

Prevalence and Antimicrobial Susceptibility of Thermophilic *Campylobacter* Isolated from Sheep at Debre Birhan, North-Shoa, Ethiopia

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ABSTRACT

Prevalence and susceptibility testing of thermophilic *Campylobacter* spp. in fecal and carcass swab samples were undertaken at Debre Birhan, North-Shoa, Ethiopia during a 9 mth period from August 2011 to April 2012. Out of 310 fecal samples, 33 (10.6%) thermophilic *Campylobacter* spp. were isolated on blood-free modified charcoal, cefoperazone, desoxycholate agar plates in a micro aerobic atmosphere. From the 33 *Campylobacter* isolates, 87.9% were *C. jejuni* and 12.1% were *C. coli*. The prevalence of *Campylobacter* was high (78.8%) from August to December on all farms, whereas low prevalence (21.2%) was observed from January to April. The isolation rate of *Campylobacter* spp. in the Awassi exotic and cross breeds (60.6%) was higher than for indigenous breeds (33.4%). No *Campylobacter* spp. were found in the Adale indigenous breeds. Fifty (21.4%) *Campylobacter* isolates were found from 70 carcass swab samples. Among the thermophilic *Campylobacter* isolates from the carcass samples, *C. jejuni* and *C. coli* accounted for 93.3 and 6.7%, respectively. A statistically significant ($P \leq 0.05$) difference was observed between the two *Campylobacter* species identified. All *Campylobacter* isolates were tested with the most commonly used antimicrobial agents in Ethiopia by the agar disc diffusion method. The resistance rates to ampicillin, tetracycline, streptomycin and nalidixic acid were 33.3, 20.8, 4.2 and 2.1%, respectively. All isolates were resistant to cephalothin. In conclusion, the sources of *Campylobacter* and their sensitivity testing in sheep feces and carcasses are useful for herd health management and sheep husbandry and in antimicrobial surveillance for public health.

Keywords: *Campylobacter* spp., *C. jejuni*, *C. coli*, sheep, antimicrobial, Ethiopia

INTRODUCTION

Campylobacter spp. are widely distributed among domestic animals. The most common infection sources are food or water. *Campylobacter jejuni* and *Campylobacter coli* are the most common

etiological agents of bacterial gastroenteritis in humans (Skirrow, 1994). However, previous studies reported that *Campylobacter* spp. are frequently and highly colonized in the intestinal tracts of animals (Nesbakken *et al.*, 2003; Pearce *et al.*, 2003; Pezzotti *et al.*, 2003). *Campylobacter*

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was found as commensals in the intestinal tract of a wide range of warm-blooded animals, both domestic and wild. Sheep and beef as well as their products have been implicated as a source of outbreaks of campylobacteriosis (Pezzotti *et al.*, 2003; Whyte *et al.*, 2004; Parisi *et al.*, 2007; Little *et al.*, 2008). Transmission can occur through direct contact with infected animals or from equipment, water or during carcass dressing in a slaughter line (Jones *et al.*, 1991; Adak *et al.*, 1995; Herman *et al.*, 2003; Whyte *et al.*, 2004). In Ethiopia, sheep carcasses were found to be more highly contaminated with *Campylobacter* spp. than goat carcasses with rates of 10.6% and 9.4%, respectively (Woldemariam *et al.*, 2009). Moreover, the studies of Dadi and Asrat (2008) also reported *Campylobacter* species isolated from sheep and goat carcasses with rates of 10.5% and 7.6%, respectively. Similar studies indicated that *Campylobacter* spp. were isolated from 38% of sheep fecal samples (Kassa *et al.*, 2007). Several studies indicated the resistance of *Campylobacter* spp. to most antimicrobial agents (Asrat *et al.*, 1999; Avrain *et al.*, 2003; Kassa *et al.*, 2007). It is probably the use of antibiotics in animals reared for human consumption—particularly in developed countries—which leads to the development of resistant pathogenic bacteria that can reach humans through the food chain (Aarestrup, 1999; Van Looveren *et al.*, 2001; Avrain *et al.*, 2003). In Ethiopia, only a few publications have reported on the occurrence and susceptibility testing of *Campylobacter* strains to antibiotics (Dadi and Asrat, 2008), while the raw meat that is widely consumed in the country increases the possibility of pathogen transmission to humans. Therefore, a cross sectional study was undertaken to determine the prevalence of *Campylobacter* spp. in different sheep breeds and carcasses at Debre Birhan North-Shoa, Ethiopia, and antimicrobial susceptibility profiles of thermophilic *Campylobacter* spp. were also investigated.

