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THESIS

DISTRIBUTION OF *CAMPYLOBACTER* SPECIES IN SHEEP OF DIFFERENT PRODUCTION AREA AT DEBRE BIRHAN, NORTH-SHOA, ETHIOPIA

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A Thesis Submitted in Partial Fulfillment of
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The prevalence of thermophilic *Campylobacter* spp. in fecal and carcass swab samples was studied at Debre Birhan, North-Shoa, Ethiopia in a 9 month period from August 2011 to April 2012. Out of 310 fecal samples 33 (10.6%) thermophilic *Campylobacter* spp. were isolated and differentiated to *C. jejuni* and *C. coli* which accounted for 87.9% and 12.1%, respectively. In addition, 15 (21.4%) *Campylobacter* species were isolated from the carcass samples swabbed and investigated (n=70) and accounted for *C. jejuni* and *C. coli*, with 93.3% and 6.7%, respectively. The prevalence of *Campylobacter* was high (78.8%) during the period from August to December in different farms while low prevalence (21.2%) was observed during the period from January to April. The isolation rate of *Campylobacter* was greater in Awassi exotic and cross breeds (60.6%) than in the indigenous breeds (39.4%).

An antimicrobial susceptibility test was carried out for 48 isolated *Campylobacter* species using the agar disc diffusion method. The results showed that resistance was found to cephalothin (100%), ampicillin (33.3%), tetracycline (22.9%), erythromycin (12.5%), streptomycin (4.2%), gentamicin (4.2%), chloramphenicol (4.2%), nalidixc acid (2.1%) and ciprofloxacin (2.1%) whereas no resistance was found to penicillin.

The 43 *C. jejuni* isolates from fecal and carcass samples by a conventional microbiological technique were confirmed as positive results using Polymerase Chaine Reaction (PCR) technique. The results showed that 100% of samples were also detected by PCR. The nucleotide sequences of *C. jejuni* gene at position 1-402 (362 bp) in this study showed 100% homology to *C. jejuni* subsp. M1 (CP 001900) and *C. jejuni* subsp. 81116 (CP000814) whereas showed 97% homology to U27272. *C. jejuni* lost their viability when frozen or refrigerated at -20 ºC and 4 ºC. Freezing may warrant consideration as a public health benefit relating to food of animal origin especially meat, to reduce the *Campylobacter* exposure level. The antimicrobial property of three types of wine against *C. jejuni* was investigated. White wine and two red wines, all with 11.5% ethanol significantly reduced the viability of *C. jejuni*. The minimum microbicidal concentration of white wine was 10% and for the two red wines was 25%.

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Yeshimebet Chanyalewu

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LIST OF ABBREVIATIONS

PCR	=	Polymerase chain reaction
NAHDIC	=	National animal health diagnostic and investigation center
CDC	=	Centers for Disease Control
EFSA	=	European food safety authority
SHF	=	Small Holder Farms
DBARC	=	Debre Birhan Agriculture Research Center
DBSBFMC	=	Debre Birhan Sheep Breeding and Forage Multiplication Center
mCCDA	=	modified Charcoal, Cefoperazone, Desoxycholate Agar
CLSI	=	Clinical and Laboratory Standards institute
VBNC	=	Viable but non-culturable

DISTRIBUTION OF *CAMPYLOBACTER* SPECIES IN SHEEP OF DIFFERENT PRODUCTION AREA AT DEBRE BIRHAN, NORTH-SHOA, ETHIOPIA

INTRODUCTION

Campylobacter is widely distributed among domestic animals, is the most frequent cases of food borne bacterial disease (CDC, 2006) in the world and is relatively 'new' zoonotic pathogens as routine culture from clinical specimens, only became possible in the late 1970s (Humphrey *et al*., 2007). Campylobacteriosis exceed the number of cases of *salmonella* as well European Food Safety Authority reported 212,064 cases for *Campylobacter* and 99,020 cases for *salmonella* (EFSA, 2012). The most common etiological agents of bacterial gastroenteritis in humans are *Campylobacter jejuni* and *Campylobacter coli*, with *C. jejuni* being the predominant causative species (Skirrow, 1994; Frost *et al*., 1998). *C. jejuni* and *C. coli* are readily isolated from the faeces of farm animals, possibly because, *Campylobacter* were found as commensals in the intestinal tract of a wide range of warm-blooded animals, almost always without any harmful effect (Stanley *et al*., 1998), including sheep. However, the direct or indirect contribution of *Campylobacter* spp. from this animal to human infections is currently unknown. There is little evidence to suggest that sheep are an important risk factor for human infections, is recognized that *Campylobacter* species are an important cause of sheep abortion.

Campylobacters generally requires 5% O_2, 10% CO_2 and 85% N_2 for growth (Hoffman and Goodman, 1982; Westfall *et al*., 1986). Previous research indicates that the incubation temperature have a considerably effect on the survival of *Campylobacter* spp (Hazeleger *et al*., 1998).The role of *C. jejuni* as a foodborne pathogen is associated with its ability to survive in food during storage and handling (Lori *et al*., 2007). The response of these organisms at low temperatures has been studied in some detail, and *C. jejuni* sws a remarkable and sudden growth rate decline

near the lower temperature limit (Anonymous, 1996; Hazeleger *et al*., 1998). Many *C. jejuni* cells lost their viability when *either frozen or refrigerated for 2 days.*

Wines have potent antimicrobial activity against *Campylobacter* which has been demonstrated under various experiments (Isohanni *et al*., 2010; Boban *et al*., 2010). Various studies describe the antimicrobial properties of wine against food-borne, bacteria some of them indicated that the strength of antimicrobial properties of wine is attributed by different components that wines contain, and given higher power of antimicrobial responsibility to red wine than white wine because of high phenolic compound that contains (Sugita-Konishi *et al*., 2001; Just & Daeschel, 2003; Moretro & Daeschel, 2004; Papadopoulou *et al*., 2005; Mónica *et al*., 2009), those studies have shown that certain phenolic compounds present in wine may affect bacterial growth and metabolism, and reported that the antimicrobial power depends on the specific compound that wine contains (Sugita-Konishi *et al*., 2001; Wen *et al*., 2003; Puupponen- Pimia *et al*., 2005; Daglia *et al*., 2007; Rodríguez-Vaquero *et al*., 2007a; Rodríguez-Vaquero *et al*., 2007b; Radovanovic *et al*., 2009). Others reported that phenol-stripped wines have considerable antimicrobial activity after intact wine and effectiveness of antimicrobial of intact wine is not attributed on its phenolic and nonphenolic compound that not can be expected on the basis of the content of particular components of wine (Weisse *et al*., 1995; Boban *et al*., 2010). implemented in the country due the shortage of molecular laboratories most/all of previous studies on *Campylobacter* are based on conventional culture method, therefore this study may encourage to further strength our laboratory work and develop simple and reliable molecular method detection of target microorganisms.

To overcome campylobacteriosis problem a number of researches have been undertake in different countries. However, no systematic investigation was made in Ethiopia to prioritize the major health risk *Campylobacter* species in sheep and conventional meat production practice, and also there are few reports on the susceptibilities of thermotolerant *Campylobacter* strains as well (Asrat *et al*., 1999; Woldemariam *et al*., 2009; Kassa *et al*., 2007; Dadi and Asrat, 2008). To readdress

this situation, we investigated the prevalence of *Campylobacter* spp. in different sheep production area and characterize *Campylobacter* spp. in sheep, and the rate of carcass contamination by *Campylobacter* in conventional slaughtering practice, survival ability of *Campylobacter* within different stress conditions and antimicrobial susceptibility profile of thermophilic *Campylobacter* spp. were also studied. Therefore cross sectional study were conducted, in Debrebirhan, North Shoa Ethiopia with the following objectives.

OBJECTIVES

1. To determine the distribution of *Campylobacter* spp. in different sheep production area at Debrebirhan, and characterize *Campylobacter* spp. in sheep.

2. To determine the effect of low temperature on the survival of *C. jejuni*.

3. To determine antimicrobial power of different types of wine against *C. jejuni*.

4. To evaluate antimicrobial susceptibility of *Campylobacter* spp. to different antimicrobial agents.

Key words: *Campylobacter*; sheep; temperature; wine; antibiotic

LITERATURE REVIEW

1. General character of *Campylobacter*

Campylobacter was first described by Theodor Escherich (Friedman *et al*., 2000). In 1886, Escherich described spiral bacteria in the colons of children who had died of what he called 'cholera infantum' (Senok and Botta, 2009). In 1919 the same microaerophilic spirillum bacterium was isolated by Smith from aborted calf tissues (Smith and Taylor, 1919). Due to its comma shaped morphology, Smith and Taylor proposed the name "Vibrio fetus" and the disease was called vibrionic abortion (Butzler, 1984; Franco, 1988; Skirrow, 1977). In 1931 Jones reported, New "vibrio" that caused dysentery in calves and its name was proposed as *Vibrio jejuni* (Jones *et al*., 1931; Butzler, 1984; Franco, 1988), Doyle described another *Vibrio* associated with swine dysentery and classified them as *Vibrio coli* in 1944 (Doyle, 1944). Three identified vibrio microorganisms were named in association with specific diseases in animals *V. jejuni*, *V. coli*, and *V. fetus* (Butzler, 1984). *Campylobacter* was classified in the genus *Vibrio* until Sebald and Veron, (1963) proposed the genus *Campylobacter*. Véron and Chatelain in 1973 published the study on the taxonomy of the microaerophilic *Vibrio* like organism and separated four species in the genus *Campylobacter*; *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter fetus* and *Campylobacter sputorum* (Véron and Chatelain, 1973). Three closely related genera, *Campylobacter*, *Arcobacter* and *Sulfospirillum*, are included in the family *Campylobacteraceae* (On, 2001; Vandamme, 2000). *Campylobacter* were recognized as fecal pathogens in humans in the late 1970s (Humphrey *et al*., 2007).

The name of *Campylobacter* is derived from the Greek word "Campylos" which means curved. *Campylobacter* are non-spore forming spiral, curved or occasionally straight rod bacteria. The size of *Campylobacter* range from 0.2 to 0.8 Mμ wide and 0.5 to 5 Mμ long, the species are oxidase and catalase positive and highly motile due to the flagella that they possess (Moren and Upton, 1987; Ursing *et al*., 1994; Simbert, 1978; Debruyne *et al*., 2008; Vandamme and De Ley, 1991).

Motility is conferred by a single flagellum at one or both ends of the bacteria (Smibert, 1984) and the corkscrew motion allows *Campylobacter* to go through the mucus layer of intestine. The flagellum has an important role in virulence because it is required for the bacteria to reach the attachment sites and penetrate into the intestinal cells. It is also reported that the flagella of *Campylobacter* appeared to have an essential role in the causation of diarrheal disease. The importance of motility as a virulence factor is demonstrated by true isogenic non-flagellated mutants, which are unable to colonize the intestine of experimental animals (Guerry *et al*., 1992). The role of flagella in the colonization of the mucous lining of the gastrointestinal tract has already been studied. Flagella are also important for invasion of host cells, as aflagellate organisms show markedly reduced internalization into host cells *in-vitro* (Wassenaar and Blaser, 1999). The flagellum is composed of two closely related proteins, encoded by two genes the major *flaA* and the minor *flaB* sharing a high degree of sequence homology (Wassenaar and Blaser, 1999).

Campylobacter when exposed to air for prolonged time periods or in old cultures transform from spiral form to coccoid morphology which are considered degenerative forms (Buck *et al*., 1983; Moran and Upton, 1987; Griffiths, 1993; Hazeleger *et al*., 1994). The coccoid form is in fact the dormant VBNC stage of *C. jejuni* (Park, 2002) however, a number of studies have suggested that the coccoid stage is merely a degenerative form that contains decreased levels of nucleic acids and peptides and also lacks cellular integrity (Moran and Upton, 1987; Beumer *et al*., 1992; Boucher *et al*., 1994). *C. jejuni* can be sometimes viable but non-culturable (VBNC) form when found in a weakened condition until they finally die. However in the form viable nonculturable (VBNC) the bacterium remains infectious but can no longer be cultured by conventional means (Rollins and Colwell, 1986). Due to exposure of low temperature or any other unfavorable environmental condition, their morphology appears to be coccoid shape but spiral VBNC can occur as well (Lazaro *et al*., 1999).

2. Culture and identification of *Campylobacter*

Campylobacter are gram-negative bacteria (Smibert, 1978) and are fastidious organisms that require enriched media and micro aerobic condition with 5% O_2, 10% CO_2 and 85% N_2 and 42 ºC optimum temperature for growth. *Campylobacter* species can be distinguished from other microorganisms by conventional method to better-understand there morphology, biochemical and physiological characteristic. However the identification of *Campylobacter* is still problematic because of their complex taxonomy and demanding growth requirement (On, 1996). The growth conditions required for the culture of *Campylobacters* are unusual and this places unique limitations on the range of food environments in which the species can multiply. For example, the organisms are generally considered to be microaerophilic, which is they are unable to grow in the presence of air and grow optimally in atmospheres containing 5% oxygen. *Campylobacters* have also restricted temperature growth range and whilst they grow optimally at 42 ºC, the organisms do not grow at temperatures below 30 ºC. Those growth characteristics place severe restrictions on the ability of *Campylobacters* to multiply outside of an animal host (Park, 2002).

