *et al.*, 2015). Working with two shelters, Acke *et al.* (2006) had two different observations where in one shelter the frequency of infection was higher in young dogs where as young and adult dogs had comparable frequencies of infections in the other shelter. In Nigeria (Salihu *et al.*, 2010) recorded comparable frequencies of infections between young and adult dogs. Surprisingly, Badlík *et al.* (2014) reported a higher occurrence of the organisms in adult dogs. With regard to sex, Badlík *et al.* (2014) observed association between the frequency of isolation of thermophilic *Campylobacter* and dog gender.

Some (2.8%) of the thermophilic *Campylobacter* isolates from dogs couldn't be specified and were simply referred to as *Campylobacter* spp. (Hald *et al.*, 2004). A similar observation surfaced in this study where five isolates (12.5%) could not be identified to species level. Also in Kenya (Turkson *et al.*, 1998) couldn't identify to species level three (7.1%) thermophilic *Campylobacter* isolated from feces of diarrhoeic dogs. Six isolates could not be assigned to a known species with probes or conventional tests (Burnens and Nicolet, 1992).

Occurrence of antimicrobial resistant phenotypes among companion animal bacteria isolates implies their role in the spread of the organisms to humans (Guardabassi *et al.*, 2004; Boerlin and White, 2006; Jackson *et al.*, 2009). Antimicrobial resistance among thermophilic *Campylobacter* isolates from dogs in this study, in which 39.0% of the isolates displayed multidrug resistance, ranged from 45.0% for gentamycin to 100.0% for nalidixic acid and cephalothin. Other frequencies of resistant isolates were 10% for ciprofloxacin, 25% for azithromycin, 15% for erythromycin and norflaxicin, and 95% for amoxicillin. Results contrary to these were obtained in Norway where Sandberg *et al.* (2002) reported susceptibility of all the thermophilic *Campylobacter* isolates from dogs (n=42) to ampicillin, ciprofloxacin, chloramphenical, gentamycin, erythromycin and tetracycline. The authors reported 45.23% resistance to streptomycin and 2.38% to nalidixic acid. In Norway Amar *et al.* (2014) also report susceptibility of all dog derived thermophilic *Campylobacter* isolates to macrolides but the authors report resistance of 20.9% of the isolates to quinolones which compares to the proportion observed for ciprofloxacin in Ireland (Acke *et al.*, 2009). Acke *et al.* (2009) found 11.8% and 37.3% of resistant thermophilic *Campylobacter* isolates to erythromycin (a macrolide) and nalidixic acid (a quinolone). Lower resistance levels observed for macrolides and quinolones in these studies support findings of the current study and underscore the potential of these classes of antimicrobial agents as drugs of choice for treatment of human campylobacteriosis. Differences in resistance seen in different investigations could be due to changes over time, but also could be an attribute of differences in exposure rates of the microbes to the different antimicrobial agents.

Results of the present study disclose the potential of dogs as sources of *Campylobacter* infections to human. Elsewhere *C. jejuni* isolates from dogs were found to be identical to those commonly found in humans (Holmberg *et al.*, 2015). Future attempts to stem human *Campylobacter* infections should take dogs into consideration. As most of the isolates were sensitive to ciprofloxacin and erythromycin, the continual use of these

antimicrobial agents in treatment of human campylobacteriosis cases is recommended.

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