MATERIALS AND METHODS

Fecal sample collection

Three hundred and ten fecal samples were randomly collected from three different sheep farm types in North-Shoa Debre Birhan Ethiopia—namely, the Debre Birhan Agriculture Research Center (DBARC; n = 138), the Debre Birhan Sheep Breeding and Forage Multiplication Center (DBSBFMC; n = 117) and small holder farms (SHF; n = 55).

Fecal samples were collected from individual healthy sheep directly from the rectum using a sterile glove and were transferred in an ice box with cooled ice packs to the National Animal Health Diagnostic and Investigation Center (NAHDIC) Sebeta, Addis Ababa on the same day of collection for laboratory examination.

Carcass samples collection

Seventy carcass swab samples were randomly collected from the surface and deep part of carcasses at five different sampling locations (neck, thorax, abdomen, breast and crutch) of conventionally slaughtered sheep at the same slaughter facilities using a sterile cotton swab for each swabbing site. Then, the swab samples were transferred to the laboratory using tubes containing 0.85% NaCl solution. All samples were transported under cool conditions to the NAHDIC.

Culture and screening of thermophilic *Campylobacter* species

Fecal and carcass swab samples were directly inoculated onto modified charcoal, cefoperazone, desoxycholate agar (mCCDA; CM739B; Oxoid Ltd.; Basingstoke, Hampshire UK), a blood free selective medium with the CCDA selective supplement SR155E and is recommended for the isolation of thermophilic *Campylobacter* spp. from clinical and environmental samples. The plates were incubated under a microaerophilic atmosphere (85% N₂, 10% CO₂, 5% O₂) using

CampyGen™ gas generating kits (Oxoid Ltd.; Basingstoke, Hampshire UK) at 42 °C for 48 hr. The *Campylobacter* spp. were identified based on the characteristics of colony appearance, Gram-staining reactions and positive testing for oxidase and catalase reactions. Species differentiation was based on hippurate hydrolysis and susceptibility to nalidixic acid (30 µg) and cephalothin (30 µg (Oxoid Ltd.; Basingstoke, Hampshire UK). These parameters formed the basis for the identification of *C. jejuni* and *C. coli* as proposed by On (1996). The type strains *C. jejuni* (NCTC 11351) and *C. coli* (LMG 6440) were included as positive controls (Kassa *et al.*, 2007; Ewnetu and Mihret, 2010).

Antimicrobial susceptibility pattern of *Campylobacter* species

Five of the most commonly available antibiotics in Ethiopia were used in the disk diffusion method for antimicrobial susceptibility testing of *Campylobacter* spp. according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2003). The inoculum was swabbed onto a Mueller-Hinton agar (Oxoid Ltd.; Basingstoke, Hampshire UK) supplemented with 5% sheep blood. The following antibiotic discs were placed onto the inoculated agar plates after drying the plates for 3–5 min: ampicillin (AMP) 10 µg, streptomycin (S) 10 µg, tetracycline (TE) 30 µg, nalidixic acid (NA) 30 µg and cephalothin (KF) 30 µg. The plates were incubated at 42 °C for 48 hr under a microaerobic-generated atmosphere (5% O₂, 10% CO₂ and 85% N₂) in an anaerobic jar (Oxoid Ltd.; Basingstoke, Hampshire UK) without a catalyst and by using CampyGen™ gas generating kits (Oxoid Ltd.; Basingstoke, Hampshire England). A standard reference strain of *E. coli* (ATCC 25922), that is sensitive to all the antimicrobial drugs tested, was used as a control strain. The susceptibility test was conducted in the NAHDIC. The breakpoint provided by NCCLS (1990) M2-A4 was used for interpretation.