As an alternative to growth bacteria on agar there are a variety of technologies which may provide rapid results, antibody/antigen interactions using immunoassay methods. Conventional detection of *Campylobacter* spp. in naturally contaminated samples (food, environmental and clinical) is very time-consuming (Engberg *et al*., 2000). On the other hand hippurate hydrolysis characterize only cultivable thermophilic *C. jejuni* but PCR can detect both culturable and nonculturable cells and also for the isolation of' other *Campylobacter* species such *C. hyointestinahs* and *C. upsaliensis* (Lawson *et al.*, 1998). Molecular methods based on PCR amplification are reliable alternatives to culture for the detection of the target microorganism in food samples with the advantages of the PCR-based detection rapidity, specificity and sensitivity (Estibaliz *et al*., 2005). The polymerase chain reaction (PCR) method provides a rapid and highly sensitive method for the detection of species specific DNA sequences is relatively uncomplicated to use and is fast and robust method to

identify *Campylobacter* at species level. PCR reaction amplifies copies of a fragment of DNA across several orders of magnitude. The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA (Linton *et al*., 1997; Dieffenbach and Dveksler, 2003; Lübeck *et al*., 2003). Sometimes combinations of these techniques are used to further enhance the speed of the result. Several studies confirm the promptness of PCR methods for the detection of *Campylobacter* in naturally contaminated retail samples (Denis *et al*., 2001; Wong *et al*., 2004; Mateo *et al*., 2005; Sallam, 2007). However, the presence of inhibitors in food material can decrease the sensitivity of PCR (Denis *et al*., 2001; Lilja and Hanninen, 2001). Analysis time for PCR can be shortened by 1 day if a 20-24 h enrichment procedure is used (Wang *et al*., 1999). Katzav *et al*. (2008) reported that PCR method shortens time compared to microbiological analyses and can be therefore used for detection of *Campylobacter* spp. However, enrichment of the samples is necessary.

To reduce the time required for detection and identification of *Campylobacter* the combination of ELISA and PCR method has been developed. The method could facilitate specific and sensitive detection of PCR amplification products. Sails *et al*. (2001) investigated five biotin-labelled probes targeted to detect digoxygenin-labelled PCR products from *C. jejuni* and *C. coli* by using the PCR-ELISA assay. The sensitivity was demonstrated to be 10-100 fold more sensitive than a gel-based PCR method and can be completed in 7 h. Lilga and Hanninen, (2001) also evaluated the automated ELISA and PCR method which included a sample preparation method based on Buoyant Density Centrifugation (BDC) for detection and identification of *C. jejuni* and *C. coli* in poultry products. The automated ELISA could easily be repeatable and reliable method and take minimum 2.5 working days, while BDC-PCR method requires 3 working days. Fingerprinting methods Terminal Restriction Fragment Length Polymorphisms (T-RFLP) and Ligase Detection Reaction (LDR) have been described and are used for the rapid detection and identification of *Campylobacter* and Arcobacter spp. (Marshall *et al*., 1999, Oreilly *et al*., 2006).

3. Mechanisms by which *Campylobacters* induce disease

The mechanisms by which *Campylobacters* induce disease are not clearly understood, but on the basis of experimental evidence two mechanisms have been postulated for gastrointestinal illness: (I) intestinal adherence and toxin production; (II) bacterial invasion and proliferation with the intestinal mucosa (Park, 2002), the enterotoxin production of *Campylobacter* is associated with illness of patient (Ruiz-palacios *et al*., 1983). Toxins play a role campylobacteriosis; indeed, a variety of toxic activities has been reported in *C. jejuni*. Cytolethal Distending Toxin (CDT) is one of *Campylobacter* cytotoxin which causes cells to become slowly distended and leads to cell death. CDT production by *Campylobacter* was first reported in 1988 (Johnson and lior, 1988). The role of CDT in *C. jejuni* pathogenesis has not been well determined yet, however, it might play a role in modulation of immune response and invasiveness (Purdy *et al*., 2000).

4. *Campylobacter* in humans

C. jejuni and *C. coli* are the most common causes of food-borne bacterial gastroenteritis in humans worldwide. *Campylobacter* spp. is known as the most common infectious agent associated with the development of Guillain–Barre´ Syndrome (GBS) which is the most common acute flaccid paralysis due to an autoimmune disorder of peripheral nervous system. It is believed in some cases to arise as a post-infectious complication (On, 1996). GBS is considered a sequela of infections caused specifically by *C. jejuni* (Steve *et al*., 2005). *C. jejuni* complications can also lead to reactive arthritis (Caughey, 1984; Nachamkin *et al*., 1998; Altekruse *et al*., 1999; Hadden and Gregson, 2001; Birk *et al*., 2004). The symptom of campylobacteriosis in humans is watery and/or bloody diarrhea, abdominal pain and cramps, fever and headache. Late onset complications such as dizziness and myalgia may occur, show severe clinical sign in 2 to 5 days, and duration of the symptoms is less than 7 days (Stern and Line, 2000; Blaser and Engberg, 2008).

The infective dose is considered to be low, from 500 to 10,000 cells, even a very small number of *Campylobacter* cells in food samples represent a potential health hazard (Robinson, 1981; Black *et al*., 1988; Liu *et al*., 2006) depending on vehicle ingested material and the susceptibility of the individual. The relatively low infective dose, the potentially serious sequelae (Moore *et al*., 2005), as well as the association between certain *Campylobacter v*irulence genes and the pattern of clinical infection (Al-Mahmeed *et al*., 2006; Rozynek *et al*., 2005), confirm the importance of this zoonotic infection as a significant health hazard.

Infected persons can be implicated as a source of campylobacteriosis (Jones *et al*., 1981; Humphrey *et al*., 2001; Jorgensen *et al*., 2002). Person-to-person transmission of *Campylobacter* from ill children to family members occurred frequently. Campylobacteriosis in pregnant women with bacteremia may pass on a severe systemic infection to fetus (Friedman *et al*., 2000; Stern and Line, 2000). Three *Campylobacter* species, *C. jejuni*, *C. coli* and *C. lari* already represent approximately 90% of all human campylobacteriosis cases (Stern and Line, 2000). Currently *C. jejuni* is the leading cause of bacterial food borne illness in the world, with *C. coli* involved in 5% of the cases (Skirrow, 1994; Frost *et al*., 1998; Nayak *et al*., 2003; Wilson, 2003). In 2000, *Campylobacter* accounted for an estimated 359,466 cases of indigenous food borne disease (Frost *et al*., 1998; Adak *et al*., 2002). In South Africa Venda region high prevalence of human campylobacteriosis was reported in HIV positive individuals (Samie *et al*., 2007). Epidemiology of campylobacteriosis remains poorly understood. Eating poultry has long been found a leading hypothesis for spread of *Campylobacter* infection. Many epidemiological studies have been conducted to assess the risks for human campylobacteriosis (Blaser *et al*., 1983; Friedman *et al*., 2004).

Campylobacter enteritis causes significant morbidity and mortality in developed countries. During the year 2005, the number of reported cases of *Campylobacter* in Germany was 60.000, higher than ever before, which made *Campylobacter* a number one bacterial pathogen responsible for food poisoning in

2005 (RKI, 2005). In Belgium, reported 63.8% cases of human campylobacteriosis per 100,000 people (Anonymous, 2005b; Ghafir *et al*., 2007). A report from the U.S. Centers for Disease Control and Prevention estimated that each year *Campylobacter* infection causes 124 deaths in the United States (Mead *et al*., 1999), and in 2003, 12.6 cases of campylobacteriosis per 100,000 people were also reported in U.S (CDC, 2004). In recent years *Campylobacter* cases have become more commonly reported than *Salmonella*. In 2010, European Food Safety Authority reported 212,064 cases of campylobacteriosis and 99,020 cases for salmonellosis from 27 member countries (EFSA, 2012). Similarly previous report indicated that campylobacteriosis are continuously increasing and have already overtaken the salmonellosis in Switzerland and some EU countries (Rautelin and Ha¨nninen, 2000; Zweifel *et al*., 2004). All reported data exhibited that the economic burden of *Campylobacter* infection is large in the United States. The estimated cost of *Campylobacter*-associated GBS has been high as $1.8 billion per year (Buzby and Roberts, 1997).

5. Source and distribution of *Campylobacter* in food animals

In sheep-raising countries *Campylobacter* species are a significant cause of sheep abortion (Poland, 2004). Study in Turkey reported that *Campylobacter* is the major problem of sheep abortion (Erganis *et al*., 2002). In New Zealand, *Campylobacter fetus subsp. fetus* is the principal cause of sheep abortion and some reports indicate that *C. jejuni* and *C. coli* have similar implication as a major cause of sheep abortions (Diker and Istanbulluoglu, 1986; Hedstrom *et al*., 1987; Delong *et al*., 1996). Sheep abort toward the end of their pregnancy, weak lambs may die within a few days and birth of dead lambs can occur (Prescott and Munroe, 1982). Abortion in the third trimester of ovine gestation results from placentitis and metritis (Thompson and Blasér, 2000), both of which may result in septicemia and death of the ewe. Losses of 10 to 20% of the lambs and 5% of the ewes that abort are common. The rate of abortions varies and depends on the proportion of susceptible ewes. If the infection is recent in the flock, the abortion rate can be quite high, at times up to 70% of the

pregnant ewes (Diker and Istanbulluoglu, 1986; Hedstrom *et al*., 1987; Diker *et al*., 1988; Delong *et al*., 1996).

The placentas of infected sheep that abort or even of those that give birth normally, as well as aborted fetuses and vaginal discharges, contain a large number of *Campylobacter* (Collins and Ross, 1984). A few infected ewes become carriers by harboring the infection in the gallbladder and shedding the agent in fecal matter. However, the epidemiology of *Campylobacter* is still poorly understood, it has been suggested that the role of sheep in the epidemiology of *Campylobacter* has been underestimated. A link between ovine and human *Campylobacter* infections has been reported in Nigeria (Raji *et al*., 2000) raising the possibility of cross-contamination between sheep and man. Thermophilic *Campylobacter* species in lambs at slaughter was also studied by Stanley *et al*. (1998). *Campylobacter jejuni* serotypes isolated from both chicken and lamb was similar to that seen in humans, suggesting that both of these food sources play a significant role in human infection (Frost, 2001).

Campylobacter spp. is widely distributed among domestic animals and it is known that many domestic and wild animals such as poultry, sheep, cattle, swine, dogs and cats carry *Campylobacters* in their gastrointestinal tracts without displaying any of the marked symptoms associated with the disease (Blaser *et al*., 1980b). Therefore, the intestinal tract of a wide range of warm-blooded animals, both domestic and wild, is thought to be the natural habitat and environmental reservoir of *Campylobacter* species. Most of *Campylobacter* infection sources are food or waterborne. The highly and frequent colonization of food animals by *Campylobacter* spp. in their intestinal tracts (Pearce *et al*., 2003; Nesbakken *et al*., 2003; Pezzotti *et al*., 2003) play an important role as source of *Campylobacter* infection. Sheep and beef (Steffen *et al*., 1986; Pezzotti *et al*., 2003; Whyte *et al*., 2004; Parisi *et al*., 2007) have been implicated as a source of outbreaks of campylobacteriosis. Transmission can also occur through direct contact with infected pet or domestic animals (Blaser *et al*., 1978; Adak *et al*., 1995; Jones *et al*., 1991). Several studies have shown *Campylobacter* contaminated fecal samples from beef, pork and poultry, meat from

pork, beef, turkey, shellfish, and sheep (Zanetti *et al*., 1996; Endtz *et al*., 1997; Whyte *et al*., 2004; Boes *et al*., 2005; Insook *et al*., 2007; Dadi and Asrat, 2008; Woldemariam *et al*., 2009; Rahimi *et al*., 2010). *C. jejuni* and *C. coli* are both commonly found in cattle, sheep and pigs (Nielsen, 2002; Stanley and Jones, 2003; Payot *et al*., 2004; Boes *et al*., 2005).

Food is involved in about 80% of cases of campylobacteriosis (Berndston *et al*., 1992; Mead *et al*., 1999; Butzler, 2004). Consumption of raw or undercooked poultry (Istre *et al*., 1984; Parisi *et al*., 2007; Son *et al*., 2007; EFSA Panel on Biological Hazards [BIOHAZ 2010]) is also implicated as a source of campylobacteriosis. Gross microbial contamination of the carcass with gut contents may occur during evisceration via hands of slaughter men or from equipment or water in a slaughter line (Jones *et al*., 1991; Herman *et al*., 2003; Whyte *et al*., 2004). Cross-contamination from raw poultry products is a more likely infection route for humans than the consumption of poultry products as such (Kapperud *et al*., 2003). In Great Britain poultry is considered the main source of campylobacteriosis (Johanne *et al*., 2009). Many other studies have shown the close relation of *C. jejuni* serogroup isolated from humans and poultry sources, there is also a general consensus that a significant reduction in human infections can be achieved by reducing *Campylobacter* infection in broiler flocks it has been estimated that a 2-log reduction of *Campylobacter* in poultry faeces could lead to a 30-fold reduction in human campylobacteriosis (Lindblom *et al*., 1986; Evans, 1992; van de Giessen *et al*., 1998; Rosenquist *et al*., 2003). That is why not surprising that epidemiological studies have revealed a strong association between *Campylobacter* infection and eating of raw or undercooked poultry in most industrialized nations (Friedman *et al*., 2000). Attribution of campylobacteriosis to specific sources may also vary between different regions (Pires *et al*., 2010).

Water has been incriminated as the source of infection in several outbreaks of *Campylobacter* infections, and in Sweden *Campylobacter* has been the most commonly identified pathogen in waterborne outbreaks (Andersson *et al*., 1997).

Contaminated drinking water is considered an important risk factor for campylobacteriosis in various places (Neal and Slack, 1995; Eberhart *et al*., 1997; Studahl and Andersson, 2000; Kapperud *et al*., 2003; Richardson *et al*., 2007). Lyngstad *et al*. (2008) reported that water from private sources is strongly associated with an increased risk of *Campylobacter* colonization. Guerin *et al*. (2007a) stated that the use of municipal water reduces the risk, however, water treatments such as disinfectants might have a protective role in spreading *Campylobacter* within a flock (Ellis-Iversen *et al*., 2009). *C. jejuni* is commonly found in natural water sources (Humphrey and Beckett, 1987). However, it is interesting to note that it has always been found in the presence of fecal coliforms, therefore, the contamination presumably stems from animals and, in some circumstances, from man. Even the low infectious dose increases the possibility of infection by drinking water containing only a few hundred viable *Campylobacters*. The important source of infection is almost always food, although it is sometimes difficult to identify the immediate source (Stern *et al*., 1985). The number of documented foodborne outbreaks associated with the consumption of salad vegetables was also found to be a risk factor for campylobacteriosis (Evans *et al*., 2003). Prevalence of *Campylobacter* spp. on fresh produce has been the subject of much research (Park and Sanders, 1992; Kumar *et al*., 2001; Thunberg *et al*., 2002; Sagoo *et al*., 2003). Outbreaks of *C. jejuni* (Blaser *et al*., 1982; Kirk *et al*., 1997; Michino and Otsuki, 2000) and *C. coli* (Ronveaux *et al*., 2000) associated with different types of fresh salads have been reported around the world. The source of *Campylobacter* infections can be divided into three categories; waterborne, foodborne or direct contact with carriers. However the importance of water in explaining sporadic *Campylobacter* infections has not been thoroughly investigated.

Extra intestinal infections with *C. jejuni* have been reported in sheep, goats, cattle and pigs. Infection can result in abortion in sheep and goats, with symptoms similar to those seen in *C. fetus* abortions, and mastitis in cattle (Doyle and Roman, 1982; Morgan *et al*., 1985). *C. jejuni* is frequently isolated from raw milk contaminated by either bovine fecal material or through mastitis infections, and is a

major cause of campylobacteriosis in countries where raw milk is consumed (Doyle and Roman, 1982; Humphrey and Beckett, 1987; Humphrey and Hart, 1988; Wood *et al*., 1992). The presence of *Campylobacters* in the intestinal tract of dairy animals will mean that milk will frequently be contaminated at milking as a consequence of faecal contamination, however, proper hygiene at milking can reduce both the incidence and level of contamination, and proper pasteurization will kill *Campylobacters* (Park, 2002). The sources of infection in cattle can be carrier bulls and also cows that remain infected from parturition to parturition.

Extensive variations in the prevalence of *Campylobacter* have been reported in both live animals and foods of animal origin. For example, infection rates in live broilers have ranged from 0 to 100% (Bryan and Doyle, 1995; Moore *et al*., 2003) with high prevalence of up to 100% also in pigs (Nesbakken *et al*., 2003) and up to 60% in cattle (Orr *et al*., 1995; Nielsen *et al*., 1997). *Campylobacter* occurrence of up to 100% has also been reported on dressed poultry carcasses (Waldroup *et al*., 1992; Atanassova and Ring, 1999; Dominguez *et al*., 2002), however, low prevalence of the organism have been reported on beef or pork carcasses (Zanetti *et al*., 1996; Madden *et al*., 2001). Other foods that *Campylobacter* has been recovered from include shellfish (Wilson and Moore, 1996; Endtz *et al*., 1997). In Ahvaz Iran 60 (29.9%) *Campylobacter* spp. was isolated from 215 meat samples, from chicken meat (61.7%), followed by turkey meat (36.0%), sheep meat (6.0%) and goat meat (4.4. %), with *C. jejuni* (88.3%) the predominant isolated species followed by *C. coli* (11.7%) (Rahimi *et al*., 2010). In Ethiopia *Campylobacter* spp. were isolated from faeces of Chicken 68.1%, sheep 38%, cattle 12.6% and pigs 50% (Kassa *et al*., 2007). Results of another study on small ruminant indicated *Campylobacter* spp. isolated from sheep and goat carcasses were 10.6% and 9.4% respectively (Woldemariam *et al*., 2009), 10.5% and 7.6% *Campylobacter* spp. was also isolated in sheep and goat carcasses respectively by Dadi and Asrat, (2008), according those reports sheep carcass found to be more highly contaminated. Thirty nine (3.54%) *Campylobacter* spp. were found In Nigeria, the highest isolation rate was 6.8% from samples of intestinal tract, *C. jejuni* were reported 79%, followed by *C. coli* 13% and 8.0% *C. laridis* (Raji *et al*., 2000). In

Switzerland about 114 (17.5%) *Campylobacter* spp. found from 653 slaughtered sheep (Zweifel *et al*., 2004). Previous study showed that free-range systems may increase the presence of *Campylobacter* spp. mostly in cattle and sheep reared in outdoor systems where there will be frequent contact with the external environment (Humphrey *et al*., 2007).

6. Seasonal occurrence of *Campylobacter*

Significant seasonal variation on the occurrence of *Campylobacter* also has been reported in different studies (Wallace *et al*., 1997; Stanley *et al*., 1998; Humphrey *et al*., 2007; Rahimi *et al*., 2010). Previous reports indicated high prevalence of *Campylobacter* in the summer season and the lowest in winter (Kapperud *et al*., 1993; Willis & Murray, 1997; Rahimi & Tajbakhsh, 2008). For example Willis and Murray, (1997) reported high rates of *Campylobacter* in retail broiler carcasses during the warmer months (87- 97%) than the winter months (7-33%). Other studies have reported seasonal periodicity in, *Campylobacter* carriage rates within dairy herds (Robinson, 1982). *Campylobacter* can survive for longer periods in water sources during winter and rapidly reduced during the summer (Korhonen and Hedlund, 1993; Obiri-Danso *et al*., 2001) as because *Campylobacter* sensitive to UV light and higher temperatures.

Another report showed higher recovery rates during July, August and September (Jore *et al*., 2010). The difference between seasonality of sporadic cases and outbreaks is unknown, but may can be due to environmental contamination or might be due to different ecological events driving those (Olson *et al*., 2008). *Campylobacter* infections show seasonal variation, with the peak occurring in the summer months (Nachamkin, 1999), 30-40% and 80% retail poultry products have been shown to be *Campylobacter* positive in Denmark and in the UK during the summer month respectively (Rosenqvist *et al*., 1999; Bolton *et al*., 1999). In Finland from July to September, 10-30% of Finnish retail poultry products were found to be positive for *Campylobacters* (Hinninen *et al*., 2000).

7. Survival of *Campylobacter* at different environmental stress

The role of *C. jejuni* as a foodborne pathogen is associated with its ability to survive in food during storage and handling. Karenlampi and Hanninen, (2004) showed that *C. jejuni* can survive on fresh produce long enough to pose a risk to the consumer. However, survival times will depend on the food matrix involved and the conditions under which the foods are stored. *Campylobacters* will not survive pasteurization treatments or proper cooking. Extensive research has been conducted on the survival of *C. jejuni* on meat (especially poultry), milk, eggs and water (Blankenship and Craven, 1982; Doyle and Roman, 1982; Hanninen *et al*., 1984; Curtis *et al*., 1995; Cools *et al*., 2003). The ability of *C. jejuni* to survive on stainless-steel surfaces and to cross-contaminate fresh produce has also been studied (Kusumaningrum *et al*., 2003; Moore *et al*., 2003), as has the survival on various food contact surfaces under different organic loads (De Cesare *et al*., 2003).

C. jejuni is unable to multiply at temperature below 30 ºC and has been demonstrated to be extremely susceptible to environmental stresses like heating, disinfectants, a wide variety of antimicrobial treatments, acidity and oxygen exposure, low pH, low temperature, food processing methods, and in addition is difficult to culture and to maintain in the laboratory. *Campylobacter* is more sensitive to heat and acid, than *Salmonellas* or *E. coli* (Solomon and Dallas, 1999; Park, 2002; Humphrey *et al*., 2007; Jasson *et al*., 2007; Smigic *et al*., 2010). *Campylobacters* are very sensitive to desiccation and accordingly do not survive well on dry surfaces (Fernandez *et al*., 1985). Similarly, *Campylobacter* are less able to tolerate other environmental stress and not grow in concentration of 2% of sodium chloride for the reason that is more sensitive to osmotic stress than other food born bacteria (Doyle and Roman, 1982) whereas *Salmonella typhimurium* and *Listeria monocytogenes* will grow in concentration of 4.5% and 10% of sodium chloride respectively (ICMSF, 1996; Park, 2002), and are generally less able to tolerate environmental stress than other foodborne pathogens. There is a need of quantitative data on survival of *Campylobacter* spp. under adverse conditions from naturally contaminated foods.

Temperature is a key factor in the survival of *C. jejuni* (Robinson, 1981; Lori *et al*., 2007). *C. jejuni* shows a notable and sudden growth rate decline at low temperature (Anonymous, 1996; Hazeleger *et al*., 1998). In 2006 Franklin *et al*. indicated the effect of freezing and duration of frozen storage on the survival of *Campylobacter*, where the level of such bacteria showed significant reduction at -20 ºC. Naturally contaminated meat stored at −22 ºC (freezing condition) also exhibits gradual reduction of *Campylobacter* Spp. (Sampers *et al*., 2010). Other studies also showed the reduction cases of *Campylobacter* by freezing (Lee *et al*., 1998; Stern and Robach, 2003; Stern *et al*., 2003; Murphy *et al*., 2006). Although during freezing *Campylobacter* spp. may be killed a fraction of the *C. jejuni* population may survive or be sub-lethally injured (Georgsson *et al*., 2006; Jasson *et al*., 2007). Vanderline *et al.* (1999) reported that some *Campylobacter* strains exhibit different survival characteristics at chill temperature.

Properly applied adequate time-temperature measures kill the organism and reduce the disease burden of human campylobacteriosis attributed to food of animal origin. Freezing as a control measure is already suggested in countries such as Sweden and Norway to reduce *Campylobacter* contamination of carcasses (Hofshagen and Kruse, 2005; Rosenquist *et al*., 2006; Lindqvist and Lindblad, 2008) and in Belgium freezing and addition of salt, are currently recommended in the processing of chicken meat preparations (Sampers *et al*., 2008).

8. Antimicrobial properties of wine against *Campylobacter*

Wine is a complex solution containing different components with strong antimicrobial properties (Mónica *et al*., 2009; Isohanni *et al*., 2010; Boban *et al*., 2010). Previous studies showed that certain phenolic compounds present in wine may affect bacterial growth and metabolism (Wen *et al*., 2003; Puupponen-Pimia *et al*., 2005; Rodríguez-Vaquero *et al*., 2007a; Rodríguez-Vaquero *et al*., 2007b). Various studies described the antimicrobial properties of wine against different relevant food-borne pathogens. Some of them indicated that the strength of antimicrobial properties

of wine is attributed to different components of wines and give higher power of antimicrobial proficiency to red wine than white wine because of high level of phenolic compound that it contains (Sugita-Konishi *et al*., 2001; Just and Daeschel, 2003; Moretro and Daeschel, 2004; Papadopoulou *et al*., 2005; Mónica *et al*., 2009). Moreover, other reports found that phenol-stripped wines have considerable antimicrobial activity that is not attributed to its phenolic and nonphenolic compound or cannot be expected on the basis of the content of particular components of wine (Weisse *et al*., 1995; Boban *et al*., 2010). Meanwhile, other studies reported that antimicrobial efficacy of the phenolic compounds in wine may be also enhanced by other components of the inherent environment factors present in wine, such as low pH (Wen *et al*., 2003).

In 2009, Mónica *et al*. reported that wines having 11.5% ethanol significantly reduced the viability of *C. jejuni*. In addition, wine with the combination of ethanol and organic acids (tartaric, malic, lactic and acetic) have more potential antimicrobial efficiency (Weisse *et al*., 1995; Just and Daeschel, 2003; Anabela *et al*., 2008). Ethanol or a solution of wine organic acids, when used separately, had a negligible influence in the survival of *C. jejuni* over the timescale studied. However, when used in a combined solution, these compounds had a similar inactivation effect as that of wine (Moretro & Daeschel, 2004). For these reasons, wines might be used as antimicrobial ingredients together with the addition of further antimicrobial agents to reduce the numbers of *Campylobacter* in naturally contaminated food products, thus lowering the risk of *Campylobacter* cross-contamination and transmission through food (Anabela *et al*., 2008; Isohanni *et al*., 2010). Ingestion of wine with food significantly decreases the number of *C. jejuni* persisting further in the alimentary tract (Anabela *et al*., 2008; Mónica *et al*., 2009; María *et al*., 2010).

9. Antibiotic susceptibility pattern of strains of *Campylobacter*

Antibiotics have a role in reducing symptoms, shortening the extent of illness and also control the transmission of disease in the community (Oberhelman and

Taylor, 2000). Since the late 1980's the resistance in *Campylobacter* isolate to fluoroquinolones has been increasing especially in Europe (Smith *et al*., 1999). Various scientific confirmations indicate that the use of antibiotics in food animals particularly in developed countries leads to the development of resistant pathogenic bacteria that can reach humans through food chain (Aarestrup, 1999; Van Looveren *et al*., 2001; Avrain *et al*., 2003). There are many reports that indicate the resistance of *Campylobacter* to different commonly used antibiotics (Engberg *et al*., 2001; Aarestrup and Engberg, 2001; Fallon *et al*., 2003).

There are a number of ways by which microorganisms are resistant to antimicrobial agents. These include: 1) the bacteria produce enzymes that either destroy the antimicrobial agent before it reaches its target or modify the drug so that it no longer is recognized by the target; 2) the cell wall becomes impermeable to the antimicrobial agent; 3) the target site is altered by mutation so that it no longer binds the antimicrobial agent; 4) the bacteria possess an efflux pump that expels the antimicrobial agent from the cell before it can reach its target; and 5) specific metabolic pathways in the bacteria are genetically altered so that the antimicrobial agent cannot exert an effect. A wide variety of efflux pumps provide antimicrobial resistance in both gram-positive and gram-negative bacteria. Active efflux of antibiotics is mediated by trans-membrane proteins inserted in the cytoplasmic membrane and, in the case of gram-negative organisms, in the outer membrane and the periplasm. These proteins form channels that actively export an antimicrobial agent out of the cell as fast as it enters (Lariviere *et al*., 1986; Barbosa and Levy, 2000; Schwarz and Chaslus-Dancla, 2001; Guo *et al*., 2010).

Erythromycin, tetracycline and quinolones have all been recommended in different clinical settings to treat *Campylobacter* gastroenteritis (Vanhoof *et al*., 1978). Earlier studies on antibiotic susceptibility pattern indicate different range of susceptibility and resistance of *Campylobacters* to ciprofloxacin, erythromycin, tetracycline, gentamycin, nitrofurantion, ampicillin, nalidixic acid, streptomycin and enrofloxacin, (Fallon *et al*., 2003; Boonmar *et al*., 2005; Sukhapesna *et al*., 2005;

Kilonzo *et al*., 2008; Little *et al*., 2008; Adekunle *et al*., 2009; Elizbieta *et al*., 2009; Tan *et al*., 2009). In 1999 Kirk *et al*. reported *C. jejuni* resistance to quinolones increase from 0.8 percent in 1996 to 3.0 percent in 1998.