Statistical analysis

Statistical analysis was conducted with the SAS statistical software package for Windows (version 9.00; Cary, NC, USA) using the chi-square (χ^2) Fisher exact test to determine the prevalence and the antimicrobial susceptibility test. A value of $P \leq 0.05$ was used to determine any statistically significant differences.

RESULTS

Isolation rate of *Campylobacter* from fecal samples

The numbers and percentages of the thermophilic *Campylobacters* isolated from sheep fecal samples obtained from the three farm types are presented in Table 1. Out of a total of 310 fecal samples, 33 (10.6%) *Campylobacter* spp. were isolated. The numbers of *Campylobacter* spp. at different farms were: 12 isolates (8.7%) from the DBARC (n = 138), 15 (12.8%) from the DBSBFMC (n = 117) and 6 (10.9%) from the SHF (n = 55). From the 33 thermophilic *Campylobacter* isolated, *C. jejuni* and *C. coli* accounted for 29 (87.9%) and 4 (12.1%), respectively (Table 1). A statistically significant difference in the rate of isolation was observed between the two *Campylobacter* species identified.

Seasonal variation versus *Campylobacter* prevalence

The prevalence of *Campylobacter* spp. differed during the different periods analyzed as shown in Figure 1. The results show that the prevalence of *Campylobacter* was high from August to December (wet and dry seasons) on different farms. The overall prevalence from August to December was 78.8% and the isolation rates during this period were 83.3% from the DBARC, 80% from the DBSBFMC and 66.7% from the SHF, whereas a low prevalence (21.2%) was found from January to April (dry season). The isolation rates during January to April in the different farm types were 16.7% from the DBARC,

33.3% from the DBSBFMC and 20% from the SHF.

Isolation rates of *Campylobacter* from carcass samples

The numbers and percentages of *Campylobacter* spp. isolated from the sheep carcass samples obtained from different parts of the carcass (neck, thorax, abdomen, breast and

crutch) are presented in Table 2. From a total of 70 carcass-swabbed samples, 15 isolates (21.4%) were found. The isolation rates of *Campylobacter* from the surface and deep parts of different carcass swab sites were 13 (37%) and 2 (5.7%) isolates, respectively (Table 2). Out of the 15 thermophilic *Campylobacter* isolated from carcasses, *C. jejuni* and *C. coli* accounted for 14 (93.3%) and 1 (6.7%), respectively.

Table 1 Prevalence of thermophilic *Campylobacter* species in sheep fecal samples obtained from different farms.

Farm type	<i>Campylobacter</i> spp.			<i>P</i> value
	<i>C. jejuni</i> No. (%)	<i>C. coli</i> No. (%)	Total No. (%)	
DBSBFMC (n = 117)	14 (11.9) ^a	1 (0.8) ^b	15 (12.8)	0.0001
DBARC (n = 138)	11 (7.9) ^a	1 (0.7) ^b	12 (8.7)	0.0005
SHF (n = 55)	4 (7.2) ^a	2 (3.6) ^b	6 (10.9)	0.0143
Total (n = 310)	29 (87.9) ^a	4 (12.1) ^b	33 (10.6)	0.0017

DBSBFMC = Debre Birhan Sheep Breeding and Forage Multiplication Center, DBARC = Debre Birhan Agriculture Research Center, SHF = Small holder farm.

^{a,b} = Values with different lower case superscript letters show significant difference between the two *Campylobacter* species identified (*P* < 0.05).