Moreover the antibiotic susceptibility patterns of *Campylobacters* seem to vary widely from country to country and from place to place. The rate of antibiotic resistant enteric infection is high in countries where the use of antimicrobial drugs in animal and human is relatively unrestricted (Coker and Adefeso, 1994; Aarestrup *et al*., 1997; Saenz *et al*., 2000; Van Looveren *et al*., 2001; Avrain *et al*., 2003; Ishihara *et al*., 2004; Adekunle *et al*., 2009). Different range of susceptibility and resistance was reported in various part of the world. In Lebanon and Iraq (69-85.7%) and (49-100%) susceptibility to ampicillin and tetracycline respectively was reported, and also (25-75%) and (32.7-70%) *Campylobacters* resistant range to tetracycline and ampicillin was found in Israel, Saudi Arabia, Ethiopia and Thailand (Schwartz *et al*., 1993; Zaman, 1992; Talhouk *et al*., 1998; Abdulameer *et al*., 1999; Asrat *et al*., 1999; Morre *et al*., 2006; Boonmar *et al*., 2005; Kassa *et al*., 2007). In Ethiopia were also found 2-6% rate resistance for amoxicillin, chloramphenicol, and erythromycin and 10-20% for ampcillin, gentamycin, kanamycin, streptomycin and tetracycline (Dadi and Asrat, 2008).

Mayrhofer *et al*. (2004) reported highest prevalence resistance to quinolones (40.7%) and tetracycline (16.4%), 0-83% and 0.3-90% range of resistance to macrolides has been also reported from food animals, particularly from pigs and from humans respectively (Pezzotti *et al*., 2003; Saenz *et al*., 2000; Praakle *et al*., 2007; Padungton. and Kaneene, 2003). Low and high level of resistance to chloramphenicol (0–60%), gentamicin (0–11.9%) streptomycin/kanamycin (0–48%) and tetracycline (0–96%) has been reported among *Campylobacter spp*. isolated from foods, food animals and humans of different parts of the world (Binotto *et al*., 2000). Several studies have been reported resistance to beta lactam antibiotics in food animals (Van Looveren *et al*., 2001; Tajada *et al*., 1996; Nachamkin *et al*., 2000). Similar findings of resistance to these drugs in human and food animal isolates have been observed in

Spain (Saenz *et al*., 2000) and Denmark (Aarestrup *et al*., 1997). The increasing rate of infections caused by antimicrobial-resistant strains of *Campylobacter* makes clinical management of cases of campylobacteriosis more difficult, worrying development is that it appears that such resistant strains may result in more adverse health outcomes.

Antimicrobial resistance has always been a major concern for human and animal health environment and has become a serious health issue recently. It is a common phenomenon in different countries and thus indicates the need for an appropriate strategy of surveillance and epidemiological monitoring to control the development of resistance. The use of antibiotics in food animals for growth promoters and as prophylactics have raised questions about the development of resistant *Campylobacter* spp in food animals followed by a possible transfer to humans via the food chain (Mayrhofer *et al*., 2004) and the resistance rate to quinolone (nalidixic acid) of *C. jejuni* and *C. coli* isolates raises the question whether this test is significant for identification purposes (Parisi *et al*., 2007).

10. Animal and human based studies conducted on *Campylobacter* in Ethiopia

Ethiopia has 23.6 million a diverse sheep population (CSA, 2006; Solomon *et al.*, 2008), at national level sheep and goat account 90% of live animal and meat (FAO, 2004) and 92% of national skin and head (FAO, 1994) export trade value however in the country there are limited information about campylobacteriosis in sheep, other food animals, food of animal origin and human.

In Ethiopia from 1992 to 2010 *Campylobacter* spp was found 196 (52.41%) in human, 352 (49.9%) animal and 90 (9.6%) in food of animal origin. The weight of *Campylobacter* infection has been described in previous studies conducted in Ethiopia and it has been estimated that *Campylobacter* infect around 548 (50.79%) human and food animals, from that 52.41 % human, 27 (38 %) sheep, 26 (12.7%) cattle, 290 (70.6%) chicken, 9 (50 %) pigs Table 1. the high prevalent *Campylobacter* species

was *C. jejuni* 64.8% followed by *C. coli* 13.9%, *C. lari* 1.1% and 111/20.8% uncharacterized *Campylobacter* species (Gedlu and Aseffa, 1996; Asrat *et al*., 1997; Getnet and Abrham, 2004; Kassa *et al*., 2007; Ewnetu and Mihret, 2010).

Table 1 Data from previous published research work results conducted in different species in Ethiopia.

Species	Isolate	*C. jejuni*	*C. coli*	*C. lari*	*Campylobacter* without species characterization	Total
Human	374	72	13	0	111	196/52.41%
sheep	71	16	11	0		27/38%
Cattle	205	14	10	2	0	26/12.7%
Chicken	411	253	33	4	0	290/70.6%
Pig	18	0	9	0	0	9/50%
Total	1079	355/64.8%	76/13.9%	6/1.1%	111/20.8%	548/50.8%

Source: (Gedlu and Aseffa, 1996; Asrat *et al*., 1997; Getnet and Abrham, 2004; Kassa *et al*., 2007; Ewnetu and Mihret, 2010)

From the total 938 isolate of *Campylobacter* species from carcass of different food animals chicken meat accounted for 13 (21.7%), sheep meat 35 (10.54%), goat meat 24 (8.8%), 4 (8.5%) and 14 (6.2%) was found from pig and cattle meat respectively with predominant prevalence of *Campylobacter* in chicken and sheep meat. From a total 938 isolate of *Campylobacter* from 1992 to 2010 in food of animal origin the overall prevalence were 90 (9.6%), the species characterized were *C. jejuni* which is the predominant, *C. coli* and *C. lari* 68 (75.6%), 20 (22.2%) and 2 (2.2%) respectively Table 2 (Dadi and Asrat, 2008; Woldemariam *et al*., 2009).

Table 2 Data from previous published research work results conducted in different food of animal origin in Ethiopia.

Meat type	Isolate	*C. jejuni*	*C. coli*	*C. lari*	Total
Sheep meat	332	27	8	0	35/10.54%
Goat meat	272	17	7	0	24/8.8%
Cattle meat	227	12	2	0	14/6.2%
Chicken meat	60	11	1	1	13/21.7%
Pig meat	47	1	2	1	4/8.5%
Total	938	68/75.6%	20/22.2%	2/2.2%	90/9.6%

Source: (Dadi and Asrat, 2008; Woldemariam *et al*., 2009)

Research Hypothesis

1. The prevalence of *Campylobacter* in different sheep production and conventionally slaughtered sheep carcass were known, and *Campylobacter* species in sheep of the study area were characterized.

2. Frozen temperature can be making to use as a control measure of *Campylobacter* spp. the growth rate of *Campylobacter* spp. reduce by freezing and also duration of freezing influence positively on the reduction level of such bacteria.

3. Red and white wine have antimicrobial power against *Campylobacter* spp.

4. Variation of antimicrobial resistances in *Campylobacter* in different geographic area was known.

MATERIALS AND METHODS

Study area

Cross sectional study were conducted on selected sheep setting area known as Small Holder Farms (SHF), Debre Birhan Agriculture Research Center (DBARC), Debre Birhan Sheep Breeding and Forage Multiplication Center (DBSBFMC) in North shoa Debrebirhan Ethiopia from July 2011to May 2012.

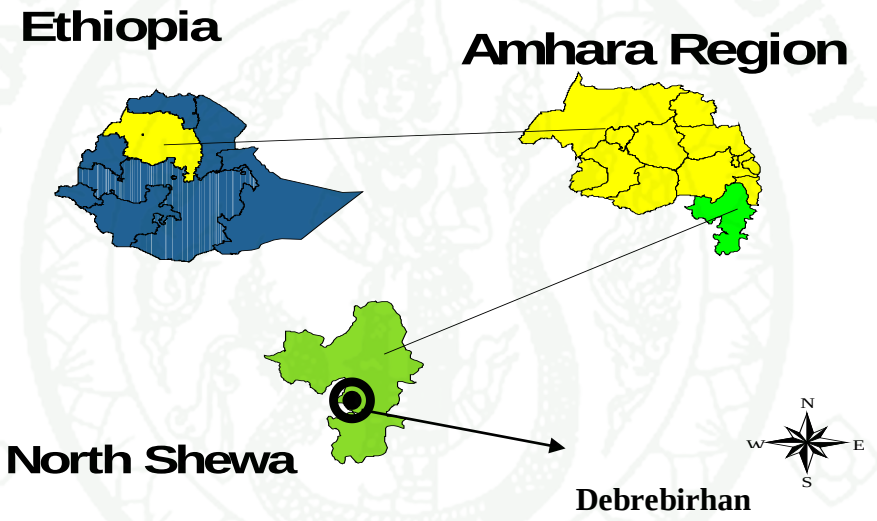

Figure 1 Map of North Shoa indicating Debrebirhan.

Description of the study area

Deberebirhan districts are found in North Shoa Zone of the Amhara Regional State. D/birhan located 130 km Northeast of Addis Ababa at an altitude 2876MASL, annual rain fall for 17 years average 911.2 mm and temperature maximum 19.67, minimum -2C degree celsius. (Ethiopia central statistics authority) (NMSA).

1. Prevalence and contamination rate of *Campylobacter* species in Sheep of North Shoa Debrebirhan Ethiopia

1.1. Fecal samples collection

In total of three hundred ten fecal samples were collected from three different sheep farms in selected centers of North Shoa Debrebirhan namely, Debre Birhan Agriculture Research Center (DBARC) (n=138), 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 to the National Animal Health Diagnostic and Investigation Center (NAHDIC) on the same day of collection in an ice box with cooled ice packs for laboratory examination.

1.2. Carcass samples collection

Of the 70 swabbed samples from carcasses, 30 were obtained from those sheep positive for *Campylobacter* spp. and 40 were obtained from those sheep negative for *Campylobacter* spp. from fecal samples. Carcass swabs were randomly collected from five different sites of conventionally slaughtered sheep (neck, thorax, abdomen, breast and crutch) using a sterile cotton swab. The swab samples were transferred to the laboratory using tubes containing 0.85% NaCl solution. All samples were transported under cool condition to National Animal Health Diagnostic and Investigation Center (NAHDIC) located at Sebeta Addis Ababa.

1.3. Culture and Identification of thermophilic *Campylobacter* species

Fecal and carcass swab samples were inoculated onto modified Charcoal, Cefoperazone, Desoxycholate Agar (mCCDA CM739B (Oxoid Ltd. Basingstoke, Hampshire UK). The mCCDA is a blood free selective medium with the CCDA selective supplement SR155E recommended for the isolation of thermophilic

Campylobacter spp from clinical and environmental samples. The plates were incubated in a microaerophilic atmosphere 5% O_2, 10% CO_2 and 85% N_2 by using CampyGen™ gas generating kits (Oxoid) at 42 ºC for 48 h.

Each *Campylobacter* spp. was identified based on the characteristics of colony appearance, Gram staining reactions and positive tests for oxidase and catalase reactions. Species differentiation was done based on hippurate hydrolysis and susceptibility to nalidixic acid (30µg) and cephalothin (30µg (Oxoid) including a positive control. These parameters formed the basis for the identification of *C. jejuni* and *C. coli* as proposed by others (On, 1996). The type strains *C. jejuni* (NCTC 11351) and *C. coli* (LMG 6440) were included as positive controls (kassa *et al*., 2007; Woldemariam *et al*., 2009).

Statistical analysis

Statistical analysis was conducted with the SAS system for windows and comparisons between the three centers and the carcass contamination sites were determined using the frequency procedure for the chi-square (χ^2) fisher exact test. A value of $p \leq 0.05$ was used as an indicative for statistically significant difference.

2. Detection of *C. jejuni* by polymerase chain reaction

The *Campylobacter jejuni* gene at position 1-402 (accession no. U27272) described by Ng *et al*. (1997) was used to amplify the target DNA fragment.

2.1. DNA isolation

Forty three *C. jejuni* samples were collected by centrifugation at 4,500 ×*g* for 15 min. The pellet was used to extract DNA using the method of Chen and Kuo, (1993) with modification. The pellet was placed in 300 µl of solution I (100 ml of solution I contained 0.2M sucrose, 0.1M NaCl, 0.03M Tris-HCl, 0.01M EDTA, pH

8.0) and 600 µl of solution II was added (100 ml of solution II contained 50mM Tris-HCl, 50mM EDTA, 5g of 10% SDS). After incubation at 65 ºC for 30 min, 300 µl of solution III (100 ml of solution III contained 5M potassium acetate 60 ml, glacial acetic acid 11.5 ml and 28.5 ml of distilled water) and 200 µl CH_2Cl, IAA were added, and were incubated at -20 ºC for 30 min and centrifuged at 16.000 ×*g* at 4 ºC for 10 min, and then the supernatant was carefully transferred into a new PCR tube followed by the addition of 400 µl isopropanol and further centrifugation at 16,000 ×*g* at 4 ºC for 10 min. The DNA pellet was washed with 70% ethanol then centrifuged at 16,000 ×*g* for 10 min at 4 ºC. Finally the DNA obtained was dried at room temperature, and was dissolved with 15-20 µl of distilled water.

2.2 Polymerase Chain Reaction (PCR)

Oligonucleotide primers specific for *Campylobacter* species 5'TGACGCTAGTGTTGTAGGAG 3' and 5'GTTGCACTTAGCGATGATGG 3' (CL2–CR3) described by Ng *et al*. (1997) for *C. jejuni* at position 1-402 (accession no. U27272) were used to amplify the DNA fragment. An amount of 25 µl of a standard PCR reaction mixture [containing 2 µl of DNA template, 2.5 µl of 10x PCR buffer (containing 50 mM KCl, 10 mM Tris– HCl, pH 8.4), 2.5 µl of 50 mM of MgCl, 1.0 µl tag DNA polymerase, 1.0 µl of 10mM dNTPs, 2.5 µl of each primer and 11 µl of deionized water] were added. The PCR tubes were placed in a thermocycler and the reaction started by denaturation for 10 min at 95 ºC followed by 25 cycles of denaturation at 95 ºC for 15 s, annealing at 48 ºC for 15 s and extension at 72 ºC for 30 s, and a final 10 min incubation at 72 ºC was allowed for the completion of the primer extension after the last cycle. The amplified product was electrophoresed in 1.2% agarose gel at 100V for 30 min stained with ethidium bromide and photographed by UV light.

2.3. DNA sequencing

The PCR products were purified using the Wizard® SV Gel and PCR Clean-UP System. The purified PCR product was used to perform long read sequencing using sequence analyzer at the DNA technology laboratory of the 1st BASE DNA Sequencing Company Malaysia. The ClustalX 1.81 and Genedoc software packages were used to analyze the DNA sequence obtained.

3. Effect of low temperature on the viability of *C. jejuni*

Approximately the same size of *C. jejuni* colonies were chosen from mCCDA blood free agar plate to be inoculated on 10 ml of 0.1% peptone broth + 0.9% NaCl. After obtaining 2.5 logCFU/ml concentration, 1ml from the original dilution was inoculated into eighteen tubes (18) of Brucella Broth (BB) (10^{-1}) and afterwaed was shacken at 50 rpm for 10 min, The cultures in BB were placed at 4 ºC and -20 ºC for 2, 7 and 14 days.

The subsequent cultivation was undertaken on plate of Muller Hinton agar with 5% sheep blood and incubated in a microaerophilic atmosphere (5% O_2, 10% CO_2 and 85% N_2) at 42 ºC for 48 h.

Statistical analysis

The SAS software was used to determine the significance differences of the effect of low temperature on the survival of *C. jejuni*, the data were determined using the mean procedure. A value of $p \leq 0.05$ was used to test statistically significant differences.

4. Antimicrobial activity of different types of wine against *C. jejuni*

Three types of wine white wine (Kemila) and two red wines (Aksumit and Gouder) with the same alcohol content of 11.5% were used in this study. *Campylobacter* inoculums were suspended in phosphate buffered solution (PBS) and the wines (red and white) each at a concentration of 1%, 10%, 25%, 50%, 75% and 100% then were serially diluted and were incubated for 10 min at 150 rpm in a shaker. Then, the inoculums was plated onto Mueller-Hinton agar with 5% sheep blood and incubated under microaerophilic conditions (85% N_2, 10% CO_2, 5% O_2) for 48 h at 42 ºC for the subsequent analysis.

5. Antimicrobial susceptibility pattern of *Campylobacter* species

Disk diffusion methods were used for the antimicrobial susceptibility testing of *Campylobacter* spp. according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2003). Standard inoculums were adjusted to 0.5 McFarland turbidity equivalents and then the cultures were swabbed onto Mueller-Hinton agar (Oxoid) 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) 10µg, erythromycin (E) 15µg**,** streptomycin (S) 10µg, tetracycline (TE) 30µg, nalidixic acid (NA) 30µg, penicillin (P) 10'IU', cephalothin (KF) 30µg, gentamicin (CN) 10µg, ciprofloxacin (CI) 5µg and chloramphenicol (C) 30µg. The plates were incubated under a microaerophilic-generated atmosphere (5% O_2, 10% CO_2 and 85% N_2) in an anaerobic jar (Oxoid) without a catalyst and using CampyGen^{TM} gas generating kits (Oxoid Ltd. Basingstoke, Hampshire England) at 42 ºC for 48 h. The diameters of the zone of inhibition were measured to the nearest millimeter. A standard reference strain of *E. coli* (ATCC 25922), that is sensitive to all antimicrobial drugs tested, was used as a control strain. The susceptibility test was conducted in the National Animal Health Diagnostic and Investigation Center (NAHDIC) Ethiopia.

Statistical analysis

The SAS software was used to determine the significance differences in antimicrobial susceptibility and the data were determined using the frequency procedure for the chi-square (χ^2) fisher exact test.

RESULTS AND DISCUSSION

Results

1. Prevalence and contamination rate of *Campylobacter* species isolated by conventional culture method

1.1. Isolation rate of *Campylobacter* from fecal samples

The numbers and percentages of the isolated thermophilic *Campylobacter* from the sheep fecal samples from three different farms were studied. Thirty-three (10.6%) *Campylobacter* spp. was isolated from 310 fecal samples. Out of the 33 isolated *Campylobacter*, 15 isolates (45.4%) were from the Debre Birhan Sheep Breeding and Forage Multiplication Center (DBSBFMC), 12 isolates (36.4%) were from the Debre Birhan Agriculture Research Center (DBARC), and the remaining 6 isolates (18.2%) were from Small Holder Farms (SHF). Of the 33 isolated thermophilic *Campylobacter*, *C. jejuni* and *C. coli* accounted for 29 (87.9%) and 4 (12.1%), respectively.

The isolation rates of the *Campylobacter* spp. in each of the three farm types are shown in Table 3, with 15 isolates (12.8%) from Debre Birhan Sheep Breeding and Forage Multiplication Center (DBSBFMC, n=117), 12 isolates (8.7%) from Debre Birhan Agriculture Research Center (DBARC, n=138) and 6 isolates (10.9%) on Small Holder Farms (SHF, n=55). A statistically significant difference was observed between the two *Campylobacter* species identified ($p < 0.05$) on all three farms.

Table 3 Prevalence of thermophilic *Campylobacter* species in sheep fecal samples obtained from three farms.

Farm	*Campylobacter* spp.		Total	P value
	C. jejuni	*C. coli*	No. (%)	
	No. (%)	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

DBSBFMC: Debre Birhan Sheep and Forage Multiplication Center

DBARC: Debre Birhan Agriculture Research Center

SHF: Small Holder Farms

[a,b] statistically significant difference between the two *Campylobacter* species identified

1.1.1. Incidence of *Campylobacter* in different indigenous and exotic breeds

The rate of isolation of *Campylobacter* in different sheep breeds is presented in Table 4. The results found that the isolation rate of *Campylobacter* was different between breeds, with 93.3% of the Awassi breed being positive for *Campylobacter* at DBSBFMC followed by 66.7% at SHF and 16.7% in DBARC.

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

Table 4 Occurrence of *Campylobacter* in different sheep breeds from three farms

Isolated *Campylobacter* spp	No. (%) *Campylobacter* isolated from different breeds at different farms					
	Awassi exotic and cross (n=116)	Bonga indigenous (n=26)	Menz indigenous (n=102)	Washera indigenous (n=15)	Adale indigenous (n=21)	Dorper exotic (n=30)
DBSBFMC (n = 15)	14 (93.3)	0	1 (6.7)	0	0	0
DBARC (n = 12)	2 (16.7)	5 (41.6)	2 (16.7)	1 (8.3)	0	2 (16.7)
SHF (n = 6)	4 (66.7)	0	2 (33.3)	0	0	0
Total (n = 33)	20 (60.6)	5 (15.2)	5 (15.2)	1 (3)	0	2 (6)

1.1.2. Seasonal variation vs. *Campylobacter* prevalence

The prevalence of *Campylobacter* spp. differed during the two different periods which were analyzed (Figure 2). The results showed that the prevalence of *Campylobacter* was high during the period from August to December in the three farm types. The overall prevalence during the period of August to December was 78.8%.

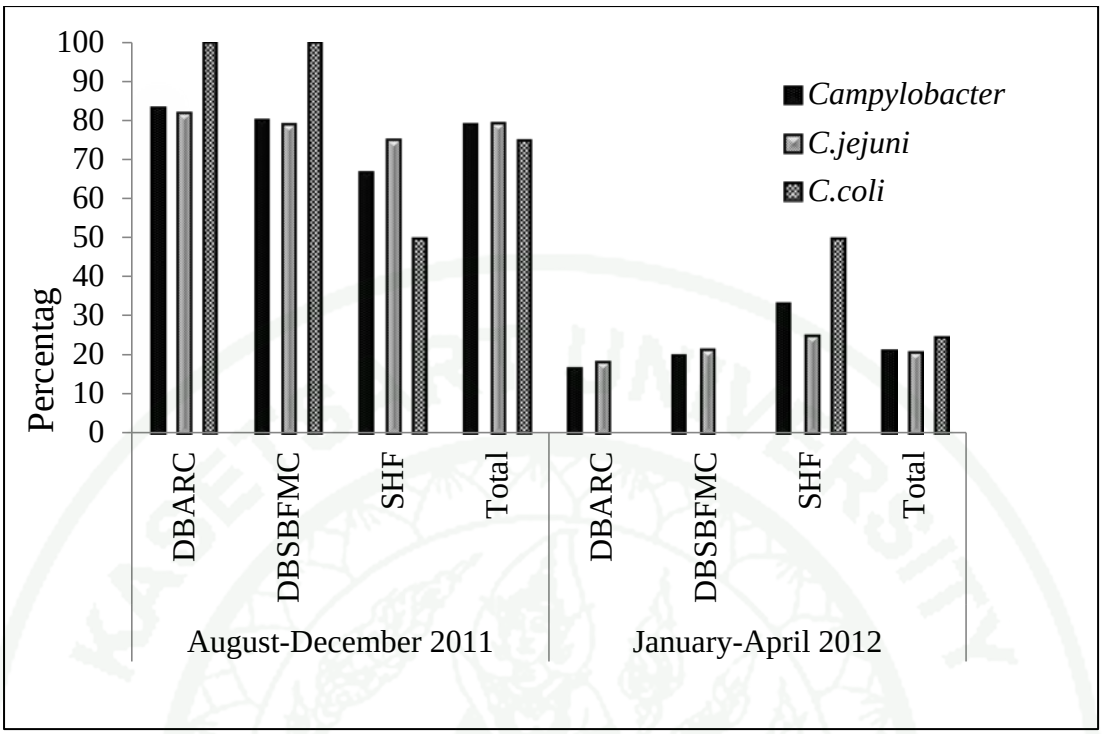

Figure 2 Prevalence of *Campylobacter* in two different periods.

The isolation rates during the period of August to December for the three farm types were 83.3% from DBARC, 80% from DBSBFMC and 66.7% from SHF, whereas a low prevalence (21.2%) was found during the period from January to April, with the isolation rates during January to April in the different farm types being 16.7% (DBARC), 33.3% (DBSBFMC) and 20% (SHF).

1.2. Isolation rate of *Campylobacter* from carcass samples

The numbers and percentages of the isolated thermophilic *Campylobacter* from the sheep carcass samples were obtained from five different sites (neck, thorax, abdomen, breast and crutch). The results in Table 5 show that 15 (21.4%) *Campylobacter* spp. was isolated from 70 carcass samples swabs. The numbers of isolates with the rates in parentheses of *Campylobacter* from the surface and deep parts of different sites were 13 (37%) and 2 (5.7%) isolates respectively. Out of the 15 thermophilic *Campylobacter* isolated from carcasses, *C. jejuni* and *C. coli* accounted

for 14 (93.3%) and 1 (6.7%), respectively. A statistically significant difference was observed between the two *Campylobacter* species identified ($p < 0.05$).

Table 5 Proportion of *Campylobacter* positive sheep carcasses according to different swabbing sites.

Swabbing site	No. of *Campylobacter* spp. (%)					
	Neck (n=7)	Thorax (n=7)	Abdomen (n=7)	Breast (n=7)	Crutch (n=7)	Total (35)
Surface swab						
C. jejuni	2 (5.7)	2 (5.7)	2 (5.7)	2 (5.7)	4 (11.4)	12 (34.2)
C. coli	0	1 (2.8)	0	0	0	1 (2.8)
Total (n=35)	2 (5.7)	3 (8.5)	2 (5.7)	2 (5.7)	4 (11.4)	13 (37)
Deep swab						
C. jejuni	1 (2.8%)	0 (0)	0 (0)	0 (0)	1 (2.8%)	2 (5.7%)
Total (n=35)						

The isolation rates of *Campylobacter* spp. from the surface swabbing sites (n=35) were 5.7%, 8.6%, 5.7%, 5.7% and 11.4% from the neck, thorax, abdomen, breast and crutch, respectively (Table 5). The isolation rate of *Campylobacter* spp. in the deep swabbing sites (n=35) was 2.8% from the neck and crutch (Table 5).

2. Screening of *C. jejuni* by polymerase chain reaction

2.1. Isolation of *C. jejuni* by conventional culture method and PCR

The amplification of *C. jejuni* genes was performed by polymerase chain reaction (PCR) with the set of primers CL2 -forward and CR3- reverse. The PCR product of *C. jejuni* (362bp) was obtained for *C. jejuni* detection as shown in Figure 3.