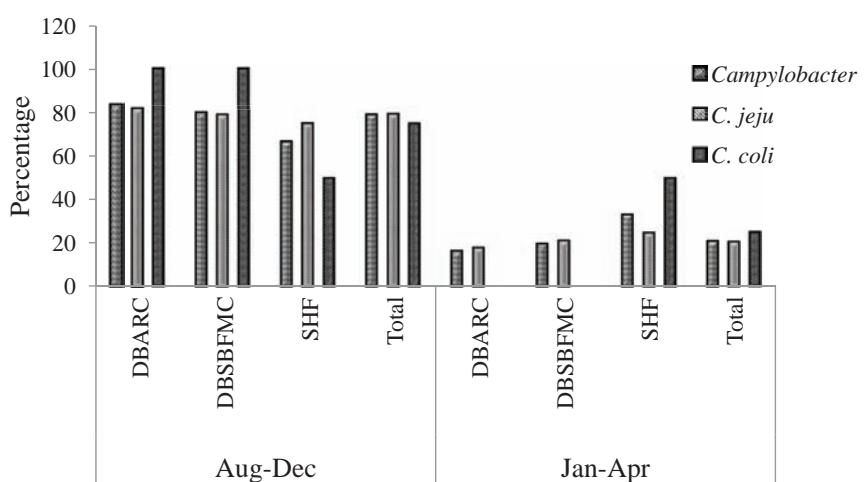


Figure 1 Graphical presentation of the prevalence of *Campylobacter* in different seasons during the study period (August 2011 to April 2012). DBARC = Debre Birhan Agriculture Research Center, DBSBFMC = Debre Birhan Sheep Breeding and Forage Multiplication Center, SHF = Small holder farm.

***Campylobacter* in different indigenous and exotic sheep breeds**

The rates of isolation of *Campylobacter* were significantly different between the breeds as shown in Table 3. Among the 33 *Campylobacter* isolates from the fecal samples, the highest isolation rates were observed in Awassi exotic and cross breeds with 20 isolates (60.6%) followed by the Bonga and Menz indigenous breeds, which accounted for 5 isolates each (15.2%) and there was 1 isolate (3%) for the Washera indigenous breed. None of the *Campylobacter* spp. was found in the Adale indigenous breed. In the Dorper exotic breed, 2 isolates (6%) of *Campylobacter* spp. were found.

Antimicrobial susceptibility pattern of *Campylobacter* species

Forty three *C. jejuni* and five *C. coli* isolates were used to test antimicrobial susceptibility for five commonly used antibiotics in Ethiopia. The number of isolates and the percentage of antimicrobial susceptibility of *C. jejuni* and *C. coli* are shown in Table 4. Out of the 48 *Campylobacter* isolates, the highest level of resistance (100%) was recorded to cephalothin. The rates of resistance to other antimicrobial agents studied were 33.3, 20.8, 4.2 and 2.1% to ampicillin, tetracycline, streptomycin and nalidixic acid, respectively. A majority of the isolated *Campylobacter* spp. was susceptible

Table 2 Proportion of *Campylobacter* positive sheep carcasses according to different swabbing sites.

Swabbing site	Neck (n = 7)	Thorax (n = 7)	Abdomen (n = 7)	Breast (n = 7)	Crutch (n = 7)	Total (N = 35)
Surface swab	2(28.6%)	3(42.9%)	2(28.6%)	2(28.6%)	4(57.1%)	13(37%)
Swabbing site	Neck (n = 7)	Thorax (n = 7)	Abdomen (n = 7)	Breast (n = 7)	Crutch (n = 7)	Total (N = 35)
Deeper swab	1(14.3%)	0(0)	0(0)	0(0)	1(14.3%)	2(5.7%)
Total	3(21.4%)	3(21.4%)	2(14.3%)	2(14.3%)	5(35.7%)	15(21.4%)

Table 3 Occurrence of *Campylobacter* spp. in different sheep breeds from three farms.

<i>Campylobacter</i> isolated	No. (%) <i>Campylobacter</i> spp. isolated in different breeds					
	Awassi exotic & cross	Bonga indigenous	Menz indigenous	Washera indigenous	Adale indigenous	Dorper exotic
DBARC (n = 12)	2(16.7)	5(41.6)	2(16.7)	1(8.3)	0(0)	2(16.7)
DBSBFMC (n = 15)	14(93.3)	-	1(6.7)	-	-	-
SHF (n = 6)	4(66.7)	-	2(33.3)	-	-	-
Total (n = 33)	20(60.6) ^a	5(15.2) ^b	5(15.2) ^a	1(3) ^c	0(0) ^c	2(6) ^b

DBSBFMC = Debre Birhan Sheep Breeding and Forage Multiplication Center, DBARC = Debre Birhan Agriculture Research Center, SHF = Small holder farm.

a,b,c = Values with different lower case superscript letters show significantly different rates of isolation between breeds ($P < 0.05$).