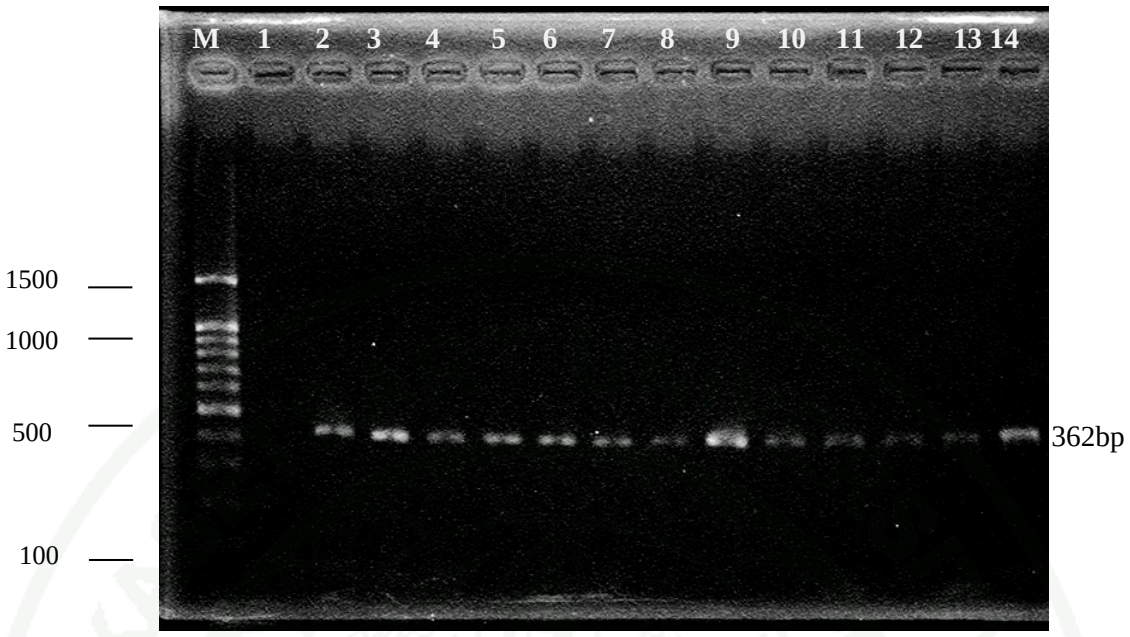

Figure 3 PCR amplification of *C. jejuni* gene on 1.2% agarose gel electrophoresis, the expected size for this gene is 362 bp. Lane M: 100 bp marker. Lane 1: Negative control. Lanes 2-14: *C. jejuni* gene isolated at position 1-402 (362 bp) from sheep feces and carcass swab samples.

In total, 43 *C. jejuni* isolates from sheep feces and carcass swab samples obtained by conventional culture method were also detected (100%) by polymerase chain reaction test, resulting in 100% positive concordance between the conventional culture and the PCR tests as shown in Table 6.

Table 6 Isolation of *C. jejuni* by conventional culture and PCR technique

Diagnostic techniques	*C. jejuni* detected
Conventional Microbiology	43
PCR	43

2.2. Nucleotied analysis

The PCR products of the *C. jejuni* gene isolates at position 1-402 (362 bp) (KC433407) were sequenced and analyzed. The nucleotide sequences of 362 bp were aligned together with the reference sequences of GenBank accession numbers CP000814, CP001900, CP000025, CP001961 and U27272 *C. jejuni* genes. The *C. jejuni* isolates (KC433407) when compared with the reference sequences were at the position 362 bp (Figure 4). The fragments from the candidate showed an exact match with the reference genes and the DNA sequences showed 97–100% similarity.

The sequence of *C. jejuni* exhibited 100% homology with the sequence from *C. jejuni* subsp. *jejuni* M1 and the *C. jejuni* subsp. *jejuni* 81116 completed genome, (362 bp compared with GenBank accession numbers CP001900 and CP000814). In addition, the sequence of *C. jejuni* (KC433407) showed 99% homology to *C. jejuni* subsp. *jejuni* S3 and RM1221 (362 bp compared with GenBank accession numbers CP001960 and CP00025) with 3 position differences and 12 position differences from U27272 which had 97% similarity (Primer reference, Ng *et al*. (1997). For the nucleotide sequences of *C. jejuni* gene 362 bp obtained in this study, the GenBank accession number given from the National Center for Biotechnology Information (NCBI) database was KC433407.

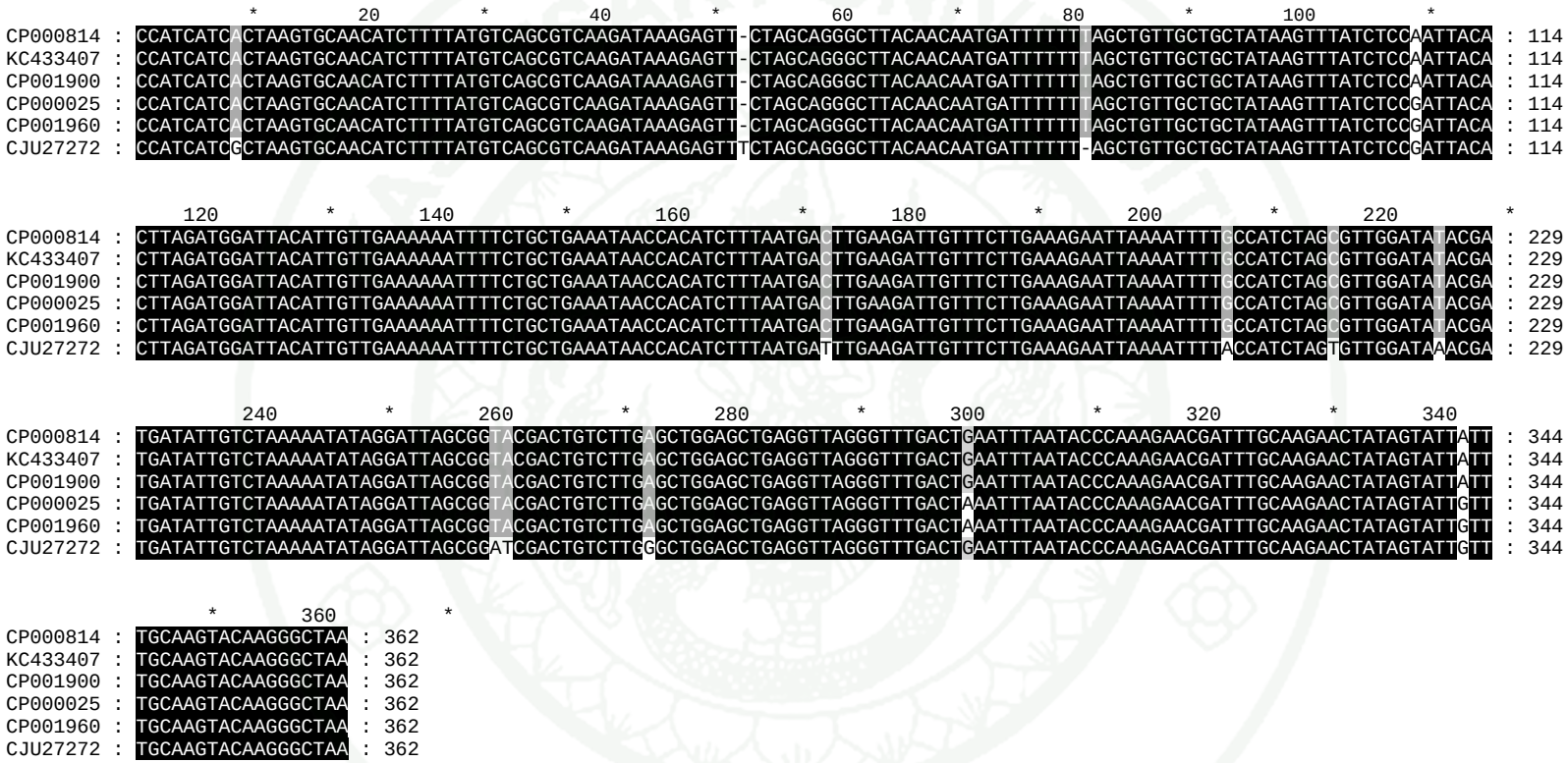

Figure 4 The nucleotide sequence alignment of *C. jejuni* subsp. *jejuni* M1, *C. jejuni* subsp. *jejuni* 81116, *C. jejuni* subsp. *jejuni* S3, *C. jejuni* subsp. *jejuni* RM1221 completed genome and *C. jejuni* subsp. *jejuni orf*1 (CP000814, CP001900, CP000025, CP001960, and CJU27272) and *C. jejuni* gene isolates at position 1-402 (362 bp) (KC433407).

3. Effect of low temperature on the viability of *C. jejuni*

Campylobacter jejuni cells lost their viability when frozen or refrigerated at -20 ºC and 4 ºC (Table 7). The number of *C. jejuni* cells was decreased to 0.54, 0.74 and 1.02 logCFU/ml during storage at 4 ºC for 2, 7 and 14 days, respectively, whereas during storage at -20 ºC for 2, 7 and 14 days, the reductions were 0.72, 1.17 and 1.52 Log CFU/ml, respectively. The results suggest that a high reduction occurred at -20 ºC. However, freezing by storage at -20 ºC for more than 2 days and refrigerating at 4 ºC for 1week produced more than a 1 logCFU/ml reduction of *C. jejuni*.

Table 7 The effect of low temperature on the viability of *Campylobacter jejuni*

	Viability of *C. jejuni* cells at low temperature			
Temperature	Day 0	Day 2	Day 7	Day 14
4 ºC	2.5±1.15	1.96±1.175	1.76*±1.097	1.48*±1.097
-20 ºC	2.5±1.255	1.78±1.072	1.33*±1.097	0.98*±1.076

Results are expressed as mean ± standard deviation Log cfu/ml (n = 3).
*High number of *C. jejuni* cell reduction

Long term freezing caused a higher level of reduction than did short term storage, the mean reduction ranges observed were from 0.54 to 1.02 logCFU/ml at 4 ºC and from 0.72 to 1.52 logCFU/ml at -20 ºC. Freezing by storage at -20 ºC for more than 2 days produced a high cell reduction rate for the *C. jejuni* count when compared with refrigeration at 4 ºC for 1 week.

4. Antimicrobial property of different wines against *C. jejuni*

We studied the effect of three wines white wine (Kemila) and 2 red wines (Aksumit and Gouder) with the same alcohol content of 11.5% at different

concentrations of 1, 10, 25, 50, 75 and 100% on the resistance capability of *C. jejuni*. All tested wines exhibited significant antimicrobial activity against *C. jejuni*

Table 8 show details of the viability of *C. jejuni* at different concentrations of wine. The white wine and red wines had a high microbicidal effect against *C. jejuni*, with the minimum microbicidal concentration of white wine and for both red wines being 10% and 25%, respectively. The results suggested that white wine has a higher microbicidal effect against *C. jejuni* than red wine because of the high count of *C. jejuni* was inactivated at 10% concentration for white wine whereas for red wines a high number of *C. jejuni* were inactivated at 25% concentration. There was no growth of *C. jejuni* in the 25% concentration and above with the white wine whereas the two red wines inhibited complete growth of *C. jejuni* at 50% concentration and above**.**

Table 8 The effect of different concentrations (1%, 10%, 25%, 50%, 75%, 100%) of white and two types of red wine on the viability of *C. jejuni*.

	Viability of *C. jejuni* at white and red wine concentration (%)						
Wine type	0	1	10	25	50	75	100
White wine /Kemila	2.37±1.03	1.95±1.08	0.75±0.91	0	0	0	0
Red wine /Axumit	2.38±1.03	1.98±1.06	1.04±1.004	0.06±0.97	0	0	0
Red wine /Gouder	2.38±0.96	2.01±1.15	1.19±1.06	0.59±0.99	0	0	0

Results are expressed as mean ± standard deviation LogCFU/ml (n = 3).

5. Antimicrobial susceptibility pattern of *Campylobacter* species

Campylobacter species chosen for the antimicrobial susceptibility test were isolated from healthy sheep feces and carcass swab samples. Forty three *C. jejuni* and

5 *C. coli* samples were used in the antimicrobial susceptibility tests. Based on the interpretation criteria shown in Table 9, out of 48 *Campylobacter* isolated, the highest level of resistance (100%) was recorded to cephalothin. The incidence of resistance to the other antimicrobial agents studied was 33.3% to ampicillin followed by 22.9% to tetracycline, 12.5% to erythromycin and 4.2% to streptomycin, chloramphenicol and gentamicin, and 2.1% to nalidixic acid and ciprofloxacin (Table 10).

Table 9 Interpretation criteria used for this study

Antibiotic	Zone diameter (mm)		
	S	I	R
Ampicillin (10 μg)	≥17	14-16	≤13
Erythromycin (15 μg)	≥23	14-22	≤13
Nalidixic acid (30 μg)	≥19	14-18	≤13
Tetracycline (30 μg)	≥19	15-18	≤14
Penicillin (10 'IU')	≥16	≥15	≤14
Streptomycin (10 μg)	≥15	12-14	≤11
Cephalothin (30 μg)	≥18	≥15-17	≤14
Gentamicin (10 μg)	≥15	13-14	≤12
Ciprofloxacin (5 μg)	≥21	16-20	≤15
Chloramphenicol (30 μg)	≥18	13-17	≤12

Zone diameter provided by CLSI for Enterobacteriaceae, Luangtongkum *et al*. (2007)
S= susceptible; I= intermediate; R= resistance.

A high level of *C. jejuni* resistance was recorded to cephalothin 43 (100%) followed by 15 (34.9%) to ampicillin, with the resistance rate of *C. jejuni* to tetracycline being 25.6%, 9.3% to erythromycin, 4.7% to streptomycin and chloramphenicol, and 2.3% to gentamicin and ciprofloxacin. There was no *C. jejuni* resistance to nalidixic acid in this study. According to the results of this study, 100%

of the isolates of *C. coli* were resistant to cephalothin, 40% to erythromycin and 20% to nalidixic acid, ampicillin and gentamicin.

Table 10 Antimicrobial resistance of *C. jejuni* and *C. coli* isolated from sheep in Debrebirhan Ethiopia

Antimicrobial agents	Number of resistant *Campylobacter* spp (%)		
	C. jejuni (n = 43)	*C. coli* (n = 5)	Total (n = 48)
Ampicillin	15 (34.9)	1 (20)	16 (33.3)
Erythromycin	4 (9.3)	2 (40)	6 (12.5)
Cephalothin	43 (100	5 (100)	48 (100)
Nalidixic acid	0	1 (20)	1 (2.1)
Penicillin	0	0	0
Streptomycin	2 (4.7)	0	2 (4.2)
Tetracycline	11 (25.6)	0	11 (22.9)
Gentamicin	1 (2.3)	1 (20)	2 (4.2)
Ciprofloxacin	1 (2.3)	0	1 (2.1)
Chloramphenicol	2 (4.7)	0	2 (4.2)

S= susceptible

I= intermediate

R= resistance.

Discussion

Previous studies from 1992 to 2010 in Ethiopia reported 70.6% *Campylobacter* in chicken followed by 52.41% in human, while the presence of *Campylobacter* in other food of animals was 50, 38 and 12.7% for pig, sheep and cattle, respectively. According to the reports for those studies, the incidence of *Campylobacter* in food of animal origin was 21.7% in chicken meat, 10.54% in sheep meat, 8.8% in goat meat, 8.5% in pig meat and 6.2% in cattle meat (Gedlu and Aseffa, 1996; Asrat *et al*., 1997; Getnet and Abrham, 2004; Dadi and Asrat, 2008; Woldemariam *et al*., 2009). Lammerding *et al*. (1988) reported that *Campylobacter* can be isolated from a high proportion of cattle, sheep and pig carcasses soon after slaughter.

Different countries have reported the presence of *Campylobacter* from different sources of samples: from humans (Anonymous, 2005b; Gedlu and Aseffa, 1996; Asrat *et al*., 1997; Getnet and Abrham, 2004; Samie *et al*., 2007; Ghafir *et al*., 2007; Ewnetu and Mihret, 2010), animals (Pearce *et al*., 2003; Nesbakken *et al*., 2003; Pezzotti *et al*., 2003; Zweifel *et al*., 2004; Kassa *et al*., 2007; Ewnetu and Mihret, 2010) and food of animal origin (Lammerding *et al*., 1988; Turnbuil and Rose, 1982; Bolton *et al*., 1985; Insook *et al*., 2007; Jozwiak *et al*., 2006; King and Adams, 2008; Mackiw *et al*., 2008; Dadi and Asrat, 2008; Woldemariam *et al*., 2009; Olivier *et al*., 2010). Throughout the world, *Campylobacte*r spp. are one of the most common pathogens of acute bacterial gastroenteritis and are transmitted predominantly via food from animals (Rautelin and Hanninen, 2000). The occurrence of human *Campylobacter* gastroenteritis has largely been attributed to the consumption of contaminated animal food products (Humphrey *et al*., 2007). The most common etiological agents of bacterial gastroenteritis in humans are *C. jejuni* and *C. coli* (Skirrow, 1994; Frost *et al*., 1998).

We investigated the prevalence of thermophilic *Campylobacter* spp. isolated from sheep feces and carcass samples during a 9 month period from August 2011 to

April 2012. The isolation rate (10.6%) of *Campylobacter* in this study in sheep feces was comparable with the value of 11.9% reported by Acik and Cetinkaya, (2006); however it was lower than the values of 38% reported by Kassa *et al*. (2007), 29.3% by Stanley *et al*. (1998) and 18% by Salihu *et al*. (2009), while Ewnetu and Mihret, (2010) recovered 72.7% *Campylobacter* from chicken in Bahir Dar Ethiopia. Nonetheless, the current results were higher than the values of 6.8% and 2.5% *Campylobacter* isolated from the intestinal contents of sheep and from their rectal swabs, respectively, in Kaduna Nigeria (Raji *et al*., 2000) and the value of 7.1% found in Lagos (Uaboi-Egbenni *et al*., 2008).

Humphrey *et al*. (2007) indicated that sheep may acquire *Campylobacter* by contact with a contaminated environment while the current study showed that the flock of DBSBFMC, which are more protected from the external environment, yielded a higher result (12.8%) than from small holder farm flocks (10.9%) where all types of food animals are kept and allowed to graze together. This may indicate that external exposure is not related with the occurrence of *Campylobacter* in the sheep flock. Similarly, Alter *et al*. (2005) reported 35% *Campylobacter* isolated from environmental samples and only 0.7% from the environment of conventional farm samples. On the other hand, Jensen *et al*. (2006) reported dissimilarity in *Campylobacter* subtypes isolated from the nearby environment and from outdoor pig rising.

The current study results provide an indication of how it is possible for contamination of the carcass by *Campylobacter* species during conventional slaughtering practice, as one possible way in which *Campylobacter* species can contaminate the carcass is by invading it from the intestinal tract since it was found in the intestinal tract by fecal examination and this was described by Pearce *et al*. (2003), Nesbakken *et al*. (2003) and Pezzotti *et al*. (2003). An additional possible option for carcass cross contamination might be via another route of invasion such as via the personnel who handle the carcass, or from equipment or water on a slaughter line as has been described by different previous studies (Jones *et al*., 1991; Herman *et*

al., 2003; Whyte *et al*., 2004). There is also support for the second option of invasion from the finding of *Campylobacter* species in the neck, thorax and crutch of the carcass of sheep that produced a negative result for *Campylobacter* by fecal examination in the current study.

The contamination rate of the sheep carcass in this study (21.4%) was in agreement with the level of 20% reported by Aquino *et al*. (2002) and relatively comparable with the value of 15.3% reported by Carbita *et al*. (1992). However our investigation into the prevalence of contamination in the sheep carcass produced results that were higher than the recorded values of 10.5% by Dadi and Daniel, (2008), 10.6% by Woldemariam *et al*. (2009), and of 11.8% and 7.4% reported by Whyte *et al*. (2004) and Little *et al*. (2008), respectively, although a high prevalence of *Campylobacter* species in different carcass samples (47.1%) was reported by Rahimi & Tajbakhsh, (2008) and a value of 29.9% by Rahimi *et al.* (2010). Insook *et al*. (2007) also reported 78.5% prevalence from broiler carcasses. The highest prevalence of *Campylobacter* in a sheep carcass in the current study may have been due to cross contamination during manual skinning and evisceration with the poor hygienic conditions of the conventional slaughter practice.

The isolation of thermophilic *Campylobacter* in the sheep carcasses of the current study provided evidence that the prevalence rate in conventionally slaughtered sheep carcasses was higher than previously reported in advanced (export) slaughterhouses in Ethiopia (Dadi and Daniel, 2008; Woldemariam *et al*., 2009). The results of the current study demonstrated that sheep carcass contamination has important implications for public health in a similar manner to that of other contaminated foods of animal origin reported previously (Berndston *et al*., 1992; Insook *et al*., 2007; Dadi and Daniel, 2008; Rahimi & Tajbakhsh, 2008; Woldemariam *et al*., 2009; Rahimi *et al.*, 2010).

Among the thermophilic *Campylobacter* isolated from both fecal and carcass samples, 87.5% were *C. jejuni* and 12.5% were *C. coli*. In all types of samples, the

most prevalent *Campylobacter* species recovered was *C. jejuni* which was in agreement with other reported findings (Tay *et al*., 1995; Nayak *et al*., 2003; Whyte *et al*., 2004; Kassa *et al*., 2007; Salihu *et al*., 2009; Rahimi *et al*., 2010) This implies that *C. jejuni* is the most common *Campylobacter* species in sheep in Ethiopia.

A high incidence (78.8%) of *Campylobacter* in sheep in the study area was found from August to December and a level of 21.2% was found from January to April. The seasonal fluctuation in the occurrence of *Campylobacter* in this study was in agreement with the values of 66.5% (Rahimi & Tajbakhsh, 2008) and 44.1% prevalence of *Campylobacter* in summer months (Rahimi *et al.*, 2010). In addition, Willis and Murray, (1997) reported 87–97% and 7–33% prevalence during warmer and winter months, respectively. Wallace *et al*. (1997) and Stanley *et al*. (1998) also reported seasonal variation in the occurrence of *Campylobacter* in chicken and lambs, respectively.

The Awassi exotic and cross breeds, Bonga, Menz, Adale, Washera indigenous breeds and Dorper exotic breed were used to study the prevalence of *Campylobacte*r spp. in this study. The results showed that the Awassi exotic and cross breeds are more susceptible than indigenous breeds since the prevalence was higher in breeds involving Awassi. The difference in the susceptibility may be associated with the environment in which the animals are reared (Alter *et al*., 2005; Jensen *et al*., 2006) or with the familiarization of the different sheep breeds with the climate.

Forty three (100%) of the *C. jejuni* isolates from the sheep feces and carcass swab samples by the conventional culture method were also positive by polymerase chain reaction, this result is comparable with 34 *C. jejuni* isolates by both conventional culture and the PCR method by Ertas *et al*. (2003). Rahimi *et al*. (2010) also reported 88.3% *C. jejuni* identified by the conventional culture method and by Polymerase Chain Reaction (PCR). The current study appeared to show that the conventional method can be used to characterize *C. jejuni*. Marianne *et al*. (2008)

reported diagnostic specificity between PCR and the cultural detection by selective enrichment and showed good agreement between the two methods.

Magistrado *et al*. (2001) also reported the effectiveness of the combination of the conventional culture and PCR methods for the rapid and sensitive finding of such bacteria. The conventional method for isolation of *Campylobacter* generally requires 6–7 days to confirm positive results, and species characterization is based only on the hippurate hydrolysis in the case of *C. jejuni* and *C. coli.* However, this phenotypic distinction not always will be accurate, so alternative means have been investigated for the detection of *Campylobacter* spp. Polymerase Chain Reaction (PCR is an excellent, sensitive test and a more rapid genetic assay for the identification and differentiation of *C. jejuni* and *C. coli* which threaten human and animal health (Gonsales *et al*., 1997; Linton *et al*., 1997; Lawson *et al*., 1998; Ertas *et al*., 2003).

The sequence of *C. jejuni* (362 bp) in the current study exhibited 100% homology with the sequence from *C. jejuni* subsp. *jejuni* M1 (1616648 bp) and the *C. jejuni* subsp. *jejuni* 81116 (1628115 bp) completed genome. However, the nucleotide analysis of those results did not suggest that the *C. jejuni* isolated was sup-species M1 or 81116 because it represented only a small region of 362 bp when compared to the completed genome. This finding is interesting for the further study of *C. jejuni* sub-species in Ethiopia.

The results of this study showed that the number of *C. jejuni* isolates decreased greatly during freezing. This observation is in agreement with earlier studies (Hanninen, 1981; Stern *et al*., 1985; Franklın *et al*., 2006). Our results showed a higher reduction rate during freezing at -20 ºC than during refrigeration at 4 ºC similar to the observations of Franklın *et al*. (2006). Different studies have shown *Campylobacter* cell reduction in beef, lamb and poultry meat storage at -19 ºC and -20 ºC from 1 to 2 log units during short and long term storage (Barrel, 1984; Blankenship and Craven, 1982; Franklin *et al*., 2006).

In the present study, we found that wines have potent antimicrobial activity which has been demonstrated in various experiments (Wen *et al*., 2003; Puupponen-Pimia *et al*., 2005; Rodríguez-Vaquero *et al*., 2007a; Rodríguez-Vaquero *et al*., 2007b; Monica *et al*., 200; Isohanni *et al*., 2010; Boban *et al*., 2010) where white and red wines were shown to have a very high bactericidal effect against *Campylobacter.* The report of Murray *et al*. (2002) showed that the consumption of wine reduced the health risk of pathogen contamination by 6%, demonstrating that wines have potent antimicrobial activity against food borne pathogens. In this study, we found that white and red wines with 11.5% alcohol had high antimicrobial activity against *C. jejuni* with the minimum microbicidal concentrations of 10% dilution for white and 25% for the two red wines, while Monica *et al*. (2009) reported the effect of white, rose and red wines with 11.5% ethanol and showed minimum microbicidal concentrations for red wine and white wine of 10% and 25%, respectively, by giving credit to the high level of phenolic compounds that red wine contains. However, Boban *et al*. (2010) confirmed that phenol-stripped wine has significant antimicrobial activity as did the intact wine and the potency of the antimicrobial power of intact wine could not be attributed to its phenolic and non-phenolic compound constituents, or perhaps it cannot be predicted on the basis of the contents of its particular components.

The higher antimicrobial activity of white wine with 11.5% ethanol in this study may indicate that phenolic compounds cannot be supposed to take the major role in the antimicrobial activity of wines, Weisse *et al*., (1995) also demonstrated that white wine dilutions reduced the number of organisms more rapidly than did red wine dilutions. Different components of wine have been proposed to contribute to its antimicrobial activity some studies placing emphasis on the role of wine phenolic compounds, while others have accentuated the role of the non-phenolic constituents of wine. The exact mechanisms responsible for the antimicrobial activity of wine are not yet fully understood (Wen *et al*., 2003; Puupponen-Pimia *et al*., 2005; Rodríguez-Vaquero *et al*., 2007a; Rodríguez-Vaquero *et al*., 2007b; Monica *et al*., 2009; Isohanni *et al*., 2010; Boban *et al*., 2010).

In Ethiopia, there have been few studies on the antimicrobial susceptibility of *Campylobacter* (Asrat *et al*., 1999). In the current study, 43 *C. jejuni* and 5 *C. coli* isolates were investigated in order to estimate the antimicrobial resistance of *Campylobacter* species isolated from different districts located in Debrebirhan, Ethiopia. All isolated (100%) thermophilic *Campylobacter* were resistant to cephalothin. Similar findings were reported by Asrat *et al*. (1999) and Kassa *et al*. (2007). While all *Campylobacter* isolated in this study showed susceptibility to penicillin, some previous studies reported differences in the susceptibility between cephalothin and penicillin; for example, 100% resistance to cephalothin and a high level of susceptibility to penicillin were reported by Therry *et al*. (1986), while Cliodna *et al*. (1985) similarly reported high susceptibility of all isolates of *Campylobacter pyloridis* to penicillin. Edmonds *et al*. (1985) also reported that 11 (78.6%) of 14 isolates of *Campylobacter fetus* were resistant to cephalothin and all of them (100%) were susceptible to penicillin. Other reports have shown a susceptibility difference between β-lactam antibiotics; Mohammed *et al*. (2007) reported 97% resistance of *Campylobacter* to cephalothin and only 11.7% to ampicillin. Sjorgren *et al*. (1992) showed 98.2% resistance to cephalothin and 20.9% to ampicillin.