Table 4 Antimicrobial sensitivity of *C. jejuni* and *C. coli* isolated from sheep in Debre Birhan Ethiopia.

Campylobacter	Number (%)				
	AMP	KF	NA	S	TE
<i>C. jejuni</i> (43)					
S	28/65.1	-	43/100	41/95.3	32/74.4
I	-	-	-	-	1/2.3
R	15/34.9	43/100	-	2/4.7	10/23.3
<i>C. coli</i> (5)					
S	4/80	-	4/80	5/100	5/100
I	-	-	-	-	-
R	1/20	5/100	1/20	-	-
Total (48)					
S	32/66.7	-	47/97.9	46/95.8	37/77.1
I	-	-	-	-	1/2.1
R	16/33.3	48/100	1/2.1	2/4.2	10/20.8

AMP = Ampicillin, KF = Cephalothin, NA = Nalidixic acid, S = Streptomycin, TE = Tetracycline, S = Susceptible, I = Intermediate, R = Resistant

to the four antimicrobial agents studied, with rates of 97.9, 95.8, 77.1 and 66.7% to nalidixic acid, streptomycin, tetracycline and ampicillin, respectively. The level of *C. jejuni* resistance recorded to ampicillin was 34.9% followed by tetracycline (23.3%). *C. jejuni* was not resistant to nalidixic acid whereas 20% resistance of *C. coli* was found to nalidixic acid and ampicillin.

DISCUSSION

The occurrence of human *Campylobacter* gastroenteritis has largely been attributed to the consumption of contaminated food of animal origin (Humphrey *et al.*, 2007). In the present study, the prevalence of *Campylobacter* spp. in sheep from different farming systems was investigated with 10.6% and 21.4% prevalence in sheep feces and carcass swab samples, respectively. There were 33 (10.6 %) thermophilic *Campylobacter* spp. isolated from sheep feces in this study comparable to 11.9% reported by Acik and Cetinkaya (2006). In Kaduna, Nigeria, Raji *et al.* (2000) reported 6.8% of *Campylobacter* spp.

from sheep intestinal contents and 2.5% from rectal swabs and a figure of 7.1% was reported from Lagos (Uaboi-Egbenni *et al.*, 2008), which was lower than in the present report. However, a high prevalence of *Campylobacter* spp., (18, 38 and 29.3%) was reported by Salihu *et al.* (2009), Kassa *et al.* (2007) and Stanley *et al.* (1998), respectively.

The contamination rate of *Campylobacter* spp. in the sheep carcasses in the present study was 21.4% (15 isolates) which is comparable to the isolate rates of 15.3% and 20% reported by Aquino *et al.* (2002) and Carbrita *et al.* (1992), respectively. However, the present finding was higher than the rates of 11.8% recorded by Whyte *et al.* (2004), 10.6% by Woldemariam *et al.* (2009), 10.5% by Dadi and Asrat (2008) and 7.4% by Little *et al.* (2008), although higher rates of *Campylobacter* species in different carcass samples were reported with 47.1% by Rahimi and Tajbakhsh, (2008) and 29.9% by Rahimi *et al.* (2010). Among the thermophilic *Campylobacter* species isolated from both fecal and carcass samples, 87.5% were *C. jejuni* and 12.5% were *C.*

coli. In both fecal and carcass samples, the highest prevalence of *Campylobacter* species recovered was *C. jejuni*. This was in agreement with those findings reported by Kassa *et al.*, (2007), Salihu *et al.* (2009) and Rahimi *et al.* (2010). This implies that *C. jejuni* is the most common *Campylobacter* species in sheep in Ethiopia.