These findings may indicate that the resistance of *Campylobacter* to β-lactam antibiotics may not always be related to the production of beta-lactamase. For example, in the findings of Aboderin *et al*. (2004), all isolates of *C. jejuni/coli* did not produce beta-lactamase, while Wright and Knowles (1980) isolated only 5.3% *C. jejuni* which produced β-lactamase. On the other hand, Lariviere *et al*. (1986) found 89% clinical isolates of *C. jejuni* produced β-lactamase, although only 14.6% were ampicillin resistant and the same study also reported that the production of β-lactamase is not always associated with resistance to β-lactams. It may be that the bacteria resist by any other mechanism other than hydrolyzed the antibiotic by β-lactamase, if the bacteria possess an efflux pump that expels the antimicrobial agent from the cell, or the cell wall becomes impermeable (changes in the bacterial cell wall permeability) (Barbosa and Levy, 2000; Schwarz and Chaslus-Dancla, 2001). Guo *et*

al. (2010) reported that the efflux mediated by cmeABC plays a significant role in the resistance to cephalosporin antibiotics in different *Campylobacter* spp.

The level of resistance of thermophilic *Campylobacter* to ampicillin in this study was 33.3% which is comparable with the level of 30% reported from Ireland by Fallon *et al*. (2003). However the current study finding was lower than 60% reported in Ethiopia by Asrat *et al*. (1999), 47.6% in Thailand by Sukhapesna *et al*. (2005), 62% in Malaysia by Tan *et al*. (2009), and 40 and 42% reported from Egypt and Singapore, respectively (Mikhail *et al*., 1989; Lim and Tay, 1989). Nonetheless, the results of the current study were higher than 10% reported by Dadi and Asrat, (2008) and 20% by Kassa *et al*. (2007). The incidence of resistance to tetracycline in the current study was 22.9%, which was comparable with the level of 20% reported by Fallon *et al*. (2003) and higher than other levels reported such as 10% by Dadi and Asrat, (2008) and 6% by Tan *et al*. (2009) and was much lower than the level of 77.94% reported by Sukhapesna *et al*. (2005). Different countries have reported low and high levels of resistance among *Campylobacter* species isolated from food and food of animals; 11.1–48.5% to ampicillin, 0–96% to tetracycline and 0–48% to streptomycin (Padungton and Kaneene, 2003; Taremi *et al*., 2006; Corcoran *et al*., 2006; Praakle *et al*., 2007). In Lebanon, 69% susceptibility to ampicillin and 49% to tetracycline was reported; in Israel 70% resistance was found to tetracycline and Saudi Arabia reported 32.7% resistance to tetracycline, while in Thailand, 25, 34, 56 and 75%, and 38, 50 and 60% resistance to ampicillin and tetracycline, respectively, was reported (Schwartz *et al*., 1993; Zaman, 1992; Talhouk *et al*., 1998; Abdulameer *et al*., 1999; Boonmar *et al*., 2005; Moore *et al*., 2006). Similar findings of those drugs in humans and food animal's isolates have been observed in Ethiopia (Asrat *et al*., 1999; Dadi and Asrat, 2008; Kassa *et al*., 2007; Ewnetu and Mihret, 2010).

According to the results of this study, 12.5% resistance of *Campylobacter* was recorded to erythromycin which is higher than the 0.7% reported by Kassa *et al*. (2007) and the 10.2% by Fallon *et al*. (2003) but lower than 55.5 and 18.8% reported by Boonmar *et al*. (2005) and Ewnetu and Mihret, (2010), respectively. The resistance

rate to streptomycin in the current study (4.2%) was higher than the 2% reported by Fallon *et al*. (2003) and much lower than the 42.65% reported by Sukhapesna *et al*. (2005). Our results showed 2.1% resistance to nalidixic acid, which was much lower than the reported levels of 92.2% by Han *et al*. (2007), of 20.5% by Tan *et al*. (2009) and of 10.2% by Ishihara *et al*. (2004). Moore *et al*. *(*2001) also reported resistance of *Campylobacter* to nalidixic acid, while a different range (14–98.7%) of resistance to fluoroquinolones was observed in various countries (Padungton and Kaneene, 2003).

The resistance rate of 34.9% of thermophilic *C. jejuni* to ampicillin was comparable with the rate of 35.9 % reported from Ireland by Fallon *et al*. (2003) and of 34%, (Boonmar *et al*. 2005). However, the current study finding was higher than those reported 18.8% by Little *et al*. (2008), 18.8% and 17.5% by Ewnetu and Mihret, (2010), 17.6% by Milfin *et al*. (2007) and 12.8% and 17% reported in Ethiopia by Dadi and Asrat, (2008) and Kassa *et al*. (2007), respectively. The resistance of *C. jejuni* to tetracycline was 25.6%, which was comparable with the levels of 22.2, 20.5 and 18.4% reported by others in Ethiopia, Ireland and Thailand (Ewnetu and Mihret, 2010; Fallon *et al*. 2003; Milfin *et al*. 2007) and higher than 5.1% reported by Dadi and Asrat, (2008) and 6% by Tan *et al*. (2009); however, it was lower than the 38, 50, and 60% findings by Boonmar *et al*. (2005) and the 77.94% reported by Sukhapesna *et al*. (2005). Our results showed 9.3% resistance of *C. jejuni* to erythromycin which was higher than 0.7% reported by Kassa *et al*. (2007) and lower than the 55.5% reported by Boonmar *et al*. (2005). Furthermore 4.7% resistance of *C. jejuni* was found to streptomycin, which was comparable to the level of 3.7%, reported by Kassa *et al*. (2007), while higher than 2.5 % (Fallon *et al*., 2003) and much lower than 42.65 % (Sukhapesna *et al*., 2005).

All 43 *C. jejuni* isolates were sensitive to nalidixic acid. This result was consistency with 97.8% and 100% reported in Ethiopia by Kassa *et al*. (2007) and Ewnetu and Mihret (2010), respectively. Smith *et al*. (1999) also reported the susceptibility of 71 *C. jejuni* isolates to nalidixic acid. On the other hand, Boonmar *et*

al. (2005) and Tan *et al*. (2009) reported 83.3% and 48% resistance of *C. jejuni* to nalidixic acid in Thailand and Malaysia, respectively.

In the present study, we found 100% *C. coli* resistance to cephalothin comparable with the 100% reported by Kassa *et al*. (2007), while the resistance rate of 40% of *C. coli* to erytromycine in the current study was higher than those findings of 3.9% and 16.7% reported by Kassa *et al*. (2007) and Little *et al*. (2008), respectively, Little *et al*. (2008) and Kassa *et al*. (2007) also reported 66.7 % and 27.5% resistance to ampicillin, respectively, which were higher than the 20% finding of the current study. Furthermore, our results showed 20% resistance of *C. coli* to gentamicine which was lower than the level of 55.6% reported by Dadi and Asrat (2008).

CONCLUSION AND RECOMMENDATION

Conclusion

This study indicates that *Campylobacter* is one of the most common manifestations of bacterial food borne disease with significant risk factors and economic and social effects in Ethiopia. The results of this study showed the presence of *C. jejuni* (87.5%) and *C. coli* (12.5%) in fecal and carcass swab samples and the risk of infection to humans through consumption of contaminated sheep meat.

Seasonal variation in the occurrence of *Campylobacter* was observed from August to December (78.8%) and from January to April (21.2%), which may direct further study into the impact of the season on the prevalence of such bacteria in Ethiopia. The seasonal variation may be associated with unidentified sources. In addition, our results showed that exotic breeds are more susceptible than cross and indigenous breeds, since the prevalence was higher (60.6%) in the Awassi breed. This difference in susceptibility may be associated with the familiarization of different sheep breeds with the climate.

Our results also revealed that raw meats originating from food animals in the traditional slaughter system are often contaminated with thermophilic *Campylobacter* spp. (21.4%). This problem in advanced (export) slaughterhouses can be reduced by implementing good manufacturing practices and a food safety assurance program but in the traditional slaughtering practices this will be difficult because of the very poor and clean less condition of animals at the time of slaughter, which in conjunction with the poor hygienic situation, represent a high risk of contamination of the carcass. Based on this argument, it is important to better understand the behavior of *C. jejuni* and *C. coli* in the traditional and advanced food production environment. The findings of this study showed also that freezing reduced *C. jejuni* cells by more than 1 log.

The current study showed that wines (white and red) had high antimicrobial activity against *C. jejuni* with the minimum microibicidal concentrations of 10% and 25%, respectively. The exposure of *Campylobacter* to white and red wines significantly reduced the number of viable cells. Additionally, the results of this study have significant importance in Ethiopia, where raw meat consumption is widely accustomed increasesing the possibility of pathogen transmission to humans.

This study revealed that antimicrobial resistance is found only at low frequencies for most of the antimicrobial agents tested. 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 food animals is low or that there has been no usage of these agents as growth promoters.

It is fact that the conventional culture method is laborious and consumes time and species characterization is based only on the hippurate hydrolysis. In contrast, isolation on the selective enriched media followed by identification of morphology, biochemical tests, hippurate hydrolysis, and nalidixic acid and cephalothin sensitivity testing is not considered at all cumbersome for the detection and differentiation of thermophilic *Campylobacter*, however the polymerase chain reaction method is fast, simple, more reliable and a suitable alternative method to conventional identification.

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testing is not considered at all cumbersome for the detection and differentiation of thermophilic *Campylobacter*, however the polymerase chain reaction method is fast, simple, more reliable and a suitable alternative method to conventional identification.

Recommendation

Further investigations are needed to clarify the incidence of the disease in food animals and in food of animal origin and to elucidate the importance for human infection in Ethiopia, where raw meat consumption is customary.

We recommend that organized action is needed to reduce the risks posed by *Campylobacter* in conventional slaughter systems in Ethiopia including an active hygienic exercise awareness program in all meat supply stages.

To reduce the disease burden of human campylobacteriosis that can be attributed to the meat production stage, it may be feasible to implement multiple barriers along the farm-to-table continuum of the product. This finding may warrant consideration of the public health benefit related to freezing food of animal origin—especially meat—to reduce the *Campylobacter* exposure level. Wines could be used as antimicrobial ingredients in meat. Wine consumption, especially with food, may protect humans against food borne illness. Further study is also needed on the continued surveillance of resistance patterns to guide the use of antimicrobial agents in the food of animals.

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APPENDICES

Appendix A

Campylobacteriosis in Ethiopia data from previous publish journals

Appendix Table A Campylobacteriosis in Ethiopia data from previous publish journals

Species	Isolate	Positive to *Campylobacter*					Sources	Year of study	Outer
		Jejuni	*coli*	*lari*	*Fetus*	Total			
Children 0-5	96	-	-	-	-	50	Ethiop.J.Health Dev. 18(3):185-189	Sep.2002-Jun.2003	Getnet and Abrham 2004
Children 0-4		-	-	-	-	60	Ann.trop.pediat. 3:207-12	Jun.1994-May 1995	Gedlu and Aseffa 1996
All age group	68	56	12	-	-	66	Epidemiol. infect. 118:91-95	Feb.1992-Jan1993	Daniel *et al* 1997
						1	>>	>>	>>
	210	16	1	-	-	17	Foodborne pathogen and dis. 7(6):667	Oct.2007-Apr.2008	Ewunetu and Mihret 2010
Sheep	71	16	11	-	-	27	Vet. Microbial. 119:82-87	Jan.-Apr. 2004	Kassa *et al* 2007
Cattle	205	14	10	2	-	26	>>	>>	>>
Chicken	220	148	12	-	-	160	Foodborne pathogen and dis. 7(6):667	Oct.2007-Apr.2008	Ewunetu and Mihiret 2010
	191	105	21	4	-	130	Vet. Microbial. 119:82-87	Jan.-Apr. 2004	Kassa *et al* 2007
Pig	18	-	9	-	-	9	>>	>>	>>

Appendix Table A (continued)

Species or food type	Isolate	Positive to *Campylobacter*				Sources	Year of study	Outer
		Jejuni	*coli*	*lari*	Total			
Sheep meat	114	10	2	-	12	Ethiop.J.Health Dev. 22(2):195-200	Nov.2006-Apr.2007	Dadi and Asrat 2008
	218	17	6	-	23	Ethiop.J.Health Dev. 23(3):229-233		Woldemariam *et al* 2009
Goat meat	92	5	2	-	7	Ethiop.J.Health Dev. 22(2):195-200	Nov.2006-Apr.2007	Dadi and Asrat 2008
	180	12	5	-	17	Ethiop.J.Health Dev. 23(3):229-233		Woldemariam *et al* 2009
Cattle meat	227	12	2	-	14	Ethiop.J.Health Dev. 22(2):195-200	Nov.2006-Apr.2007	Dadi and Asrat 2008
Chicken meat	60	11	1	1	13	>>	>>	>>
Pig meat	47	1	2	1	4	>>	>>	>>

Appendix B

Mean concentrations (mg/L) of metals in the Ethiopian Red and White wines used for antimicrobial property test against *C. jejuni*

Appendix Table B Mean concentrations (mg/L) of metals in the Ethiopian Red and White wines used for antimicrobial property test against *C. jejuni*

Metal	Ethiopian wine brand		
	Axumite red wine	Gouder red wine	Kemila white wine
K	767 ± 15	694 ± 15	735 ± 13
Mg	79.2 ± 5.5	66.0 ± 5.0	58.1 ± 4.3
Ca	37.1 ± 2.8	28.4 ± 2.2	28.4 ± 2.2
Na	24.4 ± 1.9	24.3 ±1.7	24.0 ± 1.9
Fe	3.16 ± 0.25	1.49 ± 0.06	2.33 ± 0.15
Zn	2.14 ± 0.15	2.70 ± 0.15	1.82 ± 0.12
Mn	1.46 ± 0.03	1.56 ± 0.12	1.04 ± 0.02
Cu	1.50 ± 0.08	0.55 ± 0.04	0.50 ± 0.03
Pb	0.25 ±0.02	0.16 ± 0.01	0.14 ± 0.01
Cr	0.19 ± 0.01	< MDL	< MDL
Ni	0.19 ± 0.01	0.19 ± 0.01	0.2 ± 0.01
Cd	< MDL	< MDL	< MDL
Co	< MDL	< MDL	< MDL

MDL = Method detection limit.

Source: Daniel and Bhagwan, (2011)

Appendix C

Sheep breeds used for this study

Appendix C Sheep breeds used for this study

Awassi 100% extotic breed

Awassi cross (Meanz + Awassi)

Bonga indigenous breed

Dorper 100% extotic breed

Washera indigenous breed

Menzie indigenous breed

Adale indigenous breed

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