The prevalence of *Campylobacter* spp. in different seasons was studied. The rate of *Campylobacter* spp. in sheep was 78.8% during August–December (wet and dry seasons) while it was 21.2% during January–April (dry season). The seasonal fluctuation in the occurrence of *Campylobacter* spp. in this study was in agreement with previous reports which found 66.5% (Rahimi and Tajbakhsh, 2008) and 44.1% (Rahimi *et al.*, 2010) prevalence during the summer season. In addition, Willis and Murray (1997) reported 87–97% and 7–33% prevalence during warmer and winter months, respectively. The Awassi exotic and cross breed, the Bonga, Menz, Adale, Washera indigenous breeds and the Dorper exotic breed were used to study the prevalence of *Campylobacter* spp. The results showed that the Awassi exotic breed and its crosses were more susceptible than indigenous breeds, since the prevalence was higher in the former. The difference in susceptibility may be associated with the environment where they are reared (Alter *et al.*, 2005; Jensen *et al.*, 2006) or with the familiarization of the different sheep breeds to the climate.

Forty-three *C. jejuni* and five *C. coli* isolates were investigated in order to estimate antimicrobial resistance and susceptibility of *Campylobacter* spp. All thermophilic *Campylobacter* spp. were resistant to cephalothin. Similar findings were reported by Asrat *et al.* (1999), Kassa *et al.* (2007) and Boonmar *et al.* (2005). The resistance rate of 33.3% of thermophilic *Campylobacter* to ampicillin in the present study was different from other reports in Ethiopia; in 1999, Asrat *et al.* (1999) reported 60% resistance and recently Dadi and Asrat (2008) and Kassa *et al.* (2007) reported 10% and

20% resistance to ampicillin, respectively. The results of the present study were comparable with the 30% resistance reported from Ireland by Fallon *et al.* (2003). Feizabadi *et al.* (2007) reported 97% resistance to cephalothin and only 11.7% to ampicillin, while Sjorgren *et al.* (1992) showed 98.2% resistance to cephalothin and 20.9% to ampicillin and Boonmar *et al.* (2005) reported 34% resistance to ampicillin and 100% to cephalothin. Lariviere *et al.* (1986) also found 14.6% resistance to ampicillin. The resistance rates of *Campylobacter* isolates (20.8%) to tetracycline in the present study were higher than the rate of 10% reported by Dadi and Asrat (2008) and the rate of 6% in Tan *et al.* (2009), but lower than the rate of 77.94% reported by Sukhapesna *et al.* (2005). The resistance rate to streptomycin in the current study was 4.2% which was higher than previously reported in Ireland and Thailand by Fallon *et al.* (2003) and Sukhapesna *et al.* (2005), respectively. The results of the present study also showed 2.1% resistance to nalidixic acid, which was much lower than the rate of 92.2% reported by Han *et al.* (2007), of 20.5% by Tan *et al.* (2009) and 10.2% by Ishihara *et al.* (2004). The low percentages of resistance to most antimicrobial agents tested in this study may indicate that the usage of those agents as growth promoters and in treatments for animals for human consumption is low if at all. However, the available information on the antimicrobial susceptibility patterns of *Campylobacter* spp. seems to vary widely from country to country and from place to place (Coker and Adefeso, 1994).

CONCLUSION AND RECOMMENDATION

The results of this study showed the presence of *Campylobacter* spp. in sheep feces and carcasses. Seasonal variation in the occurrence of *Campylobacter* spp. was also found. The results revealed that raw meats originating from animals grown for human consumption using a traditional slaughter system are often contaminated with

thermophilic *Campylobacter* spp. which represent a high risk of contamination of the carcass. The low percentages of resistance to most antimicrobial agents tested in this study may indicate that the usage of those agents as growth promoters and for treating animals grown for human consumption is low if at all. It is recommended that organized action is needed to reduce the risks posed by *Campylobacter* spp. in conventional slaughter systems in Ethiopia including a concerted hygienic exercise awareness program for all meat supply stages.

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